METHODOLOGIES FOR THE SYNTHESIS OF NOVEL MODIFIED NUCLEOSIDES WITH THERAPEUTIC POTENTIAL

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ABSTRACT

The discovery of synthetic nucleosides with antiviral activity has ushered in a new era in chemistry and medicine, one that can be equated to the evolvement of antibiotics to treat bacterial infections. Innovative methodologies for nucleoside synthesis have played and will continue to play important roles in this development. In the last fifteen years, the major emphasis of the research efforts of the candidate has been the development and application of methodologies for the synthesis of new congeners, isosteres and analogues of the natural nucleosides and related heterocyclic systems with potential anticancer and/or antiviral activities and with potential as biological probes.

Some important new methodologies developed by him for the synthesis of biologically relevant nucleosides may be summarized as follows: thermal radical approaches for reductive deaminations, deamination-halogenation reactions, and deamination-thioalkylations; photochemical methodologies for carbon-carbon bond formation including arylations and heteroarylations and the regiospecific introduction of synthons; photochemical hydrations, alkylthiolations, and reductive dehalogenations; and regiospecific metal-mediated functionalization reactions. Other approaches include a deoxygenation methodology, the use of modifying reagents for the synthesis of novel isosteres and ring-extended bases and nucleosides, and the synthesis of new fluorescent dihydropyridines as potential nucleoside drug delivery systems. Contributions to the chemistry of heterocycles related to the nucleic acid bases were also made.

The major impact of all of this work has been in the

creation of new avenues of investigative research in synthetic nucleoside and related chemistry, and in the availability of novel therapeutically interesting nucleosides previously unknown and inaccessible or hypothesized and inaccessible or known and poorly accessible because of severe limitations in synthetic approaches and methodologies. These investigations have also changed substantially the way in which other researchers view the field as radical, photochemical and metal-mediated synthetic methodologies were rarely used previously in nucleoside and related chemistry.

STATEMENTS

This thesis contains no material which has been submitted by the candidate for another degree in any University.

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Vasu Nair

ACKNOWLEDGEMENT

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Anticancer evaluation studies were carried out at the National Cancer Institute, RNA antiviral screenings were performed through the U. S. Army Medical Research and Development Command, and anti-HIV evaluations were performed at the Burroughs Wellcome Company and at the Rega Institute for Medical Research, Leuven.

The candidate also expresses his gratitude to the many graduate and undergraduate students and postdoctoral fellows who have worked with him over the last two decades at the University of Iowa.

Finally, very special thanks go his wife, Barbara Baker Nair, and to his daughter, Alison Baker Nair, for their constant encouragement and moral support of his research efforts. This thesis is dedicated to them.

RESEARCH ACCOMPLISHMENTS

A. General Statement

Professor Nair's research contributions have been in the general areas of synthetic organic, bioorganic and heterocyclic chemistry. His scientific contributions that are being submitted as his credentials for the D.Sc. degree include research work in his chosen major field of nucleoside and related chemistry. This work was performed almost entirely at the University of Iowa with graduate students and postdoctoral fellows working under his direct supervision. Additionally, it should be mentioned that almost all of the fundamental ideas on design of target molecules, biological rationale, synthetic methodologies and approaches were conceived by Professor Nair and originated from him. Some of the synthetic work and enzymology as well as a major part of the data interpretation also were carried out by him. All of the manuscripts were largely written by him. His scientific contributions in nucleoside and related chemistry are summarized in the following pages. The originality of these contributions and their relationship to the work of others are discussed. Relevant references which address these items in more detail are cited. A list of Professor Nair's publications is included, with notations on joint publications of the extent of his contributions to the nearest ten percent. Copies of selected publications are included in the Appendix.

Professor Nair has been recognized nationally and internationally for his research work in the nucleoside area through many research grants and contracts, through faculty

scholar awards, through a science medal, through four major current consultantships (three with pharmaceutical companies and one with the National Institutes of Health), and through many invited lectures and presentations including a planery lecture at the 1988 International Symposium on "Nucleosides, Nucleotides, and their Biological Applications." He has been invited to chair sessions in his research area at National American Chemical Society Meetings for the last ten years and he is currently on an American Chemical Society National Awards Committee. In 1990, he was invited to write an authoritative research-oriented book on "New Methodologies in Nucleoside Synthesis" in the series "Best Synthetic Methods," edited by A. R. Katritzky, C. W. Rees and O. Meth-Cohn. He was a Distinguished Visiting Scholar at the University of Adelaide in July, 1987 (for additional information, see curriculum vitae).

B. Background

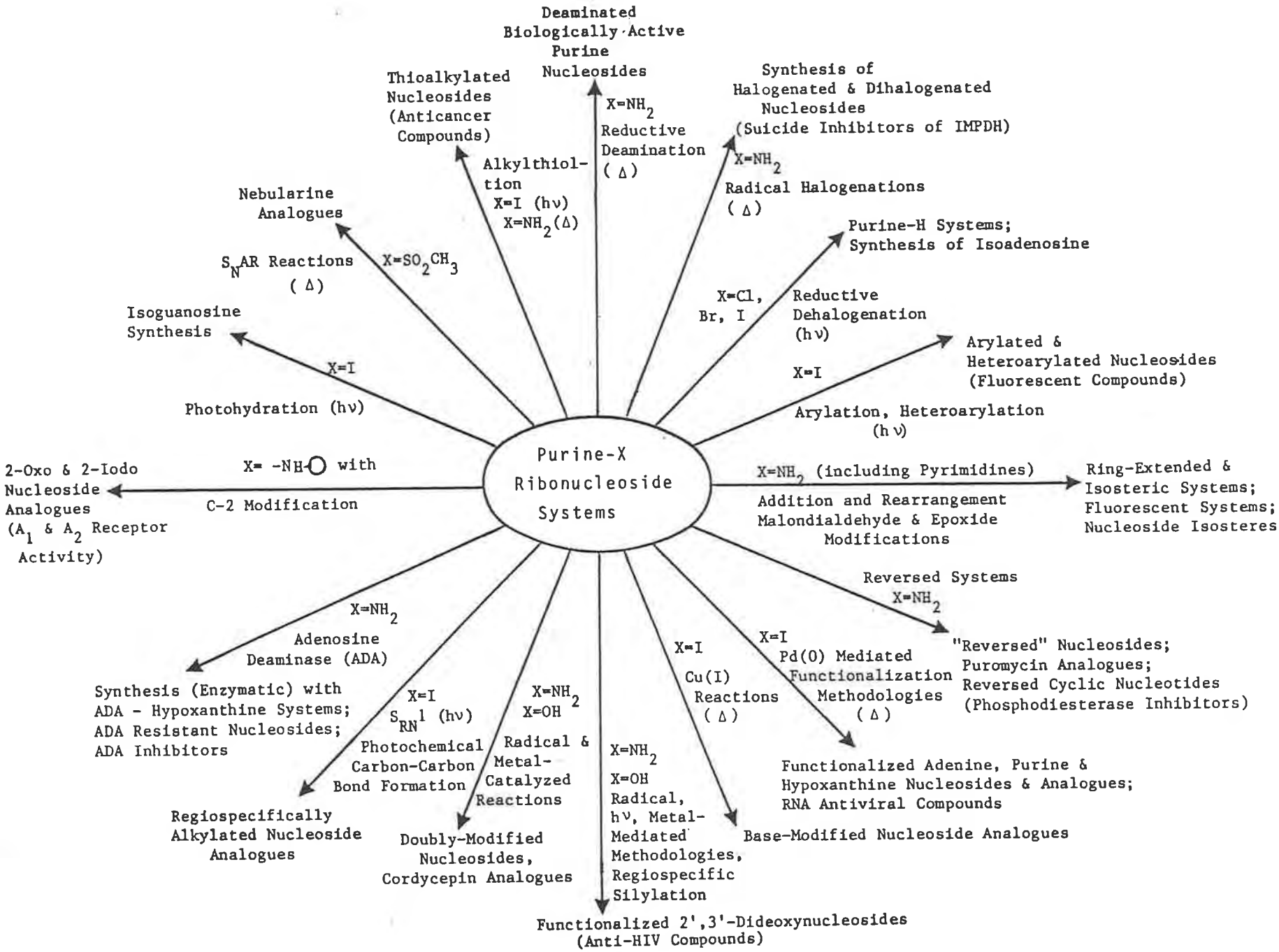
Nucleosides and nucleotides are important molecules, not only because they are components of the basic macromolecules of life (DNA and RNA), but also because of their critical role in many fundamental enzyme-catalyzed reactions in nature. These molecules have played an essential role in the understanding of the structures and biosynthesis of the nucleic acids and have contributed to the birth of the field of molecular biology. Many synthetic analogues of the natural nucleosides and nucleotides have also contributed to the scientific understanding of various aspects of cellular biochemistry including intermediary metabolism, enzyme mechanism, and hormonal action. Some

analogues of the natural purine and pyrimidine nucleosides have been found to have antibacterial and anticancer activities. The discovery of nucleosides with antiviral activity has given the field of nucleoside chemistry a great impetus in the last decade and has ushered in a new era in chemistry and medicine, one that can be compared to the development of antibiotics to treat bacterial infections. Innovative methodologies for the synthesis of novel and unique nucleosides has played and will continue to play an important role in this new era of nucleoside chemistry. With respect to this, it should be very clearly stated that nucleosides are complex multifunctional molecules and synthetic methodologies in most cases have to be specifically developed for this area even if related synthetic chemistry is known in other areas.

C. Development of Methodologies and Synthesis of Novel, Strategically Modified, Biologically Relevant Purine Nucleosides

In the last 15 years, a major emphasis of the research efforts in the candidate's laboratory has been devoted to the development and application of methodologies for the synthesis of novel congeners and analogues of the natural nucleosides. Several hundred such compounds have been successfully synthesized. The biological rationale for this work has been that these compounds would have antiviral and/ or anticancer activity and would also be useful as biological probes for the study of specific enzyme-catalyzed reactions. Some of his contributions in this area are presented in a generalized format in Scheme 1.

The major impact of this work has been in the creation of



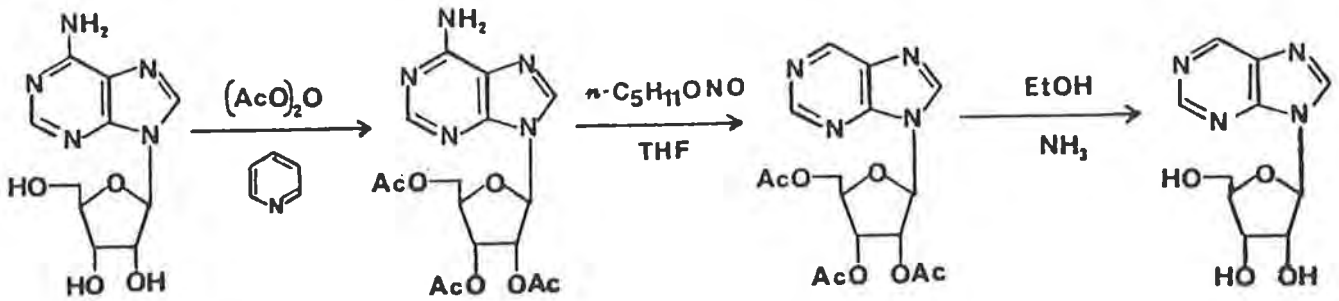
Scheme 1. Examples of the Creation of New Avenues of Research in the Nucleoside Field by Professor Nair

new avenues of investigative research in synthetic nucleoside chemistry and in the availability of novel therapeutically interesting nucleosides previously unknown and inaccessible or hypothesized and inaccessible or known and poorly accessible because of severe limitations in synthetic approaches and methodologies. These investigations have opened a significant new chapter in nucleoside chemistry and has changed substantially the way in which other researchers view the field. The use of radical, photochemical and metal-mediated synthetic methodologies were rarely or never used in the field of nucleoside chemistry until the candidate opened avenues in this area. Specific examples of these and other new methodologies in nucleoside synthesis follow.

(i) New Methodology for the Deamination of Aminopurine Nucleosides

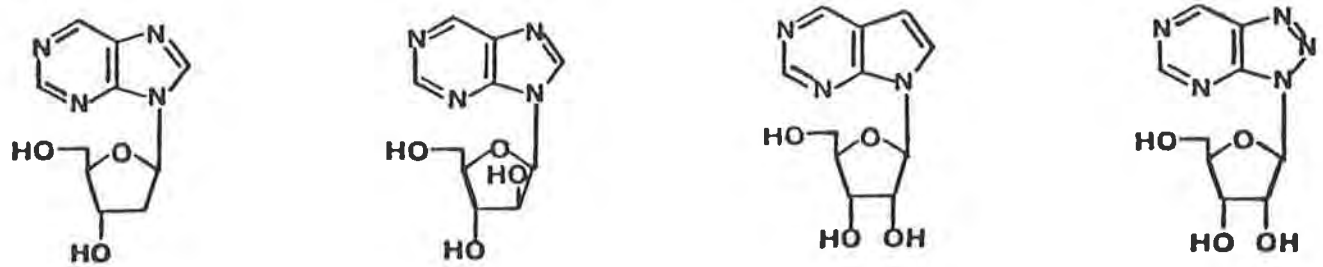
Although the deamination of aromatic amines with replacement by hydrogen of the amino group has been accomplished in numerous systems, such diazotization-deamination reportedly fails under a variety of conditions for adenine containing compounds.[1,2] While 6-diazonium salts of purines have never been isolated, conversion of adenine to hypoxanthine in nitrous acid,[3] and of 6-aminopurine derivatives to 6-fluoropurines in low yields in the presence of tetrafluoroboric acid and sodium nitrite,[4,5] are presumptive of diazonium intermediates where the counterion acts as a nucleophile. Nair, Richardson and Chamberlain have discovered an excellent new methodology for the reductive deamination of adenine nucleosides (Scheme 2).[6-8] In the development of this approach, they utilized information on the known ability of alkyl nitrites

to produce aryl radicals from arylamines.[9] For example, the nucleoside antibiotic, nebularine, was synthesized from readily available adenosine by the thermal reaction of adenosine triacetate with n-pentyl nitrite or t-butyl nitrite in tetrahydrofuran. The solvent acts as the hydrogen atom donor. The generality of the procedure was established by the synthesis of a number of biologically-active nucleosides utilizing this approach including 2'-deoxynebularine, arabinonebularine, 7-deazanebularine, and 8-azanebularine.[7] Non-nucleoside heterocyclic systems can also be reductively deaminated. Details of the approach appear as an invited contribution in the book "Nucleic Acid Chemistry", Part 4, 1989.[10]



Adenosine

Nebularine



2'-Deoxynebularine Arabinonebularine 7-Deazanebularine 8-Azanebularine

Preferred Conformations Not Implied In All Structural Representations

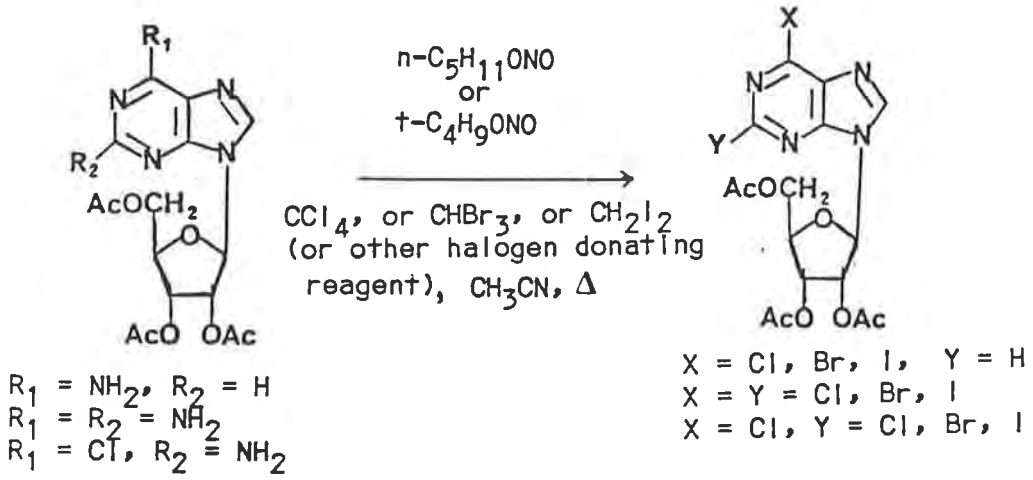
Scheme 2. New Methodology for the Reductive Deamination of Amino Purine Nucleosides and Related Compounds.

(ii) New Methodology for the Synthesis of Halogenated Nucleosides

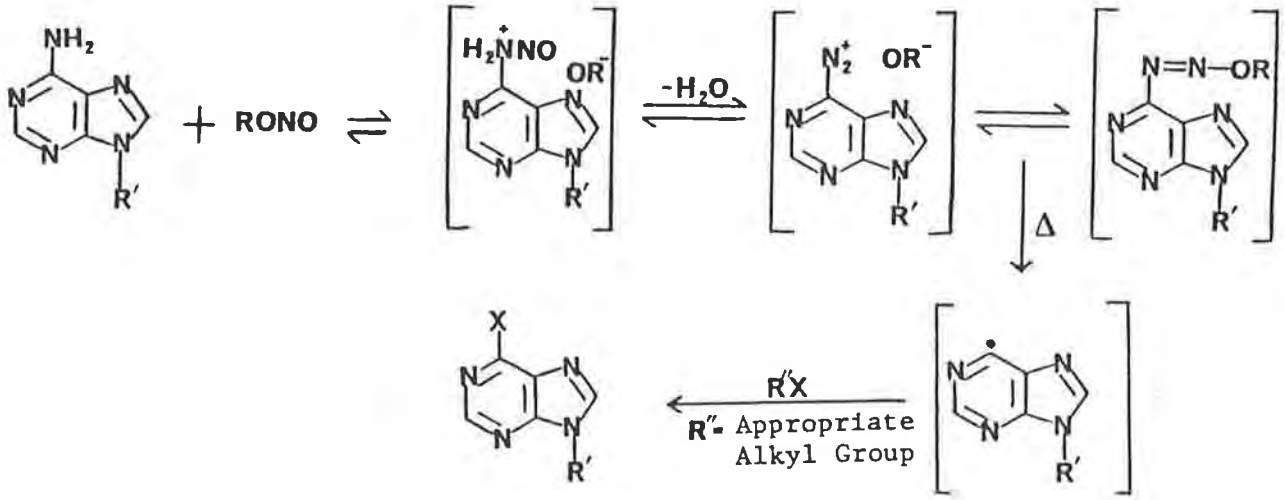
Modification of the purine base through the regiospecific introduction of halogens is of considerable significance in nucleoside chemistry. Halogenated purines and their nucleosides are important because of their possible utilization as probes in cell proliferation studies and in enzyme-catalyzed reactions (e.g. 6-halogenated purine ribonucleosides are suicide inhibitors of the purine metabolizing enzyme, inosine monophosphate dehydrogenase). They also find utility as synthetic intermediates for the preparation of alkyl, aryl, thio, thioalkyl, azido, seleno, and other purines. Synthetic access to halogenated purine bases and their derived nucleosides had been limited in general by rather harsh reaction conditions, low product yields, and/or difficulties associated with obtaining starting compounds.[11-13]

A new methodology for the specific halogenation of nucleosides through radical intermediates was discovered in Professor Nair's laboratory. As previously mentioned, Nair discovered that purinyl radicals are apparently formed when 6-diazonium salts (or azo compounds) generated under non-aqueous diazotization conditions from 9-substituted adenines are subjected to thermolytic homolysis. These transient radicals can abstract hydrogen atoms from tetrahydrofuran or other donating solvents (as previously described for reductive deaminations) or they can abstract halogen atoms from halocarbon solvents or reagents (Scheme 3).[8,14] Regiospecificity was seen in all of the cases studied. Hydrogen atom abstraction was not a complication in halogenations where halocarbon solvents containing hydrogen(s)

were used because of the large difference in bond dissociation energies between carbon-hydrogen and carbon-halogen bonds. The reactions were extended to include dihalogenated nucleosides.[15] These transformations, discovered in his laboratory,[6-8,14-16] represent the first examples of the generation and utilization of neutral purinyl radicals in nucleic acid chemistry. It should be mentioned that this radical halogenation methodology is currently the method of choice for the synthesis of halogenated nucleosides from appropriate amino nucleoside precursors.



Plausible Mechanism (6-Halonebularine Case):

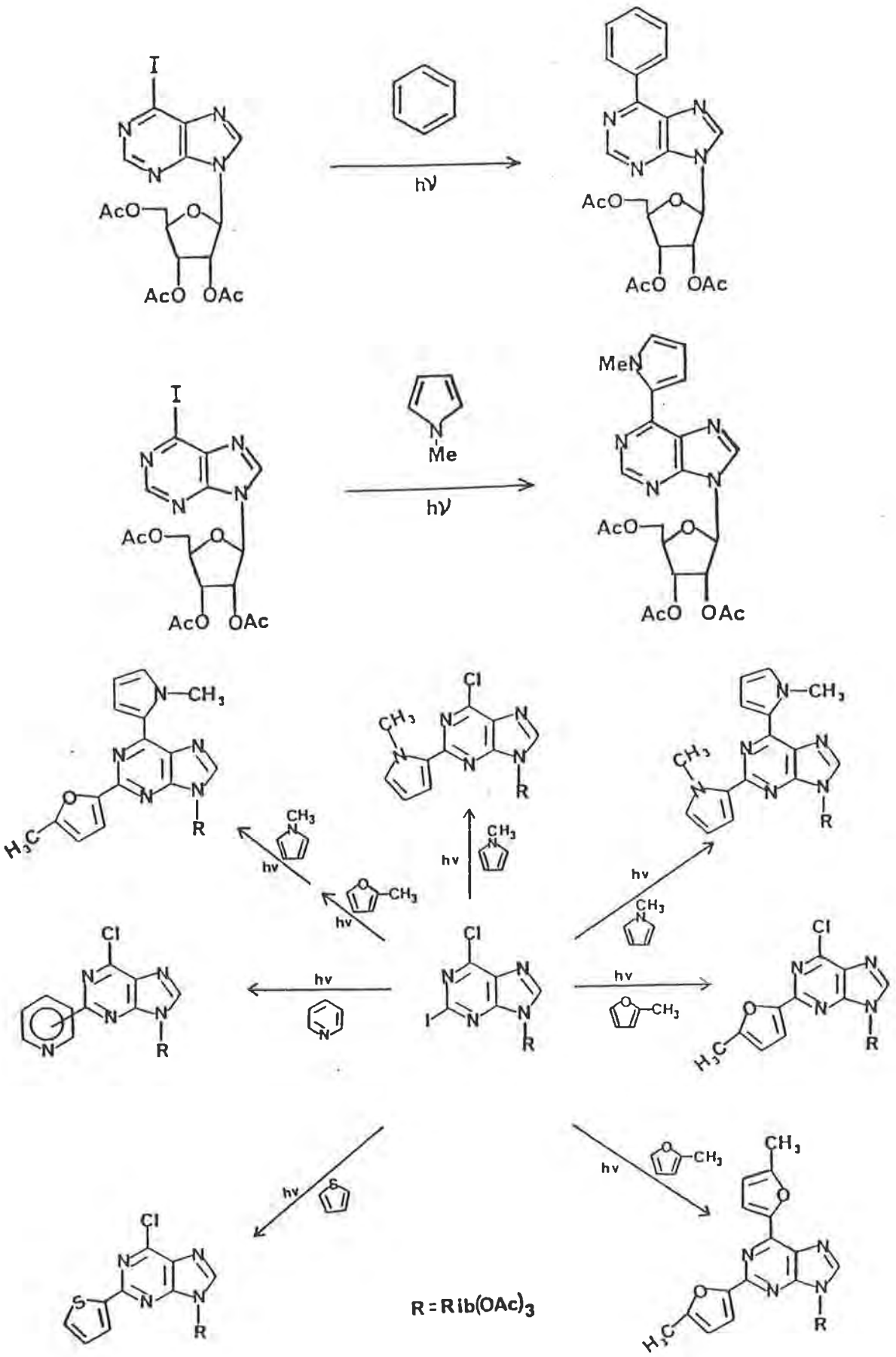


Scheme 3. **A New Methodology for the Synthesis of Halogenated and Dihalogenated Nucleosides.**

(iii) Photochemical Carbon-Carbon Bond Formation

Nitrogen-carbon bond forming reactions have received considerable interest in the chemistry and biology of purine nucleosides and nucleotides because of the presence of N-alkylated bases in certain tRNA and because of studies on the mechanism of action of some mutagenic and anticancer agents (see for example references 17 and 18). However, the same cannot be said for carbon-carbon bond forming reactions. Although carbon-carbon bond forming reactions are potentially effective approaches to the synthesis of a wide variety of structurally and biologically interesting nucleosides, this methodology has been of limited synthetic utility in the chemistry of purine nucleosides.[19-23]

Nair, Richardson, Young and Coffman discovered that purinyl radicals appeared to be generated in the photolysis of 6-iodopurines and that these transient radicals could be regiospecifically trapped by aromatic and heteroaromatic compounds to give the corresponding arylated and heteroarylated purines (Scheme 4).[24-26] At the time that this investigation was done, there were very few examples of arylated or heteroarylated nucleosides known and there were very few general methods for the introduction of aryl or heteroaryl groups into nucleic acid bases of nucleosides.[27-29] The photoarylated methodology described by Nair and coworkers represents a new, efficient and novel approach for the introduction of such groups into the 6-position of purine nucleosides. In addition, the methodology can be extended to include 2-arylated and 2-heteroarylated nucleosides, as well as the corresponding 2,6-

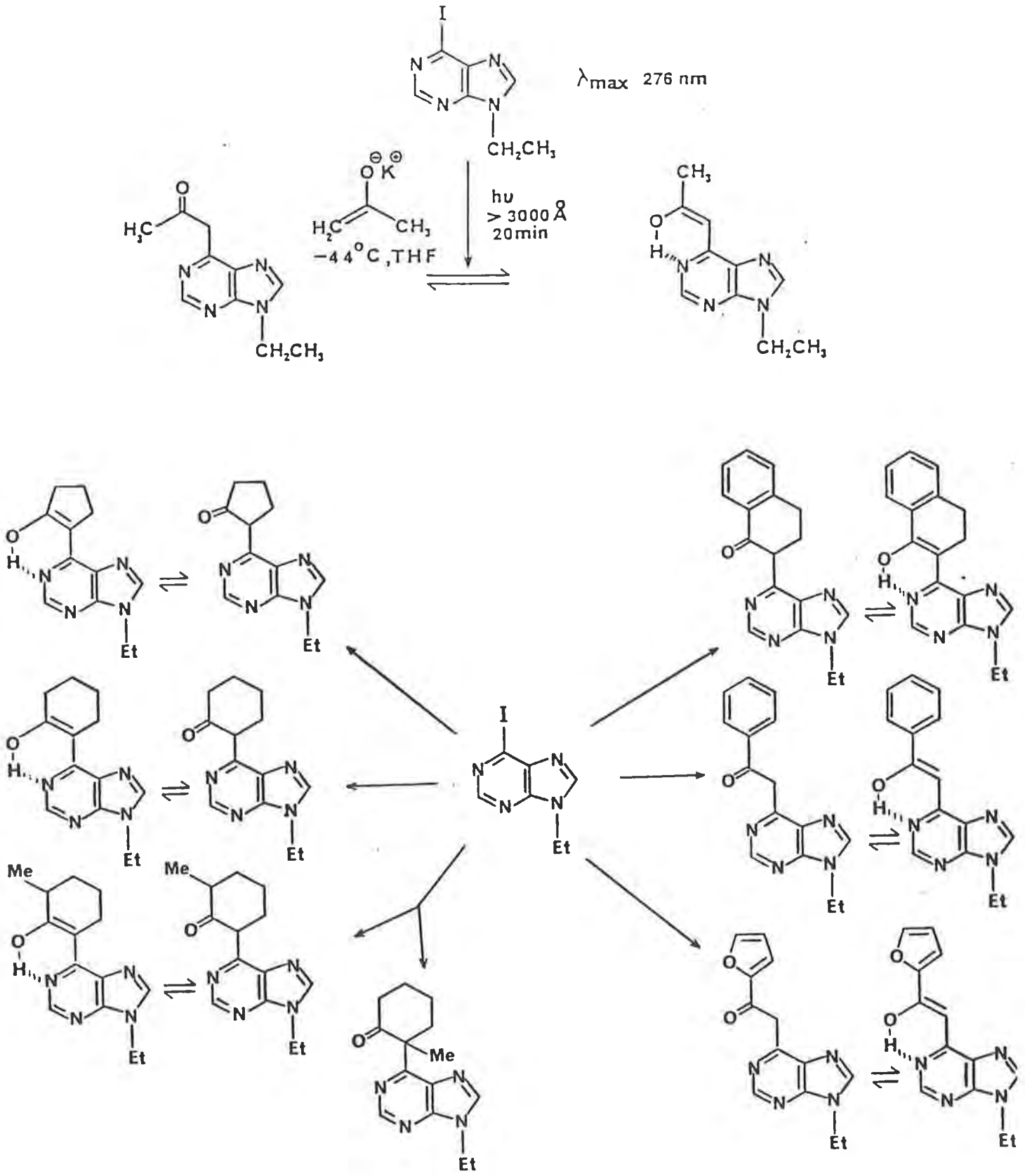


Scheme 4. Photochemical Carbon-Carbon Bond Formation: A New Methodology for the Synthesis of Novel Arylated and Heteroarylated Nucleosides.

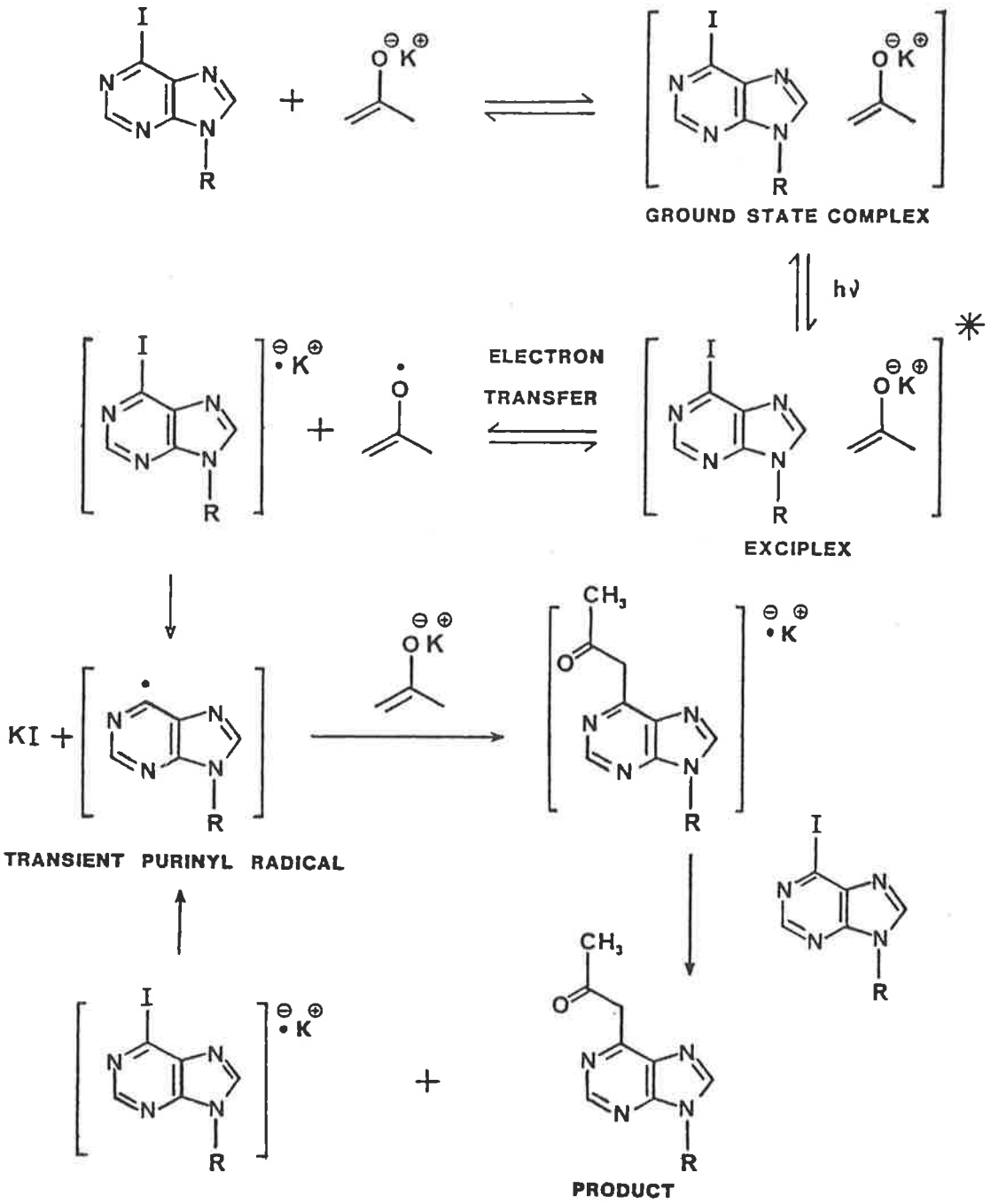
disubstituted nucleosides.[26] The reactivity of the aromatic or heteroaromatic system in these reactions follow the order: pyrrole > furan > thiophene > benzene > pyridine. Except in the case of pyridine, the reactions were regiospecific. Some of the heteroarylated nucleosides exhibit interesting fluorescence properties and specifically designed analogues of these compounds may be useful as biological probes in the interface area of chemistry and molecular biology.[30-33]

In another major advancement in this area, Nair and Chamberlain successfully developed a new, synthetically useful method of carbon-carbon bond formation in purines through a photochemical $S_{RN}1$ reaction.[34-36] Such photochemical reactions have not been previously developed for purine nucleoside chemistry. This approach has wide applicability particularly in the synthesis of nucleosides with a variety of functionalized C-alkylation at the 6-position (Scheme 5). A plausible mechanistic interpretation is shown in Scheme 6 and is supported by some experimental data.

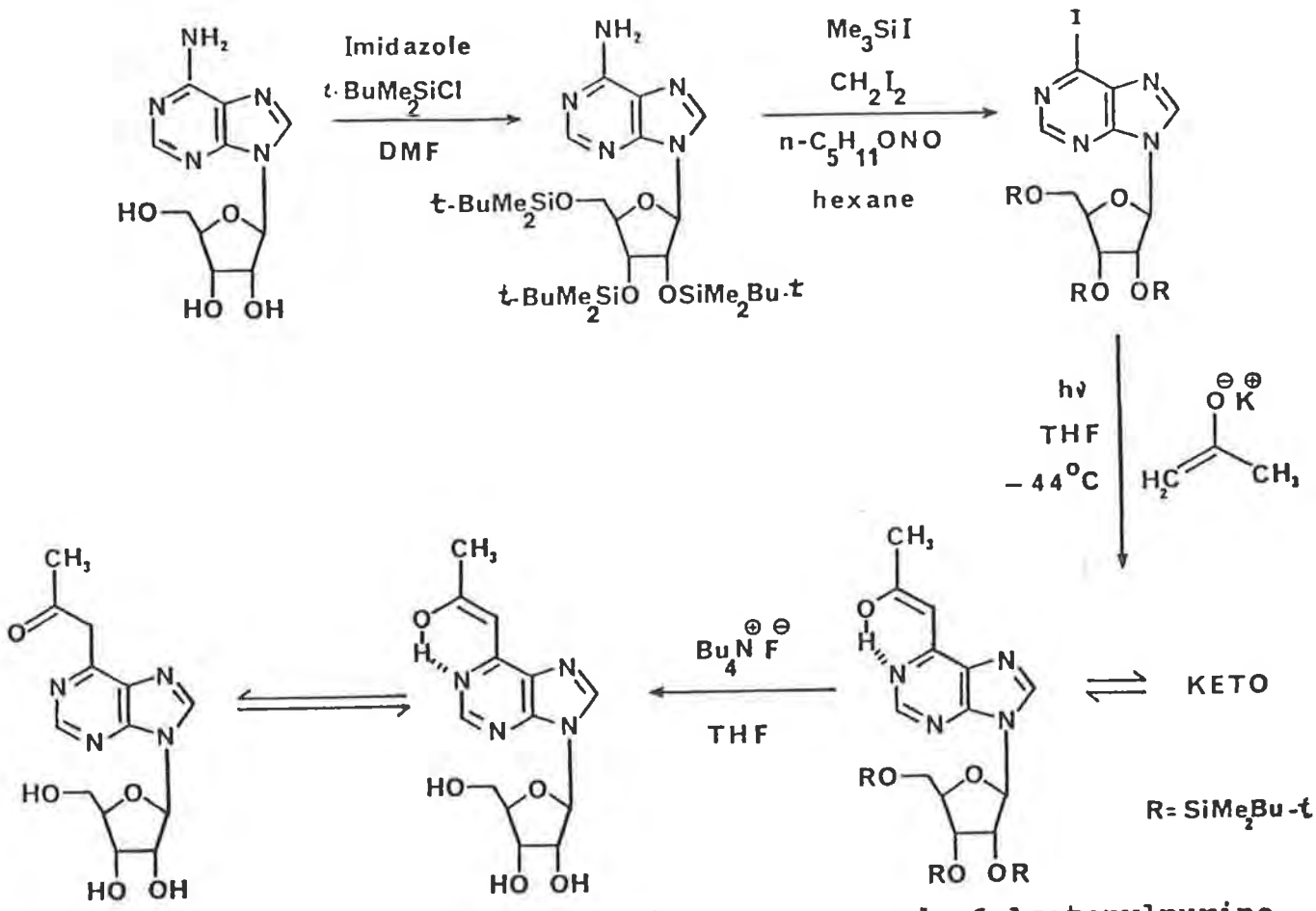
In almost all of the many cases studied, if the metal enolate or other carbanion was successfully generated under the reaction conditions, the carbon-carbon bond forming reaction proceeded efficiently and regiospecifically. 6-Acetonylpurine ribonucleoside, synthesized in the early phases of the development of this work (Scheme 7), has antitumor activity. Many other biologically relevant nucleosides may be approached using this methodology. In addition, by proper choice of nucleofugic group, apparent dark $S_{RN}1$ reactions may also be possible.[37]



Scheme 5. A New Photochemical Methodology for Functionalized Carbon-Carbon Bond Formation in Purine Systems.



Scheme 6. Plausible Mechanism for the Photochemical Reaction of the 6-Iodopurine System with Acetone Enolate

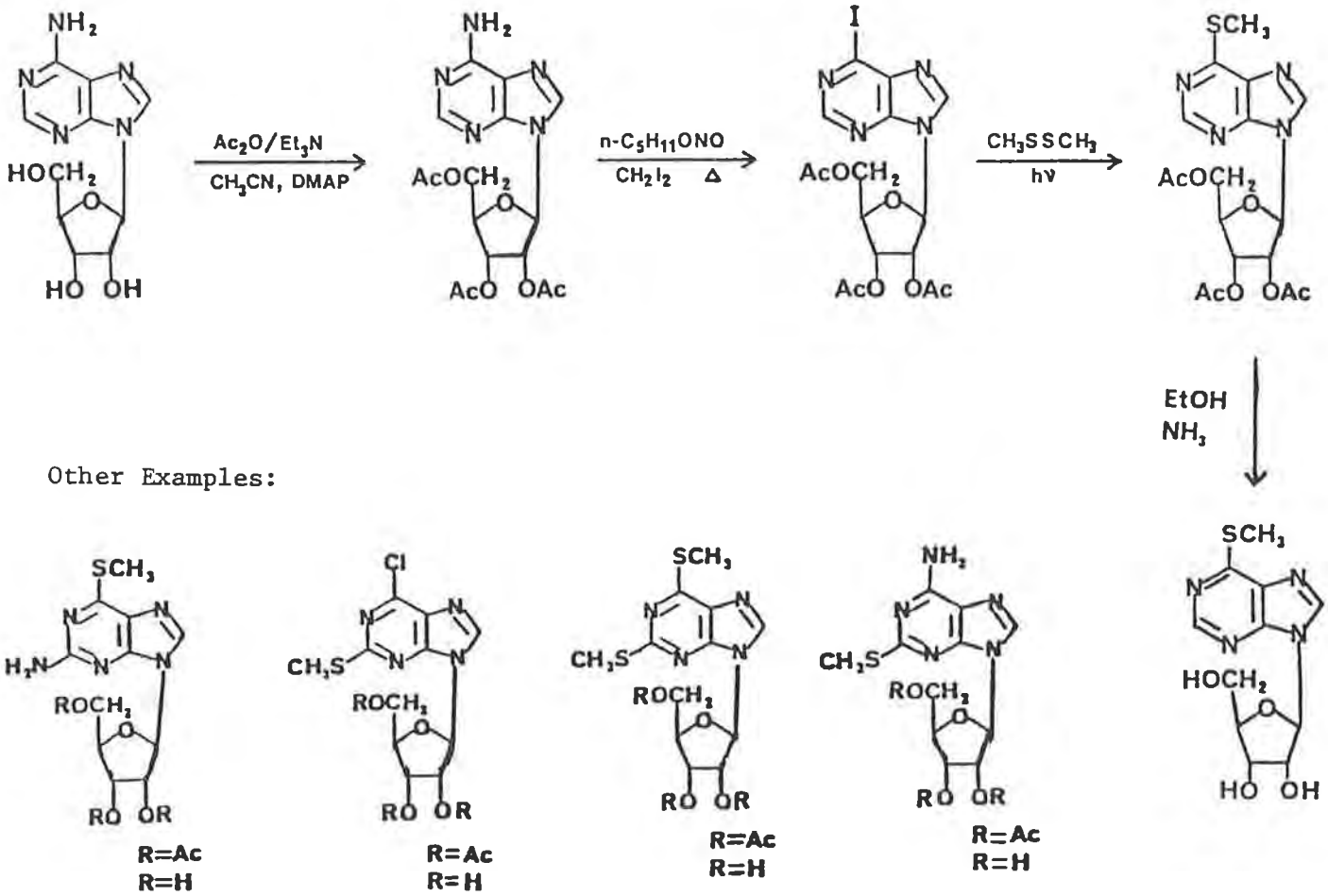


Scheme 7. Synthesis of the Antitumor Compound, 6-Acetonylpurine Ribonucleoside, by the Photochemical $S_{RN}1$ Reaction.

(iv) Photochemical and Thermal Approaches to Thioalkylated Purine Nucleosides

A number of thioalkylated purine nucleosides have been found to have interesting biological activity. For example, 6-methylmercaptopurine ribonucleoside is one of the most potent known inhibitors of de novo purine synthesis.[38] This compound and its 2-amino analogue exhibit antitumor activity.[38] 2-Methylmercaptoadenosine analogues are excellent aggregators of mammalian blood platelets.[39] Previous syntheses of 6-methylmercaptopurine nucleosides from protected inosine or guanosine via the 6-thio compound generally gave low yields of product.[13,40-44] The syntheses of 2-methylmercapto- and 2,6-dimethylmercaptopurine nucleosides have been achieved previously,

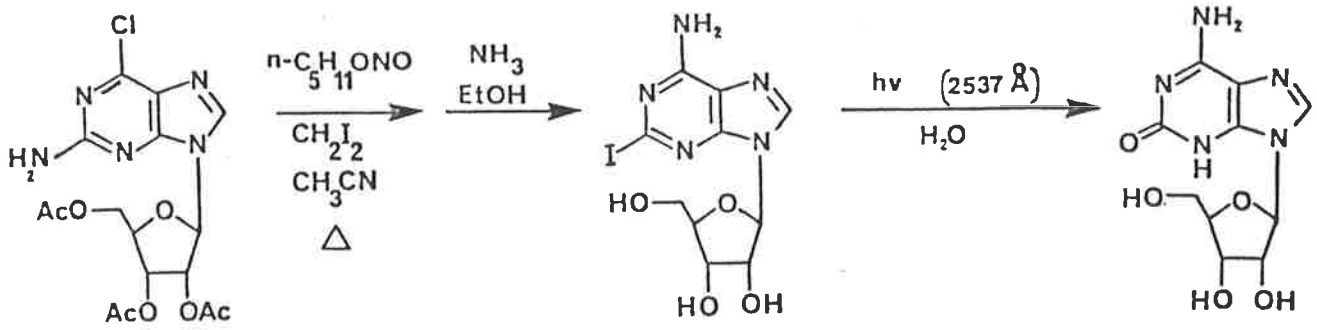
but in low yields from ring closure of appropriate imidazole derivatives with carbon disulfide, followed by alkylation with methyl iodide.[27,29,45-47] Nair and Young discovered a new, efficient, and general methodology for the synthesis of biologically important methylmercaptopurine nucleosides (Scheme 8).[48] This photochemical approach represents a substantial improvement over earlier reported methods for this class of compounds. A thermal radical approach to thioalkylated purine nucleosides is also possible <u>via</u> a deamination/alkylthiolation of aminopurine nucleosides.[49]



Scheme 8. A New Photochemical Methodology for Alkylthiolation.

(v) Photochemical Hydration Reaction: A New Synthesis of Isoguanosine and Modified Hypoxanthine Nucleosides

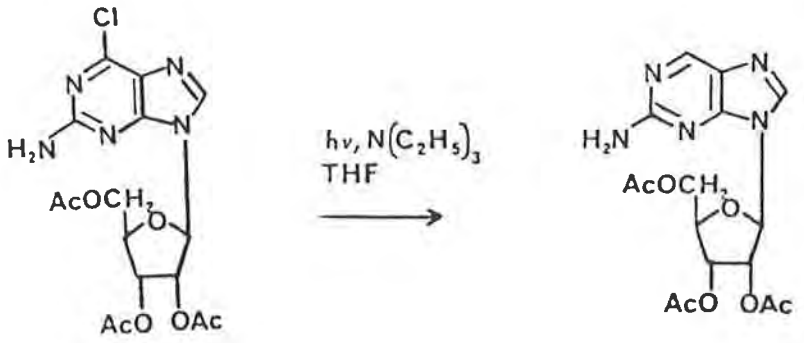
In some cases in the synthesis of biologically active nucleosides, there is need for the direct introduction of a hydroxyl group (actually existing as a lactam moiety) in a specific position of a purine or pyrimidine moiety. For example, isoguanosine, which contains such a hydroxyl group in the 2-position, is one of a few naturally occurring analogues of guanosine. The synthesis of isoguanosine was originally achieved by the reaction of 2,6-diaminopurine ribonucleoside with nitrous acid.[50] However, the overall yield was low. Isoguanosine has also been prepared in low yields from a 4,5-dicyanoimidazole nucleoside precursor.[51] It was also reported as a side product in the synthesis of 2-fluoroadenosine.[52] Rearrangement of adenosine N^1-oxide has been reported to give isoguanosine, but this approach gives variable results.[53] Nair and Young used the novel intermediate, 2-iodoadenosine, for a new, reproducible, and efficient synthesis of isoguanosine (Scheme 9).[54] The key step was a photoinduced hydration reaction. This hydration reaction is general and can also be applied to the 6-position of the purine ring. An example is the synthesis of 2-substituted inosine analogues where the lactam system is developed from a 6-halogeno group.[26] Thus, the halogen at these positions may also be viewed as a lactam equivalent which can be photochemically converted to the lactam functionality after all other synthetic conversions are completed. The synthetic ramifications of this in nucleoside chemistry are significant.



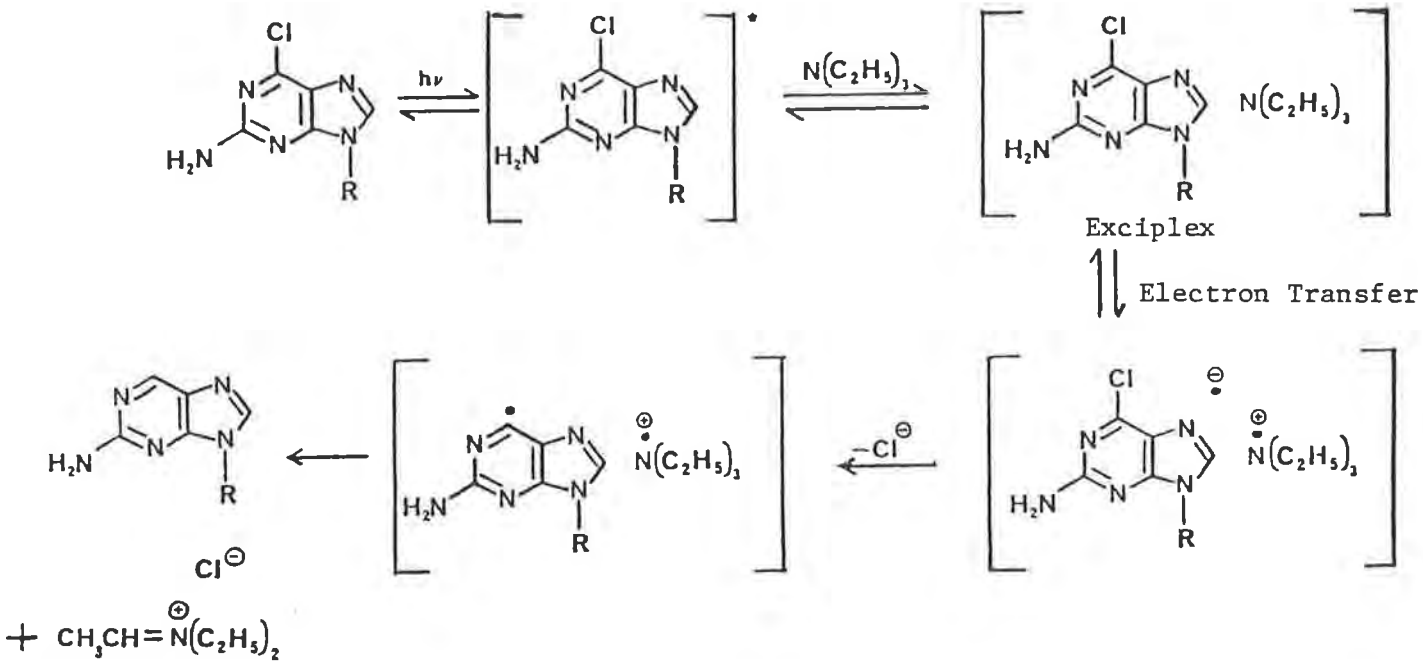
Scheme 9. A New Photochemical Methodology for the Introduction of a Hydroxyl Group (Lactam Moiety) in Nucleosides.

(vi) Photochemical Reductive Dehalogenation

In addition to providing entry into a variety of novel and unique nucleoside systems, the halogen group on purine (and pyrimidine) bases may also be utilized to remove functionality from nucleosides under certain circumstances. For example, 2-amino-9-(β-D-ribofuranosyl)purine (2-aminonebularine or isoadenosine) is a synthetically important and biologically active nucleoside.[55] However, an efficient and general method for the synthesis of this compound and others of this class was not available.[41,56-58] Nair, Young and DeSilvia discovered an excellent method for the synthesis of this compound through the photoinduced reductive dehalogenation of the readily available 6-halo-2-amino precursor (Scheme 10).[59] This is another new methodology in nucleoside synthesis with many potential synthetic applications. The full details of the experimental procedure appear in an invited contribution in the book "Nucleic Acid Chemistry", Part 4, 1989.[60]



Plausible Mechanism:



Scheme 10. Photochemical Reductive Dehalogenation: A New Methodology in Nucleoside Synthesis.

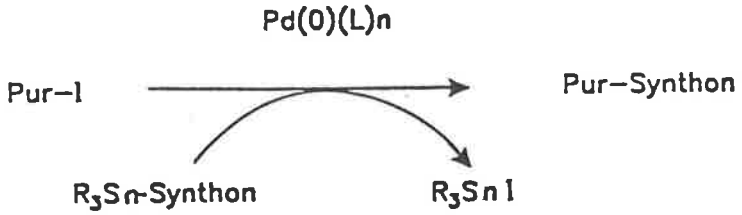
(vii) Development of Metal-Mediated Methodologies in Nucleoside Synthesis

The transition metal-catalyzed cross-coupling reactions of organometallic systems with organic halides and other suitably constructed organic precursors are of considerable potential importance for the regiospecific introduction of specific functionality into organic compounds (61-64). Organometallic systems such as organomagnesium and organocopper compounds have been used as coupling partners (65-70). However, the reactivity

of these organometallics towards other functionalities present in both partners is a serious limitation of this approach. In some recent work, Stille and his coworkers reported the palladium-catalyzed cross-coupling of organic halides with organotin compounds (71-75). Other reports have also appeared (76). Low nucleophilicity of the organostannanes as well as the high stereospecificity and regiospecificity of the reaction offered advantages over previous methods. However, the development and application of this methodology to complex natural systems and related compounds had not been investigated. With the exception of a few reports in the glycosylation area, very little metal-mediated chemistry had been investigated in the nucleoside field (77,78).

Professor Nair's interest in the synthesis of biologically relevant C-2 functionalized hypoxanthine and related purine and adenine nucleosides was the original impetus for the development of the palladium-catalyzed cross-coupling with organostannanes as a new methodology in nucleoside synthesis. For example, although a few C-2 alkylated hypoxanthine and other purine nucleosides have been synthesized, there are very few known C-2 functionalized compounds of the aforementioned families. This is largely because of limitations in synthetic methodology that allow access to these compounds. Virtually all of the 2-substituted inosines known have been synthesized from imidazole nucleosides through ring closure reactions.[47,79] Other methods known for entry into this general class of compounds appear to be of more limited scope.[80,81] The photochemical $S_{RN}1$ reaction,[34-36] although highly successful at the 6-position of the purine ring, is generally difficult at the 2-position. Nair

and coworkers discovered that palladium inserts into the carbon-iodine bond of protected 2-iodohypoxanthine nucleosides and the resulting organopalladium complexes participate smoothly in cross-coupling reactions with synthon bearing organostannanes to give C-2 functionalized hypoxanthine nucleosides (Scheme 11).[82-85]



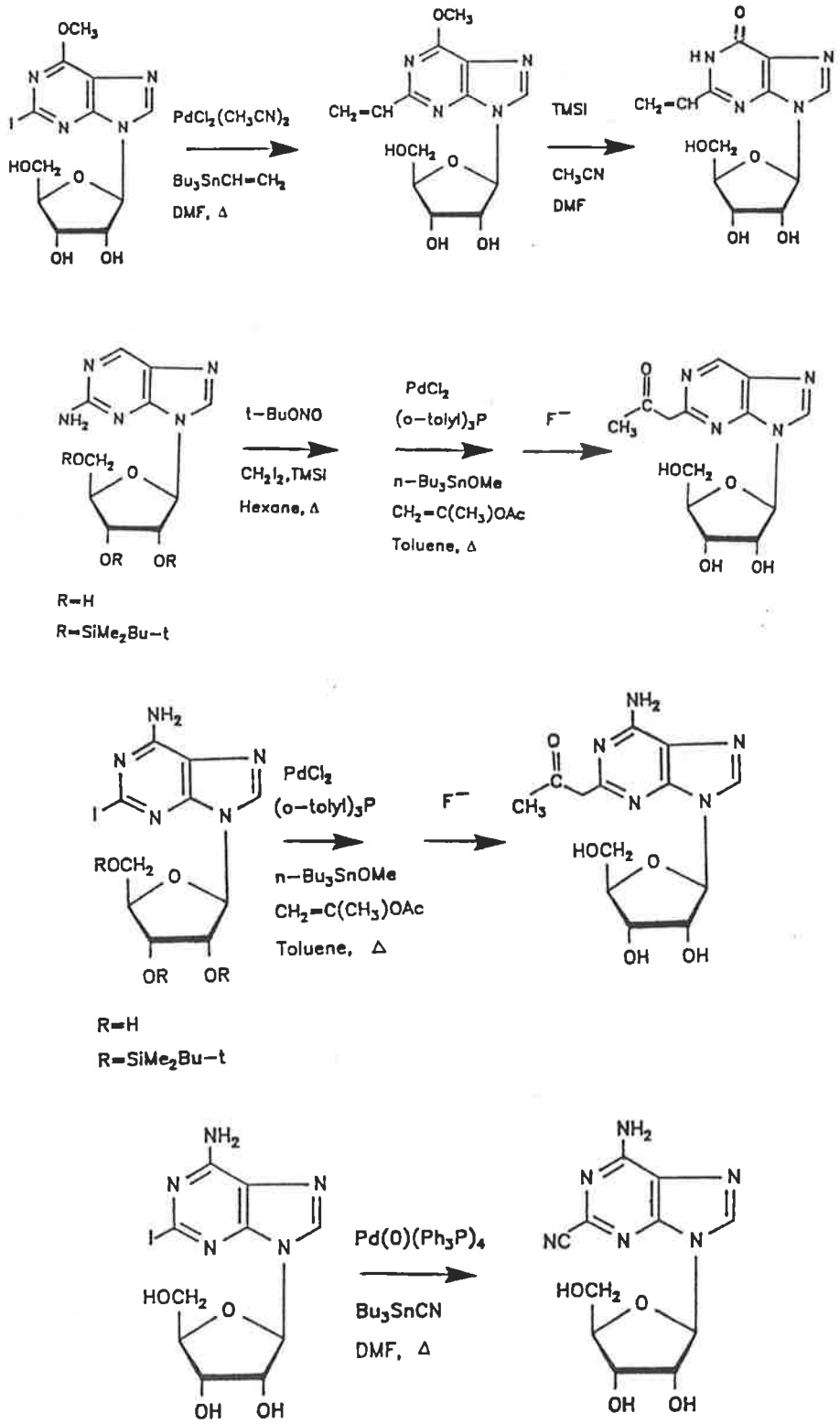
Pur–I = Iodopurine Nucleoside

Scheme 11. Generalized Representation of the New Palladium-Catalyzed Cross-Coupling Methodology in Nucleoside Synthesis

Their initial publication[82] represented the first example of the use of organotin reagents in palladium-catalyzed cross-coupling reactions involving nucleosides. These transformations have considerable generality and can be applied to other positions in the purine ring.[86] In addition, besides functionalization of hypoxanthine nucleosides, the procedure has been used by Nair and coworkers for the strategic alteration of nebularine and adenosine (Scheme 12).[87-89]

This methodology represents one of the most powerful approaches for the functionalization of purine nucleosides. In some cases, unprotected nucleosides may be used in these transformations. The methodology only seems to be restricted by the availability of the synthon bearing organostannane reagent. There are numerous other possible applications including

glycosylations and modification of carbohydrates.

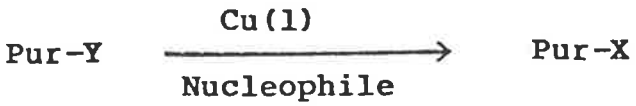


Scheme 12. Selected Examples of the Application of the Palladium-Catalyzed Cross-Coupling Methodology to the Synthesis of Novel Base-Modified Nucleosides

Incorporation of the methodology in the total synthesis of many novel biologically interesting nucleosides is easily done. For example, 2-vinylinosine, a novel, broad-spectrum RNA antiviral compound discovered in Professor Nair's laboratory, was synthesized using the palladium-catalyzed methodology as the key transformation. The metal-mediated step proceeded in almost quantitative yield. Another compound, 2-acetonylinosine, synthesized by a palladium-catalyzed cross-coupling reaction as the key step, has very specific RNA antiviral activity against a Phlebovirus with a therapeutic index of >1000.[90] Professor Nair has received a U. S. patent for this work. The C-2 functionalized adenosines synthesized by these metal-mediated methodologies, are totally resistant to the important purine metabolizing enzyme, adenosine deaminase.[88].

Other metal-mediated methodologies in nucleoside synthesis have been and are being investigated. For example, although copper mediated reactions have played a significant role in aromatic nucleophilic displacements,[92] such transformations are nearly non-existent in synthesis involving nucleosides. The reaction of a halogenated nucleoside with Cu(I)X and an appropriate nucleophile potentially allows for the introduction of a wide range of useful functional groups or synthons into specific positions of nucleosides. Nair and Sells have developed such copper-mediated reactions for the regiospecific functionalization of the base moiety of purine nucleosides.[93,94] Major emphasis in this work was placed on conversions involving the 2-position of the purine ring where normal thermal substitution reactions are usually the most difficult. The

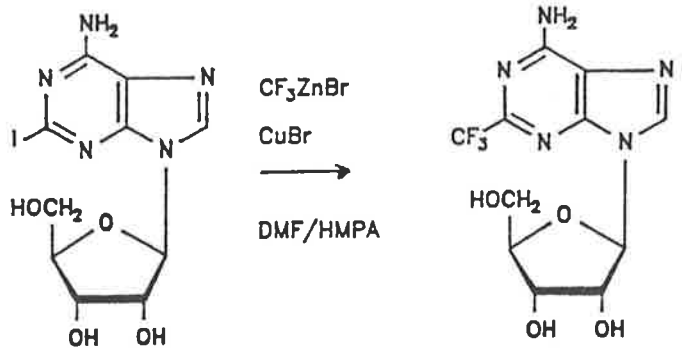
various classes of nucleophiles studied include carbon, nitrogen, oxygen, halogen, sulfur, and their combinations. The general synthetic methodology can be represented as shown in Scheme 13.



Pur-Y = Silyl Protected Purine Nucleoside, Y = Halogen or Displaceable Group at the 2-position, X = Functional Group or Synthon, e.g. CN, SCN, NH_2, N_3, NHOH, Halogen, Functionalized Alkyl Groups, and others.

Scheme 13. Novel Copper (I) Mediated Nucleophilic Reactions in Nucleoside Synthesis

Other copper-mediated syntheses have also been developed. For example, the introduction of the trifluoromethyl group in unprotected purine nucleosides can be exemplified with the synthesis of 2-trifluoromethyladenosine (Scheme 14).[88,89]



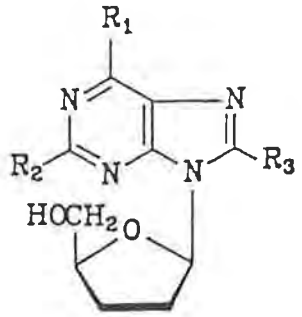
Scheme 14. Regiospecific Introduction of the Trifluoromethyl Group in Adenosine Using a Copper-Mediated Reaction

The metal-mediated reactions described in this section and their ramifications have opened a major new avenue of investigative research in synthetic nucleoside chemistry.

(viii) Other Examples of Applications of Thermal Radical, Photochemical, and Metal-Mediated Methodologies: Synthesis of Novel Antiretroviral Nucleosides and Novel RNA Antiviral Nucleosides

2',3'-Dideoxy analogues of the natural ribonucleosides have generated considerable interest recently because of the ability of some of these compounds to inhibit the cytopathic effect of the human immunodeficiency virus (HIV-1), the etiologic agent of acquired immunodeficiency syndrome (AIDS).[95] For example, 2',3'-dideoxyadenosine (ddA), as its cellularly produced triphosphate form (ddATP), is an inhibitor of HIV reverse transcriptase, an enzyme which plays a vital role in the life cycle of this virus.[96,97] In addition, cellular deamination of ddA by adenosine deaminase furnishes ddI which is also active against HIV-1.[98] The precise mechanism of the action of ddI is not fully understood. Both ddA and ddI are very unstable with respect to cleavage of the glycosidic bond.

Professor Nair and his graduate students have been involved in a major synthetic effort, which has as its goal the rational design and synthesis of stable congeners of ddA and ddI which would have high therapeutic potential against HIV-1 and HIV-2. Considerable success has been achieved in this area both in synthesis and biological activity and many congeners of ddA and ddI modified in the 2-, 6-, or 8-positions have been synthesized by them (Scheme 15).[99-103] Some of these compounds have been found to be active against HIV-1. Isomeric analogues of ddA and

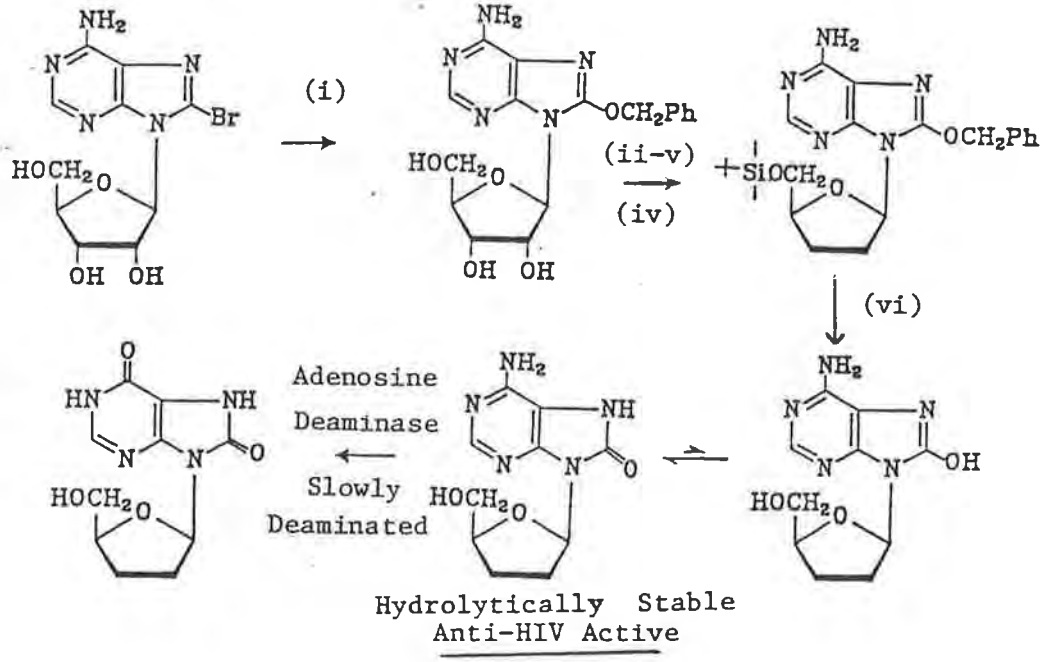


Target Compounds Synthesized:

$R_1 = R_2 = R_3 = H$
$R_1 = I,\ R_2 = R_3 = H$
$R_1 = OMe,\ R_2 = R_3 = H$
$R_1 = Cl,\ R_2 = NH_2,\ R_3 = H$
$R_1 = NH_2,\ R_2 = CN,\ R_3 = H$
$R_1 = R_2 = NH_2,\ R_3 = H$
$R_1 = NH_2,\ R_2 = Cl,\ R_3 = H$
$R_1 = NH_2,\ R_2 = Br,\ R_3 = H$
$R_1 = NH_2,\ R_2 = I,\ R_3 = H$

$R_1 = NH_2,\ R_2 = C_2H_5,\ R_3 = H$
$R_1 = NH_2,\ R_2 = CF_3,\ R_3 = H$
$R_1 = NH_2,\ R_2 = SCH_3,\ R_3 = H$
$R_1 = NH_2,\ R_2 = H,\ R_3 = SCH_3$
$R_1 = NH_2,\ R_2 = H,\ R_3 = OCH_3$
$R_1 = NH_2,\ R_2 = H,\ R_3 = OCH_2Ph$
$R_1 = NH_2,\ R_2 = H,\ R_3 = OH$
$R_1 = NH_2,\ R_2 = H,\ R_3 = NH_2$
$R_1 = NH_2,\ R_2 = H,\ R_3 = SH$

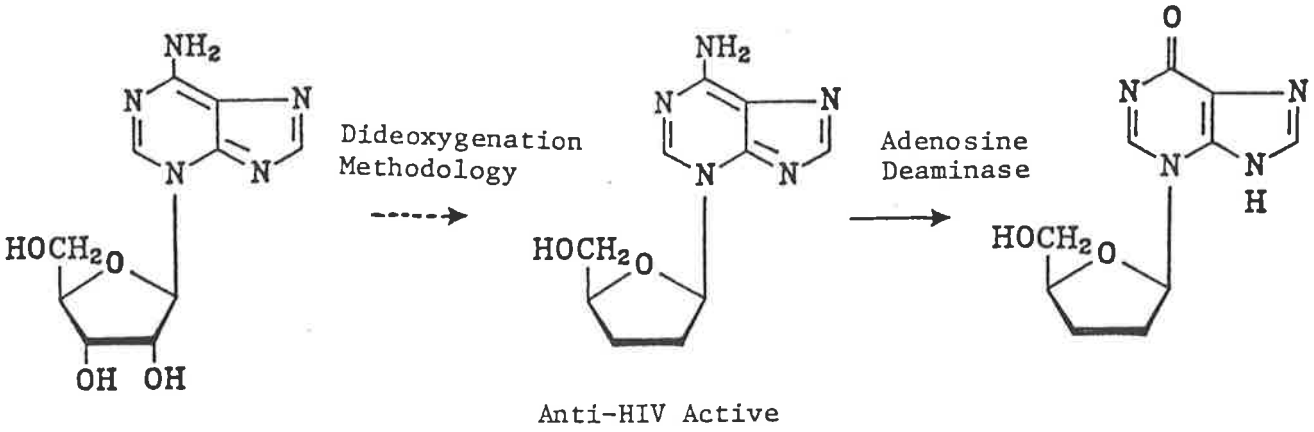
Example: 8-Hydroxydideoxyadenosine



(i) $NaOCH_2Ph$, DMF, $PhCH_2OH$, Δ; (ii) t-$Bu(CH_3)_2SiCl$, 4-dimethylaminopyridine, $(C_2H_5)_3N$, DMF, CH_2Cl_2; (iii) 1,1'-thiocarbonyldiimidazole, DMF, 25 °C; (iv) Bu_3SnH, AIBN, toluene, 110 °C; (v) 1,1'-thiocarbonyldiimidazole, DMF, 90 °C; (vi) Et_4NF, CH_3CN.

Scheme 15. Synthesis of 2',3'-Dideoxynucleosides of Anti-HIV Potential.

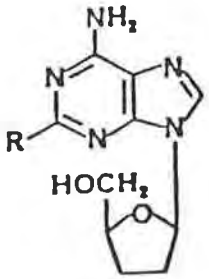
ddI have also been synthesized and exhibit some anti-HIV activity (Scheme 16).[104] A patent has been filed by Dr. Nair on this work.[105] In the course of the synthetic effort, a new procedure for the regiospecific 5'-silylation of both natural and unnatural purine and pyrimidine nucleosides was developed.[106]

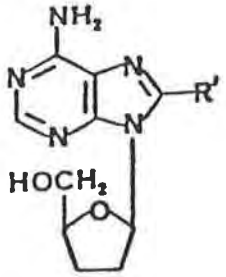


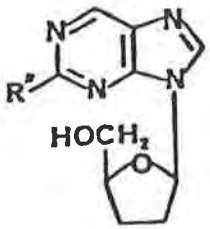
Scheme 16. Synthesis of the Novel Isomeric Analogues of Dideoxyadenosine and Dideoxyinosine.

The inherent instability of ddA and ddI towards hydrolytic cleavage of the glycosidic bond limits considerably the usefulness of these compounds as biological probes and antiviral agents. The rational design and synthesis of new analogues that are hydrolytically more stable than the parent compounds require some information on the effect of structural modification on hydrolytic stabilities. Although the hydrolytic stabilities of ribonucleosides have received considerable attention, the same cannot be said for dideoxynucleosides. Nair and graduate student Buenger have studied the glycosidic bond stabilities of dideoxynucleosides by differential UV spectroscopy (Table 1).[107] They have discovered that the most dramatic effect on the rates of glycosidic bond cleavage of dideoxyadenosine analogues is seen

Table 1. Glycosidic Bond Stabilities of 2',3'-Dideoxynucleosides at pH 3 Studied by Differential UV Spectroscopy







	Compound	pKa[a]	Relative Rate at pH 3[b]	λ (nm)[c]
1	R = H	3.7	100	254.5
2	R = NH_2	4.3	20	256.5
3	R = CN	0.6	47	259
4	R = I	1.4	55	259
5	R = SCH_3	3.3	64	264
6	R = CH_2CH_3	4.2	75	259
7	R = CF_3	0.7	79	255
8	R' = OH	3.2	0[d]	-
9	R' = OCH_2Ph	3.8	39	254.5
10	R' = SCH_3	3.6	40	269
11	R' = OCH_3	3.9	61	253
12	R' = NH_2	e	2050	263
13	R'' = H	2.1	177	244.5
14	R'' = NH_2	3.2	110	244.5

a. Determined by UV spectrophotometric methods.

b. Rates of hydrolysis are relative to dideoxyadenosine (rate = 100). The apparent first order rate constant for the hydrolysis of ddA at pH 3 is 8.23×10^{-4} min.$^{-1}$

c. Rate of change in absorbance monitored at this wavelength by differential UV spectroscopy. The monitoring wavelength represents the wavelength of maximum difference at pH 13 between the intact dideoxynucleoside and its cleaved heterocyclic base (see discussion above).

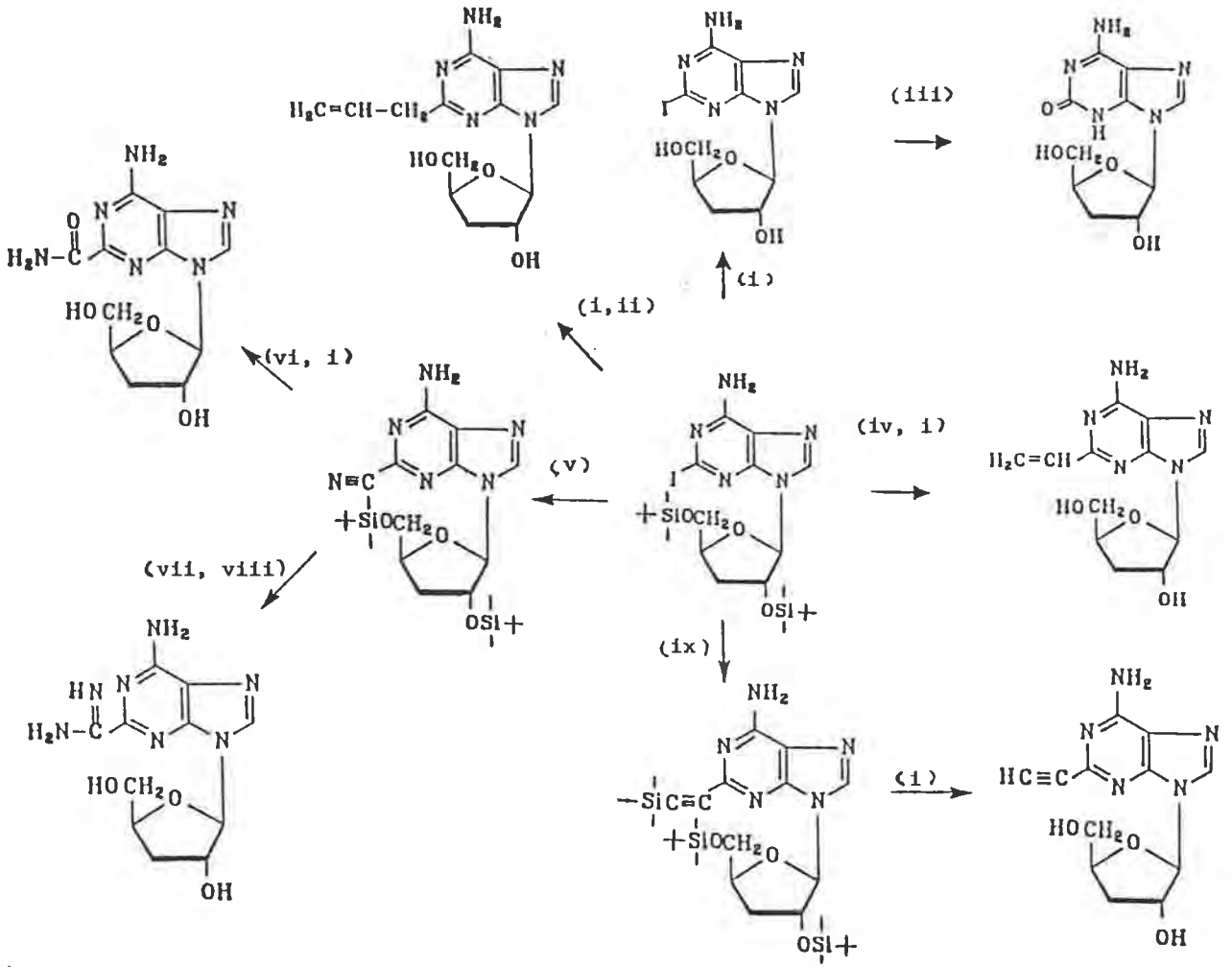
d. No detectable hydrolysis even at pH 1.

e. The pKa_1 of 12 could not be reliably determined because of its rapid breakdown under acidic conditions.

with appropriate substitution at the 8-position. These findings, are not only of significance in the design of new biologically active analogues of dideoxynucleosides, but they also contribute to the understanding of the mechanism of glycosidic bond hydrolysis of nucleosides.

It should be mentioned that Professor Nair was a consultant on work on the anti-AIDS drug, 3'-azidothymidine (AZT), at Wellcome Research Laboratories, Burroughs Wellcome Company, North Carolina.

As previously mentioned, Professor Nair has been interested also in the design and synthesis of novel and unusual nucleosides that would have potential as broad-spectrum antiviral agents against RNA arboviruses.[108-110] Alpha-, Arena-, Bunya-, and Flavi- viruses are RNA viruses that cause serious fevers including the often fatal hemorrhagic fevers. Some of these viruses also cause a variety of encephalitis. The viruses exist in one strain or another worldwide. Based in part on some RNA antiviral leads from his previous work in this area described above,[82-85,90] Professor Nair has investigated the synthesis of analogues of the naturally occurring nucleoside antibiotic, cordycepin (Scheme 17). Cordycepin is known to have antiviral activity against a number of RNA viruses.[111] The biochemical basis for this mechanism of action is thought to be the inhibition of the viral RNA polymerase activity by cordycepin 5'-triphosphate. Analogues of cordycepin are therefore of considerable potential antiviral interest. However, very few compounds of this natural nucleoside family have been investigated, in part because of previous limitations in



(i) Et_4NF, CH_3CN; (ii) n-Bu_3SnCH_2-CH=CH_2, Pd°$(Ph_3P)_4$, DMF, Δ; (iii) H_2O, hν (2537 A°);
(iv) n-Bu_3SnCH=CH_2, $PdCl_2(CH_3CN)_2$, DMF, Δ; (v) n-Bu_3SnCN, Pd°$(Ph_3P)_4$, DMF, Δ;
(vi) Bu_4NHSO_4, CH_2Cl_2, NaOH, H_2O_2; (vii) NaOMe, MeOH; (viii) NH_3, EtOH.
(ix) $(CH_3)_3SiC\equiv CH$, Et_3N, CuI, Pd°$(Ph_3P)_4$, DMF, Δ

Scheme 17. New Doubly Modified Nucleosides: Congeners of Cordycepin

methodologies that provided access to these and related deoxygenated nucleosides. The key steps used by Nair and coworkers for the synthesis of these doubly modified nucleosides of the cordycepin family were bis-silylation, radical deoxygenation, radical iodination, and metal-mediated functionalizations (Scheme 17).[112,113]

In related studies, Professor Nair and coworkers have been interested in the synthesis of the hypoxanthine nucleoside counterpart of cordycepin.[114,115] Analogues of 3'-deoxyinosine, although of potential RNA antiviral interest, are virtually unknown.

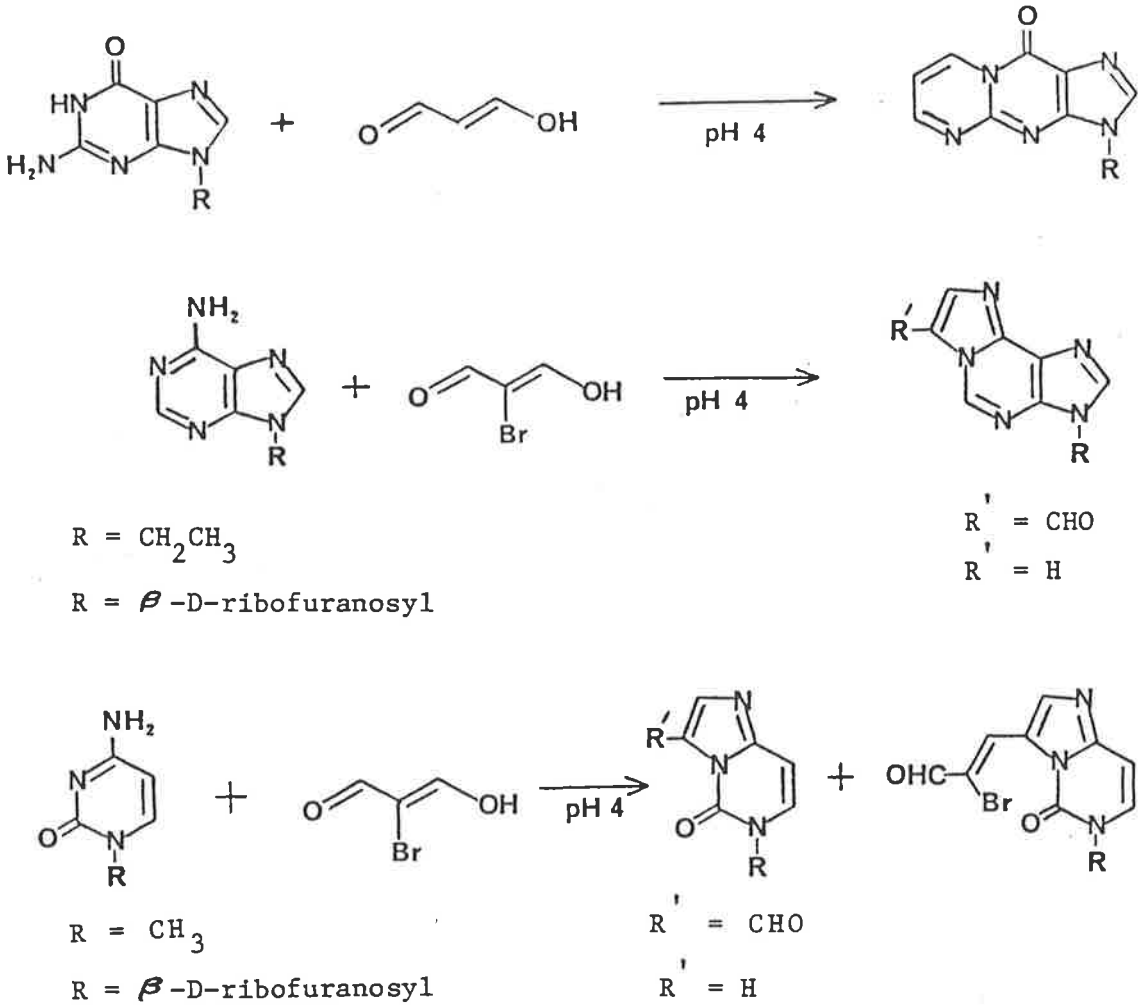
(ix) Synthesis of Isosteric and Ring-Extended Bases and Nucleosides

In addition to the development of thermal radical, photochemical, and metal-mediated methodologies for the synthesis of biologically active nucleosides, Professor Nair has also contributed to the synthesis of isosteric and ring-extended bases and their nucleosides. Nucleosides containing such bases have been of considerable interest in the anticancer field and are now finding renewed interest in the antiviral nucleoside area and also in the emerging field of molecular recognition. The genesis of this work in Professor Nair's laboratory was the modifying reagent, malondialdehyde. The ubiquitous malondialdehyde (MDA), is produced in animal tissues as an end product of unsaturated lipid peroxidation and as a side product of prostaglandin and thromboxane biosynthesis.[116,117] It is readily formed in the γ-irradiation of carbohydrates.[118] The reported toxicity and degenerative chemistry of MDA may be a result of its ability to

covalently bond and to cross-link a variety of biological macromolecules.[119,120] Malondialdehyde was found to be reactive towards nucleic acids but the structural details of these alterations were not fully understood.[120-122] The initial studies carried out by Nair and coworkers on the ability of MDA and other aldehydes to modify biomolecules were focused on amino acids and amines.[123-125] In subsequent studies in Nair's laboratory, it was shown that MDA modified the bases adenine, guanine and cytidine to give novel ring extended products.[126]

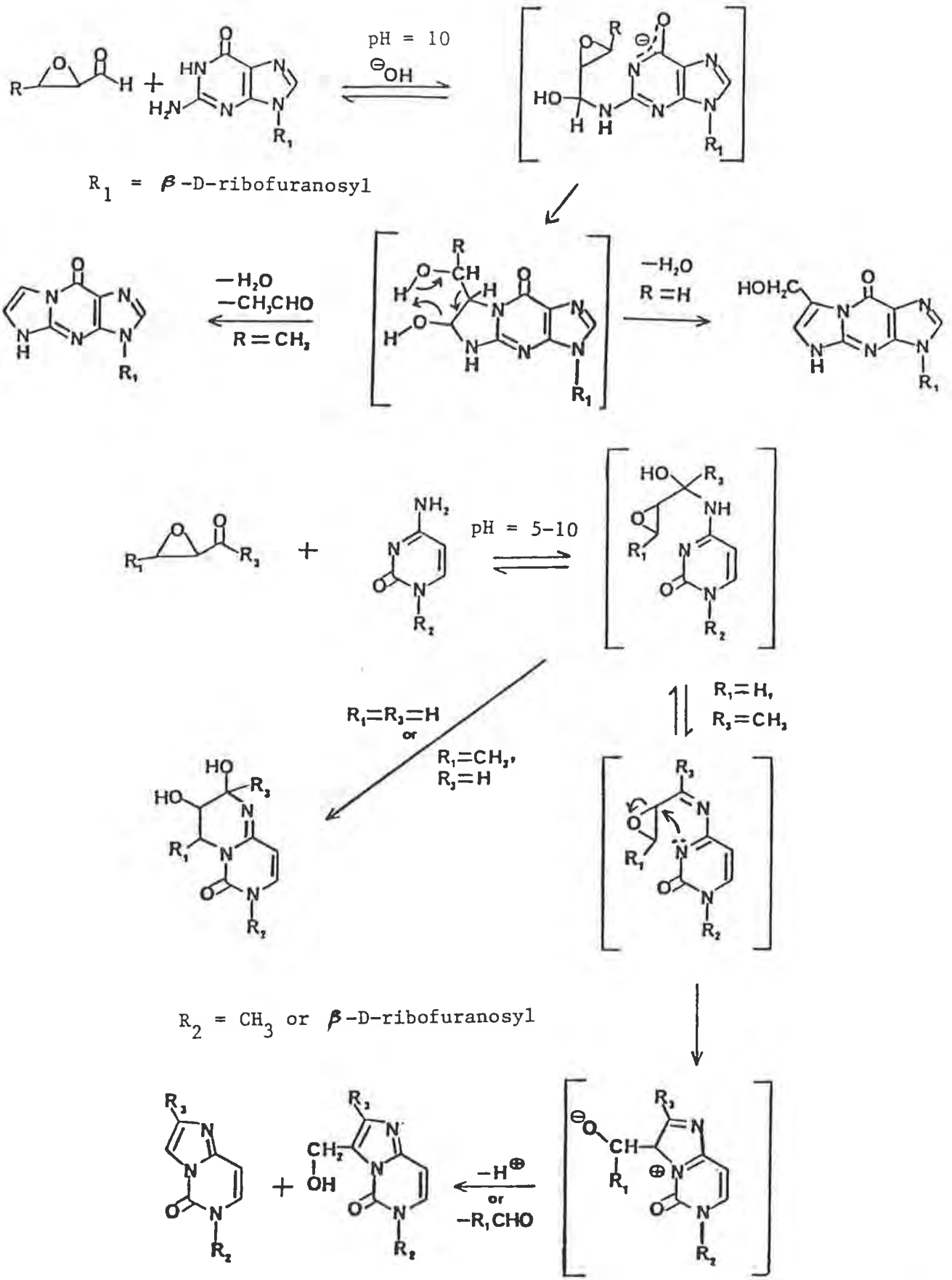
For purposes of comparison, for extension of the scope of these studies to the area of development of methodology for the synthesis of isosteric and ring-extended bases and nucleosides, and because of the considerable amount of biological interest in fluorescent modified bases, Nair and coworkers examined the reaction of 2-halogenated MDA with nucleic acid bases.[127] It was found that cytidine, 1-methylcytosine, and cytosine were all readily modified by bromomalondialdehyde (BMDA) and gave imidazo[1,2-c]pyrimidin-5(6H)-ones and the corresponding carboxaldehydes (Scheme 18). Adenosine was altered to etheno-adenosine and its carboxaldehyde derivative. The imidazo-pyrimidine carboxaldehyde was found to have very interesting fluorescence properties.

The parent imidazopyrimidine base system synthesized is isosteric with hypoxanthine and is currently being developed in Professor Nair's laboratory for the synthesis of isosteric anti-HIV analogues of dideoxyinosine.



Scheme 18. Synthesis of Novel Isosteric and Hypermodified Bases and Nucleosides

In a continuation of the aforementioned synthetic studies, and in relation to the possible modification of nucleic acid bases by epoxides to produce new biosynthetically interesting structures with an imidazo[1,2-a]purine ring system related to the important natural Y bases of t-RNA,[128] Nair and his graduate assistants examined the reaction of glycidaldehyde with guanosine (Scheme 19).[129,130] Glycidaldehyde alters the guanine moiety of guanosine to give a ring-extended tricyclic system related to the natural Y bases. Other epoxides also modify nucleic acid bases to give novel ring extended structures.[130] Interestingly, the synthesis of 1,N^6-ethenoguanosine had been reported previously



Scheme 19. Synthesis of Hypermodified and Ring-Extended Bases and Nucleosides by Reaction with Epoxides.

from guanosine but only in a few percent yield.[131] The epoxide procedure of Nair produced this hypermodified nucleoside in 41% yield. Dideoxynucleoside analogues containing these hypermodified and ring-extended bases are currently being investigated.

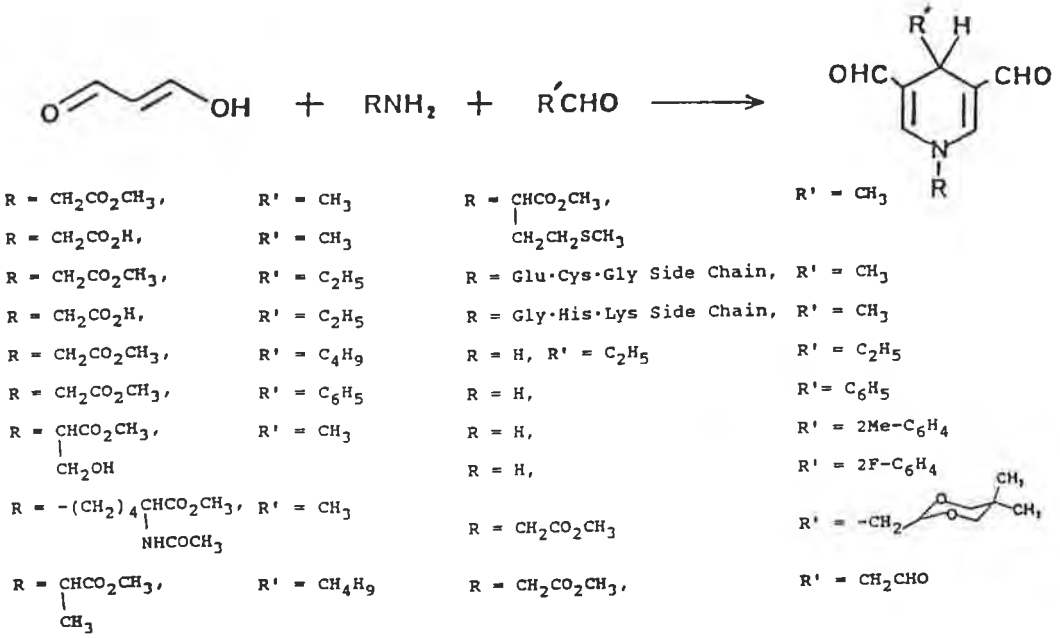
An interesting extension of these studies included the synthesis of some novel model epoxides related to natural hepoxilin A by Nair and Jahnke.[132]

Other synthetic "spin-offs" of these studies were also realized. For example, in the aforementioned work with malondialdehyde, it was discovered that this dialdehyde modified the amino groups of biomolecules to form 1:2 adducts bearing 1,5-diazapentadienium (vinamidinium) linkages. Investigation of the reactivity of this linkage by Nair and graduate students Cooper and Jahnke led to some useful and new carbon elongation reactions with the vinamidinium synthon.[133-136] These studies resulted in the synthesis of novel, highly reactive dienamines, dienaminones, γ,δ-unsaturated-β-ketoesters, and spiro compounds. Although reaction of vinamidinium salts with the activated methylene of nitriles had been previously reported,[137,138] there was only one paper at the time of Nair's publications in this area, of the alkylation of other types of activated methylene compounds by vinamidinium salts.[139]

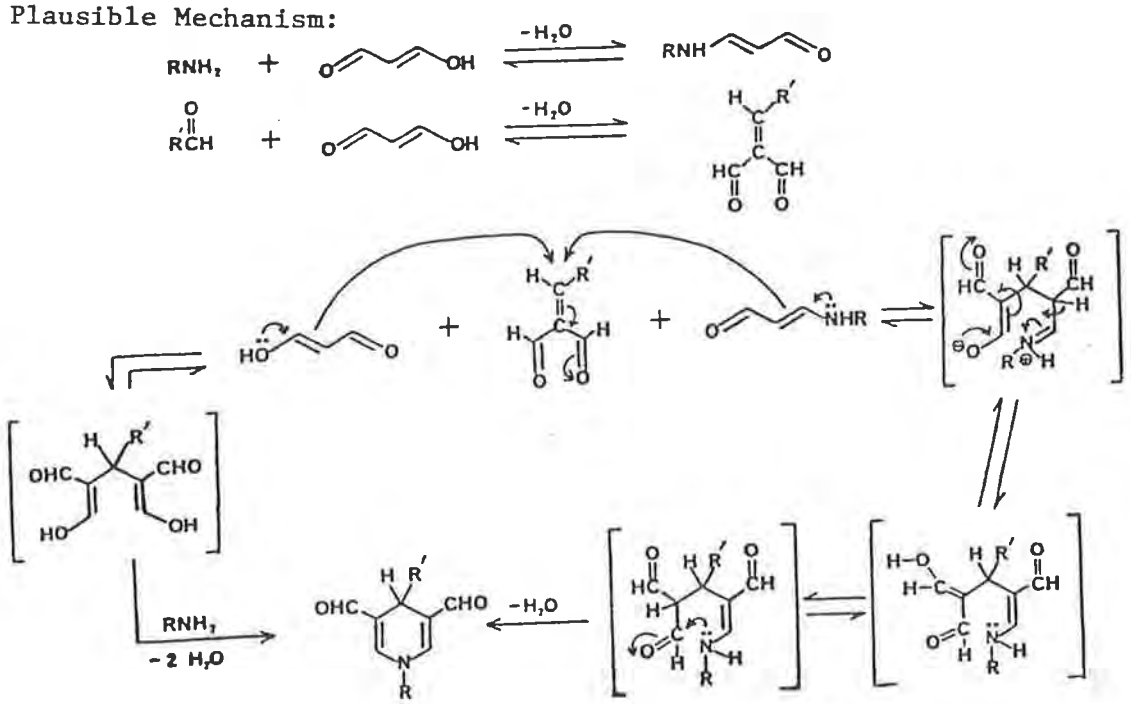
(x) Synthesis of Novel Fluorescent Dihydropyridines: Potential Drug Carrier Delivery Systems for Nucleosides

Nair and graduate students Offerman and Turner discovered that, under suitable conditions, malondialdehyde is capable of modifying amino acid residues to novel, highly fluorescent 1,4-

dihydropyridines.[140] These findings may be of significance in explaining some of the biological chemistry of malondialdehyde previously mentioned. In addition, the transformation led to the development of a new approach to the synthesis of a wide range of novel light-stable 4-arylated-1,4-dihydropyridines (Scheme 20).[140-142] These compounds are of considerable potential



Plausible Mechanism:

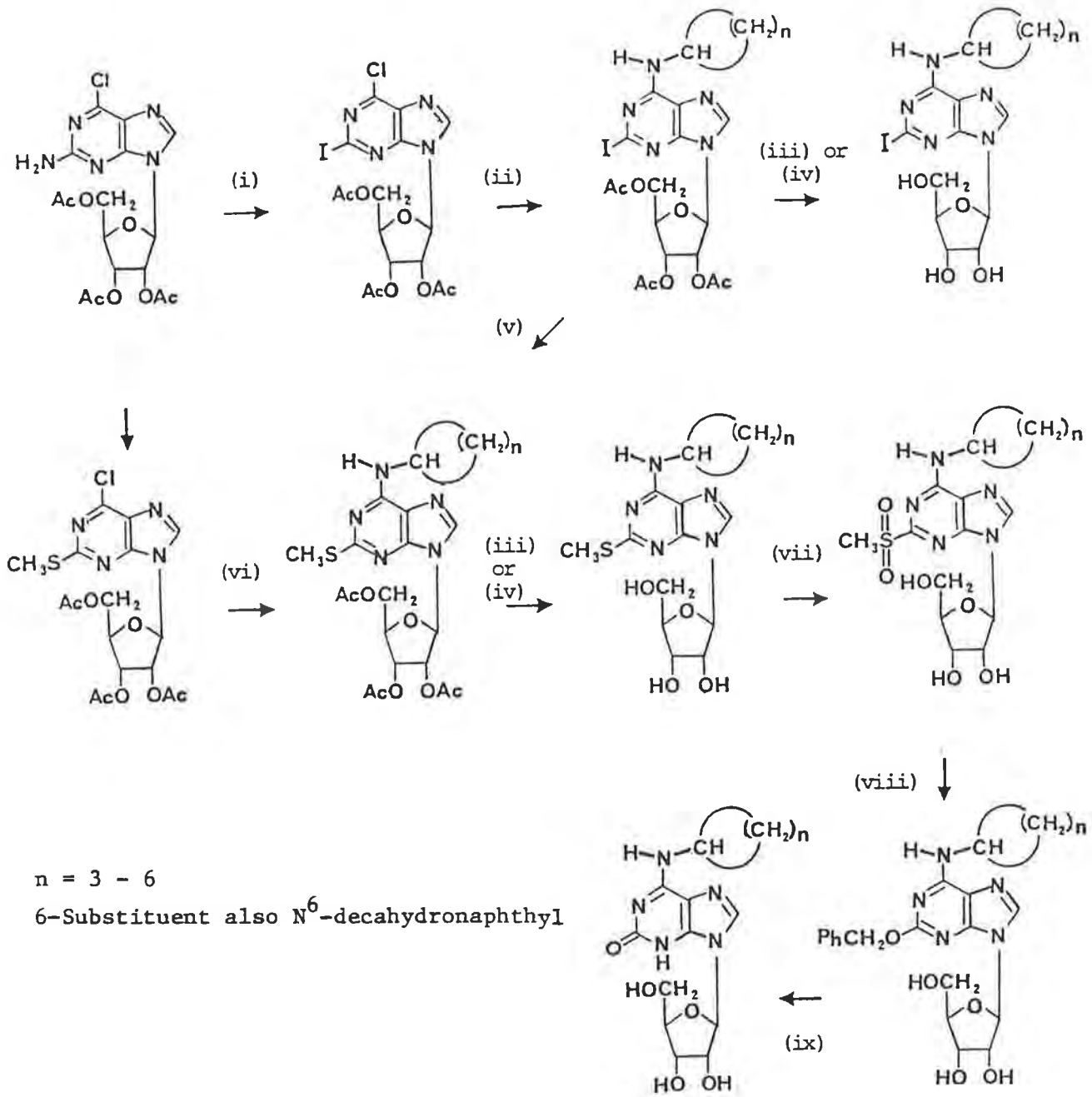


Scheme 20 **Synthesis of Novel 1,4-Dihydropyridines: Drug Carrier Delivery Systems.**

interest as calcium channel antagonists.[143-145] Another interesting aspect of this work is that derivatives of these stable dihydropyridines may be synthetically attached to antiviral nucleosides and are potential drug carrier delivery systems for nucleoside drug delivery to the brain.[146,147] This synthetic work is currently being investigated in Professor Nair's laboratory in relation to his RNA antiviral project.

(xi) Development of Methodologies for the Synthesis of Novel Nucleosides as Potential Agonists for Adenosine Receptors

Although the cardiovascular effects of adenosine were first described by Drury and Szent-Gyorgyi in 1929, it was not until much later that the biochemical basis for the physiological effects of adenosine began to be understood.[148,149] Adenosine apparently exerts its effects via the extracellular receptors, A_1 and A_2, distributed throughout a wide variety of tissues in the mammalian body. Thus, there has been interest in developing adenosine analogues that mimic the pharmacological properties of adenosine but with high A_1 or A_2 receptor specificity and with resistance to rapid metabolic degradation.[150] Work in Professor Nair's laboratory has focused on the synthesis of 2-iodo- and 2-oxo- N^6-cyclosubstituted adenosine analogues (Scheme 21).[49] The methodology for 2-oxo compounds represents a new and general synthetic approach to isoguanosine analogues. Very few examples of isoguanosine analogues of cardiovascular interest are known. Some of the compounds synthesized do exhibit potent adenosine receptor agonist activity.



(i) CH_2I_2, $n\text{-}C_5H_{11}ONO$, CH_3CN; (ii) RNH_2, TEA, $CHCl_3$; (iii) $NaOCH_3$, MeOH; (iv) NH_3, EtOH; (v) CH_3SSCH_3, CH_3CN, hv; (vi) RNH_2, TEA, DMF; (vii) oxone, acetate buffer; (viii) sodium benzyloxide, DMF; (ix) H_2, Pd/C 10%, EtOH.

Scheme 21. Synthesis of Novel Agonists for Adenosine Receptors

(xii) Design and Synthesis of Reversed Nucleosides and Nucleotides

Prior to and during his involvement in the investigations described in items (i-xi), Professor Nair made contributions in another area of synthesis of biologically active nucleosides and nucleotides, i.e. the area of "reversed" nucleosides and nucleotides. In this novel class of nucleosides, the purine ring has been moved from the 1'-position to the 5'-position,[151] leading to increased stability both with respect to glycosidic bond cleavage and degradation by mammalian adenosine deaminase.[152] An amino nucleoside of the reversed class was also synthesized.[153] Synthesis of a novel reversed analogue of the aminoacyl nucleoside antibiotic, puromycin, which incorporated all of the features of puromycin necessary for biological activity but which was devoid of biologically detrimental structural components, was achieved by Nair and Emanuel.[154] Nair and coworkers also synthesized structural analogues of adenosine 3',5'-cyclic monophosphate (cyclic AMP or cAMP) based on the reversed nucleoside concept. One of these compounds was totally resistant to degradation by mammalian cAMP phosphodiesterases and was a potent inhibitor of this enzyme.[155] This inhibitor activity was as good as that observed for one of the most potent known inhibitors of this enzyme, 1-methyl-3-isobutylxanthine (MIX).[156] A related novel cyclic nucleotide synthesized in his laboratory activated mammalian protein kinase and also inhibited the proliferation of human cancer cells.[157]

(xiii) High-Field NMR Studies of Novel Nucleosides

Professor Nair has also contributed to carbon-13 NMR studies of novel nucleoside analogues.[158]

D. Selected Accomplishments in Carbohydrate Chemistry of Relevance to the Aforementioned Synthesis of Modified Nucleosides

(i) Development of Deoxygenation Reaction

In many areas of natural products chemistry and especially in carbohydrate and related chemistry, there is occasionally need for the deoxygenation of a secondary hydroxyl group, selectively and quantitatively. Deoxy sugars are components of such aminoglycoside antibiotics as bividomycin, tobramycin, gentamicins, sagamycin, and deoxykanamycins.[159] Those such as D-desosamine and D-mycosamine are components of macrolide antibiotics.[159] Deoxygenated nucleosides are of considerable interest currently as antiviral agents as previously discussed. Nair and Sinhababu discovered that secondary hydroxyl groups in carbohydrates could be selectively and highly efficiently deoxygenated *via* their tosylhydrazones through the utilization of sodium cyanoborohydride.[160] This was the first example of the use of this deoxygenation procedure in carbohydrate chemistry. The method complements other literature procedures such as Barton's tributyltin hydride approach,[161] and also other reported deoxygenations with sodium cyanoborohydride.[162]

(ii) Functionalization of Natural Carbohydrates

The α-methylene-γ-butyrolactone moiety is a characteristic component of a large class of sesquiterpenes many of which possess marked biological activity.[163] The cytotoxic and antitumor activity of these lactones apparently derives from their chemical affinity for the thiol group of sulfhydryl

enzymes.[164] Although the enone component is essential for biological activity, there are factors that may enhance these properties. Enhancement factors include the presence of hydroxyl groups in stereochemically strategic positions and the presence of conjugated ester side chains.[165] Nair utilized the built-in functionality of carbohydrates for the synthesis of a novel carbohydrate α-methylene- γ-butyrolactone.[166] The reaction sequence developed is general and could be easily adapted for the synthesis of other carbohydrate analogues of α-methylene-γ - butyrolactone. The biomimetic reaction of the synthesized compound with sulfhydryl groups of the amino acid, cysteine, and the peptide, glutathione, proceeded rapidly, quantitatively, and stereospecifically.

(iii) Synthesis of Amino Sugars

The syntheses of amino sugars have also been described by Nair and coworkers.[154,167]

E. Accomplishments in Other Heterocyclic Chemistry

In addition to contributions to the areas of nucleoside and related carbohydrate chemistry, Professor Nair has also been very active in the area of other heterocyclic chemistry, in particular, small and medium ring heterocycles. Some of this work is of direct relevance to nucleic acid base chemistry. His contributions in this area occurred during the early stages of his professional career at the University of Iowa. This research work eventually resulted in an invited contribution to a book on "Small Ring Heterocycles" in the well-known original Weissberger-Taylor Series on "Heterocyclic Compounds." [168] A review of this contribution is also cited.[169] His research achievements in this area involved primarily synthesis and symmetry-allowed cycloadditions of the 1-azirine ring system.[170] These investigations established that 1-azirines could act as precursors to many novel and useful nitrogen containing compounds such as azepines, isoquinolines, dihydroisoquinolines, azanorcaranes, carbodiimides, triazepines, thiadiazepinones, pyrimidines, indoles, and thiazoles.[171-180]

F. Summary of Accomplishments in Subject for Thesis

The discovery of nucleosides with antiviral activity has ushered in a new era in chemistry and medicine, one that can be equated to the evolvement of antibiotics to treat bacterial infections, and innovative synthetic methodologies for biochemically based nucleoside drug design and synthesis have played and will continue to play important roles in this development. In the last one and a half decades, the major emphasis of the research efforts in Professor Nair's laboratory has been devoted to the development and application of methodologies for the synthesis of new congeners and analogues of the natural nucleosides and related heterocyclic systems. The rationale for this work has been that these compounds would have antiviral and/ or anticancer activity and would also be useful as biological probes for the study of specific enzymes or specific receptors.

Some of the important new methodologies for the synthesis of biologically useful nucleosides and related systems developed by him are presented in Scheme 1. For example, a methodology for the synthesis of base-modified nucleosides from intact natural or synthetic ribonucleonucleosides using thermally generated transient radical intermediates was developed in his laboratory. These were the first examples of the generation and synthetic utilization of nucleic acid base radicals in nucleoside chemistry. These developments led to the synthesis of a number of novel and known nucleosides of anticancer, antiviral, and enzymological interest such as nebularine and its congeners and isosteres, halogenated and dihalogenated nucleosides, and

thioalkylated nucleosides.

Photochemical methodologies have rarely been used to elaborate nucleoside structures. Nair and coworkers have found that purinyl radicals appear to be generated as transient intermediates in the photolysis of iodopurine nucleosides and these intermediates can be regiospecifically trapped to give novel arylated and heteroarylated nucleosides some of which exhibit interesting fluorescence properties. Photochemical hydration reaction of halogenated purine nucleosides to give the corresponding lactam system is new in nucleoside chemistry and was developed in Nair's laboratory. It led to a new synthesis of the natural minor nucleoside, isoguanosine, among other compounds. The photochemical thioalkylation reaction was also developed and applied to the synthesis of biologically important thioalkylated nucleosides. Reductive dehalogenation reactions were also be carried out photochemically and this methodology was applied to a new synthesis of isoadenosine. The first photochemical $S_{RN}1$ reactions in nucleoside chemistry were also developed in Professor Nair's laboratory.

Another new methodology in nucleoside synthesis which has seen a considerable amount of success is the palladium-catalyzed cross-coupling reaction of halogenated purine, adenine, and hypoxanthine nucleosides with organostannanes. This methodology represents one of the most powerful methods of functionalization of the base moiety of nucleosides. Copper (I) catalyzed functionalization of halogenated nucleosides has also been studied for the first time. Radical deoxygenation reactions have also been studied.

The combination of the radical, photochemical, and metal-mediated reactions were applied to the synthesis of a number of novel nucleosides modified in both the carbohydrate and base moieties, e.g. C-2 functionalized hypoxanthine nucleosides of RNA antiviral activity and potential, congeners of 2','3'-dideoxyadenosine with expected and observed anti-HIV activity, doubly modified nucleoside analogues of the natural nucleoside antibiotic, cordycepin, and adenosine and deoxygenated adenosine analogues as competitive inhibitors of the purine metabolizing enzyme, adenosine deaminase. Other methodologies developed in his laboratory were utilized in the synthesis of specifically designed C-2 functionalized N^6-cyclosubstituted adenosine analogues with adenosine receptor agonist activities.

Studies on the modification of nucleic acid bases and their corresponding nucleosides by malondialdehyde, its isomeric epoxide and their derivatives led to ring extended and isosteric bases and their nucleosides. Isosteres of the natural nucleic acid bases also provide entry to unique nucleosides through glycosylation reactions and this is currently being investigated for entry to unique dideoxynucleosides of anti-HIV potential.

Ramifications of base modification with MDA and epoxides led to the discovery of some interesting carbon elongation reactions and the synthesis of novel highly fluorescent dihydropyridines. The dihydropyridines were of interest not only as calcium channel antagonists, but also as potential drug carrier delivery systems for nucleoside drug delivery to the brain.

Contributions were also made to a new class of nucleosides called "reversed nucleosides." Some of his accomplishments in the area of small and medium ring heterocyclic chemistry are of

relevance to nucleic acid base chemistry.

The major impact of all of this work has been in the creation of new avenues of investigative research in synthetic nucleoside chemistry, and in the availability of novel therapeutically interesting nucleosides previously unknown and inaccessible or hypothesized and inaccessible or known and poorly accessible because of severe limitations in synthetic approaches and methodologies. These investigations have also changed substantially the way in which other researchers view the field. The use of radical, photochemical and metal-mediated synthetic methodologies were rarely or never used previously in the field of nucleoside and related chemistry.

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Note: Professor Nair's contributions for all publications, except those involving his Ph.D. thesis work, are shown in terms of percent at the end of each of these citations.

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40. V. Nair, S. G. Richardson and R. E. Coffman, Arylation and Heteroarylation of Photochemically Generated Purinyl Radicals, J. Org. Chem. **1982**, 47, 4520-4524. [70%]

41. V. Nair and S. G. Richardson, Modification of Nucleic Acid Bases via Radical Intermediates: Synthesis of Dihalogenated Purine Nucleosides, Synthesis **1982**, 670-672. [70%]

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44. V. Nair, Azirines, An Invited Contribution in "Small Ring Heterocycles", Vol. 1, A. Hassner, Ed., Wiley, New York, 1983, pp 215-332. [100%]
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47. V. Nair and S. D. Chamberlain, Reductive Deamination of Aminopurine Nucleosides, Synthesis **1984**, 401-403. [70%]

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52. V. Nair and D. A. Young, Synthetic Transformations of Transient Purinyl Radicals: Formation of Mono- and Di-Arylated and Heteroarylated Nucleosides, J. Org. Chem. **1984**, 49, 4340-4344. [70%]

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54. V. Nair and S. D. Chamberlain, Novel Photoinduced Carbon-Carbon Bond Formation in Purines, J. Am. Chem. Soc. **1985**, 107, 2183-2185. [70%]

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60. V. Nair, D. A. Young, and R. G. DeSilvia, 2-Halogenated Purine Nucleosides: Synthesis and Reactivity, J. Org. Chem. **1987**, 52, 1344-1347. [80%]

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63. V. Nair, G. A. Turner and S. D. Chamberlain, Novel Approaches to Functionalized Nucleosides via Palladium-Catalyzed Cross-Coupling with Organostannanes, J. Am Chem. Soc. **1987**, 109, 7223-7224. [75%]

64. V. Nair, S. D. Chamberlain, R. G. DeSilvia, and G. S. Buenger, Synthetic Approaches to Rare 2-Substituted Purine Nucleosides, Nucleosides and Nucleotides, **1987**, 6, 229-232. [80%]

65. V. Nair, Endogenous Synthesis of Dihydropyridines, Miles Workshop on Endogenous DHP Ligands, Miles Laboratory Publication, **1987**. [100%]

66. V. Nair, R. J. Offerman, G. A. Turner, A. N. Pryor, and N. C. Baenziger, Fluorescent 1,4-Dihydropyridines: The Malondialdehyde Connection, Tetrahedron, **1988**, 44, 2793-2803. [70%]

67. V. Nair, G. A. Turner, G. A. Buenger and S. D. Chamberlain, New Methodologies for the Synthesis of C-2 Functionalized Hypoxanthine Nucleosides, J. Org. Chem., **1988**, 53, 3051-3057. [70%]

68. V. Nair and G. S. Buenger, Rare Purine Nucleosides: Congeners of the Antibiotic, Nebularine, Synthesis, **1988**, 848-850. [70%]

69. V. Nair and B. J. Hettrick, Sulfone of the Antibiotic, Nebularine: Synthesis and Conversion to Novel Analogues of Nebularine, Tetrahedron, **1988**, 44, 7001-7006. [80%]

70. V. Nair, 2-Substituted Inosines and Their Use as Antiviral Agents, Serial No. 366,425, U. S. Patent Office, **1989** (Patent Granted). [100%]

71. V. Nair, S. G. Richardson, and S. D. Chamberlain, 9-(β -D-ribofuranosyl)purine (Nebularine) and Related Deaminated Nucleosides, Nucleic Acid Chemistry, Part 4, L. B. Townsend, Editor, **1989**. [90%]

72. V. Nair, D. A. Young, and R. DeSilvia, 2-Amino-9-(β -D-ribofuranosyl)purine, Nucleic Acid Chemistry, Part 4, L. B. Townsend, Editor, **1989**. [90%]

73. V. Nair and A. G. Lyons, Novel Unsaturated Purine Nucleosides, Tetrahedron, **1989**, 45, 3653-3662. [70%]

74. V. Nair and G. S. Buenger, Novel Stable Congeners of the Antiretroviral Compound, 2',3'-Dideoxyadenosine, J. Am. Chem. Soc., **1989**, 111, 8502-8504. [70%]

75. V. Nair, D. A. Purdy, and T. B. Sells, Synthesis of Congeners of Adenosine Resistant to Deamination by Adenosine Deaminase, Chem. Commun., **1989**, 878-879. [70%]

76. V. Nair, Development of Methodologies for the Strategic Modification of Purine Ribonucleoside Systems, Nucleosides and Nucleotides, **1989**, 8, 699-708. [100%]

77. V. Nair, Stable Congeners of 2',3'-Dideoxyadenosine as Potential Anti-AIDS Agents, Serial No. 343,334, U. S. Patent Office, **1989** (Pending). [100%]

78. V. Nair, 6-Iodo-9-(2',3',5'-tri-O-acetyl- β -D-ribofuranosyl)purine, Organic Synthesis, **1990**, Invited Contribution. [100%]

79. V. Nair and G. S. Buenger, Regiospecific 5'-Silylation of Nucleosides, Org. Prep. Proc. Intl., **1990**, 22, 57-61. [80%]

80. V. Nair and G. A. Buenger, Hydrolysis of Dideoxygenated Purine Nucleosides: Effect of Modification of the Base Moiety, J. Org. Chem., **1990**, 55, 3695-3697. [80%]

81. V. Nair and M. A. Ussery, RNA Antiviral Activity of Some Functionalized Hypoxanthine Systems, Antiviral Chem. Chemother., **1990**, Submitted. [80%]

82. V. Nair and T. B. Sells, Copper-Mediated Transformations in Nucleoside Synthesis, Tetrahedron Lett., **1990**, 31, 807-810. [75%]

83. V. Nair, G. S. Buenger, D. F. Purdy and T. B. Sells, Synthesis and Enzymology of New Dideoxynucleosides with Anti-HIV Potential, Antiviral Res., **1990**, Suppl. I, 47. [70%]

84. V. Nair and D. A. Purdy, Synthetic Approaches to New Doubly Modified Nucleosides: Congeners of Cordycepin and Related 2'-Deoxyadenosine, Tetrahedron, **1990**, In Press. [70%]

85. V. Nair and G. S. Buenger, Competitive Inhibition of Mammalian Adenosine Deaminase by Analogues of Dideoxyadenosine, Biochem. Biophys. Acta, **1990**, Submitted. [70%]

86. V. Nair and G. S. Buenger, Dideoxygenated Purine Nucleosides Substituted at the 8-Position: Chemical Synthesis and Stability, Synthesis, **1990**, In Press, October Issue. [70%]

87. V. Nair and Todd B. Sells, Metal Mediated Reactions in Nucleoside Synthesis, Nucleosides and Nucleotides, **1990**, In Press. [80%]

88. V. Nair and A. G. Lyons, Hypoxanthine Nucleoside Counterparts of the Antibiotic, Cordycepin, Tetrahedron, **1990**, In Press. [70%]

89. V. Nair, A. G. Lyons, and D. F. Purdy, Novel Analogues of the Nucleoside Antibiotic, Cordycepin, Nucleosides and Nucleotides, **1990**, In Press. [80%]

90. V. Nair and G. A. Buenger, Approaches to New Dideoxynucleosides, Nucleosides and Nucleotides, **1990**, In Press. [80%]

91. V. Nair, N. J. Leonard, E. De Clercq, J. Balzarini, and G. S. Buenger, 3-Iso-3'-dideoxyadenosine: Synthesis, Stability and Anti-HIV Activity, Antiviral Res., **1990**, Submitted. [60%]

92. V. Nair and A. J. Fasbender, Novel 2,6-Disubstituted Adenosine Analogues: Potential Agonists for Adenosine Receptors, Nucleosides and Nucleotides, **1990**, In Press. [80%]

93. V. Nair and T. B. Sells, Novel Deoxyarabino and Deoxyxylo Nucleosides, Nucleosides and Nucleotides, **1990**, In Preparation. [70%]

PATENTS

V. Nair, 2-Substituted Inosines and Their Use as Antiviral Agents, Patent Serial No. 366,425, U. S. Patent Office, Patent Granted, 1989.

V. Nair, Stable Congeners of 2',3'-Dideoxyadenosine as Potential anti-AIDS Agents, Serial No. 343,334, U. S. Patent Office, 1989 (Pending).

BOOKS AND MONOGRAPHS

V. Nair, Co-Author of "Heterocyclic Compounds", Volume 42, A. Hassner, Ed., Wiley: New York, 1983

V. Nair, Author of "New Methodologies in Nucleoside Synthesis," A. R. Katritzky, C. W. Rees, O. Meth-Cohn, Eds., Academic Press: London, 1992 (In Preparation)

PUBLICATIONS, ABSTRACTS, AND PROCEEDINGS CONTRIBUTIONS

Total = 160

INVITED AND CONTRIBUTED PAPERS AND LECTURES

Total = 136

CURRICULUM VITAE

VASU NAIR

Professor of Chemistry

and

University Faculty Scholar

University of Iowa

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Phone: (319) 335-1364 (Office)
(319) 351-7648 (Home)
FAX: (319) 335-2951

I. Personal Data

Born: Suva, Fiji Islands

Citizenship: U. S. A. (since April 18, 1975)

Social Security Number: 322 46 0386

Home Address: 1754 Winston Drive, Iowa City, Iowa 52245

Marital Status: Married, to Barbara Baker Nair (June 24, 1978)

Number of Children: 1, Alison Baker Nair (10)

Church Affiliation: First Presbyterian Church, Iowa City
(Board of Directors and Education Council)

II. Education

Year	Degree	Institution
1963	B.Sc. (Hons.)	University of Otago Dunedin, New Zealand
1966	Ph.D. (Organic Chemistry, Natural Products)	University of Adelaide Adelaide, Australia

III. Postdoctoral Training

Year	Position	Institution
1966-67	Postdoctoral Fellow	University of Sydney Sydney, Australia (with Prof. A. V. Robertson)
1967-68	Research Associate (Organic Chemistry)	University of Illinois Urbana, Illinois, U. S. A. (with Prof. N. J. Leonard)
1968-69	Research Fellow (Organic Chemistry)	Harvard University Cambridge, Mass., U. S. A. (with Nobel Laureate R. B. Woodward)

IV. Academic Appointments

Year	Position	Institution
1969-79	Assistant Professor-Associate Professor (Chemistry)	University of Iowa Iowa City, Iowa
1980-	Professor (Chemistry)	University of Iowa
1982-86	Director	University of Iowa High-Field NMR Facility
1981-85, 1989-90	Executive Committee	Department of Chemistry University of Iowa
1987	Distinguished Visiting Scholar	University of Adelaide Australia
1989-92	Faculty Scholar	University of Iowa

V. Professional Affiliations (Memberships and Offices)

American Chemical Society (Iowa Section - Executive Committee, Iowa Award Committee Chairman, Midwest Meeting Program Chairman, National ACS Award Committee)

American Association for the Advancement of Science

Iowa Academy of Science (Chairman, Organic and Biological Chemistry Section)

International Union of Pure and Applied Chemistry

International Society for Antiviral Research

VI. General Areas of Research Interest

Synthetic Organic and Bioorganic Chemistry: Heterocyclic Compounds, Carbohydrate and Nucleoside Chemistry, Biologically-Active Nucleosides and Nucleotides, Antiviral and Anticancer Activities

VII. Current Research Projects

(1) Development and Application of Synthetic Methodology in Carbohydrate and Nucleoside Chemistry

(2) Synthesis and Enzymology of Unique Nucleosides with Antiviral Activity against RNA Viruses and Retroviruses (HIV-1 and HIV-2)

VIII. Teaching Activities

Freshman Level Organic Chemistry and Biochemistry (4:8); General Chemistry Laboratory (4:9); Undergraduate Organic Chemistry (4:122); Undergraduate Organic Laboratory (4:141); Graduate Level Introduction to Organic Research (4:221); Beginning Graduate Level Organic Chemistry (4:172); Mechanisms of Organic Reactions, Graduate Level (4:228); Graduate Organic Seminar (4:285); Special Topics in Organic Chemistry (4:223); Research Seminar (4:291); Research in Chemistry (4:290)

IX. Research Activities

Ph.D. and M.S. Thesis Work Supervised Previously

Total = 23

Ph.D. Thesis Work Currently Being Supervised

(1) Greg S. Buenger

(2) Arthur G. Lyons

(3) David Purdy

(4) Al Fasbender

(5) Todd Sells

(6) Zoraida Nuesca

(7) Lawrence Zintech

(8) Steven A. Adah

(9) Pascal J. Bolon

(10) R. Brian Hamilton

Current Postdoctoral Fellows

One
Second Position Open

Service as Member of Ph.D. Committees

1969-88 Total = 120

Undergraduate Research Work Supervised

Total = 17

Visiting Faculty or Scientist Research Work Supervised

Total = 3

X. Professional Service

Year	Agency/ Organization	Nature of Service
1978-to present	N. S. F.	Reviewer of Research Grant Proposals
1970-to present	American Chemical Society, Petroleum Research Fund	Reviewer of Research Grant Proposals
1983-to present	N. I. H.	Ad Hoc Reviewer of Research Grant Proposals
1970-to present	J. Am. Chem. Soc. J. Org. Chem. Tetrahedron Tetrahedron Letters Synthesis Can. J. Chem. Bioorganic Chem. Lipids Proc.Iowa Acad. Sci. Free Radicals in Biology & Medicine Biochem. Biophys. Methods J. Med. Chem. Chem. Res. Toxicol. (ACS) Nucleic Acid Research	Referee for Manuscripts Submitted for Publication
1979	American Chemical Society, Great Lakes Meeting, Rockford, Illinois	Invited Session Chair

1979	American Chemical Society, 178th National Meeting, Washington, D.C.	Invited Session Chair
1981	American Chemical Society, 17th Midwest Meeting, Columbia, Missouri	Invited Session Chair
1982	Iowa Academy of Science, 94th Session, Fort Dodge, Iowa	Invited Section Chair and Invited Symposium Chair Organic and Biological Chemistry
1982	American Chemical Society, 184th National Meeting, Kansas City, Missouri	Invited Session Chair
1983	American Chemical Society, 186th National Meeting, Washington, D.C.	Invited Session Chair
1984	American Chemical Society, 188th National Meeting, Philadelphia, Pennsylvania	Invited Session Chair
1985	American Chemical Society, 190th National Meeting, Chicago, Illinois	Invited Session Chair
1986	American Chemical Society, 192nd National Meeting, Anaheim, California	Invited Session Chair
1987	American Chemical Society, 194th National Meeting, New Orleans, Louisiana	Invited Session Chair
1988	23rd Midwest Meeting, American Chemical Society	Program Chairman
1989	American Chemical Society, 198th National Meeting, Miami Beach, Florida	Invited Session Chair
1983-to present	Many U.S. Universities & Colleges	External Reviewer for Promotions and Tenure
1990-93	American Chemical Society	Member, Review Committee for National Awards in Carbohydrate and Related Chemistry

XI. Special Honors, Awards, and Professional Recognition

(1) The Fiji Scholarship, 1959-62

(2) Monsanto Scholarship, 1963

(3) Australian Commonwealth Fellowship, 1964-66

(4) USPHS Fellowship, 1966

(5) Postdoctoral Fellowship, NSF Sponsored, 1967-68

(6) Research Fellowship, NIH Sponsored, 1968-69

(7) Previous Major Extramural Research and Equipment Grants and Awards:

Total = 13

(8) Current and Pending Grants and Awards (1989, 1990): V. Nair, Sole Principal Investigator

(i) U.S. Army Medical Research Command, 1985-89 ($220,000, Funded)
(ii) National Institutes of Health, 1990-93 ($400,000, Funded)
(iii) U.S. Army Medical Research Command, 1989-92 ($450,000, Funded)
(iv) Defense-University Research Instrumentation Program, 1989-90 ($75,000, Funded)
(v) Burroughs Wellcome Company, 1989-90 (Unsolicited Gift for Research, $25,000, Funded)
(vi) Burroughs Wellcome Company, 1990-91 (Unrestricted Research Grant for Research, $55,000 Funded)
(vii) National Institutes of Health, 1991-95 (Pending)
(viii) University Faculty Scholar Award, 1989-92 (Funded)

(9) Member and Research Investigator, Iowa Cancer Center, 1981-to present

(10) Member, NIH Funded University Biocatalysis Program

(11) Mentioned in "American Men and Women of Science"

(12) Mentioned in "Who's Who in Technology Today"

(13) Mentioned in "Leading Consultants in Technology"

(14) Elected Fellow, Iowa Academy of Science, 1986

(15) Consultant, Eastman Kodak Research Laboratories, Rochester, New York, 1980

(16) Consultancy Visit, Miles Laboratory, West Haven, Connecticut, 1987

(17) Distinguished Visiting Scholar Award, University of Adelaide, Australia, July, 1987

(18) Leading Consultant, Nucleoside Chemistry, Burroughs Wellcome Co., Research Triangle Park, North Carolina, 1984-to present

(19) Consultant, Nucleoside Chemistry, Integrated DNA Technologies, Inc., Iowa City, 1988-present

(20) Consultant, Nucleoside and Related Chemistry, Gensia Pharmaceuticals, Inc., San Diego, California, 1988-present

(21) Consultant, National Institutes of Health, 1983-present

(22) Planery Lecturer, 8th International Symposium on Nucleosides, Nucleotides, and their Biological Applications, Alabama, 1988

(23) Faculty Scholar Award, Unique Nucleosides with Therapeutic Potential in AIDS, University of Iowa, 1989-92 (One of the Highest Awards for Research)

(24) Distinguished Fellow Award (Science Medal), Iowa Academy of Science, 1989
(Highest Award of the Academy)

XII. Departmental and University Committees

(1) Pre-Medical Curriculum Advisor, University of Iowa, 1972-76

(2) Faculty Participant in NSF funded Secondary Science Training Program, University of Iowa, 1973-76

(3) Colloquium Committee, Department of Chemistry, 1971-75, Chairman, 1972-75

(4) Equipment Committee, Department of Chemistry, 1974-76, Chairman, 1974-76

(5) Graduate Study Committee on Student Evaluation, Department of Chemistry, 1979-80

(6) Instrument Services Committees, Department of Chemistry, 1975-77

(7) Graduate Admissions Committee, Department of Chemistry, 1977-79, Chairman, 1978-79

(8) Faculty Search Committees, Department of Chemistry, 1975-to present

(i) Inorganic Search, Junior Faculty, 1975-76
(ii) Organic Search, Junior Faculty, 1976-77
(iii) Organic Search, Senior Faculty, 1976-77, Chairman
(iv) Organic Search, Junior Faculty, 1977-78
(v) Organic Search, Junior Faculty, 1980-81
(vi) Organic Search, Junior Faculty, 1981-82
(vii) Organic Search, Senior Faculty, 1983-84
(viii) Organic Search, Junior Faculty, 1983-84
(ix) Organic Search, Junior Faculty, 1984-85, Chairman
(xi) Search for Department Chair, 1987-89

(9) Professional Staff Search Committees, Chemistry

(i) NMR Technician, 1973-74, Chairman
(ii) NMR Technician, 1984-85, Chairman
(iii) Aided in Search for Technology Liason Officer, University of Iowa Research Foundation

(10) Member, Faculty Forum on Science Courses for Nursing Students, School of Nursing, 1981

(11) Academic Advisor, Special Support Services for Disadvantaged Students, University of Iowa, 1978-83

(12) Committee D, Research Involving Human Subjects, University of Iowa, 1978-80

(13) Faculty Scholars Review Committee, University of Iowa, 1983-84

(14) Executive Committee, Department of Chemistry, 1981-85, 1989-90

(15) Organized First Ida Beam Lecture in Chemistry, 1983

(16) Chair, University High-Field NMR Committee, 1986-

(17) Chair, Junior Faculty Review Committee, Department of Chemistry 1986

(18) Chair, Department Stockroom Committee, 1986-87

(19) Member, University Committee for Academic Review of College of Pharmacy, 1987

(20) Chair, First Stanley Wawzonek Lecture, 1987

(21) Mentor, Undergraduate Scholarship Assistant Program, 1987-

(22) Member, University Committee for Review of Proposal for Establishing Pharmacy Manufacturing Facility, 1988

(23) Member, Special Proposal Review Committee designated by the Office of the President, 1988

(24) Ad Hoc Member, Committee to Review Candidates for Vice President for Research, University of Iowa, 1989

XIII. Publications, Abstracts and Proceedings Contributions

Total = 160

XIV. Patents

V. Nair, 2-Substituted Inosines and Their Use as Antiviral Agents, Patent Serial No. 366,425, U. S. Patent Office, Patent Granted, 1989.

V. Nair, Stable Congeners of 2',3'-Dideoxyadenosine as Potential anti-AIDS Agents, Serial No. 343,334, U. S. Patent Office, Filed 1989 (Pending).

XV. Books and Monographs

V. Nair, Co-Author of "Heterocyclic Compounds", Volume 42, A. Hassner, Ed., Wiley: New York, 1983

V. Nair, Author of "New Methodologies in Nucleoside Synthesis," A. R. Katritzky, C. W. Rees, O. Meth-Cohn, Eds., Academic Press: London, 1992 (In Preparation)

XVI. Invited and Contributed Papers and Lectures

Total = 136

Some **Representative Examples** of **Invited** and Contributed Papers and Lectures

- V. Nair, The Total Synthesis of Vitamin B_{12}, Special Invited Seminar, The University of Iowa, Iowa City, Iowa, 1970.
- V. Nair, Synthesis and Biological Evaluation of an Analogue of Puromycin, Massachusetts Institute of Technology, Cambridge, Massachusetts, 1976.
- V. Nair and D. J. Emanuel, Synthesis, ^{13}C NMR and Stereochemistry of a Reversed Aminoacyl Nucleoside: An Analogue of Puromycin, National (Bicentennial) Meeting of the American Chemical Society, San Francisco, California, 1976.

- V. Nair, Puromycin Analogues, Rice University, Houston, Texas, 1976.

- V. Nair, Inhibitors of Adenosine Deaminase, M. D. Anderson Hospital and Tumor Institute, Texas Medical Center, Houston, Texas, 1976.

- V. Nair and A. K. Sinhababu, Selective Transformation of Sugar Tosylhydrazones to Deoxy and Unsaturated Sugars, 175th National Meeting of the American Chemical Society, Anaheim, California, 1978.

+ V. Nair, R. H. Stevens, D. E. Vietti, and A. J. Lawson, Synthesis and Biological Evaluation of a Reversed Analogue of Adenosine 3',5'-Cyclic Monophosphate, 176th National Meeting of the American Chemical Society, Miami Beach, Florida, 1978.

- V. Nair and C. S. Cooper, The Chemistry of Vinamidinium Salts: Alkylation Reactions to Multifunctional Dienaminones, 180th National Meeting of the American Chemical Society, Las Vegas, Nevada, 1980.

- V. Nair, Invited Lecture, Synthesis of Biologically-Active Modified Nucleosides and Nucleotides, Eastman Kodak Research Laboratories, Rochester, New York, 1980.

- V. Nair and R. J. Wiechert, Novel Inhibitors of 3',5'-Cyclic Nucleotide 3'-Phosphohydrolase, 182nd National Meeting of the American Chemical Society, New York, New York, 1981.

- V. Nair and S. G. Richardson, Purinyl Radicals in Nucleic Acid Chemistry, 5th International Round Table on Nucleosides, Nucleotides, and Their Biological Applications, Research Triangle Park, North Carolina, 1982.

- V. Nair, Synthetic Modifications of the Furan Ring System with Vinylogous Amidines, John Stuart Research Laboratories, Barrington, Illinois, 1982.

- V. Nair, C. S. Cooper, T. S. Jahnke, and G. A. Turner, Carbon Elongations with Vinylogous Amidines, 184th National Meeting of the American Chemical Society, Kansas City, Missouri, 1982.

- V. Nair, Synthetic Cyclic Nucleotides in Cancer Chemotherapy, Fiji School of Medicine, Fiji, 1982.

- V. Nair and G. A. Turner, Structure and Stereochemistry of Purine - Malondialdehyde Adducts, 186th National Meeting of the American Chemical Society, Washington, D.C., 1983.

- V. Nair and D. A. Young, Synthesis of Arylated and Heteroarylated Nucleosides <u>via</u> Purinyl Radicals, 187th National Meeting of the American Chemical Society, St. Louis, Missouri, 1984.

- V. Nair and R. J. Offerman, Structure, Stereochemistry, and Regiospecificity of Cytosine-Malondialdehyde Adduct Formation, 187th National Meeting of the American Chemical Society, St. Louis, Missouri, 1984.

- V. Nair, Purinyl Radicals in Nucleoside Synthesis, Wellcome Research Laboratories, Burroughs Wellcome Co., Research Triangle Park, North Carolina, 1984.

- V. Nair, Carbon Elongations with Azadienes: The Malondialdehyde Connection, Duke University, Durham, North Carolina, 1984.

- V. Nair and S. D. Chamberlain, Novel Carbon-Carbon Bond Formation in Purines Involving the $S_{RN}1$ Reaction, 188th National Meeting of the American Chemical Society, Philadelphia, Pennsylvania, 1984.

- V. Nair, D. A. Young, and S. D. Chamberlain, Novel Photoinduced C-Alkylations in Purines, 10th International Congress of Heterocyclic Chemistry, Wateloo, Canada, 1985.

- V. Nair and S. D. Chamberlain, A New Approach to the Functionalization of Purine Systems, 190th National Meeting of the American Chemical Society, Chicago, Illinois, 1985.

- V. Nair and T. S. Jahnke, Model Multifunctional Epoxides: The Chemistry of Epoxy Enols, 190th National Meeting of the American Chemical Society, Chicago, Illinois, 1985.

- V. Nair, S. D. Chamberlain, R. G. DeSilvia, and G. S. Buenger, Synthetic Approaches to Rare 2-Substituted Purine Nucleosides, 7th International Round Table Symposium on Nucleosides, Nucleotides and their Biological Applications, Konstanz, West Germany, 1986.

- V. Nair, Novel Approaches to the Synthesis of Biological Active Purine Nucleosides, Royal Institute of Technology, Stockholm, Sweden, 1986.

- V. Nair, Novel Approaches to the Synthesis of Potential Antiviral Nucleosides, Research and Development Laboratories, Antiviral Chemotherapy, Astra Alab AB, Sodertalje, Sweden, 1986.

- V. Nair, R. J. Offerman, and G. A Turner, Novel Fluorescent 1,4-Dihydropyridines, 192th National Meeting of the American Chemical Society, Los Angeles, California, 1986.

- V. Nair, Endogenous Synthesis of Dihydropyridines, Miles Institute for Preclinical Pharmacology Workshop, New York, N.Y., 1987.

- V. Nair, Eight Lectures, In the Distinguished Scholar Lecture Series on The Search for New Antiviral Compounds, Universities of Adelaide, Sydney, Flinders, La Trobe, and the Victorian College of Pharmacy, 1987.

- V. Nair, United States Army Medical Research in Infectious Diseases, Department of Antiviral Studies, Rare Functionalized Purine Nucleosides, Washington, D. C., 1987.

- V. Nair, Novel Approaches to the Synthesis of Biologically Active Purine Nucleosides, School of Chemical Sciences, University of Illinois, Urbana, 1987.

- V. Nair, G. A. Turner, G. S. Buenger, and A. G. Lyons, Synthetic Approaches to New Biologically Active Purine Nucleosides, The Third Chemical Congress of North America, Toronto, Canada, 1988.

- V. Nair, Planery Lecture, Development of Methodologies for the Strategic Modification of Purine Ribonucleoside Systems, 8th International Symposium on Nucleosides, Nucleotides, and their Biological Applications, Perdido Beach, Alabama, 1988.

- V. Nair, Synthesis of Biologically Active Nucleosides, Gensia Pharmaceuticals, San Diego, California, 1988.

- V. Nair, Design of Antiretroviral Molecules, 100th Annual Meeting of the Iowa Academy of Science, Ames, Iowa, 1988.

- V. Nair, A. G. Lyons and D. A. Purdy, Novel Functionalized 2'-Deoxynucleosides, 197th National American Chemical Society Meeting, Dallas, Texas, 1989.

- V. Nair and G. S. Buenger, Synthesis of New Congeners of Dideoxyadenosine, 197th National Meeting of the American Chemical Society, Dallas, Texas, 1989.

- V. Nair, The Quest for Effective Anti-AIDS Compounds, Iowa Section of the American Chemical Society, Cedar Rapids, Iowa, 1989.

- V. Nair, The Quest for Effective Anti-AIDS Compounds, Ozark (Missouri) Section of the American Chemical Society, Springfield, Missouri, 1989.

- V. Nair, Methodologies for the Modification of Purine Ribonucleoside Systems, National Cancer Institute, Bethesda, Maryland, 1989.

- V. Nair, Chemistry, Enzymology, and Anti-HIV Activity of New Congeners of 2',3'-Dideoxyadenosine, Third International Conference on Antiviral Research, Brussels, Belgium, 1990.

- V. Nair, Synthesis of Analogues and Pro-Drugs of the Antiretroviral Compound, 2',3'-Dideoxyinosine, Ninth International Round Table Symposium on Nucleosides, Nucleotides, and their Biological Applications, Uppsala, Sweden, 1990.

+ Paper received special ACS news coverage

XV. Invited Scientific Commentary

WSUI Faculty Commentary on Research
"The Quest for Effective Antiviral Agents: Contributions of A Basic Scientist", 1989.

INFORMATION ON THE UNIVERSITY OF IOWA

The University of Iowa, established in 1847, is a State University built on the banks of the Iowa River in Iowa City. It is one of the Big Ten Universities. The current student enrollment is 30,000. Major programs at the University of Iowa include, among others, Liberal Arts and Sciences, Medicine, Nursing, Law, Pharmacy, Engineering, Business, Journalism, Education, and Graduate Studies. The Medical School has the largest teaching hospital in the nation.

The Department of Chemistry has twenty eight faculty members, 110 graduate students, 25 postdoctoral fellows, 5 visiting scientists, and 3 temporary teaching faculty members. The department has millions of dollars worth of state-of-the-art research equipment including five high-field NMR spectrometers (one of which is a 600 MHz instrument), four mass spectrometers (including FAB HRMS, MS/MS and LC/MS), two X-ray diffractometers, three FTIR spectrometers, two ESR instruments, one fluorescence spectrometer, numerous lasers, many modern UV spectrometers, many HPLC and GC instruments, numerous computers, and many other smaller items of research equipment. Professor Nair has played a major role in the acquisition of state-of-the-art NMR equipment for this department. He is currently chair of the University High-Field NMR Committee.

APPENDIX

COPIES OF SELECTED PUBLICATIONS

Nucleoside Chemistry and Ramifications

(See Section on Research Accomplishments)

Publications Listed in Chronological Order

- N. J. Leonard, F. C. Sciavolino, and V. Nair, Stereochemistry of the Anomers of Methyl 2-Deoxy-D-ribofuranoside. Synthesis of Methyl 5-(6-Aminopurin-9-yl)-2,5-dideoxy-β-D-ribofuranoside, A "Reversed" Nucleoside, J. Org. Chem. **1968**, 33, 3169-3174.

- V. Nair and R. H. Walsh, 4-Amino-1,4-Dideoxyribofuranoside: A New Amino Sugar with Nitrogen in the Ring, Carbohyd. Res. **1974**, 36, 131-137.

- V. Nair and R. H. Walsh, Synthesis of 1-(6-Aminopurin-9-yl)-2,5-Anhydro-1,2-Dideoxy-DL-Ribitol: A New Reversed Amino Nucleoside, J. Org. Chem. **1974**, 39, 3045-3047.

- V. Nair and D. J. Emanuel, Synthetic Design, Stereochemistry, and Enzymatic Activity of a Reversed Aminoacyl Nucleoside: An Analog of Puromycin, J. Am. Chem. Soc. **1977**, 99, 1571-1576.

- B. H. Jo, V. Nair, and L. Davis, Carbon-13 NMR Studies of Vitamin B_6 Schiff Base and Carbinolamine Formation in Aqueous Solution. The Adduct of Pyridoxal 5'-Phosphate and DL-Alanine. J. Am. Chem. Soc. **1977**, 99, 4467-4471.

- V. Nair and A. K. Sinhababu, Selective Transformations of Sugar Tosylhydrazones to Deoxy and Unsaturated Sugars, J. Org. Chem. **1978**, 43, 5013-5017.

- V. Nair and S. G. Richardson, Conversion of 9-Substituted Adenines to the Corresponding Purines: A Simple Deamination Method for Adenine Derivatives, Tetrahedron Lett. **1979**, 1181-1184.

- V. Nair and R. J. Wiechert, Substrate Specificity for Adenosine Deaminase-Function of the 5'-Hydroxyl Group of Adenosine, Bioorganic Chemistry, **1980**, 9, 423-433.

- V. Nair, R. J. Wiechert, D. E. Vietti, D. Singh, and R. H. Stevens, Methyl 5'-(6-Aminopurin-9-yl)-5'-deoxy-β-D-ribofuranoside 2',3'-Cyclic Monophosphate: A Novel, Biologically-Active, Structural Analogue of Cyclic AMP, J. Cyclic Nucleotide Res. **1980**, 6, 189-200.

- V. Nair and C. S. Cooper, Selective Alkylation Reactions with Vinamidinium Salts, Tetrahedron Lett. **1980**, 21, 3155-3158.

- V. Nair and S. G. Richardson, The Utility of Purinyl Radicals in the Synthesis of Base-Modified Nucleosides and Alkyl Purines: 6-Amino Group Replacement by H, Cl, Br, and I. J. Org. Chem. **1980**, 45, 3969-3974.

- V. Nair and A. K. Sinhababu, Carbohydrate Models of α-Methylene- γ-butyrolactones, J. Org. Chem. **1980**, 45, 1893-1897.

- V. Nair, D. E. Vietti and C. S. Cooper, The Degenerative Chemistry of Malondialdehyde: Structure, Stereochemistry, and Kinetics of Formation of Enaminals from Reaction with Amino Acids, J. Am. Chem. Soc. **1981**, 103, 3030-3036.

- V. Nair and C. S. Cooper, Chemistry of 1,5-Diaza-pentadienium (Vinamidinium) Salts: Alkylation Reactions to Multifunctional Dienamines and Dienaminones, J. Org. Chem. **1981**, 46, 4759-4765.

- V. Nair, S. G. Richardson and R. E. Coffman, Arylation and Heteroarylation of Photochemically Generated Purinyl Radicals, J. Org. Chem. **1982**, 47, 4520-4524.

- V. Nair and S. G. Richardson, Modification of Nucleic Acid Bases via Radical Intermediates: Synthesis of Dihalogenated Purine Nucleosides, Synthesis **1982**, 670-672.

- V. Nair, R. J. Wiechert and D. A. Young, Inhibition of 3',5'-Cyclic Nucleotide 3'-Phosphohydrolase by a Novel 1',2'-Cyclic Nucleotide, J. Cyclic Nucleotide Res. **1982**, 8, 181-190.

- V. Nair and S. D. Chamberlain, Reductive Deamination of Aminopurine Nucleosides, Synthesis **1984**, 401-403.

- V. Nair, G. A. Turner and R. J. Offerman, Novel Adducts from the Modification of Nucleic Acid Bases by Malondialdehyde, J. Am. Chem. Soc. **1984**, 106, 3370-3371.

- V. Nair, R. J. Offerman and G. A. Turner, Structural Alteration of Nucleic Acid Bases by Bromomalondialdehyde, J. Org. Chem. **1984**, 49, 4021-4025.

- V. Nair and D. A. Young, Synthetic Transformations of Transient Purinyl Radicals: Formation of Mono- and Di-Arylated and Heteroarylated Nucleosides, J. Org. Chem. **1984**, 49, 4340-4344.

- V. Nair and T. S. Jahnke, Carbon Elongation with 1,5-Diaza-pentadienium Salts: Reaction with Aralkyl Ketones, Synthesis **1984**, 424-426.

- V. Nair and T. S. Jahnke, Novel Intermediates for the Synthesis of Carbocyclic Spiro Compounds, Tetrahedron Lett. **1984**, 25, 3547-3550.

- V. Nair and G. A. Turner, The Thiobarbituric Acid Test for Lipid Peroxidation: Structure of the Adduct with Malondialdehyde, Lipids **1984**, 19, 804-805.

- V. Nair and D. A. Young, A New Synthesis of Isoguanosine, J. Org. Chem. **1985**, 50, 406-408.

- V. Nair and S. D. Chamberlain, Novel Photoinduced Carbon-Carbon Bond Formation in Purines, J. Am. Chem. Soc. **1985**, 107, 2183-2185.

- V. Nair and S. D. Chamberlain, Novel Photoinduced Functionalized C-Alkylations in Purine Systems, J. Org. Chem. **1985**, 50, 5069-5075.

- V. Nair and R. J. Offerman, Ring Extended Products from the Reaction Epoxy Carbonyl Compounds with Nuclei Acid Bases, J. Org Chem.. **1985**, 50, 5627-5631.

- V. Nair and D. A. Young, Photoinduced Alkylthiolation of Halogenated Purine Nucleosides, Synthesis. **1986**, 450-453.

- V. Nair, C. S. Cooper, D. E. Vietti, and G. A. Turner, The Chemistry of Lipid Peroxidation Metabolites: Crosslinking Reactions of Malodialdehyde, Lipids. **1986**, 21, 6-10.

- V. Nair, R. J. Offerman, G. A. Turner, Novel, Fluorescent 1,4-Dihydropyridines, J. Am. Chem. Soc. **1986**, 108, 8283-8285.

- V. Nair, D. A. Young, and R. G. DeSilvia, 2-Halogenated Purine Nucleosides: Synthesis and Reactivity, J. Org. Chem. **1987**, 52, 1344-1347.

- V. Nair and T. S. Jahnke, Model Multifunctional Epoxides Related to Hepoxilin A, Tetrahedron. **1987**, 43, 4257-4264.

- V. Nair and D. A. Young, Conformational Correlation of Purine Nucleosides by High-Field Carbon-13 NMR Data, Mag. Res. Chem. **1987**, 25, 937-940.

- V. Nair, G. A. Turner and S. D. Chamberlain, Novel Approaches to Functionalized Nucleosides via Palladium-Catalyzed Cross-Coupling with Organostannanes, J. Am. Chem. Soc. **1987**, 109, 7223-7224.

- V. Nair, R. J. Offerman, G. A. Turner, A. N. Pryor, and N. C. Baenziger, Fluorescent 1,4-Dihydropyridines: The Malondialdehyde Connection, Tetrahedron, **1988**, 44, 2793-2803.

- V. Nair, G. A. Turner, G. A. Buenger and S. D. Chamberlain, New Methodologies for the Synthesis of C-2 Functionalized Hypoxanthine Nucleosides, J. Org. Chem., **1988**, 53, 3051-3057.

- V. Nair and G. S. Buenger, Rare Purine Nucleosides: Congeners of the Antibiotic, Nebularine, Synthesis, **1988**, 848-850.

- V. Nair and B. J. Hettrick, Sulfone of the Antibiotic, Nebularine: Synthesis and Conversion to Novel Analogues of Nebularine, Tetrahedron, **1988**, 44, 7001-7006.

- V. Nair and A. G. Lyons, Novel Unsaturated Purine Nucleosides, Tetrahedron, **1989**, 45, 3653-3662.

- V. Nair and G. S. Buenger, Novel Stable Congeners of the Antiretroviral Compound, 2',3'-Dideoxyadenosine, J. Am. Chem. Soc., **1989**, 111, 8502-8504.

- V. Nair, D. A. Purdy, and T. B. Sells, Synthesis of Congeners of Adenosine Resistant to Deamination by Adenosine Deaminase, Chem. Commun., **1989**, 878-879.

- V. Nair, Development of Methodologies for the Strategic Modification of Purine Ribonucleoside Systems, Nucleosides and Nucleotides, **1989**, 8, 699-708.

- V. Nair and G. S. Buenger, Regiospecific 5'-Silylation of Nucleosides, Org. Prep. Proc. Intl., **1990**, 22, 57-61.

- V. Nair and G. A. Buenger, Hydrolysis of Dideoxygenated Purine Nucleosides: Effect of Modification of the Base Moiety, J. Org. Chem., **1990**, 55, 3695-3697.

- V. Nair and T. B. Sells, Copper-Mediated Transformations in Nucleoside Synthesis, Tetrahedron Lett., **1990**, 31, 807-810.

- V. Nair and D. A. Purdy, Synthetic Approaches to New Doubly Modified Nucleosides: Congeners of Cordycepin and Related 2'-Deoxyadenosine, Tetrahedron, **1990**, In Press.

- V. Nair and G. S. Buenger, Dideoxygenated Purine Nucleosides Substituted at the 8-Position: Chemical Synthesis and Stability, Synthesis, **1990**, In Press, October Issue.

- V. Nair and A. G. Lyons, Hypoxanthine Nucleoside Counterparts of the Antibiotic, Cordycepin, Tetrahedron, **1990**, In Press.

- V. Nair and A. J. Fasbender, Novel 2,6-Disubstituted Adenosine Analogues: Potential Agonists for Adenosine Receptors, Nucleosides and Nucleotides, **1990**, In Press.

Other Heterocyclic Chemistry

(Listed in Chronological Order)

- R. H. Andreatta, V. Nair, A. V. Robertson, and W. R. J. Simpson, Synthesis of cis and trans Isomers of Chloro-L-proline, 4-Bromo-L-proline, and 4-Amino-L-proline, Aust. J. Chem. **1967**, 20, 1493-1509.

- V. Nair and A. V. Robertson, Solvolytic Oxidation of O-Tosyl-4-hydroxyprolines in Dimethyl Sulphoxide, Aust. J. Chem. **1967**, 20, 1767-1769.

- R. H. Andreatta, V. Nair, and A. V. Robertson, Proton Magnetic Resonance Spectra, Configuration, and Conformation of 4-Substituted Prolines, Aust. J. Chem. **1967**, 20, 2701-2713.

- V. Nair, Cycloaddition of 1-Azirines with Nitrile Oxides. Formation of Carbodiimides, Tetrahedron Lett. **1971**, 4831-4833.

- V. Nair, Ring Expansion of 1-Azirines to Azepines via Cycloaddition, J. Org. Chem. **1972**, 37, 802-804.

- V. Nair, The Reaction of 1-Azirines with 1,3-Diphenylisobenzofuran. Ring Expansion to Isoquinoline, Dihydroisoquinoline, and Azanorcarane Derivatives, J. Org. Chem. **1972**, 37, 2508-2510.

- V. Nair and K. H. Kim, Thiadiazepinones: Synthesis and Thermal Stability, Tetrahedron Lett. **1974**, 1487-1490.

- V. Nair and K. H. Kim, Regioselective [4+2] and [2+2] Cycloadditions of 1-Azirines to Heterocumulenes. Formation and Thermal Rearrangements of the Cycloadducts, J. Org. Chem. **1974**, 39, 3763-3767.

- V. Nair and K. H. Kim, Carbon Disulfide as a 2 -Component in Its Cycloaddition with 1-Azirines, J. Org. Chem. **1975**, 40, 1348-1349.

- V. Nair and K. H. Kim, Transformation of 1-Azirines to 1H-Indoles with Benzyne. Evidence for the Intermediacy of the 3H-Indole System. J. Org. Chem. **1975**, 40, 3784-3786.

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- V. Nair, K. H. Kim, and R. H. Walsh, Ozonolysis of 3,4-Dehydroproline, J. Heterocycl. Chem. 1977, 14, 313-315.

- V. Nair, Azirines, Invited Contribution in "Small Ring Heterocycles," Vol. 1, A. Hassner, Ed., Wiley, New York, 1983, pp 215-232.

Stereochemistry of the Anomers of Methyl 2-Deoxy-D-ribofuranoside. Synthesis of Methyl 5-(6-Aminopurin-9-yl)-2,5-dideoxy-α-D-ribofuranoside, a "Reversed" Nucleoside[1]

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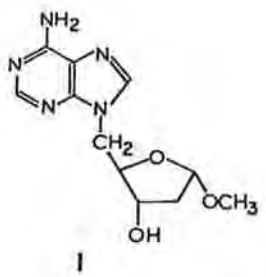
Received February 6, 1968

Methyl 2-deoxy-5-*O*-triphenylmethyl-α-D-ribofuranoside (**2**) and methyl 2-deoxy-5-*O*-triphenylmethyl-β-D-ribofuranoside (**3**) were synthesized and the stereochemistry of their anomeric centers was established unambiguously by chemical means and by complete analysis of their nmr spectra. The results are in agreement with those predicted by the Hudson isorotation rules. The syntheses of related ribofuranosides and of methyl 5-(6-aminopurin-9-yl)-2,5-dideoxy-α-D-ribofuranoside (**1**) are also described.

A route to the synthesis of ribose derivatives of adenine bonded at C-5 of the sugar moiety ("reversed" nucleosides) has been described[2] as part of a cooperative program with Professor Skoog at the University of Wisconsin[3] to determine the cytokinin activity[4,5] and chemical properties of compounds closely related to kinetin.[6,7] In providing a synthetic route to 2-deoxyribose derivatives of "reversed" nucleoside type, as exemplified by methyl 5′-(6-aminopurin-9-yl)-2′,5′-dideoxy-α-D-ribofuranoside (**1**), we found it desirable and also necessary to establish the stereochemistry of the anomeric centers for a series of useful intermediates.



1

A mixture of the α and β forms of methyl 2-deoxy-D-ribofuranosides[8] was treated with 1 equiv of triphenylmethyl chloride. Chromatography on silica gel afforded a separation of methyl 2-deoxy-5-*O*-triphenylmethyl-α-D-ribofuranoside (**2**) (28%), $[\alpha]^{26}D$ 64.4° (*c* 1.2, $CHCl_3$), and methyl 2-deoxy-5-*O*-triphenylmethyl-β-D-ribofuranoside (**3**) (24%), $[\alpha]D^{26}$ −43.8° (*c* 1.3, $CHCl_3$). The stereochemistry of the anomeric centers was temporarily assigned on the basis of Hudson's rules of isorotation[9] which correlate optical rotation and anomeric configuration. However, it has recently been discovered that several pyrimidine[10−12] and purine[13] 2-deoxy-D-ribonucleosides constitute exceptions to Hudson's rules. Although there is consistency among the rotations of a wide variety of 2-deoxy-D-ribofuranose esters and glycosides and there is no evidence currently available that Hudson's rules are not applicable to such substances,[14] it was desirable to confirm the assignments by further physical and chemical means. Accordingly, the configuraton of the anomeric center in **2** and **3** was rigorously established by an unambiguous chemical synthesis and by a complete analysis of their nmr spectra.

(1) The support of this work by a research grant (USPHS-GM-05829) from the National Institutes of Health, U. S. Public Health Service, is gratefully acknowledged.

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(3) F. Skoog, H. Q. Hamzi, A. M. Szweykowska, N. J. Leonard, K. L. Carraway, T. Fujii, J. P. Helgeson, and R. N. Loeppky, *Phytochemistry*, **6**, 1169 (1967).

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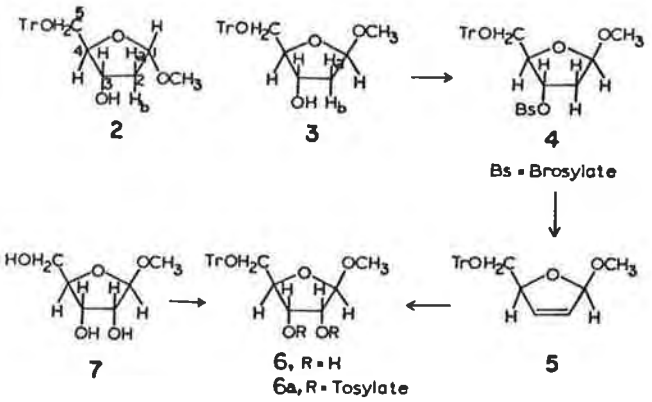
(13) R. H. Iwamoto, E. M. Acton, and L. Goodman, *J. Med. Chem.*, **6**, 684 (1963).

(14) D. L. MacDonald and H. G. Fletcher, Jr., *J. Amer. Chem. Soc.*, **84**, 1262 (1962).

The chemical determination of the stereochemistry of the anomeric center consisted in the conversion of methyl 2-deoxy-5-*O*-triphenylmethyl-β-D-ribofuranoside (**3**) into a substance of known anomeric configuration, methyl di-2,3-*O*-*p*-toluenesulfonyl-5-*O*-triphenylmethyl-β-D-ribofuranoside (**6a**). Thus, treatment of **3** with *p*-bromobenzenesulfonyl chloride in pyridine provided the *p*-bromobenzenesulfonate **4**, which was transformed into the olefin **5** in 63% yield by means of excess sodium methoxide in anhydrous DMF (Scheme I).[15,16]

SCHEME I



Osmylation of **5** followed by alkali–mannitol hydrolysis afforded the diol **6** in 74% yield. It was predicted that the diol would have the ribose configuration since osmium tetroxide should attack the double bond of **5** from the less hindered side, *i.e.*, from the side opposite the trityl and methoxyl groups. The diol was converted into its crystalline ditosylate derivative **6a**. That **6** and consequently **6a** did have the ribose configuration was shown by the intersecting conversion of methyl β-D ribofuranoside (**7**),[17] of known configuration, into the 5-*O*-trityl compound **6** and thence to the ditosylate derivative **6a**. Identity of the samples of **6a** prepared by the separate routes was established by melting point and mixture melting point, infrared and nmr spectra, and optical rotation. Since the stereochemistry at the anomeric center of **7** was known,[17] the methyl 2-deoxy-5-*O*-triphenylmethyl-D-ribofuranoside with the negative specific rotation necessarily had the β configuration (**3**) and the dextrorotatory isomer had the α configuration (**2**).[18,19]

(15) This substance is crystalline and stable at room temperature, and the method offers a convenient route for the introduction of 2,3 double bonds into the pentofuranosides; *cf.* J. Hildesheim, J. Cléophax, and S. D. Géro, *Tetrahedron Lett.*, 1685 (1967).

(16) Compound **5** may be of related biochemical interest in view of the recent investigations on 2′,3′-unsaturated nucleosides: (a) DHFUDR, see T. A. Khwaja and C. Heidelberger, *J. Med. Chem.*, **10**, 1066 (1967); (b) blasticidin S, see N. Otake, S. Takeuchi, T. Endo, and H. Yonehara, *Tetrahedron Lett.*, 1411 (1965); (c) J. R. McCarthy, Jr., M. J. Robins, L. B. Townsend, and R. K. Robins, *J. Amer. Chem. Soc.*, **88**, 1549 (1966); (d) J. P. Horwitz, J. Chua, M. Noel, and J. T. Donatti, *J. Org. Chem.*, **32**, 817 (1967); (e) J. P. Horwitz, J. Chua, M. A. DaRooge, M. Noel, and I. L. Klundt, *ibid.*, **31**, 205 (1966); (f) J. P. Horwitz, J. Chua, J. A. Urbanski, and M. Noel, *ibid.*, **28**, 942 (1963); (g) J. P. Horwitz, J. Chua, I. L. Klundt, M. A. DaRooge, and M. Noel, *J. Amer. Chem. Soc.*, **86**, 1896 (1964); (h) J. J. Fox and N. C. Miller, *J. Org. Chem.*, **28**, 936 (1963); (i) P. Reichard, *J. Biol. Chem.*, **237**, 3513 (1962).

(17) R. Barker and H. G. Fletcher, Jr., *J. Org. Chem.*, **26**, 4005 (1961).

(18) Catalytic reduction of 2,3-didehydro-2,3-dideoxy compounds leads to dideoxyribose derivatives, which are of interest particularly in purine nucleoside combination.[19]

(19) (a) M. J. Robins and R. K. Robins, *J. Amer. Chem. Soc.*, **86**, 3585 (1964); (b) M. J. Robins, J. R. McCarthy, Jr., and R. K. Robins, *Biochemistry*, **5**, 224 (1966); (c) G. L. Tong, W. W. Lee, and L. Goodman, *J. Org. Chem.*, **30**, 2854 (1965).

The configuration at C-1 of the α and β anomers of methyl 2-deoxy-5-*O*-triphenylmethyl-D-ribofuranoside (**2** and **3**), predicted first on optical rotation and then based firmly on chemical interconversion, was correlated with the nmr spectra of these anomers by a full analysis for the C-1, C-2, and C-3 protons, which is in itself of interest. This study is relevant to correlations between nmr spectra and configuration of the anomeric proton reported by Jardetzky,[20] Lemieux,[11,21] Leonard and Laursen,[22] and Robins and Robins.[23] In particular, Jardetzky[20] and Robins and Robins[23] have suggested a correlation for a series of α and β anomers of 2′-deoxyribofuranosyl nucleosides based purely on the appearance of the resonance due to the anomeric proton, its peak width, and vicinal coupling constants abstracted on a first-order basis from these signals. Our purpose in presenting the full nmr analysis of this part of the molecule is to establish accurate values of vicinal coupling constants of the anomeric proton and to emphasize the variation, with configuration, of the chemical-shift difference between the C-2 protons in **2** and **3**.

Chemical-shifts and coupling constants of the furan ring protons are tabulated (see below). The chemical shift of the C-5 protons and the overlapping methyl signals of the methoxyls are included. Assignments of multiplets to protons on C-1, C-2, C-3, and C-5 were obvious from the relative chemical shifts, integrated areas, and amount of fine structure. Difficulties with the C-3 and C-4 protons included considerable overlapping in the case of the α anomer and complexity of splitting patterns in the β anomer.

The two compounds gave an ABMX system[24] for H_{2a}, H_{2b} (AB part), H_1 (X), and H_3 (M), the M multiplet being further split by H_4. As usual, A is defined as the downfield part of the AB multiplet. The ABMX patterns can be analyzed by the general treatment of Pople and Schaefer[24] or by the procedure of Abraham and McLauchlan.[25] We chose to use the latter but with slight modifications. The AB part (showing 16 lines in the 100-Mc spectrum) was simplified by double irradiation at the position of the X resonance, which reduced this to an 8-line pattern (AB of ABM) which was analyzed by the general procedure for ABX analysis.[26] The 16-line AB part can be treated as the 8-line ABX pattern with each line doubled by M. The doublings, "d_{AM}" and "d_{BM}" (due to but not equal to J_{AM} and J_{BM} obtained in above analysis) are line separations in the M doublet of doublets. Subtraction of these doublings from the full AB part left the AB of ABX. The X part in both anomers appeared as a multiplet of four lines which gave $|J_{AX} + J_{BX}|$ for comparison with AB analysis. The parameters obtained from the analysis were used for the calculation of splitting patterns and intensities. In every case, excellent agreement was obtained between the calculated and the observed spectrum.

(20) C. D. Jardetzky, *J. Amer. Chem. Soc.*, **83**, 2919 (1961).

(21) R. U. Lemieux, *Can. J. Chem.*, **39**, 116 (1961).

(22) N. J. Leonard and R. A. Laursen, *Biochemistry*, **4**, 354 (1965).

(23) M. J. Robins and R. K. Robins, *J. Amer. Chem. Soc.*, **87**, 4934 (1965).

(24) J. A. Pople and T. Schaefer, *Mol. Phys.*, **3**, 547 (1960).

(25) R. J. Abraham and K. A. McLauchlan, *ibid.*, **5**, 195 (1962).

(26) J. A. Pople, W. G. Schneider, and H. J. Bernstein, "High-resolution Nuclear Magnetic Resonance," McGraw-Hill Book Co., Inc., New York, N. Y., 1959, pp 132–135.

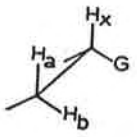
TABLE I

CHEMICAL-SHIFT AND COUPLING CONSTANT DATA FOR METHYL 2-DEOXY-5-O-TRIPHENYLMETHYL-D-RIBOFURANOSIDES

		Chemical shifts[a] δ scale, ppm									Splitting patterns	Coupling constants, cps					
		X	A[b]	B[b]	M					δ_{AB},	for H_1,						$J_{AX}/$
Compound	Solvent	H_1	H_{2a}	H_{2b}	H_3	H_4	H_5	Me	OH	cps	H_2, H_3	J_{AB}	J_{AX}	J_{BX}	J_{AM}	J_{BM}	J_{BX}
α anomer 2	$CDCl_3$ TMS	5.12	2.17	1.98	4.15	4.21	3.17	3.35	2.84	19.2	ABMX	13.4	4.8	0.7	5.8	1.3	6.85
β anomer 3	$CDCl_3$ TMS	5.01	2.10	1.96	3.82–4.40		3.25[c]	3.25	2.10	13.6	ABMX	13.0	1.9	5.5	6.4	6.4	0.34

[a] Aromatic protons absorbed between δ 7.08 and 7.54. [b] Assignment of A and B between H_{2a} and H_{2b} discussed in text. [c] Approximate value.

Parameters were obtained above in terms of A, B, M, and X, and the problem that remained was the assignment of A and B to H_{2a} and H_{2b} or *vice versa*. We resorted to empirical analogy using rigid cyclic molecules containing the monosubstituted ethane fragment **8**. There are several examples of this where the proton



8

H_b, *cis* to and eclipsed by the substituent group G (such as Cl, Br, OH, CN, N_3), is upfield from H_a, *trans* to G.[27,28] Chemical-shift theory is at present inadequate to make such predictions with confidence. Chemical shifts for H_{2a} and H_{2b} in these two compounds have been assigned by selecting the upfield component as the proton *cis* to the hydroxyl group after consideration of possible conformations. In the α anomer this shielding will occur both from the hydroxyl group and the methoxyl group but in the β anomer the shielding effects from these groups are in opposition. Recourse had to be taken then in the values of the coupling J_{AX} and J_{BX} and an approach in terms of small and large J_{vic} and the general Karplus equations.[29,30] Internal support for our assignment is discussed below (see Table I).

In the α anomer (**2**), the resonance of the C-1 proton was a clear doublet of doublets with $J_{AX} + J_{BX} = 5.5$ cps. The resonance of the C-3 proton was partly obscured by that of the C-4 proton but its splitting pattern was easily recognized in the overlapping sets of multiplets. The absorption of H_4 was a ragged doublet of doublets. Protons on C-5 were found to be magnetically equivalent and appeared as a clean doublet. The absorption of the C-2 protons (AB) appeared as a multiplet of 16 lines with some transitional degeneracies in the higher field part. The coupling constants $J_{AX} > J_{BX}$ and $J_{AM} > J_{BM}$ are of sufficient magnitude to use the ideas mentioned above on *cis* shielding by the hydroxyl and methoxyl groups and to assign H_{2a} as A and H_{2b} as B. An internal cross-check for self-consistency is provided by the larger observed value of δ_{AB} in this compound compared with that in the β anomer **3**.

In the spectrum of the β anomer the resonance of the C-1 proton appeared as a quartet with $J_{AX} + J_{BX} = 7.4$ cps. The signals due to H_3 and H_4 were a complex set of multiplets and were not analyzed. Analysis of the resonance of the C-5 protons was not possible because the methylene signal was obscured by the methoxyl group, but the former appeared to be magnetically nonequivalent. This is also the situation in the 2,3-didehydro compound **5**. The absorption of the methylene protons (H_{2a}, H_{2b}) appeared as a multiplet of 16 lines with little overlapping. The assignment of A and B as H_{2a} and H_{2b} was again made on the basis of the value of δ_{AB} and J_{vic}. The observation that $J_{AM} = J_{BM}$ is merely a reflection that changes in the conformation of the ring and orientation of substituents can produce gross changes in coupling constants.[31] In the two compounds the observed values of geminal coupling constants (J_{AB}) fit well their environment on both theoretical[32] and empirical grounds.[33] No sign determinations have been carried out but these values are presumed to be negative. The many factors which influence the magnitude of vicinal coupling constants in molecules of such complexity (in relation to their nmr spectra) cannot be dissected out in any quantitative fashion. Finally, the observed correlations between nmr spectra and configurations are as follows: (1) in the α anomer $J_{AX} > J_{BX}$, whereas in the β anomer $J_{AX} < J_{BX}$; (2) the value of δ_{AB} is larger in the α anomer.

Returning to the original goal, a series of five transformations converted the α anomer **2**, now of established configuration, into the "reversed" deoxynucleoside **1**. On treatment with *p*-bromobenzoyl chloride in pyridine, methyl 2-deoxy-5-*O*-triphenylmethyl-α-D-ribofuranoside was converted into methyl 3-*p*-bromobenzoyl-2-deoxy-5-*O*-triphenylmethyl-α-D-ribofuranoside (**9**). Aqueous acetic acid brought about detritylation. The resulting alcohol **10** was transformed by the action of *p*-bromobenzenesulfonyl chloride in pyridine into methyl 5-*p*-bromobenzenesulfonyl-3-*p*-bromobenzoyl-2-deoxy-α-D-ribofuranoside (**11**). The brosylate **11** reacted smoothly with sodium adenide in anhydrous DMF to give the blocked nucleoside, methyl 5-(6-aminopurin-9-yl)-3-*p*-bromobenzoyl-2,5-dideoxy-α-D-ribofuranoside (**12**), and methanolic ammonia transformed this into the "reversed" deoxynucleoside, methyl 5-(6-aminopurin-9-yl)-2,5-dideoxy-α-D-ribofuranoside (**1**) (Scheme II).

(27) (a) L. M. Jackman and S. Sternhell, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon Press, London, in press, Chapter 2-2; (b) A. D. Broom, M. P. Schweizer, and P. O. P. Ts'o, *J. Amer. Chem. Soc.*, **89**, 3612 (1967).

(28) For a full discussion of this point, see R. H. Andreatta, V. Nair, and A. V. Robertson, *Aust. J. Chem.*, **20**, 2701 (1967).

(29) M. Karplus, *J. Chem. Phys.*, **30**, 11 (1959).

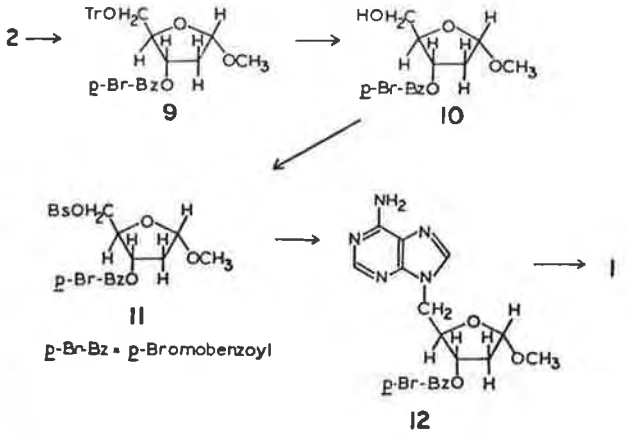
(30) M. Karplus, *J. Amer. Chem. Soc.*, **85**, 2870 (1963).

(31) It is of interest to note that no long range coupling was evident in the two spectra.

(32) A. A. Bothner-By, *Advan. Magnetic Resonance*, **1**, 195 (1965).

(33) R. C. Cookson, T. A. Crabb, J. J. Frankel, and J. Hudec, *Tetrahedron, Suppl.*, No. 7, 355 (1966).

SCHEME II



Experimental Section[34]

Methyl 2-Deoxy-5-*O*-triphenylmethyl-α- and -β-D-ribofuranosides (2 and 3).—To a solution of 9.60 g (67.5 mmol) of 2-deoxy-D-ribose in 200 ml of dry methanol was added 4.0 ml of saturated methanolic hydrochloric acid. After standing at room temperature for 30 min, the pale yellow solution was neutralized with Dowex-1 (HCO_3^-), filtered, and evaporated to dryness *in vacuo*. The residual oil was dissolved in 50 ml of dry pyridine and evaporated to dryness under high vacuum. This process was repeated once and the residue was dissolved in 200 ml of dry pyridine and treated with 19.0 g (68.0 mmol) of triphenylmethyl chloride. The solution was stirred at room temperature for 3 days and then poured into 2 l. of ice-cold 5% hydrochloric acid. The pH was adjusted to 3 by the further addition of ice-cold 10% hydrochloric acid. The mixture ws extracted with 700 ml of ether, and the aqueous phase was separated and extracted again with 300 ml of ether. The combined organic layers were shaken with three 100-ml portions of 5% potassium bisulfate, three 100-ml portions of water, and two 100-ml portions of saturated sodium chloride solution and were dried over anhydrous sodium sulfate. The ether was evaporated under reduced pressure. The residue was dissolved in 25 ml of methanol and this was placed in the icebox for 3 days. The precipitated triphenylcarbinol was removed by filtration (3.10 g) and washed thoroughly with cold methanol, and the methanol solution was evaporated *in vacuo*. The residue (23.0 g) was dissolved in ether, 15 g of silica gel was added, and the mixture was evaporated to dryness. The solid was applied to the top of a column of 400 g of silica gel packed in pentane–ether (9:1). The progress of the column was conveniently followed by tlc using the solvent system with which the column was being eluted. Elution was continued with pentane–ether (9:1) until all the triphenylcarbinol was removed. Polarity was gradually increased to pentane–ether (6:4), and 7.3 g (28%) of methyl 2-deoxy-5-*O*-triphenylmethyl-α-D-ribofuranoside (**2**) was eluted, $[\alpha]^{26}D$ 64.4° (*c* 1.3, $CHCl_3$), as a colorless gum. The material was homogeneous by tlc but could not be induced to crystallize. Continued elution of the column with pentane–ether (6:4) afforded 6.3 g (24%) of methyl 2-deoxy-5-*O*-triphenylmethyl-β-D-ribofuranoside (**3**), $[\alpha]^{26}D$ −43.8° (*c* 1.2, $CHCl_3$). This material was also homogeneous by tlc but could not be induced to crystallize. Separation of the two anomers was practically quantitative; only three of the fractions contained mixtures and these were discarded.

Methyl 3-*p*-Bromobenzenesulfonyl-2-deoxy-5-*O*-triphenylmethyl-β-D-ribofuranoside (4).—A solution of 1.10 g (2.80 mmol) of methyl 2-deoxy-5-*O*-triphenylmethyl-β-D-ribofuranoside (**3**) in 25 ml of anhydrous pyridine was treated with 1.07 g (4.20 mmol) of *p*-bromobenzenesulfonyl chloride in one portion and the solution was stirred at room temperature for 24 hr. It was poured into 150 ml of 5% sodium bicarbonate solution and extracted with 300 ml of ether. The ether was washed with four 100-ml portions of 5% potassium bisulfate solution, water to neutrality, and saturated sodium chloride solution. The ethereal solution was dried over anhydrous sodium sulfate; then the ether was evaporated under reduced pressure. The residue crystallized from methyl alcohol–ethyl acetate (10:2) to give 1.65 g of crude product. Recrystallization from methanol gave 425 mg, mp 94–95°, of a first crop and 350 mg, mp 93–95°, of a second crop (45%) of **4**: $[\alpha]^{23}D$ −8.0° (*c* 1.3, $CHCl_3$); ν_{max}^{Nujol} 1580, 1555 cm^{-1} (phenyl nuclei); nmr δ 7.76–7.15 (19 H, multiplet, aromatic protons), 5.16–4.88 (2 H, multiplet, H_1 and H_3), 4.30–4.05 (1 H, multiplet, H_4), 3.24 (3 H, singlet, CH_3O–), 3.10 (2 H, doublet, J = 6 cps, $2H_5$) and 2.31–2.15 (2 H, multiplet, $2H_2$)

Anal. Calcd for $C_{31}H_{29}BrO_6S$: C, 61.08; H, 4.79; S, 5.25. Found: C, 61.18; H, 5.01; S, 5.57.

Methyl 2,3-Didehydro-2,3-dideoxy-5-*O*-triphenylmethyl-β-D-ribofuranoside (5).—A solution of 230 mg (0.01 g-atom) of sodium in 25 ml of anhydrous methanol was evaporated to a small volume under reduced pressure. The solution was diluted with 20 ml of anhydrous dimethylformamide and evaporation was continued for 30 min to ensure complete removal of the methanol. A solution of 1.00 g (1.65 mmol) of methyl 3-*p*-bromobenzenesulfonyl-2-deoxy-5-*O*-triphenylmethyl-β-D-ribofuranoside (**4**) in 10 ml of dry dimethylformamide was added dropwise during 5 min at room temperature. The solution was stirred for 45 min and poured into a two-phase mixture of 400 ml of water and 200 ml of ether. The aqueous phase was separated and extracted again with 100 ml of ether. The combined organic extracts were washed with water and saturated sodium chloride solution and dried over anhydrous sodium sulfate. Evaporation under reduced pressure afforded 500 mg of a colorless gum which was applied to the top of a column of 20 g of silica gel packed in pentane–ether (9:1). The column was eluted with pentane–ether (8:2) and 20 ml fractions were collected. Fractions 3–7 were combined, evaporated, and recrystallized from ether–pentane to give 386 mg (63%) of **5** as colorless needles: mp 82–83°; $[\alpha]^{23}D$ −72.2° (*c* 1.1, $CHCl_3$); ν_{max}^{Nujol} 1625 (C=C) and 1590 cm^{-1} (phenyl nuclei); nmr δ 7.61–7.30 (15 H, multiplet, aromatic protons), 6.20–5.70 (3 H, multiplet, H_1, H_2, H_3), 5.01 (1 H, multiplet, H_4), 3.40 (3 H, singlet, CH_3O), and 3.20 (2 H, multiplet, $2H_5$).

Anal. Calcd for $C_{25}H_{24}O_3$: C, 80.61; H, 6.49. Found: C, 80.48; H, 6.49.

Methyl 5-*O*-Triphenylmethyl-β-D-ribofuranoside (6). A. From Olefin 5.—A solution of 1.46 g (3.93 mmol) of methyl 2,3-didehydro-2,3-dideoxy-5-*O*-triphenylmethyl-β-D-ribofuranoside (**5**) in 35 ml of anhydrous ether was treated with a solution of 1.00 g (3.93 mmol) of osmium tetroxide in 35 ml of the same solvent. A solution of 0.62 g (7.86 mmol) of anhydrous pyridine in 30 ml of dry ether was added, and the resulting solution was allowed to stand at room temperature for 24 hr. The light brown precipitate was collected by filtration, washed with ether, and dissolved in 50 ml of methylene chloride. A solution of 7.5 g of mannitol in 75 ml of 1% aqueous potassium hydroxide was added, and the two-phase system was stirred vigorously until the organic layer became colorless (*ca.* 5 hr). The methylene chloride layer was separated, washed with water, dried over anhydrous sodium sulfate, and evaporated. The resulting gum was filtered through a column of 20 g of silica gel with ether–pentane (4:1) and the eluate was evaporated under reduced pressure to give 1.18 g (74%) of **6** as a colorless glass: $[\alpha]^{23}D$ −18.8° (*c* 1.8, $CHCl_3$), homogeneous on tlc plates in solvent systems A, B, C, and D. It was characterized as the 2,3-ditosylate derivative.

A solution of 250 mg (0.61 mmol) of the compound described above in 10 ml of anhydrous pyridine, together with 285 mg (1.50 mmol) of *p*-toluenesulfonyl chloride, was stirred at room temperature for 5 days. The solution was poured into 100 ml of ice-cold 5% sodium bicarbonate, and the crystals were collected by filtration and washed well with water. One recrystallization from methanol–ethyl acetate gave 200 mg (46%) of methyl 2,3-di-*O*-*p*-toluenesulfonyl-5-*O*-triphenylmethyl-β-D-ribofuranoside (**6a**) as colorless needles: mp 140–141°; $[\alpha]^{23}D$ 63.8° (*c* 0.76' $CHCl_3$); ν_{max}^{Nujol} 1600 cm^{-1} (phenyl nuclei); nmr δ 7.96–7.03 (23 H, multiplet, aromatic protons), 5.15–4.91 (3 H, multiplet,

(34) Melting points were determined on a Thomas–Hoover capillary melting point apparatus and are corrected. The infrared spectra were recorded on a Perkin-Elmer Model 337 grating spectrophotometer. The ultraviolet spectra were recorded on a Cary Model 15 spectrophotometer. Thin layer chromatography was performed on Eastman silica gel strips with a fluorescent indicator. Solvent system A refers to pentane–ether (1:1); B, to pentane–ether (1:4); C, to pentane–ethyl acetate (7:3); and D, to 4% methanol in chloroform. Routine nmr spectra were recorded on a Varian Associates A-60A or A-56/60 spectrometer at ordinary probe temperatures. Unless otherwise noted nmr spectra were run in $CDCl_3$. Nmr analyses were carried out on expanded traces of the decoupled and undecoupled spectra recorded on a Varian HA-100 instrument. Experimental errors are estimated at ±0.1 cps for coupling constants and ±0.01 ppm for chemical shifts. We are indebted to Mr. J. Nemeth and his associates at the University of Illinois for the microanalyses.

H_1, H_2, H_3), 4.46–4.16 (1 H, multiplet, H_4), 3.33 (3 H, singlet, CH_3O), and 2.45 and 2.36 (3 H each, singlets, *p*-CH_3).

Anal. Calcd for $C_{39}H_{38}O_9S_2$: C, 65.52; H, 5.35; S, 8.97. Found: C, 65.63; H, 5.38; S, 9.04.

B. From Methyl β-D-Ribofuranoside (7).[17]—A solution of 5.0 g (33 mmol) of D-ribose in 100 ml of anhydrous methanol was cooled to 0° and treated with 0.5 ml of concentrated sulfuric acid. The colorless solution was allowed to stand at 4° for 16 hr and was passed through a column of Amberlite IR-45 (OH^-). The filtrate was evaporated to a pale yellow oil under reduced pressure. The nmr spectrum of this oil in D_2O showed the anomeric proton as a singlet at δ 4.90, indicating the β orientation of the methoxyl group at that center. The crude oil (4.5 g, 27.4 mmol) was dissolved in 25 ml of anhydrous pyridine and evaporated to dryness under reduced pressure. The process was repeated once, the residue was dissolved in 50 ml of anhydrous pyridine and treated with 7.6 g (27.4 mmol) of triphenylmethyl chloride, and the solution was stirred at room temperature for 3 days. It was poured into 500 ml of ice-cold water, and the aqueous solution was extracted with three 200-ml portions of ether. The ether was washed with four 100-ml portions of 5% potassium bisulfate, water to neutrality, and a saturated solution of sodium chloride. The ethereal solution was dried over anhydrous sodium sulfate; the ether was evaporated under reduced pressure to give 11.2 g of colorless gum. It was dissolved in ether, 5 g of silica gel was added, and the suspension was evaporated to dryness. The solid was applied to the top of a column of 100 g of silica gel packed in pentane–ether (9:1). Elution with the same solvent system removed the triphenylcarbinol present and increasing the polarity to pentane–ether (2:8) afforded 8.6 g (77%) of **6** as a colorless foam, $[\alpha]^{23}D$ −7.5° (*c* 1.9, $CHCl_3$). The material was homogeneous in solvent systems A, B, and C, but showed the presence of a very slight contaminant in system D. It was characterized as the 2,3-ditosylate derivative.

A solution of 500 mg (1.23 mmol) of the foam in 20 ml of anhydrous pyridine was treated with 570 mg (3.0 mmol) of *p*-toluenesulfonyl chloride and stirred at room temperature for 5 days. The solution was poured into 200 ml of ice-cold 5% sodium bicarbonate solution, and the crystals were collected by filtration and washed well with water. One recrystallization from methanol–ethyl acetate gave 425 mg (48%) of **6a** as colorless needles: mp 140–141°; $[\alpha]^{23}D$ 61.4° (*c* 0.64, $CHCl_3$). The infrared and nmr spectra were identical with the material prepared in section A. On admixture with a specimen from that section the mixture melted at 140–141°.

Anal. Calcd for $C_{39}H_{38}O_9S_2$: C, 65.52; H, 5.35; S, 8.97. Found: C, 65.38; H, 5.51; S, 9.25.

Methyl 3-*p*-Bromobenzoyl-2-deoxy-5-*O*-triphenylmethyl-α-D-ribofuranoside (9).—A solution of 3.1 g (7.95 mmol) of methyl 2-deoxy-5-*O*-triphenylmethyl-α-D-ribofuranoside (**2**) in 25 ml of anhydrous pyridine was evaporated to dryness under reduced pressure. This process was repeated twice, and the resulting residue was dissolved in 25 ml of anhydrous pyridine, treated with 5.75 g (26.75 mmol) of freshly prepared *p*-bromobenzoyl chloride, and another 10-ml portion of anhydrous pyridine was added. The solution was stirred at room temperature for 18 hr, and the resulting pink suspension was poured into 200 ml of ice-cold 5% sodium bicarbonate solution. The precipitate was suspended in 200 ml of water, stirred for 1 hr, and filtered. The resulting light tan powder was suspended in 150 ml of ether, stirred for 1 hr, and filtered. The residue was extracted in the same manner with a second 150-ml portion of ether and filtered again. The combined ether extracts were washed with water and a saturated sodium chloride solution, dried over anhydrous sodium sulfate, and evaporated. The yellow semisolid was recrystallized from methanol–ethyl acetate (3:1) to give 3.9 g of **9** contaminated with a second component. The material was dissolved in 100 ml of ether, 10 g of silica gel was added, and the suspensions were evaporated to dryness and applied to the top of a column of 200 g of silica gel. Elution with pentane–ether (8.5:1.5) gave 3.5 g (77%) of **9** as a white crystalline solid, mp 123–126°, sufficiently pure for use in the preparation of **10**. An analytical specimen was recrystallized from methanol as colorless prisms: mp 125–126°; $[\alpha]^{26}D$ 101.3° (*c* 1.05, $CHCl_3$); ν_{max}^{KBr} 1720 (C=O) and 1595 cm^{-1} (phenyl nuclei); nmr δ 8.01–7.10 (19 H, multiplet, aromatic protons), 5.55–5.15 (2 H, multiplet, H_1 and H_3), 4.53–4.25 (1 H, multiplet, H_4), 3.50–3.28 (5 H, multiplet, CH_3O and $2H_5$), and 2.63–1.93 (2 H, multiplet, $2H_2$).

Anal. Calcd for $C_{32}H_{29}BrO_6$: C, 67.01; H, 5.09; Br, 13.93. Found: C, 66.98; H, 5.28; Br, 13.63.

Methyl 5-*p*-Bromobenzenesulfonyl-3-*p*-bromobenzoyl-2-deoxy-α-D-ribofuranoside (11).—A suspension of 10.25 g (0.018 mol) of methyl 3-*p*-bromobenzoyl-2-deoxy-5-*O*-triphenylmethyl-α-D-ribofuranoside (**9**) in 160 ml of glacial acetic acid was warmed on a steam bath until solution was complete, *ca.* 5 min. Water (25 ml) was added, and the solution was warmed for an additional 5 min. Water (15 ml) was added, and the warming was continued for 10 min. The colorless solution was cooled, and the solvents were evaporated *in vacuo*. The white crystalline residue was dissolved in 300 ml of ether, extracted with two 100-ml portions of 5% sodium bicarbonate solution, water, and saturated sodium chloride solution, and was then dried over anhydrous sodium sulfate. Evaporation of the ether under reduced pressure gave 12.5 g of semisolid residue which was suspended on 15 g of silica gel and applied to the top of a column of 225 g of silica gel packed in pentane–ether (9:1). Elution with pentane–ether (8:2) gave 6.10 g of triphenylcarbinol. The polarity was gradually increased to pentane–ether (2:8), and 2.85, g (48%) of **10** was obtained as a pale yellow syrup. The syrup (2.75 g, 8.30 mmol) was dissolved in 25 ml of anhydrous pyridine and the solution was evaporated to dryness *in vacuo*. This process was repeated once again, and the residue was dissolved in 50 ml of dry pyridine and treated with 3.18 g (12.15 mmol) of *p*-bromobenzenesulfonyl chloride in one portion. The solution was stirred at room temperature for 24 hr, poured into 400 ml of ice-cold 5% sodium bicarbonate solution, and stirred for 15 min, and the precipitate was collected by filtration. One recrystallization from methanol–ethyl acetate (1:1) gave 3.2 g (70%) of **11**, sufficiently pure for use in the preparation of **12**. An analytical specimen crystallized from methanol–ethyl acetate (1:1) as long colorless rods: mp 137–138° dec (insertion at 135°); $[\alpha]^{23}D$ 99.7° (*c* 0.71, $CHCl_3$); ν_{max}^{KBr} 1720 (C=O), 1600 and 1585 cm^{-1} (phenyl nuclei); nmr δ 7.96–7.45 (8 H, multiplet, aromatic protons), 5.28–4.98 (2 H, multiplet, H_1 and H_3), 4.43–4.20 (3 H, multiplet, H_4 and $2H_5$), 3.33 (3 H, singlet, CH_3O), and 2.41–2.11 (2 H, multiplet, $2H_2$).

Anal. Calcd for $C_{19}H_{18}Br_2O_7S$: C, 41.47; H, 3.29; Br, 29.04. Found: C, 41.33; H, 3.46; Br, 28.67.

Methyl 5-(6-Aminopurin-9-yl)-3-*p*-bromobenzoyl-2,5-dideoxy-α-D-ribofuranoside (12).—A suspension of 162 mg (1.20 mmol) of adenine in 5 ml of anhydrous dimethylformamide was treated with 60 mg (*ca.* 1.20 mmol) of a 50% oil dispersion of sodium hydride, and the mixture was stirred at room temperature for 1 hr. It was warmed to 50°, maintained there for 30 min, and cooled to room temperature. A solution of 550 mg (1.00 mmol) of methyl 5-*p*-bromobenzenesulfonyl-3-*p*-bromobenzoyl-2-deoxy-α-D-ribofuranoside (**11**) in 15 ml of anhydrous dimethylformamide was added over a 10-min period, and the suspension was stirred at room temperature for 90 min. It was warmed to 50° and maintained at that temperature for 3 hr. After cooling to room temperature, the dimethylformamide was evaporated under high vacuum at a bath temperature of 40°. The white solid residue was extracted with two 25-ml portions of warm chloroform and the filtered chloroform extracts were combined, shaken with water, and dried over anhydrous sodium sulfate. Evaporation of the chloroform afforded 225 mg (42%) of **12**, mp 219–220°. Two recrystallizations from methanol afforded an analytical sample of **12** as small colorless rods: mp 221.5–222°; $[\alpha]^{23}D$ 142.9° (*c* 0.70, $CHCl_3$); ν_{max}^{KBr} 1715 (C=O), 1670 (purine nucleus), 1610 and 1575 cm^{-1} (purine and phenyl nuclei); nmr δ 8.18 and 7.21 (1 H each, singlets, purine H_2 and H_8), 7.76 (4 H, broad singlet, phenyl protons), 5.41–5.06 (2 H, multiplet, H_1 and H_3), 4.76–4.38 (2.7 H, multiplet, H_4 and $-NH_2$), 3.32 (3 H, singlet, CH_3O), 3.28 (2 H, singlet, $2H_5$). The $2H_2$ protons are obscured by DMSO-d_6.

Anal. Calcd for $C_{18}H_{18}BrN_5O_4$: C, 48.22; H, 4.04; Br, 17.82; N, 15.62. Found: C, 48.50; H, 4.21; Br, 18.11; N, 15.36.

Methyl 5-(6-Aminopurin-9-yl)-2,5-dideoxy-α-D-ribofuranoside (1).—Methyl 5-(6-aminopurin-9-yl)-3-*p*-bromobenzoyl-2,5-dideoxy-D-ribofuranoside (**12**) (700 mg, 1.56 mmol) was dissolved in 600 ml of anhydrous methanol at room temperature, and ammonia was bubbled through the solution for 30 min. After 36 hr at room temperature, the solution was evaporated to dryness under reduced pressure. The solid was triturated with ether to remove the methyl *p*-bromobenzoate and was collected by filtration. One recrystallization from a small volume of methanol afforded 386 mg (93%) of **1** as a white microcrystalline solid: mp 200–201°; $[\alpha]^{23}D$ 97.6° (*c* 1.09, $CHCl_3$); $\lambda_{max}^{H_2O}$ 260 mμ (ε 14,600), λ_{min} 227 (2200), $\lambda_{max}^{0.1\ N\ HCl}$ 258 (14,100), λ_{min} 230 (2900), $\lambda_{max}^{0.1\ N\ NaOH}$ 260 (14,500), λ_{min} 227 (2200); ν_{max}^{KBr} 1660,

1600, and 1585 cm^{-1} (purine nucleus); nmr δ (D_2O)[34] (2 H, singlet, purine H_2 and H_8), 5.34 (1 H, quartet, $J_{AX} + J_{BX} = 7.5$ cps, H_1), 4.50–4.21 (3 H, multiplet, H_4 and $2H_5$), 3.45 (3 H, singlet, CH_3O), and 2.16–1.97 (2 H, multiplet, $2H_3$). The ultraviolet spectra confirmed 9 substitution on the adenine nucleus.[35]

(35) N. J. Leonard and J. A. Deyrup, *J. Amer. Chem. Soc.*, 84, 2148 (1962).

Anal. Calcd for $C_{11}H_{15}N_5O_3$: C, 49.80; H, 5.69; N, 26.40. Found: C, 49.46; H, 5.77; N, 26.10.

Registry No.—**1**, 16803-00-2; **2**, 16801-99-3; **3**, 16802-00-9; **4**, 16802-01-0; **5**, 16802-02-1; **6a**, 16802-03-2; **9**, 16802-04-3; **11**, 16802-05-4; **12**, 16802-06-5.

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2-AMINO-2,5-ANHYDRO-2-DEOXY-DL-RIBITOL: AN AMINO SUGAR DERIVATIVE HAVING NITROGEN IN THE RING[1]

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ABSTRACT

A direct, high-yielding route for synthesis of a pyrrolidine analog of a 2-deoxy-*erythro*-pentose is reported. The synthesis involves modification of pyrrole-2-carboxylic acid by reduction followed by a hydroxylation step. The structure and stereochemistry of 2,5-anhydro-2-deoxy-2-*p*-toluenesulfonamido-DL-ribitol (**5a**) was established by chemical transformations and by ^{13}C n.m.r. data.

INTRODUCTION

There has been considerable interest in recent years in the synthesis of monosaccharide derivatives in which the ring oxygen atom is replaced by another heteroatom. Much of this work has been motivated by the hope that this type of structural change would be accompanied by biological activity of significance in both the modified sugar and in derived nucleosides.

In the course of work on synthesis of puromycin analogs, we required the hitherto unknown pyrrolidine derivative **5**. Although several examples of furanose sugars having nitrogen as the ring atom have been reported[2–9], they have all been obtained by multi-step transformations from naturally occurring monosaccharides. We report here a direct, high-yielding synthesis of **5** from a non-carbohydrate precursor.

RESULTS AND DISCUSSION

The point of departure was pyrrole-2-carboxylic acid (**1**), a compound available from natural sources and which possesses the framework necessary for modification to a simple amino sugar. The first step involved reduction of **1** with gaseous hydrogen iodide and aqueous hypophosphorous acid in acetic acid[10] to give the imino acid, dehydro-DL-proline (**2**). 3,4-Dehydro-*N*-*p*-tolylsulfonyl-DL-proline methyl ester (**3**) was prepared in almost quantitative yield from **2** by *N*-*p*-tolylsulfonylation followed

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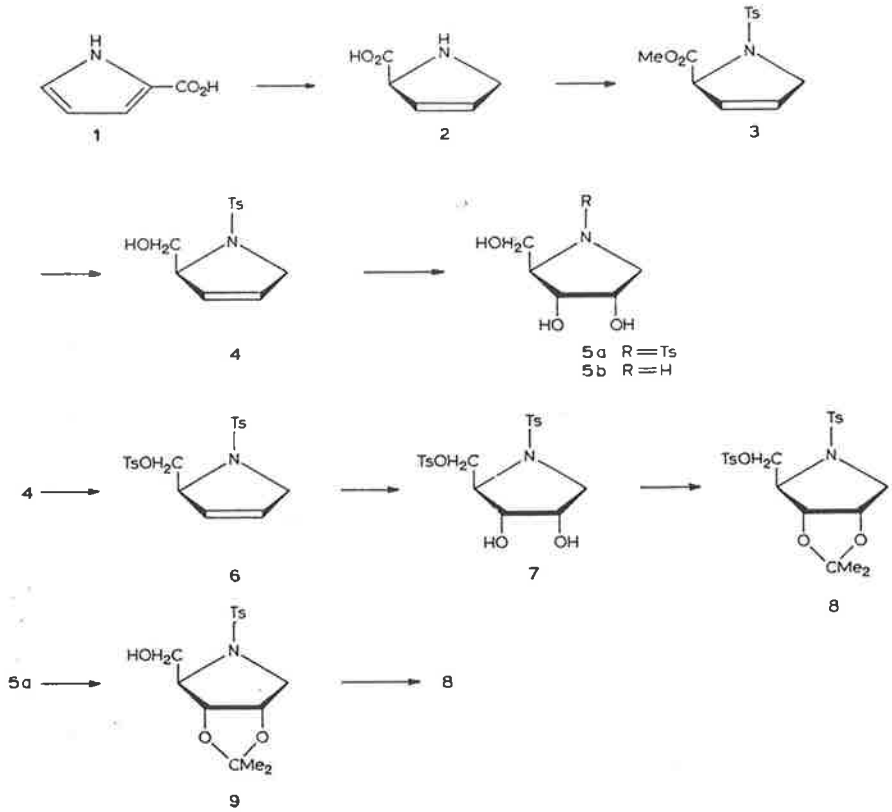
by methylation with diazomethane[11]. The ester group in **3** was then reduced almost quantitatively by lithium borohydride in tetrahydrofuran to give 3,4-dehydro-*N*-*p*-tolylsulfonyl-DL-prolinol (**4**) as a viscous oil. Hydroxylation of **4** with osmium tetraoxide gave a single compound, the diol **5a**, in quantitative yield. Desulfonylation of (**5a**) with sodium metal in liquid ammonia gave **5b**, which appeared to be extremely unstable. Electrolytic desulfonylation[12] was unsuccessful. The data recorded for this pyrrolidine sugar are therefore those of its derivative **5a**. Interestingly, the solubility and chromatographic properties of **5a** resembled closely those of mono-*p*-toluenesulfonylated ribofuranosides.



(All products are DL forms)

On mechanistic grounds, *cis*-hydroxylation with the sterically large osmium tetraoxide–pyridine complex would be expected to occur preferentially from the less-hindered face of dehydroprolinol to give 1,3-*trans*, 3,4-*cis* isomer (**5a**). Favored attack from the less-hindered face has been observed for both proline and dehydroproline derivatives[13,14]. Further confirmation of the stereochemistry was provided by the fact that acetonation gave only one product. As *cis*-hydroxylation of alkenes with osmium tetraoxide is well documented, the only possible stereochemistry in the final product other than **5a** would be the 1,3-*cis*, 3,4-*cis* isomer. The 1,3-*cis*, 3,4-*cis*

isomer would be expected to give a mixture of the 1,3- and 3,4-isopropylideneacetals, whereas **5a** would form only the one acetal, as was observed. In addition, hydroxylation of the highly hindered di-*p*-tolylsulfonyldehydroprolinol (**6**), followed by acetonation, gave a product identical with compound **8**, which had been obtained from **5a** in several steps. Indirect evidence from ^{13}C n.m.r. spectroscopy also confirmed the stereochemistry. It is known that the geometry of hydroxyl groups in sugars affects their ^{13}C chemical shifts. For example, the chemical shifts of the 2′ and 3′ carbon atoms in adenosine vary noticably from those in 9-β-D-arabinofuranosyladenine. As the chemical shifts of sugar carbon atoms in nucleosides are relatively invariant with respect to the nitrogen heterocycle[15], the chemical-shift difference must be due to geometry. Thus, the formation of more than one isomer in the hydroxylation reaction should be detectable by ^{13}C n.m.r. spectroscopy. This evidence, in conjunction with that provided by the acetonation reaction, clearly indicates that hydroxylation results in the formation of only one stereoisomer (**5a**).

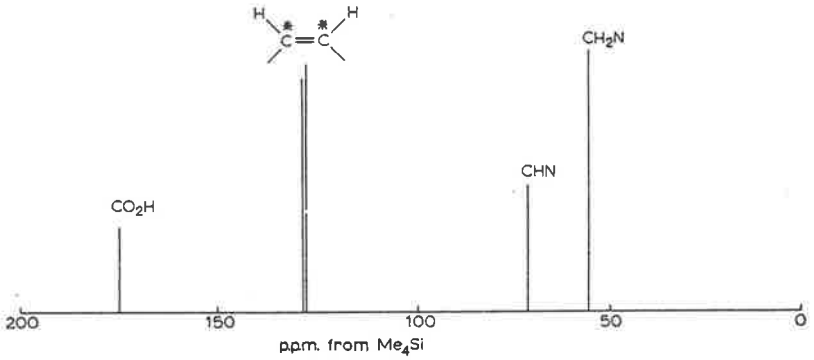


Fig. 1. The ^{13}C n.m.r. spectrum of compound **2**.

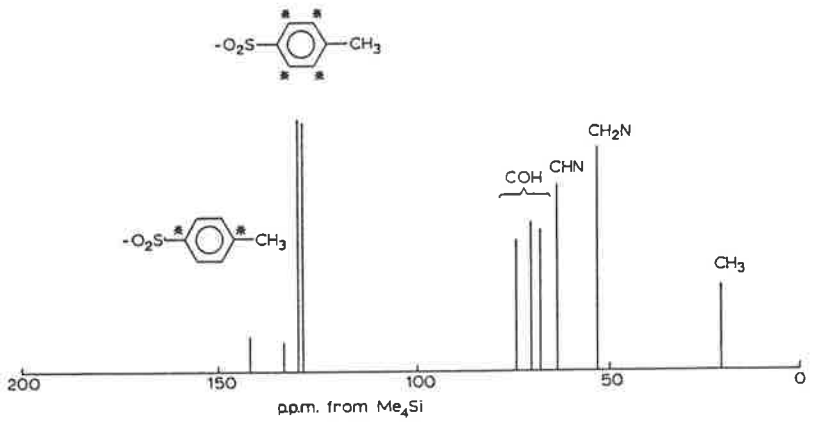


Fig. 2. The ^{13}C n.m.r. spectrum of compound **5a**.

The ^{13}C n.m.r. spectra for these compounds are simple to interpret and evaluate, as compared with the corresponding proton spectra and provide excellent evidence of structure and purity. For example, the p.m.r. spectra of dehydroproline and derivatives of it are extremely complex[16,17], and analysis of the spectrum of the amide of **2** shows it to be an ABMXX′ spin-system containing over 100 lines. However, the broad-band ^{1}H-decoupled ^{13}C n.m.r. spectrum of **2** (Fig. 1) exhibits five lines, corresponding to the five carbon atoms in the molecule. The ^{13}C spectrum of **5a** (Fig. 2) confirmed both its assigned structure and purity[18].

EXPERIMENTAL

General methods. — Melting points are uncorrected. The i.r. spectra were recorded on a Beckman IR-20A spectrometer. The p.m.r. spectra were obtained with Varian A-60 and HA-100 instruments. The carbon-13 spectra were recorded on a Bruker HX-90E Pulse Fourier Transform instrument interfaced with a Nicolet 1080 computer.

*3,4-Dehydro-*DL*-proline* (**2**). — A stirred mixture of acetic acid (150 ml) and 50% hypophosphorus acid (60 g) was cooled to −10° in an ice–salt bath. Gaseous hydrogen iodide was bubbled into the mixture until saturation was attained and the solution had turned dark brown (~30 min). Pyrrole-2-carboxylic acid (**1**, 25.28 g, 224 mmol) was then added with stirring to the reaction mixture and a continuous, gentle stream of hydrogen iodide was bubbled into the reaction mixture for 4 h. The reaction mixture was then filtered and the filtrate evaporated *in vacuo*. The residue was dissolved in water and passed through a column packed with CGC-240 cation-exchange resin (H^+ form) (Baker Chemical Co.). The column was washed with water until the eluate was neutral, and then the product was removed by elution with 2M ammonium hydroxide. Removal of the solvent from the ammoniacal eluate and crystallization of the residue from 10:1 ethanol–water gave 3,4-dehydro-DL-proline (**2**); yield 15.37 g (62%); m.p. 236° (lit.[10] m.p. 236–237°); ^{13}C n.m.r. $\delta^{D_2O}_{Me_4Si}$: 53.30, 69.44, 121.38, 126.52, and 173.17.

*3,4-Dehydro-*N*-p-tolylsulfonyl-*DL*-proline methyl ester* (**3**). — 3,4-Dehydro-DL-proline (**2**, 5.65 g, 50 mmol) dissolved in M sodium hydroxide was sulfonylated with *p*-toluenesulfonyl chloride. Treatment of the crude product with ethereal diazomethane gave 13.49 g (96%) of 3,4-dehydro-*N*-*p*-tolylsulfonyl-DL-proline methyl ester (**3**) as white prisms, m.p. 97.5–99° (lit.[11] m.p. 97.5–98.5°).

*3,4-Dehydro-*N*-p-tolylsulfonyl-*DL*-prolinol* (**4**). — Lithium borohydride (1.32 g, 60 mmol) in 40 ml of dry tetrahydrofuran was placed in a three-necked flask fitted with a condenser and a magnetic stirrer. Compound **3** (5.62 g, 20 mmol) in tetrahydrofuran (50 ml) was added slowly, and when the addition was complete, the reaction mixture was heated for 7 h at reflux with constant stirring. The solution was then cooled, the solvent was removed *in vacuo*, and 100 g of ice containing 10 ml of concentrated hydrochloric acid was added. After the bubbling had ceased, 100 ml of dichloromethane was added, followed by 100 ml of saturated sodium chloride

solution, and the entire mixture was then transferred to a separatory funnel and shaken. The organic layer was removed, and the aqueous layer was extracted with dichloromethane (3 × 100 ml). The organic extracts were then combined, dried (sodium sulfate) and evaporated *in vacuo* to give a pink oil. The product was purified by chromatography on silica gel to give 4.39 g (87%) of the 3,4-dehydro-*N*-*p*-tolylsulfonyl-DL-prolinol (**4**) as a clear yellow oil, ν_{max} 3500, 1660, 1330, 1160, and 815 cm^{-1}; n.m.r. $\delta^{CDCl_3}_{Me_4Si}$: 2.42 (s, 3 H), 2.74–3.12 (s, 1 H, exchanged by D_2O), 3.70–3.87 (d, unresolved, 2H), 4.08–4.30 (m, 2 H), 4.30–4.65 (m, 1 H), 5.45–5.87 (m, 2 H), and 7.22–7.90 (q, 4 H).

Anal. Calc. for $C_{12}H_{15}NO_3S$: C, 56.90; H, 5.97; N, 5.53. Found: C, 56.69; H, 5.94; N, 5.83.

*2,5-Anhydro-2-deoxy-2-*p-*toluenesulfonamido-*DL-*ribitol* (**5a**). — Compound **4** (1.50 g, 6 mmol) was dissolved in 25 ml of dry pyridine and stirred overnight with 1.09 g (4.30 mmol) of osmium tetraoxide in a sealed container. A solution of 10 ml of pyridine, 30 ml of water and 1.8 g of sodium hydrogen sulfite was then added, and the reaction mixture was stirred until a clear orange color appeared (5–30 min). This solution was then extracted with chloroform (4 × 50 ml)[19]. The combined chloroform layers were dried (sodium sulfate). After removal of solvent *in vacuo*, the residual brown oil was crystallized from dichloromethane–pentane to give 1.23 g (99%) of white, crystalline **5a**, m.p. 139–139.5°; ν^{Nujol}_{max} 3590, 3300, 1600, 1360, 1160, and 818 cm^{-1}; n.m.r. $\delta^{CDCl_3}_{Me_4Si}$: 2.42 (s, 3 H), 2.90–4.48 (m, 1OH, 3 H exchanged by D_2O), and 7.24–7.90 (q, 4 H); mass spectrum, 70 eV, direct inlet, 130°, *m/e* 256 ($M-CH_2OH$), 223 ($M-SO_2$), 155 (Ts), 124, 101 ($M-Ts-CH_2OH$), 91 (tropylium ion), 58 ($HN{=}CH{-}CH{=}OH$), 43 ($\dot{C}H_2{-}NH^+{=}CH_2$), 42, and 28 $(CH{\equiv}NH)^+$; ^{13}C n.m.r. $\delta^{(CD_3)_2CO}_{Me_4Si}$: 20.79, 52.46, 62.94, 67.57, 69.70, 73.32, 128.02, 129.47, 134.76, and 143.67.

Anal. Calc. for $C_{12}H_{17}NO_5S$: C, 50.20; H, 5.96; N, 4.87. Found: C, 50.24; H, 5.93; N, 4.81.

*3,4-Dehydro-*N,O-*di-*p-*tolylsulfonyl-*DL-*prolinol* (**6**). — 3,4-Dehydro-*N*-*p*-tolylsulfonyl-DL-prolinol (**4**) (500 mg, 1.98 mmol) and *p*-toluenesulfonyl chloride (416 mg, 2.18 mmol) were dissolved in 15 ml of dry pyridine and stirred in a closed vessel for 45 h. Hydrochloric acid (2M, 100 ml) was then added and the mixture was extracted with ethyl acetate (5 × 50 ml). The organic extracts were dried (sodium sulfate) and evaporated to a yellow oil. This oil was then purified by preparative-layer chromatography on silica gel (PF-254), with 10% dichloromethane in ether as developer. After removal of the solvent and drying (vacuum pump), 598 mg (75%) of compound **5** was isolated as a brown oil; n.m.r. $\delta^{CDCl_3}_{Me_4Si}$ 2.40 (s, 3 H), 2.45 (s, 3 H), 3.96–4.82 (m, 5 H), 5.67 (bs, 2 H), and 7.18–8.00 (m, 8 H).

Anal. Calc. for $C_{19}H_{21}NO_5S_2$: C, 56.00; H, 5.19; N, 3.44. Found: C, 56.24; H, 5.31; N, 3.51.

*2,5-Anhydro-2-deoxy-2-*p-*toluenesulfonamido-5-*O-p-*tolylsulfonyl-*DL-*ribitol* (**7**). — Compound **6** (1.95 g, 4.79 mmol) was dissolved in 25 ml of dry pyridine and stirred with 1.00 g (3.94 mmol) of osmium tetraoxide in a sealed vessel for ~12 h. To this mixture was added, with stirring, a solution of 1.8 g of sodium hydrogen

sulfite, 30 ml of water, and 10 ml of pyridine, so that the ratio of sodium hydrogen sulfite, water, and pyridine in the final solution was about 2:30:35. When a clear orange solution was obtained (5–30 min) it was extracted with chloroform (5 × 50 ml). The combined chloroform layers were dried (sodium sulfate), the solvent was removed, and the remaining oil was further dried (vacuum pump). Purification was effected by preparative-layer chromatography on silica gel plates with acetone as the developing solvent. The diol **6** (1.86 g, 87%) was isolated after removal of solvent as a clear viscous oil: n.m.r. $\delta^{CDCl_3}_{Me_4Si}$ 2.37 (s, 3 H), 2.43 (s, 2 H), 2.85–3.82 (m, 5 H, exchanged by D_2O removes 2 H), 3.98–4.52 (m, 4 H), and 7.17–8.00 (m, 8 H).

Anal. Calc. for $C_{19}H_{23}NO_7S_2$: C, 51.69; H, 5.25; N, 3.17. Found: C, 51.47; H, 4.92; N, 3.20.

*2,5-Anhydro-2-deoxy-3,4-*O*-isopropylidene-2-*p*-toluenesulfonamido-1-*O*-p-tolylsulfonyl-*DL*-ribitol* (**8**) *from* **7**. — To 120 mg (0.272 mmol) of compound **7** dissolved in 5 ml of acetone was added 0.5 ml of dimethoxypropane and one drop of concentrated sulfuric acid. After stirring in a sealed vessel for 23.5 h, 250 mg of sodium carbonate was added to neutralize the acid. After 15 min of stirring the sodium carbonate was filtered off and the filtrate concentrated to a brown oil that crystallized from ether–pentane giving 80 mg (61%) of compound **8** as light tan crystals, m.p. 141.5–144°. A mixed melting point with compound **8** prepared from **5a** (m.p. 142.5–143.5°) showed no depression, indicating that the samples were identical. The i.r. and n.m.r. spectra of the compounds obtained by both routes were also identical.

*2,5-Anhydro-2-deoxy-3,4-*O*-isopropylidene-2-*p*-toluenesulfonamido-*DL*-ribitol* (**9**). — Compound **5a** (441 mg, 1.57 mmol) was dissolved in 12 ml dry acetone together with two drops of concentrated sulfuric acid and 1.3 ml of dimethoxypropane, and the mixture was stirred overnight. An excess of sodium carbonate (1 g) was then added to the solution to neutralize the acid. After stirring for 10 min, the sodium carbonate was filtered off, the acetone extracts evaporated *in vacuo*, and the residue dried (vacuum pump). Crystallization from ether–pentane gave 451 mg (87%) of tan colored crystals: m.p. 110–111°; n.m.r. $\delta^{CDCl_3}_{Me_4Si}$ 0.85 (s, 3 H), 1.18 (s, 3 H), 2.41 (s, 3 H), 2.60 (bs, 1 H, exchanged by D_2O), 3.52–4.07 (m, 5 H), 4.44–4.86 (m, 2 H), and 7.20–7.95 (q, 4 H); *m/e* (70 eV): 327 (M^+).

Anal. Calc. for $C_{15}H_{21}NO_5S$: C, 55.03; H, 6.47; N, 4.28. Found: C, 55.24; H, 6.56; N, 4.30.

*2,5-Anhydro-2-deoxy-3,4-*O*-isopropylidene-2-*p*-toluenesulfonamido-5-*O*-p-tolylsulfonyl-*DL*-ribitol* (**8**) *from* **9**. — Compound **9** (327 mg, 1 mmol) was dissolved in 15 ml of pyridine, and then *p*-toluenesulfonyl chloride (210 mg, 1.1 mmol) was added and the reaction mixture was stirred at room temperature for 65 h in a closed flask. After quenching the reaction by adding 100 ml of 2M hydrochloric acid mixture was extracted with ethyl acetate (4 × 50 ml). The extracts were dried (sodium sulfate) and then evaporated *in vacuo* to a yellow oil. Crystallization from dichloromethane–pentane gave 268 mg (57%) of white crystals, m.p. 142.5–143.5; n.m.r. $\delta^{CDCl_3}_{Me_4Si}$ 0.82 (s, 3 H), 1.15 (s, 3 H), 2.40 (s, 3 H), 2.46 (s, 3 H), 3.47–4.84 (m, 7 H), and 7.15–7.93 (m, 8 H); ν^{KBr}_{max} 2980, 2940, 1600, 1405, 1385, 1365, 1193, 1180, 1165, and 820 cm^{-1}.

Anal. Calc. for $C_{22}H_{27}NO_7S$: C, 54.87; H, 5.65; N, 2.91. Found: C, 54.52; H, 5.73; N, 2.89.

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Synthesis of 1-(6-Aminopurin-9-yl)-2,5-anhydro-1,2-dideoxy-DL-ribitol, a New "Reversed" Amino Nucleoside[1]

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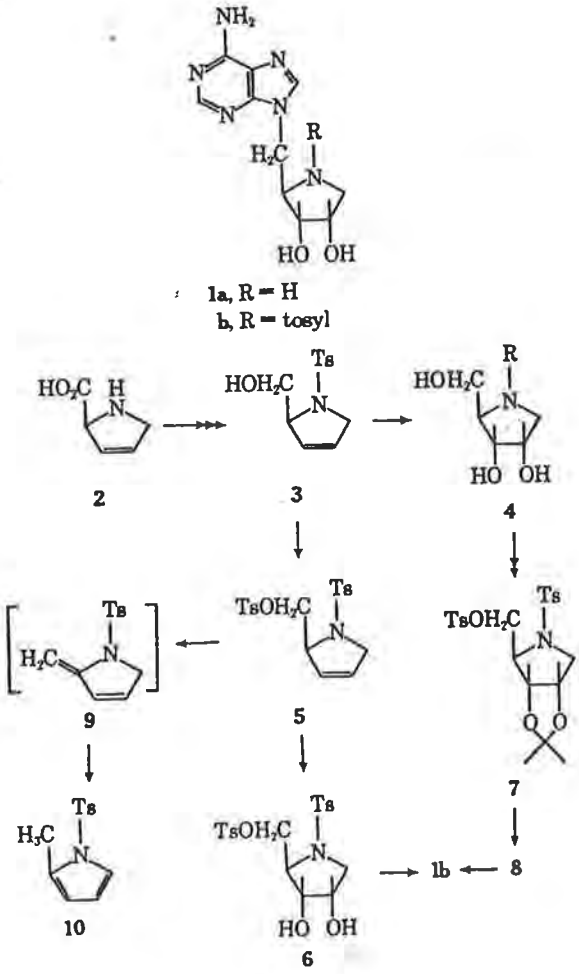
Many nucleosides which are effective agents in inhibiting the growth of malignant cells become ineffective *in vivo* because they are rapidly destroyed by enzymatic cleavage into a purine or pyrimidine and a carbohydrate moiety.[2,3] A reversed nucleoside, however, does not possess the normal linkage between the nitrogen of the base and the anomeric carbon of the sugar, and is more stable with respect to hydrolytic cleavage. A number of reversed nucleosides have already been synthesized.[4-9] Some have elicited interest in connection with cytokinin activity.[10,11] Recently, two patents have been filed which list several reversed nucleosides as antiviral and anticancer drugs.[12,13]

Our research interests in the area of amino and aminoacyl nucleosides prompted the synthesis of 1, the first example of a reversed amino nucleoside. Central to any of the several possible chemical strategies for obtaining 1 is the synthesis of the pyrrolidine sugar 4. The biologically active and synthetic imino acid dehydroproline can be modified by reduction and hydroxylation to give 4 in high yields.[14] Conversion of the amino sugar 4 to 7, subsequent coupling with the sodium salt of adenine, and removal of the isopropylidene group with formic acid gave 1b as a stable, white, crystalline compound, mp 212–213°. The detosylated com-



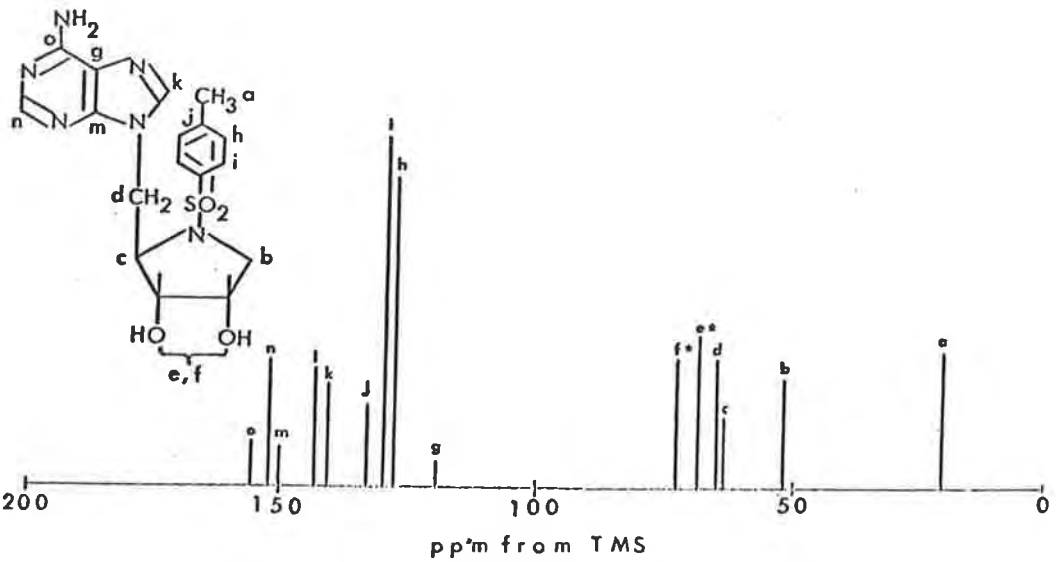


Figure 1. ^{13}C nmr spectrum of 1-(6-aminopurin-9-yl)-2,5-anhydro-1,2-dideoxy-2-(*p*-toluenesulfonamido)-DL-ribitol.

pound **1a** was found to be extremely unstable and difficult to handle. A superior route to the reversed nucleoside is direct coupling of **6** with the sodium salt of adenine, which gives **1b** in 73% yield. Confirmation of the structure of **1b** was provided by its pulsed Fourier transform (PFT) ^{13}C nmr (Figure 1).

Nucleosides containing unsaturation in the sugar moiety have aroused biochemical interest in recent years.[15] Because of this we attempted the displacement of the *p*-toluenesulfonyloxy group of **5** with the sodium salt of adenine. The product of this reaction was *N*-*p*-toluenesulfonyl-2-methylpyrrole (**10**), presumably arising from a base-induced elimination to **9** followed by a facile 1,5-sigmatropic hydrogen shift.

Experimental Section

***N*-Tosyl-3,4-dehydro-DL-prolinol (3)**[14] was prepared as a clear yellow oil from dehydro-DL-proline[17] by tosylation,[18] methylation with diazomethane,[18] and reduction of the *N*-tosyl-3,4-dehydro-DL-proline methyl ester with lithium borohydride.[14]

2,5-Anhydro-2-deoxy-3,4-isopropylidene-2-(*p*-toluenesulfonamido)-1-*O*-(*p*-toluenesulfonyl)-DL-ribitol (7). The dehydroprolinol **3** can be hydroxylated[19] in almost quantitative yield with osmium tetroxide to give **4** as white crystals, mp 139°. The triol **4** can be converted to **7** (mp 143°) by reaction with 2,2-dimethoxypropane and subsequent tosylation with tosyl chloride and pyridine.[14]

1-(6-Aminopurin-9-yl)-2,5-anhydro-1,2-dideoxy-3,4-isopropylidene-2-(*p*-toluenesulfonamido)-DL-ribitol (8). Adenine (233 mg, 1.5 mmol) was dissolved in 10 ml of dry DMF. Sodium hydride (50% in mineral oil, 70 mg, 1.65 mmol) was added to the solution and it was stirred for 0.5 hr. The suspension was then placed in an oil bath at 60° for an additional 0.5 hr to ensure completion of the reaction. After cooling to room temperature 241 mg (0.5 mmol) of 2,5-anhydro-2-deoxy-3,4-isopropylidene-2-(*p*-toluenesulfonamido)-1-*O*-(*p*-toluenesulfonyl)-DL-ribitol in 8 ml of DMF was added to the white suspension of the sodium salt of adenine. This mixture was then stirred at 60° for 12 hr. The DMF was then stripped off to give a light-brown residue that was extracted with methylene chloride. After filtering off the insoluble portion that remained, the methylene chloride was evaporated *in vacuo* to give a yellow oil that was chromatographed on preparative layer silica gel plates to give 104 mg (47%) of product as white crystals: mp 232–233°; nmr spectrum δ_{TMS} ($CDCl_3$) 0.80 (s, 3 H), 1.08 (s, 3 H), 2.41 (s, 3 H), 3.22–3.68 (m, 2 H), 4.02–5.03 (m, 5 H), 6.17–6.38 (br s, 2 H), 7.20–7.90 (m, 4 H), 8.05 (s, 1 H), 8.36 (s, 1 H); mass spectrum (70 eV, direct inlet 200°) *m/e* 444 (M^+).

Anal. Calcd for $C_{20}H_{24}N_6O_4S \cdot 1H_2O$: C, 51.95; H, 5.19; N, 18.18. Found: C, 51.79; H, 5.29; N, 17.98.

2,5-Anhydro-2-deoxy-2-(*p*-toluenesulfonamido-1-*O*-(*p*-toluenesulfonyl)-DL-ribitol (6) was prepared by tosylation of **3** followed by hydroxylation.[14]

1-(6-Aminopurin-9-yl)-2,5-anhydro-1,2-dideoxy-2-(*p*-toluenesulfonamido)-DL-ribitol (1b). Adenine (127 mg, 0.941 mmol) and sodium hydride (50% in mineral oil, 50 mg, 1.035 mmol) were dissolved in 10 ml of dry DMF and stirred for 1.5 hr to form a white suspension of the sodium salt of adenine. To this was added 415 mg (0.941 mmol) of 2,5-anhydro-2-deoxy-2-(*p*-toluenesulfonamido)-1-*O*-(*p*-toluenesulfonyl)-DL-ribitol in 18 ml of DMF. The above mixture was then heated in an oil bath at 60° for 21 hr. The DMF was then stripped off *in vacuo* and further pumped down on a vacuum pump. Addition of a small amount of CH_2Cl_2 resulted in a beige-colored precipitate which was filtered and recrystallized twice from hot methanol to give a 73% yield (279 mg) of the stable, white, crystalline adduct (**1b**): mp 212–213°; uv spectrum λ_{max} (pH 7) 233 nm (ϵ 13,925), 266 (10,735); ^1H nmr spectrum δ_{TMS} (DMSO-d_6) 2.38 (s, 3 H), 3.21–4.63 (m, 9 H), 7.24 (s, 2 H), 7.30–7.95 (m, 4 H), 8.10 (s, 1 H), 8.22 (s, 1 H); ^{13}C nmr spectrum δ_{TMS} (DMSO-d_6) 20.97, 51.78, 63.52, 64.79, 68.47, 72.75, 119.30, 127.79, 129.44, 132.90, 140.90, 143.20, 150.1, 152.4, 155.8; mass spectrum (70 eV, direct inlet 175°) *m/e* 404 (M^+).

Anal. Calcd for $C_{17}H_{20}N_6O_4S$: C, 50.49; H, 4.98; N, 20.86. Found: C, 50.38; H, 5.23; N, 20.95.

***N*-Tosyl-2-methylpyrrole (10).** *N,O*-Ditosyl-3,4-dehydro-DL-prolinol (194 mg, 0.476 mmol) in 3 ml of DMF was added to a suspension of the sodium salt of adenine formed by treating 64 mg (0.476 mmol) of adenine with 71 mg (0.704 mmol) of sodium hydride (50% in mineral oil) in 2 ml DMF for 2.5 hr. After 6 hr of stirred heating at 50°, and an additional 12 hr of reaction time at room temperature, the DMF was removed *in vacuo*. The brown residue remaining was extracted with chloroform (3 × 20 ml) and filtered. After washing the chloroform extracts with water and drying (Na_2SO_4), the solvent was removed to give 99 mg of brown product. This product was purified by preparative layer chromatography on silica gel plates to give 55 mg (49% yield) of the *N*-tosyl-2-methylpyrrole: mp 87.5–89° (lit. mp 93–94°);[16] nmr spectrum δ_{TMS} ($CDCl_3$) 2.29 (s, 3 H), 2.42 (s, 3 H), 5.85–6.03 (m, 1 H), 6.17 (t, 1 H), 7.28 (m, 1 H), 7.20–7.82 (m, 4 H); mass spectrum (70 eV) *m/e* 235 (M^+).

Anal. Calcd for $C_{12}H_{13}NO_2S$: C, 61.25; H, 5.57; N, 5.95. Found: C, 61.55; H, 5.57; N, 5.80.

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Registry No.—**1b**, 51932-88-8; **3**, 51932-89-9; **4**, 52019-89-3; **5**, 51932-90-2; **6**, 51932-91-3; **7**, 51932-92-4; **8**, 51932-93-5; **10**, 17900-53-7; adenine, 73-24-5.

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Synthetic Design, Stereochemistry, and Enzymatic Activity of a Reversed Aminoacyl Nucleoside: An Analogue of Puromycin[1]

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Abstract: The aminoacyl nucleoside puromycin (**1**), produced by *Streptomyces albo-niger*, is a broad-spectrum antibiotic with antitumor activity. It inhibits protein synthesis by accepting the nascent peptide chain of ribosome-bound peptidyl tRNA. A molecular system which incorporates all the features of puromycin necessary for biological activity but which is devoid of structural components detrimental to its use in humans or animals is the reversed aminoacyl nucleoside, 3-L-phenylalanylamino-5-(6-aminopurin-9-yl)-3,5-dideoxy-D-ribofuranose (**2**). The stereospecific synthesis of **2** starting from D-xylose is described. Structural confirmation came from spectroscopic studies, particularly natural abundance ^{13}C NMR. A conformation for **2** based on spin-lattice relaxation (T_1) data and biological precedent is proposed. The aminoacyl nucleoside **2** is not a substrate for adenosine deaminase, and the aminonucleoside derived from **2** is not expected to be a substrate for adenosine kinase.

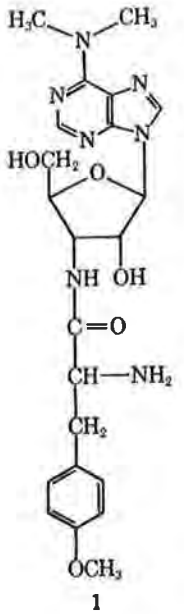
Introduction

The aminoacyl nucleoside puromycin (**1**), produced by *Streptomyces albo-niger*, is a broad-spectrum antibiotic with antitumor activity.[2,3] Its structure bears a close resemblance to the aminoacyl-adenyl terminus of aminoacyl tRNA,[3] and it can therefore act as an acceptor of the peptide chain of ribosome-bound peptidyl tRNA. Puromycin therefore inhibits protein synthesis by substituting for the incoming coded aminoacyl tRNA. As puromycin acts as a codon-independent functional analogue of aminoacyl tRNA, it is likely that there is a binding site on an enzyme such as peptidyl transferase for the adenine system.[4] Investigation has also shown that there are certain structural requirements of puromycin that are necessary for biological activity. The aminonucleoside and an aromatic L-amino acid are required for maximum inhibition of protein biosynthesis. However, the methyls of the dimethyl group, the methoxyl group, the furanosyl oxygen, and the 5′-OH appear to be unnecessary for biological activity.[5-10] Additionally, the removal of the 5′-OH group from the puromycin structure appears to be desirable from the standpoint of toxicity. The nephrotoxicity of puromycin[10] has been ascribed to the enzymatic release of 6-dimethylamino-9-(3′-amino-3′-deoxy-β-D-ribofuranosyl)purine (PAN) by hydrolysis of the *p*-methoxyphenylalanyl group.[11] Metabolic studies have demonstrated that PAN can be monodemethylated by liver enzymes both in vitro and in vivo and subsequently converted to the 5′-nucleotide. Since PAN itself is not a substrate for adenosine kinase, it has been suggested that the demethylated PAN from liver is made available to the kidney where nucleotide formation can occur, and that this nucleotide may be the active metabolite of PAN which induces kidney toxicity.[11]

A molecular system which incorporates all the features of puromycin necessary for biological activity but which is devoid of structural components detrimental to its use in mammalian systems is the "reversed" aminoacyl nucleoside,[14] 3-L-phenylalanylamino-5-(6-aminopurin-9-yl)-3,5-dideoxy-D-ribofuranose (**2**). We wish to report on the synthesis and enzymatic activity of **2** and on the establishment of its stereochemistry by natural abundance PFT carbon-13 NMR techniques.

Results and Discussion

Central to any of the several chemical strategies for obtaining **2** is the synthesis of the appropriate 3-amino sugar. A convenient and readily available starting material is xylose (**3**)



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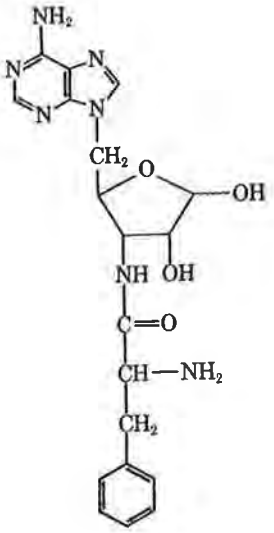

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(represented in the furanose form for convenience). Its initial modification at carbon-3, the transformation of a hydroxyl group to an amino group, requires protection of the other hydroxy groups. This was done by first converting xylose to its 1,2:3,5-di-*O*-isopropylidene derivative **4** with acetone/concentrated H_2SO_4/anhydrous $CuSO_4$ and subsequently selectively and quantitatively hydrolyzing the 1,3-dioxane ring with 0.2% aqueous HCl at room temperature.[15] The selective hydrolysis of the 1,3-dioxane ring is the result of a more favorable stereoelectronic arrangement for attack of water and subsequent ring cleavage in the case of the 1,3-dioxane ring as compared with the 1,3-dioxolane ring. Although D-xylose exists almost predominantly in the pyranose form in solution, the furanose form has been detected by ^1H and ^{13}C NMR techniques.[12,13] Both the di- and monoisopropylidene derivatives of D-xylose exist in the furanose form. The hydrolysis of **4** to **5** can be conveniently followed by monitoring the disappearance of two of the singlets (due to the methyl groups) in the δ 1.0–1.5 ppm region. Additionally, the doublet at δ 5.99 ($J_{1,2}$ = 4 Hz) for the anomeric hydrogen remained. The 5-hydroxyl group was then selectively protected by taking advantage of the greater reactivity of the primary 5-OH compared with the secondary 3-OH toward methyl chloroformate

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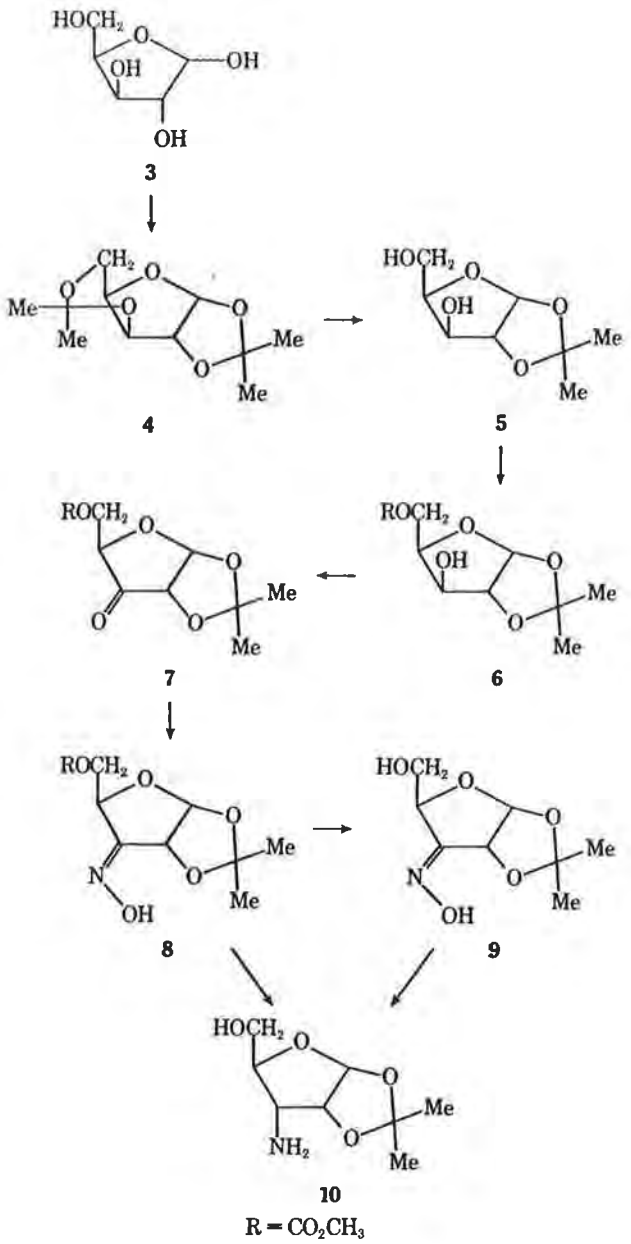
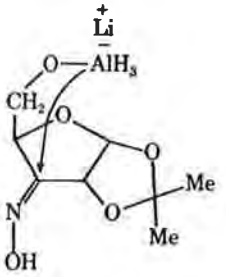
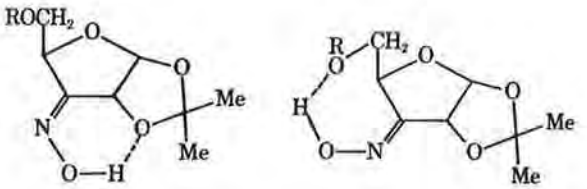


in pyridine at 0 °C.[16] It was anticipated that later in the synthetic scheme (after the 3 position had been modified) it would be necessary to remove selectively the 5-protecting group for further elaboration at that position. Since the isopropylidene group is acid labile, a base labile blocking group such as the carbonate was chosen.

In earlier studies we had examined the possibility of converting the 3β-hydroxyl group to the 3α-amino group via initial displacement of the 3β-tosylate with azide ions under a variety of conditions. However, only very low yields of product were obtained because of the difficulty in the S_N2 displacement reaction arising from adverse steric and dipolar effects.[17,18] An alternative approach involved stereospecific reduction of the 3-oxime. Oxidation of **6** with $Me_2SO/DCC/H_3PO_4$[19,20] gave the 3-keto compound **7** in almost quantitative yield. The keto group of **7** undergoes facile hydration, a feature which made structural verification of the oxidation product difficult. However, careful preparative layer chromatography gave unhydrated ketone which showed carbonyl absorptions in the infrared at 1755 cm^{-1} (3-keto) and 1735 cm^{-1} (carbonate). Two peaks were also observed in the ^{13}C NMR spectrum for these carbons and were unequivocally identified as 5-carbonate carbon (155.1 ppm) and 3-keto carbon (207.4 ppm). Conversion of the ketone **7** to the oxime **8** occurred smoothly in the presence of hydroxylamine hydrochloride and pyridine at 55 °C. Reduction of oxime **8** with $LiAlH_4$ in THF occurred stereospecifically to give the α-amino sugar **10.** It should be noted that the conversion of **8** to **10** involves not only the reduction of the oxime but also the removal of the carbonate protecting group. Carbonates are reductively cleaved to alcohols by $LiAlH_4$ at a much faster rate than oximes are reduced to amines, and it was anticipated that after its formation, the 5-CH_2OH would direct approach of the reducing agent stereospecifically to the 3 position. That this induced asymmetric



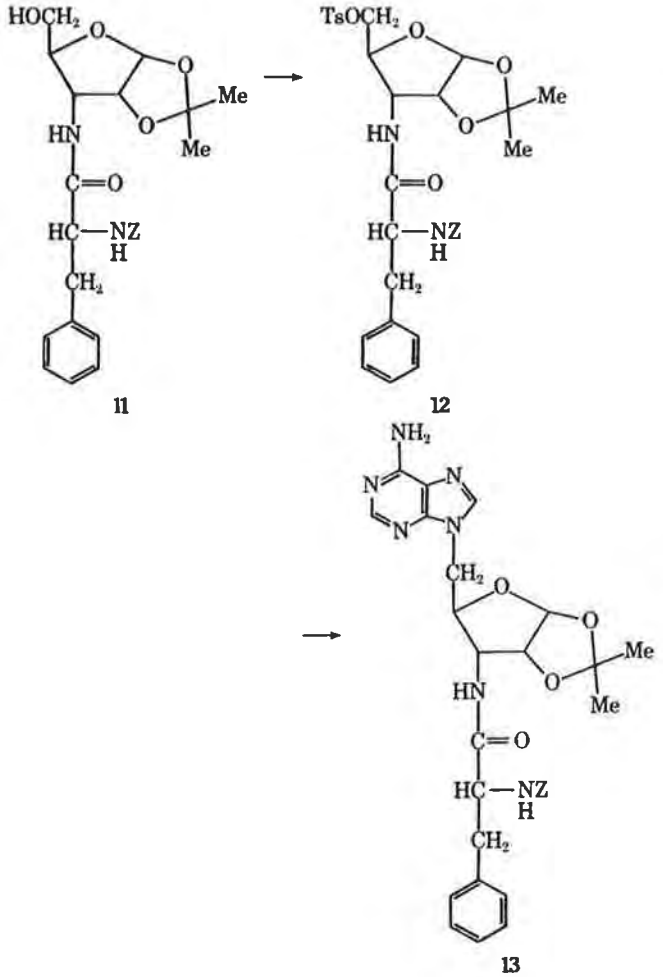
conversion produced one isomer almost exclusively was shown unequivocally by chromatographic analysis and NMR studies. The ^1H NMR spectrum of **10** exhibited coupling constants ($J_{1,2} = J_{2,3} \simeq 4.0$ Hz) consistent with the α-stereochemistry of the amino group.[21] Its ^1H noise-decoupled PFT ^{13}C NMR spectrum showed *only* eight resonances for the eight carbons of the α-amino sugar **10** with carbon-3 showing a single resonance at δ 54.6. Compound **10** can also be obtained by initial cleavage of the carbonate protecting group with $NaOCH_3/CH_3OH$, followed by reduction of the oximino alcohol **9** with $LiAlH_4$. An interesting observation, hitherto undetected in the sugar series, arose from our efforts to establish unequivocally the complete structures of all intermediates in this synthesis. The oximes **8** and **9** exist in two forms, syn and anti as evi-



9, R = H
8, R = CO_2CH_3

denced by ^{13}C NMR spectral studies which showed two peaks for almost all of the carbons in these compounds. The syn:anti ratio was about 2:1.

The synthetic plan then involved further modification at C-3 and subsequent tailoring at C-5 for the attachment of the purine base. Selective peptide bond formation at the 3-NH_2, without esterification of the 5-OH, and racemization of the amino acid was achieved by reaction of the amino sugar **10** with *N*-benzyloxycarbonyl-L-phenylalanine in the presence of *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ).[22] The relatively slow formation and rapid consumption of the intermediate carbonic anhydride ensures against its accumulation which minimizes side reactions such as esterification and racemization. Under our reaction conditions this conversion is almost quantitative if unreacted starting material is taken into account. The aminoacyl sugar **11** is smoothly converted to its tosylate **12** by reaction with *p*-toluenesulfonyl chloride and pyridine. Introduction of the purine ring at the 5 position was achieved through nucleophilic displacement of the tosyloxy group with sodium adenine (prepared by reaction of adenine with sodium hydride) to give the protected reversed nucleoside **13** in 62% purified yield. That no racemization was occurring under the conditions of this coupling reaction was confirmed by the observation that at the



11 12 13

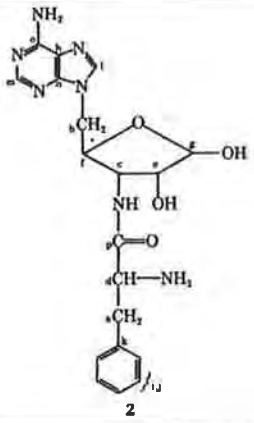
termination of the reaction the unreacted starting material (about 8%) recovered from the reaction showed a rotation almost identical with that found at the commencement of the run. Interestingly also, little change in optical rotation was noted in going from **12** ($[\alpha]^{25}D$ +47° (*c* 0.49, $CHCl_3$)) to **13** ($[\alpha]^{25}D$ +44° (*c* 1.6, CH_3OH)). The structure of **13** was confirmed by infrared, ^1H NMR, and ^{13}C NMR spectral data. Restricted rotation of the peptide bond to the 3′-amino group was clearly evident in the ^{13}C NMR spectrum which showed coalescence of the cis and trans forms at 125 °C. That attachment of the purine ring involved the 9 position was substantiated by correlation of the UV absorption maxima in ethanol at 265 nm (ε 14 700) with those known for 9-substituted adenines.[21,23-27] Removal of the isopropylidene group was achieved almost quantitatively by careful treatment with HCl. Treatment of the resulting product under conditions of hydrogenolysis with 10% Pd on charcoal and 2 atm of H_2 gave the reversed aminoacyl nucleoside **2** as its dihydrochloride salt, which when lyophilized appeared as a white powder, mp 172–175 °C dec. Like puromycin, the reversed nucleoside is also unstable at room temperature.

The structure of **2** was confirmed by ^1H NMR, ^{13}C NMR, and UV data. The ultraviolet spectrum showed λ_{max} at 263 nm (ε 15 300). The optical rotation $[\alpha]^{25}D$ +50° (*c* 0.21, H_2O) was quite similar to the protected reversed nucleoside $[\alpha]^{25}D$ +44° (*c* 1.6, CH_3OH). The carbon-13 spectrum clearly showed all the carbons of **2** and that the ribose ring had the two anomeric forms α and β in the ratio of 1:3 (see Table I).

Chemical shift assignments were aided by ^{13}C spin-lattice relaxation (T_1) measurements. Further, as relaxation data relate closely to overall and segmental motions, bonded and nonbonded interactions, and related factors controlling molecular motions, some correlations between T_1 data and conformation in solution can be made. Spin-lattice relaxation

Table I. ^{13}C NMR Data for the Dihydrochloride of **2** in D_2O

Carbon	Ppm from Me_4Si	T_1, s[a]
a	37.8	0.23
b	46.6	0.12
c	53.4, 53.8	0.28
d	55.2	0.18
e	74.1, 74.6	0.24
f	78.0, 78.5	0.23
g	97.6, 102.2	0.25
h	118.5	2.60
i	128.8	0.21
j	130.2	0.29
k	134.7	1.89
l	145.2	0.22
m	145.9	0.29
n	149.7	3.32
o	150.4	3.32
p	170.3	2.65



2

[a] The estimated maximum errors in the T_1 values vary from ±10 to ±20%.

measurements of 0.2 M **2** in D_2O were made using the inversion-recovery technique[28-30] with the pulse sequence $(T\text{-}180°\text{-}\tau\text{-}90°)_x$. The similarity of T_1 values for the ribosyl carbons and their magnitudes are consistent with a relatively rigid ring undergoing some anisotropic rotation. These values are close to those reported for adenosine-5′-monophosphate (AMP) at a concentration of 1.0 M.[31,36] The relatively short T_1 value for the 5′-CH_2 suggests that the relaxation is dominated by ^{13}C-^1H dipole-dipole interactions with the directly bonded hydrogens. The nonprotonated purine ring carbons which are directly bonded to nitrogens (and therefore strongly affected by ^{13}C-^{14}N dipolar interactions)[33] have T_1 values close to those observed for these carbons in 1.0 M AMP. As expected, the protonated purine carbons C(2) and C(8) show much shorter T_1 values and again similar to those observed for 1.0 M AMP where it has been suggested[31,32] that the AMP molecules are aggregated with stacking of the bases. This arrangement allows for a greater degree of freedom for the ribosyl ring through segmental motion about the glycosyl bond. The phenlalanyl methine carbon undergoes relaxation faster than the methylene carbon which may imply contribution from the attached NH_2 to the relaxation of the methine carbon. The magnitude of both T_1 values, however, suggests that segmental motion is not significant here. The protonated aromatic carbons show T_1 values of 0.21 and 0.29 s implying restriction to spin rotation of the phenyl ring. As expected, the nonprotonated carbon has a long T_1 value.

The reversed aminoacyl nucleoside is a structural analogue of puromycin. It has been suggested[4] that the mechanism of action of puromycin requires it to assume a U-shaped conformation where the nucleic acid base and the aromatic ring are stacked. The x-ray structure of puromycin[34] reveals the association of the methylated base and the aromatic ring. The two rings are stacked at an interplanar spacing of 3.4 Å. Measurement of ^{13}C spin-lattice relaxation times for 0.2 M puromycin dihydrochloride suggests[35] that in solution the puromycin molecule acquires a preferred conformation where segmental and internal rotational motion is limited. This conformation may indeed be similar to that found in the crystalline state of puromycin and also that found in general with relatively concentrated solutions of nucleic acid components such as AMP. The ^{13}C T_1 values observed for the reversed puromycin analogue are close to those observed for puromycin, and it is suggested that the analogue may also take up a preferred conformation in solution with association and stacking of the purine and aromatic rings. When this confor-

mation is assumed during inhibition of protein biosynthesis, the free amino group of the phenylalanyl moiety of the analogue would be sterically well positioned to make a nucleophilic attack on the carbonyl of the peptide. An arrangement such as this would also involve a continuous stack of four hydrophobic rings.

For the puromycin analogue **2** to be an effective inhibitor of protein biosynthesis, it is important that it not undergo in vivo degradation by enzymes such as adenosine deaminase. Mammalian adenosine deaminase reacts with adenosine and structural analogues of adenosine converting them to the corresponding inosines.[37,38] In general, substrate binding and significant substrate activity requires the presence of a 9-substituted unhindered adenine ring and the 5′-hydroxyl of the carbohydrate moiety.[37-40] Dramatic changes occur with respect to substrate activity when the 5′-OH is altered as in the reversed aminoacyl analogue **2** of puromycin, where the 5′-OH has been replaced by the purine ring. Indeed when **2** was treated with adenosine deaminase (calf doudenal mucosa) in 0.05 M phosphate buffer and the reaction assayed spectrophotometrically, no deamination was observed even after 20 h.

The 5′-OH of puromycin has been implicated in its toxicity. Toxic manifestations, including renal lesions, apparently result from small amounts of aminonucleoside produced by the hydrolytic removal of the amino acid moiety from administered puromycin. The aminonucleoside is first monodemethylated and then converted to the 5′-nucleotide by adenosine kinase, and evidence suggests that it is the 5′-nucleotide that is responsible for the cytotoxicity associated with puromycin.[9,41] In the reversed nucleoside **2**, hydrolytic cleavage of the phenylalanine would release an aminonucleoside which would not be a substrate for adenosine kinase.

Biological evaluation of **2** as an inhibitor of protein biosynthesis is currently in progress.

Experimental Section

The melting points reported are uncorrected and were taken on a Thomas-Hoover melting point apparatus fitted with a microscope. The infrared spectrometer used was a Beckman IR 20A. The ^{1}H NMR spectra were recorded on a Varian A-60 spectrometer. The ^{13}C NMR spectra were obtained using a Bruker HX-90E pulse Fourier transform NMR spectrometer interfaced with a Nicolet 1080 computer and disk unit. The mass spectrometer employed was a Hitachi RMU-6E instrument with direct inlet capability at an ionizing energy of 70 eV. The optical rotations were measured with a Perkin-Elmer 141 polarimeter. The ultraviolet data were taken with a Beckman Model 24 ultraviolet spectrometer. Lyophilizations were done with a Virtis Automatic Freeze-Dryer Model 10-010. The catalytic hydrogenation was done on a Parr Model 3911 low-pressure hydrogenation apparatus. Solvents were evaporated under reduced pressure using a Buchi Model R rotary evaporator. Reactions requiring constant shaking were run on an Eberbach Shaker Bath Model 6250. Elemental analyses were performed by the University of Iowa Microanalytical Service on a Hewlett-Packard F and M Scientific 185 C, H, and N analyzer.

Preparative layer chromatography plates were prepared by coating seven 20 × 20 cm glass plates with a slurry made from 150 g of E. Merck PF 254 or PF 254 + 366 silica gel in 390 ml of water. The plates were air dried overnight, baked for 4 h at 110 °C, and then allowed to equilibrate to room temperature. The plates were then stored in a desiccator. Separations were accomplished in glass developing tanks and were followed by ultraviolet light using a Chromato-Vue light box equipped with long and short wavelength ultraviolet lamps.

1,2:3,5-Di-*O*-isopropylidene-α-D-xylofuranose (4) was prepared in 90% yield as an oil from xylose: bp 120–125 °C (1 mm) [lit.[15] bp 90–92 °C (0.2 mm)]; ^{1}H NMR δ Me_4Si ($CDCl_3$) 1.28 (s, 3 H), 1.36 (s, 3 H), 1.42 (s, 3 H), 1.47 (s, 3 H), 3.95–4.10 (m, 3 H), 4.28 (d, 1 H), 4.50 (d, $J_{2,1}$ = 4 Hz, 1 H), 5.99 (d, $J_{1,2}$ = 4 Hz, 1 H).

1,2-*O*-Isopropylidene-α-D-xylofuranose (5)[15] was prepared from **4** in 97% yield by controlled hydrolysis with 0.2% aqueous HCl: ^{1}H NMR δ Me_4Si ($CDCl_3$) 1.32 (s, 3 H), 1.50 (s, 3 H), 3.98–4.40 (m, 6 H), 4.52 (d, $J_{2,1}$ = 4 Hz, 1 H); mass spectrum *m/e* 175 ($M^+ - CH_3$), 159 ($M^+ - CH_2OH$), 129, 127 ($M^+ - CH_3, - CH_2OH, - OH$), 115 ($M^+ - CH_3, - CH_3COOH$).

1,2-*O*-Isopropylidene-5-*O*-methoxycarbonyl-α-D-xylofuranose (6) was prepared from **5** by reaction with methyl chloroformate and pyridine.[16] The carbonate crystallized from 1:1 benzene–hexane as white crystals (70%): mp 133–134 °C; $[\alpha]^{25}D$ −12.5° (*c* 2, CH_3OH); IR ν_{max} (Nujol) 3420 (OH), 1730 (carbonate) cm^{-1}; ^{1}H NMR δ Me_4Si ($CDCl_3$) 1.32 (s, 3 H), 1.50 (s, 3 H), 2.98 (s, br, 1 H), 3.82 (s, 3 H), 4.28–4.48 (m, 4 H), 4.57 (d, $J_{2,1}$ = 4.0 Hz, 1 H), 5.97 (d, $J_{1,2}$ = 4.0 Hz, 1 H); ^{13}C NMR δ Me_4Si ($CDCl_3$) 26.2, 26.8, 55.2, 64.7, 74.6, 78.1, 85.2, 104.8, 112.0, 156.2.

Anal. Calcd for $C_{10}H_{16}O_7$: C, 48.4; H, 6.5. Found: C, 48.5; H, 6.7.

1,2-*O*-Isopropylidene-5-*O*-methoxycarbonyl-α-D-*erythro*-3-pentosulofuranose (7). To a stirred solution of 1,2-*O*-isopropylidene-5-*O*-methoxycarbonyl-α-D-xylofuranose (4.96 g, 20.0 mmol) and dicyclohexylcarbodiimide (12.17 g, 59.1 mmol) in 25 ml of Me_2SO and 30 ml of ethyl acetate was added 1 ml of pyridine, followed by a solution of phosphoric acid (0.98 g, 10.0 mmol) in 5 ml of Me_2SO. The reaction mixture was stoppered and cooled in an ice bath for 20 min. The ice bath was removed and the reaction mixture stirred at room temperature for 18 h. Ethyl acetate was added (50 ml) followed by a solution of oxalic acid (5 g) in methanol (10 ml). After gas evolution has ceased (about 30 min), 75 ml of a saturated aqueous solution of NaCl was added and the solution was filtered. The filtrate layers were separated, and the aqueous phase was extracted with ethyl acetate (50 ml) and CH_2Cl_2 (50 ml). The organic layers were combined, washed with 5% aqueous $NaHCO_3$ (50 ml) and saturated aqueous NaCl (50 ml), and dried over Na_2SO_4. The solvent was removed in vacuo. Ethyl acetate (25 ml) was added to the residue and the solution filtered. The filtrate was chromatographed on a column (2.5 × 20 cm) of silica gel. The column was eluted with 200 ml of $CHCl_3$, leaving, after evaporation of the solvent, 4.42 g of crude yellow oil. This oil was chromatographed on preparative layer silica gel plates. The plates were developed with 1:1 ether/CH_2Cl_2, and the band with R_f 0.8 was cut out and eluted with CH_2Cl_2, giving 4.03 g (16.4 mmol, 82%) of 1,2-*O*-isopropylidene-5-*O*-methoxycarbonyl-α-D-*erythro*-3-pentosulofuranose as a pale-yellow oil. An analytical sample was prepared by crystallization. The purified sugar was dissolved in a small amount of chloroform. Ether was then slowly added dropwise until the solution became turbid. The solution was then allowed to stand several days in the refrigerator. The solution was filtered, and the white crystals were collected and dried. An alternate method of crystallization involved dissolving the purified sugar in methylene chloride, followed by freeze drying. The residue was washed with ether and the product isolated as white crystals: mp 57–58 °C; $[\alpha]^{25}D$ +62° (*c* 1.55, CH_3OH); IR ν_{max} (Nujol) 1755 (C=O, ring), 1735 (carbonate) cm^{-1}; ^{1}H NMR δ Me_4Si ($CDCl_3$) 1.41 (s, 3 H), 1.48 (s, 3 H), 3.78 (s, 3 H), 4.12–4.70 (m, 4 H), 6.10 (d, $J_{1,2}$ = 4 Hz, 1 H); ^{13}C NMR δ Me_4Si ($CDCl_3$) 27.1, 27.4, 55.2, 66.2, 76.2, 77.0, 103.3, 114.5, 155.1, 207.4.

Anal. Calcd for $C_{10}H_{14}O_7$: C, 48.8; H, 5.7. Found: C, 48.6; H, 5.9.

1,2-*O*-Isopropylidene-5-*O*-methoxycarbonyl-α-D-*erythro*-3-pentosulofuranose Oxime (8). Hydroxylamine hydrochloride (27.76 g, 399.0 mmol) and 1,2-*O*-isopropylidene-5-*O*-methoxycarbonyl-α-D-*erythro*-3-pentosulofuranose (15.12 g, 61.46 mmol) were dissolved in 75 ml of dry pyridine. The reaction mixture was protected from moisture and heated in an oil bath at 55 °C for 12 h with stirring. The pyridine was then removed under reduced pressure at a temperature of 30–35 °C. Water (50 ml) and CH_2Cl_2 (50 ml) were added to the residue, and the mixture was stirred until all the solid dissolved. The phases were separated, and the aqueous layer was extracted with CH_2Cl_2 (4 × 50 ml). The CH_2Cl_2 extracts were combined, dried over Na_2SO_4, and the solvent evaporated in vacuo (bath temperature, 30 °C). After drying 1–2 h on a vacuum pump, a gummy residue remained. This gum was dissolved in a few milliliters of $CHCl_3$ and chromatographed on a column of silica gel (40 × 3.5 cm). The column was first washed with 200 ml of Skelly B. Then 600 ml of ether was passed through the column and the ether fraction was evaporated to dryness to give 12.20 g (46.74 mmol, 76%) of 1,2-*O*-isopropylidene-5-*O*-methoxycarbonyl-α-D-*erythro*-3-pentosulofuranose oxime as a white solid. This was recrystallized from ether/pentane to yield the product as white prisms: mp 90–91 °C; $[\alpha]^{25}D$ +120° (*c* 2.0,

CH_3OH); IR ν_{max} (Nujol) 3390 (N–OH), 1735 (C=O), 1640 (C=N, weak) cm^{-1}; ^1H NMR δ Me_4Si ($CDCl_3$) 1.41 (s, 3 H), 1.49 (s, 3 H), 3.76 (s, 3 H), 4.25–4.81 (m, 2 H), 5.00 (2 doublets, syn, anti forms, $J_{2,1}$ = 4 Hz, 1 H), 5.15–5.39 (m, 1 H), 5.96 and 5.98 (2 doublets, syn, anti forms, $J_{1,2}$ = 4 Hz, 1 H), 10.30 (s, br, 1 H); ^{13}C NMR δ Me_4Si ($CDCl_3$) 27.5, 27.7, 55.1, 67.1, 67.9, 73.5, 75.3, 77.6, 78.4, 105.1, 105.4, 113.8, 114.3, 155.5, 156.7, 157.6.

Anal. Calcd for $C_{10}H_{15}NO_7$: C, 46.0; H, 5.8; N, 5.4. Found: C, 45.6; H, 6.0; N, 5.5.

1,2-*O*-Isopropylidene-α-D-*erythro*-3-pentosulofuranose Oxime (9). To a stirred solution of 1,2-*O*-isopropylidene-5-*O*-methoxycarbonyl-α-D-*erythro*-3-pentosulofuranose oxime (6.10 g, 23.4 mmol) in 20 ml of CH_3OH and cooled in an ice bath was added a solution of CH_3ONa (1.36 g, 25.2 mmol) in 100 ml of cold CH_3OH. The reaction flask was sealed and stirred an additional 15 min in the cooling bath. The flask was then placed in the refrigerator for 48 h. Then 12 ml of 2 N HCl was added and the solution stirred at room temperature for 15 min. The solvent was removed in vacuo (bath temperature, 25 °C) to give 4.30 g (91%) of crude product. Recrystallization from $CHCl_3$/pentane yielded 3.94 g (19.4 mmol, 83%) of 1,2-*O*-isopropylidene-α-D-*erythro*-3-pentosulofuranose oxime as white crystals: mp 139–140 °C; $[\alpha]^{25}D$ +231° (*c* 1.0, CH_3OH); IR ν_{max} (Nujol) 3450 (N–OH), 3260 (CH_2–OH) cm^{-1}; ^1H NMR δ Me_4Si (acetone-d_6) 1.33 (s, 3 H), 1.39 (s, 3 H), 3.41–4.12 (m, br, 3 H), 4.86–5.08 (m, 2 H), 5.96 and 5.98 (2 doublets, syn, anti forms, $J_{1,2}$ = 3.9 Hz, 1 H), 10.41 (s, br, 1 H); ^{13}C NMR δ Me_4Si (CH_3OD) 27.6, 27.8, 28.0, 62.4, 64.2, 75.1, 79.8, 80.1, 80.8, 106.1, 106.4, 114.0, 114.5, 157.7, 159.2.

Anal. Calcd for $C_8H_{13}NO$: C, 47.3; H, 6.5; N, 6.9. Found: C, 47.1; H, 6.5; N, 6.9.

1,2-*O*-Isopropylidene-3-amino-3-deoxy-α-D-ribofuranose (10). A solution of 1,2-*O*-isopropylidene-α-D-*erythro*-3-pentosulofuranose oxime (**8**) (2.33 g, 11.6 mmol) in 20 ml of dry THF was added dropwise to a stirred suspension of lithium aluminum hydride (1.09 g, 28.7 mmol) in 30 ml of THF cooled in an ice bath. After the addition was complete, the reaction mixture was refluxed for 3 h and then stirred at room temperature 12 h. The excess lithium aluminum hydride was decomposed by the slow, dropwise addition of 15 ml of a 1:1 THF/water solution while the reaction vessel was cooled in an ice bath. The reaction mixture was filtered and the filter cake was washed with additional THF/water solution (20 ml) and then refluxed in CH_2Cl_2 to remove any occluded product. The CH_2Cl_2 solution was combined with the THF filtrate, and the solvents were removed in vacuo (bath temperature, 25 °C), giving a crude yellow oil. This oil was chromatographed on preparative layer silica gel plates. The plates were developed with 5% CH_3OH/CH_2Cl_2. The band with R_f 0.25 was cut out and eluted with 15% CH_3OH/CH_2Cl_2. 1,2-*O*-Isopropylidene-3-amino-3-deoxy-α-D-ribofuranose was isolated (1.19 g, 6.30 mmol, 54%) as a pale-yellow oil: $[\alpha]^{25}D$ +41° (*c* 1.15, CH_3OH); IR ν_{max} (Nujol) 3100–3500 (br, NH_2, OH) cm^{-1}; ^1H NMR δ Me_4Si ($CDCl_3$) 1.32 (s, 3 H), 1.52 (s, 3 H), 2.32–2.59 (br, 3 H), 3.02–3.81 (m, 4 H), 4.52 (t, $J_{2,1} = J_{2,3}$ = 3.95 Hz, 1 H), 5.80 (d, $J_{1,2}$ = 3.95 Hz, 1 H); ^{13}C NMR δ Me_4Si ($CDCl_3$) 26.5, 26.6, 54.6, 62.2 80.7, 81.4, 104.2, 112.0; mass spectrum *m/e* 174 ($M^+ - CH_3$), 157 ($M^+ - CH_3, - NH_3$), 127 ($M^+ - CH_3, - CH_2OH, - NH_2$), 114 ($M^+ - CH_3, - CH_3COOH$), 100, 85, 71, 57, 43, 28.

Anal. Calcd for $C_8H_{15}NO_4$: C, 50.8; H, 8.0; N, 7.4. Found: C, 50.6; H, 8.2; N, 7.1.

A second band with R_f 0.35 was also cut out from the preparative layer plates. After elution with 15% CH_3OH/CH_2Cl_2 44 mg (0.23 mmol, 2%) of 1,2-*O*-isopropylidene-3-amino-3-deoxy-α-D-xylofuranose was recovered: ^1H NMR δ Me_4Si ($CDCl_3$) 1.32 (s, 3 H), 1.50 (s, 3 H), 2.30–2.65 (br, 3 H), 3.00–4.00 (m, 4 H), 4.26 (d, $J_{2,1}$ = 4 Hz, 1 H), 5.91 (d, $J_{1,2}$ = 4 Hz, 1 H).

1,2-*O*-Isopropylidene-3-*N*-benzyloxycarbonyl-L-phenylalanylamino-3-deoxy-α-D-ribofuranose (11). *N*-benzyloxycarbonyl-L-phenylalanine (3.30 g, 11.0 mmol)[42] and *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (2.87 g, 11.6 mmol) were dissolved in 95 ml of dry benzene. The solution was stirred and heated at 40 °C for an hour. Then a solution of 1,2-*O*-isopropylidene-3-amino-3-deoxy-α-D-ribofuranose (2.09 g, 11.0 mmol) in 25 ml of dry methanol was added to the benzene reaction mixture which was then stirred and heated at 40 °C for 20 h. After cooling to room temperature, the solvent was removed in vacuo (bath temperature, 25 °C). The residue was a yellow oil. This oil was chromatographed on a column of silica gel (3.5 × 35 cm). The column was washed with 900 ml of ether to remove unreacted starting materials and quinoline, a by-product of the reaction. The column was then eluted with 400 ml of ether, followed by 750 ml of 1:1 ether/CH_2Cl_2, yielding 2.95 g (6.28 mmol, 57%) of 1,2-*O*-isopropylidene-3-*N*-benzyloxycarbonyl-L-phenylalanylamino-3-deoxy-α-D-ribofuranose as a white foam that flowed into a liquid at 62–64 °C: $[\alpha]^{25}D$ +43° (*c* 0.78, CH_3OH); IR ν_{max} (KBr) 3420 (OH), 3325 (NH), 1705 (C=O, urethane), 1660 (C=O, amide) cm^{-1}; ^1H NMR δ Me_4Si (CD_3OD) 1.29 (s, 3 H), 1.44 (s, 3 H), 3.01 (d, br, 2 H), 3.45–4.20 (m, 8 H), 5.08 (s, 2 H), 5.82 (d, 1 H), 5.95 (d, 1 H), 7.30 (s, 5 H), 7.34 (s, 5 H); ^{13}C NMR δ Me_4Si ($CDCl_3$) 26.3, 26.8, 39.1, 51.1, 56.5, 60.4, 60.7, 67.1, 76.2, 79.5, 80.1, 85.7, 104.1, 104.9, 111.6, 112.5, 126.8–129.2, 136.2, 136.4, 155.9, 171.9.

Anal. Calcd for $C_{25}H_{30}N_2O_7 \cdot CH_3OH$: C, 62.1; H, 6.8; N, 5.6. Found: C, 62.4; H, 6.9; N, 5.4.

1,2-*O*-Isopropylidene-3-*N*-benzyloxycarbonyl-L-phenylalanylamino-3-deoxy-5-*O*-*p*-toluenesulfonyl-α-D-ribofuranose (12). 1,2-*O*-Isopropylidene-3-*N*-benzyloxycarbonyl-L-phenylalanylamino-3-deoxy-α-D-ribofuranose (4.20 g, 8.95 mmol) was dissolved in pyridine (30 ml) and cooled in an ice bath. Then a solution of *p*-toluenesulfonyl chloride (1.86 g, 9.77 mmol) in 10 ml of pyridine was added dropwise with stirring. The reaction flask was sealed, and the solution was stirred 10 min in the ice bath. The reaction mixture was then allowed to stand at room temperature for 4 days. The solution was then stirred in an ice bath and 100 ml of cold 2 N HCl was slowly added. The aqueous layer was extracted with CH_2Cl_2 (3 × 75 ml). The CH_2Cl_2 extracts were combined, dried over Na_2SO_4, and evaporated to dryness in vacuo (bath temperature, 30 °C). The residue was a pale-yellow foam. It was chromatographed on preparative layer silica gel plates. The plates were developed with ether. The band with R_f 0.6 was cut out and eluted with 10% CH_2Cl_2/ether. 1,2-*O*-Isopropylidene-3-*N*-benzyloxycarbonyl-L-phenylalanylamino-3-deoxy-5-*O*-*p*-toluenesulfonyl-α-D-ribofuranose (4.25 g, 6.81 mmol, 76.1%) was isolated as a brittle white foam that flowed into a liquid at 56–57 °C: $[\alpha]^{25}D$ +47° (*c* 0.49, $CHCl_3$); IR ν_{max} (Nujol) 3310 (NH), 1700 (C=O, urethane), 1665 (C=O, amide), 1250 and 1170 (SO_2) cm^{-1}; ^1H NMR δ Me_4Si ($CDCl_3$) 1.23 (s, 3 H), 1.32 (s, 3 H), 2.42 (s, 3 H), 3.09 (d, 2 H), 3.20–4.55 (m, 6 H), 5.12 (s, 2 H), 5.58–5.64 (br, 1 H), 5.70 (d, $J_{1,2}$ = 4 Hz, 1 H), 5.80–6.05 (br, 1 H), 7.15–7.50 (m, 12 H), 7.87 (d, 2 H); ^{13}C NMR δ Me_4Si ($CDCl_3$) 21.6, 26.3, 26.5, 39.4, 51.7, 56.6, 67.0, 69.1, 74.3, 77.7, 78.1, 78.5, 104.4, 105.1, 111.9, 112.8, 127.3–129.9, 132.9, 136.3, 136.5, 144.9, 155.9, 171.0.

Anal. Calcd for $C_{32}H_{36}N_2O_9S$: C, 61.5; H, 5.8; N, 4.5. Found: C, 61.4; H, 6.2; N, 4.6.

1,2-*O*-Isopropylidene-3-*N*-benzyloxycarbonyl-L-phenylalanylamino-5-(6-aminopurin-9-yl)-3,5-dideoxy-α-D-ribofuranose (13). Sodium hydride (343 mg, 7.15 mmol of a 50% oil dispersion) was placed in a flask and rinsed with a few milliliters of Skelly B. The liquid was removed and the process repeated a second time. The sodium hydride was then dried on a vacuum pump. Adenine (965 mg, 7.15 mmol) was then added to the flask, followed by 15 ml of DMF. The suspension was stirred for 1 h at room temperature and subsequently for 1 h at 50 °C. A solution of 1,2-*O*-isopropylidene-3-*N*-benzyloxycarbonyl-L-phenylalanylamino-3-deoxy-5-*O*-*p*-toluenesulfonyl-α-D-ribofuranose in 10 ml of DMF was then added to the stirring suspension of the sodium salt of adenine. The reaction mixture was then heated 10 h at 95–100 °C. The DMF was then evaporated under reduced pressure at 30 °C. The gummy residue was washed with 100 ml of a hot solution of 10% CH_3OH/CH_2Cl_2 and filtered. The filtrate was evaporated in vacuo at 25 °C and the resulting yellow/orange gum was purified on preparative layer silica gel plates. The plates were developed with 10% CH_3OH/CH_2Cl_2. The band at R_f 0.6 was cut out and eluted with 20% CH_3OH/CH_2Cl_2 yielding 2.47 g (4.22 mmol, 62%) of 1,2-*O*-isopropylidene-3-*N*-benzyloxycarbonyl-L-phenylalanylamino-5-(6-aminopurin-9-yl)-3,5-dideoxy-α-D-ribofuranose as a white foam that liquefied at 109–110 °C: $[\alpha]^{25}D$ +44° (*c* 1.6, CH_3OH); IR ν_{max} (neat) 3320 (br, NH), 1705 (C=O, urethane), 1650 (C=O, amide) cm^{-1}; uv λ_{max} (95% EtOH) 265 nm (ϵ 14 700); ^1H NMR δ Me_4Si (CD_3OD) 1.30 (s, 3 H), 1.47 (s, 3 H), 3.05 (d, 2 H), 3.50–4.90 (m, 7 H), 5.08 (s, 2 H), 5.75–5.82 (br, 1 H), 5.95 (d, *J* = 4 Hz, 1 H), 6.80–7.15 (br, 2 H), 7.30 (s, 5 H), 7.32 (s, 5 H), 8.14 (s, 1 H), 8.26 (s, 1 H); ^{13}C NMR δ Me_4Si (CH_3OD), 26.1, 26.5, 26.8, 27.0, 38.3, 39.5, 45.6, 55.0, 55.4, 58.8, 61.0, 67.6, 74.7, 77.9, 79.4, 80.1, 105.3, 105.7, 113.6, 113.8, 119.7, 127.8–130.9, 136.2, 136.5, 138.1, 138.5, 143.2, 150.8, 153.7, 157.2, 158.0, 158.6, 175.5, 175.8.

Anal. Calcd for $C_{30}H_{33}N_7O_6$: C, 61.3; H, 5.7; N, 16.7. Found: C, 60.3; H, 5.9; N, 15.8.

3-L-Phenylalanylamino-5-(6-aminopurin-9-yl)-3,5-dideoxy-D-ribofuranose (2) Dihydrochloride. 1,2-*O*-Isopropylidene-3-*N*-benzyloxycarbonyl-L-phenylalanylamino-5-(6-aminopurin-9-yl)-3,5-dideoxy-α-D-ribofuranose (755 mg, 1.29 mmol) was dissolved in 3 ml of concentrated HCl and left to stand at room temperature for 2.5 h. Then 12 ml of water were added, and the solution was evaporated in vacuo (bath temperature, 22 °C). A tan gummy compound was isolated: mp 178–181 °C dec; $[\alpha]^{25}D$ +32° (*c* 0.41, H_2O); IR ν_{max} (Nujol) 3410 (br, OH), 3300 (br, NH), 1690 (C=O, urethane), 1660 (C=O, amide); ^1H NMR δ Me_4Si external (D_2O) 3.20–4.00 (m, br, 8 H), 4.20–5.00 (m, br, 8 H), 5.65–5.85 (br, 1 H), 7.75 (s, 5 H), 7.89 (s, 5 H), 8.84 (s, 1 H), 8.97 (s, 1 H). This compound was identified as 3-*N*-benzyloxycarbonyl-L-phenylalanylamino-5-(6-aminopurin-9-yl)-3,5-dideoxy-D-ribofuranose and used directly without further purification. This sugar (302 mg, 0.55 mmol) was dissolved in 40 ml of water. Then 205 mg of 10% Pd on charcoal was added and the mixture subjected to catalytic hydrogenation for 2 h at 28 psi and room temperature. The catalyst was removed by filtration through a column of cotton. The cotton was washed with an additional 30 ml of water. The filtrate was poured into a 500-ml, round-bottom flask and frozen with liquid nitrogen. The solution was then lyophilized and 215 mg (0.44 mmol, 80%) of 3-L-phenylalanylamino-5-(6-aminopurin-9-yl)-3,5-dideoxy-D-ribofuranose dihydrochloride was isolated as a white powder. An analytical sample was prepared by crystallization from cold water/ethanol: mp 172–175 °C dec; $[\alpha]^{25}D$ +50° (*c* 0.21, H_2O); IR ν_{max} (Nujol) 3240–3550 (br, NH and OH), 1665 (C=O) cm^{-1}; UV λ_{max} (H_2O, pH 7), 263 nm (ϵ 15 300); ^1H NMR δ Me_4Si external (D_2O) 3.10–4.00 (m, 4 H), 4.10–5.00 (br, 7 H), 5.10–5.40 (br, 4 H), 5.64–5.74 (s, br, 1 H), 7.88 (s, 5 H), 8.87 (s, 1 H), 9.00 (s, 1 H); ^{13}C NMR δ Me_4Si (D_2O, dioxane internal standard) 37.8, 46.6, 53.4, 53.8, 55.2, 70.1, 74.6, 78.0, 78.5, 97.6, 102.2, 118.5, 128.8, 130.2, 134.7, 145.2, 145.9, 149.7, 150.4, 170.3.

Anal. Calcd for $C_{19}H_{25}Cl_2N_7O_4 \cdot 5H_2O$: C, 39.6; H, 6.1; N, 17.0. Found: C, 40.0; H, 6.2; N, 17.0.

Enzymatic Evaluation. Adenosine deaminase (E.C. 2.5.4.4) Type I from calf intestinal mucosa was purchased from Sigma Chemical Co. Substrate activity of **2** was monitored spectrophotometrically by observation of the absorbancy at 263 nm. The enzymatic reaction was carried out in 0.05 M phosphate buffer (pH 7.4) at 25 °C.[37,38] No deamination occurred even after 20 h. Under similar conditions adenosine was rapidly converted to inosine.

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Carbon-13 Nuclear Magnetic Resonance Studies of Vitamin B_6 Schiff Base and Carbinolamine Formation in Aqueous Solution.[1] 1. The Adduct of Pyridoxal 5′-Phosphate and DL-Alanine

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Abstract: The Schiff base and carbinolamine formation from pyridoxal 5′-phosphate and DL-alanine in aqueous solution was investigated by carbon-13 nuclear magnetic resonance spectroscopy. The pK_a value for the deprotonation of the pyridinium nitrogen was found to be less than that of free pyridoxal 5′-phosphate. At pH 7.1 two pH-dependent forms of the Schiff base and three species of carbinolamines which have different configurations were detected, while at pH 10.5 the pH-dependent forms of the Schiff base predominate. At pH 6.3 the Schiff base is equally distributed between the pH-dependent forms and increased concentration of the three carbinolamine species were detected. Evidence presented suggests that the Schiff bases allow for no intramolecular interactions between the iminium proton and either the phenolate anion of C-3 of pyridoxal 5′-phosphate or the carboxyl group of the amino acid. At pH 12.8, the equilibrium is shifted from the Schiff base toward free components and the carbinolamine intermediate was not clearly detected.

Vitamin B_6 compounds are known to be essential in enzymatic metabolism of amino acids. The early studies by Braunstein et al.[3] and Snell et al.[4] suggested that the initial step in the metabolic mechanism of amino acids is Schiff base formation between the formyl group of pyridoxal 5′-phosphate and the amino group of the amino acid.

Considerable information on the equilibrium of Schiff bases formed by pyridoxal or pyridoxal 5′-phosphate with amino acids or amines has been obtained from UV–visible and ^{1}H NMR studies in aqueous and nonaqueous media,[5-14] but structural evidence for these dynamic states has not been conclusive. Furthermore, the tetrahedral intermediate (carbinolamine) formed through the addition process between the carbonyl and the amino group of the two components has received little structural attention.

Recent studies of vitamin B_6 and derivatives[15,16] and amino acids[17,18] by carbon-13 nuclear magnetic resonance spectroscopy (^{13}C NMR) have led us to pursue the application of ^{13}C NMR methods for the derivation of dynamic structural information in the formation of Schiff bases and carbinolamine complexes from pyridoxal 5′-phosphate and amino acids.

This study is the first comprehensive treatment of ^{13}C NMR application to Schiff base and carbinolamine formation from pyridoxal 5′-phosphate and amino acids, although a preliminary study on pyridoxal 5′-phosphate–amine systems was reported very recently.[19,20] We wish to establish a correlation of chemical shifts with Schiff base structures and to provide ^{13}C NMR evidence for the intermediacy of carbinolamines.

Experimental Section

Pyridoxal 5′-phosphate was purchased from Sigma Chemical Co. and DL-alanine was obtained from Merck and Co. Reagents were used without further purification. D_2O obtained from Diaprep was 99.7% pure. The NaOD was prepared from D_2O and metallic sodium under dry nitrogen.

^{13}C NMR spectra were obtained at 25 °C on a Bruker HX90E pulse Fourier transform NMR spectrometer (22.63 MHz) interfaced with a Nicolet 1080 computer. Typical parameters for ^{13}C NMR experiments follow: spectral width of 6024 Hz with acquisition of 8 K data points, 7-μs pulse corresponding to a tip angle of 30°, and a recovery time of 2 s. The number of spectral accumulations was in the range of 5000–7500 depending on sample conditions. Chemical shifts are given in parts per million (ppm) downfield from external tetramethylsilane (capillary with 5-mm o.d. concentric tube within the 10-mm sample tube). The digital reproducibility is ±0.1 ppm. The probe temperature was 25 °C. D_2O solvent was the source of an internal deuterium lock. Broad band proton noise decoupling and gated decoupling experiments were carried out by standard methods.

The sample solution was prepared by first dissolving the amino acid in the 0.35 M pyridoxal 5′-phosphate (pH 6.0) stock solution and then adjusting to the final concentration and pH. Sample concentrations were 0.3 M in each component. Before preparing the stock solution,

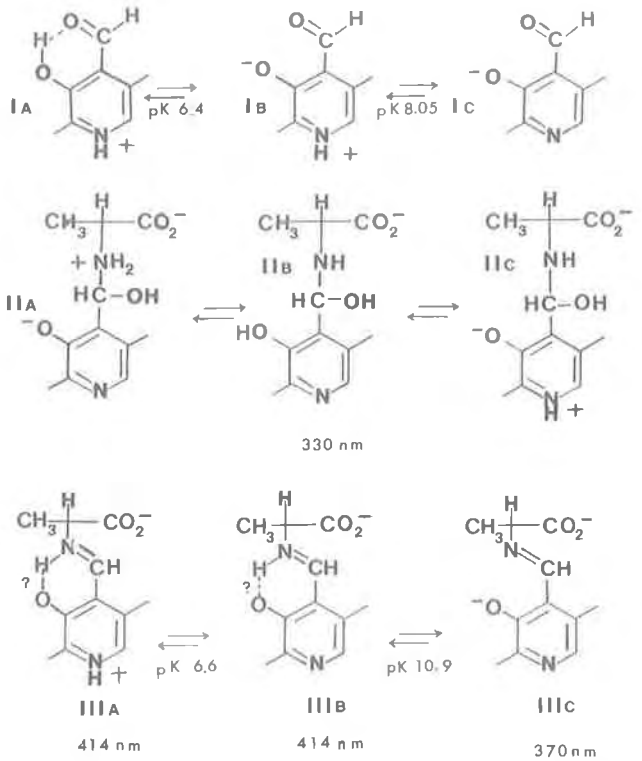

Figure 1. Structural and spectral species present in a mixture of pyridoxal 5′-phosphate and alanine.

D_2O was purged with nitrogen gas. Spectral data were collected within 5 h after sample preparation. The time limitation was included to avoid slower side reactions such as vitamin B_6 catalyzed reactions of amino acids,[20] which were ascertained by ^1H NMR spectroscopy. Acidity was varied by the addition of NaOD. Under these conditions and at these concentrations pH is a working concept and are direct readings of the pH meter without corrections to pD. Therefore, results reported here must be viewed as approximations of pH.

Results and Discussion

UV and visible spectroscopy of aqueous solutions of pyridoxal phosphate and amino acids shows that extensive imine or Schiff base formation occurs over a wide pH range.[5]

In Table I, we have compared the ^{13}C NMR data of the equilibrium states formed with 0.3 M pyridoxal 5′-phosphate and 0.3 M DL-alanine in aqueous solution at three different pHs. The resonances arising from free components were easily assigned in each case from the pH-dependent spectra of pyridoxal 5′-phosphate and DL-alanine.

The spectra at pH 6.3 and 7.1 are more complex than those at pH 10.5 (Figure 1), indicating greater Schiff base formation at the higher pH value. The Schiff bases formed in the neutral pH range are yellow and absorb light maximally at about 400 nm (Figure 1). An obvious indication of Schiff base formation in the NMR case is the decrease in intensity of the formyl carbon (197.1 ± 0.3 ppm) of pyridoxal 5′-phosphate. The Schiff base formed shows two pK values. The first pK of 6.6 is associated with ionization of the pyridine nitrogen and results in formation of the anion IIIB (Figure 1). No UV visible spectral changes are reported or associated with this first dissociation, which seems surprising.[5] The anion form IIIB (Figure 1) has a proton associated with or residing on the imine nitrogen which may or may not be hydrogen bonded to the phenolic oxygen at the 4 position over the pH range 7.1–10.5. The anion IIIB is the major UV–visible spectral species over the pH range 7.1–10.5 and has a λ_{max} of 414 nm.

Our approach to studying the pH dependence on Schiff base formation was to correlate ^{13}C chemical shift data with the structures of various possible species. The intense lines observed

Table I. ^{13}C NMR Chemical Shifts of Pyridoxal 5′-Phosphate–DL-Alanine System in Aqueous Solution

pH		C-2	C-2′	C-3	C-4	C-4′	C-5	C-5′	C-6	C-α	C-β	CO_2^-
	IA	152.7	17.4	*a*	127.2	197.1	137.5	62.7	125.4	51.7	17.4	177.1
	II	146.6										
7.1		145.9	16–17[c]	163–164[c]	125–138[c]	68.7	130–136	61–64[c]	125–137	61–64[c]	16–17[c]	*a*
		145.4		(triplets)								
	IIIB⇄ IIIC	157.1	19.1	168.3	116.9	164.9	133.5 132.8	62.7	133.1	63.5	20.7	178.2
	IB	157.4	*a*	*a*	125.2	*a*	133.6 133.3	*a*	130.6	52.2	*a*	*a*
10.5	I[b]	157.4	20.0	168.1	125.2	197.6	134.0 133.0	63.5	129.3			
	IIIB⇄ IIIC	157.4	19.6	168.1	116.8	165.1	132.6 132.3	62.6	132.3	63.7	20.7	178.5
	IA	152.6	17.0	164.5	127.3	197.1	136–137	61.8	125.2	51.7		177–178[c] (doublets)
	II	146.5										
6.3		146.1	15–18[c]	163–165[c]	124–137[c]	68.5						
		145.5		(triplets)			124–137[c]	61–64[c]	124–137[c]	61–64[c]	16–18[c]	
	IIIA	156.3	20.5	168.7	117.7	163.9						

[a] The concentration is not enough to be detected. [b] Resonances from the binary system of aqueous pyridoxal 5′-phosphate solution at pH 10.5. [c] Approximately estimated range; the resonances cannot be distinguished because of the overlapped complexity.

in the spectrum at pH 7.1 became more intense at pH 10.5 while those with low intensity at pH 7.1 diminished further at pH 10.5 (Figure 2a and b) which is indicative of greater Schiff base formation. The reverse situation is true at pH 6.3 (Figure 2c). It is suggested that the lines of high intensity at pH 10.5 are due to the time-averaged chemical shifts of the equilibrium state between imine anion and dianion (IIIB ⇄ IIIC).

In the spectrum taken at pH 10.5 the formyl carbon resonance of pyridoxal 5′-phosphate completely disappeared, which indicates that Schiff base formation is favored at a moderate alkaline range. The dianion which also exists at these pH values is a weak acid with a pK of 10.9.[21] The dianion form has an absorption band at about 370 nm (Figure 1). The increased Schiff base formation is consistent with the increased concentration of the nucleophile (the deprotonated amino acid) under alkaline conditions. Furthermore, under these conditions, base-catalyzed dehydration of carbinolamine is favored (further discussion will be given on this intermediate). The resonances of the equilibrium state between the imine anion (IIIB) and the imine dianion (IIIC) are pH independent in the pH range 7.1–10.5, and the equilibrium is sustained in the mixture even as low as pH 7.1. Assignments of the resonance lines for the equilibrium state of these two Schiff bases (IIIB and IIIC) were made not only on the basis of expected chemical shifts but also from gated decoupling data. Carbons CO_2^-, C-2, C-3, C-4, and C-5 appeared as singlets, C-α, C-4′, and C-6 as doublets, C-5′ as a triplet, and C-β and C-2 as quartets. At lowest field (178.5 ppm) is the carboxyl group and the strong signals at 169.1 and 157.4 ppm must be due to C-3 and C-2, respectively, based on the deshielding of phenolate anion and the pyridine nitrogen. Assignment of C-4 at 116.8 ppm and C-5 at 132.0 ppm was done on the basis of the expected influence on chemical shifts upon Schiff base formation. In the ^1H noise-decoupled spectrum, C-5 appeared as a doublet at 132.4 ppm. The observed coupling constant ($^3J_{POCC}$ = 8.0 Hz) is consistent with a trans arrangement about C5′–O bond.[16] Incidental overlap of C-6 (132.3 ppm) and one of the peaks of C-5 was confirmed by gated decoupling and noise-modulated off-resonance decoupling experiments. Assignment of C-4′ at 165.1 ppm (J_{C-H} = 174.3 Hz) is based on the observation of similar values for imine carbons.[20] Appearance of C-α at 63.7 ppm (J_{C-H} = 141.9 Hz) is in the range expected for such a carbon. An unambiguous differentiation between C-β and C-2′ was not possible and the assignments made in Table I and Figure 2 for these resonances may be reversed.

As previously stated, the two Schiff bases (IIIB and IIIC) are in equilibrium as the major species at pH 7.1. In Table I, it was shown that the chemical shift of pyridoxal 5′-phosphate at pH 10.5 is very similar to that of the equilibrium state Schiff base formed at pH 7.1, which suggests that the pK_a of the pyridinium nitrogen of pyridoxal 5′-phosphate has decreased upon Schiff base formation. While we were preparing this article, we were informed that the pK_a is 6.6 as determined by UV–visible spectroscopy.[22] A very recent report[23] on the nature of binding of pyridoxal 5′-phosphate and the corresponding Schiff bases to glycogen phosphorylase lends further support to the above.

On the basis of the chemical shift correlation established for the equilibrium state Schiff base (IIIB ⇄ IIIC) structural assignments of the species observed at pH 6.3 can be made. Evidence for the Schiff base formation under these conditions comes from the observation of the C-4′ resonance at 163.9 ppm. This iminium nitrogen has a pK_a of 10.9.[22] The ^{13}C chemical shift of the C-4′ resonance shows little variation over the pH range of 7.1–10.5, even up to 12.8. This observation would suggest that the iminium proton remains associated over a wide range of pH which may extend up to a pH of 13.9 if we rely on the above value for the pK_a. Unfortunately, the ^{13}C chemical shift of the C-4′ could not be examined at such a high

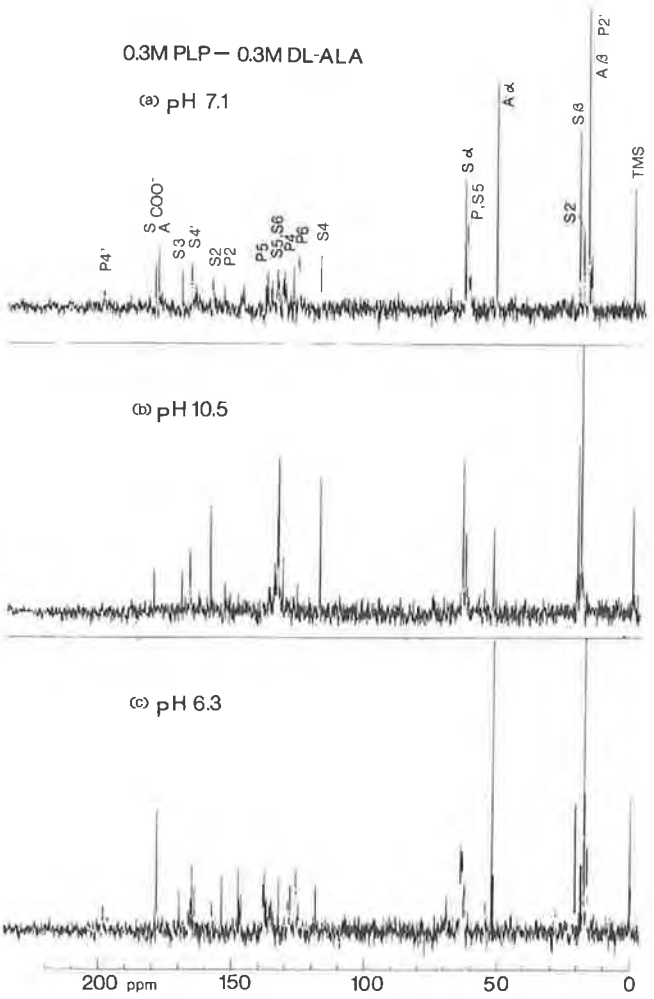

Figure 2. ^{13}C NMR spectra of 0.3 M pyridoxal 5′-phosphate (PLP)–0.3 M DL-alanine (DL-ALA) at three different pHs. P and A: resonances of pyridoxal 5′-phosphate and DL-alanine in free states in the system. S: resonance of dianion Schiff base.

alkaline pH because of the low concentration of Schiff base under these conditions. Consequently, we are led to assume that the resonances observed at pH 10.5 and 7.1 are due to the equilibrium state Schiff base (IIIB ⇄ IIIC).

Observation of the C-2 and C-2′ resonances of the Schiff base at 168.3 and 20.5 ppm, respectively, is a clear indication that the pyridine nitrogen of the Schiff base formed at pH 6.3 is in the deprotonated state as both these resonances would be several parts per million upfield if the pyridine nitrogen were protonated. On the basis of the resonances of C-4′, C-2, C-2′, and C-3 (168.7 ppm) a plausible structure for the species is IIIA. The C-3 resonance at 168.7 ppm shows little change from that detected at pH 10.5 (168.1 ppm) and furthermore at pH 12.8 (168.3) which suggests that the phenolate anion at C-3 in IIIA is not involved in hydrogen bonding to the iminium proton in spite of the extremely high pK_a of this proton which would be explained by hydrogen bonding. The x-ray structure of the corresponding chelated Schiff base shows a metal–oxygen distance of 1.9–2.1 Å,[24,25] a distance too large for the imine proton to assume a hydrogen bond with the phenolate anion, although the crystal structure is not always extrapolable to the corresponding solution structure. As previously mentioned, close examination of the resonances over the range 15–20 ppm where C-2′ resonance occurs provides information on whether the pyridine ring nitrogen is protonated or deprotonated. In the deprotonated form (IIIA), the C-2′ resonance occurs at 20.5 ppm. All of the other peaks in this region at pH 6.3 occur upfield from 18 ppm. One of the peaks in this 15–

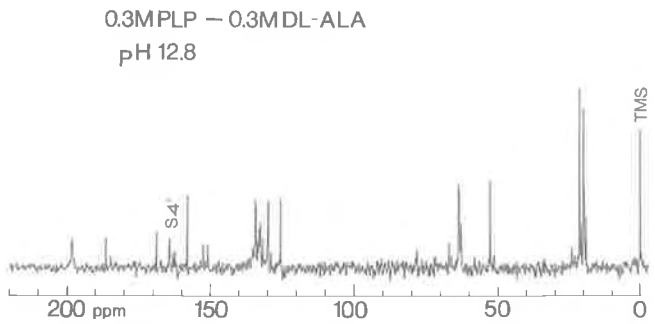

Figure 3. ^{13}C NMR spectrum of 0.3 M PLP–0.3 M DL-ALA at pH 12.8.

18-ppm region is due to C-β of IIIA and the other resonances in this region suggest the presence of species where both the α-amino group and the pyridine nitrogen are protonated. Alanine with a protonated α-amino group has its C-β resonance at 16–17 ppm at pH 6.3.[18] Also as protonation at N-1 of the pyridine ring of vitamin B_6 compounds results in an upfield shift of the C-2′ resonance to 16–17 ppm,[15,16] two of the lines may result from C-β and C-2′ of the free components. The remaining lines, then, should be attributed to some other plausible species, some of whose carbons exhibit triple resonances at 160–163 and also at 145–147 ppm. Those resonances were also detected at pH 7.1 with less enhanced intensity than at pH 6.3. The C-2 resonance at 145–147 ppm and C-3 at 160–165 ppm are common for some vitamin B_6 compounds where C-4′ is in an sp^3-hybridized state such as pyridoxine, pyridoxamine, and pyridoxamine 5′-phosphate.[15,16] This trend is also present in amine adducts of pyridoxal 5′-phosphate where C-4′ is sp^3 hybridized. In addition, the range 145–147 ppm is much further upfield than one would expect for pyridoxal 5′-phosphate and its Schiff base derivatives. The reasons outlined above lead us to suggest that at pH 6.3 and 7.1, tetrahedral intermediates such as three different species of carbinolamine may account for the spectral observations. The closeness of the triplets of C-2 and C-3 observed at 145–150 and 160–165 ppm provides some support for the similarity in the structures.

One might anticipate this observation to be due to the existence of the three possible carbinolamine species which differ by protonation (IIA, IIB, and IIC) as intermediates. Some recent kinetic studies of carbinolamine and imine formation between carboxyl compounds and highly basic amines lend support to the existence of these forms.[26] However, this possibility is not likely to be applicable here since protonation is fast on an NMR time scale and only average chemical shifts can be observed. Another possible explanation for three species of carbinolamine could be configurational forms arising from hydrogen bonding involving the phosphate group and the amino group.

The existence of the carbinolamine (IIC) formed in the condensation process between pyridoxal 5′-phosphate and amines was speculated by Honikel and Madsen[27] although the original proposal for the presence of this species was implied by Kent et al.[28] The resonance at 68.5 (pH 6.3) and 68.7 ppm (pH 7.1) is probably due to the C-4′ of one of the carbinolamine species. Several other observations should be clarified. The resonance of the carboxyl carbon of the pyridoxal 5′-phosphate–alanine complexes does not show much variation in chemical shift in the range pH 10.5–6.3. At pH 10.5, the Schiff base is in the dianion form and no hydrogen bond formation with the imine proton is possible. At pH 6.3, however, the imine moiety of the Schiff base is protonated. From the chemical shift data, it appears therefore that the carboxyl carbon is not involved in intramolecular hydrogen bonding with the imine proton even at pH 6.3. The x-ray results[24,25] provide some support for our conclusion by showing that the chelate Schiff base has a distance of 1.9–2.2 Å from metal to carboxyl group removing the possibility of intramolecular hydrogen bonding. Our results are in contrast to the speculation made by Martell and his co-workers.[11,29] Further, the ^{13}C chemical shift for C-3 in the Schiff bases is almost invariable (within experimental error) over the pH range 6.3–10.5, implying no hydrogen bonding to the iminium proton. Suggestions made previously on the basis of UV–visible[10] and ^1H NMR data[11] are not in agreement with this. Evidence for the keto enamine suggested by early studies of Metzler[5,10] and Heinert and Martell[29] was not clear in these studies. However, the present detection of carbinolamine intermediates makes the possible existence of keto enamine structure somewhat unlikely. Pyridine derivatives which lack a double bond in conjugation with the aromatic ring absorb at wavelengths below 330 nm[22] whereas the free aldehyde form of pyridoxal phosphate and its Schiff bases absorb above 390 nm.[22]

In the Schiff base studies of pyridoxal 5′-phosphate and tris(hydroxymethyl)aminomethane, it was reported that a carbinolamine was in equilibrium with free components.[14] In the ^{13}C NMR spectrum obtained at pH 12.8 (Figure 3), the appearance of the C-4′ resonance at 197.6 ppm is an indication of an equilibrium shift to free pyridoxal 5′-phosphate. The peak at 163.9 ppm due to C-4′ of the Schiff base (IIIB) is still present. This is consistent with our ^1H NMR results[30] which showed that the Schiff base IIIC was in dynamic equilibrium with free components in high pH values. The resonance pairs observed at 150–153 and 160–163 ppm for the C-2 and C-3 carbons, respectively, may arise from two carbinolamines or one carbinolamine and a high pH-dependent Schiff base. However, the concentration of carbinolamines were not sufficient to be detected by ^1H NMR experiments.[30] Observation of six ^{13}C resonances in the CH_3 region (19–22 ppm) is indicative of the presence of multiple species such as free Schiff bases of the C-α deuterated and undeuterated, and carbinolamine. The downfield shifts of the carboxyl resonances to 184–186 ppm at this high pH for both free and Schiff base forms are probably due to the reorganization of the solvation state around this group.

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(2) Author to whom correspondence should be addressed.
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Selective Transformations of Sugar Tosylhydrazones to Deoxy and Unsaturated Sugars[1]

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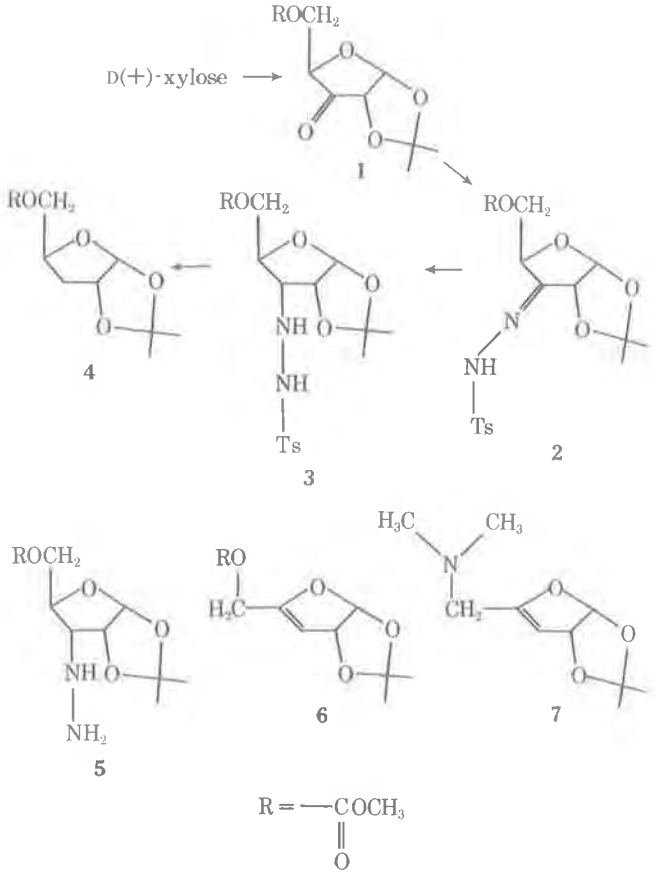
In many areas of synthesis of natural products and especially in carbohydrate chemistry, there is occasionally need for deoxygenation of a secondary hydroxyl group selectively and quantitatively. In connection with some work in our laboratory on the synthesis of nucleoside antibiotics, we needed a relatively simple, high yielding, synthetic accessibility to the naturally occurring pentose 3-deoxy-D-*erythro*-pentose.[2] The existing methods of synthesis of 3-deoxypentoses are in general cumbersome, involved procedures which have limitations due to low product yields, complexity of product mixtures, and difficulties associated with obtaining starting compounds.[2-7] The major difficulty in deoxygenation of secondary hydroxyl groups in carbohydrate chemistry arises because S_N2 processes are generally hindered at these carbons both sterically and through dipolar effects.

The availability of mild and specific oxidation methods in carbohydrate chemistry suggested the desirability of deoxygenation via keto sugars. Recent reports[8] suggest that a wide variety of aldehydes and ketones can be deoxygenated via their tosylhydrazones with sodium cyanoborohydride ($NaBH_3CN$) under acidic conditions. Noteworthy features of $NaBH_3CN$[9] which are of particular interest to carbohydrate chemistry include its acid stability[10] and its reported ability to reduce tosylhydrazones selectively to methylene derivatives without the formation of side products in the presence of a host of otherwise sensitive functional groups. These observations are significant in view of the fact that glycofuranosidulose and glycopyranosidulose derivatives can be produced in high yields, and these in turn, we have found, can be converted to the corresponding crystalline tosylhydrazones almost quantitatively. In this report we wish to describe a mild and high yielding procedure for the synthesis of protected 3-deoxy sugars from their tosylhydrazones. We also developed in the process one of the most efficient methods for the introduction of 3,4 unsaturation in furanoid sugars.[3,11]

The keto sugar **1** served as the source for our starting compound **2**. This ketone can be prepared in high yield from D(+)-xylose.[12] In order that the final product be produced in

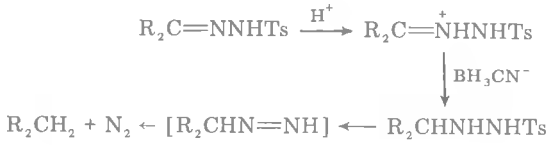
such a form that it could be utilized for further elaborations, two different protecting groups were used to mask the other hydroxyl groups of D(+)-xylose, namely, the acid-labile isopropylidene group and the base-labile carbonate protecting



group. The reaction of the keto sugar **1** with *p*-toluenesulfonylhydrazine in ethanol afforded crystalline tosylhydrazone **2**, mp 174–175 °C, in 96% conversion. The reaction of **2** with $NaBH_3CN$ in DMF at 110 °C in the presence or absence of *p*-toluenesulfonic acid gave a mixture of products as determined by ^1H NMR. The ratio and complexity of products were dependent on the amount of *p*-toluenesulfonic acid used. In the absence of acid, the unsaturated sugars **6** and **7**, the hydrazine **5**, and the deoxy sugar **4** (4%) were all produced, with the unsaturated sugar **6** (40%) being the major product. In the presence of catalytic amounts of *p*-toluenesulfonic acid, the same product mixture was obtained, except that in this case the deoxy sugar **4** was the major product (20%). At relatively high concentrations of acid (pH ~3.8, $NaBH_3CN$ is unstable below pH 3), no unsaturated products were formed and the final mixture consisted of **3**, **4**, **5**, and their 5-dimethylamino derivatives. Lower reaction temperatures did not alter significantly the ratios of the products. Although the unsuitability of DMF as a solvent for selective deoxygenation was apparent, an important observation from these studies was that at lower pH values not only was the amount of unsaturated sugar minimized, but the tosylhydrazine **3** was isolated. This result, together with the demonstrated propensity of $NaBH_3CN$ to reduce iminium systems,[9] suggested the possibility of deoxygenation through the intermediacy of the tosylhydrazine. In contrast to earlier reports,[8] the tosylhydrazine formed is relatively stable under our reaction conditions (pH 3–4), and its decomposition needs then to be induced separately (Scheme I).

Thus, when the tosylhydrazone **2** in THF–CH_3OH (1:1 by volume) was treated with $NaBH_3CN$ and anhydrous HCl (pH ~3.8) at room temperature, the tosylhydrazine **3** was isolated as a crystalline solid, mp 144–147 °C dec, in quantitative

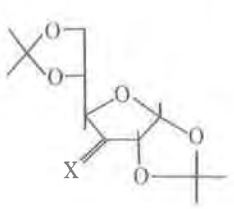
Scheme I



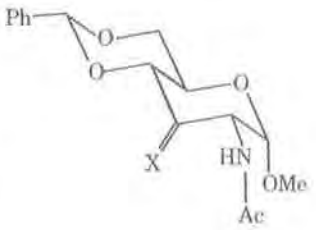
conversion. Interestingly, this reduction is stereospecific as shown by NMR studies. The ^1H NMR spectrum of **3** exhibited coupling constants $J_{1,2} = J_{2,3} \simeq 4.0$ Hz, consistent with the α stereochemistry of the hydrazine group.[13a] Its ^1H noise-decoupled PFT ^{13}C NMR spectrum showed the presence of a single compound with carbon-3 resonance at δ 64.0. The tosylhydrazine **3** can be converted to 3-deoxy-1,2-*O*-isopropylidene-5-*O*-methoxycarbonyl-α-D-*erythro*-pentofuranose (**4**), mp 70–71 °C, in quantitative yield by heating in refluxing ethanol for 5 h in the presence of sodium acetate. The identity of **4** was easily established by ^{13}C and ^1H NMR spectroscopy and elemental analysis, and by its conversion to the known 3-deoxy-1,2-*O*-isopropylidene-α-D-*erythro*-pentofuranose (**4**; R = H).[13b]

To examine the generality of this deoxygenation procedure, we extended this study to glucose. The keto sugar **8** was converted to the tosylhydrazone **9** almost quantitatively. The reduction of **9** in THF–CH_3OH at pH ~3.8 with $NaBH_3CN$ proceeded stereospecifically and quantitatively to give **10**, and the latter was then converted quantitatively to the deoxy sugar **11** by treatment with sodium acetate in ethanol under reflux.

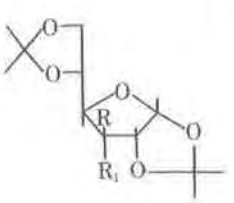
One further example was studied. The deoxy sugar **16** was of special interest because 2-amino-2,3-dideoxy sugars and related modified ones occur in a number of aminoglycoside antibiotics. For example, 2-amino-2,3-dideoxy-D-*ribo*-hexopyranose occurs in lividomycin B[14] and 2,6-diamino-2,3,6-trideoxy-D-*ribo*-hexopyranose is a component of tobramycin.[15] Although syntheses of these aminodeoxy sugars have already been described,[16–18] a superior approach to their preparation would be via 2-amino-2-deoxy-D-glucose, a commercially available starting material which has the 2-amino group in the required configuration. This scheme would involve application of our deoxygenation procedure. For example, for the synthesis of **16** the keto sugar **13** was prepared



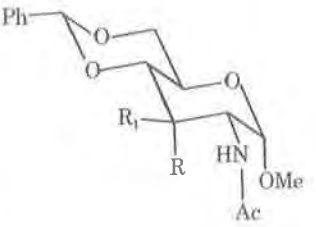
8, X = O
9, X = NNHTs



13, X = O
14, X = NNHTs



10, R = H; R_1 = NHNHTs
11, R = R_1 = H



15, R = H; R_1 = NHNHTs
16, R = R_1 = H

in excellent yields from 2-amino-2-deoxy-D-glucose.[19,20] Compound **13** was found, however, to be sparingly soluble in methanol or ethanol, the most commonly employed solvents for hydrazone formation without acid catalysis,[21] and the most convenient procedure for this conversion was found to be in

DMF at 70 °C under *p*-toluenesulfonic acid catalysis. Without acid catalysis, the reaction is extremely sluggish. However, hydrolysis of the labile benzylidene protecting group cannot be completely eliminated under the conditions used for hydrazone formation. The hydrazone **14** was converted to the deoxy sugar **16** in almost quantitative yield, as expected, through the intermediacy of **15**. The stereochemistry at carbon-3 in **15** has not been established unequivocally because of considerable overlapping in the ^{1}H NMR spectrum in the region of the proton on this carbon.

The persistant formation of unsaturated sugar, the Bamford–Stevens[22] product from the tosylhydrazone **2**, by the action of as weak a base as $NaBH_3CN$ ($K_b \approx 10^{-10}$)[23] in DMF or even in the presence of catalytic amounts of acid ($NaBH_3CN$, *p*-toluenesulfonic acid, DMF) is surprising. In their detailed investigation of deoxygenations via tosylhydrazones induced by $NaBH_3CN$, Hutchins and co-workers[8] observed no unsaturated products even with highly hindered tosylhydrazones. We discovered that the Bamford–Stevens product could be maximized by use of sodium acetate. Thus, when the tosylhydrazone **2** was treated with sodium acetate in DMF at 100 °C for 0.75 h, the 3,4-unsaturated sugar **6** was produced in almost quantitative yield. A similar result was observed in the conversion of the tosylhydrazone **9** into 1,2:5,6-di-*O*-isopropylidene-α-D-*erythro*-hex-3-enofuranose (**12**). An interesting and puzzling feature of the ^{1}H NMR spectrum of **6** was the deceptive simplicity of the appearance of the C_2 and C_3 protons. This was also found to be the case with the unsaturated sugars **7** and **12**.[24] Resolution enhancement,[25] however, showed extensive coupling of these protons. The ^{13}C NMR spectrum of **6** (in $CDCl_3$) confirmed its structure with C_3 and C_4 resonances appearing at 101.0 and 155.3 ppm, respectively.

Experimental Section

1,2-*O*-Isopropylidene-5-*O*-methoxycarbonyl-α-D-*erythro*-3-pentosulofuranose (1) was prepared as described previously.[12]

1,2-*O*-Isopropylidene-5-*O*-methoxycarbonyl-α-D-*erythro*-3-pentosulofuranose *p*-Toluenesulfonylhydrazone (2). A mixture of 1,2-*O*-isopropylidene-5-*O*-methoxycarbonyl-α-D-*erythro*-3-pentosulofuranose (24.6 g, 100 mmol) and *p*-toluenesulfonylhydrazine (20.5 g, 110 mmol) in absolute ethanol (150 mL) was heated with stirring at 70 °C for 2 h and then left overnight at room temperature. Ether (200 mL) was added, and the solid was collected by filtration and washed thoroughly with ether (4 × 50 mL). Recrystallization from a 9:1 mixture of ethanol–CH_2Cl_2 gave a white crystalline solid (39.5 g, 96%): mp 174–175 °C; $[\alpha]^{25}_D$ +259° (*c* 1, $CHCl_3$); IR ν_{max} (Nujol) 3220 (NH), 1760 (C=O), 1620 (C=N) cm^{-1}; ^{1}H NMR (60 MHz) δ Me_4Si ($CDCl_3$) 1.13 (s, 3 H), 1.35 (s, 3 H), 2.42 (s, 3 H), 3.75 (s, 3 H), 4.2–5.12 (m, 4 H), 5.95 (d, $J_{1,2}$ = 4.7 Hz, 1 H), 7.34 (d, $J \simeq$ 8.5 Hz, 2 H), 7.86 (d, $J \simeq$ 8.5 Hz, 2 H), 8.57 (s, 1 H).

Anal. Calcd for $C_{17}H_{22}N_2O_8S$: C, 49.27; H, 5.31; N, 6.76. Found: C, 49.43; H, 5.33; N, 6.76.

1,2-*O*-Isopropylidene-3-deoxy-3-(*p*-toluenesulfonylhydrazino)-5-*O*-methoxycarbonyl-α-D-ribofuranose (3). To a stirred solution of the tosylhydrazone **2** (4.14 g, 10 mmol) in a mixture of 1:1 THF–MeOH (80 mL) was added a trace of methyl orange (indicator) and sodium cyanoborohydride (630 mg, 10 mmol). Methanol saturated with hydrogen chloride was then added dropwise, keeping the color of the solution at the red-yellow transition point (orange, pH ~3.8). The mixture was stirred at room temperature for 1 h. A second portion of sodium cyanoborohydride (315 mg, 5.0 mmol) was added followed by the dropwise addition of methanol saturated with hydrogen chloride to maintain the pH at ~3.8. The mixture was then stirred for 1 h at 25 °C and at pH ~3.8. A saturated solution of $NaHCO_3$ was then added, and the mixture (pH ~7) was concentrated in vacuo at 40 °C to 10 mL. Water (60 mL) was added, and the solution was extracted with CH_2Cl_2 (3 × 40 mL). The combined organic phases were washed with 6 N HCl (1 × 30 mL), saturated aqueous $NaHCO_3$ (1 × 30 mL), and saturated aqueous NaCl (1 × 30 mL), respectively, dried over anhydrous Na_2SO_4, and then evaporated in vacuo at 40 °C to dryness to give 4.1 g (~100%) of **3** as a white crystalline solid: mp 144–146 °C dec; $[\alpha]^{25}_D$ +98.9° (*c* 1, $CHCl_3$); ^{1}H NMR δ Me_4Si ($CDCl_3$) 1.30 (s, 3 H), 1.45 (s, 3 H), 2.43 (s, 3 H), 3.0–3.30 (m, 1 H), 3.77 (s, 3 H), 3.93–4.50 (m, 4 H), 4.64 (t, $J_{1,2} = J_{1,2} = J_{2,3} \simeq$ 4.0 Hz, 1 H), 5.78 (d, $J_{1,2} \simeq$ 4.0 Hz, 1 H), 6.75 (s, 1 H), 7.36 (d, J = 8.5 Hz, 2 H), 7.90 (d, J = 8.5 Hz, 2 H); ^{13}C NMR δ Me_4Si ($CDCl_3$) 21.6, 26.3, 26.5, 54.9, 64.0, 66.4, 75.9, 77.4, 104.7, 112.5, 128.4, 129.6, 134.7, 144.2, 155.4.

Anal. Calcd for $C_{17}H_{24}N_2O_8S$: C, 49.04; H, 5.77; N, 6.72. Found: C, 49.07; H, 5.61; N, 6.71.

3-Deoxy-1,2-*O*-isopropylidene-5-*O*-methoxycarbonyl-α-D-*erythro*-pentofuranose (4). A mixture of the tosylhydrazine **3** (2.08 g, 5.0 mmol) and sodium acetate trihydrate (2.72 g, 20 mmol) in 60 mL of absolute ethanol was refluxed for 5 h. Ethanol was removed in vacuo, and the residue was dissolved in 50 mL of water. The aqueous solution was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic phases were washed with water (1 × 20 mL), dried over anhydrous Na_2SO_4, and then evaporated to dryness to give 1.16 g (~100%) of **4** as a pale yellow syrup. Column chromatography of the crude syrup on silica gel with 7:3 hexane–ether as eluent (250 mL) followed by evaporation of the eluent in vacuo gave 1.05 g (91%) of an analytically pure solid: mp 70–71 °C; $[\alpha]^{25}_D$ −1.53° (*c* 6, $CHCl_3$); ^{1}H NMR δ Me_4Si ($CDCl_3$) 1.30 (s, 3 H), 1.48 (s, 3 H), 1.60–2.27 (m, 2 H), 3.78 (s, 3 H), 3.98–4.82 (m, 4 H), 5.84 (d, $J_{1,2} \simeq$ 3.8 Hz, 1 H); ^{13}C NMR δ Me_4Si ($CDCl_3$) 26.2, 26.8, 35.0, 54.9, 68.0, 75.5, 80.4, 105.7, 111.4, 155.7.

Anal. Calcd for $C_{10}H_{16}O_6$: C, 51.72; H, 6.9. Found: C, 51.63; H, 7.07.

3-Deoxy-1,2-*O*-isopropylidene-α-D-*erythro*-pentofuranose (4; R = H). To a stirred solution of 3-deoxy-1,2-*O*-isopropylidene-5-*O*-methoxycarbonyl-α-D-*erythro*-pentofuranose (610 mg, 2.6 mmol) in 10 mL of methanol was added a solution of sodium methoxide (216 mg, 4.0 mmol) in 10 mL of methanol. The reaction flask was stoppered, and the mixture was stirred at 25 °C for 16 h. The mixture was then neutralized (pH 7) with Dowex 50W-X8 (H^+ form) and filtered immediately, and the filter was washed with methanol (3 × 5 mL). The combined filtrates were evaporated in vacuo to dryness to give 435 mg (96%) of the title compound as a pale yellow solid. Recrystallization from cyclohexane gave white plates: mp 79–80 °C; $[\alpha]^{27}_D$ −10.3° (*c* 0.8, $CHCl_3$) [lit.[13b] mp 79–80 °C $[\alpha]^{20}_D$ −10° (*c* 0.8, 1,2-dichloroethane)]; ^{1}H NMR δ Me_4Si ($CDCl_3$) 1.32 (s, 3 H), 1.50 (s, 3 H), 1.73–2.10 (m, 2 H), 2.57 (broad s, OH, 1 H), 3.37–3.96 (m, 2 H), 4.13–4.53 (m, 1 H), 4.66–4.82 (m, 1 H), 5.82 (d, $J_{1,2}$ = 3.8 Hz, 1 H).

1,2-*O*-Isopropylidene-3-deoxy-3-hydrazino-5-*O*-methoxycarbonyl-α-D-ribofuranose (5). To a solution of 1,2-*O*-isopropylidene-3-deoxy-3-(*p*-toluenesulfonylhydrazino)-5-*O*-methoxycarbonyl-α-D-ribofuranose (2.91 g, 7.0 mmol) in 50 mL of *p*-dioxane was added sodium cyanoborohydride (882 mg, 14.0 mmol). The mixture was refluxed for 9 h and treated upon cooling with 100 mL of 10% aqueous NaCl solution. The mixture was extracted with CH_2Cl_2 (3 × 30 mL), and the combined organic phases were washed with saturated aqueous NaCl solution (1 × 50 mL), dried over anhydrous Na_2SO_4, and then evaporated to dryness. The syrupy residue (1.8 g) crystallized from chloroform–hexane as white crystals (1.1 g, 60%): mp 141–142 °C; ^{1}H NMR δ Me_4Si (CD_3CN) 1.40 (s, 3 H), 1.57 (s, 3 H), 2.33–2.61 (br, 1 H), 3.15–3.57 (m, 1 H), 3.80 (s, 3 H), 3.87–4.58 (m, 3 H), 4.80 (t, $J_{2,1} = J_{2,3} \simeq$ 4.0 Hz, 1 H), 5.88 (d, $J_{1,2} \simeq$ 4.0 Hz, 1 H), 6.20 (br, 2 H); ^{13}C NMR δ Me_4Si (CD_3OD) 26.7, 55.4, 62.9, 67.3, 77.4, 77.9, 106.3, 113.8, 157.0.

Anal. Calcd for $C_{10}H_{18}N_2O_6 \cdot H_2O$: C, 42.92; H, 7.14; N, 10.00. Found: C, 42.60; H, 6.60.

3-Deoxy-1,2-*O*-isopropylidene-5-*O*-methoxycarbonyl-α-D-*glycero*-pent-3-enofuranose (6). A solution of 1,2-*O*-isopropylidene-5-*O*-methoxycarbonyl-α-D-*erythro*-3-pentosulofuranose *p*-toluenesulfonylhydrazone (1.035 g, 2.5 mmol) and sodium acetate (0.8 g) in 10 mL of dry DMF was heated at 100 °C with stirring for 40 min. After cooling to room temperature, water (20 mL) was added and the solution was extracted with CH_2Cl_2 (3 × 30 mL). All of the organic layers were combined, dried over Na_2SO_4, and then evaporated in vacuo to dryness. The crude oily residue was treated with hexane (30 mL) under reflux and the solution was collected by decantation. This process was repeated twice. All of the hexane solutions were combined, and evaporation of solvent gave 529 mg (92%) of a colorless oil. The product was essentially pure. An analytical sample was prepared by preparative layer chromatography on silica gel plates (R_f 0.6, developing solvent CH_2Cl_2): IR ν_{max} (neat) 1775 (C=O), 1690 (C=C) cm^{-1}; ^{1}H NMR δ Me_4Si ($CDCl_3$) 1.43 (s, 6 H), 3.8 (s, 3 H), 4.67 (s, 2 H), 5.23–5.34 (complex m, 2 H), 6.12 (d, $J_{1,2}$ = 5 Hz, 1 H); ^{13}C NMR δ Me_4Si ($CDCl_3$) 27.8, 28.0, 55.2, 62.0, 83.3, 101.0, 106.5, 112.5, 155.3, 155.8.

Anal. Calcd for $C_{10}H_{14}O_6$: C, 52.70; H, 6.09. Found: C, 51.85; H, 6.45.

1,2:5,6-Di-*O*-isopropylidene-α-D-*ribo*-hexofuranos-3-ulose *p*-Toluenesulfonylhydrazone (9). A mixture of 1,2:5,6-di-*O*-isopropylidene-α-D-*ribo*-hexofuranos-3-ulose (8)[26] (5.16 g, 20 mmol) and *p*-toluenesulfonylhydrazine (4.46 g, 24 mmol) in 30 mL of absolute ethanol was heated at 70 °C with stirring for 2 h and then left at room

temperature overnight. The mixture was filtered, and the precipitate was washed several times with ether. Recrystallization from ethanol–CH_2Cl_2 (9:1) gave a white crystalline solid (7.7 g, 91%): mp 181–183 °C dec; ^1H NMR δ Me_4Si ($CDCl_3$) 1.38 (s, 9 H), 1.60 (s, 3 H), 2.44 (s, 3 H), 3.63–4.20 (m, 3 H), 4.45–4.63 (m, 1 H), 4.86 (d of d, $J_{2,1} \simeq 4.3$ Hz, $J_{2,4} \simeq 1.3$ Hz, 1 H), 5.78 (d, $J_{1,2} \simeq 4.3$ Hz, 1 H), 7.31 (d, $J = 8.5$ Hz, 2 H), 7.82 (d, $J = 8.5$ Hz, 2 H), 10.30 (s, 1 H).

Anal. Calcd for $C_{19}H_{26}N_2O_7S$: C, 53.52; H, 6.10; N, 6.57. Found: C, 53.62; H, 6.14; N, 6.59.

1,2:5,6-Di-*O*-isopropylidene-3-deoxy-3-(*p*-toluenesulfonylhydrazino)-α-D-*ribo*-hexofuranose (10). To a solution of 1,2:5,6-di-*O*-isopropylidene-α-D-*ribo*-hexofuranos-3-ulose *p*-toluenesulfonylhydrazone (**9**) (426 mg, 1.0 mmol) in 15 mL of THF–CH_3OH (1:1 by volume) was added sodium cyanoborohydride (95 mg, 1.5 mmol) and a trace of methyl orange indicator. To the stirred mixture was added methanol containing ~5% hydrogen chloride, and the color of the solution was maintained at the red-yellow transition point (orange, pH ~3.8). The mixture was stirred for 1 h at 25 °C and at pH ~3.8. Saturated aqueous $NaHCO_3$ solution was then added, and the mixture (pH ~7) was evaporated in vacuo to dryness. Water (15 mL) was added to the residue, and the solution was extracted with CH_2Cl_2 (3 × 15 mL). The combined organic phases were washed with 6 N HCl (1 × 10 mL), saturated aqueous $NaHCO_3$ (1 × 10 mL), and saturated aqueous NaCl (1 × 10 mL), respectively, and were dried over anhydrous Na_2SO_4 and then evaporated in vacuo to dryness to give 420 mg (~100%) of the title compound as a white crystalline solid: mp 66–70 °C; ^1H NMR δ Me_4Si ($CDCl_3$) 1.31 (s, 3 H), 1.41 (s, 6 H), 1.47 (s, 3 H), 2.42 (s, 3 H), 2.75–3.15 (m, 1 H), 3.53–4.23 (br m, 5 H), 4.6 (t, $J_{2,1} = J_{2,3} \simeq 4.0$ Hz, 1 H), 5.70 (d, $J_{1,2} \simeq 4.0$ Hz, 1 H), 7.30 (s, obscured by C_6H_4, 1 H), 7.36 (d, $J = 8.5$ Hz, 2 H), 7.91 (d, $J = 8.5$ Hz, 2 H).

Anal. Calcd for $C_{19}H_{28}N_2O_7S$: C, 53.27; H, 6.54; N, 6.54. Found: C, 53.09; H, 6.58; N, 6.54.

3-Deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-*ribo*-hexofuranose (11). A mixture of 1,2:5,6-di-*O*-isopropylidene-3-deoxy-3-(*p*-toluenesulfonylhydrazino)-α-D-*ribo*-hexofuranose (**10**) (428 mg, 1.0 mmol) and sodium acetate trihydrate (544 mg, 4.0 mmol) in 15 mL of absolute ethanol was refluxed for 5 h. Ethanol was removed in vacuo, and the residue was dissolved in 15 mL of water. The aqueous solution was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic phases were washed with saturated aqueous NaCl (1 × 10 mL), dried (Na_2SO_4), and then evaporated to dryness to give 240 mg (~100%) of 3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-*ribo*-hexose as a pale yellow syrup:[27] ^1H NMR δ Me_4Si ($CDCl_3$) 1.31 (s, 3 H), 1.34 (s, 3 H), 1.41 (s, 3 H), 1.49 (s, 3 H), 1.61–2.46 (m, 2 H), 3.63–4.35 (br m, 4 H), 4.75 (t, $J_{2,1} = J_{2,3} \simeq 4.0$ Hz, 1 H), 5.83 (d, $J_{1,2} \simeq 4.0$ Hz, 1 H); $[\alpha]^{25}_D$ −7.4° (*c* 10, EtOH) [lit.[4] −7.5° (*c* 10, EtOH)].

3-Deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-*erythro*-hex-3-enofuranose (12). A mixture of 1,2:5,6-di-*O*-isopropylidene-α-D-*ribo*-hexofuranos-3-ulose *p*-toluenesulfonylhydrazone (852 mg, 2.0 mmol) and sodium acetate trihydrate (1.1 g, 8.0 mmol) in 15 mL of DMF was heated with stirring at 100 °C for 1 h. Water (15 mL) was then added, and the solution was extracted with CH_2Cl_2 (3 × 15 mL). The combined organic phases were washed with saturated aqueous NaCl (2 × 15 mL) and dried (Na_2SO_4), and the solvent was removed in vacuo. To the brown viscous residue was added hexane (15 mL), and the mixture was heated to reflux. The hexane solution was collected by decantation of the hot mixture. This process was repeated twice with 15-mL portions of hexane. The combined hexane solutions were evaporated to dryness to give 440 mg (91%) of the title compound as a pale yellow syrup which solidified on standing. An analytical sample was prepared by chromatography on a preparative layer silica gel plate. The plate was developed with 1:1 ether–hexane. The band with R_f 0.77 upon elution with ether followed by removal of solvent gave white solid: mp 46–48 °C (lit.[28] mp 46–48 °C); ^1H NMR δ Me_4Si ($CDCl_3$) 1.36 (s, 3 H), 1.44 (s, 9 H), 3.79–4.26 (m, 2 H), 4.57 (t, $J \simeq 6.2$ Hz, 1 H), 5.20–5.35 (m, 2 H), 6.06 (d, $J_{1,2} \simeq 5.0$ Hz, 1 H).

Anal. Calcd for $C_{12}H_{18}O_5$: C, 59.50; H, 7.44. Found: C, 59.44; H, 7.77.

Methyl 2-Acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-*ribo*-hexopyranosid-3-ulose *p*-Toluenesulfonylhydrazone (14). A mixture of methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-*ribo*-hexopyranosid-3-ulose[19,20] (**13**) (642 mg, 2.0 mmol), *p*-toluenesulfonylhydrazine (744 mg, 4.0 mmol), and *p*-toluenesulfonic acid monohydrate (76 mg, 0.4 mmol), in 20 mL of dry DMF was stirred at 70–72 °C for 1 h. The solution was cooled, neutralized (pH 7–8) with aqueous $NaHCO_3$, and then evaporated in vacuo at <40 °C to dryness. Water (20 mL) was added to the solid residue and then CH_2Cl_2 (20 mL). The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (2 × 15 mL). The combined organic phases were washed with 6 N HCl (2 × 20 mL), saturated aqueous $NaHCO_3$ (1 × 20 mL), and saturated aqueous NaCl (1 × 20 mL), respectively, dried (Na_2SO_4), and then evaporated in vacuo to dryness to give 656 mg of pale yellow solid (67%). Recrystallization from ether–ethanol gave 580 mg of **14** as white plates: mp 179–181 °C; IR ν_{max} 3320 (NH), 1635, 1535 (amide I and II), 1605 (C=N) cm^{-1}, and no C=O near 1745 cm^{-1}; ^1H NMR δ Me_4Si ($CDCl_3$) 2.00 (s, 3 H), 2.37 (s, 3 H), 3.28 (s, 3 H), 3.70–4.70 (m, 5 H), 4.87 (d, $J_{1,2} = 3.5$ Hz, 1 H), 5.47 (s, 1 H), 6.42 (d, $J_{N,H} = 9$ Hz, 1 H), 7.25 (d, $J = 8.5$ Hz, 2 H), 7.4 (s, 5 H), 7.70 (d, $J = 8.5$ Hz, 2 H), 10.05 (s, 1 H).

Anal. Calcd for $C_{23}H_{27}N_3O_7S$: C, 56.44; H, 5.32; N, 8.58. Found: C, 56.11; H, 5.46; N, 8.27.

Methyl 2-Acetamido-4,6-*O*-benzylidene-2,3-dideoxy-α-D-*ribo*-hexopyranoside (16). To a stirred solution of the tosylhydrazone **14** (489 mg, 1.0 mmol) in a mixture of 1:1 THF–CH_3OH (12 mL) was added sodium cyanoborohydride (63 mg, 1.0 mmol) and a trace of methyl orange. Methanol saturated with hydrogen chloride was then added dropwise, keeping the color of the solution at the red-yellow transition point (orange, pH ~3.8). The mixture was stirred at ~25 °C at this pH for 1.5 h. A second portion of sodium cyanoborohydride (63 mg, 1.0 mmol) was added followed by methanol saturated with hydrogen chloride to maintain the pH at 3.8. The mixture was then stirred for 1.5 h at ~25 °C at pH ~3.8. The mixture was neutralized (pH ~7) with aqueous $NaHCO_3$ and then evaporated in vacuo to dryness. To the residue was added water (20 mL), followed by CH_2Cl_2 (20 mL). The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (2 × 20 mL). The combined organic phases were washed with 6 N HCl (1 × 20 mL), saturated aqueous $NaHCO_3$ (1 × 20 mL), and saturated aqueous NaCl (1 × 20 mL), respectively, dried (Na_2SO_4), and then evaporated in vacuo to dryness to give 480 mg (98%) of **15** as a white solid which was utilized in the next step without further purification: ^1H NMR δ Me_4Si ($CDCl_3$) 1.93 (s, 3 H), 2.38 (s, 3 H), 2.77–3.36 (m, partly obscured by the OCH_3 signal, 1 H), 3.27 (s, 3 H), 3.62–4.32 (m, 5 H), 4.57 (d, $J_{1,2} = 3.5$ Hz, 1 H), 4.65–4.83 (broad, 1 H), 5.45 (s, 1 H), 6.5 (d, $J_{N,H} = 9$ Hz, 1 H), 6.96 (s, 1 H), 7.27 (d, $J = 8.5$ Hz, 2 H), 7.4 (s, 5 H), 7.72 (d, $J = 8.5$ Hz, 2 H).

A mixture of the tosylhydrazine **15** (491 mg, 1.0 mmol) and sodium acetate trihydrate (544 mg, 4.0 mmol) in 10 mL of absolute ethanol was refluxed for 5 h. Ethanol was removed in vacuo, and water (15 mL) was added to the residue. The aqueous mixture was extracted with CH_2Cl_2 (3 × 15 mL). The combined organic phases were washed with saturated aqueous NaCl (1 × 20 mL), dried (Na_2SO_4), and then evaporated in vacuo to dryness to give 300 mg (98%) of the deoxy sugar **16** as a light yellow solid. Recrystallization from ether–ethyl acetate gave a white solid (292 mg): mp 244–245 °C (subl); $[\alpha]^{24}_D$ +54.0° (*c* 1, $CHCl_3$) [lit.[18] mp 245 °C (subl), $[\alpha]_D$ +55.5° (*c* 0.95); lit.[16] mp 263–264 °C, $[\alpha]_D$ +52° (*c* 1.0); lit.[17] mp 224 °C (subl), $[\alpha]_D$ +53.7° (*c* 1.0)]; ^1H NMR δ Me_4Si ($CDCl_3$) 1.78–2.27 (m, partly hidden under $COCH_3$, 2 H), 1.95 (s, 3 H), 3.40 (s, 3 H), 3.53–4.47 (m, 5 H), 4.58 (d, $J_{1,2} = 3.5$ Hz, 1 H), 5.51 (s, 1 H), 5.63–5.95 (broad d, $J_{N,H} = 9$ Hz, 1 H), 7.2–7.8 (m, 5 H); ^{13}C NMR δ Me_4Si ($CDCl_3$) 22.3, 29.8, 46.4, 53.9, 63.0, 68.2, 75.4, 96.7, 100.7, 125.1, 127.3, 128.0, 136.4, 168.4.

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Registry No.—**1**, 62789-42-8; **2**, 68014-59-5; **3**, 68014-60-8; **4** (R = C(=O)OMe), 68014-61-9; **4**, (R = H), 3396-71-2; **5**, 68014-62-0; **6**, 68014-63-1; **8**, 2847-00-9; **9**, 18265-29-7; **10**, 68014-64-2; **11**, 4613-62-1; **12**, 2774-28-9; **13**, 4288-74-8; **14**, 68014-65-3; **15**, 68014-66-4; **16**, 22595-95-5.

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CONVERSION OF 9-SUBSTITUTED ADENINES TO THE CORRESPONDING PURINES: A SIMPLE DEAMINATION METHOD FOR ADENINE DERIVATIVES

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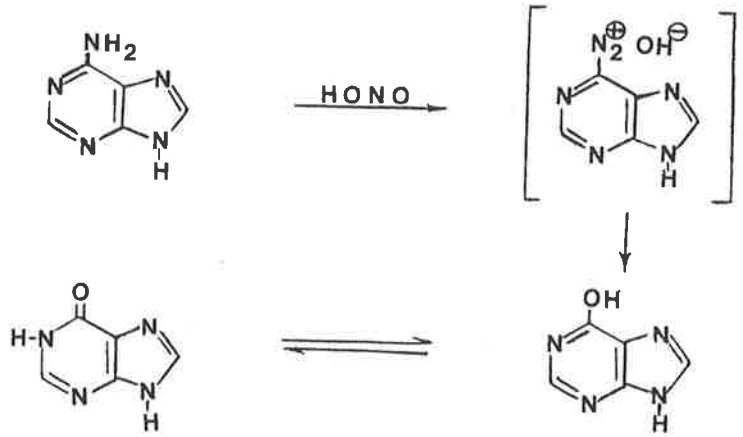
Summary: An effective procedure for the replacement of an amino group in nucleic acid bases with hydrogen is described. The method provides a direct synthesis of the antibiotic, nebularine, from adenosine.

Although the deamination of aromatic amines with replacement by hydrogen of the amino group has been accomplished in numerous systems,[1] such diazotization-deamination reportedly fails under a variety of conditions for 6-aminopurine (adenine) derivatives.[2-4] While 6-diazonium salts of purine have never been isolated, conversion of adenine to hypoxanthine in nitrous acid (Scheme 1)[5] and of 6-aminopurine derivatives to 6-fluoropurines[6,7] are presumptive of diazonium intermediates where the counterion also acts as a nucleophile. We now wish to

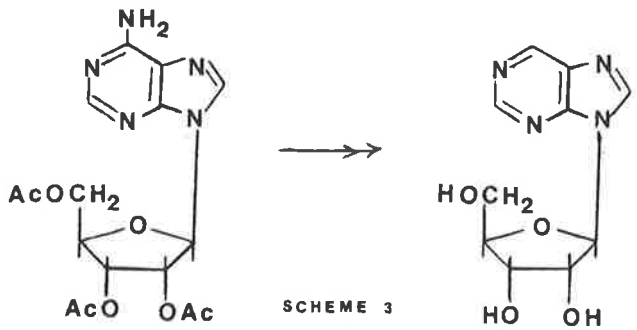


SCHEME 1

report the successful deamination of 9-substituted 6-aminopurines under mild conditions to give the corresponding purines, and extension of this method to the synthesis of the antitumor antibiotic, 9-β-D-ribofuranosylpurine (nebularine)[8] from adenosine.

We utilized in this deamination the known ability of alkyl nitrites to produce aryl radicals from arylamines and the subsequent possibility of hydrogen abstraction by the radical from a suitable hydrogen atom donor.[9,10] The latter can be the solvent. Of the various solvents or solvent combinations with potentially abstractable hydrogen atoms, we found dry tetrahydrofuran to be the most satisfactory. As radical processes are inhibited by the presence of scavengers such as O_2 or NO, the solvent was initially purged with nitrogen and the reaction was conducted in an atmosphere of nitrogen. Diazonium salts and azo compounds (which predominate in neutral solution) are decomposed homolytically by blue light,[11] so irradiation to aid homolytic dissociation was also included. Thus when a solution of 9-ethyladenine in dry distilled THF was treated with n-pentyl nitrite under nitrogen and refluxing temperatures and with constant illumination from a 200 watt tungsten filament lamp for 6 hr, chromatographic separation of the product mixture gave 9-ethylpurine in 68% yield (Scheme 2). The structure of the product was confirmed by ^{13}C, ^{1}H NMR and UV spectroscopic data comparison with an authentic sample prepared from sodium purinide and ethyl tosylate.[12,13] Interestingly, no coupling products or hypoxanthine were detected. 9-Benzyladenine was similarly deaminated to the corresponding purine in good yield.

SCHEME 2



SCHEME 3

In order to examine the generality of this deamination method, we extended the study to nucleosides. Thus when 2',3',5'-tri-O-acetyladenosine in THF was deaminated as described above, 2',3',5'-tri-O-acetylnebularine was isolated in 41% conversion. The protected compound can be converted easily to nebularine by deacylation with methanolic ammonia (Scheme 3). This represents an excellent direct synthesis of this nucleoside antibiotic from readily available adenosine.[14-16] We are currently examining the application of this simple deamination method to the synthesis of more complex modified nucleosides.

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Substrate Specificity of Adenosine Deaminase—Function of the 5′-Hydroxyl Group of Adenosine

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Nucleosides in which the adenine ring has been moved from the 1′ position to the 5′ position are resistant to degradation by the enzyme, adenosine deaminase. This study provides further evidence for the importance of the 5′-hydroxyl group as a structural requirement for significant substrate activity.

INTRODUCTION

The ubiquitous enzyme adenosine deaminase (adenosine aminohydrolase, EC 3.5.4.4) catalyzes the hydrolytic deamination of adenosine to inosine. This deamination is an important factor in limiting the usefulness of adenosine analogs in chemotherapy (*1*). The design of synthetic analogs of adenosine that would be more resistant to degradation by adenosine deaminase should take into account the structural requirements for overall substrate activity. Determination of these structural requirements has been reported in a number of investigations (*2–20*). In general, substrate binding and significant substrate activity require the presence of the adenine ring (*3*, *8–11*). A number of structural changes are permitted in the sugar moiety and the minimum requirements appear to be a tetrahydrofuran ring bearing the 5′-CH_2OH (*3*, *4–7*, *9*, *17–20*). The stereochemical requirement is β-D or α-L with respect to the anomeric position and the 5′-CH_2OH (*6*, *9*, *12–14*). Dramatic changes in substrate activity occur when the 5′-CH_2OH is altered (*6*, *12*). We wish to report that examination of a class of nucleosides called "reversed" nucleosides where the purine ring has been moved from the 1′ position to the 5′ position shows that these are remarkably stable compared to adenosine toward the deaminase.

METHODS AND MATERIALS

General Methods

The melting points reported were uncorrected and were taken on a Thomas–Hoover melting point apparatus equipped with a microscope. The 60-MHz ^1H nmr spectra were taken on a Varian A-60 nmr spectrometer. The 90-MHz ^1H nmr spectra and the ^{13}C nmr spectra were recorded on a Bruker HX-90E pulse Fourier

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transform nmr spectrometer. Lyophilizations were done on a Labconco Freeze Dry 5 lyophilization unit. Elemental analyses were performed by the University of Iowa Micoranalytical Service. Ultraviolet absorption spectra and all ADA kinetic assays were done on a Cary Model 118CX recording UV-VIS spectrophotometer. All kinetic measurements were conducted at 25.0 ± 0.1°C. This temperature was maintained through use of a Forma Scientific Masterline Model 2095 constant temperature water bath and circulator. All assay solutions (with the exception of the ADA solution) were preincubated in the water bath for at least 30 min prior to use. Water from the water bath was circulated through the thermostatable cell holder in the Cary 118 sample compartment. The ADA solution was made up in ice-cold buffer (0.05 *M* potassium phosphate, pH 7.40), and the resulting solution was kept on ice until use. The ADA solutions were used within 2 hr after dilution of a stock suspension of ADA crystals in 3.2 *M* $(NH_4)_2SO_4$. The deaminations of the adenine nucleosides by ADA were followed spectrophotometrically at 265 nm. Solutions of substrates of appropriate concentrations in 0.05 *M* potassium phosphate buffer (pH 7.40) were used. The deamination reactions were initiated by addition of ADA solution, the concentration of which had been previously determined to give a workable reaction rate. Photolyses were carried out in a Rayonet photochemical reactor. Purine was purchased from ICN Pharmaceuticals, Cleveland, Ohio. The purine ribofuranoside, Sephadex, and adenosine deaminase (Type I from calf intestinal mucosa) were purchased from Sigma Chemical Company, St. Louis, Missouri.

Data Processing

The kinetic data were fitted to the linear transformation of the Michaelis–Menten equation that follows by means of a linear least-squares analysis:

$$1/v = (K_m/V_{max})(1/s) + 1/V_{max}$$

(the Lineweaver–Burk plot). All calculations were performed on an IBM 360/65 digital computer or on a CDC Cyber digital computer with programs written in FORTRAN IV by the authors. Plots presented are derived from computer-generated Versatec electrostatic plots, using subroutines analogous to those available in the CalComp system. Double precision was used throughout. In addition, the nonparametric statistical analysis of Cornish-Bowden and Eisenthal (*21*, *22*) was employed using a FORTRAN IV program written by these authors and modified by the authors of this work to generate Versatec plots.

Synthesis

*5'-(Purin-9-yl)-5'-deoxy-(α),β-*D*-ribofuranose* (*3*). A suspension of purine (250 mg, 2.08 mmol) and sodium hydride (100 mg, 2.08 mmol of a 50% oil dispersion) in 7 ml of DMF was stirred at 25°C for 75 min and then at 55°C for 15 min. A solution of methyl 2,3-*O*-isopropylidene-5-*O*-*p*-toluenesulfonyl-β-D-ribofuranoside in 13 ml dry DMF was added to it, and the solution was heated at 95°C for 15 hr. The reaction mixture was then stripped of solvent under reduced pressure and the resulting solid was extracted with hot $CHCl_3$. The filtered extract solution was

TABLE I

^{13}C NUCLEAR MAGNETIC RESONANCE DATA FOR NUCLEOSIDES AND PHOTOADDUCTS

Compound	Solvent (concn)	Chemical shifts (δ) from TMS											
		C2	C4	C5	C6	C8	C1′	C2′	C3′	C4′	C5′	OCH_3	CH_2OH
1 α	D_2O (0.1 *M*)	153.1	149.7	118.7	156.0	143.9	97.3	71.9	71.4	80.9	46.0	—	—
1 β	D_2O (0.1 *M*)	153.1	149.7	118.7	156.0	143.9	102.0	75.8	72.2	80.5	46.5	—	—
2	D_2O (0.1 *M*)	151.6	149.4	118.5	154.8	144.0	109.1	74.9	72.3	80.6	46.3	56.3	—
3 α	D_2O (0.15 *M*)	152.6	152.0	133.5	148.2	149.4	97.4	72.2	71.4	80.6	46.3	—	—
3 β	D_2O (0.15 *M*)	152.6	152.0	133.5	148.2	149.4	102.0	75.7	72.2	80.3	46.3	—	—
4 α	CH_3OD (0.05 *M*)	135.6	135.6	117.7	67.2	149.2	97.9	72.7	72.2	82.9	47.8	—	56.8
4 β	CH_3OD (0.05 *M*)	135.6	135.6	117.7	67.2	149.2	103.5	77.1	73.6	82.1	48.2	—	56.8
5	D_2O (0.25 *M*)	152.6	151.2	134.4	148.7	146.6	89.2	74.6	71.2	86.4	62.2	—	—
6	CH_3OD (0.14 *M*)	134.8	135.4	119.7	67.0	148.8	91.4	75.9	73.0	88.3	63.8	—	56.6
Adenosine (*32*)	DMSO-d_6 (0.9 *M*)	152.4	149.1	119.4	156.1	140.1	88.1	73.6	70.8	86.0	61.8	—	—

evaporated to dryness and the resulting yellow oil was chromatographed on preparative silica gel PF 254 plates using 5% CH_3OH/CH_2Cl_2 for development. The major product was the 9-substituted purine (306 mg, 52%), mp 67.5–69°C: uv λ_{max} (CH_2Cl_2) 264 nm; (CH_3OH, pH 9) 263 nm; ^1H nmr δ Me_4Si ($CDCl_3$) 1.30 (s, 3H), 1.46 (s, 3H), 3.42 (s, 3H), 4.21–5.05 (m, 6H), 8.30 (s, 1H), 9.00 (s, 1H), 9.20 (s, 1H).

Anal. Calcd for $C_{14}H_{18}N_4O_4$: C, 54.89; H, 5.92; N, 18.29. Found: C, 54.68; H, 5.99; N, 17.97.

The 7-substituted purine was also isolated as a minor product (26%), and could easily be distinguished from the 9-isomer by its uv spectrum: λ_{max} (CH_2Cl_2) 270 nm; (CH_3OH, pH 9) 267 nm (see Refs. (*23*, *24*) for uv data comparison of 7- and 9-substituted purines). Further confirmation of structure was provided by ^{13}C nmr data (Table 2).

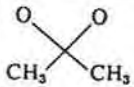
Deprotection of the major product (306 mg) was carried out in 10 ml of 0.18 *M* HCl at 80°C for 2.75 hr. After cooling, the solution was passed through a Dowex 1-X8 column (HCO_3^- form) and lyophilized to give **3** as a highly hygroscopic amorphous solid (265 mg, ~100%): uv λ_{max} (H_2O) 264.0 nm (log ε 3.84). ^{13}C and ^1H nmr spectral data provided excellent structural confirmation (Tables 1 and 3). The ratio of α to β forms was 1:6.

Anal. Calcd. for $C_{10}H_{12}N_4O_4 \cdot \frac{2}{3}H_2O$: C, 45.46; H, 5.09; N, 21.20. Found: C, 45.28; H, 5.03; N, 21.26.

*5′-(Purin-9-yl)-5′-*deoxy-(α),β-D-*ribofuranose methanol photoadduct* (*4*). A solution of 80 mg (0.32 mmol) of **3** in 170 ml of oxygen-free dry methanol was photolyzed in a Rayonet photochemical reactor with 2537-Å lamps for 50 min

TABLE 2

^{13}C NUCLEAR MAGNETIC RESONANCE DATA DIFFERENTIATING 7- AND 9-SUBSTITUTION IN SUBSTITUTED PURINES

Compound	Solvent (concn)	C2	C4	C5	C6	C8	C1′	C2′	C3′	C4′	C5′			OCH_3	CH_3
7-methyl purine (*33*)	DMSO-d_6 (0.6 *M*)	152.0	159.8	125.7	140.7	149.7	—	—	—	—	—	—	—	—	31.6
Protected 3 7-isomer[a]	$CDCl_3$ (0.5 *M*)	153.2	160.7	125.3	140.2	148.5	110.5	84.9	84.6	81.8	49.4	113.2	24.9 26.4	56.1	—
9-Methyl purine	DMSO-d_6 (1.3 *M*)	151.8	151.3	133.4	147.4	147.4	—	—	—	—	—	—	—	—	29.3
Protected 3 9-isomer[a]	$CDCl_3$ (0.5 *M*)	152.1	150.9	133.4	148.1	145.2	109.8	84.6	84.1	81.5	46.4	112.4	24.5 25.9	55.3	—

[a] Protecting groups were β-methyl acetal and 2′,3′-*O*-isopropylidene (see Methods and Materials.

TABLE 3

^{1}H NUCLEAR MAGNETIC RESONANCE DATA FOR NUCLEOSIDES AND PHOTOADDUCTS

Compound	Solvent (concn)	Chemical shifts (δ) and multiplicities										
		H2	H6	H8	H1′	H2′	H3′	H4′	H5′	OCH_3	CH_2OH	NH_2
1 α and β	D_2O (0.2 *M*) ext TMS	8.58(s) or 8.52(s)	—	8.58(s) or 8.52(s)	5.72(d) and 5.85(s)	4.35 to 5.64 (m)			4.30(q)	—	—	—
2	DMSO-d_6 (1 *M*)	8.16(s) or 8.09(s)	—	8.16(s) or 8.09(s)	4.64(s, br)	3.39 to 4.26 (m)				3.22(s)	—	7.20(s)
3 α and β	D_2O (0.15 *M*) ext TMS	9.52(s)	9.65(s)	9.16(s)	6.08(s, br) and 6.06(d)	4.42 to 5.87 (m)				—	—	—
4 α and β	CD_3OD (0.2 *M*)	7.32(d) or 7.00(s)	3.76(t)	7.32(d) or 7.00(s)	5.13(d) and 5.06(s, br)	3.51 to 4.56 (m)				—	3.88	—
5	D_2O (0.25 *M*) ext TMS	9.21(s)	9.36(s)	9.04(s)	6.53(d)	4.63 to 5.86 (m)			4.31(m)	—	—	—
6	CD_3OD (0.2 *M*)	7.53(s) or 7.04(s)	~3.71(t)	7.53(s) or 7.04(s)	5.64(d)	4.10 to 4.65 (m)			3.68(m)	—	3.71	—
Adenosine	DMSO-d_6 (0.2 *M*)	8.34(s) or 8.18(s)	—	8.34(s) or 8.18(s)	5.88(d)	4.68(q)	4.14(q)	3.96(q)	3.62(m)	—	—	7.33(s)

(*25, 26*). The reaction was monitored by following the change in the uv spectrum from 262 to 296 nm as the reaction progressed. The methanol was then removed carefully under reduced pressure and room temperature to give 73 mg (81%) of a pale yellow viscous material. The compound was unstable and its structure and purity was established by its mass spectrum (M^+ 284) and by its ^{13}C and ^1H nmr spectra (Tables 1 and 3).

Nebularine–methanol photoadduct (*6*). A solution of 81 mg (0.32 mmol) of nebularine **5** was photolyzed as described above for **3** to give 95 mg (~100%) of a colorless, unstable oil (*27*). Its structure confirmation was provided by its mass spectrum (M^+ 284) and by NMR data (Tables 1 and 3).

*5′-(6-Aminopurin-9-yl)-5′-deoxy-(α),β-*D*-ribofuranose* (*1*). This compound was prepared as previously described (*28*) and purified by gel permeation chromatography on a column of Sephadex G-25–80. The nucleoside **1** was obtained as a white solid: mp 181–182°C (lit. (*28*) mp 168–169°C); uv λ_{max} (H_2O) 259.7 nm (log ϵ 4.17); ^{13}C and ^1H nmr data (Tables 1 and 3).

*Methyl 5′-(6-aminopurin-9-yl)-5′-deoxy-β-*D*-ribofuranoside* (*2*). The protected reversed nucleoside methyl 5′-(6-aminopurin-9-yl)-2′,3′-*O*-isopropylidene-5′-deoxy-β-D-ribofuranoside (3.22 g, 10 mmol) (*29*) was dissolved in a solution of 250 ml of CH_3OH containing 10 ml of 2 *M* HCl and heated under reflux for 24 hr. The CH_3OH was removed *in vacuo* and the residue was taken up in 10 ml of H_2O. The aqueous solution was immediately neutralized with Dowex 1 (HCO_3^- form). Filtration of the resin and evaporation of the filtrate gave **2** (2.65 g, 95%) as a white solid which was recrystallized from methanol, mp 180–181°C: uv λ_{max} (H_2O) 261.5 nm (log ϵ 4.11); ^{13}C and ^1H nmr (see Tables 1 and 3).

Anal. Calcd for $C_{11}H_{15}N_5O_4 \cdot \frac{1}{2}H_2O$: C, 45.52; H, 5.56; N, 24.13. Found: C, 45.56; H, 5.48; N, 24.32.

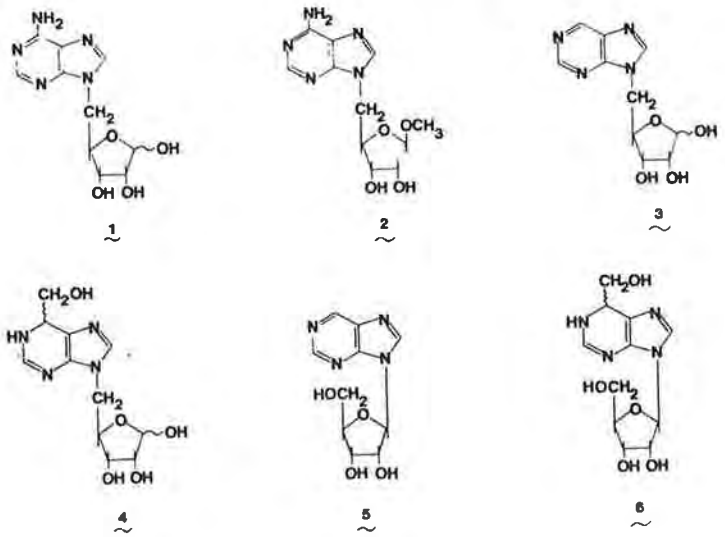
RESULTS AND DISCUSSION

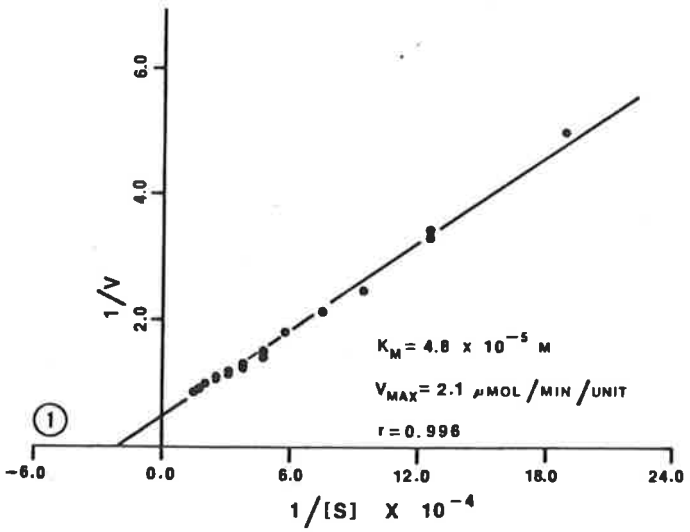
The reversed nucleosides **1** and **2** were synthesized by thermally induced coupling of the sodium salt of adenine and methyl 2,3-*O*-isopropylidene-5-*O*-*p*-toluenesulfonyl-β-D-ribofuranoside followed by removal of protecting groups (*28–30*). The carbon-13 nmr spectra of **1** and **2** provided excellent confirmation of their structures (Table 1). The ratio of the two isomers $\alpha : \beta$ was 1 : 6 for **1** (*31*), and the stereochemistry at C1′ of the acetal **2** was inferred from the appearance of the H1′ resonance as a singlet. The nebularine analog **3** was synthesized by reaction of methyl 2,3-*O*-isopropylidene-5-*O*-*p*-toluenesulfonyl-β-D-ribofuranoside with sodium purinide, subsequent separation of the mixture of 7-isomer (26%) and 9-isomer (52%) and finally removal of the protecting groups. Assignment of structure for the 7- and 9-isomers from the coupling reaction came from uv data and particularly from ^{13}C nmr spectral analysis (see Table 2). Photolysis (quartz tube, 254-nm irradiation) of **3** in dry oxygen-free methanol solution gave **4** as a pale yellow unstable oil in 81% yield. Its mass spectrum confirmed that a 1 : 1 photoadduct was formed and its ^{13}C nmr spectrum provided

excellent evidence of gross structure. The nebularine photoadduct **6** was prepared in a similar manner from **5** and methanol (*25–27*).

The deaminations with adenosine deaminase were followed spectrophotometrically by monitoring of the uv absorption at 265 nm (*34*). The kinetic data were treated as described under Methods and Materials. The Lineweaver–Burk plots of adenosine, its reversed analog, and the β-methyl acetal of reversed adenosine are shown in Figs. 1, 2, and 3. Michaelis constants (K_m), maximal velocities (V_{max}), and correlation coefficients (r) are included with the plots. The results indicate that **1** and **2** exhibit much slower substrate activity with the enzyme than adenosine.

The contribution made to overall substrate activity by the 5′-hydroxyl group is apparent from these studies. The results presented are also consistent with the possibility that the anomeric β-hydroxyl group (86% β from ^1H and ^{13}C nmr data) may be capable of assuming the role of the 5′-hydroxyl group. This interpretation has some precedent for it had been suggested previously that the function of the 5′-hydroxyl group could be assumed effectively by a hydroxyl group on C3′ in a configuration *cis* to the adenine moiety (*5, 6, 12*). Thus, 5′-deoxyxylosyladenine is a substrate for adenosine deaminase whereas 5′-deoxyadenosine and 5′-deoxyarabinofuranosyladenine are not deaminated (*5, 12*). A possible explanation for this activity was given by Shah *et al.* (*12*) who suggested that the hydrogen of the β-hydroxyl group at C3′ of 5′-deoxyxylosyladenine can occupy an almost identical position as does the hydroxyl group at C5′ in one of the conformations of adenosine. The possible involvement of the β-hydroxyl group at C1′ in substrate activity in our studies is further supported by the observation that when the reversed nucleoside **1** was converted to its acetal **2**, V_{max} diminished dramatically. In fact, the observed V_{max} for adenosine in these studies was 26,500 times faster than that for **2**! The K_m values are more difficult to interpret. If it is assumed that the K_m values are an indication of enzyme–substrate affinities, then the acetal **2** (K_m = 2.3

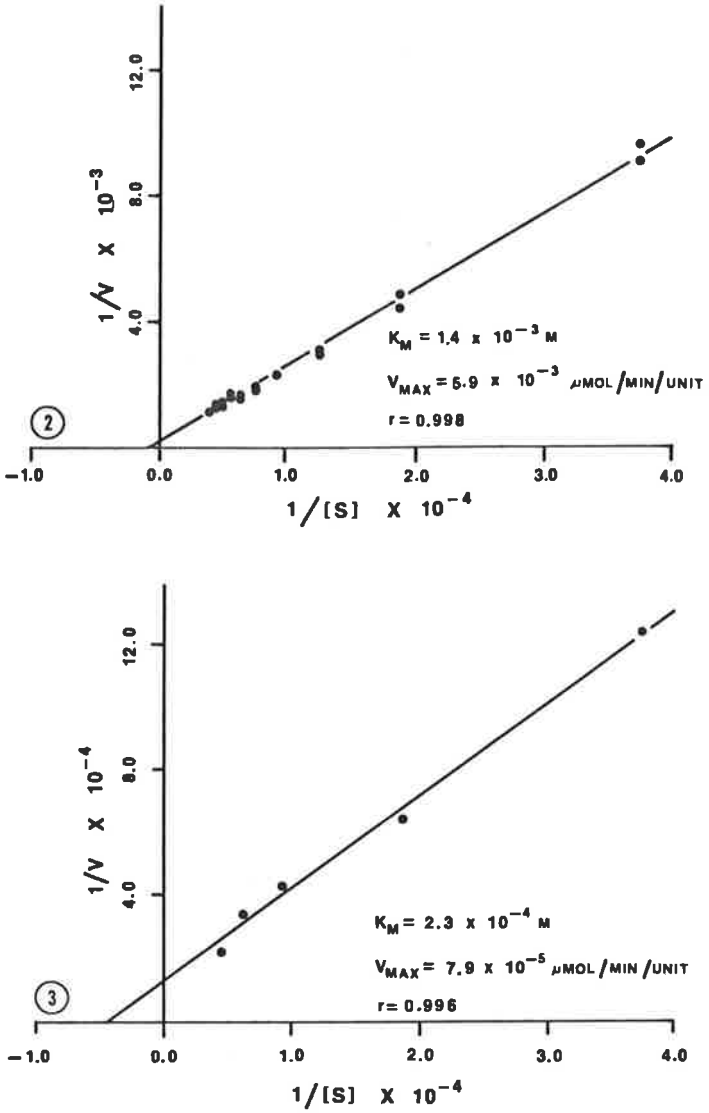




FIGS. 1–3. Lineweaver–Burk plots of activity of adenosine (Fig. 1), reversed adenosine 1 (Fig. 2), and reversed adenosine acetal 2 (Fig. 3). Substrate concentration [S] is expressed as mol/liter and initial velocity (V) as μmol/min/unit of ADA. A unit is defined as an amount of enzyme that will catalyze the conversion to product of 1 μmol of substrate/min. Final concentrations of ADA in the reaction mixture were 9.40×10^{-3} U/ml for the adenosine analysis, 0.94 U/ml for the reversed adenosine analysis, and 18.8 U/ml for the reversed adenosine acetal analysis.

$\times 10^{-4}$ M) may be binding more effectively than **1** ($K_m = 1.4 \times 10^{-3}$ M). The observed differences in V_{max} may then be attributed to the catalytic effectiveness of the enzyme–substrate complex formed.

Results of the inhibition studies with compounds **1** and **2** were compared with known inhibitors of ADA such as nebularine **5** and its methanol photoadduct **6**, a "transition-state" analog (*35*). Both **5** and **6** were found to be strongly inhibitory in our studies, and **2** showed weak inhibitory activity. Compound **1** showed no measurable inhibitor activity (Table 4). At relatively high concentrations of **1** (> 10:1 of **1**:adenosine), the apparent rate shows an increase above the control value due to the substrate activity of **1**. The difference in inhibitor activity between **1** and **2** is consistent with the relative magnitudes of K_m for these compounds. For further comparison, we synthesized and examined the reversed analog of nebularine and its methanol photoadduct. Interestingly, compounds **3** and **4** do not appear to have any inhibitory effect.



FIGS. 2 AND 3

In summary, we suggest that although the β-hydroxyl group at C1′ may be contributing to the binding and activity of **1**, this contribution is not significant enough in **1** (or the other reversed analog) for very effective substitution of the functional role of the 5′-hydroxyl group in either substrate or inhibitor activity.

TABLE 4

INHIBITION STUDIES WITH ADENOSINE DEAMINASE[a]

Inhibitor	Inhibitor concn (M)	Initial velocity[b]
Control— No inhibitor added	—	0.369
1	3.24×10^{-5}	0.379
1	5.40×10^{-5}	0.370
2	1.13×10^{-4}	0.293
2	2.26×10^{-4}	0.132
3	1.25×10^{-4}	0.358
3	2.50×10^{-4}	0.348
4	1.13×10^{-4}	0.358
4	2.25×10^{-4}	0.365
5	1.25×10^{-4}	0.022
6	1.13×10^{-4}	—[c]

[a] ADA concn = 9.4 units/liter; adenosine concn = 1.04×10^{-5} M.

[b] μmol/min/unit of enzyme.

[c] No detectable substrate conversion.

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METHYL 5′-(6-AMINOPURIN-9-YL)-5′-DEOXY-β-D-RIBOFURANOSIDE 2′,3′-CYCLIC MONOPHOSPHATE: A NOVEL, BIOLOGICALLY ACTIVE, STRUCTURAL ANALOGUE OF CYCLIC AMP[1]

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SUMMARY

A novel structural analogue of cyclic AMP has been synthesized. This compound has been found to activate protein kinase from skeletal muscle (K_a 5.0 μM). It is virtually resistant to degradation by beef heart cAMP phosphodiesterase. It is an inhibitor of this enzyme with an $[I]_{50}$ of 47.0 μM. The proliferation of cancer cells (HT-29) is inhibited by this compound. It represents the first example of a 2',3'-cyclic nucleotide with marked biological activity.

INTRODUCTION

The ubiquitous compound, adenosine 3',5'-cyclic monophosphate (cAMP) plays an essential role in the regulation of some fundamental cellular processes (3). Protein phosphorylation by cAMP-dependent protein kinase (E.C. 2.7.1.37; ATP: protein phosphotransferase) is a major pathway by which cAMP influences cellular metabolism (4). Cyclic AMP activates protein kinase by dissociation of the inactive holoenzyme (R_2C_2) to yield free C subunits (which catalyze the transfer of γ-phosphate of ATP to certain proteins), and a cAMP regulatory subunit complex (5–10). At least two types of cAMP-dependent protein kinase exist in various mammalian tissues. These have been referred to as Type I and Type II according to their order of elution from DEAE-cellulose with increasing salt concentration.

Regulation of cyclic nucleotide levels in biological systems is complex, but two major factors controlling endogenous levels of cAMP appear to be the rate of synthesis and the rate of degradation. The hydrolytic degradation of cAMP is catalyzed by cyclic 3',5'-nucleotide phosphodiesterases (E.C. 3.1.4.17) (3,11). The propensity of the enzyme to exhibit multiple forms in many mammalian tissues has been repeatedly observed in many laboratories (11). The implications of the hydrolytic destruction of cAMP with respect to the synthesis of biologically useful compounds can be appreciated when one examines the actions

of a number of experimental drug substances such as theophylline, MIX, quazodine, papaverine and others which exert their effects through inhibition of destruction of endogenous cAMP.

Synthetic analogues of cAMP are useful agents for studying the biochemistry of cAMP and may prove effective in controlling physiological disorders caused by defects in the metabolism of the natural cyclic nucleotide. We wish to report on the synthesis and remarkable biological activity of methyl 5'-(6-aminopurin-9-yl)-5'-deoxy-β-D-ribofuranoside 2',3'-cyclic monophosphate, a novel, stable, structural analogue of cAMP. This synthetic cyclic nucleotide where the point of attachment of the adenine ring has been changed from the 1'-position to the 5'-position will be referred to as "reversed" cyclic-AMP or RcAMP.

MATERIALS AND METHODS

General

The ^{1}H, ^{13}C, and ^{31}P NMR spectra were recorded on JEOL FX90Q and Bruker HX-90E pulse Fourier transform spectrometers. All thin layer chromatographies on cellulose were conducted using Bakerflex Cellulose F plates (2.5 x 7.5 cm) and using ethanol:1M ammonium acetate (5:2) as the developing solvent. Ultraviolet absorption spectra, all static ultraviolet and visible absorbance measurements, and all dynamic spectrophotometric enzyme assays were accomplished using a Varian Associates Cary 219 recording UV-VIS spectrophotometer. All kinetic measurements were conducted, unless otherwise stated, at 25.0 ± 0.1°C. The UV absorbance of column effluents was monitored with a Pharmacia UV-2 UV monitor.

Adenosine deaminase (Type I from calf intestinal mucosa), adenine, ribose, 5'-AMP deaminase, *E. coli* alkaline phosphatase, theophylline, histone type IIA, Malachite Green (free base), DEAE Sephadex, and Tween 20 were purchased from Sigma Chemical Co., St. Louis, Mo. Beef heart 3',5'-cyclic AMP phosphodiesterase was purchased from Boehringer Mannheim, Fed. Republic of Germany. 3',5'-Cyclic AMP, 2',3'-cAMP, $N^6,O^{2'}$-dibutyryl cAMP were purchased from Aldrich Chemical Co., Milwaukee, Wi. [γ-^{32}P]ATP was purchased from New England Nuclear Co., Boston, Mass.

Synthesis

Methyl 5'-(6-aminopurin-9-yl)-5'-deoxy-β-D-ribofuranoside(1). This compound was prepared in four steps (13-16) from D-ribose. Crystallization of the lyophilized material from this preparation gave colorless prisms, m.p. 185-186° (lit. (12) 195° (dec.)); ^{1}H NMR δ TMS (0.1M, DMSO-d_6) 8.16, 8.09, (1H, 1H, s, s, H2, H8), 4.64 (1H, broad s, H1'), 4.26-3.39 (5H, m, H2', H3', H4', 2H5'), 3.22, (3H, s, OCH_3), 7.20 (2H, s, NH_2); ^{13}C NMR δ TMS (p-dioxane, δ 67.4 ppm, int. ref.) (0.1M, D_2O) 154.8 (C6), 151.6 (C2), 149.4 (C4), 144.0 (C8), 118.5 (C5), 109.1 (C1'), 80.6 (C4'), 74.9 (C2'), 72.3 (C3'), 56.3 (OCH_3), 46.3 (C5').

Anal. Calcd. for $C_{11}H_{15}N_5O_4 \cdot 1/2\ H_2O$: C, 45.52; H, 5.56; N, 24.13. Found: C, 45.56; H, 5.48; N, 24.32.

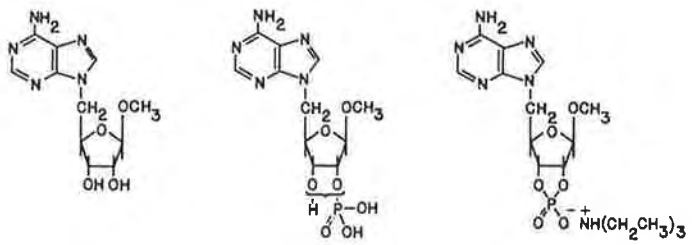
Methyl 5'-(6-aminopurin-9-yl)-5'-deoxy-β-D-ribofuranoside 2'(3')-monophosphate(2). The phosphorylation of 1 was conducted by an adaptation of the method of Saffhill (17) for the synthesis of 2'(3')-nucleoside monophosphates. The nucleoside (1, 281 mg, 1 mmol) and tri(tetramethylammonium)trimetaphosphate (4.59 g, 10 mmol) were dissolved in 1M aqueous NaOH (10 mL, 10 mmol), and the solution was allowed to stand at room temperature for four days. The solution was then passed through a column of activated charcoal (1.5 x 35 cm, 20-30 mesh) and the column was washed with 150 mL of water. Compound 3 and unreacted 1 were eluted from the column with 50% aqueous pyridine. Solvent was removed from the effluent *in vacuo* (bath temperature ~ 40°C). The residue was dissolved in 50 mL of water, and this solution was evaporated *in vacuo* and then redissolved in 3 mL H_2O. This solution was applied to a column of Dowex 1 (1.9 x 35 cm, $HCOO^-$ form). The column was washed with 500 mL H_2O, which recovered unreacted 1. Compound 2 was eluted with 500 mL of 0.5M HCOOH (aq). Solvent was removed from the effluent *in vacuo*. The residue was dissolved in H_2O and re-evaporated several times then dried under high vacuum over P_2O_5 to give 253 mg (67%) of the nucleotide free acid as a clear, glassy solid, homogeneous by thin layer chromatography on cellulose (R_f 0.32); UV $\lambda_{max}^{H_2O}$ = 260 nm (ε = 15,000 M^{-1} cm^{-1}); ^1H NMR δ TMS (TMS capillary, ext. ref.) (0.1M, D_2O) 9.00 (2H, broad s, H2, H8), 5.2-4.0 (6H, m, H1', H2', H3', H4', 2H5'), 3.87, 3.78 (1H, s, s, OCH_3); ^{13}C NMR δ TMS (p-dioxane, δ 67.4 ppm, int. ref.) (0.1M, D_2O) 150.6 (C6), 149.7 (C4), 146.3 (C2), 145.2 (C8), 118.6 (C5), 101.8, 100.7 (C1'), 80.2, 79.9, 79.4 (C2'), 75.6, 75.1 (C3'), 71.5, 71.3 (C4'), 56.5 (OCH_3), 46.5, 46.8 (C5'); ^{31}P NMR δ H_3PO_4 (85% H_3PO_4, concentric capillary, ext. ref.) (0.1M, H_2O) -0.04 (s).

Anal. Calcd. for $C_{11}H_{16}N_5O_7P \cdot H_2O$: C, 34.82; H, 4.75; N, 18.47. Found: C, 34.71; H, 4.36; N, 18.26.

Methyl 5'-(6-aminopurin-9-yl)-5'-deoxy-β-D-ribofuranoside 2',3'-cyclic monophosphate(3). Compound 3, the triethylammonium salt of the 2',3'-cyclic nucleotide, was synthesized from 1 *via* the trichloromethyl phosphonic ester. This compound was not isolated but was cyclized in the workup to yield the cyclic nucleotide (3). The conditions employed in the synthesis are similar to those used by Marumoto, *et al.* (18), for the production of nucleoside 2'(3')-trichloromethylphosphonates. Compound 1 (281 mg, 1 mmol) was dissolved in 21 mL dry DMF. This solution was cooled to 0°C, then trichloromethyl phosphonyl dichloride (19,20) (1.67 g, 4.52 mmol) was added, and the resulting solution kept at 4°C for 20 hr. Ice-cold distilled water (55 mL) was added and the resulting solution was neutralized with cold 5M NaOH. This solution was passed over a column of activated charcoal (1.4 x 35 cm, 20-50 mesh). The column was washed with 500 mL H_2O, and was eluted with 500 mL ethanol:H_2O:NH_4OH/9:10:1. The effluent was evaporated *in vacuo* (bath temperature 40°C). The residue was dissolved in 10 mL of 0.05M triethylammonium bicarbonate buffer, pH 8.1 (21), and applied to a DEAE Sephadex A-25-120 column (2.5 x 31 cm, HCO_3^- form) equilibrated with the same TEA bicarbonate buffer. Fractions were collected, and

the absorbance at 254 nm of the effluent was continuously monitored. Fractions corresponding to the cyclic nucleotide triethylammonium salt (3) peak were pooled, evaporated in vacuo (bath temperature 40°C), then dissolved in 10 mL H_2O, frozen and lyophilized to yield 236 mg (50% yield from 1) of 3 dihydrate as a clear, colorless beaded glass, mp 120-121°C; homogeneous by thin layer chromatography on cellulose (Rf 0.66); UV $\lambda_{max}^{H_2O}$ = 260 nm (ε = 14,990 M^{-1} cm^{-1}, for anhydrous 3); ^1H NMR δ TMS (TMS capillary, ext. ref.) (0.2M, D_2O), 8.57 (2H, broad s, H2, H8), 5.58 (1H, s, H1'), 5.49-4.75 (5H, m, H2', H3', H4', 2H5'), 3.75 (3H, s, OCH_3), 3.60 (6H, q, J = 7.32 Hz, $[NH(CH_2{*}CH_3)_3]^+$), 1.68 (9H, t, J = 7.32 Hz, $[NH(CH_2CH_3{*})_3]^+$); ^{13}C NMR δ TMS (p-dioxane, δ 67.4 ppm, int. ref.) (0.3M, D_2O) 155.3 (C6), 152.1 (C2), 149.3 (C4), 143.6 (C8), 110.3 (C5), 110.0 (C1'), 84.5 (C2'), 82.9 (C3'), 80.1 (C4'), 56.7 (OCH_3), 47.6 ($[NH(C{*}H_2CH_3)_3]^+$), 47.0 (C5'), 9.1 ($[NH(CH_2C{*}H_3)_3]^+$); ^{31}P NMR δ H_3PO_4 (50% H_3PO_4 in D_2O, concentric capillary, ext. ref.) (0.36M, H_2O) 19.7 (s).

Anal. Calcd. for $C_{17}H_{29}N_6O_6P \cdot 2H_2O$: C, 42,50; H, 6.92; N, 17.49. Found: C, 42.48; H, 6.42; N, 17.44.



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Enzymology

All 3',5'-cAMP phosphodiesterase (PDE) assays conducted in this work were coupled enzyme assays. The action of PDE on cAMP produces 5'-AMP which is then dephosphorylated by alkaline phosphatase to produce adenosine and inorganic phosphate. Experiments to determine the specific rates of hydrolysis of our new cyclic nucleotide and of adenosine 2',3'-cyclic monophosphate relied on determination of the free phosphate released as a result of consecutive action of PDE and of alkaline phosphatase. This method was also used to demonstrate that reaction of alkaline phosphatase with compound 2, the 2'(3')-phosphate monoester, resulted in complete dephosphorylation of this compound. The second assay method employed is a method of our own design and involves coupling to a third enzyme, adenosine deaminase. Adenosine deaminase (ADA) catalyzes the hydrolytic deamination of adenosine to produce inosine. This method was used for examining the substrate activity of cAMP toward PDE (K_m 17.8 µM), the inhibition by RcAMP of the hydrolysis of cAMP by PDE, and the inhibition by theophylline of the hydrolysis of cAMP by PDE.

In the PDE assays employing phosphate determinations to assess activity (23,24), the phosphate assay procedure of Itaya and Ui (25), as modified by Stull (26) was used, with modifications of our own. In the phosphate assays, 20 µL of a PDE solution (2 mg/mL) was pipetted into 0.98 mL of a solution containing 40 µmol TRIS, 25 µmol Mg acetate, 0.1 to 1.0 µmol cAMP or 1.0 µmol RcAMP or 1.0 µmol 2',3'-cAMP, and 32 µg alkaline phosphatase. The final pH was 7.5 (due to adjustment of the stock TRIS solution pH). The solutions were mixed while on ice. After mixing, 20 µL samples of each solution were removed and assayed for initial free phosphate content. The solutions were then placed into a 30°C water bath (with rapid swirling to avoid a possible lag due to slow attainment of thermal equilibrium). After incubation for 10 to 30 minutes, another 20 µL aliquot was removed from each solution and assayed for free phosphate. For phosphate determination, each 20 µL aliquot was added to a solution comprised of 60 µL 1M HCl, 20 µL 2M HCl, and 200 µL 0.2% Malachite Green, 2.7% ammonium molybdate in 1M HCl. The absorbance at 660 nm was determined relative to the absorbance of an appropriate reagent blank. The absorbance observed was compared to a standard curve made under identical conditions with precisely-known phosphate content. Activity was expressed as nanomoles of phosphate produced, normalized with respect to the amount of PDE present and with respect to time.

Assays by the ADA-coupled method were employed to generate the Lineweaver-Burk plot of cAMP and the RcAMP and theophylline inhibition data. In these assays, 20 µL (containing 0.01 unit) of a 1 mg/mL PDE solution is added to 0.98 mL of an otherwise complete reaction mixture containing 40 µmol TRIS, 25 µmol Mg acetate, appropriate amounts of cAMP and/or RcAMP and/or theophylline (see Figures for exact concentrations), 32 µg alkaline phosphatase, and 0.94 unit ADA. The solution was rapidly mixed, placed in the spectrophotometer in a 1 cm light path, 1 mL cell, and the absorbance at 265 nm recorded with respect to time for two minutes. No reaction was observed in the absence of PDE. The reaction rate was demonstrated to be linear for at least five minutes. To determine whether inosine, the final product of the coupled enzyme reaction sequence, had an inhibitory effect on any of the enzymatic reactions, the reaction rate at 92 µM cAMP in the presence of 10 µM inosine was determined. This cAMP concentration corresponds to the highest cAMP concentration used in the Lineweaver-Burk study. The actual rate of production of inosine at this cAMP concentration in the Lineweaver-Burk study is 3.46 µM min^{-1}. In observing the reaction for two minutes (as was done for every assay), the inosine concentration approaches 7.0 µM at the end of the observation. In the test assay with inosine added to an initial concentration of 7.0 µM, the rate observed is 90% that of the rate observed in the absence of added inosine. This information, combined with the fact that the observed reaction rate is linear for at least five minutes, indicates that errors due to feedback inhibition by inosine are probably not significant. In the PDE inhibition studies, it was found that doubling the concentrations of either alkaline phosphatase or adenosine deaminase in an assay in which the concentration of inhibitor was equal to its $[I]_{50}$ value had no effect on the observed rate of reaction. Thus, it can be inferred that neither theophylline nor RcAMP inhibit the alkaline phosphatase or ADA steps in the coupled PDE assay.

We also attempted to use the 5'-AMP deaminase assay of Dedman and Means (27a), and Drummond and Perrott-Yee (27b) with the exception that 5 µg rather than 5 mg of 5'-AMP deaminase per 1.0 mL assay volume was used. However, in our hands, this assay provided results that were not nearly as reproducible or as sensitive as those of our ADA assay.

Rabbit (New Zealand white) skeletal muscle protein kinase was purified through the first DEAE cellulose (Whatman DE-52) step (28). Our column elution profile was almost identical to that reported by Beavo, Bechtel, and Krebs (28). Peak I from this separation was used to obtain our data. Protein concentraton was determined to be 3.13 mg/mL. Kinase assays were carried out in a total volume of 0.1 mL which contained 50 mM sodium acetate (pH 6.1 ± 0.1), 5 mM sodium fluoride, 5 mM theophylline, 80 µg histone Type IIA, 5 mM $MgCl_2$, 100 µM [γ-^{32}P] ATP (20-60 cpm/picomole), 10 µL of rabbit skeletal muscle protein kinase, and appropriate amounts of cyclic AMP (or the synthetic cyclic nucleotide). The reactions were initiated by adding the enzyme, and allowed to proceed at 37°C for 5 minutes. The reactions were terminated by addition of 2.0 mL of ice-cold 20% trichloracetic acid. The measurement of [^{32}P]-incorporation into the protein substrate was made by separating the [^{32}P]-labeled TCA precipitated phosphoprotein by the Millipore filtration method and counting the radioactivity in 10 mL of toluene based scintillation fluid containing 0.375% (w/v) PPO and 0.01% (w/v) POPOP.

Tumor Antiproliferative Studies

The human colon adenocarcinoma (HT-29) cell line was kindly provided for this study by Dr. J. Fogh of the Memorial Sloan-Kettering Institute for Cancer Research, New York. The cells were grown in McCoy's 5a medium (Grand Island Biological Co., Grand Island, N.Y.) supplemented with 15% heat-inactivated fetal calf serum, penicillin (100 U/mL) and streptomycin (0.1 mg/mL) in a humidified 95% air - 5% CO_2 atmosphere. Single cell suspensions were prepared enzymatically from the culture with 0.25% trypsin in PBS at 37°C for 2-5 min and resuspended in the culture medium at the desired cell density. For the growth studies, these single cell suspensions (5-10 x 10^3 cells) were added to T-25 Falcon flasks (Falcon Plastics, Oxnard, Calif.). The control and treated cells were cultured concomitantly under identical conditions; treatment involved growing the cells in the presence of 0.1 mM cAMP, dibutyryl cAMP, or 0.01-0.1 mM RcAMP within the culture medium. These reagents were added 24 hr. following initial seeding of the culture flask. This allowed assessment of their effect on cellular proliferation and not cell attachment. Cultures were terminated by decantation of the medium and detached by the addition of 500 µL of saline containing 10 mM KCN. Cell enumeration was accomplished by an electronic cell counter (Coulter Electronics, Hialeah, Fla.). Mean percent change was derived from a minimum of 5 flasks per point for all groups, and reflected either stimulation (+%) or inhibition (-%) of cell proliferation.

RESULTS

The starting point for our synthesis was D-ribose, which was converted in several steps to compound 1 (13-16,29). Maintaining O-alkylation at the 1'-position was desirable from the viewpoint of both stability and biological activity. Phosphorylation of 1 was carried out with trichloromethyl phosphonyl chloride (18-20) to give the trichloromethyl phosphonic ester which cyclized readily to give the cyclic nucleotide 3. Compound 3 was obtained in high purity by anion exchange chromatography on DEAE Sephadex A-25-120 (HCO_3^- form). Complete structural confirmation was provided by ^1H, ^{13}C, and ^{31}P NMR spectroscopy and UV spectroscopy. The UV spectrum provided confirmation for 9-substitution [λ_{max} 260 nm (ε 14,990 corrected for anhydrous compound)], and the ^{31}P NMR data in D_2O showed it to be a 2',3'-cyclic monophosphate (δ 19.7 ppm downfield from H_3PO_4). The ^1H noise-decoupled ^{13}C NMR spectrum in D_2O showed the presence of only one compound with resonances consistent with the structure and substitution pattern depicted in 3. The ^1H NMR spectrum showed the proton at C-1' as an apparent singlet ($J_{1',2'}$ < 0.5 Hz) indicating a β-stereochemistry for the methyl acetal.

Protein kinase (Type I) from rabbit skeletal muscle was purified through the first DEAE cellulose step. Activation studies with the kinase were carried out by determining radiochemically the phosphorylation of histone Type IIA with [γ-^{32}P] ATP. The results are presented graphically (Fig. 1). Each point represents the average of three determinations. The concentrations of cyclic nucleotide needed to give half-maximal activation of protein kinase under the described assay conditions (referred to as K_a) were determined by visual inspection of the dose-response curves obtained. This method was used because of the non-linearity of plots in other methods for determining this parameter (30,31). The apparent K_a of 20 nM obtained for cAMP is close to values previously reported (32). The synthetic cyclic nucleotide is clearly capable of activating the protein kinase (apparent K_a 5.0 μM) and the V_{max} obtained experimentally is 0.88 relative to cAMP.

Phosphodiesterase assays were performed by coupled enzyme methods. Determination of the rate of hydrolysis of the cyclic nucleotide 3 relied on determination of the free phosphate produced as a result of the consecutive action of PDE and alkaline phosphatase. The results are shown in Table 1. A second assay procedure which involved coupling to a third enzyme, adenosine deaminase, was also used in these studies. The reaction rates were followed spectrophotometrically at 265 nm, which corresponds to the wavelength of maximum difference between the absorbance of adenosine and that of inosine, the product of the ADA conversion of adenosine. This method was used for inhibition studies with the PDE. The results are shown in Fig. 2.

The synthetic cyclic nucleotide 3 was also evaluated for anti-tumor activity with HT-29 cells. The data for this are summarized in Table 2. These studies demonstrate that this novel cyclic nucleotide was capable of inhibiting *in vitro* proliferation of human adenocarcinoma cells by 19%. Under identical conditions 3',5'-cAMP was ineffective, while N^6,2'-O-dibutyryl-cAMP inhibited the proliferative activity by almost 30%, a value similar to that previously reported by others (33).

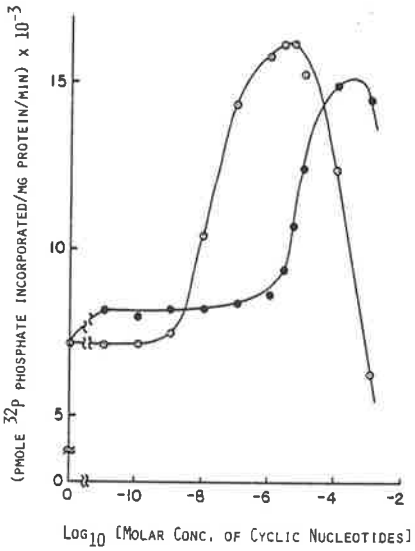

Figure 1. Activation of rabbit skeletal muscle protein kinase (Type I) by cAMP (⊙—⊙) and RcAMP (●—●). The value at the Y-axis intercept represents the control value containing no exogenous cyclic nucleotide. K_a for cAMP = 0.02 μM; K_a for RcAMP = 5 μM.

Table 1

Relative Rates of Hydrolysis of Cyclic Nucleotides by 3',5'-cAMP Phosphodiesterase

Compound	Relative Rate (%)
Cyclic 3',5'-AMP[a]	100 (5)
Cyclic 2',3'-AMP[a]	~0.0 (5)
Cyclic Nucleotide 3[a]	2.7±0.5 (5)
N^6,2'-O-Dibutyryl-cAMP[34]	7
N^6-Butyryl-cAMP[34]	8
2'-O-Butyryl-cAMP[34]	81

a. The number of determinations are shown in parenthesis. Enzyme concentrations were adjusted to give observable rates. The rates were finally expressed as μMol/min/mg from which relative rate data (%) were obtained. Substrate concentrations were 100 μM.

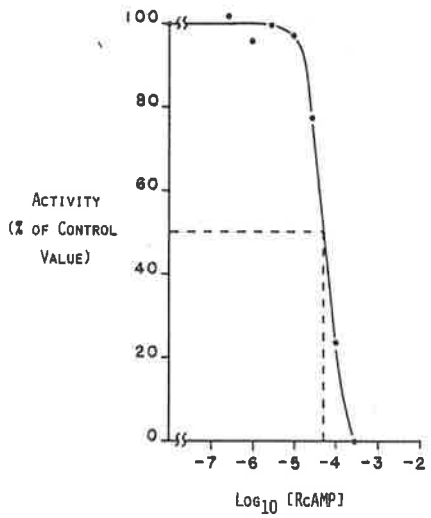

Figure 2. Inhibition by RcAMP of the beef heart high K_m PDE catalyzed hydrolysis of cAMP as determined by the ADA-coupled PDE assay. The cAMP concentration was 98.7 μM. $[I]_{50}$ for RcAMP = 47.0 μM.

Table 2

The Effect of cAMP and its Analogues on the Proliferation of HT-29 Cells

Compound	Concentration	Mean % Change[a]
Control	-	-
3',5'-cAMP	0.1 mM	+0.2
DB-3',5'-cAMP	0.1 mM	-30.0[b]
Cyclic Nucleotide 3	0.05 mM	-18.3
Cyclic Nucleotide 3	0.1 mM	-19.0[c]

a. Percent increase (+) or decrease (-) in cell population 8 days after addition of the specified agent.

b. $p < 0.05$

c. $p < 0.01$

DISCUSSION

The synthetic cyclic nucleotide 3 is the first example of a new, totally different class of cAMP analogues, which we have referred to as the "reversed" cyclic nucleotides. Its facile synthesis established in our laboratory resulted in a very pure final product isolated as the triethylammonium salt of the cyclic phosphate diester. Phosphorylation of the nucleoside β-methyl acetal (1), the last step in the synthetic pathway, represents a new method for the synthesis of 2',3'-cyclic nucleotides. The 2'(3')-trichloromethyl phosphonate is not isolated but spontaneously cyclizes under our work-up conditions to yield the cyclic phosphate diester.

It is remarkable that RcAMP displays activity with both protein kinase and with PDE. 2',3'-Cyclic AMP is ineffective in activating protein kinase (37) and is not hydrolyzed to any observable extent by beef heart high K_m PDE (see Table 1). The fact that RcAMP can interact with the allosteric cAMP-binding site of protein kinase and with the active site of PDE may be indicative of an important underlying similarity between cAMP and RcAMP. It can be seen, from a comparison of molecular models of cAMP and RcAMP, that conformations of these two molecules can be achieved in which the intramolecular spatial arrangement of the adenine moiety with respect to the phosphate group is such that a close superposition is possible. The similarity between the two compounds with respect to their adenine ring-to-phosphate distances may explain the ability of RcAMP to interact with protein kinase and with PDE.

The adenosine deaminase-coupled assay described is a new assay method for high K_m PDE. This is a dynamic assay and, as such, provides advantages over static assay methods such as the inorganic phosphate assay procedure (23-26). The validity of our results obtained by the ADA-coupled assay is supported by observation of a value for K_m (17.8 μM) for the PDE-catalyzed hydrolysis of cAMP which is in agreement with published values (11). An additional cross-check of this assay system was provided by examining inhibition of the PDE hydrolysis of cAMP with theophylline. Our $[I]_{50}$ value of 160 μM is close to values previously reported for theophylline (32,36). The $[I]_{50}$ of 47 μM obtained for RcAMP indicates that it is a potent inhibitor of the PDE especially when considered in light of the fact that the inhibition study was conducted at cAMP concentrations (100 μM) much higher than the K_m of the enzyme for cAMP.

In summary, this study describes the synthesis and some of the biological properties of a novel cyclic nucleotide. This analogue is the first example of a 2',3'-cyclic nucleotide capable of interacting with mammalian protein kinase and phosphodiesterase. The n-fold stimulation values of protein kinase by cAMP and by RcAMP are nearly identical. RcAMP is hydrolyzed to a very small extent by beef heart PDE and is capable of inhibiting the PDE-catalyzed hydrolysis of cAMP. The synthetic cyclic nucleotide is capable of inhibiting *in vitro* the proliferation of human tumor (HT-29) cells.

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SELECTIVE ALKYLATION REACTIONS WITH VINAMIDINIUM SALTS

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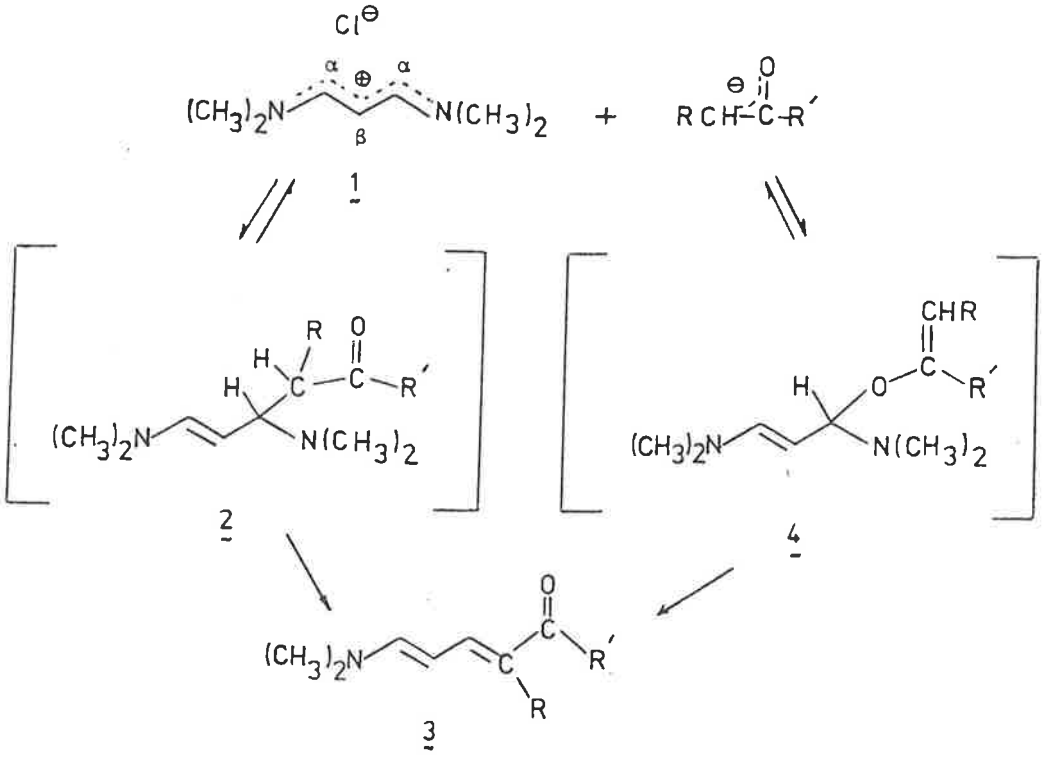
Summary: Vinamidinium salts react readily with enolates of ketones, esters, and lactones to produce multifunctional, synthetically-useful, dienaminones.

Selective alkylation of the carbon α to a carbonyl group with a variety of reagents is a major synthetic application of compounds containing activated methylene groups.[1] Vinamidinium salts,[2] vinylogs of amidinium compounds are possible alkylating agents. They normally exhibit regenerative character and are susceptible to substitution rather than addition reactions. The meneidic character of vinamidinium salts has been demonstrated in both electrophilic substitution reactions such as halogenation and nitrations, and in nucleophilic substitution reactions with amines.[3] Although reaction of vinamidinium salts with activated methylenes of nitriles has been reported,[4,5] there is only one report of the alkylation of other types of activated methylene compounds.[6] We wish to report on the utilization of vinamidinium salts in the alkylation of carbonyl enolates of cyclic and acyclic ketones, mono- and di- esters, keto esters, and lactones to produce multifunctional dienaminones 3.

In previous work with vinamidines, perchlorate salts were used.[7] We have found a convenient method for the preparation of the tetramethylvinamidinium chloride salt 1 in good yield by reaction of β-dimethylamino acrolein[8] with dimethylamine hydrochloride. The ^{13}C NMR spectrum of 1 (in $CDCl_3$) showed the resonance for the α-carbons at 164.2 ppm and the resonance for the β-carbon at 90.3 ppm depicting the expected alternation of electron density in this push-pull system. The single resonance observed for the $C_\alpha H$ and C_α in the ^1H and ^{13}C NMR spectra (in $CDCl_3$) and the vicinal proton coupling constant of 12.0 Hz are consistent with an all-trans or W conformation for 1.[9,10] The UV max (ethanol) at 309 nm for 1 provided a very convenient marker for monitoring its reactions. All substrates absorbed at wavelengths shorter than that of 1, and in the range 250-300 nm, and all the products absorbed at wavelengths longer than 1, in the range 350-420 nm. The actual absorbance of the dienaminones could be readily predicted by using the Woodward Rules for absorption of unsaturated carbonyl compounds.[11]

Reactions of the vinamidinium salt 1 were carried out with the enolates generated in situ by reaction of sodium hydride with the carbonyl compound in triethylamine or pyridine (Scheme 1). The results with several different classes of activated methylene containing compounds were a variety of dienaminone products (Table 1). Close inspection of the spectral data of the



SCHEME 1

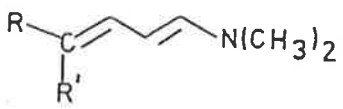
dienaminones revealed a number of common features which could also be used to identify new dienaminones. The ^{1}H NMR data (in $CDCl_3$) indicate that all of the dienaminones exist in the all-trans or W conformation (J ~ 12 Hz). The chemical shifts for the proton on the carbon β to the amino group were all similar with the resonance observed as a triplet at 4.87-6.41 ppm. The C_α proton characteristically appeared as a doublet at 6.32-7.33 ppm. Similar trends were found in the ^{13}C NMR data. Further, in the case of cyclopentanone (and other cyclic ketones), the structure was shown to be 9a and not its stereoisomer 9b by observation of a trans allylic coupling constant of 2.0 Hz between the C-3 protons and the exocyclic methylene proton.[12] The chemical shift of the exocyclic methylene proton at 7.08 ppm (in $CDCl_3$) is also consistent with 9a.[13,14]

When an unsymmetrical ketone such as 2-butanone was treated with 1, two products 8a and 8b were isolated by preparative gas chromatography in a 70:30 ratio, respectively. The predominance of 8a is consistent with the expected greater stability of primary versus secondary anions. This type of regioselectivity could be exploited in a synthetic scheme involving unsymmetrical compounds.

A plausible mechanism for formation of the dienaminones involves initial nucleophilic attack of the enolate on 1 to generate the σ-complex 2 which subsequently eliminates a molecule of dimethylamine with the assistance of a tertiary amine, such as triethylamine (Scheme 1). Elimination of amine from the σ-complex displaces the equilibrium and produces a new push-pull

Table 1: Method and Yield for the Preparation of the Dienaminones

Reaction of 1 With:	Method[18]	Reaction Conditions		Product	Yield (%)	MP (°C)
		Temp °C	(Time)			
Diethyl Malonate	A	Reflux	(10 hrs)	5	73	51-3
Ethyl Acetoacetate	B	25	(25 hrs)	7a,7b	57	88-90
Cyclopentanone	B	25	(26 hrs)	9a	88	88-91
Cyclohexanone	B	25	(30 hrs)	10	73	86-9
γ-Butyrolactone	B	25	(48 hrs)	11	91	99-102
dℓ-Camphor	C	Reflux	(40 hrs)	12	33	oil
3-Pentanone	C	25	(33 hrs)	6	66	oil
2-Butanone	B	25	(46 hrs)	8a,8b	78	oil
Estrone-3-Methyl Ether	B	25	(5 days)	13	56	209-11



5 R=R'=$CO_2CH_2CH_3$

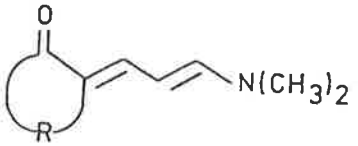
6 R=$COCH_2CH_3$ R'=CH_3

7a R=$COCH_3$ R'=$CO_2CH_2CH_3$

7b R=$CO_2CH_2CH_3$ R'=$COCH_3$

8a R=$COCH_2CH_3$ R'=H

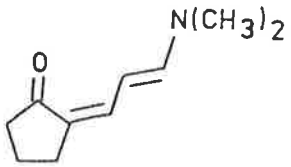
8b R=$COCH_3$ R'=CH_3



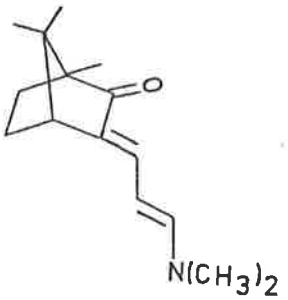
9a R=$(CH_2)_3$

10 R=$(CH_2)_4$

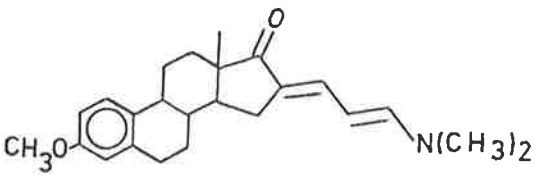
11 R=$O(CH_2)_2$



9b



12



13

system. O-Alkylation, a troublesome side reaction in many direct alkylations of activated methylenes,[15-17] is not important in these reactions because the σ-complex 4 if formed can undergo a Claisen rearrangement and $(CH_3)_2NH$ elimination to give 3.

The dienaminones (5-13) produced in these interesting transformations are of interest in themselves but also provide functionally substituted carbonyl compounds which are potentially useful intermediates in natural products synthesis. We are presently extending the scope of this work by examining the latter.

Acknowledgment: We thank the National Science Foundation for CHE 79-058865.

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Utility of Purinyl Radicals in the Synthesis of Base-Modified Nucleosides and Alkylpurines: 6-Amino Group Replacement by H, Cl, Br, and I[1]

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When 9-substituted adenines are treated with *n*-pentyl nitrite in hydrogen atom donating solvents and the resulting reaction mixtures are warmed and photolyzed with visible light, the corresponding 9-substituted purines are isolated. The conversion apparently involves homolysis of the intermediate 6-diazonium salts or azo compounds to produce purinyl radical intermediates. These purinyl radicals can subsequently abstract hydrogen atoms from solvent molecules. We have utilized our deamination procedure for the direct synthesis of the antitumor antibiotic nebularine from adenosine. When the deaminations of 9-substituted adenines are conducted in dry CCl_4, $CHBr_3$, or CH_2I_2, the corresponding 6-chloro-, 6-bromo-, and 6-iodopurines are isolated in good yields. There appears to be no detectable hydrogen abstraction in competition with halogen abstraction in the cases of $CHBr_3$ and CH_2I_2 solvents. These transformations provide shortened preparative pathways to intermediates useful in the synthesis of other base-modified purines. Under appropriate reaction conditions, conversions to the 6–6′ dimers also may be possible. The type of transformation in this report represents one of the first examples of the use of neutral purinyl radicals in nucleic acid chemistry.

Modified nucleosides and nucleic acid bases have been extensively investigated due to their potential activity as antibiotics, enzyme inhibitors, and antitumor agents. For this reason, improved and abbreviated syntheses of such materials or their precursors are of considerable interest.

Recently we have communicated a new and direct synthesis for the adenosine deaminase inhibitor and nucleoside antibiotic nebularine (**1a**),[2,3] from readily available adenosine (**2a**), via the intermediacy of purinyl radicals.[4] These previously unreported purinyl radicals were generated in an anhydrous diazotization/deamination procedure using *n*-pentyl nitrite as the nitrosating agent. We now supply complete details for the synthesis of nebularine and 9-ethylpurine and extend the work to demonstrate the general utility of purinyl radical intermediates in the synthesis of 6-chloronebularine triacetate (**3b**), and its bromo and iodo congeners **4b** and **5b**, respectively, from triacetyladenosine (**2b**). From 9-ethyladenine (**6**), in addition to the deaminated compound **7**, 6-halo-9-ethyl-

(1) Presented in part at the 178th National Meeting of the American Chemical Society, Washington, DC, Sept 13, 1979.

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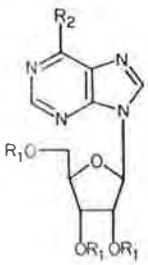
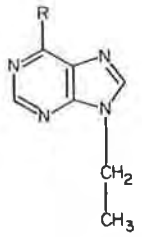
purines 8–10 have also been prepared.



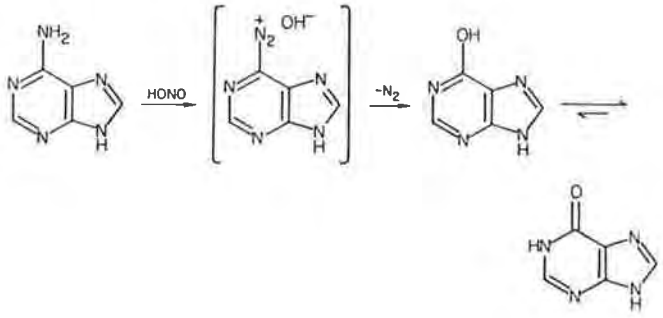


1a, R_1 = H; R_2 = H
1b, R_1 = $COCH_3$; R_2 = H
2a, R_1 = H; R_2 = NH_2
2b, R_1 = $COCH_3$; R_2 = NH_2
3a, R_1 = H; R_2 = Cl
3b, R_1 = $COCH_3$; R_2 = Cl
4a, R_1 = H; R_2 = Br
4b, R_1 = $COCH_3$; R_2 = Br
5a, R_1 = H; R_2 = I
5b, R_1 = $COCH_3$; R_2 = I

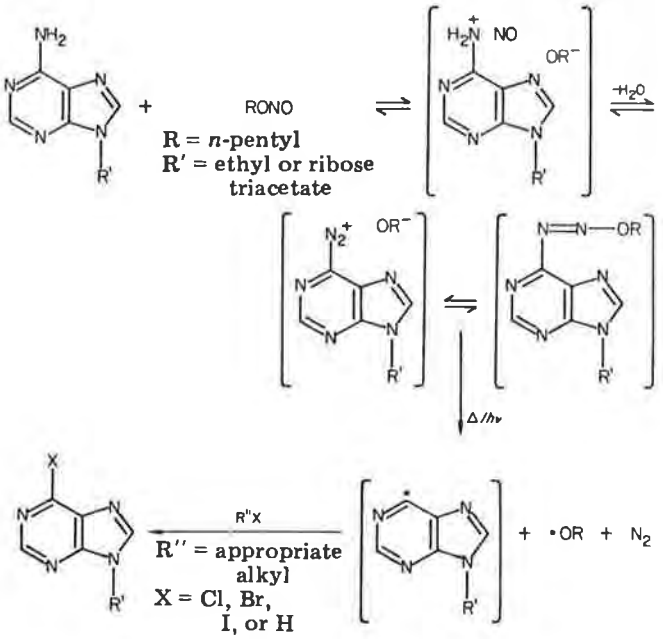
6, R = NH_2
7, R = H
8, R = Cl
9, R = Br
10, R = I

Older synthetic procedures for nebularine (**1a**) involved chloromercuripurinide fusion reactions with sugar derivatives such as chlorotriacetoribofuranose,[5] modified fusion reactions,[6] and longer syntheses involving catalytic hydrogenation of ring-halogenated materials[7] or of thioinosine.[8] Access to 9-alkylpurines from 9-alkyladenines or from other corresponding purines was previously limited. Such routes included alkylation of purine directly,[4] vinylation of purine followed by reduction,[9] reductive dehalogenation of appropriate halopurines,[10,11] or elaboration of 4,5-diaminopyrimidines.[11] Similarly, utilization of inconvenient starting materials or the use of comparatively severe reaction conditions was required for preparation of 6-chloro- and other 6-halo-9-alkylpurines or 6-halonucleosides. The halopurines are key intermediates in the synthesis of alkyl-,[12] aryl-,[12] thio-,[13] thioalkyl-,[14] azido-,[15] seleno-,[16] and most other C-6 substituted[17] purines. The chloro derivatives possess activity as chemotherapeutic agents[18] and are useful as biochemical probes for enzyme-catalyzed reactions.[19] Most chloropurines and the corresponding nucleosides were previously accessible only from hypoxanthine precursors through reaction with phosphorus halides, via sulfopurines by reaction with thionyl chloride, or from the corresponding chloropyrimidines.[20–22]

Scheme I



Scheme II



Results and Discussion

Although reductive deamination of aromatic amines with replacement by hydrogen of the amino group has been accomplished for many systems,[23] such diazotization/deamination reportedly fails under a variety of conditions for 6-aminopurine (adenine) derivatives.[15,24,25] While 6-diazonium salts of purine have not been isolated,[26] conversion of adenine to hypoxanthine in nitrous acid (Scheme I)[27] and of 6-aminopurine derivatives to the corresponding 6-fluoropurines[28,29] are presumptive of diazonium intermediates where the counterion acts as a nucleophile in an

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Table I. ^{13}C NMR Data for Substituted Purines

no.	compd	solvent	chemical shifts (δ) from Me_4Si C2	C4	C5	C6	C8	9-substituent carbons
1a	nebularine	D_2O	152.6	151.2	134.4	148.7	146.6	89.2, 86.4, 74.6, 71.2, 62.2
1b	2′,3′,5′-triacetylnebularine	$CDCl_3$	152.6	151.0	134.5	148.8	144.0	170.3, 169.6, 169.4, 86.5, 80.4, 73.0, 70.5, 63.0, 20.7, 20.5, 20.3
3b	2′,3′,5′-triacetyl-6-chloronebularine	$CDCl_3$	152.2	151.3	132.2	149.0	144.2	170.3, 169.6, 169.4, 86.9, 80.5, 73.1, 70.5, 63.0, 20.7, 20.5, 20.4
4b	2′,3′,5′-triacetyl-6-bromonebularine	$CDCl_3$	152.0	150.1	134.8	144.2	143.2	170.3, 169.6, 169.4, 86.9, 80.4, 73.1, 70.5, 63.0, 20.7, 20.5, 20.3
5b	2′,3′,5′-triacetyl-6-iodonebularine	$CDCl_3$	152.0	147.5	122.4	144.2	139.1	170.2, 169.6, 169.4, 86.9, 80.4, 73.0, 70.4, 62.9, 20.7, 20.5, 20.4
7	9-ethylpurine	$CDCl_3$	152.5	149.9	134.2	148.6	144.8	38.9, 15.3
8	6-chloro-9-ethylpurine	$CDCl_3$	151.9	151.0	131.7	149.2	144.7	39.6, 15.3
9	6-bromo-9-ethylpurine	$CDCl_3$	151.7	150.5	134.2	144.8	143.0	39.7, 15.3
10	6-iodo-9-ethylpurine	$CDCl_3$	151.8	148.0	122.1	144.2	138.7	39.6, 15.3

Table II. ^1H NMR Data for Substituted Purines

no.	compd	solvent	chemical shifts (δ) from Me_4Si H2	H6	H8	9-substituent protons
1a	nebularine	D_2O	8.63	8.97	8.81	6.1 (d), 4.1–4.9 (m), 3.85 (d)
1b	2′,3′,5′-triacetylnebularine	$CDCl_3$	8.40	9.18	9.01	6.33 (d), 6.03 (t), 5.73 (t), 4.45 (s), 2.17 (s), 2.11 (s), 2.09 (s)
3b	2′,3′,5′-triacetyl-6-chloronebularine	$CDCl_3$	8.53		8.79	6.33 (d), 6.01 (t), 5.71 (t), 4.46 (s), 2.18 (s), 2.12 (s), 2.11 (s)
4b	2′,3′,5′-triacetyl-6-bromonebularine	$CDCl_3$	8.51		8.74	6.35 (d), 6.01 (t), 5.70 (t), 4.47 (s), 2.17 (s), 2.12 (s), 2.10 (s)
5b	2′,3′,5′-triacetyl-6-iodonebularine	$CDCl_3$	8.46		8.79	6.29 (d), 5.98 (t), 5.67 (t), 4.45 (s), 2.17 (s), 2.12 (s), 2.10 (s)
7	9-ethylpurine	D_2O	8.29	8.77	8.63	4.25 (q), 1.45 (t)
8	6-chloro-9-ethylpurine	$CDCl_3$	8.17		8.76	4.38 (q), 1.60 (t)
9	6-bromo-9-ethylpurine	$CDCl_3$	8.30		8.70	4.43 (q), 1.63 (t)
10	6-iodo-9-ethylpurine	$CDCl_3$	8.24		8.63	4.39 (q), 1.61 (t)

ionic process. Interestingly, 8-diazonium salts of purines prepared from 8-amino substituted purines have been isolated and easily undergo many of the usual reactions of diazonium salts.[30]

We have discovered that 9-substituted 6-aminopurines when diazotized in an appropriate anhydrous medium under suitable reaction conditions are converted to the corresponding 9-substituted purines (Table IV). The procedure employs the known ability of alkyl nitrites to produce aryl radicals from arylamines and the subsequent ability of those radicals to react with suitable donor molecules.[31,32] The latter can be solvent. Among possible hydrogen atom donating solvents, dry tetrahydrofuran proved superior to dioxane, 2-propanol, cyclohexene, xylene, and various solvent mixtures. 1,1-Di-*o*-xylylethane solvent[33] also performed adequately as a hydrogen atom donor. Anhydrous carbon tetrachloride served satisfactorily as a chlorine atom donating solvent. Chloroform and methylene chloride were not employed due to their relatively low boiling points and the probability of competition between H abstraction and Cl abstraction by the purinyl radicals. The bond energy for C–H of 96–99 kcal/mol is close to the C–Cl bond energy of 79 kcal/mol.[35] Bromoform and diiodomethane proved to be satisfactory sources of bromine and iodine, respectively, for these 6-halopurine preparations (Scheme II). The energies of C–Br and C–I bonds, 66 and 52 kcal/mol,[35] respectively, are apparently sufficiently less than that of the C–H bond to avoid detectable competition of hydrogen with halogen abstraction. The apparent relative rates of reaction follow the order iodo > bromo > chloro for these three halomethanes.

In general, for adenine derivatives with appreciable solubility in the solvent, a solution containing a minimum of solvent was added dropwise to a warm, stirred mixture of solvent and dry distilled *n*-pentyl nitrite, under nitrogen in a vessel equipped with a bubbler. Sparingly soluble adenine derivatives were combined under nitrogen with solvent and *n*-pentyl nitrite at room temperature and warmed in the apparatus while the suspension was stirred. In all cases, constant illumination was supplied by a 200-W unfrosted tungsten lamp supported an inch from the reaction flask. Upon completion of the reaction (which may be monitored by gas chromatography or thin-layer chromatography of small aliquots), the solvent and unreacted pentyl nitrite were removed on a rotary evaporator. The resulting material was dissolved in 1:9 methanol–dichloromethane, dried (Na_2SO_4), and separated on silica gel

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Table III. Electronic Spectra for Substituted Purines

no.	compd	solvent	λ_{max}, nm	$\epsilon \times 10^{-3}$
1b	2′,3′,5′-triacetyl-nebularine	MeOH	262.0	7.5
3b	2′,3′,5′-triacetyl-6-chloronebularine	MeOH	263.5	9.7
4b	2′,3′,5′-triacetyl-6-bromonebularine	MeOH	266.2	9.3
5b	2′,3′,5′-triacetyl-6-iodonebularine	MeOH	273.5	10.4
7	9-ethylpurine	H_2O	264.5	8.0
8	6-chloro-9-ethyl-purine	H_2O	265.5	9.4
9	6-bromo-9-ethyl-purine	H_2O	267.5	9.0
10	6-iodo-9-ethyl-purine	H_2O	276.5	10.8

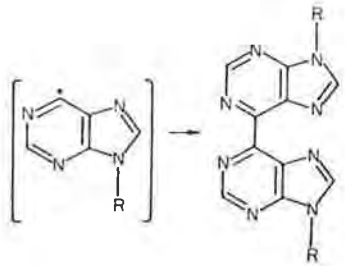
PF-254 plates. In the case of adenosine derivatives, the ribosyl hydroxyl groups were protected as the triacetate,[34] and this avoided possible formation of nitrite esters of the sugar in the subsequent diazotization reaction. The acetyl derivative gave enhancement of nucleoside solubility in organic solvents as well. After reaction, smooth convenient deprotection can be achieved by the methanolic ammonia method of Brown and Weliky.[5]

The structures of H-substituted products 1 and 7 were confirmed by ^{13}C, ^1H NMR, and UV spectrometric data comparison with authentic samples (see Tables I–III). Compound 7 was independently prepared from sodium purinide and ethyl tosylate in dimethylformamide.[36,37] Confirmation of the identities of the halogenated purines **3b**, **4b**, and **5b**, and **8**–**10** was obtained by ^{13}C and ^1H NMR spectroscopy and mass spectrometry (see Tables I–III).

9-Ethyl-6-chloropurine (8) obtained from our halogenation procedure was identical in all respects with authentic 8 prepared from 6-chloropurine and ethyl iodide in dimethyl sulfoxide.[10] Also, as radical processes are inhibited by the presence of scavengers such as O_2 and NO,[39] we found it desirable to purge the solvent with nitrogen and conduct the conversions under an atmosphere of nitrogen.

The mechanism of these transformations requires explanation. 6-Diazonium salts of purine may exist in equilibrium with the corresponding azo compounds (Scheme II). Both heat and certain frequencies of light are known to homolytically dissociate aryldiazonium salts and monoaryl azo compounds to yield aryl radicals.[40,41] The behavior of 6-diazonium salts and the corresponding azo compounds of purines may be analogous. Thus photolysis of our warmed reaction mixture would presumably result in the formation of purinyl radicals which could then abstract hydrogen atoms from tetrahydrofuran or halogen atoms from halocarbon solvents or undergo other radical reactions. Neutral purinyl radicals have not been reported previously although theoretical calculations on them have been undertaken.[38] Substantiating evidence for the intermediacy of purinyl radicals was provided by detection of hexachloroethane in the deamination reaction run in CCl_4. Hexachloroethane is the dimerization product of trichloromethyl radicals which are formed by the abstraction of a chlorine atom from CCl_4.[39]

Scheme III



11

Although dimerization of phenyl radicals or diazonium salts to form biphenyls is well-established,[42] no 6–6′ purine dimers have been reported. Under appropriate conditions, it may be possible to produce 6–6′ dimeric purines such as 11 (Scheme III).[43] Products corresponding to donation of the 1′ or 4′ hydrogen atoms of ribose have not been observed, although hydrogens at these sites correspond to the easily abstractable α-H atoms in tetrahydrofuran. Similarly, radical combination to produce 6-alkoxypurine was not observed.

The explanation for the failure of conventional reductive deamination methods with purines (e.g., $NaNO_2$ in aqueous acetic acid or HCl) presumably centers around attack of the intermediate diazonium compound by water, producing the hypoxanthine (Scheme I). Such aqueous deamination to form hypoxanthines has become a common method for preparing them. Although 1 equiv of water is produced under the conditions of our reactions here (Scheme II), no hypoxanthine products have been noted.[44] Apparently the rate of decomposition to form purinyl radicals by the diazonium or azo intermediate is much greater than the rate of attack by water upon the diazonium intermediate, or the extent of reaction of purinyl radical with the solvent is very much greater than the extent of reaction between the radical and water to generate hypoxanthine products.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Nuclear magnetic resonance spectra employing tetramethylsilane as an internal standard were recorded on a Bruker Model HX90E Pulse Fourier transform spectrometer and also on a Varian A-60 spectrometer. Low-resolution mass spectra were obtained on a Hitachi RMU-6 mass spectrometer. The ultraviolet spectra were obtained on a Varian Cary Model 219 spectrophotometer. Tetrahydrofuran was distilled over calcium hydride prior to use and stored over 4-Å molecular sieves under nitrogen. *n*-Pentyl nitrite[45] was dried over sodium sulfate, distilled, and stored at 10 °C over 4-Å molecular sieves. Carbon tetrachloride and bromoform were distilled prior to use. Diiodomethane was used without purification. Di-

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(42) Cohen, T.; LeWarchik, R. J.; Tarino, J. Z. *J. Am. Chem. Soc.* **1974**, *96*, 7753 and references therein.
(43) When 6-iodo-9-ethylpurine is heated and/or photolyzed, a crystalline compound, which is possibly the dimer 11, R = C_2H_5, is isolated.
(44) When 2′,3′,5′-tri-*O*-acetylcytidine is reacted under similar conditions to effect replacement of NH_2 by H, Cl, Br, and I, good yields of the corresponding uridine are obtained in all cases. In the cytidyl case, the pyrimidine base possesses little or no aromatic stability due to N-alkyl substitution. Reaction may proceed via an ionic mechanism where generated water serves as the nucleophile. Extension of the bromination reaction to aromatic 2-aminopyrimidine, however, results in isolation of 52.8% 2-bromopyrimidine.
(45) "Vogel's Textbook of Practical Organic Chemistry", 4th ed.; Furniss, B. S., et al., Eds.; Longman: London, 1978; p 409.

Table IV. Method and Yields in Deamination Reactions

no.	compd	RONO equiv	% yield	mp, °C
1b	2′,3′,5′-triacetylnebularine	20	45.8	oil
3b	2′,3′,5′-triacetyl-6-chloronebularine	2.24	65.5	oil
3b	2′,3′,5′-triacetyl-6-chloronebularine	20	71.8	
4b	2′,3′,5′-triacetyl-6-bromonebularine	2.24	55.8	oil
4b	2′,3′,5′-triacetyl-6-bromonebularine	20	72.7	
5b	2′,3′,5′-triacetyl-6-iodonebularine	20	68.6	oil
7	9-ethylpurine	method A	21	51–53
7	9-ethylpurine	20	68.2	
8	6-chloro-9-ethylpurine	2.24	68.1	79–80
8	6-chloro-9-ethylpurine	40	65.3	
9	6-bromo-9-ethylpurine	2.24	37.9	94
9	6-bromo-9-ethylpurine	20	56.8	
10	6-iodo-9-ethylpurine	2.24	23.7	142
10	6-iodo-9-ethylpurine	20	35.2	

methylformamide was dried over barium oxide (72 h), distilled, and stored over 4-Å molecular sieves. Starting purines and purine nucleosides were dried prior to use. Dixylylethane was provided courtesy of Gulf Oil Chemicals and used without purification. Preparative layer chromatography was carried out on EM silica gel PF_{254} plates.

9β-D-Ribofuranosylpurine (Nebularine, 1a). Under a nitrogen atmosphere, 0.393 g (1.0 mmol) of desiccated **2b**[34] in 20 mL of dry tetrahydrofuran was added over 20 min to a stirred refluxing solution of 2.7 mL (20 mmol) of *n*-pentyl nitrite in 20 mL of THF. Constant illumination during reflux was provided by an unfrosted 200-W tungsten lamp supported 2 in. from the apparatus. Gas evolution began. Reflux was continued for 96 h. The final reaction mixture was yellow-orange. Solvent was removed on a rotary evaporator. The residue was dissolved in 1:9 methanol–dichloromethane, dried (Na_2SO_4), and chromatographed on preparative silica gel plates with 1:19 ethanol–chloroform as developing solvent. The band at R_f 0.28 gave 0.173 g of 9β-(2′,3′,5′-tri-*O*-acetyl)-D-ribofuranosylpurine, **1b** (0.458 mmol, 45.8%), as a pale yellow oil:[6] ^{13}C NMR, see Table I; ^1H NMR (Me_4Si, $CDCl_3$) δ 2.09 (s, 1 H), 2.11 (s, 1 H), 2.17 (s, 1 H), 4.45 (s, 2 H), 5.73 (t, 1 H), 6.03 (t, 1 H), 6.33 (d, 1 H), 8.40 (s, 1 H), 9.01 (s, 1 H), 9.18 (s, 1 H). To 0.795 g (2.1 mmol) of triacetylnebularine (**1b**) was added saturated methanolic ammonia[5] at 0 °C to give, following recrystallization from methanol, 0.159 g (0.63 mmol, 31.5%) of **1a** as tan crystals: mp 178–179 °C [lit.[5] mp 181–182 °C]; ^{13}C NMR, see Table I; ^1H NMR δ Me_4Si (D_2O) 3.85 (d, 2 H), 4.1–4.9 (m, 3 H), 6.1 (d, 1 H), 8.63 (s, 1 H), 8.81 (s, 1 H), 8.97 (s, 1 H); UV, see Table III.

6-Amino-9-ethylpurine (9-Ethyladenine, 6). To a suspension of 5.07 g of adenine (37.5 mmol) in 80 mL of dimethylformamide was added 1.8 g (37.5 mmol) of a 50% dispersion of sodium hydride in mineral oil at room temperature with moisture protection. The reaction mixture was stirred for 30 min and the resulting white mass of sodium adeninide was warmed to 60 °C for an additional 30 min. A solution of 7.5 g (37.5 mmol) of ethyl *p*-toluenesulfonate[46] in 10 mL of DMF was added over 10 min at 25 °C to the sodium adeninide, and the mixture was stirred for an additional 10 min and warmed to 60 °C for 10 h. The solvent was removed on a rotary evaporator, and the residue was dissolved in 100 mL of 1:9 methanol–dichloromethane. Insoluble sodium *p*-toluenesulfonate was filtered off. After removal of solvent from the filtrate, recrystallization of the portion soluble in hot 2-butanone gave 2.45 g (15 mmol, 40%) of **6** as a fine white powder: mp 192–193 °C [lit.[11] mp 194–195 °C]; ^{13}C NMR (Me_4Si, $CDCl_3$) δ 15.5, 38.9, 120.1, 139.9, 150.3, 153.1, 155.8; ^1H NMR (Me_4Si, Me_2SO-d_6) δ 1.40 (t, 3 H), 4.20 (q, 2 H), 7.05–7.3 (br s, 2 H), 8.18 (s, 2 H); UV (H_2O) λ_{max} 262 nm (ε 1.3 × 10^4).

Anal. Calcd for $C_7H_9N_5$: C, 51.5; H, 5.5; N, 42.9. Found: C, 50.9; H, 5.5; N, 43.1.

9-Ethylpurine (7). Method A. From Purine. To 0.72 g (6.0 mmol) of purine dissolved in 15 mL of dimethylformamide was added with stirring under moisture protection 0.288 g (6.0 mmol) of a 50% dispersion of sodium hydride in mineral oil. After gas evolution ceased, the solution was heated to 50 °C for 45 min. After the solution was cooled to 0–5 °C, a solution containing 1.2 g (6.0 mmol) of ethyl *p*-toluenesulfonate[46] in 5 mL of dimethylformamide was added rapidly. The reaction vessel was allowed to warm to 25 °C and stirred for 10 h. Solvent was removed (<35 °C) at reduced pressure and sodium *p*-toluenesulfonate was removed as for **6**. Preparative layer chromatography on silica gel using 1:9 methanol–dichloromethane for development gave 0.186 g (1.26 mmol, 21%) of **7** (mp 44–48 °C). Sublimation at 60 °C under aspirator vacuum yielded a flocculent white sublimate of **7**, mp 51–53 °C [lit.[11] mp 53–56 °C].

Method B. From 6. A solution of 0.163 g (1.0 mmol) of **6** was dissolved in 30 mL of tetrahydrofuran and added slowly to a refluxing solution of 2.7 mL (20 mmol) of *n*-pentyl nitrite in 30 mL of tetrahydrofuran, as in **1b**. After 5 h, the reaction was worked up as before and chromatographed on silica gel, using 1:9 methanol–dichloromethane. Upon elution the band at R_f 0.26 yielded 0.101 g (0.682 mmol, 68.2%) of crude **7**, as a pale yellow material which was identical in all respects with that obtained by alkylation of purine in method A.

Method C. From 6. The procedure of method B was repeated with 20 mL of 1,1′-ethylidenebis(3,4-dimethylbenzene)[33] (dixylylethane) in place of tetrahydrofuran. After 1 h at 100 °C a solution formed. Heat and light were continued for an additional 18 h. The reaction mixture was placed on a column consisting of 60–200 mesh silica gel and flushed with hexane (100 mL). The column was then eluted with 1:9 methanol–methylene chloride. Preparative layer chromatography of the evaporated eluant as before gave 0.056 g (0.375 mmol, 37.5%) of **7**, identical in all respects with the product from method B: ^{13}C NMR, see Table I; ^1H NMR, see Table II; UV, see Table III; mass spectrum, *m/z* 148 (M^+), 133, 120, 106, 93, 66.

Anal. Calcd for $C_7H_8N_4$: C, 56.8; H, 5.4; N, 37.8. Found: C, 56.6; H, 5.4; N, 38.2.

6-Chloro-9-ethylpurine (8). Method A. From 6-Chloropurine. 6-Chloropurine, 1.08 g (7.0 mmol), was converted by the method of Montgomery and Temple[10] in dimethyl sulfoxide with potassium carbonate and ethyl iodide to give **8** as an oil which solidified upon chilling. The solid product was recrystallized once from Skellysolve C to give a fluffy white solid, 0.55 g (3.01 mmol, 43%), mp 78–80 °C [lit.[10] mp 81–84 °C].

Method B. From 6. A mixture of 0.163 g (1.0 mmol) of **6**, 40 mL of carbon tetrachloride, and 0.3 mL (2.24 mmol) of *n*-pentyl nitrite was reacted as for **3b**, except that the system here was heated to reflux temperature (see Table IV). After 12 h, the brown reaction mixture was worked up as before and chromatographed on silica gel, using 1:9 methanol–dichloromethane. From the band at R_f 0.53 was obtained, after elution, 0.124 g (0.681 mmol, 68.1%) of **8**, which was recrystallized from Skellysolve C to give a fluffy white solid identical in every respect with **8** isolated from method A: mp 78–80 °C, which showed no depression upon mixture with material from method A; ^{13}C NMR, see Table I; ^1H NMR see Table II; UV, see Table III; mass spectrum, *m/z* 184 ($^{37}ClM^+$), 182 ($^{35}ClM^+$), 156, 154, 129, 127, 119, 92.

Anal. Calcd for $C_7H_7N_4Cl$: C, 46.0; H, 3.8; N, 30.7. Found: C, 45.6; H, 3.9; N, 29.3.

6-Bromo-9-ethylpurine (9). To 0.163 g (1.0 mmol) of **6** were added 2.7 mL (20 mmol) of *n*-pentyl nitrite and 10 mL of bromoform. The resulting suspension was reacted as for **3b**, except

(46) Tipson, R. S. *J. Org. Chem.* **1944**, *9*, 235. Purification was facilitated by washing a CH_2Cl_2 solution of crude product through a 2-in. barrel of 80–200 mesh silica gel and subsequently removing the solvent.

that the mixture was slowly warmed from 60 to 120 °C. After 3 h the reaction was worked up as before. Development of preparative layer plates with 1:9 methanol–dichloromethane gave a trace of starting **6** and 0.129 g (0.568 mmol, 56.8%) of **9**, which was recrystallized from 10 mL of Skellysolve C to give white scales of **9**: mp 93–95 °C; ^{13}C NMR, see Table I; ^{1}H NMR, see Table II; UV, see Table III; mass spectrum, m/z 228 (^{81}BrM$^+$), 226 (^{79}BrM$^+$), 199, 197, 147, 119, 92, 65.

Anal. Calcd for $C_7H_7N_4Br$: C, 37.0; H, 3.1; N, 24.7. Found: C, 37.4; H, 3.2; N, 24.6.

6-Iodo-9-ethylpurine (10). A mixture of 0.163 g (1.0 mmol) of **6** and 2.7 mL (20 mmol) of *n*-pentyl nitrite was treated with 5 mL of diiodomethane as in **5b** and warmed to 80 °C. After 10 h, the reaction was worked up and treated to remove iodine color as before. Preparative layer chromatography on silica gel (1:9 methanol–methylene chloride for development) gave at R_f 0.49 0.0964 g (0.345 mmol, 34.5%) of **10**, which was recrystallized from Skellysolve C to give a dense pale yellow powder: mp 141–143 °C; ^{13}C NMR, see Table I; ^{1}H NMR, see Table II; UV, see Table III; mass spectrum, m/z 274 (M$^+$), 147, 119, 92, 65.

Anal. Calcd for $C_7H_7N_4I$: C, 30.7; H, 2.6; N, 20.5. Found: C, 31.5; H, 2.7; N, 20.0.

6-Chloro-9β-(2′,3′,5′-tri-*O*-acetyl)-D-ribofuranosylpurine (3b). Under a nitrogen atmosphere, 0.393 g (1.0 mmol) of dry **2b** was added to a solution of 0.3 mL (2.24 mmol) of *n*-pentyl nitrite in 40 mL of carbon tetrachloride. The suspension was stirred and illuminated as for **1b** and warmed to 80 °C. Reaction was discontinued after 23 h and the red-brown mixture, worked up as for **1b**, gave, after development of preparative layer plates with 1:9 methanol-methylene chloride, 0.270 g of **3b** (0.656 mmol, 65.5%) as a yellow oil (R_f 0.58). Recovery of 0.093 g (0.236 mmol, 23.6%) of **2b** was made. Similar reaction using 2.7 mL (20 mmol) of *n*-pentyl nitrite gave a trace of starting compound **2b** (R_f 0.27) and 0.296 g (0.718 mmol, 71.8%) of **3b**: ^{13}C NMR, see Table I; ^{1}H NMR, see Table II; UV, see Table III.

Anal. Calcd for $C_{16}H_{17}N_4O_7Cl\cdot H_2O$: C, 44.6; H, 4.4; N, 13.0. Found: C, 43.9; H, 4.2; N, 11.9.

6-Bromo-9β-(2′,3′,5′-tri-*O*-acetyl)-D-ribofuranosylpurine (4b). A mixture of 0.393 g (1.0 mmol) of **2b**, 2.7 mL (20 mmol) of *n*-pentyl nitrite, and 15 mL of bromoform was reacted as for **1b**, with the solution maintained at 80 °C. The reaction mixture turned a golden color; gas evolution ceased and reaction was stopped after 3.5 h. After workup using 1:9 methanol–dichloromethane as developing solvent for silica gel preparative layer plates, 0.030 g (0.077 mmol, 7.7%) of **2b** was recovered (R_f 0.30) and 0.332 g (0.727 mmol, 72.7%) of **4b** was isolated as a pale yellow oil (R_f 0.59): ^{13}C NMR, see Table I; ^{1}H NMR, see Table II; UV, see Table III.

Anal. Calcd for $C_{16}H_{17}N_4O_7Br\cdot H_2O$: C, 40.4; H, 3.6; N, 11.8. Found: C, 40.6; H, 3.8; N, 11.6.

6-Iodo-9β-(2′,3′,5′-tri-*O*-acetyl)-D-ribofuranosylpurine (5b). A mixture of 0.393 g (1.0 mmol) of **2b** and 2.7 mL (20 mmol) of *n*-pentyl nitrite was stirred at 60 °C under nitrogen. Diiodomethane (5 mL) was added at once, under illumination as for **1b**, with stirring. After 2 h the red reaction mixture was cooled and worked up as before except that the methylene chloride solution of the residue was treated with saturated aqueous sodium sulfite solution to remove the free iodine before drying. Development with 1:9 methanol–methylene chloride gave a 0.056-g (0.143 mmol, 14.3%) recovery of **2b** (R_f 0.30) and 0.346 g (0.686 mmol, 68.6%) of **5b**, as a yellow oil which darkens upon standing (R_f 0.61): ^{13}C NMR, see Table I; ^{1}H NMR, see Table II; UV, see Table III; mass spectrum, m/z 504 (M$^+$).

Anal. Calcd for $C_{16}H_{17}N_4O_7I$: C, 38.1; H, 3.4; N, 11.1. Found: C, 38.3; H, 3.5; N, 11.0.

Registry No. 1a, 550-33-4; **1b**, 15981-63-2; **2b**, 7387-57-7; **3b**, 5987-73-5; **4b**, 74465-47-7; **5b**, 5987-74-6; **6**, 2715-68-6; **7**, 5427-23-6; **8**, 5462-86-2; **9**, 74465-48-8; **10**, 74465-49-9; adenine, 73-24-5; sodium adeninide, 40428-86-2; purine, 120-73-0; 6-chloropurine, 87-42-3.

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Carbohydrate Models of α-Methylene-γ-butyrolactones[1]

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The α-methylene-γ-butyrolactone moiety is a characteristic component of a large class of sesquiterpenes many of which possess marked cytotoxic, antitumor, and other biological activities. The activity of these lactones apparently derives from their chemical affinity for the thiol groups of sulfhydryl enzymes. Although the enone component is essential for biological activity, there are additional factors which may enhance these properties. These enhancement factors include the presence of hydroxyl groups in stereochemically strategic positions and the presence of various conjugated ester side chains. The built-in functionality of carbohydrates was utilized for the synthesis of such analogues. The target molecule was 2-deoxy-2-*C*-methylene-D-*threo*-pentono-1,4-lactone (**2**), the D-xylose analogue of α-methylene-γ-butyrolactone. Synthesis of **2** commenced with the protection of D-xylose at the 1, 3, and 5 positions to give methyl 3,5-*O*-isopropylidene-α-D-xylofuranoside (**3**). Compound **3** was oxidized with RuO_4 to the 2-keto sugar which was condensed with $NaCH_2NO_2$. Treatment of the resulting nitro alcohols with Ac_2O in Me_2SO followed by quantitative reduction with $NaBH_4$ gave the protected 2-deoxy-2-*C*-nitromethyl derivative of D-xylose. Removal of the protecting groups followed by oxidation with bromine in water–acetic acid and then treatment with $BaCO_3$ gave the target molecule **2** as evidenced by IR, ^1H NMR, and ^{13}C NMR data. The reaction of **2** was carried out with the model sulfhydryl compounds cysteine and glutathione. In each case the reaction was complete in less the 15 min and gave crystalline adducts quantitatively. In addition, these sulfhydryl compounds added stereospecifically, as evidenced by ^{13}C NMR data.

The potent cytotoxic action of many sesquiterpene plant products and their ability to inactivate certain selected enzymes have been attributed to the presence of the α-methylene-γ-butyrolactone moiety.[2-5] The activity of these compounds apparently derives from the extreme ease

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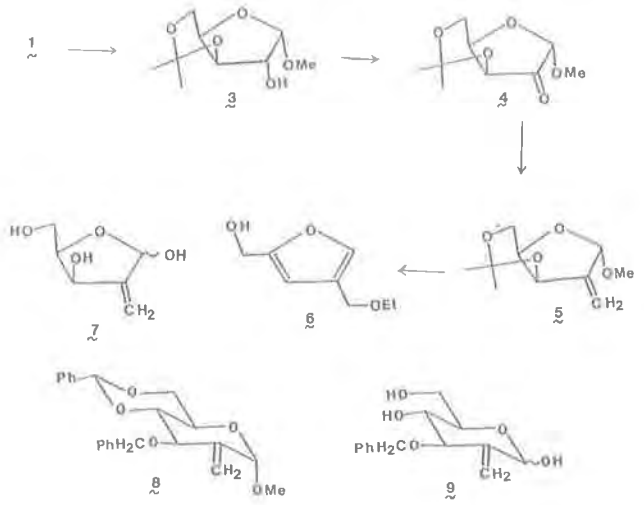
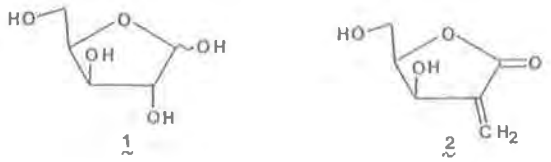
Scheme I



with which this functionality reacts with thiols.[3,6,7] Interest in α-methylene-γ-butyrolactones as medicinal agents has been stimulated by the possibility that some of these might show enough selective toxicity against neoplastic cells to be of therapeutic value as anticancer agents.[4,7-11] Although the presence of the α-methylene-γ-butyrolactone unit is essential for biological activity,[3,12] there are other factors which may enhance these properties.[7] These enhancement factors include the presence of hydroxyl groups in stereochemically strategic positions and the presence of various conjugated ester side chains. A conjugated ester moiety, if present in a sesquiterpene lactone, not only increases lipophilicity of the molecule but also may constitute an additional functionality reactive toward thiols.[3,4] Although their exact role is not clear, the presence of hydroxyl groups adjacent to the α-methylene group is a common feature among a number of sesquiterpene lactones showing in vivo antitumor activity.[3,4,7] Presence of such hydroxyl groups apparently increases the rate of cysteine addition to these molecules.[3,12] It has been suggested that the hydroxyl groups might be involved in direct binding of these compounds to receptor sites in tumor cells.[7,13] It should be mentioned, however, that the biological activity of α-methylene-γ-butyrolactones is not confined to the complex polyfunctional sesquiterpene lactones only. For example, it has been shown that synthetic α-methylene-γ-butyrolactone derivatives containing no other reactive functionality can in some instances have growth-inhibitory activity comparable to that of multifunctional natural products.[14,15]

Although intense interest has been shown in the synthesis of α-methylene-γ-butyrolactone derivatives,[16-18] no study involving the effects of the enhancement factors on synthetic models has been made. As α-methylene-γ-butyrolactones, either synthetic or natural, are expected to have some biological properties,[7] a rational approach toward the development of new SH alkylating antitumor agents is the synthesis of model compounds which incorporate both active centers and enhancement factors. Compounds which by virtue of their built-in functionality appear to be ideal starting materials for the synthesis of such model compounds are the carbohydrates. For example, the α-methylene-γ-butyrolactone derivative that is obtainable by the minimum structural alteration of D-xylose (1) (shown in the furanose form) is 2. Either of the



remaining hydroxyl groups of 2 can be suitably esterified to give model compounds incorporating the active center as well as enhancement factors. In this report we wish to describe the synthesis of the D-xylose model of α-methylene-γ-butyrolactone and to discuss its reactions with model biological thiols.

Results and Discussion

In terms of functional group transformation, conversion of 1 to 2 involves oxidation of a lactol to a lactone and replacement of a secondary hydroxyl group by an exocyclic methylene group. A reaction sequence that appears to be relatively simple involves introduction of the methylene group by the Wittig reaction of a suitably protected 2-keto sugar followed by deprotection and oxidation at C-1. Another reasonable sequence involves generation of the exocyclic methylene group in the last step of the synthesis from a suitable branched-chain lactone. In either pathway, a 2-keto sugar is the key intermediate.

Synthesis of the 2-keto sugar necessitates prior protection of the other hydroxyl groups of D-xylose. This was done by reacting D-xylose with methanolic hydrogen chloride followed by treatment with acetone/*p*-TsOH·H_2O/$CuSO_4$ to give methyl 3,5-*O*-isopropylidene-α,β-D-xylofuranosides.[19] The mixture was separated by fractional distillation to give α-(3) and the β isomer in 33 and 21% overall yield, respectively. The structure of 3 and its β isomer was confirmed by ^1H NMR data. In the α isomer (3), H-1 occurred as a doublet with coupling constant $J_{1,2}$ = 4.0 Hz, characteristic of vicinal cis coupling in furanose rings, while H-1 of the β isomer, where H-1 and H-2 are trans, occurred as expected as a singlet.[20] Although either isomer could be used in the subsequent steps, only the α isomer was used here mainly because it was produced in higher yields. Oxidation of the xyloside 3 with RuO_2/KIO_4/K_2CO_3 in water–carbon tetrachloride proceeded smoothly to give the keto sugar 4 in 80% yield (Scheme I). The product obtained initially was a mixture of 4 and its hydrate. Pure ketone was obtained by azeotropic distillation of the water of hydration with benzene. Pure

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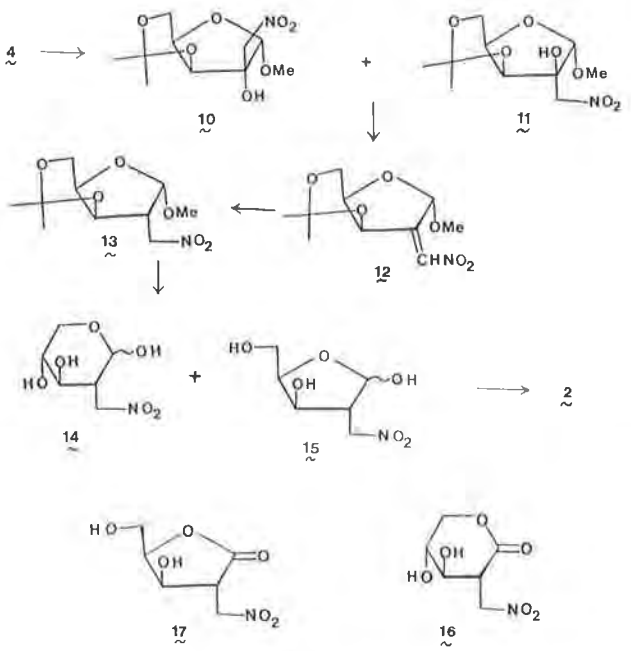
Scheme II



4 showed absorption in the infrared at 1790 cm^{-1}. As the keto sugar was unstable and darkened on storage, it was utilized immediately after its synthesis. Interestingly, dimethyl sulfoxide based reagents such as Me_2SO/Ac_2O and $Me_2SO/DCC/H_3PO_4$/pyridine were found to be unsatisfactory for this oxidation reaction.[31]

Treatment of the keto sugar **4** with methylenetriphenylphosphorane,[21] generated in situ, in toluene at 0 °C gave the unsaturated sugar **5** in 28% yield. The yields were lower in THF or ether or at higher temperatures. Removal of the protecting groups of **5**, both of which are acid labile, was then attempted. However, treatment of **5** in ethanol–water with dilute HCl led to the formation of the furan derivative **6**. The desired hydrolysis product **7** was not detected. This is in contrast to our earlier observation[22] that compound **8** can be hydrolyzed quantitatively in ethanol–water which is 0.1 N in HCl at 85 °C for 1 h to give the expected hydrolysis product **9**. It is apparent that had the 3-OH been protected by an acid-stable protecting group, formation of the furan derivative **6** could have been avoided. However, the nature of the Wittig reaction[21] and the Wittig product precluded the use of other common protecting groups, namely, those labile to hydrogenolysis or base.

Attention was then turned toward the reaction sequence involving generation of the exocyclic methylene group in the last step of the synthesis from a branched-chain lactone. The branched chain that appeared suitable for this purpose was a nitromethyl group. The problem then was the replacement of the 2α-OH of **3** by CH_2NO_2. The keto sugar **4** again served as the starting material. Condensation of **4** with $NaCH_2NO_2$ in excess CH_3NO_2 gave an isomeric mixture of nitro alcohols **10** and **11** in 83% yield (Scheme II). The ratio of xylo (**10**) to lyxo (**11**) was 70:30 as evidenced by ^1H NMR data. As the 2β-OH of **11** is cis to H-1, a shielding is expected in the chemical shift of H-1 of **11** compared to that of **10**. Thus the singlet at δ 4.98 in the NMR spectrum was assigned to the lyxo isomer (**11**) and the singlet at δ 5.20 to the xylo isomer (**10**). The nitro alcohols were dehydrated with Ac_2O/Me_2SO at 25 °C to give the nitro alkene **12**. The crude nitro alkene was reduced with $NaBH_4$ in ethanol–water at 0 °C for 2 h whereby the nitro alkane **13** was obtained in 81% yield as a syrup. Interestingly, this reduction is stereospecific, as shown by NMR studies.[31,32] The ^1H noise-decoupled PFT ^{13}C NMR spectrum of **13** showed the presence of a single compound with C-2 resonance at δ 49.6. Its ^1H NMR spectrum exhibited a coupling constant, $J_{1,2}$ = 5.0 Hz, consistent with the α stereochemistry of the nitromethyl group.[20]

The synthetic plan then involved removal of the protecting groups (of **13**), oxidation at C-1, and subsequent generation of the exocyclic methylene group at C-2. Removal of protecting groups was accomplished quantitatively by treating **13** with Dowex 50-W (H^+ form) in ethanol–water at 65 °C for 3 h. The syrupy product appeared to be a mixture consisting of the pyranose (**14**) and furanose (**15**) forms of 2-deoxy-2-*C*-(nitromethyl)-D-xylose as evidenced by ^{13}C NMR data. As expected, the pyranose forms **14** were predominant, and detailed ^{13}C NMR data analysis suggested that compounds **14** accounted for about 77% of the product mixture.[23] Oxidation of the free sugar (**14** + **15**) was carried out with bromine in 30% acetic acid for 24 h in the dark. After removal of excess bromine, the mixture was stirred with excess $BaCO_3$ for 2 h to neutralize the acids. Extractive workup gave not the expected nitromethyl lactones **16** and **17** but the target D-xylose model of α-methylene-γ-butyrolactone (**2**) directly in 52% yield. The structure of the α-methylene lactone **2** was confirmed by its IR, ^1H NMR, and ^{13}C NMR spectral data. The infrared spectrum showed the presence of a strong absorption at 1768 cm^{-1}, characteristic of γ-lactones.[24] The absorption due to a NO_2 group was absent, and the occurrence of two peaks at 915 and 1650 cm^{-1} suggested the presence of an exocyclic methylene group.[24] The ^{13}C NMR spectrum indicated the presence of only one compound with a characteristic C-4 resonance at δ 83.4 similar to that found in other γ-lactone structures.[23] In the ^1H NMR spectrum, each hydrogen of the exocyclic methylene group occurred as a doublet with J_{gem} = 2.0 Hz.

The direct formation of **2** can be explained by considering the mechanistic details of bromine oxidation and the stabilities of γ- and δ-aldonolactones toward acids and bases. In neutral and acidic media, bromine oxidizes aldoses directly to the corresponding aldonolactones and in the process 2 mol of HBr are produced for each mole of aldose oxidized.[25] This HBr probably catalyzed the equilibration of **16** and **17**, ultimately favoring the γ-lactone, **17**, because of its greater thermodynamic stability.[26] During the treatment with $BaCO_3$, the δ-lactone **16** (or the α-methylene δ-lactone derived from **16**) is removed from the solution as an insoluble barium salt because of its greater reactivity toward base.[27] Elimination of the elements of HNO_2 from **17** was possibly mediated by $BaCO_3$. This is reasonable as nitromethyl groups when positioned α to an ester or lactone carbonyl carbon undergo facile elimination reactions to give unsaturated esters or lactones.[28]

As mentioned earlier, the biological activity of α-methylene lactones apparently derives from their affinity for

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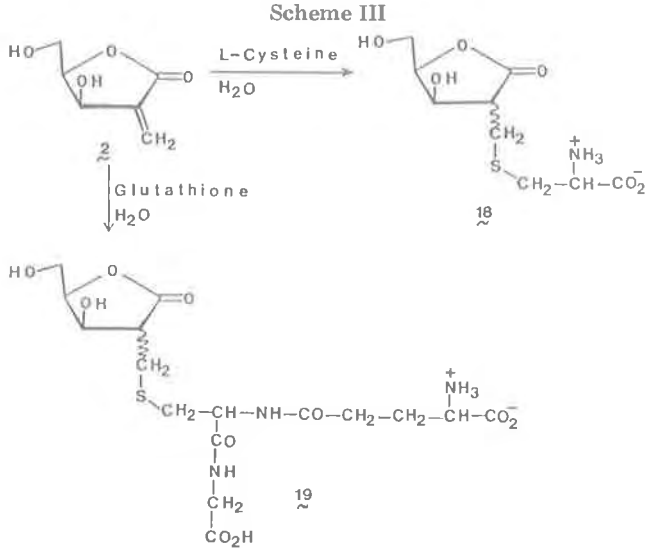
Scheme III



the thiol groups of sulfhydryl enzymes. Accordingly, biomimetic reactions of 2-deoxy-2-*C*-methylene-D-*threo*-pentono-1,4-lactone (**2**) with model sulfhydryl systems such as cysteine and glutathione were next attempted. In both cases, the reaction was quantitative and complete in less than 15 min in neutral aqueous medium to give the cysteine adduct **18** and the glutathione adduct **19**, respectively, as crystalline solids (Scheme III). The reactions were very conveniently monitored by ^{1}H NMR by observing the disappearance of the signals due to the exocyclic methylene group of **2**. The reaction time compares very favorably with that of some of the reactive naturally occurring α-methylene-γ-butyrolactones. For example, the reaction of eupatundin with cysteine at pH 7.4 was complete in 30 min, whereas reaction of helenalin with glutathione in water was complete in 4 h.[6,29] The assignment of peaks in the ^{1}H NMR spectra of the adducts was not possible due to considerable overlapping of signals. Thus it was not possible to deduce the stereochemistry of the adducts. However, ^{13}C NMR data indicated the formation of a single isomer in each case and that it was the SH group that had added to the C=CH_2 group of the α-methylene lactone **2**.

Since the reaction sequence developed for the synthesis of **2** appears to be of general applicability, this approach should lead to other carbohydrate analogues of α-methylene-γ-butyrolactones with various stereostructural characteristics. Synthesis of various esters of **2** and the biological evaluation of **2** and its esters are currently in progress.

Experimental Section

Methyl 3,5-*O*-Isopropylidene-α-D-xylofuranoside (3) and Its β Anomer. The crude syrup obtained in two steps from D-xylose by the method of Baker and co-workers[19] was distilled through a Vigreux column to give the α anomer **3** as a colorless syrup (33%): bp 83–88 °C (0.1 mm) [lit.[19] bp 85–88 °C (0.1 mm)]; $[\alpha]^{25}_D$ +17.6° (*c* 2, H_2O); ^{1}H NMR ($CDCl_3$) δ 1.35 (s, 3 H), 1.41 (s, 3 H), 3.28 (br s, OH), 3.52 (s, 3 H, OMe), 3.92–4.18 (m, 5 H), 5.15 (d, 1 H, $J_{1,2}$ = 4.0 Hz, H-1); ^{13}C NMR (Me_4Si, $CDCl_3$): δ 19.6, 28.5, 56.3, 60.5, 71.3, 75.5, 76.8, 97.7, 103.4. The Vigreux column was replaced by a Claisen head, and the residue was distilled to give the β anomer (21%): bp 107–109 °C (0.1 mm); $[\alpha]^{25}_D$ –64.2° (*c* 2, H_2O) [lit.[19] bp 108–110 °C (0.1 mm); $[\alpha]^{24}_D$ –64.2° (*c* 2, H_2O)]; ^{1}H NMR ($CDCl_3$) δ 1.36 (s, 3 H), 1.38 (s, 3 H), 3.40 (s, 3 H, OMe), 3.82–4.27 (m, 6 H), 4.86 (s, 1 H, H-1); ^{13}C NMR (Me_4Si, $CDCl_3$): δ 21.0, 27.0, 55.2, 60.9, 75.0, 75.3, 80.2, 98.4, 110.5.

Methyl 3,5-*O*-Isopropylidene-α-D-*threo*-pentofuranosid-2-ulose (4). To a well-stirred solution of methyl 3,5-*O*-isopropylidene-α-D-xylofuranoside (**3**; 14.1 g, 70 mmol) in 200 mL of carbon tetrachloride were added water (200 mL), ruthenium dioxide (225 mg, 50–60% hydrated reagent, Engelhard Industries), potassium carbonate (2.48 g, 18 mmol), and potassium metaperiodate (23 g, 100 mmol). The mixture was stirred vigorously at 25 °C for 15 h. The oxidation was then terminated by adding 2-propanol (10 mL) and stirring the mixture for 10 min. The mixture was then filtered through a pad of Celite, and the filter was washed with two 20-mL portions of carbon tetrachloride. The organic layer was separated, and the aqueous layer was evaporated in vacuo at >40 °C to dryness. The residue was stirred with 100 mL of warm (50 °C) chloroform for 5 min and filtered. The process was repeated once. The chloroform solutions were combined with the carbon tetrachloride solution, dried (Na_2SO_4), and evaporated in vacuo to dryness to give the keto sugar predominantly as its hydrate as a syrup to partly solidified material (12.02 g). The syrup or the partly solidified material was crystallized from ether–hexane (40:60 by volume) to give pure hydrate, mp 69–70 °C (lit.[30] mp 69–70 °C). Anhydrous keto sugar was obtained by dissolution of the hydrate in boiling dry benzene and evaporation of the solution in vacuo to dryness. This process, when repeated two to three more times, gave essentially pure keto sugar as a syrup: 11.32 g (80%); $[\alpha]^{25}_D$ +111° (*c* 1, $CHCl_3$); IR (neat) 1790 (C=O) cm^{-1}; ^{1}H NMR δ 1.46 (s, 6 H), 3.56 (s, 3 H), 3.94–4.27 (m, 4 H), 4.92 (s, 1 H, H-1); ^{13}C NMR (Me_4Si, $CDCl_3$): δ 19.4, 28.7, 56.7, 60.8, 71.4, 73.6, 98.2, 106.4, 203.9.

Methyl 3,5-*O*-Isopropylidene-2-deoxy-2-*C*-methylene-α-D-*threo*-pentofuranoside (5). To a stirred suspension of methyltriphenylphosphonium bromide (5 g, 14 mmol) in dry toluene (70 mL) under nitrogen was added through a septum port 9 mL of 1.56 M *n*-butyllithium in hexane. The bright yellow mixture was stirred at 25 °C for 30 min. A solution of methyl 3,5-*O*-isopropylidene-α-D-*threo*-pentofuranosid-2-ulose (**4**; 1.42 g, 7 mmol) in 30 mL of dry toluene was then added dropwise through the septum port. The reaction mixture was then stirred at 25 °C under nitrogen for 18 h. Excess methylenetriphenylphosphorane was destroyed by addition of acetone (2 mL) and then stirring for 30 min. Evaporation of solvent gave a light red gum. The gum was extracted with boiling hexane (5 × 30 mL). The hexane solutions were combined and evaporated in vacuo to dryness. The residue was chromatographed on a column of silica gel (30 g) and eluted with 1:1 ether–hexane to give the title compound as a colorless oil (395 mg, 28%): ^{1}H NMR ($CDCl_3$) δ 1.41 (s, 3 H), 1.45 (s, 3 H), 3.41 (s, 3 H), 3.90–4.31 (m, 4 H), 4.53 (br s, 1 H), 5.45 and 5.51 (2 s, 2 H); ^{13}C NMR (Me_4Si, $CDCl_3$) δ 19.8, 28.5, 55.4, 60.3, 71.2, 78.6, 97.8, 103.7, 115.8, 148.4.

Attempted Hydrolysis of Methyl 3,5-*O*-Isopropylidene-2-deoxy-2-*C*-methylene-α-D-*threo*-pentofuranoside (5). To a solution of **5** (280 mg, 1.4 mmol) in 15 mL of ethanol was added 15 mL of 0.2 N hydrochloric acid. The mixture was heated with stirring at 65 °C for 20 min. The mixture was cooled to 25 °C, neutralized with Dowex 1-X8 (HCO_3^- form) to pH 7, and then evaporated in vacuo to dryness to give 4-(ethoxymethyl)-2-(hydroxymethyl)furan (**6**) as a pale yellow oil: 210 mg (~98%); ^{1}H NMR ($CDCl_3$) δ 1.18 (t, J = 7 Hz, 3 H), 3.1 (br s, 1 H, OH), 3.5 (q, J = 7 Hz, 2 H), 4.31 (s, 2 H), 4.51 (s, 2 H), 6.30 (s, 1 H), 7.35 (s, 1 H).

Methyl 3,5-*O*-Isopropylidene-2-*C*-(nitromethyl)-α-D-xylo- and -lyxofuranosides (10 and 11, Respectively). To a stirred solution of methyl 3,5-*O*-isopropylidene-α-D-*threo*-pentofuranosid-2-ulose (**4**; 5.05 g, 25 mmol) in a mixture of 50 mL of dry nitromethane and 25 mL of dry methanol at –50 to –40 °C (dry ice–acetone bath) under nitrogen was added a solution of sodium methoxide (1.35 g, 25 mmol) in 25 mL of dry methanol. The cooling bath was then allowed to attain room temperature in ca. 1.5 h. The mixture was then stirred at 25 °C for 4 h. The light yellow solution was neutralized to pH 7 with Dowex 50W-X8 (H^+ form), and the resin was removed by filtration immediately

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after neutralization. The filtrate was evaporated in vacuo to dryness to give a light red syrup. The syrup was dissolved in 30 mL of ether and applied on a column of neutral alumina (activity I, 20 g). The column was eluted with 200 mL of ether. The ether solution on evaporation to dryness gave the isomeric nitro alcohols as a very pale yellow syrup: 5.4 g (82.4%); IR (neat) 3450 (OH), 1550 (NO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.38 and 1.45 (2 s, 6 H, CMe_2), 3.40 and 3.60 (2 s, 3 H, OMe of lyxo and xylo isomer, respectively), 3.8–5.8 (m, 7 H, OH, H-3, H-4, H-5, CH_2NO_2), 4.98 and 5.20 (2 s, 1 H, H-1 of lyxo and xylo isomer, respectively), approximate ratio of xylo to lyxo isomer of 70:30; ^{13}C NMR (Me_4Si, $CDCl_3$) xylo isomer: δ 21.9, 26.7, 55.4, 60.3, 70.9, 71.6, 77.7, 79.9, 99.7, 108.6; lyxo isomer: δ 18.8, 28.8, 57.0, 60.5, 71.6, 75.0, 76.9, 80.5, 97.9, 104.2.

Anal. Calcd for $C_{10}H_{17}NO_7$: C, 45.63; H, 6.46; N, 5.32. Found: C, 45.85; H, 6.36; N, 5.10.

Methyl 2-Deoxy-3,5-*O*-isopropylidene-2-*C*-(nitromethyl)-α-D-xylofuranoside (13). To a solution of methyl 3,5-*O*-isopropylidene-2-*C*-nitromethyl-α-D-xylo- and -lyxofuranosides (**10** and **11**; 7.89 g, 30 mmol) in 65 mL of dry dimethyl sulfoxide was added 50 mL of freshly distilled acetic anhydride. The mixture was stirred at 25 °C for 24 h while being protected from moisture. The pale yellow solution turned light red at the end of the reaction. The solvent was then removed by distillation at reduced pressure (boiling point up to 48 °C at 0.1 mm). The residue, a red gum, containing the nitroalkene **12**, was dissolved in 80 mL of absolute ethanol and the solution was cooled in an ice–water bath. To the cooled, stirred solution was added a solution of sodium borohydride (2.28 g, 60 mmol) in 20 mL of water in small aliquots over a period of 5 min. The mixture was stirred at 0–5 °C for 1 h and then at 25 °C for 1 h. The color of the reaction mixture changed from light red to pale yellow at the end of the reaction. The reaction mixture was then neutralized to pH 6–7 by careful addition of glacial acetic acid and filtered, and the filtrate was evaporated in vacuo to dryness. To the residue was added water (50 mL) and $CHCl_3$ (50 mL). The organic layer was separated, and the aqueous layer was extracted with $CHCl_3$ (3 × 30 mL). The combined organic layers were dried (Na_2SO_4) and passed through a column of neutral alumina (activity I, 25 g). The column was then washed with 100 mL of $CHCl_3$. The combined $CHCl_3$ solutions were evaporated in vacuo to dryness to give **13** as a colorless syrup: 6.0 g (81%); $[\alpha]^{26}_D$ +111.5° (*c* 0.7, C_2H_5OH); IR (neat) 1550 (NO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.33 (s, 3 H), 1.39 (s, 3 H), 2.87–3.09 (m, 1 H, H-2), 3.88 (s, 3 H, OMe), 3.69–4.92 (m, 6 H), 4.96 (d, 1 H, $J_{1,2}$ = 5.0 Hz, H-1); ^{13}C NMR (Me_4Si, $CDCl_3$): δ 19.0, 28.6, 49.6, 56.4, 60.4, 69.8, 70.7, 72.9, 97.9, 106.6.

Anal. Calcd for $C_{10}H_{17}NO_6$: C, 48.57; H, 6.80; N, 5.64. Found: C, 48.84; H, 6.74; N, 5.68.

2-Deoxy-2-*C*-(nitromethyl)-D-xyloses 14 and 15. To a solution of methyl 2-deoxy-3,5-*O*-isopropylidene-2-*C*-(nitromethyl)-α-D-xylofuranoside (**13**; 2.47 g, 10 mmol) in 20 mL of ethanol were added 50 mL of water and 15 g of Dowex 50W-X8 (H^+ form). The mixture was heated with stirring at 65 °C for 3 h. The mixture was then filtered, and the resin was washed with ethanol (3 × 15 mL). The combined filtrates were evaporated in vacuo at 35 °C to dryness. The residue was then dried over phosphorous pentoxide overnight to give 2-deoxy-2-*C*-nitromethyl-D-xylose as a pale yellow gum: 1.89 g (97%); IR (neat) 3350 (OH), 1550 (NO_2) cm^{-1}; ^1H NMR (D_2O) δ 2.66–3.16 (m, 1 H, H-2). ^{13}C NMR data indicated presence of anomeric mixtures of furanose and pyranose forms. The major form had the following chemical shifts in parts per million from Me_4Si in D_2O: 42.3, 65.0, 69.1, 72.0, 73.8, 93.6.

Anal. Calcd for $C_6H_{11}NO_6$: C, 37.30; H, 5.70; N, 7.25. Found: C, 37.10; H, 5.82; N, 7.10.

2-Deoxy-2-*C*-methylene-D-*threo*-pentono-1,4-lactone (2). 2-Deoxy-2-*C*-(nitromethyl)-D-xyloses (**14** and **15**; 775 mg, 4.0 mmol) were dissolved in 30 mL of 30% acetic acid in water. Bromine (1 mL) was added, and the mixture was swirled to dissolve the bromine. The flask was stoppered and allowed to stand in the dark at 25 °C for 24 h. Excess bromine was then removed by aeration, and the resulting clear colorless solution was diluted with 30 mL of water. Barium carbonate (17.73 g, 90 mmol) was then added in small portions with stirring over a period of 30 min. After the addition was over, the mixture was stirred at 25 °C for 2 h and filtered, and the filtrate was evaporated in vacuo at 35 °C to dryness. The residue was taken up in 100 mL of acetone, the resulting mixture was stirred at 25 °C for 15 min and filtered, and the precipitate was washed with acetone (3 × 50 mL). The acetone solutions were combined and evaporated to dryness in vacuo. The residue was redissolved in 100 mL of acetone, the solution was filtered to remove inorganic materials, and the filtrate was evaporated to dryness. This process was repeated until a clear pale yellow syrup was obtained. The syrup was then dissolved in 10 mL of acetone, and CH_2Cl_2 was added until the precipitation of a fluffy white solid was complete. The mixture was filtered, and the filtrate was evaporated to dryness to give 2-deoxy-2-*C*-methylene-D-*threo*-pentono-1,4-lactone (**2**) as a very pale yellow syrup: 297 mg (52%); IR (neat) 3365 (OH), 1768 (C=O), 1650 and 915 (C=CH_2) cm^{-1}; ^1H NMR (D_2O) δ 4.18–4.60 (m, 3 H, H-4, H-5), 5.60 (2 t, $J_{3,4}$ = 6.47 Hz, $J_{3,5}$ = 1.83 Hz, 1 H, H-3), 6.62 (d, J_{gem} = 2 Hz, 1 H, H-2′), 6.93 (d, J_{gem} = 2 Hz, 1 H, H-2′); ^{13}C NMR (Me_4Si, D_2O): δ 60.6, 68.5, 83.4, 128.2, 138.4, 172.6.

Anal. Calcd for $C_6H_8O_4$: C, 50.00; H, 5.55. Found: C, 49.75; H, 5.45.

Reaction of 2 with L-Cysteine. L-Cysteine (120.7 mg, 1.0 mmol) was dissolved in hot water (0.6 mL) and then cooled to 25 °C. A solution of 2-deoxy-2-*C*-methylene-D-*threo*-pentono-1,4-lactone (**2**; 144 mg, 1.0 mmol) in 0.4 mL of water was then added under nitrogen. (The reaction was complete in less than 15 min as evidenced by ^1H NMR in a run carried out in D_2O.) The mixture was allowed to stand at 25 °C for 1 h. Acetone was added to the solution until precipitation was complete. The solid was collected by filtration and recrystallized from acetone–ethanol–water. The recrystallized product was dried over P_2O_5 at 0.1 mm overnight to give the cysteine adduct **18** as white plates: 238 mg (90%); mp 180–185 °C dec; ^{13}C NMR (Me_4Si, D_2O): δ 25.5, 26.1, 33.6, 54.4, 60.5, 69.9, 85.0, 173.4, 179.5.

Anal. Calcd for $C_9H_{15}NO_6S$: C, 40.75; H, 5.66; N, 5.28. Found: C, 40.52; H, 5.23; N, 4.99.

Reaction of 2 with Glutathione. Glutathione (184.3 mg, 0.6 mmol) was dissolved in hot water (0.4 mL) and then cooled to 25 °C. A solution of 2-deoxy-2-*C*-methylene-D-*threo*-pentono-1,4-lactone (**2**; 86.4 mg, 0.6 mmol) in 0.3 mL of water was then added under nitrogen. (The reaction was complete in less than 15 min as evidenced by ^1H NMR in a run carried out in D_2O.) The mixture was allowed to stand at 25 °C for 1 h and was then evaporated in vacuo to dryness. The residue was scratched under 20 mL of acetone and filtered. The precipitate was dried over P_2O_5 at 0.1 mm overnight to give the glutathione adduct **19** as a granular solid: 264 mg (~99%); mp 145 °C dec; ^{13}C NMR (Me_4Si, D_2O): δ 26.3, 26.9, 31.1, 32.0, 42.3, 54.5, 56.4, 60.6, 70.3, 82.4; 173.1, 174.3, 175.6, 179.0.

Anal. Calcd for $C_{16}H_{25}N_3O_{10}S$: C, 42.57; H, 5.54; N, 9.09. Found: C, 42.20; H, 5.84; N, 9.12.

Registry No. **1**, 58-86-6; **2**, 73230-64-5; α-**3**, 7045-40-1; β-**3**, 51754-99-5; **4**, 65247-31-6; **5**, 73230-65-6; **6**, 73230-66-7; **10**, 70448-59-8; **11**, 73230-67-8; **12**, 73230-68-9; **13**, 70448-61-2; α-**14**, 73230-69-0; β-**14**, 73230-70-3; α-**15**, 73230-71-4; β-**15**, 73230-72-5; **18**, 73230-73-6; **19**, 73230-74-7; L-cysteine, 52-90-4; glutathione, 70-18-8.

Degenerative Chemistry of Malondialdehyde. Structure, Stereochemistry, and Kinetics of Formation of Enaminals from Reaction with Amino Acids[1]

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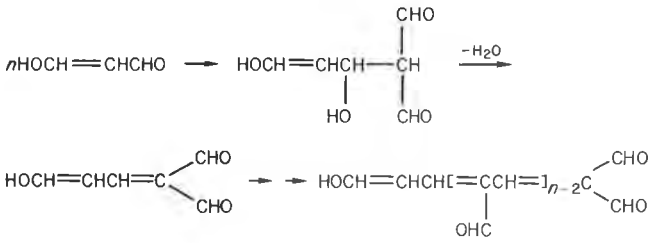
Abstract: Malondialdehyde (MDA) is a naturally occurring compound produced in biological materials as a result of polyunsaturated lipid oxidation or from irradiation of certain compounds. Malondialdehyde chemistry has long been of interest in food science and has received attention recently because of suggestions that it may play a role in cellular aging and in a host of other degenerative biological transformations associated with lipid peroxidation. The detrimental effects of MDA are believed to result from its reaction with primary amino groups of biological macromolecules. So that further insight into the specific nature of these interactions could be gained, the reaction of pure MDA with several amino acid derivatives was studied at pH 4.2. The products from the reaction were found to be enaminals, and ^1H and ^{13}C NMR spectroscopy showed evidence of both *s*-cis and *s*-trans conformations in $CDCl_3$ while only the *s*-trans form was evident in D_2O. This represents the first detailed structural analysis of these products. The rate and equilibrium constants for enaminal formation were determined with the use of UV spectroscopy. With histidine, tyrosine, arginine, and tryptophan there was no evidence for reaction of MDA at positions other than the α-amino group, and the possible implications of this finding in MDA–protein interactions are discussed. The reactions of the more stable methylmalondialdehyde were examined as well.

Malondialdehyde (MDA) (**1**) is a naturally occurring three-carbon dialdehyde produced in the oxidation of polyunsaturated lipids.[2-7] It is generated also in the irradiation of carbohydrates,[8] and certain amino acids.[9] The measurement of MDA has been used by the food industry as a measure of oxidative rancidity.[7,10-12] It has been suggested that the production of MDA in foods alters their nutritive value.[13-16] Recent reports that MDA is toxic,[17,18] carcinogenic,[17] and mutagenic[18-20] and may be involved in a number of age-related disorders[21-24] have generated wide interest in its chemistry.

The detrimental effects of MDA are likely to result from its ability to covalently bond and to cross-link a variety of biological macromolecules by reaction with functional groups such as primary amino groups. Products such as **2** and **3** have been isolated

$$\underset{\mathbf{1}}{\mathrm{OHCCH{=}CHOH}} + \mathrm{RNH_2} \rightarrow \underset{\mathbf{2}}{\mathrm{RNHCH{=}CHCHO}} \xrightarrow{\mathrm{RNH_2}} \underset{\mathbf{3}}{\mathrm{RNHCH{=}CHCH{=}NR}}$$

Scheme I



from the reaction of MDA and glycine.[25,26] The 1-amino-3-iminopropenes exhibit fluorescence with an excitation maximum at 370 nm and an emission maximum at 450 nm. The reaction of MDA with proteins has been said to result in cross-linking and the products have fluorescence spectra similar to 1-amino-3-iminopropenes. This suggests that the cross-linking of these macromolecules may occur through formation of 1-amino-3-iminopropene linkages. It should be noted, however, that MDA is unstable and undergoes an aldol-type self condensation reaction to produce a mixture of polymers (Scheme I).[27] Some of these polymers exhibit fluorescence similar to those of 1-amino-3-iminopropenes and also are capable of cross-linking proteins.[27-30] Interestingly, an age skin pigment called lipofuscin which has been suggested as being derived from the reaction of MDA with skin proteins also has a fluorescence spectrum similar to the 1-amino-3-iminopropenes.[21,26]

Although MDA is known to react with proteins, the reaction products have not been characterized adequately.[14,25,26,31-34] Valuable information on both the reactive sites and the structural nature of protein modification and cross-linking can be obtained through investigation of simple model systems that represent the

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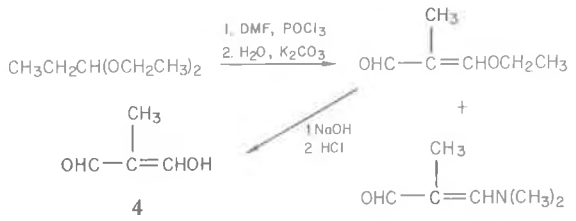
Scheme II



vulnerable protein components. We report here the first complete account of the structure, stereochemistry, and kinetics of the initial reaction of MDA with representative amino acids. Studies of MDA chemistry are complicated by its tendency to undergo self-condensation reactions. Methylmalondialdehyde (MMDA) (**4**) is a much more stable yet closely related derivative of malondialdehyde. The reactions of MMDA with amino acid derivatives were therefore studied as well to determine the feasibility of its use in model studies of MDA reactions. The focal point of this study is the initial reaction of MDA with the amino acids to form enaminals. The rates and equilibria involved in the reactions were examined by UV spectroscopy, and the stereochemical features of the products in solution were analyzed by ^1H and ^{13}C NMR spectroscopy.

Results and Discussion

The choice of amino acid derivatives for the study was based on several considerations. Initially, the reactions of glycine and alanine derivatives were examined as their structural simplicity facilitated isolation and identification of the products. Lysine, histidine, tyrosine, arginine, and tryptophan derivatives were chosen to determine if the dialdehydes react at positions other than the α-amino group. The only simple primary amino groups present in protein are the ϵ-amino groups of lysine and the N-terminal amino groups. Crawford et al. determined that MDA does, in fact, react with the ϵ-amino group of lysine and the N-terminal amino acid, asparagine, of bovine plasma albumin.[14] Subsequently, a number of enzymes,[25,26,30] food proteins,[13] and collagen[35-37] were reacted with MDA, hydrolyzed, and subjected to amino acid analysis. The results of these studies vary depending on the protein studied and the reaction conditions, but the amino acids which consistently appear to be altered to some degree are histidine, tyrosine, arginine, lysine, and methionine. Cysteine and tryptophan are destroyed in protein hydrolysis, but tryptophan residues of protein have been shown to react with triose reductone (hydroxymalondialdehyde).[38] Methionine– and cysteine–malondialdehyde interactions are currently under study in our laboratory, and these results will be reported elsewhere.

Malondialdehyde was prepared by hydrolysis of its bis(dimethyl acetal) and was purified and stored as its relatively stable enolic sodium salt.[39,40] The sodium salt of MDA crystallizes from ethanol/ether as white needles, mp 246 °C dec. Its UV spectrum in 0.01 M HCl showed λ_{max} 245 nm (ϵ 12 800). A bathochromic shift to λ_{max} 267 nm (ϵ 29 400) occurred in 0.01 M NaOH. Its ^1H NMR spectrum in D_2O (external Me_4Si) showed resonances at δ 9.08 (2 H, d, J = 10.1 Hz) and 5.73 (1 H, t, J = 10.1 Hz), suggesting an *s*-trans stereochemistry.[40] Interestingly, the triplet at δ 5.73 slowly disappeared, and examination of its ^{13}C NMR spectrum showed C_2 as a triplet at δ 110.1, suggesting slow exchange of the hydrogen at C_2 with deuterium.

For purposes of comparison and for extension of the scope of these studies, a substituted malondialdehyde also was utilized. The choice of a specific substituted malonadialdehyde for use in these studies was governed by the requirements not only of stability but also of inherent structural closeness to the parent compound. The substituted compound that best fulfilled these requirements was methylmalondialdehyde (MMDA). This was prepared by a modified literature procedure involving the Vilsmeier–Haack–Arnold acylation of propionaldehyde diethyl acetal as outlined in Scheme II.[41-43] We have discovered in our laboratory that phosphorus oxychloride is a better reagent to use than phosgene or oxalyl chloride. The ethyl ether of **4** is produced as a side product, and it can be easily converted to **4** by hydrolysis. Analytically pure samples of **4** can be prepared by taking advantage of its amphoteric character. Thus, purification can be achieved through conversion of **4** to its hydrochloride salt.[43] Methylmalondialdehyde is a solid mp 89 °C. It exhibits a strong UV absorption in neutral solution at 274 nm (ϵ 29 800). This provides a convenient method for monitoring some reactions of this compound. Additionally, the methyl group on this compound provides an excellent marker for following its reaction by ^1H and ^{13}C NMR spectroscopy.

In general, the reactions were carried out by combining aqueous solutions of the dialdehyde enolic sodium salt and a molar

Table I. Products from the Reaction of Malondialdehyde and Methylmalondialdehyde with Amino Acid Derivatives

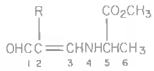
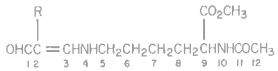
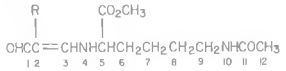
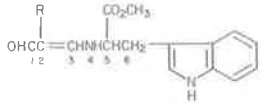
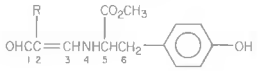
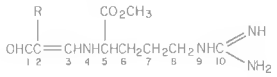
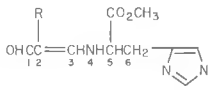
amino acid derivative	product
glycine	 **5**, R = H, R′ = Na **6**, R = CH_3, R′ = H
glycine methyl ester	**7a**, R = H, R′ = CH_3; **7b**, R = R′ = CH_3
alanine methyl ester	 **8a**, R = H; **8b**, R = CH_3
α-acetyllysine methyl ester	 **9a**, R = H; **9b**, R = CH_3
ε-acetyllysine methyl ester	 **10a**, R = H; **10b**, R = CH_3
tryptophan methyl ester	 **11a**, R = H; **11b**, R = CH_3
tyrosine methyl ester	 **12a**, R = H; **12b**, R = CH_3
arginine methyl ester	 **13a**, R = H; **13b**, R = CH_3
histidine methyl ester	 **14a**, R = H; **14b**, R = CH_3
α-acetylhistidine methyl ester	no reaction
α-acetyltyrosine methyl ester	no reaction

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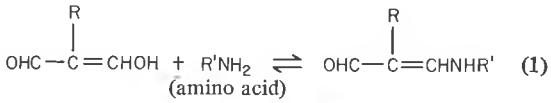
Table II. Physical and Spectral Properties of Enaminal Products[a]

compd	method[b]	R_f[b]	yield, %	mp, °C	UV_{max} (log ε)[c]
6	B[d]		56	170–171	289 (4.51)
7a	A	0.53	50	103–104	277 (4.52)
7b	A	e	63	oil[e]	288 (4.55)
8a	A	0.53	32	oil	279 (4.53)
8b	A	0.63	18	oil	288 (4.54)
9a	B	0.23	10	oil	282 (4.53)
9b	B	0.27	12	oil	289 (4.56)
10a	B	0.42	10	oil	280 (4.52)
10b	B	0.50	10	oil	289 (4.56)
11a	A	0.60	97	89–90	280 (4.46)[f]
11b	A	0.60	88	57–59	285 (4.56)[f]
12a	B	0.41	30	164–165	282 (4.53)
12b	B	0.50	25	59–61	290 (4.50)
13a	B	g	20	165 dec	283 (4.53)
13b	B	g	50	165 dec	292 (4.57)
14a	B	0.15	45	45–46	279 (4.52)
14b	B	0.30	29	42–43	288 (4.51)

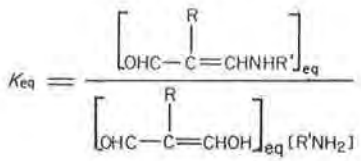
[a] All compounds gave satisfactory elemental analyses. [b] Methods A and B and chromatographic techniques are described in the Experimental Section. [c] In 0.2 M, pH 4.2 acetate buffer solution. [d] Purified by recrystallization from 2-propanol. [e] Purified by distillation on a micromolecular still at 0.2 torr, 180–185 °C (bath temperature). [f] UV spectrum was taken in ethanol because of low solubility in aqueous buffer. [g] Purified by high pressure liquid chromatography.

equivalent of the amino acid methyl ester hydrochloride. One equiv of HCl was added to the solutions for the reactions of the dialdehydes with the α-acetyl amino acid methyl esters. So that MDA polymerization, could be minimized, reactions involving it were carried out in solutions low in MDA concentration. The results of the reaction between the dialdehydes and the amino acid derivatives are summarized in Table I. The use of the methyl ester derivatives of the amino acids simplified isolation, purification, and structural analysis of the products. The procedures used and the yields, physical properties, and UV data for the products are shown in Table II. In every case, only the 1:1 adduct of amino acid and MDA or MMDA was observed. This is in contrast to the work of Chio and Tappel[25] who observed both 1:1 and 1:2 adducts in strongly acidic aqueous solutions. Chio and Tappel prepared MDA by the acidic hydrolysis of its bis(acetals). Malondialdehyde prepared by this method is generally contaminated with its partial hydrolysis products (3,3-dialkoxypropionaldehyde and β-alkoxyacrolein) and its polymers. These side products also are reactive toward amino acids. We have used highly purified MDA (as its sodium salt) in our studies. Thus, we believe that the reasons for this difference must be associated with the purity of the MDA and the mildly acidic conditions used in our work. Unambiguous evidence for the formation of the 1:1 adducts came from spectral data and elemental analysis. The mass spectra provided particularly convincing data. In all cases, parent ion currents were present in the mass spectra of these 1:1 adducts.

The UV absorption maximum and absorptivity of the dialdehydes vary with pH changes.[43-46] At low pH values, the UV absorption maximum is 30–40 nm lower than that of the enaminal products. Ultraviolet spectroscopy, therefore, provides a convenient means of examining the rate and equilibria involved in the dialdehyde–amino acid reaction. The reaction of MDA with glycine has been shown to be second order, first order in both amino acid and MDA, and the rate varied greatly with changes of pH having a maximum rate at pH 4.2.[33] This is also the optimum pH for the reaction of MDA with protein[30,47] and is just below the malondialdehyde acid dissociation constant of 4.5.[48] The *s*-trans protonated enol form of MDA predominates at pH 4.2. Methylmalondialdehyde with an acid dissociation constant of 4.7 shows similar behavior.[43-46] Rate studies were carried out at pH 4.2 under pseudo-first-order conditions with a large excess of amino acid derivative. The disappearance of MDA and the appearance of enaminal product were monitored at their absorption maxima of approximately 249 and 280 nm, respectively. An isosbestic point was present at 258–261 nm, and this behavior was typical of all the MDA–amino acid reactions studied here. Methylmalondialdehyde reactions were monitored at 252 and 288 nm. The MMDA reactions exhibit an isosbestic point at 265–269 nm. The observed pseudo-first-order rate constants (k_{obsd}) for MDA disappearance and for enaminal formation agreed within experimental error. Where applicable, absorbance values of A_∞ at these wavelengths were corrected for absorbance due to amino acid derivative. These values along with the molar absorptivity of the dialdehydes (MDA 249 nm, log ε 4.10; MMDA 252 nm, log ε 4.27) and the products (280 nm, log ε 3.53; 288 nm, log ε 3.35) were substituted into the appropriate Beer's law relationships and the two equations solved for the concentrations of product and dialdehyde at equilibrium. From this information, the equilibrium constant (K_{eq}), the second-order rate constant (k_f) for enaminal formation, and k_r, the pseudo-first-order rate constant for the hydrolysis were obtained by using eq 1–4.[49] The results are summarized in Table III.



(1)

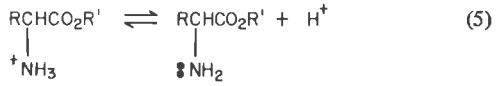


(2)

$$k_{obsd} = k_f[R'NH_2] + k_r \quad (3)$$

$$k_f/k_r = K_{eq} \quad (4)$$

Several classes of amino acids are represented in the study, and there appear to be several features which affect the reaction rate and equilibrium constant. For the reaction of MDA with the α-amino group of glycine, alanine, and ε-*N*-acetyllysine, there appears to be a decrease in K_{eq} and k_f with increase in the size of the alkyl substituent. However, arginine and tyrosine react as fast as glycine despite the presence of large groups so that the position of the equilibrium (eq 5) at pH 4.2 must also be taken



(5)

into account. The observation that ε-*N*-acetyllysine shows a higher K_{eq} and k_f than α-*N*-acetyllysine is consistent with this (pK_2 of α-N^+H_3 is 8.95 and pK_3 of ε-N^+H_3 is 10.53).[50] The reaction of MDA with the α-amino group of histidine is exceptionally favorable. The exact reason for this effect is unclear. The possibility of participation of the imidazole ring of histidine in the rate acceleration observed at the α-amino group of this amino acid was examined. However, addition of α-*N*-acetylhistidine to solutions of other amino acids does not accelerate their reaction with MDA. It is conceivable that the facile reaction with histidine is the result of an intramolecular rate acceleration process where initial binding of MDA to the imidazole nitrogen is followed by transfer of the MDA from this nitrogen to the α-amino group.[51] The behavior of MMDA parallels that of MDA except that the

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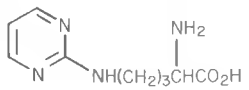
Table III. Rate and Equilibrium Constants[a] and Related Ultraviolet Spectral Data[b] for the Reaction of Dialdehyde and Amino Acid

compd	log ε (249 nm)	log ε (280 nm)	log K_{eq}	k_f, M^{-1} min^{-1}	k_r, 10^3 min^{-1}	compd	log ε (252 nm)	log ε (288 nm)	log K_{eq}	k_f, 10^2 M^{-1} min^{-1}	k_r, 10^4 min^{-1}
7a	3.65	4.51	2.87	2.51	3.35	7b	3.38	4.55	2.20	2.76	1.76
8a	3.55	4.53	2.38	0.82	3.39	8b	3.30	4.54	1.90	1.24	1.55
9a	3.57	4.53	2.09	0.14	1.12	9b	3.65	4.55	1.29	0.16	0.82
10a	3.48	4.54	2.55	1.09	3.07	10b	3.18	4.56	2.10	1.21	0.97
12a	3.43	4.50	2.78	2.23	3.73	12b	3.34	4.49	2.72	2.64	0.50
13a	3.48	4.53	2.82	2.26	3.46	13b	3.25	4.57	2.47	2.68	0.91
14a	3.58	4.51	3.81	9.27	1.45	14b	3.39	4.51	3.48	13.4	0.44

[a] The rate constant k_f is the second-order rate constant for the formation of the enaminals; k_r is the first-order rate constant for the reverse reaction, i.e., enaminal hydrolysis; K_{eq} is the equilibrium constant for enaminal formation. [b] The molar absorptivity (ε) for the enaminals is given at wavelengths near the absorption maxima and the molar absorptivity (ε) for the dialdehydes is given at the absorption maxima, in 0.2 M pH 4.2 buffer. These values along with the absorptivity of the dialdehydes were used to calculate the concentrations of dialdehyde and enaminal at equilibrium.

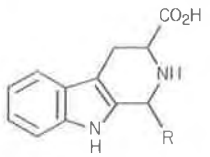
reactions are generally about 100 times slower and the values of K_{eq} are somewhat smaller.

The kinetic studies also show that, under the conditions employed, MDA reacts with the amino acid methyl esters exclusively at the α-amino group. The observation of an isosbestic point and the fact that identical values of k_{obsd} were calculated from MDA disappearance and enaminal appearance precludes any significant amount of side reactions. It is notable that there is no evidence for reaction of the guanidino group of arginine. In strongly acidic solution, arginine has been reported to react with MDA to give δ-*N*-(2-pyrimidinyl)-L-ornithine (**15**).[34] Tryptophan was not



15

included in the kinetic studies because of the low solubility of the product in water. However, the enaminal, **11a**, was isolated in nearly quantitative yield from the reaction of tryptophan methyl ester with MDA in acetate buffer at pH 4.2. Interestingly, under somewhat more forcing conditions (1 N H_2SO_4, 50–60 °C, 1 h), tryptophan has been reported to give the cyclic product **16** on



16

reaction with simple aldehydes.[52,53] However, there was no evidence of a product such as **16** from the MDA reaction.

The α-*N*-acetyl derivatives of histidine, tyrosine, and lysine methyl esters were also exposed to MDA in acetate buffer, under the same conditions as in the rate studies. Except in the case of lysine, there was no evidence for a decrease in MDA concentration even after several days at 25 °C. Even at higher temperatures (pH 4.2, 60 °C, 7 days) and higher pH values (pH 7.0, 25 °C, 4 days), there was no apparent reaction between MDA (or MMDA) and α-*N*-acetyltyrosine or α-*N*-acetylhistidine methyl esters. Several studies have suggested that MDA reacts with histidine and tyrosine residues of proteins, but the nature of this interaction remains unclear. It has also been suggested that the product from the reaction of MDA with the ε-amino group of lysine residues in proteins is quite resistant to hydrolysis.[35] However, the relatively low values of k_f and K_{eq} obtained for the reaction of MDA with the ε-amino group of α-*N*-acetyllysine indicate that this product is fairly susceptible to hydrolysis even under conditions chosen to favor enaminal formation rather than hydrolysis. Apparently, protein provides an environment more

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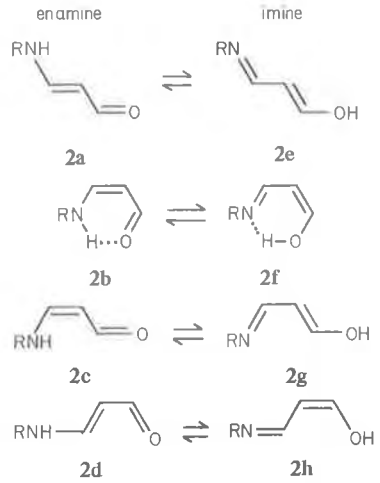
Scheme III



favorable to some of these interactions, or the complexities involved in studying MDA protein interactions hinder one from obtaining a clear picture of the nature of the reactions on the basis of the simple model systems utilized in this study.

The ^1H and ^{13}C NMR spectra of most of the products were obtained in $CDCl_3$ and provided insight into the stereochemical features of the products in that solvent. The NMR spectra of the glycine methyl ester enaminals (**7**) were determined in both $CDCl_3$ and D_2O for purposes of comparison. Due to their insolubility in nonhydroxylic solvents, the NMR spectra of **5**, **6**, **13**, and **14** were run in D_2O. The products shown in Table I in the enaminal form are capable of enamine–imine tautomerism and may assume any of the four tautomeric pairs of isomers illustrated in Scheme III. These isomers are all interconvertable by the two processes of tautomerism and hindered rotation about a carbon–carbon single bond. By analogy to related compounds, it was expected that the trans-enamine form **2a** would be the most preferred conformation in hydrogen-bonding solvents and that **2a** and the cis-chelated form **2b** would both be important in non-hydrogen-bonding solvents. The NMR spectra of the products supported these assumptions. Our subsequent use of the terms trans and cis isomers will refer to forms **2a** and **2b**.

Table IV lists the ^1H NMR data for the products **5–14**. So that our interpretation and correlation of this data with structure (enamine vs. imine) and stereochemistry (cis and trans) could be exemplified, a discussion of the 90-MHz ^1H NMR spectrum in $CDCl_3$ of **7a**, the product from the reaction of MDA with glycine methyl ester, is presented. The spectrum (Figure 1) shows two complete sets of resonances which can be assigned to the trans and cis forms. The chemical shift of the NH and its splitting of H_3 and H_5 provide evidence for the enamine rather than the imine tautomer. The predominant isomer is the trans form with $J_{1,2}$ = 8.0 Hz and $J_{2,3}$ = 13.2 Hz. The cis isomer shows values of $J_{1,2}$ = 2.1 Hz and $J_{2,3}$ = 7.4 Hz. Because of intramolecular hydrogen bonding in the cis form, the NH resonance appears at δ 10.0, much further downfield than that of the trans isomer at δ 5.8.[54] These

Table IV. ^{1}H NMR Data for Enaminals[a]

compd	solvent[b]	isomer[c] (%)	H_1	H_2	H_3	H_4	H_5	OCH_3	$J_{1,3}$	$J_{2,3}$	$J_{3,4}$	$J_{1,3}$[d]	$J_{4,5}$	H_{others}
5	D_2O	trans (100)	9.30	5.76	8.13		4.30		9.0	13.0				
6	D_2O	trans (100)	8.95		7.77		4.63							2.10 (s, 3 H, R = CH_3)
7a	$CDCl_3$	trans (78)	9.16	5.23	7.24	5.80	3.88	3.82	8.0	13.2	7.4		5.1	
7a	$CDCl_3$	cis (22)	9.20	5.12	6.70	10.0	3.97	3.78	2.1	7.3	12.5	3.2	6.3	
7a	D_2O	trans (100)	9.25	5.67	8.05		4.57	4.22	9.0	13.0				
7b	$CDCl_3$	trans (100)	8.97		6.78	5.45	4.07	3.80			13.2		4.8	1.67 (s, 3 H, R = CH_3)
7b	D_2O	trans (100)	9.02		7.62		4.63	4.20						2.04 (s, 3 H, R = CH_3)
8a	$CDCl_3$	trans (70)	9.08	5.26	7.22	6.7	4.1	3.78	8.3	13.2	8.3			1.49 (d, J = 7.1, 3 H, H_6)
8a	$CDCl_3$	cis (30)	9.20	5.09	*e*	10.0	4.1	3.78	2.2	7.1	*e*	*e*		1.52 (d, J = 7.3, 3 H, H_6)
8b	$CDCl_3$	trans (100)	9.01		6.79	5.3	4.0	3.80			13.7			1.54 (d, J = 7.4, 3 H, H_6), 1.68 (s, 3 H, R = CH_3)
9a	$CDCl_3$	trans (51)	9.06	5.26	7.1	5.9	3.2	3.75	8.5	13.0	*f*			1.2–1.9 (m, 6 H, H_6, H_7, H_8), 2.03 (s, 3 H, H_{12}), 4.6 (m, 1 H, H_9), 6.4 (m, 1 H, H_{10})
9a	$CDCl_3$	cis (49)	9.08	4.98	6.76	10.0	3.2	3.75	2.3	6.9	13.4	3.0		1.2–1.9 (m, 6 H, H_6, H_7, H_8), 2.03 (s, 3 H, H_{12}), 4.6 (m, 1 H, H_9), 6.4 (m, 1 H, H_{10})
9b	$CDCl_3$	trans (100)	8.80		6.93	6.2	3.4	3.74			13.3			1.2–1.9 (m, 6 H, H_6, H_7, H_8), 1.67 (s, 3 H, R = CH_3), 2.03 (s, 3 H, H_{12}), 4.7 (m, 1 H, H_9), 6.74 (d, $J_{9,10}$ = 7.9, 1 H, H_{10})
10a	$CDCl_3$	trans (57)	9.2	5.29	7.19	6.1	3.90	3.77	5.1	13.2	8.5			1.2–1.9 (m, 6 H, H_6, H_7, H_8), 1.98 (s, 3 H, H_{12}), 3.2 (m, 2 H, H_9), 6.40 (t, $J_{9,10}$ = 7.3, H_{10})
10a	$CDCl_3$	cis (43)	9.2	5.10	6.74	10.0	3.90	3.77	2.1	7.6	12.4	2.9		1.2–1.9 (m, 6 H, H_6, H_7, H_8), 1.98 (s, 3 H, H_{12}), 3.2 (m, 2 H, H_9), 6.40 (t, $J_{9,10}$ = 7.3, H_{10})
10b	$CDCl_3$	trans (100)	8.98		6.75	5.3	3.90	3.79			13.5			1.2–1.9 (m, 6 H, H_6, H_7, H_8), 1.70 (s, 3 H, R = CH_3), 1.97 (s, 3 H, H_{12}), 3.2 (m, 2 H, H_9), 5.8 (br m, 1 H, H_{10})
11a	$CDCl_3$	trans (63)	9.0	5.29	*e*	5.7	4.30	3.73	8.2	13.1	*e*			3.3 (m, 2 H, H_6), 6.7–7.7 (m, 5 H, indole), 8.4 (br m, 1 H, indole NH)
11a	$CDCl_3$	cis (27)	9.1	4.92	6.42	10.0	4.30	3.73	2.0	7.1	8.8	2.9		3.3 (m, 2 H, H_6), 6.7–7.7 (m, 5 H, indole), 8.4 (br m, 1 H, indole NH)
11b	$CDCl_3$	trans (100)	8.78		6.48	5.3	4.30	3.78			13.7			1.56 (s, 3 H, R = CH_3), 3.32 (t, $J_{5,6}$ = 6.0, 2 H, H_6), 6.9–7.6 (m, 5 H, indole), 8.7 (br m, 1 H, indole NH)
12a	$(CD_3)_2CO$	trans (100)	8.96	5.31	7.31		4.33	3.69	8.4	12.4				3.13 (m, 2 H, H_6), 6.75 (d, J = 9.0, 2 H, aromatic), 7.05 (d, J = 9.0, 2 H, aromatic)
12b	$CDCl_3$	trans (100)	8.80		6.41	5.5	4.30	3.80			14.0			1.61 (s, 3 H, R = CH_3), 3.11 (d, 2 H, H_6), 6.8 (m, 5 H, phenol)
13a	D_2O	trans (100)	9.24	5.80	8.00		4.8	4.23	8.5	13.2				2.0–2.5 (m, 4 H, H_6, H_7), 3.66 (t, J = 6.4, H_8)
13b	D_2O	trans (100)	8.60		7.10		4.2	3.80						1.52 (s, 3 H, R = CH_3), 1.7 (m, 4 H, H_6, H_7), 3.2 (m, 2 H, H_8)
14a	D_2O	trans (100)	9.15	5.71	7.90		4.95	4.21	7.8	13.0				3.6 (m, 2 H, H_6), 7.48 (s, 1 H, imidazole), 8.33 (s, 1 H, imidazole)
14b	$CDCl_3$	trans (100)	8.75		7.25	6.6	4.35	3.80						1.55 (s, 3 H, R = CH_3), 3.20 (d, J = 7.0, 2 H, H_6), 6.80 (s, 1 H, imidazole), 7.6 (s, 1 H, imidazole), 7.25 (s, 1 H, imidazole NH)

[a] Chemical shift values are in δ from internal Me_4Si except for D_2O solutions where external Me_4Si (capillary) was used as reference. Because of broadening and overlap, chemical shifts in some cases could be determined only to one decimal place. Coupling constants are given in Hz to one decimal place. See Table I for numbering. [b] The concentration of enaminal is 0.1 M. [c] Trans and cis refer to forms **2a** and **2b**. The percent of isomers present in solution was calculated from integrated intensities of peaks. [d] The magnitude of the coupling constants is given but the signs were not determined. [e] Obscured by overlapping peaks. [f] Unresolved multiplet.

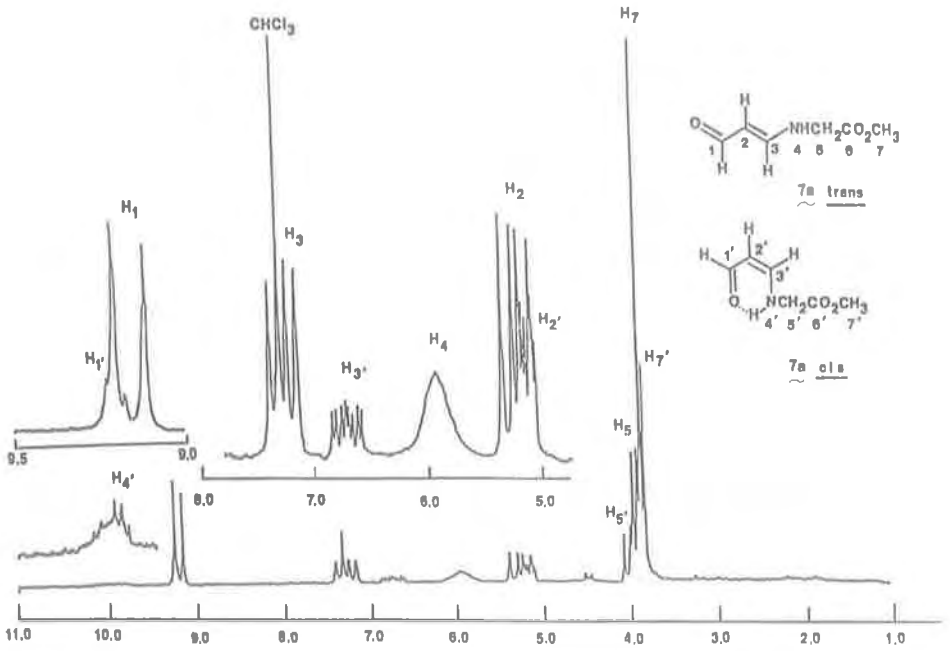


Figure 1. ^{1}H NMR Spectrum of **7a** in $CDCl_3$. Chemical shift values are in δ from internal Me_4Si.

broad peaks disappear on exchange with D_2O, and the splittings between H_3 and H_4 and between H_4 and H_5 are no longer present. An interesting feature of the spectrum of the cis isomer was the presence of a four-bond coupling $J_{1,3}$ = 3.2 Hz while no such coupling was observed for the trans isomer. This difference can be attributed to a favorable (*W*) stereochemical relationship for long-range coupling of H_1 and H_3 in the cis form which was not present in the trans isomer.[55] On the basis of integrated intensities, the trans isomer was 78% of the isomeric mixture. The ^{13}C NMR data of **7a** in $CDCl_3$ (Table V; see paragraph concerning supplementary material) also show a mixture of isomers, and assignment of resonances to the carbons of cis and trans forms was based partly on the relative intensities of the pairs of peaks. The NMR spectra of the other products were interpreted with the use of similar reasoning. The percent of cis isomer present in $CDCl_3$ solutions showed an increase with increasing size of the nonpolar amino substituent. This trend has also been observed in related compounds.[54] The MMDA products gave much simpler ^{1}H NMR spectra with only one isomer in evidence. In the $CDCl_3$ spectra, the splitting of H_5 by the NH again confirms the presence of the enamine tautomer, and H_4 chemical shifts of δ 5–6 are consistent with the expected trans form. The ^{13}C NMR spectra of these enaminals in $CDCl_3$ also show only one isomer. For both MDA and MMDA adducts **5–14**, the spectra in D_2O provide evidence for a single isomer. For the MDA products, the trans structure can be assigned based on the magnitude of splittings between H_1, H_2, and H_3. Although unequivocal assignments cannot be made for the MMDA products in D_2O, it is reasonable to assume that the trans form predominates for these compounds as well.

The MDA-induced cross-linking of proteins has been proposed to occur through formation of 1-amino-3-iminopropene linkages.[25,26] The failure to observe the formation of this type of product from the reaction of MDA with these amino acid derivatives under mild conditions was somewhat surprising. After completion of the rate studies, the reaction solutions (which contained a large excess of amino acid) were allowed to stand at room temperature for 30 days. After this time, the UV spectrum showed only a slight decrease in enaminal absorbance (less than 10%) and there was no significant change in the absorption maximum or in the shape of the UV spectrum. Interestingly, significant protein cross-linking by MDA has been reported to occur within 24 h.[26,30] It is possible that protein molecules provide a more favorable environment for MDA reaction than is present with simple amino acid derivatives. In relation to this, it should be mentioned that in studies in our laboratory using a "less hydrophilic" solvent (e.g., methanol), 2:1 or cross-linked adducts from MDA and amino acids have been isolated. Alternatively, MDA polymers may play a role in the observed inter- and intra-molecular cross-linking of proteins. The polymers have free aldehyde groups,[27–29] and it is possible that to some extent the observed cross-linking and the fluorescence of MDA–protein products result from reaction of the polymers with proteins.[30]

In summary, with the use of carefully controlled conditions of pH, temperature, and concentration, detailed information on the reaction of MDA with amino acid derivatives was obtained. These results serve as simple models for MDA–protein interactions, and the physical data obtained for the products should be useful in examining MDA interactions in more complex systems. Studies of MMDA show that although it reacts much slower than MDA, its behavior parallels that of MDA in other respects. The results suggest that MMDA may be useful in defining some aspects of the biological chemistry of MDA where the instability of the latter precludes acquisition of useful experimental data. Further studies are in progress regarding the formation, structure, and chemistry of the 1-amino-3-iminopropenes (**3**) and on the interaction of malondialdehyde with sulfur-containing amino acids.

Experimental Section

Melting points are uncorrected and were determined on a Thomas-Hoover melting point apparatus fitted with a microscope. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker HX-90E or a Joel FX-90Q pulse Fourier Transform NMR spectrometer. Mass spectra were determined on a Hitachi RMU-6-E mass spectrometer at an ionizing energy of 70 eV. Elemental analyses were performed by the University of Iowa microanalytical service. Ultraviolet spectra were recorded on a Cary model 219 spectrophotometer. Molar extinction coefficients were determined from the slope of plots of absorbance vs. concentration, and absorbance values were obtained from freshly made solutions of the analytically pure products (10^{-5}–10^{-6} M) in 0.2 M, pH 4.2 acetic acid–sodium acetate buffer.

Materials. Amino acid derivatives and malondialdehyde bis(dimethyl acetal) were purchased from Aldrich Chemical Co., Milwaukee, WI, or from Sigma Chemical Co., St. Louis, MO. α-*N*-Acetylhistidine,[51,56] α-*N*-acetyltyrosine,[57] α-*N*-acetyllysine,[58–60] and ϵ-*N*-acetyllysine[61] methyl

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esters were prepared by literature methods. *N*-Prop-2-enal amino acetic acid (**5**) was prepared by the method of Crawford.[33] Acetic acid–sodium acetate buffer (0.2 M, pH 4.2) was made from 19.2 g (0.32 mole) of glacial acetic acid and 6.56 g (0.08 mol) of anhydrous sodium acetate which was diluted to 2 L with deionized, degassed, distilled water. Merk silica gel PF_{254} was used for preparative layer chromatography.

Sodium malondialdehyde was prepared by a modified literature procedure.[39,40] Malondialdehyde bis(dimethylacetal) (5 mL, 30 mmol) was added to 32 mL of 1 N HCl. The flask was stoppered tightly and the suspension stirred at room temperature for several minutes until a homogeneous solution was obtained. The solution was then kept at 0 °C for 24 h. The resulting clear pale yellow solution was neutralized (pH 7–8) with 5 N NaOH and the solvent removed under reduced pressure (bath temperature <40 °C). The residue was slurried with 75 mL of CH_3OH and treated with Norit decolorizing carbon. The Norit and NaCl were filtered and the filtrate evaporated under reduced pressure. The residue was recrystallized twice from ethanol–ether and dried for 24 h at room temperature at 0.1 torr over P_2O_5 to give 1.87 g (47%) of MDA sodium salt hydrate as white needles: mp 245 °C dec; ^{1}H NMR(D_2O) δ 9.08 (d, J = 10.1 Hz, 2 H, CHO), 5.73 (t, J = 10.1 Hz, 1 H); ^{13}C NMR(D_2O) δ 193.6, 110.3. After 24 h H_2 exchanges with D_2O: δ 193.6, 110.1 (t, J_{CD} = 24.9 Hz).

Anal. Calcd for the sodium salt of $C_3H_3O_2{\cdot}H_2O$: C, 32.13; H, 4.50. Found: C, 32,36; H, 4.43.

Methylmalondialdehyde (4) was prepared by a modification of literature procedures.[41–43] Dimethylformamide (187 mL, 2.4 mol) was added dropwise to 322 g (2.2 mol) of phosphoryl chloride with vigorous stirring and cooling in ice. During the addition the temperature was kept below 30 °C. After the addition was complete, 132 g (1.0 mol) of propionaldehyde diethylacetal was added slowly with vigorous stirring and slight warming. When the exothermic reaction began, the heat was removed and the reaction temperature was maintained at 60–70 °C by the slow dropwise addition of the acetal. The solution changed from light orange to dark brown as the reaction progressed. When addition of the acetal was complete, the solution was warmed to 70 °C for 2 h, cooled to room temperature, poured into 1500 g of ice, and allowed to stand overnight. Anhydrous potassium carbonate was added until the solution was basic, and 1000 mL of water was added to dissolve the precipitated salts. The aqueous solution was then extracted with CH_2Cl_2 (5 × 200 mL). The combined organic extracts were dried over anhydrous K_2CO_3 and evaporated under reduced pressure. The liquid residue was fractionally distilled. After DMF was removed, a yellow liquid was collected at 75–80 °C (0.3–0.5 torr). The liquid was redistilled at 40 °C (0.26 torr), giving 29.6 g (26%) of 2-methyl-3-ethoxyprop-2-enal as a colorless liquid: ^{1}H NMR($CDCl_3$) δ 1.34 (t, J = 7.2 Hz, 3 H), 1.60 (s, 3 H), 4.25 (q, J = 7.2 Hz, 2 H), 7.37 (s, 1 H), 9.25 (s, 1 H); ^{13}C NMR($CDCl_3$) δ 6.3, 15.3, 71.0, 119.6, 168.6, 191.5. A second fraction was obtained at 110–115 °C (0.3 torr) which was identified as 2-methyl-3-(dimethylamino)prop-2-enal[41] (30.7 g, 27%): ^{1}H NMR($CDCl_3$) δ 1.93 (s, 3 H), 3.15 (s, 6 H), 6.58, (s, 1 H), 8.88 (s, 1 H). The 2-methyl-3-(dimethylamino)prop-2-enal (23.7 g, 0.21 mol) and 9.2 g (0.23 mol) of sodium hydroxide in 30 mL of water were stirred vigorously and warmed to 70 °C until a homogeneous solution was obtained from the original two layers (5 min). The solution was concentrated almost to dryness under reduced pressure (bath temperature 50 °C) and the sodium salt of MMDA precipitated by means of a 1:1 ethanol–acetone mixture. The product was filtered and the mother liquor evaporated almost to dryness. In the same manner a further portion of the sodium salt was isolated and the previous operation repeated once more. The residue was recrystallized from ethanol–ether and dried overnight over $CaSO_4$ at room temperature (0.2 torr), giving 14.0 g (62% from 2-methyl-3-(dimethylamino)prop-2-enal) of the sodium salt. The salt was also prepared from the 2-methyl-3-ethoxyprop-2-enal in the same manner. The free acid of MMDA was prepared and purified by the method of Moschel and Leonard.[43] The sodium salt was a fine white powder: mp 360 °C dec; ^{1}H NMR(D_2O) δ 8.90 (s, 2 H, CHO), 2.00 (s, 3 H, CH_3).

General Procedure for the Preparation of the Enaminals 6–14. Method A. The amino acid methyl ester hydrochloride was dissolved in pH 4.2 acetate buffer, and 1 equiv of MDA or MMDA sodium salt was added. The solution were diluted with buffer to give reactant concentrations of 0.02–0.20 M. The solutions were allowed to stand at room temperature, and samples were withdrawn periodically and diluted with pH 4.2 buffer for analysis of the UV spectrum. When the absorbance of product reached a maximum, the solutions were treated with a slight excess of 1 N $NaHCO_3$ and the products extracted from the aqueous solution with CH_2Cl_2 ((3–6) × 15 mL). The organic extracts were dried over anhydrous Na_2SO_4, and the solvent was evaporated under reduced pressure to give the crude enaminal. The product was purified by preparative layer chromatography on silica gel. The plates were developed with 10% methanolic CH_2Cl_2 and, the product was eluted from the gel with 25% methanolic CH_2Cl_2. The pure enaminal was obtained by evaporation of the solvent under reduced pressure and was dried over P_2O_5 overnight at room temperature (0.1 torr).

Method B. The solutions were made up and monitored by their UV spectra as in method A. When the reaction was complete, the solution was neutralized with $NaHCO_3$ and solvent evaporated under reduced pressure. The dry residue was taken up in a few milliliters of methanol, and sodium chloride was filtered. The filtrate was applied to silica gel preparative layer plates and the plates developed with 2–3 immersions in 10% methanolic CH_2Cl_2. The products were eluted from the silica gel, isolated, and dried as in method A.

The products **13a** and **13b** were purified by high pressure liquid chromatography by using an Altex model 100 pump with preparative heads, and Altex injector model numbers 905-19 with a 10.2-mL loop, and Altex model 153 analytical UV (280 nm) detector with an 8-μL analytical cell, and a Texas Instruments (servo-riter) recorder. For **13a** a column 20.5-mm i.d. × 32 cm packed with Amberlite XAD-4 (75–105-μm particle size) was used.[62] A 2-mL sample of 0.1 M neutralized reaction solution was injected with water used as eluting agent at a flow rate of 16.8 mL/min (350–500 psi). Product fractions were collected at 12–22 min, pooled, and lyophilized, giving **13a** as a white solid. A 1-mL sample of 0.1 M neutralized solution of product **13b** was injected onto a column of Amberlite XAD-4 (37–44-μm particle size 6.6-mm i.d. × 25 cm. Water was used as elutant at a flow rate of 4.2 mL/min (1500 psi), and the product fraction was collected at 8–16 min, pooled, and lyophilized, giving **13b** as a white solid.

Kinetics. The amino acid derivatives were dissolved in 0.2 M pH 4.2 acetate buffer solution at concentrations of 0.001–0.06 M and the ionic strength adjusted to 0.10 M with NaCl. The solutions were placed in a cuvette and allowed to equilibrate at 25° ± 0.5 °C in the thermostated cell holder of the spectrometer. Constant temperature was maintained by a Forma Scientific model 2095 water bath and circulator. The reaction was initiated by adding 20 μL of 5 × 10^{-3} M MDA sodium salt solution. Alternatively, solutions of the amino acid derivatives were made up in volumetric flasks, equilibrated at 25 °C in the water bath, and an aqueous solution of MDA sodium salt added to give an MDA concentration of 0.001 M. Aliquots (100 μL) were withdrawn periodically and diluted to 10 mL with acetate buffer for observing the UV spectrum. The ultraviolet spectra were recorded with buffer solution in the reference beam. The disappearance of MDA was monitored at 249 nm and of MMDA at 252 nm; the appearance of their enaminal products was followed at 279 and 288 nm, respectively. The reactions were generally followed for at least three half-lives and infinity readings taken after ten half-lives. The observed first-order rate constants, k_{obsd}, were calculated from the slopes of linear plots of ln $(A_\infty - A_t)$ vs. time. The plots had correlation coefficients of >0.995. During the first 3–4, min there is a rapid decrease in the absorbance at 249 nm due to equilibration of the isomeric forms of MDA which occurs with protonation of the enolate anion. Points taken before 4 min were therefore not included in the calculations of the slopes. This behavior could be eliminated by using solutions of MDA equilibrated with buffer. However, because of its greater stability, it was generally more convenient to use aqueous solutions of the sodium salt. The rate studies were repeated at three or more different concentrations of amino acid derivative, maintaining at least a 20-fold excess of amino acid derivative over dialdehyde. Absorbance values from solutions at A_∞ were used to calculate the equilibrium constants K_{eq} in Table V. The values have an average deviation of ±10%. The rate constants k_f and k_r for the forward and reverse reaction (eq 1) were calculated from k_{obsd} values and K_{eq} and have a deviation of less than 5%.

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Supplementary Material Available: Listing of ^{13}C NMR data for enaminals (Table V) (2 pages). Ordering information is given on any current masthead page.

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Chemistry of 1,5-Diazapentadienium (Vinamidinium) Salts: Alkylation Reactions to Multifunctional Dienamines and Dienaminones[1]

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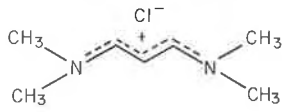
1,5-Diazapentadienium chloride **1** is a push–pull 6-π-electron system. It reacts selectively and in high yields with enolates of cyclic and acyclic ketones, esters, lactones, and lactams to produce multifunctional dienaminones. Heterocyclic systems containing activated methylene groups such as 2-ethyl-2-oxazoline, 2-picoline, and 2-methylfuran are converted to reactive dienamines. Derivatives of γ,δ-unsaturated β-keto esters, useful intermediates in organic synthesis, can be synthesized directly by selective alkylation with **1** of the dianion derived from ethyl acetoacetate. Cyclopentane-1,3-dione methyl ether reacts with **1** to produce both *E,E* and *Z,E* alkylated products.

One of the major synthetic applications of compounds containing activated methylene groups is the selective alkylation of the carbon α to the carbonyl group.[2,3] The enols and enolates of these carbonyl compounds have been alkylated with a variety of alkyl and allyl halides, as well as alkyl sulfonates, tosylates, oxonium ions and other reagents. We report the use of vinamidinium salts[4] in the alkylation of carbonyl enolates to produce dienaminones. The reaction appears to have generality, and we have applied it successfully to diesters, keto esters, cyclic and acyclic ketones, lactones, lactams, and other compounds containing activated methylene groups. The dienaminones and dienamines formed in these reactions are multifunctional compounds that have potential as intermediates in the synthesis of some natural products.

The vinamidine system (1,5-diaza-1,3-pentadiene) is present in natural products such as the betacyanin pigments found in red beets, many cacti, pokeberry and other plants[5] and in the porphyrin and corrin ring systems of chlorophyll, hemoglobin, cytochromes, and vitamin B_{12}.[6] Vinamidinium salts such as **1** are examples of push–pull alkenes, compounds that are stabilized by groups which can donate or accept electrons. They are vinylogues of amidinium salts and have an alternation of electron density; the α-carbons are electron poor and are attacked by nucleophilic reagents, and the β-carbon is electron rich and is attacked by electrophilic reagents.[7,8] The enhanced stability and push–pull nature of the vinamidinium system gives it regenerative character which makes it prone to substitution rather than addition reactions. The regenerative character of the vinamidinium salts has been demonstrated in both electrophilic reactions such as halogenation, nitration, and Vilsmeier type alkylations,[9] and in nucleophilic reactions with amines and carbon nucleophiles. The nucleophilic reactions have been exploited the most and have led to the synthesis of some polycyclic aromatic and heterocyclic compounds.[10–12] Vinamidinium salts have been used to alkylate the activated methylenes of various nitriles,[12,13] but there is only one report of the alkylation of other types of activated methylene compounds.[14]

Results and Discussion

In previous work with vinamidines, the perchlorate salts were used.[15] In this work we found a convenient method for the preparation of the tetramethylvinamidinium chloride salt **1** in good yield using readily available com-



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mercial reagents. This procedure involved the preparation of β-(dimethylamino)acrolein by a Vilsmeier reaction on

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Table I. Procedures and Product Yields in the Reaction of the Vinamidinium Salt 1 with Various Substrates

substrate	method	reaction conditions: temp, °C	reaction conditions: time	product	yield, %	mp, °C
diethyl malonate	A	reflux	10 h	**2**	73	51–53
ethyl acetoacetate	B	25	25 h	**4a, 4b**	57	88–90
3-pentanone	C	25	33 h	**3**	66	102–103
2-butanone	B	25	46 h	**5a, 5b**	78	84–87
2-butanone	D	0	4.5 h	**5a, 5b**	78	84–87
cyclopentanone	B	25	26 h	**9a**	88	89–90
cyclopentanone	D	25	5 h	**9a**	88	89–90
cyclohexanone	B	25	30 h	**10**	73	86–89
cycloheptanone	C	25	51 h	**11**	60	oil
cycloheptanone	D	0	4 h	**11**	60	oil
dl-camphor	C	reflux	40 h	**16**	33	oil
estrone 3-methyl ether	B	25	5 days	**15**	56	209–210
estrone 3-methyl ether	D	25	18 h	**15**	80	209–210
γ-butyrolactone	B	25	48 h	**12**	91	100–101
γ-butyrolactone	D	25	6 h	**12**	91	100–101
δ-valerolactone	C	25	38 h	**13**	86	101–103
δ-valerolactone	D	25	4 h	**13**	86	101–103
1-ethyl-2-pyrrolidinone	D	0	3 h	**14**	51	75–78
2-ethyl-2-oxazoline	D	−70	4 h	**6**	44	97–100
2-picoline	D	0	1.5 h	**7**	50	oil

ethyl vinyl ether.[16] The β-(dimethylamino)acrolein was isolated in 57% yield and was characterized by ^1H and ^{13}C NMR data and by mass spectrometry. The vinamidinium salt 1 was obtained by heating the β-(dimethylamino)-acrolein and dimethylamine hydrochloride in absolute ethanol under reflux. The reaction was monitored by UV spectroscopy and was stopped when the UV absorption maximum reached 309 nm. The product, a white solid which was isolated in 70% yield, must be recrystallized twice to avoid contamination with dimethylamine hydrochloride. The salt 1 was found to be hygroscopic, similar to the vinamidinium perchlorates, was dried over P_2O_5, and was stored in a desiccator. Once dried, the salt is stable indefinitely and can be handled in air for short periods of time. Its ^{13}C NMR spectrum showed the resonance for the α-carbons at 164.2 ppm and the resonance for the β-carbon at 90.3 ppm typical of the alternation of electron density in push–pull alkene systems.[17] The ^1H NMR spectrum also had only one resonance for H_α, a doublet at 8.60 ppm (J = 12.0 Hz). The β-carbon proton was observed as a triplet at 5.26 ppm (J = 12.0 Hz). The single resonance observed for H_α and C_α in the ^1H and ^{13}C NMR spectra and the coupling constant of 12.0 Hz are consistent with an all-trans or W form for the stereochemistry of 1 in nonpolar solvents. This geometry is typical of tetrasubstituted vinamidinium salts.[17,18] As the salt 1 contains a cation, the mass spectrum does not produce a parent peak, but the parent minus the chloride anion is observed and is, in fact, the base peak. The UV max (EtOH) at 309 nm of 1 provided a very convenient marker for monitoring the alkylation reactions.

When the vinamidinium salt 1 was treated with enolates generated in situ by reaction of sodium hydride with cyclic or acyclic carbonyl compounds in triethylamine or pyridine, dienaminones were isolated in good yields. The results are shown in Table I. It is essential to keep these reactions free from water in that sodium hydroxide produced by reaction of sodium hydride with water rapidly converts salt 1 to β-(dimethylamino)acrolein. The sodium hydride itself does not appear to attack the vinamidinium system even at elevated temperatures for several days.

In our preliminary work,[1] the reactions with 1 were carried out with enolates generated in situ by reaction of sodium hydride with the carbonyl compounds. In subsequent work, we investigated the possibility of using lithium diisopropylamide (LDA) as an alternative to sodium hydride particularly for substrates with low acidity. We found LDA to be an excellent base which generated anions readily where sodium hydride did not (e.g., lactam, oxazoline, picoline). In addition, there was no evidence of amine exchange between LDA and the vinamidinium salt under the reaction conditions used, a consideration that had dictated initially our use of sodium hydride in this work. We then reinvestigated with LDA several of the reactions with carbonyl compounds in which sodium hydride was used as base. An improvement in yield was noted in only one case (15). However, dramatic reductions in reaction times were observed in all cases. For example, the reaction time for the conversion of cyclopentanone to its dienaminone decreased from 26 h to 5 h, and the reaction time for the conversion of δ-valerolactone was reduced from 38 to 4 h. The greater effectiveness of LDA as the base in these alkylation reactions is probably due to its higher solubility in the reaction medium.

The reactions were extended to include the alkylation of heterocyclic systems containing activated methylene groups such as 2-ethyl-2-oxazoline, 2-picoline, and 2-methylfuran. The anion of 2-ethyl-2-oxazoline reacts with the salt 1 at −70 °C to give the dienamine **6**. It is necessary to run this reaction at low temperature because of the known tendency of oxazoline anions to rearrange thermally.[19] The anion of 2-picoline generated by reaction with LDA rapidly turns dark blue after addition of 1, and at the termination of the reaction (~1 h), the solution slowly turns yellow, allowing visual monitoring of the transformation. In the presence of LDA, 2-methylfuran reacts rapidly with 1 as evidenced by the appearance of an intense peak at 414 nm in the UV–visible spectrum. However, the instability of the dienamine products precluded further investigation of this reaction at this time.[20]

When dry reagents and solvents are used, high yields of dienamines and dienaminones are obtained in all of the

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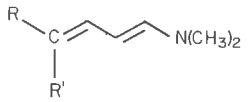
reactions except in cases of inherent product instability. All of the reactions referred to above could be monitored very conveniently by UV spectroscopy. The substrates absorbed at wavelengths shorter than those for 1 (in the range 250–300 nm), and the products absorbed at wavelengths longer than those for 1 (in the range 350–420 nm). The absorption maxima of the products could be predicted readily by using Woodward's UV rules.[21] However, as no value was available for a dimethylamino group δ to a carbonyl, a value of 135 nm was assigned. This gave good agreement between the predicted and observed wavelengths of the products in all cases.

Close inspection of the spectral data for the dienamines and the dienaminones revealed several common characteristics. The ^{1}H NMR data (in $CDCl_3$) indicate that these compounds exist in the all-trans or W stereochemistry ($J \approx 12$ Hz). The chemical shifts for the proton on the carbon β to the amino group were all similar, with the resonance observed as a triplet at 4.87–6.41 ppm. The proton on the carbon α to the amino group characteristically appeared as a doublet at 6.32–7.33 ppm. Similar trends were found in the ^{13}C NMR data. The mass spectra of all of these compounds gave parent ions (by EI or CI methods) and fragmentation patterns consistent with their structures.

Symmetrical compounds such as diethyl malonate and 3-pentanone formed single dienaminone products. In the case of 3-pentanone, only one geometric isomer (*E,E*) was observed (rationale for the stereochemical assignments is discussed below for the cyclopentanone case). Ethyl acetoacetate reacted with 1 to produce the *Z,E* and *E,E* isomers **4a** and **4b** in a 44:56 ratio, respectively. When an



2, R = R′ = $CO_2CH_2CH_3$
3, R = $COCH_2CH_3$; R′ = CH_3
4a, R = $COCH_3$; R′ = $CO_2CH_2CH_3$
4b, R = $CO_2CH_2CH_3$; R′ = $COCH_3$
5a, R = $COCH_2CH_3$; R′ = H
5b, R = $COCH_3$; R′ = CH_3

6, R =  R′ = CH_3

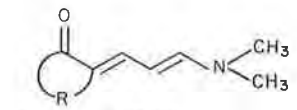
7, R =  R′ = H

unsymmetrical ketone such as 2-butanone was treated with the vinamidinium salt 1, two products, **5a** and **5b**, were isolated by HPLC on silica gel in a 70:30 ratio respectively. The products are formed in a ratio consistent with the operation of kinetic control in this reaction. This type of regioselectivity could be exploited in a synthetic scheme involving unsymmetrical compounds.

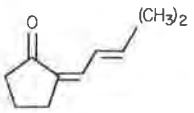
The reaction of cyclopentanone with the vinamidinium salt 1 was of particular interest to us because of the possible ramifications of the resulting dienaminone **9a** (Chart I) in natural product synthesis. The structure of **9a** was established by ^{1}H and ^{13}C NMR data and by its mass spectrum. A detailed ^{1}H NMR analysis showed that only one compound was formed. The coupling constant of 12.5 Hz was consistent with the all-trans or W arrangement seen in the other dienaminones. Further, in the case of cyclopentanone (and other cyclic carbonyl containing systems), the structure was shown to be **9a** (*E,E*) and not its stereoisomer **9b** (*Z,E*) by comparison of chemical shift data with related ring systems containing exocyclic double

(21) Woodward, R. B. *J. Am. Chem. Soc.* **1941**, *63*, 1123.

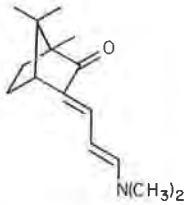
Chart I



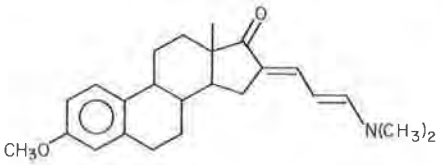
9a, R = $-(CH_2)_3-$
10, R = $-(CH_2)_4-$
11, R = $-(CH_2)_5-$
12, R = $-O(CH_2)_2-$
13, R = $-O(CH_2)_3-$
14, R = $CH_3CH_2N(CH_2)_2-$



9b



16



15

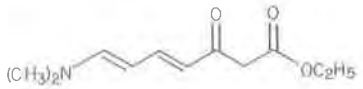
bonds.[22,23] On this comparative basis the exocyclic methylene proton of structure **9a** would be expected to have chemical shift of 6.8 ppm or more. The observed value was 7.08 ppm. The *Z,E* isomer **9b** would be expected to have a chemical shift for the exocyclic methylene proton of 5.6 to 5.9 ppm, much less than our observed value. Also present was a trans allylic coupling constant of 2.0 Hz between the C-3 ring protons and the exocyclic methylene proton (doublet of triplets).[24] The ^{13}C NMR spectrum exhibited one resonance for each carbon. Assignment of these resonances was aided by off-resonance and delayed ^{1}H decoupling experiments. The carbon α to the amine appeared at 152.3 ppm, and the carbon β to the amine appeared at 95.3 ppm. The resonance at 123.8 ppm (singlet in off-resonance decoupled spectrum) was assigned as the carbon δ to the amine. The resonance at 136.3 ppm (doublet) was assigned to the γ-carbon. The carbon resonances of the dienaminone appear to have an alternation of electron density similar to that observed in the vinamidinium system. This alternation of electron density is consistent with the generation of a new meneidic system in dienaminone formation.

Another interesting carbonyl system studied was γ-butyrolactone. We were particularly interested in this compound because of the possibility of entry into analogues of natural products containing the α-methylene-γ-butyrolactone moiety.[25] The γ-butyrolactone was an excellent substrate for 1 and gave the dienaminone **12** in 91% yield. As observed for the dienaminones of cyclopentanone, cyclohexanone, and cycloheptanone, the products from the reaction with γ-butyrolactone and δ-valerolactone also had the stereochemistry represented in **12** and **13** as evidenced from ^{1}H and ^{13}C NMR data.

(22) Crandall, J. K.; Arrington, J. P.; Hen, J. *J. Am. Chem. Soc.* **1967**, *89*, 6208.
(23) Paquette, L. A.; Eizember, R. F. *J. Am. Chem. Soc.* **1967**, *89*, 6205.
(24) Jackman, L. M.; Sternhell, S. "Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry", 2nd ed.; Pergamon: Sydney, 1969; p 316.
(25) Kupchan, S. M. *Pure Appl. Chem.* **1970**, *21*, 227.

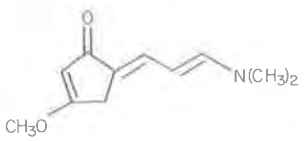
An example of the alkylation reaction with a bicyclic ketone was the transformation of camphor to **16**. The yield obtained is comparable to the yields observed with other alkylations of camphor.[26] Reaction of **1** with a more complex ketone, estrone 3-methyl ether, gave the product **15** (Chart I). It is of interest to note that estrone derivatives with alkyl substitution at position 16 have been found to have antiandrogenic properties.[27]

γ,δ-Unsaturated β-keto esters are useful intermediates in organic synthesis.[28] The direct synthesis of related keto esters can be realized by selective alkylation by **1** of the dianion from ethyl acetoacetate. This methodology allows the alkylation of the less stable of the two enolates.[29,30] Thus, when ethyl acetoacetate was converted to its dianion by reaction with sodium hydride followed by *n*-butyllithium, the resulting dianion reacted smoothly with sodium hyride followed by *n*-butyllithium, the resulting dianion reactes smoothly with the vinamidinium salt to give the unsaturated keto ester **17** in 74% conversion as determined by UV spectroscopy. The actual isolated yield was much lower than this because of product instability.

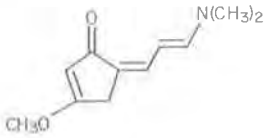


17

The methyl ether of cyclopentane-1,3-dione reacted with **1** to give **18a** (*E,E*) and **18b** (*Z,E*) in 19% and 12% pure



18a

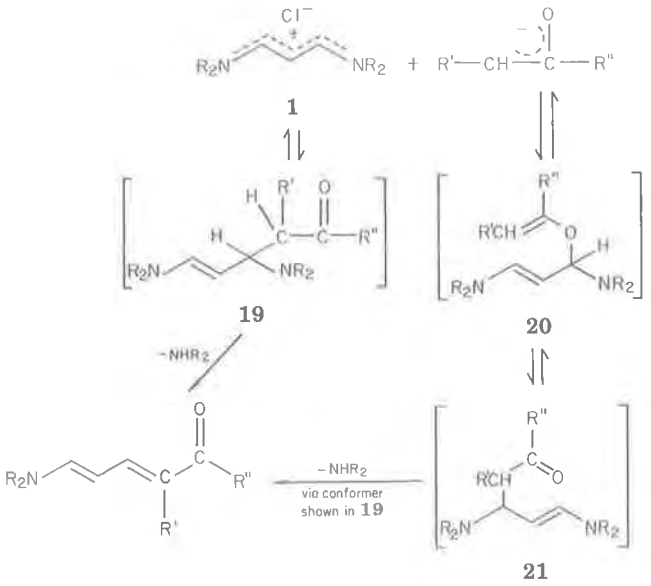


18b

isolated yields, respectively. Considerable decomposition occurred during the workup of this reaction, and the isolated yields were much lower than the spectrophotometrically determined percent conversions. The dianion of cyclopentane-1,3-dione gave even lower yields than that observed for the methyl ether. It should be mentioned with respect to **18b** that this was the only example of a dienaminone with *Z,E* stereochemistry isolated in this study.

All of the reactions discussed above involve nucleophilic attack of enolate (or other stabilized carbanionic species) on the α-carbon of the vinamidinium salt. The intermediate σ complex **19** formed, for example, in the case of the carbonyl-activated methylenes can eliminate a molecule of dimethylamine with the assistance of a tertiary amine such as triethylamine (Scheme I). Elimination of the amine from the σ complex drives the equilibrium forward and produces a new push–pull system. For elimination of the molecule of amine, the σ complex must have a hydrogen atom on the carbon β to the amino group. O-Alkylation, a troublesome side reaction in many direct alkylations of carbonyl-activated methylenes,[3] is not important in these alkylations. The intermediate formed, **20**, cannot eliminate a molecule of dimethylamine readily and probably collapses to give starting materials. This O-alkylated intermediate may undergo a [3,3]sigmatropic

Scheme I. Mechanism of Formation of Dienaminones and Dienamines



rearrangement to give **21** which could eliminate dimethylamine through a conformer similar to **19** to give the dienaminone product. The Claisen rearrangement may not be a significant pathway in most of these alkylations because of the relative low temperatures (generally 25 °C) used in this work.[31]

In summary, the reactions of the vinamidinium salt **1** with activated methylene groups result in the selective introduction of a conjugated three-carbon moiety in these molecules. We are presently examining some interesting transformations of these compounds including their utilization in natural products synthesis.

Experimental Section

The melting points reported are uncorrected and were taken on a Thomas-Hoover melting point apparatus fitted with a microscope. The infrared spectra were recorded on a Beckman IR-20A. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker HX-90E pulse Fourier transform NMR spectrometer interfaced with a Nicolet 1080 computer and disk unit or on a JEOL FX90Q pulse Fourier transform NMR spectrometer. Tetramethylsilane was the internal reference. The mass spectrometers employed were a Hitachi RMU-6E instrument and a Hewlett-Packard 5985 GC/MS system. The ultraviolet data were taken with a Cary Model 219 ultraviolet–visible spectrophotometer. Elemental analyses were performed by the University of Iowa Microanalytical Service on an automated Perkin-Elmer Model 240 carbon, hydrogen, and nitrogen analyzer. HPLC separations were done with an Altex Model 100 pump with preparative heads and an Altex Model 905-19 injector with a 10.2-mL loop and were monitored with a Tracor 970 detector with a Corasil (37–50 μm) silica gel column (1.0 cm 0.0 × 35 cm).

β-(Dimethylamino)acrolein. This compound was prepared by the method of Makin, Shavrygina, and co-workers[16] except that the product was obtained by extraction with methylene chloride instead of a benzene–alcohol mixture. The product, a clear amber liquid, was obtained in 58% yield: bp 121–123 °C (2.5 torr); IR (neat) 1680 (C=O), 1675 (C=C, trans); ^{1}H NMR ($CDCl_3$) δ 2.86 (s, 3 H), 3.15 (s, 3 H), 5.12 (dd, 1 H, J = 8.5, 12.7 Hz), 7.19 (d, 1 H, J = 12.7 Hz), 9.04 (d, 1 H, J = 8.5 Hz); mass spectrum, m/z (relative intensity) 99 (M^+, 100), 84 ($M^+ - CH_3$, 45), 71 (M^+ – CHO, 15), 55 ($M^+ - N(CH_3)_2$, 44).

1,1,5,5-Tetramethyl-1,5-diazapentadienium Chloride (1). β-(Dimethylamino)acrolein (17.5 g, 0.17 mol) and dry dimethylamine hydrochloride (14.4 g, 0.17 mol) in absolute ethanol

(26) McConaghy, J. S.; Bloomfield, J. J. *J. Org. Chem.* **1968**, *33*, 3425.
(27) Nakayama, R.; Goto, G.; Yoshioka, K.; Hiraga, K.; Masudka, M. *Chem. Pharm. Bull.* **1978**, *26*, 1718.
(28) van den Goorbergh, J. A. M.; van der Gen, A. *Tetrahedron Lett.* **1980**, *21*, 3621 and references therein.
(29) Stuart, N. H.; Weiler, L. *J. Am. Chem. Soc.* **1974**, *96*, 1082.
(30) Krapcho, A. P.; Jahngen, E. G. E.; Kashdar, D. S. *Tetrahedron Lett.* **1974**, 2721.
(31) Rhoads, S. J.; Raulins, N. R. *Org. React.* **1974**, *25*, 1–252.

(120 mL) were heated under reflux in a Soxhlet extractor, thimble charged with 3-Å molecular sieves, for 8.5 h under a positive N_2 atmosphere. The solvent was then removed under reduced pressure. The residue, an orange oil, was slurried with ether (3 × 120 mL), and the resulting solid was recrystallized twice from acetone. The product was hygroscopic and was isolated by filtration under N_2. The product was an off-white solid: 20.0 g (70%); mp 187–189 °C (lit.[32] mp 188–190 °C); UV max (EtOH) 309 nm (ε 47 115); ^{1}H NMR ($CDCl_3$) δ 3.17 (s, 6 H), 3.42 (s, 6 H), 5.26 (t, 1 H, J = 12.0 Hz), 8.60 (d, 2 H, J = 12.0 Hz); ^{13}C NMR ($CDCl_3$) δ 38.5, 46.5, 90.3, 164.2; mass spectrum, m/z (relative intensity) 127 (M^+ – Cl, 100), 112 (M^+ – Cl, CH_3, 13), 97 (M^+ – Cl, $2CH_3$, 9), 82 (M^+ – Cl, $3CH_3$, 66).

Methods for the Preparation of Dienaminones. Four different experimental procedures were used for the synthesis of the dienaminones. In all of these procedures the reactions were run in oven-dried glassware under a positive nitrogen atmosphere. The vinaminidinium salt was dried over P_2O_5 in vacuo prior to use. The solvents were also dried prior to use. THF was distilled from $LiAlH_4$. Triethylamine and pyridine were distilled from NaH under N_2. Mineral oil was removed from the NaH dispersion by rinsing with dry hexane before the addition of other reagents. The reaction progress was monitored by UV spectroscopy. The starting materials absorbed in the range of 250–310 nm, and the products absorbed in the range of 370–423 nm. The results of these reactions are summarized in Table I. All reactions were worked up by removal of the solvent under reduced pressure, addition of saturated NaCl solution (15 mL) to the residue (cautiously!), and extraction with CH_2Cl_2 (4 × 20 mL). The combined organic layers were dried over Na_2SO_4. After removal of the solvent, the products were purified by preparative layer chromatography on either E. Merck silica gel-PF-254 or aluminum oxide 60-PF-254 plates.

In method A the carbonyl compound (1.1 mmol) in THF (3 mL) was added dropwise with stirring to the vinamidinium salt (1.0 mmol) and NaH (1.5 mmol) in pyridine (7 mL) at 0 °C. This was followed by stirring at 0 °C for 30 min and then stirring under the specified conditions (Table I). The products were isolated by the standard workup procedure and were purified by the specified chromatographic procedures. In method B the carbonyl compound (1.1 mmol) in THF (3 mL) was added dropwise with stirring to the vinamidinium salt (1.0 mmol) and NaH (1.5 mmol) in triethylamine (7 mL) at 0 °C. This was followed by stirring at 0 °C for 30 min and then stirring under the specified conditions. The products were isolated by the standard workup procedure and were purified by the specified chromatographic procedures. In method C the carbonyl compound (1.1 mmol) in THF (3 mL) was added dropwise with stirring to the vinamidinium salt (1.0 mmol) and NaH (1.5 mmol) in triethylamine (7 mL) over 4-Å molecular sieves (0.3 g) at 0 °C. This was followed by stirring at 0 °C for 30 min and then stirring under the specified conditions. The products were isolated by the standard workup procedure and were purified by the specified chromatographic procedures. In method D the carbonyl compound (1.1 mmol) in dry THF (3 mL) was added dropwise to a stirred solution of lithium diisopropylamide (LDA). [The LDA was generated by adding 1.3 M *n*-BuLi (1.0 mL) to dry diisopropylamine (1.3 mmol) in dry THF (5 mL) at 0 °C and stirring the mixture at 0 °C for 20 min after the addition was complete.] To this was added dry triethylamine (4 mL) and the vinamidinium salt (1.0 mmol). The reaction was stirred at 0 °C for 30 min and then under the specified conditions. The reaction was quenched by carefully pouring the reaction mixture into saturated NaCl solution (20 mL) followed by the standard aqueous workup. The products were purified by the specified chromatographic procedures. Unless stated otherwise, all crystalline products were recrystallized from hexane or hexane/ether.

Dienaminone of Diethyl Malonate 2. Method A. The residue after the workup was chromatographed on silica gel preparative-layer plates which were developed with 3% MeOH/acetone. The band with R_f 0.52 was cut out and eluted with 5% MeOH/acetone. The dienaminone **2** was obtained as yellow crystals: UV max (EtOH) 372 nm (ε 54 990); ^{1}H NMR ($CDCl_3$) δ 1.25 (t, 3 H, J = 7.5 Hz), 1.30 (t, 3 H, J = 7.5 Hz), 3.00 (s, 6 H), 4.25 (q, 2 H, J = 7.5 Hz), 4.30 (q, 2 H, J = 7.5 Hz), 6.05 (t, 1 H, J = 13.0 Hz), 7.00 (d, 1 H, J = 13.0 Hz), 7.70 (d, 1 H, J = 13.0 Hz); ^{13}C NMR ($CDCl_3$) δ 14.5, 59.7, 59.8, 97.1, 106.3, 153.6, 156.9, 167.0; mass spectrum, m/z (relative intensity) 241 (M^+, 100), 196 (M^+ – $HN(CH_3)_2$, 85), 168 (M^+ – CO_2Et, 33), 97 (39), 94 (55), 82 (92).

Anal. Calcd for $C_{12}H_{19}NO_4$: C, 59.73; H, 7.94; N, 5.81. Found: C, 59.73; H, 7.60; N, 5.67.

Dienaminones of Ethyl Acetoacetate, 4a,b. Method B. The residue after the workup was chromatographed on silica gel preparative-layer plates which were developed with 5% acetone/EtOAc. The band with R_f 0.50 was cut out and eluted with 7% acetone/EtOAc. The dienaminones **4a,b** were obtained as a tan solid: UV max (EtOH) 393 nm (ε 54 798); ^{1}H NMR ($CDCl_3$) δ 1.30 (t, 3 H, J = 7.8 Hz), 1.35 (t, 3 H, J = 7.8 Hz), 2.39 (s, 3 H), 2.43 (s, 3 H), 3.07 (s, 12 H), 4.23 (q, 4 H, J = 7.8 Hz), 6.41 (t, 1 H, J = 12.7 Hz), 6.77 (t, 1 H, J = 12.7 Hz), 7.33 (d, 2 H, J = 12.7 Hz), 7.51 (d, 1 H, J = 12.7 Hz), 7.76 (d, 1 H, J = 12.7 Hz); isomers observed in 44% and 56% yields by NMR; ^{13}C NMR ($CDCl_3$) δ 14.5, 29.7, 31.8, 35.0, 59.6, 98.7, 99.3, 113.3, 115.0, 154.4, 155.0, 160.3, 168.3, 196.2, 198.7, both isomers observed; mass spectrum, m/z (relative intensity) 211 (M^+, 50), 196 (M^+ – CH_3, 27), 168 (M^+ – CH_3CO, 33), 167 (M^+ – $N(CH_3)_2$, 67), 124 (30), 94 (37), 45 ($(CH_3)_2NH^+$, 100), 44 ($CH_2NHCH_3^+$, 67).

Anal. Calcd for $C_{11}H_{17}NO_3$: C, 62.53; H, 8.11; N, 6.63. Found: C, 62.21; H, 7.83; N, 6.56.

Dienaminone of Cyclopentanone, 9a. Method B. The residue after the workup was chromatographed on aluminum oxide preparative-layer plates which were developed with 3% acetone/EtOAc. The band with R_f 0.50 was cut out and eluted with 5% acetone/EtOAc. The dienaminone **9a** was obtained as yellow crystals: UV max (EtOH) 396 nm (ε 42 515); ^{1}H NMR ($CDCl_3$) δ 1.88 (m, 2 H), 2.33 (m, 2 H), 2.51 (m, 2 H), 2.91 (s, 6 H), 4.91 (t, 1 H, J = 12.5 Hz), 6.73 (d, 1 H, J = 12.5 Hz), 7.08 (dt, 1 H, J = 12.5, 2.0 Hz); ^{13}C NMR ($CDCl_3$) δ 19.8, 27.2, 38.7, 40.7, 95.3, 123.8, 136.3, 152.3, 206.0; mass spectrum, m/z (relative intensity) 165 (M^+, 100), 164 (M^+ – H, 27), 150 (M^+ – CH_3, 33), 121 (–M^+ – $N(CH_3)_2$, 70), 109 (61), 108 (24), 94 (44), 93 (33).

Anal. Calcd for $C_{10}H_{15}NO$: C, 72.69; H, 9.15; N, 8.48. Found: C, 72.24; H, 8.99; N, 8.48.

Dienaminone of Cyclohexanone, 10. Method B. The residue after the workup was chromatographed on aluminum oxide preparative-layer plates which were developed with 3% acetone/EtOAc. The band with R_f 0.58 was cut out and eluted with 5% acetone/EtOAc. The dienaminone **10** was obtained as a brown solid: UV max (EtOH) 397 nm (ε 37 901); ^{1}H NMR ($CDCl_3$) δ 1.76 (m, 4 H), 2.68 (m, 4 H), 2.91 (s, 6 H), 5.00 (t, 1 H, J = 12.5 Hz), 6.76 (d, 1 H, J = 12.5 Hz), 7.41 (dt, 1 H, J = 12.5, 2.0 Hz); ^{13}C NMR ($CDCl_3$) δ 23.0, 23.2, 25.9, 38.8, 40.5, 94.1, 121.4, 140.8, 151.7, 198.1; mass spectrum, m/z (relative intensity) 179 (M^+, 91), 164 (M^+ – CH_3, 5), 136 (31), 135 (M^+ – $N(CH_3)_2$, 100), 134 (M^+ – $HN(CH_3)_2$, 33), 108 (26), 94 (28).

Anal. Calcd for $C_{11}H_{17}NO$: C, 73.69; H, 9.56; N, 7.81. Found: C, 73.18; H, 9.31; N, 7.53.

Dienaminone of 3-Pentanone, 3. Method C. The residue after the workup was chromatographed on aluminum oxide preparative-layer plates which were developed with 25% EtOAc/$CHCl_3$. The band with R_f 0.69 was cut out and eluted with 27% EtOAc/$CHCl_3$. The dienaminone **3** was obtained as yellow crystals: UV max (EtOH) 385 nm (ε 44 081); ^{1}H NMR ($CDCl_3$) δ 1.10 (t, 3 H, J = 7.5 Hz), 1.82 (s, 3 H), 2.61 (q, 2 H, J = 7.5 Hz), 2.93 (s, 6 H), 5.13 (t, 1 H, J = 12.2 Hz), 6.70 (d, 1 H, J = 12.2 Hz), 7.21 (d, 1 H, J = 12.2 Hz); ^{13}C NMR ($CDCl_3$) δ 9.9, 11.2, 29.8, 40.6, 95.0, 123.5, 142.2, 149.9, 200.7, mass spectrum, m/z (relative intensity) 167 (M^+, 15), 123 (35), 122 (M^+ – $HN(CH_3)_2$, 100), 121 (88), 107 (M^+ – CH_3, $HN(CH_3)_2$, 37), 94 (29), 93 (40), 79 (67).

Anal. Calcd for $C_{10}H_{17}NO$: C, 71.81; H, 10.25; N, 8.38. Found: C, 71.74; H, 10.15; N, 8.09.

Dienaminones of 2-Butanone, 5a,b. Method B. The residue after the workup was chromatographed on aluminum oxide preparative-layer plates which were developed with EtOAc. The band with R_f 0.60 was cut out and eluted with 2% acetone/EtOAc. The dienaminones **5a,b** were obtained as a yellow oil. The isomers **5a** and **5b** were separated by HPLC silica gel with 5% EtOAc/CH_2Cl_2. The separation was monitored at 395 nm. The flow

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rate of 5.6 mL/min was used. The first peak had a retention time of 13 min and was identified as **5b** which was 30% of the mixture. The second peak had a retention time of 22 min and was identified as **5a** which was 70% of the mixture. For the dienaminone **5a**: UV max (EtOH) 370 nm (ε 44 702); ^{1}H NMR ($CDCl_3$) δ 1.10 (t, 3 H, J = 7.5 Hz), 2.46 (q, 2 H, J = 7.5 Hz), 2.89 (s, 6 H), 5.12 (t, 1 H, J = 12.1 Hz), 5.85 (d, 1 H, J = 14.6 Hz), 6.70 (d, 1 H, J = 12.1 Hz), 7.31 (dd, 1 H, J = 12.1, 14.6 Hz); ^{13}C NMR ($CDCl_3$) δ 9.3, 29.7, 40.6, 96.8, 116.8, 146.3, 151.7, 230.9 For the dienaminone **5b**: UV max (EtOH) 380 nm (ε 44 300); ^{1}H NMR ($CDCl_3$) δ 1.81 (s, 3 H), 2.27 (s, 3 H), 2.92 (s, 6 H), 5.11 (t, 1 H, J = 12.1, 11.7 Hz), 6.69 (d, 1 H, J = 11.7 Hz), 7.15 (d, 1 H, J = 12.1 Hz); ^{13}C NMR ($CDCl_3$) δ 11.0, 29.6, 40.6, 95.1, 124.5, 143.5, 150.1, 229.5; mass spectrum, m/z (relative intensity) 108 ($M^+ - HN(CH_3)_2$, 92), 107 ($M^+ - HN(CH_3)_2 - H$, 100), 79 ($M^+ - HN(CH_3)_2 - C_2H_6$, 35), 77 ($M^+ - Et - HN(CH_3)_2$, 33), 54 (10), 52 (14), 51 ($M^+ - EtCO - HN(CH_3)_2$, 17).

Anal. Calcd for $C_9H_{15}NO \cdot H_2O$: C, 63.12; H, 10.01; N, 8.18. Found: C, 63.55; H, 9.76; N, 7.97.

Dienaminone of Estrone 3-Methyl Ether, 15. Method B. The residue after the workup was chromatographed on aluminum oxide preparative-layer plates which were developed with 1% acetone/$CHCl_3$. The band with R_f 0.50 was cut out and eluted with 3% acetone/$CHCl_3$. The dienaminone **15** crystallized as a pale yellow solid: UV max (EtOH) 390 nm (ε 45 778); ^{1}H NMR ($CDCl_3$) δ 0.90 (s, 3 H), 1.23 (m, 4 H), 1.50 (m, 4 H), 2.18 (m, 2 H), 2.87 (s, 6 H), 3.73 (s, 3 H), 4.93 (t, 1 H, J = 13.4 Hz), 6.68 (d, 1 H, J = 13.4 Hz), 7.14 (m, 4 H); ^{13}C NMR ($CDCl_3$) δ 14.9, 26.1, 26.8, 29.6, 31.9, 37.8, 40.6, 44.6, 48.1, 55.0, 95.1, 111.3, 113.8, 123.6, 126.1, 132.5, 136.4, 137.7, 151.5, 157.4, 208.6; mass spectrum, m/z (relative intensity) 365 (M^+, 100), 350 ($M^+ - CH_3$, 4), 321 ($M^+ - (CH_3)_2N$, 12), 286 ($M^+ - C_5H_5N$, 6), 186 (6), 160 (2), 110 (12), 73 (13).

Anal. Calcd for $C_{24}H_{31}O_2N \cdot 0.5H_2O$: C, 76.97; H, 8.61; N, 3.74. Found: C, 77.18; H, 8.61; N, 3.44.

Dienaminone of Cycloheptanone, 11. Method C. The residue after the workup was chromatographed on aluminum oxide preparative-layer plates which were developed with 4% EtOAc/CH_2Cl_2. The band with R_f 0.45 was cut out and eluted with 10% EtOAc/CH_2Cl_2. The dienaminone **11** was isolated as a yellow oil: UV max (EtOH) 383 nm (ε 33 969); ^{1}H NMR ($CDCl_3$) δ 1.68 (s, 8 H), 2.55 (m, 2 H), 2.89 (s, 6 H), 5.09 (t, 1 H, J = 12.5, 12.1 Hz), 6.75 (d, 1 H, J = 12.5 Hz), 7.28 (d, 1 H, J = 12.1 Hz); ^{13}C NMR ($CDCl_3$) δ 25.4, 27.2, 29.6, 34.9, 40.7, 43.5, 94.2, 126.8, 139.9, 151.8, 200.0; mass spectrum, m/z (relative intensity) 193 (M^+, 1), 163 ($M^+ - Et$, 1), 149 ($M^+ - N(CH_3)_2$, 100), 133 (18), 123 (1), 121 (4), 104 (11).

This compound was too unstable to give satisfactory elemental analysis.

Dienaminone of δ-Valerolactone, 13. Method C. The residue after the workup was chromatographed on aluminum oxide preparative-layer plates which were developed with 4% EtOAc/CH_2Cl_2. The band with R_f 0.48 was cut out and eluted with 10% EtOAc/CH_2Cl_2. After crystallization the dienaminone **13** was obtained as a pale yellow solid: UV max (EtOH) 365 nm (ε 38 422); ^{1}H NMR ($CDCl_3$) δ 1.91 (m, 2 H, J = 5.1 Hz), 2.44 (t, 2 H, J = 5.1 Hz), 2.92 (s, 6 H), 4.24 (t, 2 H, J = 5.1 Hz), 4.96 (t, 1 H, J = 12.5, 12.1 Hz), 6.72 (d, 1 H, J = 12.5 Hz), 7.51 (dt, 1 H, J = 12.1, 2.0 Hz); ^{13}C NMR ($CDCl_3$) δ 22.8, 23.5, 40.6, 67.9, 93.7, 108.6, 145.1, 151.6, 168.6; mass spectrum, m/z (relative intensity) 181 (M^+, 100), 166 ($M^+ - CH_3$, 5), 137 ($M^+ - CO_2$, 28), 108 ($C_6H_5O_2^+$, 25) 94 (43), 82 (37), 44 (11).

Anal. Calcd for $C_{10}H_{15}NO_2$: C, 66.27; H, 8.34; N, 7.73. Found: C, 66.58; H, 8.54; N, 7.46.

Dienaminone of γ-Butyrolactone, 12. Method C. The residue after the workup was chromatographed on aluminum oxide preparative-layer plates which were developed with EtOAc. The band with R_f 0.50 was cut out and eluted with 2% acetone/EtOAc. The dienaminone **12** was obtained as yellow crystals: UV max (EtOH) 358 nm (ε 33 319); ^{1}H NMR ($CDCl_3$) δ 2.80 (m, 2 H), 2.99 (s, 6 H), 4.35 (t, 2 H, J = 7.5 Hz), 4.87 (t, 1 H, J = 12.2 Hz), 6.73 (d, 1 H, J = 12.2 Hz), 7.19 (dt, 1 H, J = 12.2, 2.0 Hz); ^{13}C NMR ($CDCl_3$) δ 25.9, 40.7, 65.2, 94.5, 108.6, 139.2, 151.3, 173.7; mass spectrum, m/z (relative intensity) 167 (M^+, 100), 166 ($M^+ - H$, 62), 138 ($M^+ - C_2H_4$, 51), 122 ($M^+ - HN(CH_3)_2$, 11), 109 (22), 108 (22), 94 (43).

Anal. Calcd for $C_9H_{13}NO_2$: C, 64.65; H, 7.84; N, 8.83. Found: C, 64.50; H, 7.89; N, 8.25.

Dienaminone of *dl*-Camphor, 16. Method B. The residue after the workup was chromatographed on aluminum oxide preparative-layer plates which were developed with 8% EtOAc/$CHCl_3$. The band with R_f 0.46 was cut out and eluted with 10% EtOAc/$CHCl_3$. The dienaminone **16** was obtained as a yellow oil: UV max (EtOH) 380 nm (ε 33 602); ^{1}H NMR ($CDCl_3$) δ 0.81–1.25 (m, 9 H), 1.46–2.69 (m, 5 H), 2.87 (s, 6 H), 4.95 (t, 1 H, J = 12.2 Hz), 6.32 (d, 1 H, J = 12.2 Hz), 6.88 (d, 1 H, J = 12.2 Hz); ^{13}C NMR ($CDCl_3$) δ 9.5, 18.8, 20.4, 26.6, 31.3, 40.6, 47.2, 48.0, 57.8, 94.3, 130.5, 131.5, 131.5, 150.3, 207.2; mass spectrum, m/z (relative intensity) 233 (M^+, 22), 218 ($M^+ - CH_3$, 2), 205 ($M^+ - CO$, 16), 190 ($M^+ - CH_3$, CO, 18), 136 (90), 121 (70), 107 (100), 95 (60), 94 (68), 93 (99).

Anal. Calcd for $C_{15}N_{23}NO$: C, 71.68; H, 10.03; N, 5.57. Found: C, 71.92; H, 10.25; N, 5.31.

Dienamine of 2-Ethyl-2-oxazoline, 6. Method D. The salt **1** was added to a solution of the oxazoline anion at −70 °C, and the reaction was stirred at this temperature for 4 h. The reaction was then allowed to slowly warm to room temperature and was then quenched. The residue after the workup was chromatographed on aluminum oxide preparative-layer plates which were eluted with 4% acetone/EtOAc. The band with R_f 0.71 was cut out and eluted with 10% acetone/EtOAc. The product was then sublimed at 55–60 °C (0.02 torr), and the dienamine **6** was isolated as an off-white solid: UV max (EtOH) 336 nm (ε 41 499); ^{1}H NMR ($CDCl_3$) δ 1.89 (s, 3 H), 3.02 (s, 6 H), 4.05 (m, 2 H), 4.54 (m, 2 H), 5.19 (t, 1 H, J = 12.4 Hz), 7.14 (d, 1 H, J = 12.4 Hz), 7.85 (d, 1 H, J = 12.4 Hz); ^{13}C NMR ($CDCl_3$) δ 13.1, 40.5, 54.1, 67.1, 94.9, 109.7, 137.7, 148.3, 167.9; mass spectrum, m/z (relative intensity) 180 (M^+, 5), 137 ($M^+ - HN(CH_3)_2$, 11), 136 ($M^+ - N(CH_3)_2$, 100), 122 ($M^+ - CH_2N(CH_3)_2$, 31, 108 (7), 92 (27), 71 (10).

Anal. Calcd for $C_{10}H_{16}N_2O$: C, 66.63; H, 8.95, N, 15.55. Found: C, 66.03; H, 8.28; N, 15.06.

Dienamine of 2-Picoline, 7. Method D. The residue after the workup was distilled in a micromolecular still. The product distilled with an oil bath temperature of 130–135 °C (0.3 torr) [lit.[33] 120–122 °C (0.1 torr)]. The product rapidly decomposed on exposure to air. It is best stored in a freezer under N_2: UV max (EtOH) 369 nm (ε 29 350); ^{1}H NMR ($CDCl_3$) δ 2.97 (s, 6 H), 5.04 (dd, 1 H, J = 11.0, 13.0 Hz), 6.00 (d, 1 H, J = 15.0 Hz), 6.32 (d, 1 H, J = 13.0 Hz), 7.00 (m, 2 H), 7.13 (dd, 1 H, J = 15.0, 11.0 Hz), 7.35 (m, 1 H), 8.37 (m, 1 H).

Dienaminone of 1-Ethyl-2-pyrrolidinone, 14. Method D. The residue after the workup was chromatographed on aluminum oxide preparative layer plates which were developed with 5% acetone/EtOAc. The band with R_f 0.41 was cut out and eluted with 10% acetone/EtOAc. The dienaminone **14** was isolated as a yellow solid: UV max (EtOH) 343 nm (ε 34 143); ^{1}H NMR ($CDCl_3$) δ 1.12 (t, 3 H, J = 7.3 Hz), 2.66 (m, 2 H), 2.82 (s, 6 H), 3.43 (m, 4 H), 4.80 (t, 1 H, J = 12.6, 12.0 Hz), 6.54 (d, 1 H, J = 12.6 Hz), 6.87 (dt, 1 H, J = 12.0, 2.5 Hz); ^{13}C NMR ($CDCl_3$) δ 12.6, 22.3, 37.6, 40.5, 43.8, 94.3, 118.7, 130.8, 148.0, 169.9; mass spectrum, m/z (relative intensity) 194 (M^+, 96), 179 ($M^+ - CH_3$, 17), 165 ($M^+ - Et$, 9), 150 ($M^+ - N(CH_3)_2$, 54), 122 (39), 94 (100), 82 (60), 58 (37), 43 (50).

Anal. Calcd for $C_{11}H_{18}N_2O$: C, 68.00; H, 9.34; N, 14.43. Found: C, 67.74; H, 9.16; N, 14.28.

Dienaminone from the Ethyl Acetoacetate Dianion, 17. An oven-dried round-bottomed flask with a septum inlet, pressure-equalized addition funnel, and gas stopcock was evacuated and flushed with N_2, and the reaction was run under a positive N_2 atmosphere. The flask was charged with NaH (0.072 g, 1.5 mmol)/mineral oil which was rinsed with dry hexane to remove the oil. To this was added 5 mL of dry THF. The reaction was cooled in an ice bath, and ethyl acetoacetate (0.17 mL, 1.3 mmol) in 5 mL dry THF was added dropwise with stirring. After the addition was complete the reaction was stirred at 0 °C for 30 min, and *n*-BuLi (1.0 mL, 1.4 mmol) was added dropwise by syringe. The dianion solution was bright orange. After the addition was complete, the reaction was stirred at 0 °C for 20 min. To the dianion solution was added 0.16 g (1.0 mmol) of the vinamidinium

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salt. After being stirred at 0 °C for 3 h, the reaction mixture was slowly warmed to room temperature and was stirred for an additional 2 h. It was then poured onto 20 mL of saturated NH_4Cl solution and extracted with CH_2Cl_2 (3 × 25 mL). Solvent was removed from the dried (Na_2SO_4) extracts under reduced pressure, and the residue was chromatographed on two silica gel preparative-layer plates, which were eluted with 3% acetone/EtOAc. The band with R_f 0.47 was cut out and eluted with 6% acetone/EtOAc. The dienaminone **17** was isolated as a yellow oil: 0.035 g (17% yield); UV max (EtOH) 388 nm (ϵ 43346); ^1H NMR ($CDCl_3$) δ 1.26 (t, 3 H, J = 7.3 Hz), 2.92 (s, 6 H), 3.47 (s, 2 H), 4.13 (q, 2 H, J = 7.3 Hz), 5.15 (t, 1 H, J = 12.1, 14.5 Hz), 5.86 (d, 1 H, = 14.5 Hz), 6.76 (d, 1 H, J = 12.1 Hz), 7.34 (dd, 1 H, J = 14.5, 12.1 Hz).

Anal. Calcd for $C_{11}H_{17}NO_3$: C, 62.53; H, 8.11; N, 6.63. Found: C, 62.25; H, 7.84; 6.34.

Dienaminones of 3-Methoxy-2-cyclopentenone, 18a,b. The 3-methoxy-2-cyclopentenone was prepared by the method of House and Rasmusson.[34] The reaction residue, an amber oil, was purified by sublimation at 50–55 °C (bath temperature; 0.5 torr). The product, a white solid, was isolated in 81% yield: mp 49–51 °C (lit.[34] mp 51–52 °C); mass spectrum, m/z (relative intensity) 112 (M^+, 100), 83 (M^+ – CHO, 44), 81 (M^+ – OCH_3, 41), 69 (M^+ – C_2H_3O, 97), 57 (38).

The reaction of the vinamidinium salt **1** with 3-methoxy-2-cyclopentenone was carried out by method D. The residue after workup was chromatographed on aluminum oxide preparative-layer plates which were developed with 10% acetone/EtOAc. Two compounds were isolated, **18a** and **18b**, as yellow oils.

The band with R_f 0.40 was removed and eluted with 2% CH_3OH/EtOAc. The dienaminone **18b** was isolated as a yellow oil: 12% yield; UV max (EtOH) 395 nm (ϵ 45400); ^1H NMR ($CDCl_3$) δ 2.85 (s, 6 H), 2.96 (m, 2 H), 3.86 (s, 3 H), 4.85 (t, 1 H, J = 12.1 Hz), 5.33 (s, 1 H), 6.51 (d, 1 H, J = 12.1 Hz), 6.60 (d, 1 H, J = 12.1 Hz); mass spectrum, m/z (relative intensity) 193 (M^+, 11), 178 (M^+ – CH_3, 7), 167 (M^+ – C_2H_2, 29), 149 (M^+ – $N(CH_3)_2$, 88), 97 (21), 70 (60), 43 (100).

The band with R_f 0.30 was cut out and eluted with 2% MeOH/EtOAc. The dienaminone **18a** was isolated as a yellow oil: 19% yield; UV max (EtOH) 395 nm (ϵ 45431); ^1H NMR ($CDCl_3$) δ 2.88 (s, 6 H), 3.12 (m, 2 H), 3.82 (s, 3 H), 4.87 (t, 1 H, J = 12.1 Hz), 5.41 (s, 1 H), 6.66 (d, 1 H, J = 12.1 Hz), 7.02 (d, 1 H, J = 12.1 Hz); mass spectrum, m/z (relative intensity) 193 (M^+, 10), 178 (M^+ – CH_3, 4), 167 (M^+ – C_2H_2, 32), 149 (M^+ – $N(CH_3)_2$, 100), 97 (6), 70 (10), 43 (10).

Both of these compounds were too unstable to give satisfactory elemental analysis. However, mass, UV, and NMR spectral data provided excellent confirmation of the structure in each case.

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Registry No. (*E*)-**1**, 70669-77-1; (*E*)-**2**, 78804-79-2; (*E,E*)-**3**, 75834-03-6; (*Z,E*)-**4a**, 78804-80-5; (*E,E*)-**4b**, 78804-81-6; (*E,E*)-**5a**, 75847-94-8; (*E,E*)-**5b**, 78804-82-7; (*E,E*)-**6**, 78804-83-8; (*E,E*)-**7**, 78804-84-9; (*E,E*)-**9a**, 75833-99-7; (*E,E*)-**10**, 75834-00-3; (*E,E*)-**11**, 78804-85-0; (*E,E*)-**12**, 75834-01-4; (*E,E*)-**13**, 78804-86-1; (*E,E*)-**14**, 78804-87-2; (*E,E*)-**15**, 75834-05-8; (±)-(*E,E*)-**16**, 78804-88-3; (*E,E*)-**17**, 78804-89-4; (*E,E*)-**18a**, 78804-90-7; (*Z,E*)-**18b**, 78804-91-8; β-(dimethylamino)acrolein, 927-63-9; dimethylamine·HCl, 506-59-2; diethyl malonate, 105-53-3; ethyl acetoacetate, 141-97-9; 3-pentanone, 96-22-0; 2-butanone, 78-93-3; cyclopentanone, 120-92-3; cyclohexanone, 108-94-1; cycloheptanone, 502-42-1; *dl*-camphor, 21368-68-3; estrone 3-methyl ether, 1624-62-0; γ-butyrolactone, 96-48-0; δ-valerolactone, 542-28-9; 1-ethyl-2-pyrrolidinone, 2687-91-4; 2-ethyl-2-oxazoline, 10431-98-8; 2-picoline, 109-06-8; 3-methoxy-2-cyclopentenone, 4683-50-5.

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Arylation and Heteroarylation of Photochemically Generated Purinyl Radicals[1]

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Neutral purinyl radicals are new transient intermediates in nucleic acid chemistry. Photolysis of 9-substituted 6-iodopurines with ultraviolet light provides an excellent method for generating purin-6-yl radicals (or caged radical pairs), through homolysis of the weak carbon–iodine bond. The intermediacy of these radicals can be inferred from ESR data. When photolysis is carried out in the presence of benzene, the nascent purinyl radicals (or radical pairs) are intercepted and the corresponding 9-substituted 6-arylpurine is isolated. Heteroaromatic arylations also are possible. Thus, photolysis in the presence of *N*-methylpyrrole results in the formation of 9-substituted 6-(*N*-methylpyrr-2-yl)purine. Furan and thiophene derivatives also undergo photoarylation. The products are consistent with the preferred sites for radical attack upon these heteroaromatics. Reaction with diphenyl disulfide results in the formation of the corresponding purinyl thioether.

We have reported recently the use of a diazotization/deamination procedure for the conversion of 6-aminopurine precursors to various 6-halogenated purines.[2] This deamination procedure was utilized for the direct synthesis of the antibiotic nebularine, from readily available adenosine.[3] These reactions apparently proceed via diazotization of the 6-amino group to form unstable intermediate diazonium salts or azo compounds which decompose homolytically under the reaction conditions to generate purinyl radicals. We have discovered that 9-substituted purin-6-yl radicals or corresponding radical pairs can be relatively cleanly generated by the photolysis of 6-iodopurines. This paper reports on the generation and specific arylation and heteroarylation of transient purinyl radicals.

Direct attachment of aryl groups to the purine ring has not been reported to occur in nature. Few 6-arylpurines have been synthesized, although 6-phenylpurine and some *N*-alkyl derivatives have been prepared.[4,5] 6-(3-Methylpyrrol-1-yl)purine, a methylpyrrole attached to purine through the pyrrole nitrogen at position 6, has been prepared from zeatin.[6] Synthetic 2-arylpurines,[7] which have been evaluated as coronary vasodilators, as well as 8-phenylpurines,[8] are also known. The only literature method for direct 6-arylation of purines is nucleophilic displacement of chlorine from 6-chloropurines by phenyllithium. The 6-arylpurines, especially those possessing heterocyclic substituents in the 6-position, bear a structural resemblance to cytokinins such as kinetin and its riboside,[9] although the aminomethylene spacer group is absent in the arylpurines. Synthetic aryl and heteroaryl purines also may be useful as biochemical probes for the study of enzyme-catalyzed reactions.

Results and Discussion

The preparation of biaryls via photolysis of iodo aromatics such as iodobenzene has been the subject of a number of investigations.[10,11] These reactions proceed via homolysis of the weak aryl carbon–iodine bond.[12] Phenyl radicals generated in heteroaromatic solvents such as furan, thiophene, and pyrrole afford modest yields of phenyl-substituted heterocyclic products.[13,14] Preferred sites for radical attack on various heteroaromatics have been experimentally determined.[15] Photoarylations in which a heteroaryl iodide is employed generally proceed as for iodobenzene.[16–18] When shorter wavelength UV light is

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excluded by the use of appropriate filters, no rearrangement of arylated products is observed.[11,16] Reports where both the radical source and the substrate are heteroaromatics are rare.[19-21]

Photoarylation of purine was initially examined with the photolysis of 6-iodo-9-ethylpurine (**1a**) in dry, nitrogen-purged benzene. When ultraviolet radiation was supplied in a Rayonet photolysis apparatus by mercury lamps with the principal wavelength of 2537 Å, the reaction required 24 h. Much shorter reaction times (6 h) are required when a Hanovia 450-W mercury lamp (with Vycor filter) is used. Following removal of solvent and separation on silica gel plates, 6-phenyl-9-ethylpurine (**2a**) was obtained in 54% yield as colorless rods from hexane; mp 61 °C. The phenylpurine was identified by its mass spectrum (m/z 224, M^+), its ^1H and ^{13}C NMR resonances, and its UV data (290.5 nm, ϵ 1.8×10^4). The bathochromic shift observed in the UV spectrum is consistent with extension of conjugation through the phenyl group. Results similar to that observed for **1a** were obtained with the corresponding nucleoside **1b**.

1a, R = Et

b, R = Rib(OAc)$_3$

2a, R = Et; R′ = Ph

b, R = Rib(OAc)$_3$; R′ = Ph

3a, R = Et; R′ =

b, R = Rib(OAc)$_3$; R′ =

4a, R = Et; R′ =

b, R = Rib(OAc)$_3$; R′ =

5a, R = Et; R′ =

b, R = Rib(OAc)$_3$; R′ =

6, R = Et; R′ = SPh

Extension of this photoinduced reaction to the heteroaromatic solvents described in this paper was limited by three requirements. Such solvents must exist as liquids in the temperature range of the photolysis assembly. The solvents and their products must be photolytically stable under the reaction conditions. Such solvents must exhibit selectivity in radical reactions.

When **1a** was allowed to react with freshly distilled, dry, nitrogen-purged *N*-methylpyrrole in a Hanovia apparatus with irradiation from a Vycor-sleeved 450-W mercury lamp for 8 h, 6-(*N*-methylpyrr-2-yl)-9-ethylpurine (**3a**) was isolated in 54% yield as colorless plates (heptane); mp 93 °C. That substitution had occurred at the α-position of the pyrrole ring was evidenced by the chemical shifts, multiplicities, and coupling constants of the ^1H NMR resonances and by the off-resonance ^{13}C data of the pyrrole ring in the product. In the ^1H NMR spectrum, H-3 appeared as a doublet of doublets at δ 7.83 with $J_{3,4}$ = 3.7 Hz and $J_{3,5}$ = 1.9 Hz. The downfield shift of this proton from 6.11 ppm in *N*-methylpyrrole is similar to the observed downfield shift of the ortho (α) protons of the phenyl group in **2a** and **2b**. The observed coupling constants, particularly $J_{3,4}$, are characteristic of 2-substituted pyrroles.[22] The UV spectrum of **3a** showed absorption at 239 nm (ϵ 9.7×10^3), a broad band at 269 nm (ϵ 3.2×10^3), and an intense absorption at 343.5 nm (ϵ 3.0×10^4) indicative of conjugation of the pyrrole moiety to the purine ring. The protected nucleoside **1b** underwent facile heteroarylation to the product **3b**.

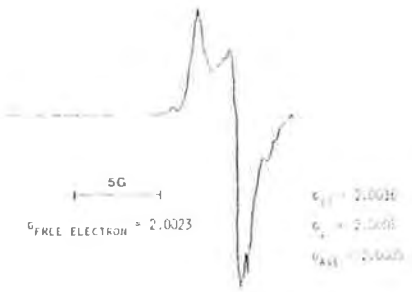

Figure 1. ESR spectrum of photoirradiated 6-iodo-9-ethylpurine in benzene at 77 K.

Under conditions of photolysis, **1a** reacted with 2-methylfuran as the solvent to give 6-(5-methylfur-2-yl)-9-ethylpurine (**4a**) in 52% yield. The position of substitution on the furan ring could be deduced from its NMR data, specifically its ^1H NMR spectrum. The proton on carbon 3 (H-3) of the furan system appeared as a clean doublet at δ 7.83 with $J_{3,4}$ = 3.3 Hz. The observed coupling constant is typical of $J_{3,4}$ for substituted furans.[22] The downfield shift of this proton is consistent with substitution of the purinyl moiety at carbon 2. Further substantiation of this assignment was evident in the chemical shift (δ 6.30) and multiplicity (doublet of doublets, $J_{4,3}$ = 3.3 Hz, $J_{allylic}$ = 1.0 Hz) of H-4. No other substitution pattern could be accommodated by the ^1H NMR spectral data. As in the case of **3a**, a bathochromic shift of the purine ring absorption was evident in the UV spectrum of **4a**. The nucleoside **1b** gave similar results.

Heteroarylation of thiophene by photolysis of **1** in the presence of this sulfur-containing heterocycle as the solvent gave **5** with substitution occurring at the α-position of the thiophene ring. Assignment of structure was made from spectral data.

Previous studies of radical attack upon pyrrole and furan derivatives show that both exhibit high regioselectivity of reaction at the 2-position.[15] Reportedly for thiophene, several percent of the 3-isomer forms along with the predominant 2 isomer. In our reactions, only one product was observed in all cases, for both the nucleosides and ethyl derivatives. Spectroscopic data of the products were consistent with the locus of attack being at the α- rather than the less favorable β-position.

The radical nature of the photoarylation reaction was further demonstrated by ESR spectroscopy. A benzene solution of 9-ethyl-6-iodopurine in a quartz ESR tube was degassed under vacuum in several freeze–thaw cycles. The sealed tube was irradiated at 25 °C with a mercury lamp (2537 Å) and rapidly cooled in liquid nitrogen. The ESR

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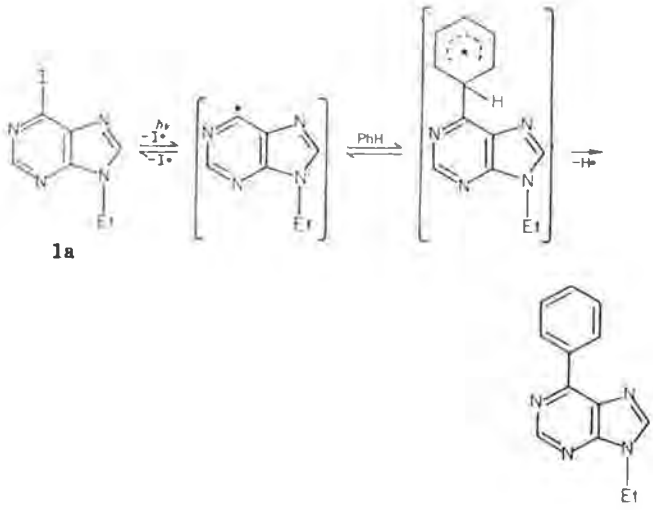
Scheme I. Mechanism of Photoarylation of 6-Iodo-9-ethylpurine in Benzene



1a

2a

spectrum of this sample obtained at 77 K is shown in Figure 1. The shape of the spectrum suggests that the radical(s) possess(es) considerable structural asymmetry. The resonance ($g_{\parallel}$ = 2.0016, $g_{\perp}$ = 2.0005, g_{av} = 2.0009) corresponds to an aromatic free radical. Complete brief thawing of the frozen sample followed by recooling to 77 K caused the ESR signal to disappear completely. A mechanistic explanation accommodated by the results of this experiment is shown in Scheme I. An alternative explanation that invokes the intermediacy of solvent-caged radical pairs is consistent also with the ESR data.[23]

That nascent purinyl radicals (either free or caged) were indeed being intercepted by aromatic and heteroaromatic substrates was further supported by another experiment, i.e. reaction with disulfide linkages. Displacement reactions in which aryl radicals attack a sulfur of weak disulfide linkages to give aryl thioethers and thienyl radicals are well-known.[24,25] The reaction between photochemically generated purinyl radicals and diphenyl disulfide was examined by photolysis of 6-iodo-9-ethylpurine in DMF solution at 3500 Å. The longer wavelength light was used because the ultraviolet absorption of the solvent is appreciable below 3000 Å. The photochemically induced displacement results in the formation of 6-(phenylthio)-9-ethylpurine.

Neutral purinyl radicals are new intermediates in nucleic acid chemistry.[2] This report presents one example of the utility of such transient species in the modification of nucleic acid bases.

Experimental Section

Irradiation was accomplished in a Hanovia 450-W mercury photolysis apparatus or in a Rayonet photochemical reactor.

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Nuclear magnetic resonance spectra employing tetramethylsilane as the internal standard were recorded on JEOL Model FX90Q and Bruker Model HX90E Pulse Fourier transform spectrometers. Electron spin resonance spectra were recorded on a Varian V-4500 X-band spectrometer. Mass spectra were obtained on a Hewlett-Packard 5985 GC/MS system. The ultraviolet spectra were recorded on a Varian-Cary Model 219 spectrophotometer. Elemental analyses were performed by the University of Iowa Microanalytical Service or by Galbraith Laboratories, Knoxville, TN. *N*-Methylpyrrole and 2-methylfuran (Aldrich) were distilled prior to use; thiophene (Aldrich), benzene, heptane, and dimethyl sulfoxide (MCB Omnisolv) were used without further purification. Preparative layer chromatography employed EM silica gel PF_{254} plates, activated for 3 h at 135 °C.

6-Phenyl-9-ethylpurine (2a). To 250 mL dry N_2-purged benzene was added 0.222 g (0.810 mmol) of 6-iodo-9-ethylpurine (**1a**). The solid dissolved on stirring, and the solution was transferred to the Pyrex immersion well of a quartz photochemical reactor. The system was flushed with nitrogen, and photolysis was carried out by employing a 450-W mercury UV source with a Vycor glass sleeve filter. The photoarylation was followed by UV spectral methods, and after 6 h the reaction was stopped. The solvent was removed under reduced pressure, and the residue was chromatographed on silica gel plates. The two principal bands that resulted from development of the plates with 1:10 ethanol–ethyl acetate occurred at R_f 0.47 and 0.59. The band at R_f 0.47 corresponded to unreacted **1a** (0.044 g, 0.16 mmol, 32.0%). After elution, the band at R_f 0.59 afforded 0.060 g (0.268 mmol, 53.6%) of **2a** as an amorphous solid. Recrystallization from Skellysolve B gave colorless tufts: mp 60.5–61 °C; ^{13}C NMR ($CDCl_3$) δ 14.9, 38.4, 128.1, 129.3, 130.4, 130.4, 135.3, 143.2, 151.8, 151.8, 154.3; ^{1}H NMR ($CDCl_3$) δ 1.59 (t, 3 H), 4.362 (q, 2 H), 8.12 (s, 1 H), 9.03 (s, 1 H), 7.5–7.6 and 8.7–8.8 (m, 5 H); UV (EtOH) λ_{max} 290.5 nm (ϵ 1.8 × 10^4), 278, 312 nm (sh); mass spectrum, *m/z* (relative intensity) 225 (21.4), 224 (M^+, 100.0), 196 ($M^+ - C_2H_4$, 89.3), 195 (28.6), 169 (41.7), 142 (14.3), 78 (10.7).

Anal. Calcd for $C_{13}H_{12}N_4$: C, 69.6; H, 5.3; N, 25.0. Found: C, 69.9; H, 5.2; N, 25.0.

6-(*N*-Methylpyrr-2-yl)-9-ethylpurine (3a). To 52 mL of distilled, dry *N*-methylpyrrole was added 0.301 g (1.10 mmol) of **1a**. The solution was thoroughly purged with nitrogen and photolyzed as described for **2a**. After 8 h, the reaction was discontinued, and the solvent was removed. The brown-black residue was chromatographed on silica gel with 1:10 ethanol–ethyl acetate as the developer. Where practicable, the reaction products were handled under a stream of nitrogen to minimize oxidation. The band at R_f = 0.63 was eluted to give 0.134 g (0.593 mmol, 53.9%) of **3a** as a tawny solid which was recrystallized from heptane to afford colorless plates: mp 93–93.5 °C; ^{13}C NMR ($CDcl_3$) δ 14.9, 38.2, 37.8, 108.4, 119.0, 126.7, 128.7, 128.8, 142.1, 149.2, 150.5, 151.0; ^{1}H NMR ($CDCl_3$) δ 1.56 (t, 3 H), 4.20 (s, 3 H), 4.31 (q, 2 H), 6.30 (dd, 1 H), 6.86 (t, 1 H), 7.85 (dd, 1 H), 8.01 (s, 1 H), 8.83 (s, 1 H); UV (EtOH) λ_{max} 239 nm (ϵ 9.7 × 10^3), 269 (3.2 × 10^3), 343.5 (3.0 × 10^4); mass spectrum, *m/z* (relative intensity) 228 (9.7), 227 (M^+, 74.0), 226 (100.0), 198 (Pur C_2H_5, 38.7), 186 (25.8), 149 (Pur, 41.9), 97 (16.1), 71 (22.6).

Anal. Calcd for $C_{12}H_{13}N_5$: C, 63.4; H, 5.8; N, 30.8. Found: C, 63.2; H, 5.9; N, 30.7.

6-(5-Methylfur-2-yl)-9-ethylpurine (4a). To 55 mL dry N_2-purged 2-methylfuran was added 0.278 g (1.01 mmol) of **1a**. The mixture was transferred to the Hanovia photochemical reactor, stirred, flushed with N_2 and photolyzed as for **2a**. After 9 h, the reaction was discontinued. Removal of solvent and silica gel chromatography (1:10 ethanol/ethyl acetate) gave 0.065 g (0.237 mmol, 24%) of recovered starting material, **1a**, and 0.120 g (0.519 mmol, 52%) of **4a**, as an off-white waxy solid. Recrystallization from heptane gave colorless needles: mp 156–157 °C; ^{13}C NMR ($CDCl_3$) δ 14.1, 15.4, 38.9, 109.5, 119.6, 128.1, 143.6, 145.9, 147.9, 151.6, 152.5, 156.6; ^{1}H NMR ($CDCl_3$) δ 1.57 (t, 3 H), 2.51 (s, 3 H), 4.33 (q, 2 H), 6.30 (dd, 1 H), 7.83 (d, 1 H), 8.09 (s, 1 H), 8.94 (s, 1 H); UV (EtOH) λ_{max} 230 nm (ϵ 9.7 × 10^3), 280 (2.0 × 10), 328.5 (2.6 × 10^4), 339 (2.6 × 10^4); mass spectrum, *m/z* (relative intensity) 229 (11.3), 228 (M^+, 79.0), 213 (8.1), 200 (Pur C_2H_5 + H, 32.3), 185 (27.4), 149 (Pur-het + H, 75.8), 83 ($MeFurH^+$, 59.7), 69 ($FurH^+$, 100.0).

Anal. Calcd for $C_{12}H_{12}N_4O$: C, 63.2; H, 5.3; N, 24.6. Found: C, 63.0; H, 5.3; N, 24.6.

6-(Thien-2-yl)-9-ethylpurine (5a). To 60 mL dry N_2-purged thiophene was added 0.211 g (0.77 mmol) of **1a**. The solution was transferred to the Hanovia photochemical reactor and treated as for **2a** for a total of 9.5 h. Removal of solvent gave a brown syrup which was chromatographed on silica gel with 1:10 ethanol–ethyl acetate as the developer. The band at R_f 0.57 upon elution yielded **5a** as an oil which solidified on standing (0.069

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g, 0.30 mmol, 39.0%). Recrystallization of this from heptane gave spiny colorless needles: mp 75–77 °C; ^{13}C NMR ($CDCl_3$) δ 15.4, 40.0, 128.7, 130.8, 131.5, 132.6, 139.9, 143.9, 150.0, 151.8, 152.3; ^{1}H NMR ($CDCl_3$) δ 1.58 (t, 3 H), 4.34 (q, 2 H), 7.27 (dd, 1 H), 7.61 (dd, 1 H), 8.11 (s, 1 H), 8.67 (dd, 1 H), 8.89 (s, 1 H); UV (EtOH) λ_{max} 226.5 nm (ϵ 8.4 × 10^3), 271 (6.2 × 10^3), 324 (2.0 × 10^4), 337 (sh); mass spectrum, *m/z* (relative intensity) 232 (^{34}SM$^+$, 0.6), 231 (1.5), 230 (^{32}SM$^+$, 8.5), 202 (M$^+$ – C_2H_4, 11.2), 149 (Et-PurH$^+$, 7.9), 97 (10.5), 85 (11.2), 32 (84.6), 31 (100.0).

Anal. Calcd for $C_{11}H_{10}N_4S$: C, 57.5; H, 4.4; N, 24.3. Found: C, 57.3; H, 4.3; N, 23.4.

6-Phenyl-9β-(2′,3′,5′-tri-*O*-acetyl-D-ribofuranosyl)purine (2b). A solution of 0.272 g (0.54 mmol) of **1b**[2] in dry benzene (250 mL) was purged with nitrogen and photolyzed as described for **2a**. The progress of reaction was monitored by thin-layer chromatography on silica gel. After 7 h, when no further changes in TLC were observed, the reaction mixture was concentrated under reduced pressure. The orange residue was chromatographed. Three developments with 7:200 2-propanol–dichloromethane gave two significant bands. After elution by 1:9 methanol–dichloromethane, the principal band at R_f 0.5 gave 0.146 g (0.295 mmol, 54.6%) of **2b** as a colorless oil: ^{13}C NMR ($CDCl_3$) δ 20.1, 20.2, 20.4, 62.8, 70.4, 72.8, 80.1, 86.2, 128.4, 129.6, 130.9, 131.4, 135.1, 142.41, 152.4, 155.1, 169.1, 169.3, 170.0; ^{1}H NMR ($CDCl_3$) δ 2.08 (s, 3 H), 2.13 (s, 3 H), 2.15 (s, 3 H), 4.45 (s, 3 H), 5.75 (t, 1 H), 6.03 (t, 1 H), 6.31 (d, 1 H), 8.31 (s, 1 H), 9.03 (s, 1 H), 7.5–7.6, 8.7–8.8 (m, 5 H); UV (EtOH) λ_{max} 290 nm (ϵ 2.0 × 10^4) 274, 308 (sh); mass spectrum, *m/z* (relative intensity) 455 (1.5), 454 (M$^+$, 4.7), 395 (M$^+$ – C_3H_7O, 20.8), 259 (sugar, 14.1), 225 (Pur + CH_2O, 20.8), 198 (12.9), 197 (90.9), 196 (16.5), 170 (26.4), 139 (100.0), 97 (59.6), 43 (79.0).

Anal. Calcd for $C_{22}H_{22}N_4O_7$: C, 58.2; H, 4.9; N, 12.3. Found: C, 57.6; H, 4.9; N, 12.4.

The band at R_f 0.62 upon elution afforded only a trace of starting material.

6-(*N*-Methylpyrr-2-yl)-9β-(2′,3′,5′-tri-*O*-acetyl-D-ribofuranosyl)purine (3b). To 50 mL of dry, distilled, N_2-purged *N*-methylpyrrole was added 0.218 g (0.433 mmol) of **1b**, and the solution was photolyzed as described for **2b**. The solution darkened during irradiation. After 11 h the reaction was stopped, and the solvent was removed on a rotary evaporator. The brown syrup remaining was chromatographed on silica gel by employing 1:50 2-propanol–dichloromethane as the developer. Two developments were required. Where practicable, the reaction products were handled under nitrogen; the mixture darkened at room temperature in air. Upon elution with 1:9 methanol–dichloromethane 0.140 g (0.306 mmol, 70.7%) of **3b**, the chief product (R_f 0.32), was obtained as a pale beige oil which darkens on exposure to air: ^{13}C NMR ($CDCl_3$) δ 20.4, 20.5, 20.8, 38.3, 63.2, 70.8, 78.6, 80.3, 86.2, 109.1, 119.9, 127.0, 129.5, 129.8, 141.2, 150.2, 150.8, 152.0, 169.4, 169.6, 170.3; ^{1}H NMR ($CDCl_3$) δ 2.07 (s, 3 H), 2.13 (s, 3 H), 2.14 (s, 3 H), 4.19 (s, 3 H), 4.42 (s, 3 H), 5.70 (t, 1 H), 6.00 (t, 1 H), 6.29 (d, 1 H), 6.33 (dd, 1 H), 6.90 (t, 1 H), 7.87 (dd, 1 H), 8.16 (s, 1 H), 8.82 (s, 1 H); UV (EtOH) λ_{max} 242 nm (ϵ 9.7 × 10^3), 268 (2.9 × 10^3), 332 (sh), 345 (2.9 × 10^4); mass spectrum, *m/z* (relative intensity) 459 (0.7), 458 (3.7), 457 (M$^+$, 15.0), 259 (sugar, 2.5), 228 (Pur + CH_2O, 5.9), 200 (15.5), 199 (41.5), 198 (Pur, 100.0), 183 (1.5), 157 (5.3), 139 (37.6), 97 (26.8), 43 (39.9).

Anal. Calcd for $C_{21}H_{23}N_5O_7 \cdot H_2O$: C, 53.0; H, 5.3; N, 14.7. Found: C, 52.7; H, 5.1; N, 14.2.

6-(5-Methylfur-2-yl)-9β-(2′,3′,5′-tri-*O*-acetyl-D-ribofuranosyl)purine (4b). To 20 mL of dry, N_2-purged 2-methylfuran was added 0.338 g 0.67 mmol) of **1b**. The nearly colorless solution was stirred and transferred to a quartz photolysis tube in a Rayonet photochemical reactor fitted with 2537-Å mercury lamps. The solution was purged with nitrogen for 10 min and then irradiated. The solution slowly turned brown. Lost solvent (~5 mL) was replenished after 16 h. After 23 h the irradiation was discontinued, and the solvent was removed under reduced pressure. The resulting tan gum was chromatographed on silica gel with 1:50 2-propanol–dichloromethane, to give a broad band at R_f 0.25 which was rechromatographed on silica gel with neat ethyl acetate as the developing solvent. The band at R_f 0.75 was starting material (0.062 g, 0.123 mmol, 18.3%). The band at R_f 0.70 yielded 0.156 g (0.340 mmol, 50.7%) of **4b** as a colorless oil: ^{13}C NMR ($CDCl_3$) δ 14.2, 20.4, 20.6, 20.8, 63.1, 70.7, 78.6, 80.4, 86.4, 109.7, 120.3, 128.1, 142.4, 146.4, 147.6, 151.3, 153.0, 157.0, 169.4, 169.6, 170.4; ^{1}H NMR ($CDCl_3$) δ 2.08 (s, 3 H), 2.12 (s, 3 H), 2.16 (s, 3 H), 2.52 (s, 3 H), 4.44 (s, 3 H), 5.74 (t, 1 H), 6.00 (t, 1 H), 6.28 (d, 1 H), 6.28 (d, 1 H), 7.85 (d, 1 H), 8.24 (s, 1 H), 8.94 (s, 1 H); UV (EtOH) λ_{max} 230 nm (ϵ 9.5 × 10^3), 270 (3.5 × 10^3), 329.5 (2.8 × 10^4), 339 (2.8 × 10^4); mass spectrum, *m/z* (relative intensity) 460 (1.0), 459 (3.6), 458 (M$^+$, 15.0), 259 (sugar, 9.0), 229 (Pur + CH_2O, 24.2); 201 (54.5), 200 (PurH, 82.5), 185 (200 – CH_3, 11.6), 171 (15.9), 157 (17.5), 139 (100.0), 97 (70.5), 43 (86.6).

Anal. Calcd for $C_{21}H_{22}N_4O_8 \cdot H_2O$: C, 52.9; H, 5.1; N, 11.7. Found: C, 53.3; H, 4.9; N, 11.2.

6-(Thien-2-yl)-9β-(2′,3′,5′-tri-*O*-acetyl-D-ribofuranosyl)purine (5b). To 60 mL dry N_2-purged thiophene was added 0.190 g (0.38 mmol) of **1b**. The solution was photolyzed in the Hanovia photochemical reactor as described for **2b**. After 8.5 h the reaction was stopped, and the solvent was removed. The resulting malodorous brown material was taken up in 2–3 mL of 1:9 methanol–dichloromethane and chromatographed on silica gel with 1:50 2-propanol–dichloromethane as the developing solvent. The principal band at R_f 0.45 upon elution and removal of solvent in vacuo afforded 0.094 g (0.204 mmol, 53.8%) of **5b** as a foam: ^{13}C NMR ($CDCl_3$) δ 20.4, 20.5, 20.8, 63.1, 70.7, 73.1, 80.5, 86.4, 128.8, 130.9, 131.1, 133.0, 139.7, 142.5, 150.7, 151.6, 152.8, 169.3, 169.6, 170.3; ^{1}H NMR ($CDCl_3$) δ 2.09 (s, 3 H), 2.13 (s, 3 H), 2.16 (s, 3 H), 4.44 (s, 3 H), 5.70 (t, 1 H), 6.00 (t, 1 H), 6.28 (d, 1 H), 7.26 (dd, 1 H), 7.63 (dd, 1 H), 8.26 (s, 1 H), 8.66 (dd, 1 H), 8.89 (s, 1 H); UV (EtOH) λ_{max} 225 nm (ϵ 8.3 × 10^3), 270 (6.2 × 10^3), 322.5 (2.1 × 10^4), 335 (sh); mass spectrum, *m/z* (relative intensity) 460 (7.9), 402 (5.7), 259 (20.0), 203 (Pur + 2 H, 56.6), 202 (32.9), 157 (20.1), 149 (60.8), 139 (98.7), 97 (100.0).

Anal. Calcd for $C_{20}H_{20}N_4O_7S \cdot 2H_2O$: C, 48.4; H, 4.9; N, 11.3. Found: C, 48.3; H, 4.7; N, 10.8.

6-(Phenylthio)-9-ethylpurine (6). To 0.236 g (0.861 mmol) of **1a** dissolved in 15 mL of dry *N,N*-dimethylformamide was added 0.262 g (1.20 mmol) of diphenyl disulfide (Aldrich Chemical Co.). The solution was purged with N_2 and irradiated in a Rayonet photochemical reactor by employing a 3500-Å mercury source for 19.5 h, at which point no further change was visible by TLC of reaction aliquots. The solvent was removed in vacuo at 85 °C. The residue was separated by preparative layer chromatography on silica gel with 1:10 ethanol–ethyl acetate as the developer. The bands were eluted with 10% MeOH in CH_2Cl_2. The band at R_f 0.44 gave 0.146 g (0.533 mmol, 61.9%) of the starting purine **1a**. The band at R_f 0.68 afforded upon elution 0.036 g (0.141 mmol, 16.4%, 43.0% conversion) of **6**, the title compound, as buff crystals which were dried in vacuo: mp 106–108 °C; ^{13}C NMR δ Me_4Si ($CDCl_3$) 15.4, 39.1, 127.5, 129.2, 129.4, 130.9, 135.5, 142.6, 148.9, 152.1, 160.5; ^{1}H NMR δ Me_4Si ($CDCl_3$) 1.55 (t, 3 H), 4.31 (q, 2 H), 7.35–7.75 (m, 5 H), 8.01 (s, 1 H), 8.62 (s, 1 H); UV (EtOH) λ_{max} 289.5 nm (ϵ 1.73 × 10^4); mass spectrum, *m/z* (relative intensity) 256 (M$^+$, 50.0), 255 (100), 241 (M$^+$ – CH_3, 24.0), 227 (M$^+$ – C_2H_5, 24.0), 162 (60.0), 147 (M$^+$ – PhS, 70.0), 129 (80.0), 119 (Pur$^+$ – H, 36.7), 93 (33.3), 71 (70.0), 57 (26.7).

Anal. Calcd for $C_{13}H_{12}N_4S$: C, 60.9; H, 4.7; N, 21.9. Found: C, 60.7; H, 4.8; N, 21.2.

6-Iodo-9-ethylpurine (1a). Method A. From 9-Ethyladenine. A stirred mixture of 0.815 g (5.00 mmol) of 9-ethyladenine[2] and 8 mL of diiodomethane was treated at 70 °C under nitrogen with 5.4 mL (40.00 mmol) of *n*-pentyl nitrite. The reaction was continued for 3 h, the solvent was removed on a rotary evaporator at 85 °C, and the residue was taken up in CH_2Cl_2. This solution was treated with aqueous sodium sulfite. The organic layer was dried with Na_2SO_4, concentrated, and chromatographed on silica gel with 1:9 methanol–dichloromethane as the developing solvent. The band at R_f 0.19 upon elution yielded 0.145 g of unreacted 9-ethyladenine (0.89 mmol, 17.8%). The band at R_f 0.55 gave 0.49 g (1.79 mmol, 35.8%) of **1a** as a yellow solid which crystallized from heptane as pale yellow plates: mp 141–143 °C (lit.[2] mp 141–143 °C).

Method B. From 6-Iodopurine. To 2.00 g (8.14 mmol) of 6-iodopurine and 1.22 g (9.00 mmol) of K_2CO_3 was added 80 mL of dry dimethyl sulfoxide followed by 1.28 mL (16.30 mmol) of iodoethane. The mixture was protected from moisture and stirred at 35 °C for 2.5 h. It was then chilled in an ice–water bath, and 48 mL of water was slowly added. The resulting solution was extracted with three 40-mL portions of ether and four 40-mL

portions of toluene. The organics were combined, dried (Na_2SO_4), and chromatographed on silica gel with 1:9 methanol–dichloromethane as the developing solvent. The principal band at R_f 0.72 afforded after elution 1.22 g (4.45 mmol, 54.7%) of solid **1a** which recrystallized from heptane as pale yellow crystals: mp 144–145 °C (lit.[2] mp 141–143 °C); UV (EtOH) λ_{max} 276.5 nm (ϵ 1.2 × 10^4).

Anal. Calcd for $C_7H_7N_4I$: C, 30.6; H, 2.6; N, 20.4. Found: C, 30.6; H, 2.6; N, 20.2.

Another band at R_f 0.49 gave 0.24 g (0.88 mmol, 10.8%) of 6-iodo-7-ethylpurine which crystallized from heptane as fluffy, pale, lemon-colored crystals: mp 160–162 °C; UV (EtOH) λ_{max} 283.5 nm (ϵ 7.4 × 10^3); ^1H NMR ($CDCl_3$) δ 1.62 (t, 3 H), 4.62 (q, 2 H), 8.37 (s, 1 H), 8.79 (s, 1 H); mass spectrum, m/z (relative intensity) 275 (6.3), 274 (M^+, 54.2), 149 (6.9), 148 (11.1), 147 (M − I, 100.0), 119 (41.7).

Anal. Calcd for $C_7H_7N_4I$: C, 30.6; H, 2.6; N, 20.4. Found: C, 30.3; H, 2.7; N, 20.6.

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Registry No. 1a, 74465-49-9; **1b**, 5987-74-6; **2a**, 83026-89-5; **2b**, 83026-93-1; **3a**, 83026-90-8; **3b**, 83026-94-2; **4a**, 83026-91-9; **4b**, 83026-95-3; **5a**, 83026-92-0; **5b**, 83026-96-4; **6**, 83026-97-5; benzene, 71-43-2; *N*-methylpyrrole, 96-54-8; 2-methylfuran, 534-22-5; thiophene, 110-02-1; diphenyl disulfide, 882-33-7; 9-ethyladenine, 2715-68-6; 6-iodopurine, 2545-26-8.

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erature (during this period the dimethoxyaldehydes are completely ydrolyzed into the α-ketoaldehydes, leaving the dimethoxyketones **2** early unchanged). The reaction mixture is extracted with dichloro-ethane (3 × 100 ml), the combined extracts washed with brine, and ater, and dried with magnesium sulfate. Evaporation of the solvent nd distillation affords **2** (Table 1).

is-acetals 4; General Procedures:

solution of the mixture of the acetals **2** and **3** (0.1 mol) in dry me-anol is treated with thionyl chloride (1.2 equiv.) during 30 min (in e case of $R^2 = i\text{-}C_3H_7$, an additional 0.5 equivalents of thionyl chlo-de and 0.5 equivalents of methyl orthoformate are added). After neu-alization with potassium carbonate, the mixture is stirred for 15 min ith 2 normal aqueous potassium hydroxide (100 ml) in order to de-mpose the dimethyl sulfite. The mixture is poured into water and tracted with dichloromethane (3 × 50 ml). Work-up by Method A nd distillation gives the bis-acetals **4** (Table 2).

-Ketoaldehydes 5; General Procedure:

ne mixture of the acetals **2** and **3** (0.1 mol) without work-up is oured into 10% phosphoric acid (100 ml) or 5% sulfuric acid (100 ml) nd the reaction mixture is refluxed for 30 min. After cooling, the action mixture is extracted with dichloromethane (4 × 50 ml) and the mbined extracts are successively washed with 5% sodium hydrogen rbonate solution (50 ml) and water (50 ml). Drying, evaporation of e solvent, and distillation furnishes **5** in moderate yields (Table 3).

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Modification of Nucleic Acid Bases via Radical Intermediates: Synthesis of Dihalogenated Purine Nucleosides[1]

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New and improved preparations of structurally modified nucleic acid bases and their corresponding nucleosides are important goals in synthetic organic chemistry because of the potential utility of these compounds as synthetic precursors, in chemotherapeutic studies, and as biochemical probes in the investigation of specific enzyme-catalyzed reactions[2]. This is particularly true for halogenated nucleosides. For example, dihalopurine ribosides, such as 2,6-dichloro-9β-D-ribofuranosylpurine, are valuable synthetic precursors to 2-chloroadenosine[3,4], and a wide variety of derivatives. Some of these compounds have shown biological activity as coronary vasodilators[3], inhibitors of blood platelet aggregation[5], antihypertensives[6], and antilipemic/hypocholesterolemic agents[7,8].

We have reported recently that thermal and adjunctive photolytic homolysis of 6-diazonium salts (or the corresponding azo forms) of 9-substituted adenines in non-hydroxylic media apparently produce purinyl radical intermediates. These radicals can abstract hydrogen or halogen atoms from appropriate solvent molecules[9,10]. This paper reports on the utilization of transient neutral purinyl radicals for the synthesis of 2,6-dihalogenated nucleosides.

The starting material for these conversions was the 2-amino-6-chloro nucleoside **(2)** prepared easily from 2′,3′,5′-tri-*O*-acetylguanosine[11] by treatment with phosphoryl chloride and *N,N*-diethylaniline[12]. When **2** was heated in tetrachloromethane in the presence of *n*-pentyl nitrite for 24 h, the 2,6-dichloro nucleoside **4** was obtained in 66% yield as a white crystalline product identical chromatographically and spectrally to authentic **4** prepared in 44% yield by established literature methods[13] (see also Refs.[14,15]). Extension of this reaction to the 2-bromo-6-chloro nucleoside **5**[3,15] was carried out by heating **2** in tribromomethane in the presence of *n*-pentyl nitrite for 8 h. Compound **5** was isolated in 55% yield. The 2-iodo-6-chlororibofuranosylpurine **(6)** is a new dihalogenated nucleoside. It can be prepared in excellent yields (83%) as a crystalline solid by warming **2** in diiodomethane and *n*-pentyl nitrite for 1 h.

The purin-2-yl radical **3** presumably is generated as the transient species in these conversions from the thermal homolysis of the 2-diazonium salt/2-azo compound intermediate. This purinyl radical abstracts halogen atoms from solvent molecules. No competition of hydrogen with halogen abstraction was observed in the preparation of the 2-bromo- and 2-iodo-substituted nucleosides, where tribromomethane and diio-

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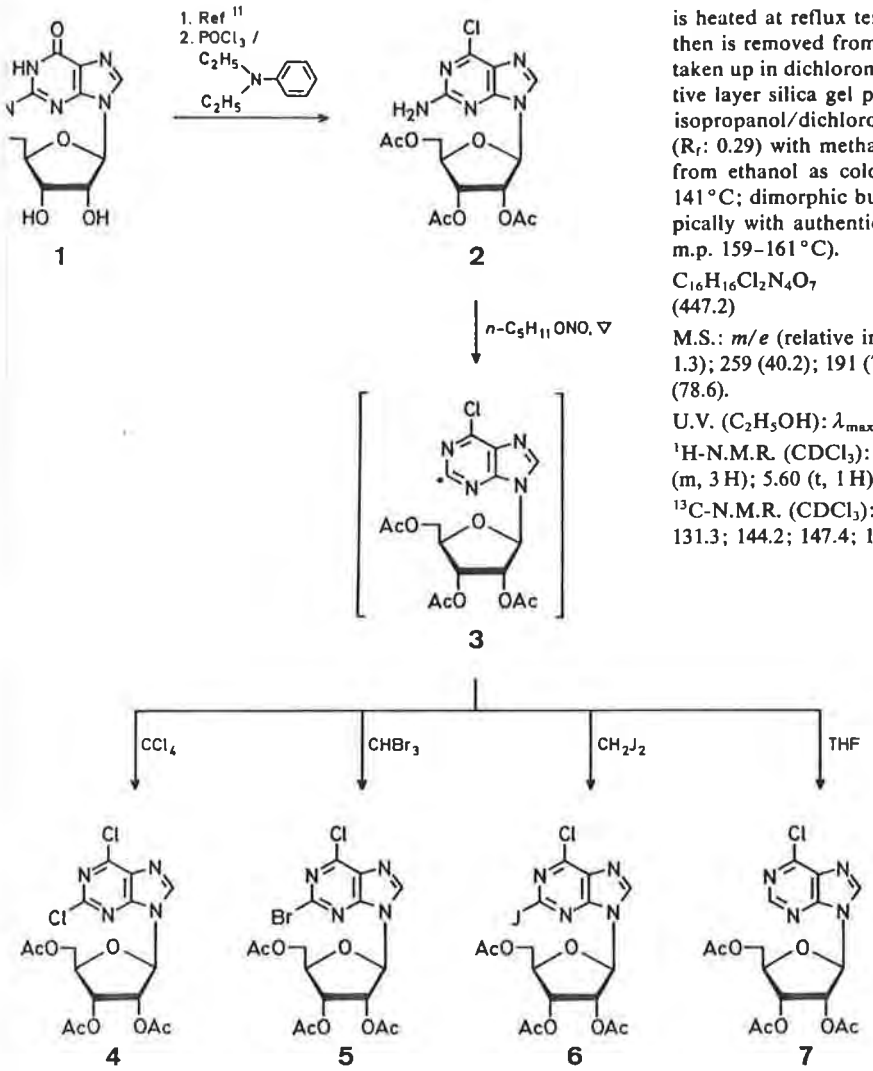

nethane, respectively, were used as solvents. This is ex-
:ted from the differences in bond energies between C—H
6 kJ/mol; 97 kcal/mol) and C—Br (276 kJ/mol; 66 kcal/
l) or C—J (218 kJ/mol; 52 kcal/mol). As expected, when
reaction was carried out in tetrahydrofuran as solvent, re-
:tive deamination to 6-chloronebularine (**7**)[10] occurred.

summary, this work provides a useful approach to the syn-
sis of dihalogenated purine nucleosides using simple, read-
available reagents. The yields are good to excellent.
ither hydrolytic deamination, hydrolysis of halogen, nor
;ar cleavage are significant side reactions under these mild,
ı-aqueous, non-acidic reaction conditions. The high cost
l limited availability of 2,6-dihalogenated purines, and the
ld and selectivity of base-sugar coupling methods suggests
t specific transformations using natural nucleosides is the
t approach for the synthesis of halogenated nucleosides.

lting points, determined on a Thomas-Hoover capillary melting-
nt apparatus, are uncorrected. N.M.R. spectra were recorded on
)L FX90Q and Bruker HX90E pulse Fourier transform spectromet-
U.V. spectra were taken on a Cary 219 spectrophotometer. Mass
ctra at 70 eV were obtained on a Hewlett Packard 5985B GC-mass
ctrometer.

·Dichloro-9β-(2′,3′,5′-tri-*O*-acetyl)-D-ribofuranosylpurine (4):
ıixture of compound **2**[11,12] (0.293 g, 0.685 mmol), dry, distilled *n*-
tyl nitrite (1.53 ml, 11.4 mmol), and dry tetrachloromethane (30 ml) is heated at reflux temperatures under nitrogen for 24 h. The solvent then is removed from the yellow solution and the resulting residue is taken up in dichloromethane (3 ml) and chromatographed on preparative layer silica gel plates. The plates are developed twice with 1:50 isopropanol/dichloromethane. Elution of the only significant band (R_f: 0.29) with methanol/dichloromethane gives **4** which crystallizes from ethanol as colorless needles; yield: 0.201 g (66%); m.p. 139–141 °C; dimorphic but identical chromatographically and spectroscopically with authentic **4** prepared by the literature method[13] (Ref.[13], m.p. 159–161 °C).

$C_{16}H_{16}Cl_2N_4O_7$	calc.	C 42.97	H 3.61	N 12.53
(447.2)	found	43.2	3.5	12.3

M.S.: *m/e* (relative intensity) = 448 ($^{35}Cl^{37}ClM^+$, 0.4); 446 ($^{35}Cl_2M^+$, 1.3); 259 (40.2); 191 (7.1); 190 (2.7); 189 (10.9); 188 (2.8); 139 (100); 97 (78.6).

U.V. (C_2H_5OH): λ_{max} = 213 (ε = 20000); 252 (5400); 273.5 nm (8300).

^{1}H-N.M.R. ($CDCl_3$): δ = 2.09 (s, 3H); 2.14 (s, 3H); 2.17 (s, 3H); 4.43 (m, 3H); 5.60 (t, 1H); 5.82 (t, 1H); 6.23 (d, 1H); 8.34 ppm (s, 1H).

^{13}C-N.M.R. ($CDCl_3$): δ = 20.3; 20.5; 20.7; 62.9; 70.5; 73.2; 80.8; 86.6; 131.3; 144.2; 147.4; 152.2; 153.3; 169.4; 169.6; 170.2 ppm.

2-Bromo-6-chloro-9β-(2′,3′,5′-tri-*O*-acetyl)-D-ribofuranosylpurine (5):
A mixture of compound **2** (0.165 g, 0.385 mmol), *n*-pentyl nitrite (0.87 ml, 6.44 mmol), and tribromomethane (10 ml) is heated under nitrogen at 85 °C for 8 h. Work-up, separation, and crystallization as described for **4** gives **5** as white crystals; yield: 0.102 g (55%); m.p. 155–156 °C (Ref.[3], m.p. 153–154 °C).

$C_{16}H_{16}BrClN_4O_7$	calc.	C 39.08	H 3.28	N 11.40
(491.7)	found	39.3	3.4	11.3

M.S.: *m/e* (relative intensity) = 494 ($^{81}Br^{37}ClM^+$, 0.2); 492 ($^{79}Br^{37}ClM^+$ and $^{81}Br^{35}ClM^+$, 0.4); 490 ($^{79}Br^{35}ClM^+$, 0.4); 259 (53.5); 235 (11.0); 234 (3.3); 233 (9.2); 232 (2.2); 139 (100.0); 97 (67.0).

U.V. (CH_3OH): λ_{max} = 216 (ε = 21800); 254 (5200); 275 nm (8600).

^{1}H-N.M.R. ($CDCl_3$): δ = 2.10 (s, 3H); 2.13 (s, 3H); 2.17 (s, 1H); 4.44 (m, 3H); 5.65 (t, 1H); 5.82 (t, 1H); 6.24 (d, 1H); 8.34 ppm (s, 1H).

^{13}C-N.M.R. ($CDCl_3$): δ = 20.4; 20.5; 20.8; 63.0; 70.6; 73.3; 80.8; 86.7; 131.7; 143.4; 144.0; 151.8; 152.5; 169.5; 169.6; 170.3 ppm.

2-Iodo-6-chloro-9β-(2′,3′,5′-tri-*O*-acetyl)-D-ribofuranosylpurine (6):
A mixture of compound **2** (0.303 g, 0.709 mmol), *n*-pentyl nitrite (1.91 ml, 14.2 mmol), and diiodomethane (5 ml) is heated at 85 °C for 1 h. Work-up, separation, and crystallization as described for **4** gives **6** as hygroscopic, white crystals; yield: 0.318 g (83%); m.p. 181–183 °C.

$C_{16}H_{16}ClIN_4O_7 \cdot 1.5H_2O$	calc.	C 33.97	H 3.39	N 9.90
(538.7)	found	34.2	3.2	9.6

M.S.: *m/e* (relative intensity) = 540 ($^{37}ClM^+$, 1.0); 538 ($^{35}ClM^+$, 2.1); 283 (6.6); 282 (2.3); 281 (15.3); 280 (2.3); 259 (68.7); 139 (100.0); 97 (75.6).

U.V. (CH_3OH): λ_{max} = 222.5 (ε = 21200); 258 (6600); 281 nm (9300).

N.M.R. ($CDCl_3$): δ = 2.11 (s, 3 H); 2.13 (s, 3 H); 2.18 (s, 3 H); 4.43 3 H); 5.65 (t, 1 H); 5.81 (t, 1 H); 6.23 (d, 1 H); 8.27 ppm (s, 1 H).
-N.M.R. ($CDCl_3$): δ = 20.4; 20.5; 20.8; 62.9; 70.5; 73.3; 80.8; 86.7; .9; 132.2; 143.4; 150.7; 151.9; 169.4; 169.5; 170.2 ppm.

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methylsilylketene: Synthesis of Coumarins via clization-Elimination

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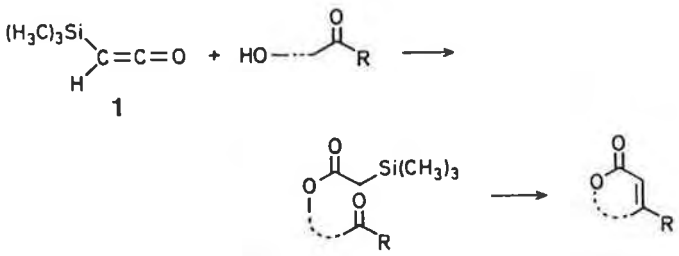
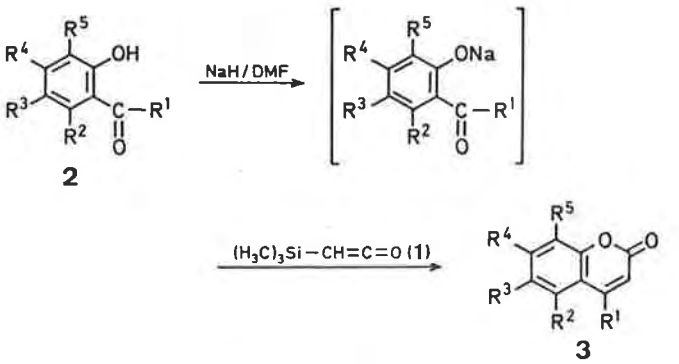
ile trimethylsilylketene (**1**) is easily prepared from ethoxytylene[1] and is stable with respect to dimerization and deposition, few synthetic applications of this reagent have n developed[2-5]. Alcohols have been added to trimethylsietene, affording alkyl α-silylcarboxylates[1]. Since enolate ons derived from such esters have been added to carbonyl pounds with resultant elimination to α,β-unsaturated es-[6,7], combination of these two reactions in an intramolecusense would afford a ready synthesis of cyclic unsaturated ones.



1

In this communication we describe application of this strategy for the one-pot conversion of *o*-acylphenols (**2**) to coumarins (**3**) in high yields.



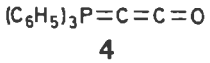
2

3

Following formation of the phenoxide with sodium hydride in dimethylformamide, the ketene (**1**) is added at room temperature. The reaction is monitored by I.R. spectroscopy until the ketene ($\nu_{C=O}$ = 2100 cm^{-1}) is consumed (~2 h). No intermediates were observed via T.L.C. analysis, indicating that, under our conditions, addition of phenoxide to ketene **1** appears to be rate-limiting.

The reaction accommodates a wide variety of substitution patterns with little variation in reaction time. Yields were observed to be substantially lower when other hydroxy groups were present, probably due to competitive nucleophilic addition. When two equivalents of ketene **1** are added, the yield is restored to the normal level. Presumably, the other hydroxy group is protected as the silylacetate which is hydrolyzed upon work-up.

The cumulated phosphorane **4** has been used to form coumarins in a conceptually similar reaction[8]. While our method requires phenoxide formation, the reaction occurs much more readily to give a somewhat higher yield. The work-up and isolation is also easier since triphenylphosphine oxide is obtained in the ylid reaction.



4

Other conversions of *o*-acylphenols to coumarins usually involve applications of the Knoevenagel reaction[9]. Phenols are frequently converted directly to coumarins via the Pechmann reaction[10]. The present method involves milder conditions, is a one-pot procedure, and gives excellent yields with a wide variety of substituents.

Coumarins (3); General Procedures:

Method A, for Coumarins **3a–d, h**: Sodium hydride (0.5 g, 22.2 mmol) and freshly distilled dimethylformamide (20 ml) are placed in a dry, 50 ml, three-necked, round-bottomed flask fitted with stirrer, argon inlet, condenser, and addition funnel. Then, a solution of the *o*-acylphenol (**2a–d, h**; 22.2 mmol) in dimethylformamide (10 ml) is added and the mixture is stirred under argon at room temperature until

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INHIBITION OF 3′,5′-CYCLIC NUCLEOTIDE 3′-PHOSPHOHYDROLASE BY A NOVEL 1′,2′-CYCLIC NUCLEOTIDE

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SUMMARY

5'-(6-Aminopurin-9-yl)-5'-deoxy-α-D-ribofuranose 1',2'-cyclic monophosphate, a novel and unique structural analogue of 3',5'-cyclic AMP, has been synthesized. It is totally resistant to degradation by beef heart cAMP phosphodiesterases. It is a potent inhibitor of this enzyme with an $[I]_{50}$ of 14.1 μM determined at a cAMP concentration of 100 μM. This inhibitor activity is as good as that observed with 1-methyl-3-isobutylxanthine (MIX).

INTRODUCTION

The major pathway for the degradation of adenosine 3',5'-cyclic monophosphate (cyclic AMP or cAMP) involves hydrolysis of the 3'-phosphate ester group which is catalyzed by 3',5'-cyclic nucleotide 3'-phosphohydrolases (3',5'-cyclic nucleotide phosphodiesterases, E.C. 3.1.4.17) (3-8). The propensity of this enzyme to exhibit multiple forms in mammalian tissues has been observed repeatedly in many laboratories (5). However, most investigators in this area agree that two main forms of the enzyme appear to exist in any homologous tissue preparation, multiple forms being characteristic of the heterogeneity of the tissues used (5,8). The two forms differ in a number of aspects but primarily in their affinity for a natural substrate, 3',5'-cyclic AMP. In most tissues, the low affinity form has a K_m of the order of 10^{-4} to 10^{-5} M, whereas the high affinity enzyme has a K_m about two orders of magnitude lower (~10^{-6} M) (5-7). The particulate nature of the low K_m form has been stressed in a number of studies (5,6). It has been demonstrated also that certain phosphodiesterases have a calcium-dependent protein activator (6). The extent of hydrolysis of cAMP analogues by the enzyme and its inhibition by a wide variety of compounds have been investigated (7,9,10).

Synthetic analogues of cAMP may be useful as biological probes for further understanding of the molecular mechanism of action of cAMP.

They may contribute also to the development of potential chemotherapeutic agents exhibiting tissue selectivity with respect to enzymes of the cAMP system. We wish to report on the synthesis and potent inhibitor activity towards cAMP-phosphodiesterase of a novel 1',2'-cyclic nucleotide. The implications of this type of study can be appreciated when one views the actions of such cAMP-phosphodiesterase inhibitors as theophylline, 1-methyl-3-isobutylxanthine (MIX), quazodine, papaverine, and a number of other drug substances purported to exert their effects through inhibition of destruction of endogenous cAMP.

MATERIALS AND METHODS

General

The ^{1}H, ^{13}C, and ^{31}P NMR spectra were recorded on JEOL FX90Q and Bruker HX-90E pulse Fourier transform spectrometers. All thin layer chromatographies on cellulose were conducted using Bakerflex Cellulose F plates (2.5 x 7.5 cm) and using ethanol:1M ammonium acetate (5:2) as the developing solvent. Ultraviolet absorption spectra, all static ultraviolet and visible absorbance measurements, and all dynamic spectrophotometric enzyme assays were accomplished using a Varian Associates Cary 219 recording UV-VIS spectrophotometer. All kinetic measurements were conducted, unless otherwise stated, at 25.0 ± 0.1°C. The UV absorbance of column effluents was monitored with a Pharmacia UV-2 UV monitor.

Adenosine deaminase (Type I from calf intestinal mucosa), alkaline phosphatase (from E. coli), adenine, ribose, theophylline, 1-methyl-3-isobutylxanthine (MIX), DEAE-Sephadex, and Malachite Green (free base) were purchased from Sigma Chemical Co., St. Louis, Mo. Beef heart 3',5'-cAMP phosphodiesterase was purchased from Boehringer, Mannheim, Fed. Republic of Germany. Adenosine 3',5'-cyclic monophosphate (3',5'-cAMP) and adenosine 2',3'-cyclic monophosphate (2',3'-cAMP) were purchased from Aldrich Chemical Co., Milwaukee, Wi.

Synthesis

5'-(6-Aminopurin-9-yl)-5'-deoxy-α(β)-D-ribofuranose (1). This compound was prepared in four steps (11-14) from D-ribose.~ It was purified by reverse phase HPLC on Amberlite XAD-4 (44-53 μm) with 5% ethanol-water as the eluting solvent. Crystallization of the purified material from water gave white prisms, m.p. 181-182° (lit. (13) 168-169°); UV $\lambda_{max}^{H_2O}$ = 261 nm (ε 15,000 M^{-1} cm^{-1}); ^{1}H NMR δ TMS (TMS capillary, ext. ref.) (0.2 M, D_2O) 4.30 (q, 2H, H5'), 4.35 to 5.64 (m, 3H, H2', H3', H4'), 5.72 (d, 0.14H, H1' α), 5.85 (s, 0.86H, H1' β), 8.52, 8.58 (s, s, 1H, 1H, H2, H8); ^{13}C NMR δ TMS (p-dioxane, δ 67.4 ppm, int. ref.) 46.0, 46.5, 71.4, 71.9, 72.2, 75.8, 80.5, 80.9, 97.3, 102.0, 118.7, 143.9, 149.7, 153.1, 156.0.

5'-(6-Aminopurin-9-yl)-5'-deoxy-α-D-ribofuranose 1',2'-Cyclic monophosphate (3) and 5'-(6-Aminopurin-9-yl)-5'-deoxy-α(β)-D-ribofuranose 2',3'-cyclic monophosphate (2). A mixture of 5'-(6-aminopurin-9-yl)-5'-deoxy-α(β)-D-ribofuranose (1, 802 mg, 3 mmol), dry pyridine (1.21 mL, 15 mmol), and dimethylformamide (20.3 mL) was made. The suspension was protected from moisture with a $CaCl_2$ drying tube and was warmed to effect dissolution. The solution was cooled in an ice-water bath to 0°C. Phosphorus oxychloride (0.28 mL, 3 mmol) was added to the cold solution with stirring. The resulting bright orange solution was allowed to stir at ambient temperature for 33 hr and then cooled in an ice-water bath to 0°C. Aqueous triethylammonium bicarbonate (1.0 M, pH 8, 10 mL) was added. The resulting solution was concentrated under reduced pressure (0.5 torr). The residue was dissolved in water (200 mL) and slowly applied to a 2.8 x 35 cm charcoal column (20-50 mesh, prepared by treating in succession with 500 mL conc. HCl, 1 L H_2O, 500 mL conc. NH_4OH, and H_2O until neutral). The column was then washed with H_2O (1 L) and eluted with ethanol: H_2O:benzene, 1:1: saturated (500 mL), followed by ethanol:H_2O:benzene, 6:3:1 (500 mL). The combined effluents were evaporated at reduced pressure (bath temperature 40°C). The residue was dissolved in H_2O (20 mL) and filtered with a 0.45 μm pore diameter filter. The filtered solution was divided into three equal volumes and each was applied to a DEAE Sephadex A-25-120 column (2.5 x 28 cm, HCO_3^- form, equilibrated with H_2O). The column was eluted with a linear gradient of 0 to 0.5 M triethylammonium bicarbonate, pH 8, 600 mL each chamber, 1.2 L total volume. Fractions of the effluent were collected, and the absorbance at 254 nm of the effluent was continuously monitored. A peak with a large shoulder eluted at 0.3-0.4 M buffer (see Fig. 1). Fractions corresponding to this peak were pooled and evaporated under reduced pressure (bath temperature 40°C). This material was further resolved on a DEAE Sephadex A-25-120 column (2.0 x 54 cm, HCO_3^- form, equilibrated with 0.15 M triethylammonium bicarbonate, pH 8). Two peaks were observed in the chromatogram. The second peak was the pure α, β equilibrium mixture of the 2',3'-cyclic phosphate 2 (triethylammonium salt) (glassy solid) (60 mg, 4.6%). The first peak was the 1',2'-cyclic phosphate, which was contaminated with a small amount of the 2',3'-cyclic phosphates (see Fig. 1). Further purification on the 2.0 x 54 cm DEAE Sephadex column (HCO_3^- form, equilibrated with 0.15 M triethylammonium bicarbonate, pH 8) gave pure 1',2'-cyclic phosphate 3 (triethylammonium salt) as a clear glassy hygroscopic solid (51 mg, 3.9%). For the 2',3'-cyclic phosphate 2 (triethylammonium salt): UV $\lambda_{max}^{H_2O}$ 261 nm (ε 15,000 M^{-1} cm^{-1}); ^1H NMR δ TMS (0.05 M, D_2O) 1.72 (t, J = 7.33 Hz, 9H, $[NH(CH_2CH^*{}_3)_3]^+$, 3.63 (q, J = 7.33 Hz, 6H, $[NH(CH^*{}_2\text{-}CH_3)_3]^+$), 4.74-5.50 (m, 5H, H2', H3', H4', 2H5'), 5.60 (d, J = 6.9 Hz, 0.14 H, H1'(α)), 5.99 (s, 0.86, H1'(β)), 8.58 (s, 2H, H2, H8); ^{13}C NMR δ TMS (0.15 M, H_2O) 9.1, 47.6, 47.7, 77.5, 78.4, 80.1, 83.7, 84.0, 97.2, 103.0, 103.3, 119.6, 144.0, 147.5, 151.6, 155.0; ^{31}P NMR δ H_3PO_4 (0.1 M, H_2O) (85% H_3PO_4 capillary, ext. ref.) 20.0 (s, 0.86 P, β), 21.5 (s, 0.14 P, α).

Anal. Calc'd for $C_{16}H_{27}N_6O_6P \cdot H_2O$: C, 42.86; H, 6.52; N, 18.72. Found: C, 43.18; H, 6.92; N, 18.90.

For the 1',2'-cyclic phosphate 3 (triethylammonium salt): UV $\lambda_{max}^{H_2O}$ 261 nm (ε 15,000 M^{-1} cm^{-1}); ^{13}C NMR δ TMS (0.4 M, H_2O) 9.2, 45.4, 47.6,

72.8, 73.4, 79.4, 102.6, 119.3, 144.7, 149.7, 151.6, 155.3; ^{31}P NMR δ H_3PO_4 (0.1 M, H_2O) (85% H_3PO_4 capillary ext. ref.) 18.3 (s).

Anal. Calc'd for $C_{16}H_{27}N_6O_6P \cdot H_2O$: C, 42.86; H, 6.52; N, 18.72. Found: C, 42.53; H, 6.91; N, 19.45.

A second method also was used to prepare compounds 2 and 3. In this procedure, a mixture of 1 (802 mg, 3 mmol), dimethylformamide (20.0 mL), and dry pyridine (1.21 mL, 15 mmol) was warmed to effect dissolution. This solution was cooled to 0°C and treated with trichloromethyl phosphonic acid dichloride (715 mg, 3 mmol) (15-18), and then kept at 4°C for 48 hr. Ice-cold distilled water (55 mL) was added and the resulting solution was neutralized with cold 5 M NaOH. This solution was further diluted to 300 mL, passed over a column of activated

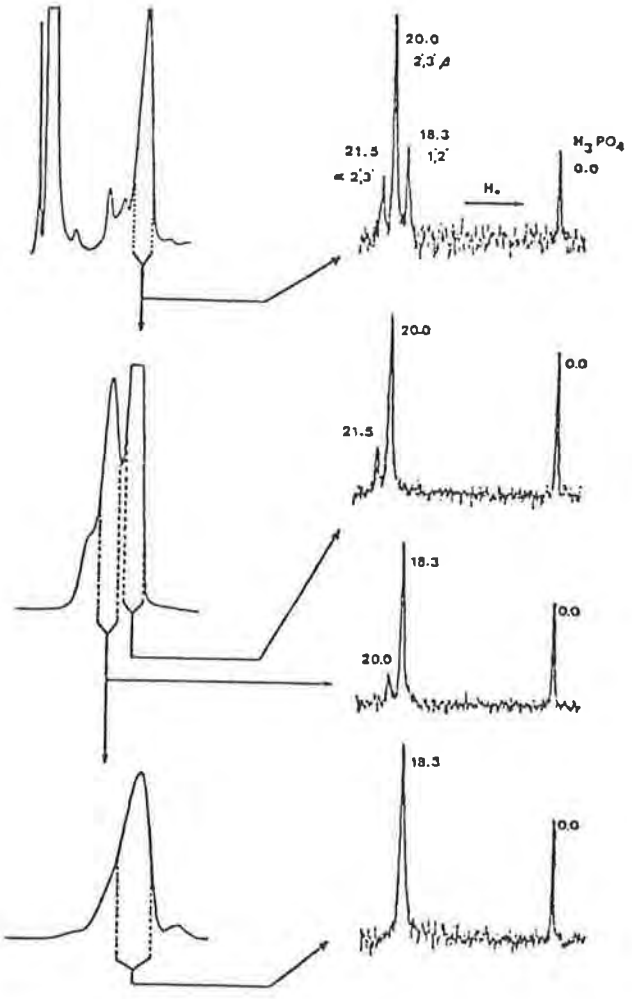


Fig. 1. Chromatograms showing purification of 2 and 3 on DEAE Sephadex monitored by UV and ^{31}P NMR spectroscopy.

charcoal (2.0 x 30 cm, 20-50 mesh), and then eluted and separated on DEAE Sephadex as described above. The % yields of pure 2 and 3 were 14.1% and 13.4%, respectively.

Enzymology

Substrate Activity. Determination of the rate of hydrolysis of the cyclic nucleotides catalyzed by beef heart 3',5'-cyclic AMP phosphodiesterase (PDE) relied on measurement of the free phosphate produced as a result of the consecutive action of PDE and of *E. coli* alkaline phosphatase (19). These phosphate assays followed the general procedure of Itaya and Ui (20) with some modifications (15,21). Substrate concentrations were 100 µM. Three phosphate determinations were made at the beginning and three at the end of each assay. The phosphate determinations were averaged before calculation of $\Delta [P_i]$. The detailed procedure has been described previously by us (15). Hydrolysis of 3',5'-cAMP was used as the standard and substrate activities of the synthetic cyclic nucleotides were expressed as a percentage of 3',5'-cAMP activity.

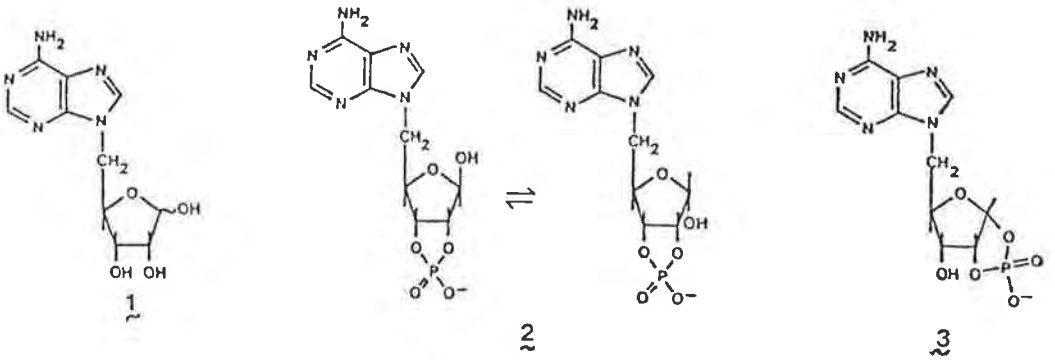
Inhibition Studies. The assays used in these studies employed a method of our own design. In this procedure, the adenosine produced by the consecutive action of PDE and alkaline phosphatase on 3',5'-cyclic AMP was deaminated with adenosine deaminase to produce inosine. The concentrations of the enzymes were established in such a way that the alkaline phosphatase and adenosine deaminase steps were not rate limiting. The conversion of adenosine to inosine was monitored spectrophotometrically. This procedure was used to generate the Lineweaver-Burk plot for 3',5'-cyclic AMP and to obtain inhibition data for theophylline, MIX and for the synthetic cyclic nucleotides 2 and 3. In these assays, 41.4 µL of an enzyme solution containing 0.929 unit (4.0 µg) of ADA, 0.352 unit (20.7 µg) of alkaline phosphatase, and 0.005 unit (20 µg) of PDE was added to 0.96 mL of a mixture containing 40 µmol TRIS, 25 µmol magnesium acetate, and appropriate amounts of cAMP or cAMP and inhibitor (see Figures for exact concentrations used). The actual kinetic experiment was conducted after all constituents for each assay, except the solution containing the enzymes (kept on ice), had been pipetted into a test tube containing the otherwise complete reaction mixture. To initiate a particular assay, the enzymes solution was added. After mixing, the solution was transferred to a narrow-path, 1 cm quartz cell of approximately 0.8 mL capacity, and the absorbance at 265 nm recorded with respect to time for five minutes. The reaction rate was demonstrated to be linear for at least five minutes.

RESULTS

The starting compound for our syntheses was natural D-ribose, which was converted in several steps to the nucleoside 1 (11-15). Phosphorylation of this compound can be carried out by two methods. Initially, this reaction was carried out with phosphorus oxychloride in dimethylformamide containing pyridine. After careful hydrolysis of excess acid chloride, neutralization, and desalting on charcoal, the crude product

was chromatographed on DEAE Sephadex with salt-gradient elution. A single phosphorus-containing peak was obtained. This peak had ^{31}P NMR chemical shifts of 21.5, 20.0, and 18.3 ppm indicative of cis, five-membered ring cyclic phosphates (22,23). The two cyclic phosphates that can be formed from 1 are 2 (α and β) and 3. Fig. 1 depicts the separation process used to purify 2 and 3. The phosphorus-containing peak from the first chromatography was subjected to two further separations on DEAE Sephadex with isocratic elution for complete separation and purification of 2 and 3. Phosphorylation of 1 to 2 and 3 in a one-step procedure and in higher yields can be achieved with trichloromethyl phosphonic acid dichloride (15-18) in dimethylformamide. The intermediate trichloromethyl phosphonic esters cyclize spontaneously under the conditions of the reaction and work-up. Complete structural confirmation for the cyclic nucleotides came from ^{1}H, ^{13}C, and ^{31}P NMR spectroscopy and UV spectroscopy.

As further evidence of structure, hydrolysis of the 1',2'-cyclic nucleotide 3 in 0.2 M HCl (aqueous) was conducted, and the progress of reaction was followed by ^{31}P NMR spectroscopy. Both the protonated 2'-phosphate monoester (δ -0.32 ppm) and the protonated α 1'-phosphate monoester (δ -1.37 ppm) were produced as intermediates. After 24 hr, the only ^{31}P NMR resonance observed was at 0.00 ppm, indicating that all esters had been hydrolyzed.



Phosphodiesterase assays were performed by coupled enzyme methods. Determination of the rate of hydrolysis of the synthetic cyclic nucleotides was carried out by coupling the PDE step to alkaline phosphatase and analyzing the inorganic phosphate thereby released (15,19-21). Data are expressed as percentage ratio of the substrate activity of the test compound to the activity of 3',5'-cyclic AMP. The results shown in Table 1 represent total enzymatic activity ("high" and "low" K_m).

Inhibition studies were performed by a dynamic assay developed in our Laboratory which involved coupling of the PDE, alkaline phosphatase steps to a third enzyme, adenosine deaminase. This reaction can be followed spectrophotometrically by observing the change in absorbance

Table 1

Relative Rates of Hydrolysis of Cyclic Nucleotides by Beef Heart 3',5'-cAMP Phosphodiesterases[a]

Compound	Relative Rate (%)
3',5-Cyclic AMP	100 (5)
2',3'-Cyclic AMP	1.6 (5)
3',5'-Cyclic GMP	120 (7)
Cyclic Nucleotide 2	1.8 (5)
Cyclic Nucleotide 3	0.0 (12)

a. The number of determinations are shown in parenthesis. Enzyme concentrations were adjusted to give observable rates. The rates were finally expressed as μMol/min/mg from which relative rate data (%) were obtained. Substrate concentrations were 100 μM.

at 265 nm, the wavelength of maximum difference between the spectrum of adenosine and that of inosine. E. coli alkaline phosphatase is a relatively non-specific enzyme, and it was determined in pilot experiments that this enzyme could quantitatively convert the phosphate monoesters of 1 to inorganic phosphate and nucleoside 1. However, this enzyme was found to display no activity with either cAMP or with the synthetic cyclic nucleotides. Concentrations of alkaline phosphatase and adenosine deaminase were not rate limiting. The nucleoside 1 is a poor substrate for adenosine deaminase and charged nucleotides are not substrates for this enzyme. Thus, the results were not complicated by observance of any hydrolysis of the inhibitor. In addition, the reaction rate was observed to be linear for at least five minutes, so that any errors due to feedback inhibition of any of the enzymes in the coupled reactions were not significant in the initial rate observations made in this study. The validity of our results obtained by the adenosine deaminase-coupled assay is supported by the observation of a value for K_m (17.8 μM, r = 0.990) for the PDE-catalyzed hydrolysis of 3',5'-cyclic AMP which is in agreement with published values (4). In the inhibition experiments, the initial concentration of cAMP in each determination was 100 μM, and the concentration of the inhibitor was varied from ~10^{-7} M to 10^{-3} M. The activity at a particular concentration of inhibitor was expressed as a percentage of the activity in the absence of inhibitor. The concentration of inhibitor at which the activity was 50% of the value obtained in the absence of inhibitor ($[I]_{50}$) was determined by graphical interpolation. The known inhibitors, theophylline and MIX were included to provide a basis for comparison. The results of the inhibition studies with compounds 2 and 3 are shown in Figs. 2 and 3.

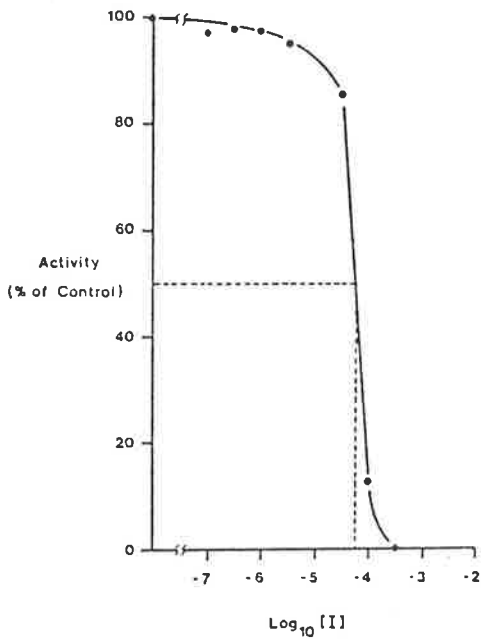


Fig. 2. Inhibition by 2 of beef heart PDE activity. [cAMP] = 100 μM, $[I]_{50}$ = 58 μM.

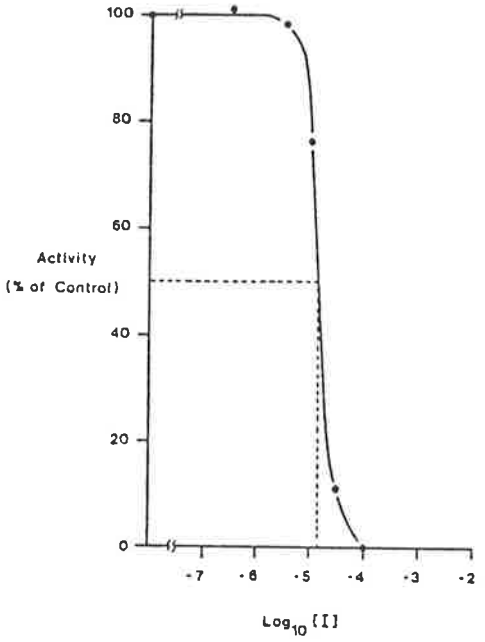


Fig. 3. Inhibition by 3 of beef heart PDE activity. [cAMP] = 100 μM, $[I]_{50}$ = 14 μM.

DISCUSSION

5'-(6-Aminopurin-9-yl)-5'-deoxy-α-D-ribofuranose 1',2'-cyclic monophosphate 3, a novel and unique structural analogue of 3',5'-cyclic AMP has been synthesized in one step by phosphorylation of the precursor nucleoside 1 with trichloromethyl phosphonic acid dichloride. Its structure was established by spectroscopic data as well as by its hydrolysis to the mixture of 1'- and 2'-phosphate monoesters. Another product formed in the phosphorylation reaction was the 2',3'-cyclic nucleotide 2, which was separated and purified from 3 by anion-exchange chromatography on DEAE Sephadex.

The stability of 2 and 3 with respect to hydrolytic degradation catalyzed by 3',5'-cyclic AMP phosphodiesterases (Table 1) was studied. Both synthetic cyclic nucleotides are resistant to such degradation. In fact, compound 3 is totally unreactive hydrolytically in the presence of this enzyme. This observation is consistent with studies on 2',3'-cyclic AMP which has been shown to display negligible substrate activity towards the PDE (4). The inhibition data, obtained using a coupled-enzyme method of our own design (cf. 24), are more significant. Compound 2 is a strong inhibitor of the PDE with an

$[I]_{50}$ of 58 μM, lower than values previously reported for theophylline (9). The $[I]_{50}$ value of 14.1 μM for compound 3 makes it an extremely potent inhibitor of the PDE. Of special significance with respect to this inhibitor activity is that these studies were conducted at concentrations of 3',5'-cyclic AMP (100 μM) much higher than the K_m of the enzyme (17.8 μM) for 3',5'-cyclic AMP. It should be mentioned also that our synthetic cyclic nucleotide 3 is as good an inhibitor of "high" K_m PDE as MIX ($[I]_{50}$ = 15.5 μM). In addition, the results of these inhibition studies are very striking when viewed in light of the fact that 2',3'-cyclic AMP displays an $[I]_{50}$ of 600 μM with cat heart PDE (25). Although our inhibition study has been limited by the absence of the effects of the synthetic cyclic nucleotides on "low" K_m PDE, the observed inhibition of "high" K_m PDE may be just as important, as it has been postulated that this enzyme is responsible for the rapid removal of the glut of 3',5'-cyclic AMP produced on adenylate cyclase activation (26).

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GEORG THIEME VERLAG · STUTTGART · NEW YORK

Methyl 2,6-Bis-*O*-tri-*n*-butylstannyl-α-D-glucopyranoside (5j):
A solution of acetylacetone (0.49 g, 4.89 mmol) is added to a stirred solution of the above product **5i** (2.19 g, 2.41 mmol) in heptane (5 ml) at room temperature. The reaction mixture immediately turns yellow (formation of **4**). After 0.5 h, the solution is concentrated (bath temperature: max. 60 °C/10^{-3} torr) to leave the colourless, viscous product; yield: 1.9 g, (~ 100 %); $[\alpha]_D^{20}$: 28.6° (*c* 1.7, DMSO).

$C_{31}H_{66}Sn_2O_6$	calc.	C 48.22	H 8.61	Sn 30.74
(772.2)	found	48.34	8.49	30.62

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Reductive Deamination of Aminopurine Nucleosides

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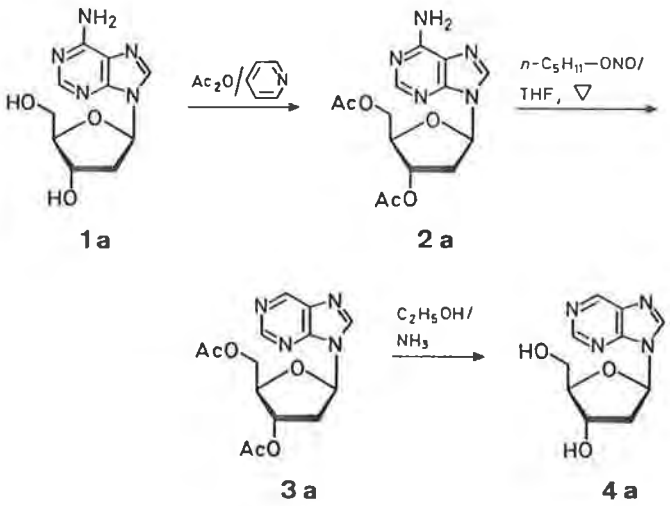
The reductive deamination of adenosine analogs provides a direct route to purine nucleosides. Some of these uncommon nucleosides have been found to have interesting enzymatic and biological properties. For example, 9-(β-D-ribofuranosyl)-9*H*-purine (**4b**; nebularine) is an antibiotic, which is a strong competitive inhibitor of adenosine deaminase[1,2,3]. It has been studied as an antileukemic agent in combination chemotherapy[4]. 7-Deazanebularine (**4d**) inhibits cellular and viral nucleic acid synthesis and is cytotoxic to mammalian cells[5,6]. Other deaminated nucleosides also have been reported to exhibit biological activity[7,8,9]. We have reported recently[10] that when protected adenosine is treated thermally with *n*-pentyl nitrite in tetrahydrofuran, the corresponding reductively deaminated product, nebularine is formed. The reaction apparently proceeds through the intermediacy of purinyl radicals which abstract hydrogen atoms from solvent molecules. This paper reports the improvement of this synthetic procedure and its application to the direct synthesis of a number of nucleoside antimetabolites.

The starting compounds for these reductive deaminations were obtained commercially with the exception of 8-azaadenosine (**1e**) which was prepared by a reported method[11]. The



diazotization procedure in non-hydroxylic organic solvents requires that the nucleoside hydroxy groups are protected to avoid cross nitrite ester formation and also to enhance solubility. The protected (acetylated) nucleosides **2** were deaminated on heating with *n*-pentyl nitrite in dry tetrahydrofuran under nitrogen. The yields for the deaminations varied between 46 and 81 % (Table 1). The optimum temperature for

Table 1. Steps and Product Yields in the Reductive Deamination of Nucleosides

Starting Compound	Yields [%] for			Final Product
	acetylation	deamination	deacetylation	
1a	74	64	71	**4a**
1b	72	75	83	**4b**
1c	94	51	74	**4c**
1d	86	46	72	**4d**
1e	72	81	90	**4e**

Table 2. Physical Data for Compounds **4a–e**

Product	m.p. [°C] (Lit.m.p.[°C])	M.S. *m/e* (relative intensity %)	U.V. (H_2O) λ_{max} (ε)	^{1}H-N.M.R. (DMSO-d_6) δ [ppm]	^{13}C-N.M.R. (DMSO-d_6) δ [ppm]
4a	180–182° (181–182°)[14]	236 (M^+, 1.1); 219 (1.6); 206 (5.7); 163 (2.3); 159 (5.0); 149 (42.6); 135 (4.4); 121 (100); 120 (26.2); 119 (12.4); 93 (16.5); 66 (8.8)	264 (7450)	2.37 (m, 1 H); 2.78 (m, 1 H); 3.53 (m, 1 H); 3.63 (m, 1 H); 3.89 (q, 1 H, J = 7.8 Hz, 4.4 Hz); 4.45 (m, 1 H); 4.99 (t, 1 H, J = 5.6 Hz); 5.37 (d, 1 H, J = 3.9 Hz); 6.49 (t, 1 H, J = 6.7 Hz); 8.82 (s, 1 H); 8.95 (s, 1 H); 9.18 (s, 1 H)	39.4; 61.6; 70.6; 83.6; 88.0; 134.2; 145.2; 148.0; 150.6; 151.9
4b	178–179° (181–182°)[12]	252 (M^+, 0.5); 235 (2.6); 222 (2.8); 175 (4.4); 163 (24.6); 159 (1.4); 149 (90.7); 134 (4.4); 133 (8.7); 121 (100); 120 (12.9); 119 (4.4); 93 (5.5); 66 (1.3)	262 (6690)	3.65 (d, 1 H, J = 11.7 Hz); 3.74 (d, 1 H, J = 11.7 Hz); 4.04 (d, 1 H, J = 3.7 Hz); 4.24 (t, 1 H, J = 4.0 Hz); 4.68 (t, 1 H, J = 5.2 Hz); 5.14 (br, s, 1 H); 5.30 (br. s, 1 H); 5.87 (br. s, 1 H); 6.11 (d, 1 H, J = 5.6 Hz); 8.87 (s, 1 H); 8.99 (s, 1 H); 9.22 (s, 1 H)	61.3; 70.4; 73.8; 85.8; 87.7; 134.2; 145.4; 148.2; 151.0; 152.1
4c	253–254° (242–243°)[15]	236 (3.1); 222 (0.6); 175 (1.3); 165 (15.7); 149 (100); 134 (3.0); 133 (7.5); 121 (74.6); 120 (20.9); 119 (1.7); 93 (5.3); 66 (100)	262 (7220)	3.16 (d, 1 H, J = 4.2 Hz); 3.68 (s, 2 H); 3.82 (s, 1 H); 4.17 (d, 1 H, J = 4.2 Hz); 4.24 (s, 1 H); 5.10 (s, 1 H); 5.57 (d, 1 H, J = 3.1 Hz); 5.66 (s, 1 H); 6.42 (d, 1 H, J = 4.4 Hz); 8.67 (s, 1 H); 8.94 (s, 1 H); 9.17 (s, 1 H)	60.7; 74.8; 75.7; 83.7; 84.4; 133.5; 146.0; 147.6; 151.0; 151.9
4d	118–119° (117°)[16]	251 (M^+, 2.5); 234 (0.8); 221 (2.8); 174 (3.3); 162 (16.7); 158 (1.3); 148 (100); 133 (7.0); 132 (13.0); 120 (58); 119 (95.5); 118 (2.8); 92 (9.8); 65 (1.5)	270 (3500)	3.57 (d, 1 H, J = 11.1 Hz); 3.62 (d, 1 H, J = 11.1 Hz); 3.94 (d, 1 H, J = 3.2 Hz); 4.14 (s, 1 H); 4.45 (s, 1 H); 5.09 (br. s, 1 H); 5.18 (br. s, 1 H); 5.37 (br. s, 1 H); 6.23 (d, 1 H, J = 6.0 Hz); 6.72 (d, 1 H, J = 3.4 Hz); 7.87 (d, 1 H, J = 3.4 Hz); 8.81 (s, 1 H); 9.03 (s, 1 H)	61.5; 70.5; 73.9; 85.1; 86.6; 100.3; 119.1; 127.6; 149.4; 150.5; 150.8
4e	101–102° (101°)[17]	253 (M^+, 4.6); 223 (2.4); 175 (2.8); 165 (5.0); 159 (4.7); 149 (44.9); 135 (9.2); 122 (6.4); 121 (11.3); 120 (11.9); 97 (36.6); 85 (57.0); 71 (88.1); 57 (100)	262 (5860)	3.60 (m, 2 H); 4.04 (m, 1 H); 4.39 (t, 1 H, J = 3.8 Hz); 4.96 (t, 1 H, J = 5.2 Hz); 6.36 (d, 1 H, J = 4.6 Hz); 9.31 (s, 1 H); 9.83 (s, 1 H)	61.6; 70.6; 73.1; 86.2; 89.7; 135.5; 148.6; 152.1; 156.3

these reactions was 50 °C. Other hydrogen atom donors were also tried but proved inferior to tetrahydrofuran although dimethylformamide gave lower but acceptable yields.

The deacetylation of the deaminated compounds **3** to the purine nucleosides **4** required a modification of the literature procedure as low yields are often reported for this reaction[12,13]. Our modified procedure and work-up conditions gave yields of 70–90% (Table 1).

Indentification of the final products were made by U.V., mass spectral, and N.M.R. data, and by comparison of physical data available in the literature on these compounds. The high -field ^{1}H- and ^{13}C-N.M.R. data reported in Table 2 represent the most complete compilation of N.M.R. data for these compounds.

In summary, the reductive deamination procedure described in this paper has proved to be an excellent and direct route for the synthesis of purine nucleosides from their more readily available 6-amino precursors. This method has generality and can be utilized for the synthesis of a wide variety of biologically active nucleosides.

Melting points, determined on a Thomas Hoover capillary melting point apparatus, are uncorrected. N.M.R. spectra were recorded on a Bruker WM 360 spectrometer. U.V. spectra were taken on a Cary 219 spectrophotometer. Mass spectra at 70 eV were obtained on a Hewlett Packard 5985B GC-mass spectrometer.

Acetylation Reaction 1 → 2; General Procedure:
A mixture of nucleoside **1** (0.5 mmol), acetic anhydride (6 mmol), and dry pyridine (5 ml) is stirred at 0 °C for 1 h protected by a calcium chloride drying tube. The mixture is then stirred at 25 °C for

5 h. Ethanol (1 ml) is added and stirring is continued for 45 min. The solvent is then removed in vacuo (< 50 °C). The residue is repeatedly taken up in ethanol and evaporated until all of the pyridine is removed. The remaining clear oil is taken up in dichloromethane and chromatographed on preparative layer silica gel plates with methanol/dichloromethane (1 : 9) as the developing solvent.

Deamination Reaction 2 → 3; General Procedure:
A solution of the acetylated nucleoside **2** (0.5 mmol), dry, distilled *n*-pentyl nitrite (0.5 ml, 3.7 mmol), and dry tetrahydrofuran is stirred at 50 °C under nitrogen for 24 h. An additional aliquot of *n*-pentyl nitrite (0.5 ml) is added each day for two more days. The solvent is then removed and the oily residue is taken up in a methanol/dichloromethane mixture and chromatographed on silica gel plates with methanol/dichloromethane (1 : 9) as the eluting solvent. The products have R_f values just greater than the starting materials. The deaminated material is normally dried on a vacuum pump and used in the deacetylation reaction.

Deacetylation Reaction 3 → 4; General Procedure:
Absolute ethanol is cooled to ice/salt bath temperatures and saturated with ammonia over a period of 0.5 h. The acetylated nucleoside **3** is then dissolved in a minimum amount of absolute ethanol and added to the saturated solution. After standing for one day at room temperature, the mixture is re-saturated with ammonia and allowed to react for one more day. Removal of the solvent in vacuo is followed by repeatedly taking up the residue in methanol and rotoevaporating off the methanol. To ensure complete removal of acetamide the residue is placed in a sublimation apparatus at 30 °C for 12 h. The products are crystallized finally from methanol/ether.

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An Improved Synthesis of N^{δ}-(Phosphonoacetyl)-L-ornithine

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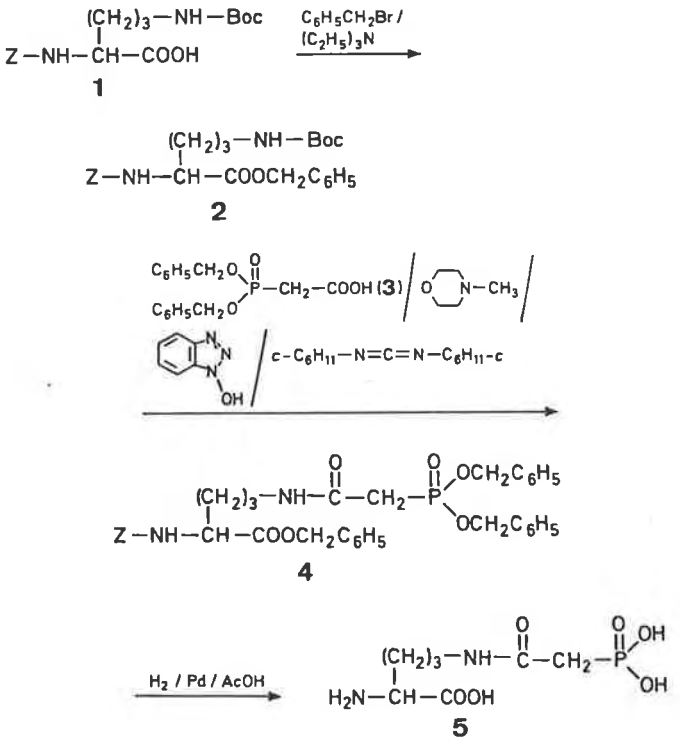
Transition-state analogues are analogues of activated complexes of substrates which are formed during enzyme-catalysed reactions. As these activated complexes bind more tightly than the substrates, their analogues are potent enzyme inhibitors and are highly specific since the activated complexes of substrates are unlikely to be identical for different enzyme reactions[1,2,3]. As part of studies on ornithine transcarbamylase, the second enzyme of the urea biosynthetic pathway, we have synthesised the transition-state analogue N^{δ}-(phosphonoacetyl)-L-ornithine (PALO; **5**)[4], and shown it to be a potent and specific inhibitor of ornithine transcarbamylase. These properties have been utilized in the development of a purification procedure using immobilised PALO, whereby ornithine transcarbamylase can be purified to homogeneity in a single step from a variety of sources[5].

The usefulness of PALO as a reagent for isolating ornithine transcarbamylase has meant that prior methods which gave poor yields were inadequate for a large scale synthesis. These were further hampered by giving products of variable purity which required extensive chromatography. We now outline a simple synthesis of **5** via the stepwise manipulation of protected ornithine intermediates. This approach has the advantage of generating substantial quantities of PALO in high purity.



Boc = *t*-C_4H_9O-CO-
Z = $C_6H_5CH_2O$-CO-

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Novel Adducts from the Modification of Nucleic Acid Bases by Malondialdehyde[1]

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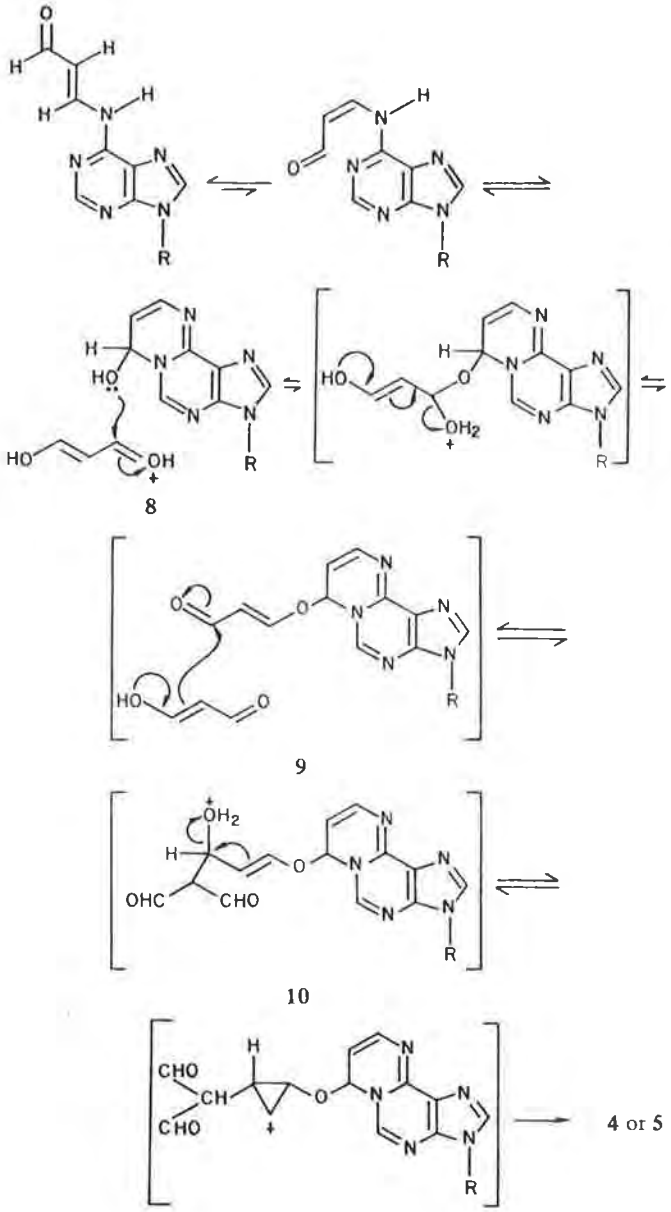
The ubiquitous natural compound, malondialdehyde (MDA) (**1**), is produced in animal tissues as an end product of unsaturated lipid peroxidation and as a side product of prostaglandin and thromboxane biosynthesis.[2] It is readily formed in the γ-irradiation of carbohydrates.[3] The reported toxicity and degenerative chemistry of MDA[4-8] may be a result of its ability to covalently bond and to cross-link a variety of biological macromolecules. Thus, the presence of MDA in foods and in living tissues in which the lipid component has undergone oxidation may be of considerable physiological importance. Malondialdehyde is reactive toward nucleic acids resulting in the loss of their template activity.[9,10] We have shown previously that MDA reacts relatively rapidly (as evidenced from kinetic data) at the α-amino group of amino acids to form both 1:1 and 1:2 adducts.[11] This communication reports on the isolation and structural elucidation of novel and unusual adducts from the reaction of MDA with adenine and cytosine.[12]

The reaction of MDA (as its sodium salt)[11] with adenosine was carried out in aqueous solution at pH 4.2[13] and 37 °C for 3 days to furnish two adducts, which were separated and purified by multiple reverse phase HPLC on Amberlite XAD-4 resin (40–50 μm) using ethanol/water as the eluting solvent. The first adduct, mp 125–127 °C, formed in about 7.0% conversion, showed UV absorption at $\lambda_{max}^{H_2O}$ 326 (ϵ = 46 000), 241 (ϵ = 8500), and 222 nm (ϵ = 9900). The presence of a molecular ion at m/z 321 and fragments in the mass spectrum and the UV data suggested the formation of a nucleoside modified at the 6-position by an α,β-unsaturated aldehyde moiety. The 360-MHz high-field ^{1}H NMR data (including homonuclear decoupling) together with the 90.6-MHz ^{13}C NMR data in Me_2SO-d_6 provided excellent supporting evidence for the complete structure and stereochemistry as **2**. The NH resonance appeared at δ 11.36 (d, J = 11.4 Hz) and the aldehyde proton at δ 9.42 (d, J = 8.5 Hz). The two vinyl protons gave resonances at δ 6.01 (d, d, $J_{c,d}$ = 8.5, $J_{b,c}$ = 13.3 Hz, H_c) and 8.71 (d, d, $J_{b,c}$ = 13.3, $J_{a,b}$ = 11.4 Hz, H_b) indicative of a trans geometry. The adenine ring protons appeared as singlets at δ 8.71 (H_2) and 8.56 (H_8). The ribose protons gave the expected resonance pattern with the anomeric proton appearing as a doublet at δ 6.01 (J = 5.7 Hz). The ^{13}C NMR spectrum of **2** showed 13 carbons with appropriate chemical shifts. The spectral data also suggest that the enaminal moiety in **2** is coplanar with the purine ring, and the marked downfield shift of the N–H is due largely to the diamagnetic anisotropic deshielding by the purine ring. An adduct similar to **2** was isolated (20%) as the single product from the reaction of methylmalondialdehyde (MMDA) and adenosine.

The second adduct (mp 149–151 °C, 11%) exhibited UV absorbances in H_2O at 327 (ϵ = 29 700), 260 (sh, ϵ = 13 960), and

Scheme I



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(13) Reaction occurs at a pH range of 4.0–7.0. However, the optimum conditions for reaction are at pH 4.2, slightly below the pK_a of MDA

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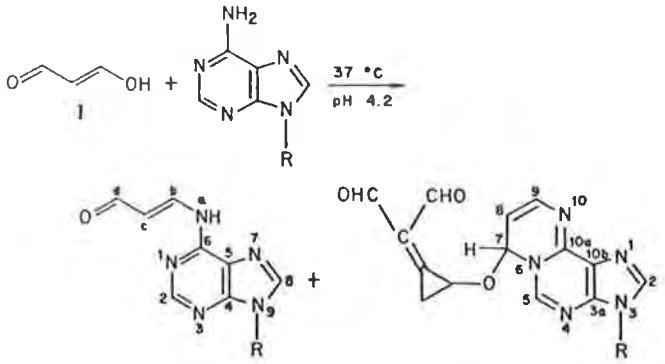

2, R = ribosyl
3, R = Et

4, R = ribosyl
5, R = Et

237 nm (ϵ = 27 060). The EI and FAB mass spectral data and elemental analysis suggested a molecular formula of $C_{19}H_{19}N_5O_7$. The delayed-decoupled high-field ^{13}C NMR spectrum (in Me_2SO-d_6) revealed the presence in the structure of the following proton substitution pattern: 2 CH_2, 12 CH, 5 C. An unusual feature in the ^{13}C spectrum was the presence of a CH at δ 16.2 and a CH_2 at δ 24.1. This information in conjunction with other ^{13}C and ^1H NMR data led to the conclusion that a methylidene cyclopropane moiety was present within the structure. Two aldehyde carbons were seen at δ 187.9 and 187.1, with the corresponding protons appearing as singlets at δ 9.38 and 9.21. The cyclopropyl CH appeared as a broadened quartet at δ 4.03 and the geminal protons at δ 2.08 and 1.97 (J_{gem} = 13.5 Hz). The ^{13}C and ^1H NMR data were also consistent with the formation of a new six-membered ring with carbon resonances at δ 77.2, 142.9, and 162.4 and corresponding proton resonances at δ 7.72 (brs), 7.63 (brd, J = 6.8 Hz), and 9.21 (brd, J = 6.8 Hz). The purine and ribose components were intact and gave expected ^{13}C and ^1H peaks. The spectral data were completely consistent with **4**, a 3:1 adduct of MDA and adenosine.

Modified bases identical with those present in **2** and **4** were formed in the reaction of 9-ethyladenine[14] with MDA. When cytidine and 1-methylcytosine[15] were treated with MDA, adducts **6** and **7** were isolated as the sole products.

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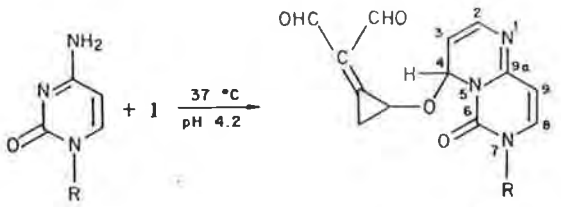

6, R = ribosyl
7, R = Me

A plausible mechanism for the formation of the intriguing 3:1 adducts is shown in Scheme I for the adenine case. The mechanism implies the intermediacy of the enaminal **2** (or **3**). Thus, cyclization of this enaminal gives a tricyclic base **8**. Reaction of **8** with another molecule of MDA followed by elimination of water results in the formation of the ether **9**. Intermediate **9** can be attacked further by a molecule of MDA to give **10**, which can undergo cyclization and 1,2-hydrogen shifts to give the observed products **4** (and **5**). Although in the formation of the 3:1 adducts two new chiral centers are introduced, the relative stereochemistry of the resulting diastereoisomeric structures is not readily discernible from the high-field NMR data.

We conclude that MDA is capable of modifying both adenine and cytosine bases at the amino group. Subsequent cyclization of these primary products followed by further reaction with MDA results in the formation of hypermodified bases with methylene cyclopropane rings. The alteration of adenine and cytosine by MDA has not been reported previously. The formation of cyclopropane rings in the degenerative chemistry of MDA is also novel. The toxic effects of MDA that involve nucleic acids could be mediated by the formation of such bicyclic and tricyclic bases or interstrand and intrastrand crosslinking involving enaminal structures.

Acknowledgment. Support of these investigations by a grant (CHE-8200818) from the NSF is gratefully acknowledged. The Bruker WM-360 high-field NMR spectrometer used in this work was purchased in part from a grant (CHE-8201836) from the NSF. We thank Dr. Curt S. Cooper for some preliminary experiments with MMDA.

Supplementary Material Available: NMR (^1H and ^{13}C), UV, and mass spectral data for all adducts (6 pages). Ordering information is given on any current masthead page.

Structural Alteration of Nucleic Acid Bases by Bromomalonaldehyde[1]

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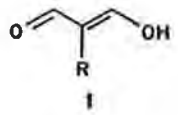
Bromomalonaldehyde (BMDA), prepared by bromination of malonaldehyde with elemental bromine, has been employed to modify a number of nucleic acid bases. These reactions transform pyrimidine and purine bases into modified systems containing etheno and etheno carboxaldehyde moieties, among other products. The structures of these modified bases were established by UV, mass spectral, and high-field NMR data. Fluorescence emission data for some of the adducts are of significance. The general mechanism of modification is discussed.

In the course of some work in our laboratory on the behavior of the ubiquitous natural compound malonaldehyde (MDA, 1, R = H) toward biomolecules,[2,3] we needed some information on the comparative reactivity of 2-substituted malonaldehydes. In particular, the structural nature of modification of nucleic acid bases was of interest in this work. The chemical modification of the base moiety of nucleic acids is also of synthetic and biological interest. For example, 1,N^6-ethenoadenosine and 3,N^4-ethenocytidine are both able to substitute for adenine nucleotides in some biological systems.[4] The observation that some modified bases are fluorescent has generated considerable interest in their use as biological probes in the structure and mechanism of action of nucleic acids and some enzymes and coenzymes.[4-15] Ethenoadenine derivatives exhibit fluorescence emission in the range of 410 nm with quantum yields of the order of 0.56. However, the use of ethenocytidine derivatives as biological probes has been limited by their inappropriate fluorescence emission wavelengths and low quantum yields.[4,16] The search therefore continues for cytidine derivatives which possess fluorescence characteristics that allow for ready detection in biological systems. In this paper we report on the interesting modifications of a number of purine and pyrimidine bases by bromomalonaldehyde (BMDA).



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Results and Discussion

Although the chemistry of halogenated malonaldehydes (1, R = Cl, Br, I, F) has been explored to some extent mainly for the synthesis of heterocycles,[17-20] little is known about the reactivity of these compounds toward nucleic acid bases. Bromomalonaldehyde can be prepared by bromination of MDA with elemental bromine as described by Trofimenko.[21] Modification of the pyrimidine and purine bases was accomplished by stirring the substrate in an aqueous acidic medium with BMDA at 60 °C. The reactions were followed by UV spectral methods and terminated when the absorption for the bathochromically shifted product peak had maximized. Separation and purification of the modified bases and derivatives were achieved by preparative-layer chromatography on silica gel or by HPLC on Amberlite XAD-4 resin.

The reaction of BMDA with cytidine afforded a yellow crystalline compound in 43% yield (mp 202–204 °C) with UV absorption shifted to 325 nm (ε 10 500) which is indicative of more extended conjugation. The molecular ion in its mass spectrum at m/z 295 suggested the formation of a 1:1 adduct in which bromine was not present. The 360-MHz ^{1}H NMR spectrum in Me_2SO-d_6 showed the presence of 4 non-ribosyl protons at δ 6.91 (d, J = 7.8 Hz), 8.12 (d, J = 7.8 Hz), 8.16 (s), and 10.57 (s). Three additional carbon resonances (compared to cytidine) at δ 130.0, 139.6, and 181.0 suggesting the presence of a bicyclic base carrying an exocyclic vinylogous amide carbonyl group were present in its high-field ^{13}C NMR spectrum in

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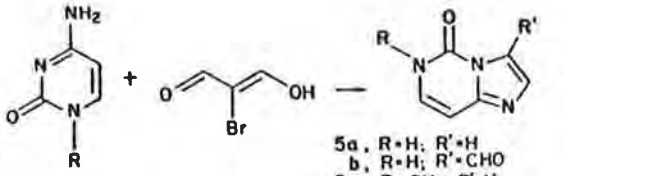
0022-3263/84/1949-4021$01.50/0

Me_2SO-d_6. Taken together, the data suggested that the modified base was 6-(β-D-ribofuranosyl)-3-formylimidazo[1,2-c]pyrimidin-5(6*H*)-one **7**. Its formation can

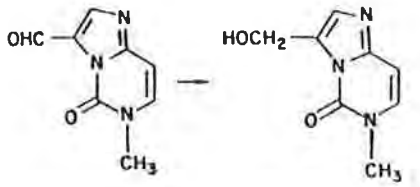


2, R=H
3, R=CH_3
4, R=β-D-ribofuranosyl

5a, R=H; R'=H
b, R=H; R'=CHO
6a, R=CH_3; R'=H
b, R=CH_3; R'=CHO
c, R=CH_3; R'=CH=CBrCHO
7 R=β-D-ribofuranosyl; R'=CHO

be suggested as occurring through initial development of a Schiff base and subsequent nucleophilic displacement of bromide ion by N-3 of cytidine to give the observed bicyclic product. Interestingly, compound **7** was found to exhibit fluorescence at 398 nm when excited at 357 nm, and this is in contrast to 3,N^4-ethenocytidine which exhibits fluorescence at 340 nm when excited at 288 nm.[4]

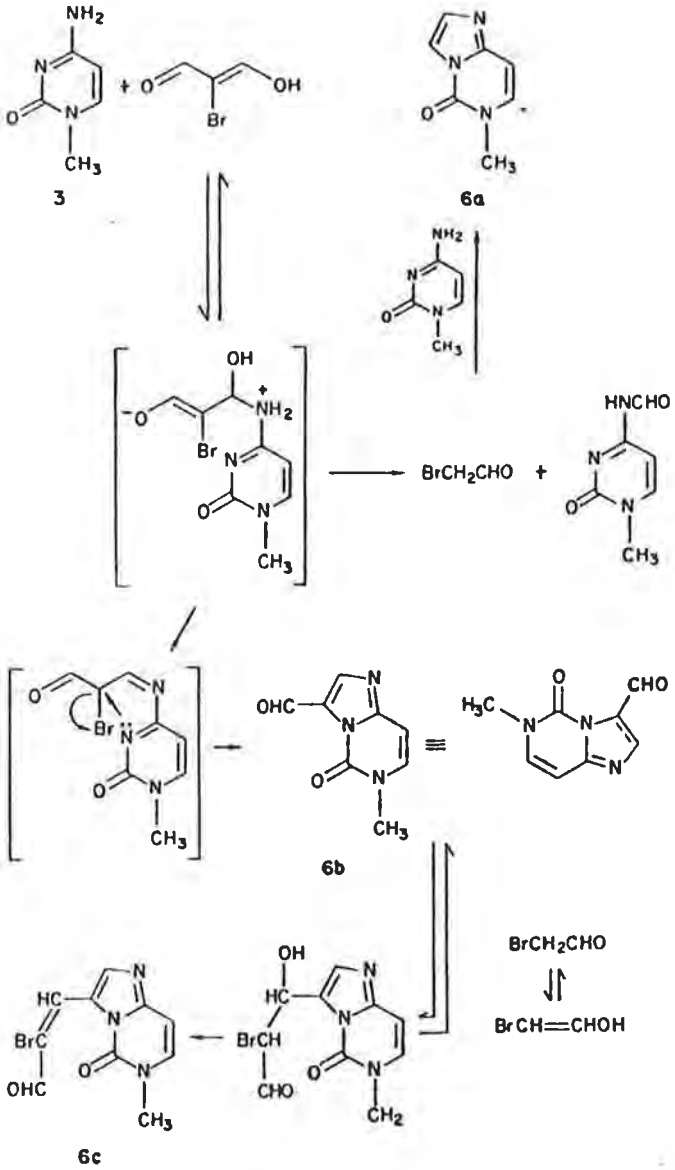
We also examined the reaction of BMDA with alkylated bases. Because of the ease of handling these base derivatives and their products, this was considered an attractive approach to these base modifications. 1-Methylcytosine can be conveniently prepared from cytosine by reaction with *N*,*N*-dimethylformamide dimethyl acetal and subsequent hydrolysis of the intermediate imine with ammonium hydroxide.[22] When 1-methylcytosine was treated with BMDA, three modified bases were isolated after preparative-layer chromatography. The first product, (mp 181–182 °C) isolated in 27% yield, was 6-methyl-3-formylimidazo[1,2-c]pyrimidin-5(6*H*)-one (ethenomethylcytosinecarboxaldehyde) (**6b**) whose spectral



6b **8**

characteristics were similar to those of **7**. The structure of **6b** was confirmed further by its facile reduction with sodium borohydride to compound **8**. The second product (21%), identified as 6-methylimidazo[1,2-c]pyrimidin-5(6*H*)-one (ethenomethylcytosine) (**6a**), was a decarbonylated product which had been reported previously.[23] The third modified base, mp 254–256 °C, was isolated in 7% yield. The mass spectral data (M^+ 283 and 281) and elemental analysis suggested a molecular formula of $C_{10}H_8N_3O_2Br$. A large shift to longer wavelength (λ_{max} 371 nm, ε 17 200) compared to methylcytosine was present in its UV spectrum in ethanol. It showed marked fluorescence at 452 nm when excited at 398 nm. The 360-MHz ^1H NMR spectrum in Me_2SO-d_6 showed three protons as singlets at δ 9.44, 9.35, and 8.73, two proton doublets at δ 7.76 and 6.81 (J = 7.7 Hz), and a singlet at δ 3.56 integrating for three protons. The total data are best accommodated by structure **6c**. A plausible mechanism for the formation of these products is shown in Scheme I. The formation of **6a** apparently results from the reaction of **3** with bromoacetaldehyde produced by the cleavage of the initial adduct resulting from **3** and BMDA. In support of this were the observations that BMDA did not produce

Scheme I

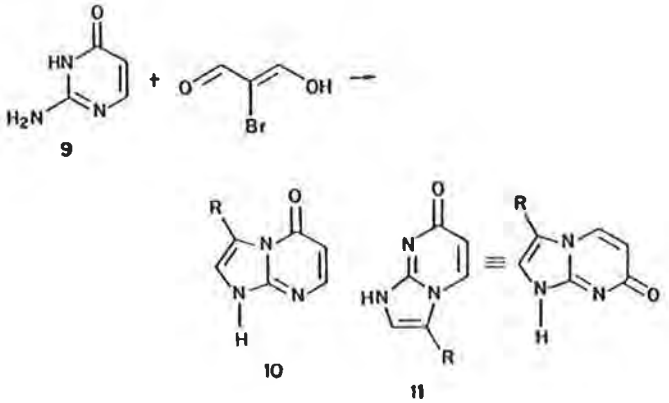


6c

bromoacetaldehyde in the absence of **3** and that of **6a** could not be produced by the thermal decarbonylation of **6b**. Compound **6b** (and not **6a**), however, is the likely precursor of **6c** through reaction with bromoacetaldehyde.

Cytosine (**2**) was also found to react with BMDA to give the imidazopyrimidinone **5a** (13% yield) and a trace amount of the formyl analogue **5b** which was detected by mass spectrometry.

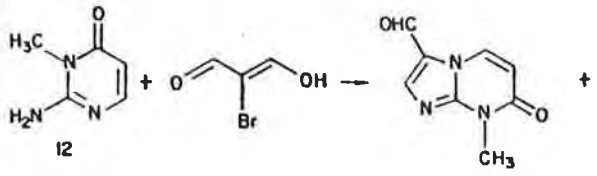
The reaction of isocytosine (**9**) with BMDA was also examined. Two ring systems, **10** and **11**, are possible depending on the regiochemistry of adduct formation. The



9 **10** **11**

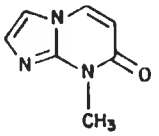
(22) Hosmane, R. S.; Leonard, N. J. *Synthesis* 1981, 118.
(23) Kochetkov, N. K.; Shibaev, V. N.; Kost, A. A. *Tetrahedron Lett.* 1971, 1993.

modified heterocyclic ring 10 would arise from initial Schiff base formation and cyclization in a linear mode, whereas the cyclization for 11 would occur in an angular fashion. The single product isolated from this reaction in 68% yield had a molecular ion in its mass spectrum at m/z 135 suggesting that it was derived from bromoacetaldehyde and not BMDA. Absorptions at 213 nm (ϵ 17 000) and 296 nm (ϵ 8500) were present in its UV spectrum in H_2O. The high-field ^1H and ^{13}C NMR data could not distinguish between the two possibilities. The method chosen to distinguish between 10 and 11 involved an unambiguous synthesis of one of the isomeric systems. Isocytosine can be methylated selectively at the 3-position in 44% yield by reaction with *N,N*-dimethylformamide dimethyl acetal followed by ammonium hydroxide.[22] Treatment of 3-methylisocytosine (12) with BMDA employing conditions identical with those used for isocytosine gave two products, 8-methyl-3-formylimidazo[1,2-*a*]pyrimidin-7(8*H*)-one (13),



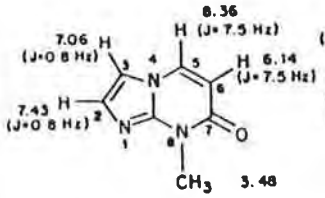
12

13

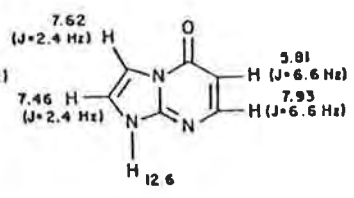


14

which was isolated in 16% yield, and 8-methylimidazo-[1,2-*a*]pyrimidin-7(8*H*)-one (14), which was obtained in 61% yield. A comparison of the 360-MHz ^1H NMR spectrum of the isocytosine adduct with that of 14 allowed



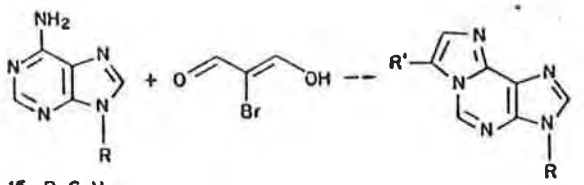
14



10, R=H

us to determine the regiochemistry of the addition and the structure of the former adduct as 10. The chemical shifts of the protons in each of these compounds were assigned by a consideration of expected values, observed coupling constants, and homonuclear decoupling data. Examination of the chemical shifts of H-2 showed a difference of 0.03 ppm as is expected for protons in similar environments. However, the resonances for H-3 exhibit a much larger difference. If the product of the reaction were 11 (R = H) only a small difference in chemical shift would have been expected from 14 (H-3 at δ 7.06). However, H-3 in the isocytosine–BMDA adduct occurs at δ 7.62. In compound 10 (R = H), H-3 would be deshielded by the carbonyl at C-5 and would be expected to appear further downfield. The product therefore is structure 10 (R = H) and not 11 (R = H). Such chemical shift differences have been exploited by us recently in the assignment of the structure and the mechanism of formation of tricyclic adducts from guanosine and glycidaldehyde.[24]

The modification of purine bases by BMDA was also investigated. Adenosine 16 reacts with BMDA to give two products, 3-ribosylimidazo[2,1-*i*]purine (ethenoadenosine) (18a) in 10% yield, and 3-ribosyl-7-formylimidazo[2,1-*i*]-purine (ethenoadenosinecarboxaldehyde) (18b) in 18% yield. Ethenoadenosine has been prepared previously and



15, R=C_2H_5
16, R=ribosyl

17, R=C_2H_5; R'=CHO
18a, R=ribosyl; R'=H
b, R=ribosyl; R'=CHO

its spectral data were consistent with literature values.[25] The reaction of 9-ethyladenine (15) with BMDA gave a single product 3-ethyl-7-formylimidazo[2,1-*i*]purine (3-ethylethenoadeninecarboxaldehyde) (17) in 24% yield. Both 17 and 18b exhibit fluorescence at 410 nm when excited at 270 nm.

In summary, the reactions of bromomalonaldehyde with a number of nucleic acid bases have been investigated. These interactions transform the base moiety through the formation of additional heterocyclic rings.[26] Etheno as well as the more novel ethenocarboxaldehyde products are formed. In one instance, a hypermodified base is the result. The ethenocarboxaldehyde cytosine derivatives exhibit fluorescence properties that are potentially more useful than ethenocytosines previously reported.

Experimental Section

The melting points reported are uncorrected and were taken on a Thomas-Hoover melting point apparatus fitted with a microscope. The ^1H NMR and ^{13}C NMR data were recorded on a JEOL FX90Q pulse Fourier transform NMR spectrometer or on a Bruker WM 360 high-field NMR spectrometer. Tetramethylsilane was the internal reference. Mass spectra at 30 eV were obtained on a Hewlett-Packard 5985 GC/MS system. The ultraviolet data were taken with a Cary Model 219 ultraviolet–visible spectrophotometer. Elemental analyses were performed by the University of Iowa Microanalytical Service on an automated Perkin Elmer Model 240 carbon, hydrogen, and nitrogen analyzer. HPLC separations were done at low pressure utilizing a column of Amberlite XAD-4 resin (270-325 mesh). Preparative-layer chromatography was done on E. Merck silica gel-PF-254. Fluorescence spectra were uncorrected and performed on an Aminco-Bowman Spectrophotofluorimeter using a xenon lamp.

Preparation of Bromomalonaldehyde (1, R = Br). This compound was prepared by the method of Trofimenko[21] and was obtained in 68% yield: mp 147–148 °C (lit.[21] mp 148 °C); UV (H_2O) λ_{max} 277 nm; ^1H NMR ($CDCl_3$) δ 9.33 (s, 2 H).

Reaction of Bromomalonaldehyde with Cytidine (4). An aqueous solution of 0.250 g (1.03 mol) of cytidine and 0.153 g (1.02 mmol) of bromomalonaldehyde was adjusted to pH 4.5 with 2 N NaOH. The reaction was heated to 60 °C under nitrogen and was discontinued when the absorbance at 325 nm maximized (72 h). The solution was then neutralized and the solvent removed in vacuo at 50 °C. The brown residue was then purified on a column of Amberlite XAD-4 using 80/20 H_2O/ethanol as the solvent. Unreacted starting materials preceded the product off the column. A total of 0.127 g (0.43 mmol, 43% yield, 69% conversion) of 6-(β-D-ribofuranosyl)-3-formylimidazo[1,2-*c*]pyrimidin-5(6*H*)-one (7) was obtained as yellow crystals from ethanol: mp 202–204 °C; UV (H_2O) λ_{max} 325 nm (ϵ 1.05 × 10^4); fluorescence (EtOH) excitation 357 nm and emission 398 nm; mass spectrum, m/z (relative intensity) 295 (M^+, 2.3), 163 ("base" + H^+, 100), 162 ("base", 19.8), 133 ("base" – CHO, 15.3), 107 (21), 73 (24.1); ^1H NMR (Me_2SO-d_6) δ 3.71–6.08 (m, 9 H, ribose), 6.91 (d, 1 H, J = 7.8 Hz), 8.12 (d, 1 H, J = 7.8 Hz), 8.16 (s, 1 H), 10.57 (s, 1

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(25) Barrio, J. R.; Secrist, III, J. A.; Leonard, N. J. *Biochem. Biophys. Res. Commun.* 1972, *46*, 597.

(26) Iodomalonaldehyde exhibited similar behavior.

H); ^{13}C NMR (Me_2SO-d_6) δ 60.2, 69.2, 74.6, 85.0, 89.7, 98.1, 130.0, 131.6, 139.6, 146.3, 149.1, 181.0.

Anal. Calcd for $C_{12}H_{13}N_3O_6 \cdot {}^1/_2\ H_2O$: C, 47.37; H, 4.64; N, 13.81. Found: C, 47.69; H, 4.61; N, 13.79.

Preparation of 1-Methylcytosine (3). This compound was prepared by the method of Hosmane and Leonard[22] and was obtained in 63% yield: mp 300–302 °C dec [lit.[22] mp 300–303 °C dec]; UV (H_2O) λ_{max} 273 nm; ^1H NMR (Me_2SO-d_6) δ 3.23 (s, 3 H), 5.52 (d, 1 H, J = 8.7 Hz), 7.60 (d, 1 H, J = 8.7 Hz), 11.16 (bs, 2 H).

Reaction of Bromomalonaldehyde with 1-Methylcytosine (3). To 0.643 g (5.14 mmol) of 1-methylcytosine dissolved in 20 mL of water was added 0.771 g (5.10 mmol) of bromomalondialdehyde. The pH was adjusted to 4.1 with 2 N NaOH and the reaction mixture was heated to 60 °C under N_2 until the UV absorbance at 330 nm reached a maximum (73 h). The reaction mixture was then neutralized and the solvent removed in vacuo at 50 °C. The brown residue was then passed through a short silica gel column using 20% $MeOH/CH_2Cl_2$ as the solvent. The solvent was again removed and the residue chromatographed on silica gel preparative-layer plates with 5% $MeOH/CH_2Cl_2$. Two bands were collected. The band with R_f 0.43 provided 0.097 g (0.34 mmol, 7%) of 6-methyl-3-(2-bromo-3-oxo-1-propenyl)-imidazo[1,2-c]pyrimidin-5(6*H*)-one (6c) as yellow prisms: mp 254–256 °C; UV (EtOH) λ_{max} 371 nm (ε 1.72×10^4); fluorescence (EtOH) excitation 398 nm and emission 452 nm; mass spectrum, m/z (relative intensity) 283, 281 (M^+, 5.4, 5.6), 202 (M^+ – Br, 100), 174 (M^+ – Br – CO, 64.7), 173 (M^+ – Br – HCO, 16.9), 159 (30.8), 149 (M^+ – C_3H_2OBr, 22.2), 131 (14.0); ^1H NMR (Me_2SO-d_6) δ 3.56 (s, 3 H), 6.81 (d, 1 H, J = 7.7 Hz), 7.76 (d, 1 H, J = 7.7 Hz), 8.73 (s, 1 H), 9.35 (s, 1 H), 9.44 (s, 1 H).

Anal. Calcd for $C_{10}H_8N_3O_2Br$: C, 42.57; H, 2.86; N, 14.90. Found: C, 42.03; H, 2.82; N, 14.70.

The band with R_f 0.29 was then rechromatographed on silica gel plates utilizing 20% CH_2Cl_2/ethyl acetate as the solvent. After two immersions two bands were isolated. The band with R_f 0.65 provided 0.156 g (1.05 mmol, 21%) of 6-methylimidazo[1,2-c]-pyrimidin-5(6*H*)-one (ethenomethylcytosine (6a)). The spectroscopic data of 6a was consistent with literature values.[23] The band with R_f 0.70 yielded 0.246 g (1.39 mmol, 27%) of 6-methyl-3-formylimidazo[1,2-c]pyrimidin-5(6*H*)-one (ethenomethylcytosinecarboxaldehyde) (6b) as yellow crystals: mp 181–182 °C; UV (H_2O) λ_{max} 328 nm (ε 5.25×10^3); fluorescence (EtOH) excitation 352 nm and emission 402 nm; mass spectrum, m/z (relative intensity) 178 (M^+ + 1, 9.6), 177 (M^+, 100), 149 (M^+ – CHO, 84.5), 122 (M^+ – C_3H_3O, 12.5); ^1H NMR ($CDCl_3$) δ 3.71 (s, 3 H), 6.76 (d, 1 H, J = 7.6 Hz), 7.24 (d, 1 H, J = 7.6 Hz), 8.22 (s, 1 H), 10.73 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 37.4, 99.2, 130.4, 135.2, 140.1, 147.0, 149.2, 181.5.

Anal. Calcd for $C_8H_7N_3O_2$: C, 54.20; H, 3.96; N, 23.72. Found: C, 53.66; H, 3.99; N, 23.60.

Reaction of Bromomalonaldehyde with Cytosine (2). To 10 mL of H_2O was added 0.115 g (1.04 mmol) of cytosine and 0.166 g (1.10 mmol) of bromomalonaldehyde. The pH was adjusted to 4.2 with 2 N NaOH and the solution was heated to 60 °C under N_2 atmosphere. The reaction was monitored by UV spectroscopy and was stopped when the absorbance at 268 nm reached a maximum (72 h). The solvent was then removed in vacuo at 50 °C and the brown residue passed through a short column of silica gel using 15% $MeOH/CH_2Cl_2$. The solvent was again removed in vacuo and the brown residue was chromatographed on silica gel plates using 7% $MeOH/CH_2Cl_2$ as the solvent. The band with R_f 0.30 was eluted and then repurified on silica gel plates with a solvent of 10% CH_2Cl_2/ethyl acetate. The band with R_f 0.30 after two immersions was found to contain 0.019 g (0.14 mmol, 13%) of imidazo[1,2-c]pyrimidin-5(6*H*)-one (5a) as white crystals: mp 234–236 °C; UV (H_2O) λ_{max} 268 nm (ε 8.67×10^3); fluorescence (EtOH) excitation 289 nm and emission 339 nm; mass spectrum, m/z (relative intensity) 136 (M^+ + 1, 7.5), 135 (M^+, 100), 107 (M^+ – H_2CN, 29.8); ^1H NMR (Me_2SO-d_6) δ 6.59 (d, 1 H, J = 7.3 Hz), 7.25 (d, 1 H, J = 7.8 Hz), 7.39 (d, 1 H, J < 1 Hz), 7.78 (d, 1 H, J < 1 Hz), 11.40 (bs, 1 H).

Anal. Calcd for $C_6H_5N_3O$: C, 53.33; H, 3.73; N, 31.10. Found: C, 52.92; H, 3.95; N, 29.29.

A trace amount of 3-formylimidazo[1,2-c]pyrimidin-5(6*H*)-one (5b) also was detected in the mass spectrum of 5a: m/z (relative intensity) 163 (M^+, 72.2), 162 (M^+ – H, 24.1), 135 (M^+ – CO, 100), 107 (M^+ – CO, HCN, 46.5).

Reduction of Ethenomethylcytosinecarboxaldehyde (6b). To 45 mL of dry ethanol was added 0.123 g (3.30 mmol) of $NaBH_4$. The flask was cooled in an ice bath and 0.048 g (0.27 mmol) of 6b was added. The flask was stirred in the ice bath for 1 h and then at room temperature for 4 h. The solvent was removed in vacuo and the white solid that remained was dissolved in 20 mL of H_2O and extracted with CH_2Cl_2 (5 × 20 mL). The organic layer was then dried with sodium sulfate and the solvent removed in vacuo. The white solid which remained was then chromatographed on silica gel using 5% $MeOH/CH_2Cl_2$ as the solvent. The band with R_f 0.30 afforded after elution 0.025 g (0.14 mmol, 51%) of 6-methyl-3-(hydroxymethyl)imidazo[1,2-c]pyrimidin-5(6*H*)-one (8) as white crystals: mp 157–159 °C; UV (H_2O) λ_{max} 277 nm (ε 1.45×10^4); fluorescence (EtOH) excitation 297 nm and emission 342 nm; mass spectrum, m/z (relative intensity) 180 (M^+ + 1, 6.4), 179 (M^+, 67.9), 162 (M^+ – OH, 100), 150 ("base" + H^+, 65.9), 133 (M^+ – CH_3 – CH_2OH, 44.2); ^1H NMR ($CDCl_3$) δ 3.47 (s, 4 H), 4.86 (s, 2 H), 6.58 (d, 1 H, J = 7.8 Hz), 6.97 (d, 1 H, J = 7.8 Hz), 7.24 (s, 1 H).

Anal. Calcd for $C_8H_9N_3O_2 \cdot {}^1/_4H_2O$: C, 52.33; H, 5.23; N, 22.88. Found: C, 52.83; H, 5.13; N, 22.39.

Reaction of Bromomalonaldehyde with Isocytosine (9). To 10 mL of water was added 0.110 g (0.99 mmol) of isocytosine (9) and 0.162 g (1.08 mmol) of bromomalonaldehyde. The pH was adjusted to 3.8 with 2 N NaOH and the reaction was heated to 60 °C under N_2 atmosphere until the absorbance at 292 nm reached a maximum (96 h). The reaction was then neutralized with 2 N NaOH and the solvent removed in vacuo at 50 °C. The residue was then passed through a short silica gel column using 20% $MeOH/CH_2Cl_2$ as the eluant. The solvent was again removed and the residue chromatographed on silica gel preparative-layer plates using 10% $MeOH/CH_2Cl_2$. The band with R_f 0.40 afforded upon elution 0.091 g (0.67 mmol, 68%) of imidazo[1,2-a]pyrimidin-5(1*H*)-one (10) as yellow crystals: mp 193–197 °C; UV (H_2O) λ_{max} 213 nm (ε 1.7×10^4), 296 (ε 8.5×10^3); fluorescence (EtOH) excitation 300 nm and emission 353 nm; mass spectrum, m/z (relative intensity) 136 (M^+ + 1, 8.1), 135 (M^+, 100), 10 7 (M^+ – H_2CN, 40.6); ^1H NMR (Me_2SO-d_6) δ 5.81 (d, 1 H, J = 6.4 Hz), 7.46 (d, 1 H, J = 2.2 Hz), 7.62 (d, 1 H, J = 2.2 Hz), 7.93 (d, 1 H, J = 6.4 Hz), 12.6 (s, 1 H); ^{13}C NMR (Me_2SO-d_6) δ 97.1, 107.1, 120.7, 146.4, 149.6, 157.0.

Anal. Calcd for $C_6H_5N_3O \cdot {}^1/_4H_2O$: C, 51.61; H, 3.61; N, 30.09. Found: C, 51.41; H, 3.84; N, 30.69.

Preparation of 3-Methylisocytosine (12). To a scrupulously dry 3-neck round-bottom flask was added 1.135 g (10.21 mmol) of dry isocytosine and 16.0 mL (120.44 mmol) of *N,N*-dimethylformamide dimethyl acetal. The solution was allowed to reflux and 0.15 mL of trifluoroacetic acid was added via syringe through a septum over 10 min. The solution was then allowed to reflux for 15 h and cooled in an ice bath and the white solid which precipitated out was filtered off and dried to afford 1.230 g (6.83 mmol, 67%) of N^2-[(*N,N*-dimethylamino)methylene]-3-methylisocytosine as white crystals: mp 139.5–141.5 °C; UV (H_2O) λ_{max} 296 nm; mass spectrum, m/z (relative intensity) 180 (M^+, 81), 165 (M^+ – CH_3, 19.3), 136 (M^+ – $N(CH_3)_2$, 100), 109 (M^+ – $NCHNMe_2$, 53.2); ^1H NMR (Me_2SO-d_6) δ 3.05 (s, 3 H), 3.09 (s, 3 H), 3.16 (s, 3 H), 5.87 (d, 1 H, J = 6.2 Hz), 7.67 (d, 1 H, J = 6.2 Hz), 8.67 (s, 1 H).

The N^2-[(*N,N*-dimethylamino)methylene]-3-methylisocytosine was then stirred with 125 mL of concentrated NH_4OH for 12 h at room temperature. Excess ammonia was removed by refluxing the solution on a steam bath for 1.5 h. The solvent was removed and a white sticky solid remained. Separation of the solid on silica gel preparative-layer plates with 10% $MeOH/CH_2Cl_2$ and elution of the band with R_f 0.30 afforded 0.551 g (4.41 mmol, 65%, 44% yield overall) of 3-methylisocytosine (12) as white crystals: mp 263–266 °C (lit.[27] mp 262–266 °C); UV (H_2O) λ_{max} 284 nm (ε 1.09 × 10^4), 226 (ε 8.15×10^3; mass spectrum, m/z (relative intensity) 126 (M^+ + 1, 10.3), 125 (M^+, 100), 96 (M^+ – CH_2NH, 30.7); ^1H NMR (Me_2SO-d_6) δ 3.25 (s, 3 H), 5.58 (d, 1 H, J = 6.4 Hz), 7.13 (s, 2 H), 7.51 (d, 1 H, J = 6.4 Hz).

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Reaction of Bromomalonaldehyde with 3-Methylisocytosine (12). To 13 mL of water was added 0.117 g (0.94 mmol) of 3-methylisocytosine (12) and 0.143 g (0.95 mmol) of bromomalonaldehyde. The pH was adjusted to 4.3 with 2 N NaOH and the reaction mixture was heated to 60 °C under nitrogen atmosphere until the absorbance at 263 nm reached a maximum (6 days). The reaction was then neutralized with 2 N NaOH and the solvent removed in vacuo at 50 °C. The residue was then chromatographed on silica gel preparative-layer plates using 5% MeOH/CH_2Cl_2 as the solvent. The band with R_f 0.35 provided 0.026 g (0.15 mmol, 16%) of 8-methyl-3-formylimidazo[1,2-*a*]pyrimidin-7(8*H*)-one (13) as pale yellow crystals: mp 178–181 °C; UV (H_2O) λ_{max} 264 nm (ϵ 1.51 × 10^4), 280 nm (ϵ 1.45 × 10^4); fluorescence (EtOH) excitation 302 nm and emission 424 nm; mass spectrum, *m/z* (relative intensity) 178 (M^+ + 1, 10.1), 177 (M^+, 100), 148 (M^+ – CHO, 18.3); ^1H NMR (Me_2SO-d_6) δ 3.54 (s, 3 H), 6.35 (d, 1 H, *J* = 7.8 Hz), 8.15 (s, 1 H), 8.80 (d, 1 H, *J* = 7.8 Hz), 9.71 (s, 1 H).

Anal. Calcd for $C_8H_7N_3O_2$: C, 54.23; H, 3.98; N, 23.72. Found: C, 53.81; H, 4.28; N, 23.49.

Another band found at R_f 0.25 afforded 0.086 g (0.58 mmol, 1%) of 8-methylimidazo[1,2-*a*]pyrimidin-7(8*H*)-one (14) as yellow crystals: mp 145–148 °C; UV (H_2O) λ_{max} 221 nm (ϵ 1.24 × 10^4); fluorescence (EtOH) excitation 309 nm and emission 455 nm; mass spectrum, *m/z* (relative intensity) 150 (M^+ + 1, 8.8), 149 (M^+, 100), 120 (64.7); ^1H NMR (Me_2SO-d_6) δ 3.48 (s, 3 H), 6.14 (d, 1 H, *J* = 7.8 Hz), 7.06 (d, 1 H, *J* < 1 Hz), 7.43 (d, 1 H, *J* < 1 Hz), 8.36 (d, 1 H, *J* = 7.3 Hz).

Anal. Calcd for $C_7H_7N_3O$: C, 56.37; H, 4.73; N, 28.17. Found: C, 56.80; H, 5.07; N, 28.28.

Reaction of Bromomalonaldehyde with Adenosine (16). To 170 mL of water was added 1.001 g (3.75 mmol) of adenosine and 0.873 g (5.78 mmol) of bromomalonaldehyde. The pH was adjusted to 4.5 with 2 N NaOH and the solution was heated to 50 °C under nitrogen atmosphere for 72 h. The solvent was then removed in vacuo at 50 °C and the brown residue which remained was separated on a column of Amberlite XAD-4 using 80:20 H_2O:ethanol as the solvent. 1,N^6-Ethenoadenosine (18a) was afforded as white crystals in 10% yield (0.109 g, 0.38 mmol, 13% conversion). The spectral data for 18a were consistent with literature values.[25] Also obtained was 1,N^6-ethenoadenosine-8-carboxaldehyde (18b) (0.218 g) (0.69 mmol, 18% yield, 24% conversion) as white crystals: mp 216–218 °C; UV (H_2O) λ_{max} 248 nm (ϵ 2.38 × 10^4), 325 nm (ϵ 1.75 × 10^4), 335 nm (ϵ 1.71 × 10^4); fluorescence (EtOH) excitation 270 nm and emission 410 nm; mass spectrum, *m/z* (relative intensity) 319 (M^+, 1.1), 187 (M^+ + H^+ – ribose, 100), 186 (M^+ – ribose, 31.0), 159 (base – CO, 6.3); ^1H NMR (Me_2SO-d_6) δ 4.59–6.12 (m, 9 H), 8.61 (s, 1 H), 8.81 (s, 1 H), 9.95 (s, 1 H), 10.02 (s, 1 H); ^{13}C NMR (Me_2SO-d_6) δ 61.0, 70.1, 74.3, 85.6, 87.9, 122.8, 124.7, 136.6, 141.4, 142.0, 144.7, 147.8, 179.1.

Anal. Calcd for $C_{13}H_{13}N_5O_5 \cdot H_2O$: C, 46.29; H, 4.48; N, 20.76. Found: C, 46.88; H, 4.43; N, 20.94.

Reaction of Bromomalonaldehyde with 9-Ethyladenine. To 60 mL of H_2O was added 0.452 g (2.77 mmol) of 9-ethyladenine (15) and 0.456 g (3.02 mmol) of bromomalonaldehyde. The pH was checked (3.3) and not adjusted. The reaction was then heated to 55 °C under nitrogen atmosphere for 72 h. The reaction was extracted with CH_2Cl_2 (3 × 40 mL) and the organic phase dried over Na_2SO_4. The solvent was removed in vacuo at 50 °C and the residue chromatographed on silica gel plates with 13% MeOH/CH_2Cl_2 as the solvent. The band with R_f 0.59 afforded 0.143 g (0.66 mmol, 24%) of 9-ethylethenoadeninecarboxaldehyde (17) as white crystals: mp 223–225 °C; UV (95% ethanol) λ_{max} 230 nm (ϵ 2.06 × 10^4), 328 nm (ϵ 1.51 × 10^4), 339 nm (ϵ 1.50 × 10^4); fluorescence (EtOH) excitation 270 nm and emission 410 nm; mass spectrum, *m/z* (relative intensity) 216 (M^+ + 1, 12.2), 215 (M^+, 100), 187 (M^+ – CO, 34.4), 186 (M^+ – Et, 34.4), 159 (M^+ – Et – CO, 13.5); ^1H NMR ($CDCl_3$) δ 1.62 (t, 3 H), 4.44 (q, 2 H), 8.13 (s, 1 H), 8.37 (s, 1 H), 10.02 (s, 1 H), 10.08 (s, 1 H); ^{13}C NMR (Me_2SO-d_6) δ 15.4, 38.4, 122.4, 124.7, 136.4, 139.6, 143.4, 145.1, 147.9, 179.0.

Anal. Calcd for $C_{10}H_9N_5O$: C, 55.80; H, 4.21; N, 32.54. Found: C, 55.89; H, 4.11; N, 32.34.

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Registry No. 1 (R = Br), 2065-75-0; 2, 71-30-7; 3, 1122-47-0; 4, 65-46-3; 5a, 55662-66-3; 6a, 45859-50-5; 6b, 91898-74-7; 6c, 91898-75-8; 7, 91898-76-9; 8, 91898-77-0; 9, 108-53-2; 10 (R = H), 55662-68-5; 12, 2417-17-6; 13, 91898-78-1; 14, 91898-79-2; 15, 2715-68-6; 16, 58-61-7; 17, 91898-80-5; 18a, 39007-51-7; 18b, 91898-81-6; $Me_2NCH(OMe)_2$, 4637-24-5; N^2-[(dimethylamino)-methylene]-3-methylisocytosine, 91898-82-7.

Synthetic Transformations of Transient Purinyl Radicals: Formation of Mono- and Diarylated and Heteroarylated Nucleosides[1]

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Photolysis of 2,6-dihalogenated purine nucleosides produced, through cleavage of the carbon–halogen bond, both purin-2-yl and purin-6-yl radicals (or caged radical pairs) which were intercepted by aromatic solvents such as benzene to produce 2-aryl- or 2,6-diarylpurine nucleosides. Heteroarylations and their selectivities involving pyrrole, thiophene, furan, and pyridine systems were also explored. In all cases, except for the "π-deficient" pyridine, the photoinduced heteroarylations were regiospecific and the products photostable. Photoinduced hydration of 2-substituted 6-chloropurine nucleosides provides an excellent approach for the synthesis of uncommon 2-substituted inosine analogues. High-field ^{13}C NMR data suggest that the 2,6-disubstituted purine nucleosides prefer the anti conformation in solution.

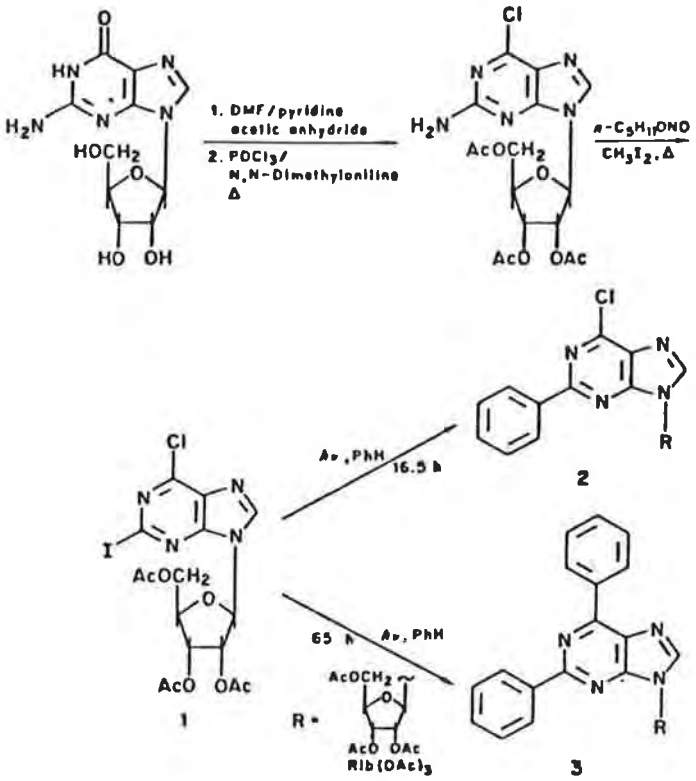
Transient purinyl radicals or the corresponding radical pairs are cleanly produced when 6-iodopurines are photolyzed with ultraviolet light.[2] The intermediacy of such radicals has also been inferred in previous studies from our laboratory on the reductive deamination and the halogenative deamination of 6-aminopurines.[3-6] Photochemically generated purinyl radicals or their equivalent provide an excellent synthetic approach to specific arylated and heteroarylated purines. This paper reports on the synthesis and the physical properties of these uncommon purine derivatives.

Few general methods for the introduction of aryl groups at carbon-6 in purine nucleosides are known. Taylor and Martin have reported that a suitable leaving group at C-6 can be displaced by an alkylidenephosphorane and the resulting ylide can be converted by hydrolysis or by reaction with a carbonyl to the 6-aralkyl or 6-aralkenyl derivative.[6] Bergstrom and Reddy recently reported[7] that the nickel-catalyzed coupling between aryl-Grignard reagents and protected 6-chloropurine nucleosides gives, in moderate yields, the corresponding 6-aryl nucleosides. A good general method for the introduction of aryl groups at the 2-position of purines is not known, although synthesis of 2-arylpurines has been accomplished by ring closure of imidazole intermediates with aldehydes.[8,9]

Results and Discussion

Homolysis of the aryl carbon–iodine bond (dissociation energy ~65 kcal/mol) to produce free or caged aryl radicals which subsequently react with aromatic or heteroaromatic substrates to produce biaryls or phenyl-substituted heterocyclic products has been investigated.[10-13] Light-induced homolysis of the carbon–halogen bond has been reported to occur for certain halopyrimidines.[14]

Scheme I. Photolytic Arylation Reactions of 2,6-Dihalogenated Purine Nucleoside



Some arylpyrimidines have been synthesized by this method.[15-18] We have reported previously that photolysis of 6-iodopurines in benzene or in heteroaromatic compounds results in the formation of 6-arylated or 6-heteroarylated purines.[2] In this paper we present examples of the selectivity that can be achieved in these reactions as well as some representative examples of 2-arylated and 2,6-diarylated and heteroarylated purine derivatives. A new approach to the synthesis of 2-substituted inosines is also mentioned.

The starting compound for these photoinduced transformations was 2-iodo-6-chloro-9β-(2,3,5-tri-*O*-acetyl-D-ribofuranosyl)purine (1) which was prepared from guanosine as previously described by us (Scheme I).[5,19]

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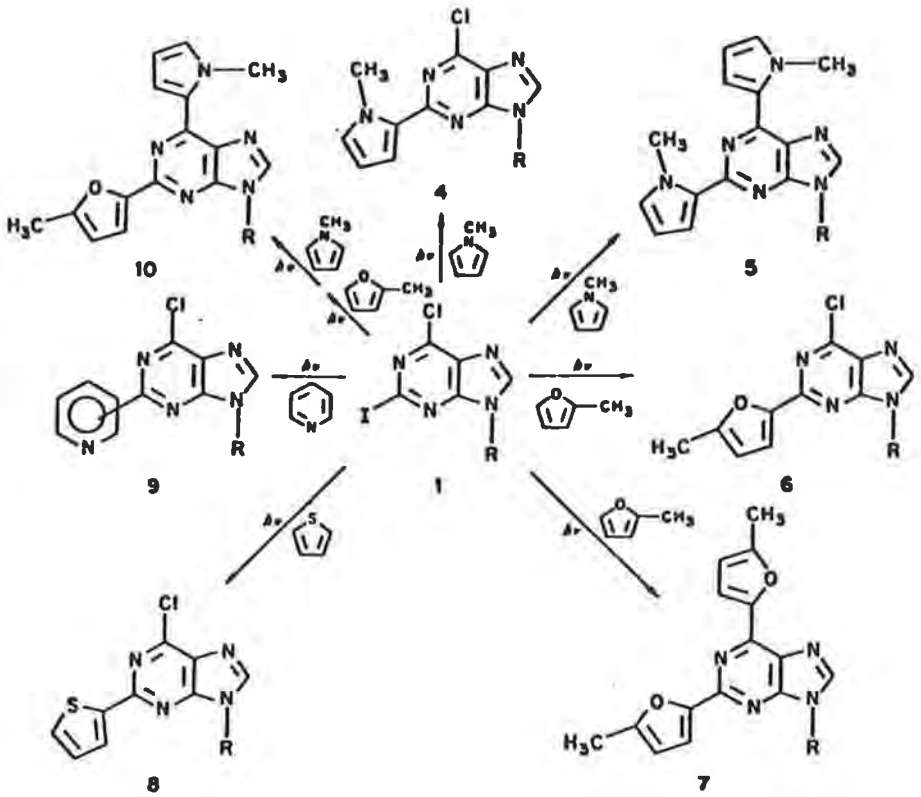
Scheme II. Photoinduced Heteroarylations Involving Purinyl Radicals[a]



[a] R = Rib(OAc)$_3$.

Photolysis of 1 in dry, nitrogen-purged benzene (Hanovia 450-W mercury lamp, Vycor filter) for 25 h gave 2-phenyl-6-chloronebularine (2) (59%) and 2,6-diphenylnebularine (3) (21%) (Scheme I). Compound 2 was characterized by its mass spectrum [m/z 488 (M^+, ^{35}Cl), 490 (M^+, ^{37}Cl)], by its high-field ^1H and ^{13}C NMR data which showed the attachment of the phenyl ring on the purine base, and by its UV spectrum which was consistent with the presence of a phenyl ring conjugated to the purine system. 2,6-Diphenylnebularine (3) was similarly characterized by its spectral data.

Selectivity in the formation of 2 or 3 can be achieved by controlling reaction times. Thus, when 1 was photolyzed in benzene for 16.5 h, compound 2 was the sole product and was isolated in 80% yield. Longer reaction times resulted in increased formation of 3, until at 65 h, compound 3 was the only isolable product. These arylations could be monitored very conveniently by mass spectrometry.

Extension of these reactions to photoinduced heteroarylations involving both π-excessive and π-deficient systems was also investigated. Thus, when 1 was allowed to react with *N*-methylpyrrole under photolysis (Hanovia 450-W mercury lamp, Vycor filter) for 2 h, 2,6-bis(*N*-methylpyrr-2-yl-9β-(2,3,5-tri-*O*-acetyl-D-ribofuranosyl)-purine (5) was isolated in 61.4% yield as the sole product (Scheme II). Compound 5 was found to be photostable as reaction times of up to 11 h gave similar yields and no photorearranged products. It was identified by its mass spectrum [m/z 536 (M^+)], by its ^1H and ^{13}C NMR spectra, and by its bathochromically shifted UV spectrum [λ_{max} 331.5 nm (ϵ 3.1 × 10^4) (ethanol)]. Compound 5 exhibited interesting fluorescence properties, emitting at 450 nm when excited at 330 nm. The absence of the monoheteroarylated product 4 in this reaction involving relatively short reaction times can be explained by the increased reactivity of the π-excessive pyrrole ring compared to benzene. However, formation of the monoheteroarylated product can be maximized with the use of lower intensity light. Thus, when 1 was photolyzed in *N*-methylpyrrole for 2 h (Rayonet, 2537 Å), 2-(*N*-methylpyrr-2-yl)-6-chloro-9β-(2,3,5-tri-*O*-acetyl-D-ribofuranosyl)purine (4) was isolated in 63% yield, along with a 24% yield of 5. The position of substitution on the pyrrole ring in 4 (and also 5) was deduced from the high-field ^1H NMR spectrum (in $CDCl_3$) which showed H-3 of the pyrrole moiety as a doublet of doublets at δ 8.19 with $J_{3,4}$ = 3.9 Hz and $J_{3,5}$ = 1.8 Hz. The downfield shift of this proton from 6.11 ppm in *N*-methylpyrrole is consistent with heteroarylation at the 2-position of the pyrrole ring.[2] The observed coupling constants, particularly $J_{3,4}$, are characteristic of 2-substituted pyrroles.[20]

Photolysis of 1 in 2-methylfuran as solvent for 6 h gave the monoheteroarylated product 6 in 71% yield together with a trace amount of the 2,6-bis(5-methylfur-2-yl)purine nucleoside 7 (Scheme II). The regiochemistry of the reaction was again apparent from the high-field ^1H NMR data. Formation of the heteroarylated product 8 (75%) was achieved by photolysis of 1 in dry thiophene in the Hanovia apparatus for 7 h. Previous studies of radical attack on the thiophene ring system has shown the formation of both the α- and β-substituted thiophenes.[21] 2-Arylthiophenes have been converted photochemically to 3-arylthiophenes.[22] The "purinyl radical" reaction on thiophene appears to be regiospecific for the α-position to form a very photostable product. The use of longer reaction times in both this and the furan case resulted in

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Table I. Correlation of Conformation with Carbon Chemical Shifts (δ) of 2,6-Disubstituted Purine Nucleosides

compound	solvent	C1′	C2′	C3′	C4′	C5′	Δ(C2′–C3′)	confmtn
adenosine	Me_2SO-d_6	87.9	73.4	70.6	85.8	61.6	2.8	anti
inosine[c]	Me_2SO-d_6	87.6	74.2	70.4	85.7	61.4	3.8	anti
8-bromoinosine[c]	Me_2SO-d_6	90.5	71.2	70.5	86.3	61.9	0.7	syn
tri-*O*-acetylinosine[a]	$CDCl_3$	86.5	73.3	70.5	80.3	63.0	2.8	anti
8-bromotri-*O*-acetyladenosine[b]	$CDCl_3$	88.9	71.8	70.4	79.8	62.8	1.4	syn
1	$CDCl_3$	86.7	73.3	70.5	80.8	62.9	2.8	anti
2	$CDCl_3$	82.3	73.2	70.1	80.1	62.6	3.1	anti
3	$CDCl_3$	87.0	73.2	70.2	80.0	62.7	3.0	anti
4	$CDCl_3$	87.0	73.1	70.0	80.8	62.6	3.1	anti
5	$CDCl_3$	86.5	73.1	70.2	79.8	62.7	2.9	anti
6	$CDCl_3$	87.5	73.5	70.3	80.4	62.8	3.2	anti
8	$CDCl_3$	87.7	73.4	70.3	80.2	62.8	3.1	anti
10	$CDCl_3$	86.9	73.5	70.5	80.2	63.1	3.0	anti
11	Me_2SO-d_6	87.2	73.9	70.3	85.5	61.2	3.6	anti

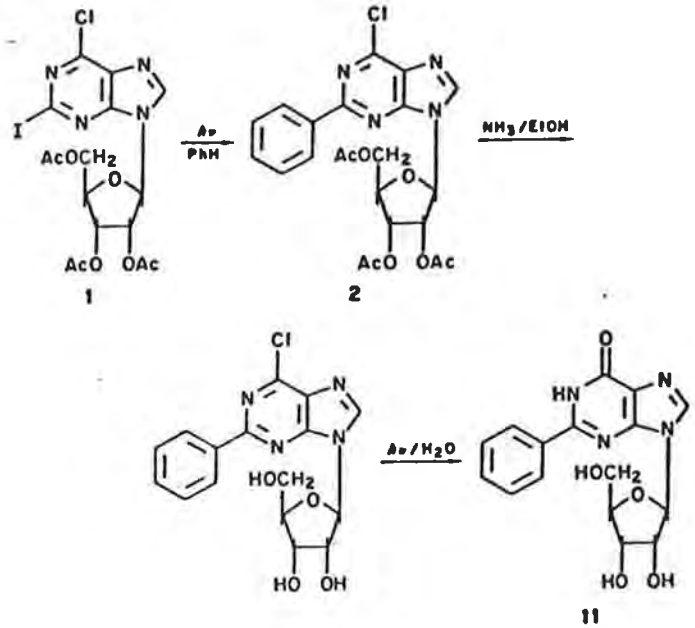
[a] Prepared from photolysis of 6-iodo-9β-(2,3,5-tri-*O*-acetyl-D-ribofuranosyl)purine in wet CH_3CN. [b] Prepared by the procedure in ref 27. [c] Data from ref 26.

considerable polymerization of the photosolvents and the diheteroarylated products could not be isolated and purified from this intractable mixture. The π-deficient heterocycle, pyridine, also participated in these heteroarylations, to give albeit a 14% yield of the isomerically mixed product 9 (Scheme II).

The 2-substituted 6-chloropurine nucleosides synthesized in this work are interesting synthetic intermediates as they can be modified further to produce some uncommon nucleosides. For example, photolysis in the same solvent produces the aforementioned symmetrical 2,6-disubstituted nucleosides. In the presence of a different solvent, unsymmetrical 2,6-substituted nucleosides are formed (e.g., Scheme II, compound 10). Photolysis in the presence of water results in the formation of inosine analogues in excellent yields (e.g., Scheme III, compound 11). This approach provides a direct method for the synthesis of a wide variety of 2-substituted inosine derivatives.

Finally, it should be mentioned that determination of the glycosidic bond conformation of purine nucleosides in solution is of considerable importance in the correlation of their stereochemistry with biological activity. The syn and anti conformations of natural nucleosides in solution have been determined by potential energy calculations,[23] circular dichroism,[24] and ^1H and ^{13}C NMR spectroscopy.[25,26] The data from these studies have shown that adenosine, guanosine, and inosine all prefer the anti conformation in solution. Most purine nucleoside analogues that have been studied are 8-substituted adenosines which have been shown to prefer the syn conformation in solution. Few examples of 2,6-disubstituted purine nucleoside analogues have been investigated. The purpose of this study was to provide such conformational information and also to emphasize that high-field ^{13}C NMR data can be used very effectively to determine the glycosidic conformation in solution. In the syn conformation, the proximity of the lone pair of electrons on N-3 to C-2′ results in an upfield shift of this carbon in syn compared to the anti conformation. The only other carbon resonance that changes significantly in the carbohydrate portion of the molecule is the anomeric carbon (C-1′), and its chemical shift is largely dependent on the structure of the base and not its conformation. When differences between the chemical shifts of C-2′ and C-3′ are examined, it is clear that in the syn conformation Δ(C2′–C3′) is <1.4 ppm whereas in the anti conformation this difference is ≥3.0 ppm. The results are shown in Table I and suggests that the 2,6-disubstituted purine nucleosides prefer the anti conformation in solution.

Scheme III. Synthesis of 2-Substituted Inosine Analogues



In summary, facile arylations and heteroarylations at the 2- and 2,6-positions of purine nucleosides can be achieved through the intermediacy of reactive transient purinyl radicals. The reactivities of the aromatic or heteroaromatic solvents used follow the order π-excessive (pyrrole, furan, thiophene) > benzene > π-deficient (pyridine). Except in the case of pyridine, the conversions were regiospecific. Although the genesis of the purinyl radicals (or caged radical pairs) is not fully understood, a plausible mechanism in the case of arylations and heteroarylations may involve initial formation of an exciplex followed by electron transfer and cleavage of the C–I bond of the resulting radical anion. This type of mechanism may not be operating in the hydration reaction.

Experimental Section

Irradiation was accomplished in a Hanovia 450-W mercury photolysis apparatus or in a Rayonent photochemical reactor.

Melting points are uncorrected and were determined on a Thomas-Hoover melting point apparatus fitted with a microscope. Nuclearmagnetic resonance spectra employing tetramethylsilane as the internal standard were recorded on JEOL Model FX90Q

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and Bruker Model WM360 pulse fourier transform spectrometers. Mass spectra at 30 eV were obtained on a Hewlett-Packard 5985 GC–mass spectrometer. The ultraviolet spectra were recorded on a Varian-Cary Model 219 spectrophotometer. Fluorescence spectra are uncorrected and were performed on an Aminco-Bowman Spectrophotofluorimeter using a xenon lamp. *N*-Methylpyrrole, 2-methylfuran (Aldrich), benzene (MCB Omnisolv), and pyridine (MCB) were distilled prior to use; thiophene (Aldrich) was used without further purification. Preparative-layer chromatography employed EM silica gel PF_{254} plates, activated for 3 h at 135 °C.

2-Iodo-6-chloro-9β-(2,3,5-tri-*O*-acetyl-D-ribofuranosyl)purine (1) was prepared by using literature procedures.[5,19] Guanosine was transformed by initial treatment with pyridine, acetic anhydride, and dimethylformamide to 2′,3′,5′-tri-*O*-acetylguanosine.[19] The protected guanosine was allowed to react with phosphoryl chloride, *N,N*-dimethylaniline, acetonitrile, and tetraethylammonium chloride to provide 2-amino-6-chloro-9β-(2,3,5-tri-*O*-acetyl-D-ribofuranosyl)purine.[19] Treatment of the 2-amino-6-chloropurine nucleoside with *n*-pentyl nitrite and diiodomethane[5] gave 1 (83% yield, 66% overall yield from guanosine) as white cyrstals: mp 181–183 °C (lit.[5] mp 181–183 °C); ^{13}C NMR ($CDCl_3$) δ 20.4, 20.5, 20.8, 62.9, 70.5, 73.3, 80.8, 86.7, 116.9, 132.2, 143.4, 150.7, 151.9, 169.4, 169.5, 170.2; ^{1}H NMR ($CDCl_3$) δ 2.11 (s, 3 H), 2.13 (s, 3H), 2.18 (s, 3 H), 4.43 (m, 3 H), 5.65 (t, 1 H), 5.81 (t, 1 H), 6.23 (d, 1 H), 8.27 (s, 1 H); UV (MeOH) λ_{max} 222.5 nm (ε 2.1 × 10^4), 258 (ε 6.6 × 10^3), 281 (ε 9.3 × 10^3); mass spectrum, *m/z* (relative intensity) 540 (M$^+$, 1.0), 538 (M$^+$, 2.1), 283 (6.6), 282 (2.3), 281 (15.3), 280 (2.3), 259 (68.7), 139 (100.0).

2-Phenyl-6-chloro-9β-(2,3,5-tri-*O*-acetyl-D-ribofuranosyl)purine (2). To 300 mL of dry benzene was added 0.284 g (0.527 mmol) of 2-iodo-6-chloro-9β-(2,3,5-tri-*O*-acetyl-D-ribofuranosyl)purine (1). The solution was transferred to a Hanovia photochemical reactor, purged with nitrogen, and photolyzed for 16.5 h by employing a 450-W mercury UV source with a Vycor glass sleeve filter. The solvent was then removed (40 °C, reduced pressure) and the residue chromatographed on silica gel plates. After elution with 7:3 ethyl acetate:hexane, the band at R_f 0.63 afforded 0.207 g (0.423 mmol, 80.3%) of 2 as a light yellow low melting solid: ^{13}C NMR ($CDCl_3$) δ 20.4, 20.5, 20.6, 62.6, 70.1, 73.2, 80.1, 82.3, 128.6, 128.7, 131.1, 136.3, 143.7, 151.5, 151.9, 159.9, 169.3, 169.4; ^{1}H NMR ($CDCl_3$) δ 1.96 (s, 3 H), 2.11 (s, 3 H), 2.18 (s, 3 H), 4.40 (m, 3 H), 5.85 (t, 1 H), 6.12 (t, 1 H), 6.24 (d, 1 H), 7.49–7.46 (m, 3 H), 8.25 (s, 1 H), 8.53–8.46 (m, 2 H); UV (EtOH) λ_{max} 236 nm (ε 1.7 × 10^4), 286 (ε 1.6 × 10^4), 274 (ε 1.5 × 10^4); fluorescence (EtOH) excitation 325 nm and emission 382 nm; mass spectrum, *m/z* (relative intensity) 490 (M$^+$, 1.1), 488 (M$^+$, 2.8), 259 (sugar$^+$, 37.8), 233 (5.4), 232 (3.4), 231 (17.1), 230 (3.8), 229 (Pur$^+$, 1.0), 199 (1.3), 196 (1.8), 195 (7.5), 194 (Pur$^+$ – Cl, 0.9), 157 (12.6), 139 (100).

2,6-Diphenyl-9β-(2,3,5-tri-*O*-acetyl-D-ribofuranosyl)purine (3). A solution consisting of 0.072 g (0.134 mmol) of 1 in 90 mL of dry benzene was photolyzed as described for 2 for 65 h. Separation on silica gel plates with 7:3 ethyl acetate:hexane as the developing solvent gave 0.042 g (0.078 mmol, 58.2%) of 3 as a light yellow low melting solid: ^{13}C NMR ($CDCl_3$) δ 20.4, 20.5, 20.6, 62.7, 70.2, 73.2, 80.0, 87.0, 128.5, 128.6, 130.0, 130.3, 131.1, 135.8, 138.0, 142.9, 152.8, 154.9, 159.2, 169.4, 169.5, 170.4; ^{1}H NMR ($CDCl_3$) δ 1.97 (s, 3 H), 2.11 (s, 3 H), 2.19 (s, 3 H), 4.41 (m, 3 H), 5.93 (t, 1 H), 6.20 (t, 1 H), 6.29 (d, 1 H), 7.59–7.48 (m, 6 H), 8.23 (s, 1 H), 8.66 (m, 2 H), 8.86 (m, 2 H); UV (EtOH) λ_{max} 266 nm (ε 2.7 × 10^4), 310 (ε 1.0 × 10^4); fluorescence (EtOH) excitation 322 nm and emission 375 nm; mass spectrum, *m/z* (relative intensity) 530 (M$^+$, 4.2), 471 (M$^+$ – $C_2H_3O_2$, 2.2), 301 (Pur$^+$ + CH_2O, 10.6), 274 (9.6), 273 (48.9), 272 (13.1), 271 (Pur$^+$, 2.0), 259 (sugar$^+$, 11.7), 199 (1.3), 157 (12.3), 139 (100).

2-(*N*-Methylpyrr-2-yl)-6-chloro-9β-(2,3,5-tri-*O*-acetyl-D-ribofuranosyl)purine (4). A N_2-purged solution of 0.103 g (0.192 mmol) of 1 in 65 mL of dry *N*-methylpyrrole was photolyzed in a quartz reaction vessel for 2 h by using a Rayonet photochemical reactor (2537-Å lamps). The solvent was removed (40 °C, reduced pressure) and the residue was chromatographed on silica gel plates (3:2 ethyl acetate:hexane).

The band at R_f 0.55 gave 0.058 g (0.118 mmol, 61.5%) of 4 as a light brown glass: ^{13}C NMR ($CDCl_3$) δ 20.4, 20.5, 20.6, 38.1, 62.6, 70.0, 73.1, 80.8, 87.0, 108.4, 116.5, 128.8, 129.1, 129.8, 142.6, 150.5, 151.7, 155.2, 169.2, 169.4, 170.3; ^{1}H NMR ($CDCl_3$) δ 2.05 (s, 3H), 2.10 (s, 3 H), 2.15 (s, 3 H), 4.10 (m, 3 H), 4.42 (s, 3 H), 5.69 (t, 1 H), 6.23–5.90 (m, 3 H), 6.79 (dd, 1 H), 8.17 (s, 1 H), 8.19 (dd, 1 H); UV (EtOH) λ_{max} 337 nm (ε 1.1 × 10^4), 298 (ε 1.0 × 10^4), 245 (ε 8.9 × 10^3); fluorescence (EtOH) excitation 355 nm and emission 440 nm; mass spectrum, *m/z* (relative intensity) 493 (M$^+$, 5.8), 491 (M$^+$, 15.9), 259 (sugar$^+$, 8.5), 235 (19.6), 234 (23.2), 233 (55.7), 232 (Pur$^+$, 32.9), 199 (2.9), 157 (14.1), 139 (100).

The band with R_f 0.72 gave 0.025 g (0.046 mmol, 24.0%) of 5.

2,6-Bis(*N*-methylpyrr-2-yl)-9β-(2,3,5-tri-*O*-acetyl-D-ribofuranosyl)purine (5). A solution consisting of 0.187 g (0.347 mmol) of 1 and 75 mL of dry *N*-methylpyrrole was made and photolyzed for 2 h as described for 2. Separation on silica gel plates with 7:3 ethyl acetate:hexane afforded 0.114 g (0.213 mmol, 61.4%) of 5 as a beige low melting solid: ^{13}C NMR ($CDCl_3$) δ 20.3, 20.5, 20.6, 37.7, 38.3, 62.7, 70.2, 73.1, 79.8, 86.5, 107.9, 108.9, 114.9, 119.5, 127.0, 127.3, 127.5, 129.2, 132.0, 140.6, 150.0, 151.0, 154.7, 169.1, 169.4, 170.3; ^{1}H NMR ($CDCl_3$) δ 2.07 (s, 3 H), 2.08 (s, 3 H), 2.13 (s, 3 H), 4.12 (s, 3 H), 4.23 (s, 3 H), 4.39 (m, 3 H), 5.61 (t, 1 H), 6.33–6.04 (m, 4 H), 6.76 (dd, 1 H), 6.86 (dd, 1 H), 7.12 (dd, 1 H), 7.80 (dd, 1 H), 8.11 (s, 1 H); UV (EtOH) λ_{max} 331.5 nm (ε 3.1 × 10^4); fluorescence (EtOH) excitation 330 nm and emission 450 nm; mass spectrum, *m/z* (relative intensity) 538 (1.1), 537 (5.5), 536 (M$^+$, 15.8), 279 (12.8), 278 (38.9), 277 (Pur$^+$, 100.0), 259 (sugar$^+$, 1.4), 199 (1.3), 197 (Pur$^+$ – C_5H_6N, 1.1), 157 (9.0), 139 (67.7).

2-(5-Methylfur-2-yl)-6-chloro-9β-(2,3,5-tri-*O*-acetyl-D-ribofuranosyl)purine (6). A solution consisting of 0.113 g (0.210 mmol) of 1 and 60 mL of dry 2-methylfuran was photolyzed for 6 h as described for 4. Silica gel chromatography with 7:3 ethyl acetate:hexane as the developer provided two bands.

The band at R_f 0.47 gave 0.073 g (0.148 mmol, 70.5%) of 6 as a golden low melting solid: ^{13}C NMR ($CDCl_3$) δ 14.1, 20.4, 20.5, 20.6, 62.8, 70.3, 73.5, 80.4, 87.5, 109.1, 116.2, 130.0, 143.4, 149.5, 151.6 151.6, 152.5, 156.3, 169.3, 169.4, 170.3; ^{1}H NMR ($CDCl_3$) δ 2.02 (s, 3 H), 2.12 (s, 3 H), 2.17 (s, 3 H), 2.46 (s, 3 H), 4.46 (m, 3 H), 6.20–5.70 (m, 4 H), 7.37 (d, 1 H, J = 3.0 Hz), 8.18 (s, 1 H); UV (EtOH) λ_{max} 324 nm (ε 1.5 × 10^4), 302 (ε 1.3 × 10^4), 245 (ε 7.8 × 10^3); fluorescence (EtOH) excitation 340 nm and emission 410 nm; mass spectrum, *m/z* (relative intensity) 494 (M$^+$, 0.7), 492 (M$^+$, 1.8), 259 (sugar$^+$, 7.0), 237 (4.7), 236 (12.5), 235 (14.1), 234 (33.7), 233 (Pur$^+$, 2.9), 199 (4.2), 157 (12.4), 139 (100.0).

The band with R_f 0.66 afforded 0.004 g of 2,6-bis(5-methylfur-2-yl)-9β-(2,3,5-tri-*O*-acetyl-D-ribofuranosyl)purine (7). Compound 7 was only characterized by its mass spectrum, *m/z* (relative intensity) 538 (M$^+$, 15.9), 282 (5.6), 281 (30.1), 280 (91.2), 279 (Pur$^+$, 4.7), 259 (sugar$^+$, 7.8), 199 (3.0), 157 (14.7), 139 (100.0).

2-(Thien-2-yl)-6-chloro-9β-(2,3,5-tri-*O*-acetyl-D-ribofuranosyl)purine (8). To 60 mL of dry thiophene was added 0.235 g (0.436 mmol) of 1. The solution was photolyzed as for 2 for 7 h. Separation gave 0.162 g (0.327 mmol, 75.0%) of 8 as a low melting light yellow glass: ^{13}C NMR ($CDCl_3$) δ 20.4, 20.5, 20.6, 62.8, 70.3, 73.4, 80.2, 87.7, 128.4, 130.0, 130.4, 141.9, 143.8, 151.3, 151.7, 156.3, 169.3, 169.4, 170.2; ^{1}H NMR ($CDCl_3$) δ 2.01 (s, 3 H), 2.12 (s, 3 H), 2.18 (s, 3 H), 4.43 (m, 3 H), 6.20–5.86 (m, 3 H), 7.14 (dd, 1 H, J = 3.0, 3.9 Hz), 7.40 (d, 1 H, J = 3.9 Hz), 8.10 (d, 1 H, J = 3.0 Hz), 8.23 (s, 1 H); UV (EtOH) λ_{max} 316 nm (ε 1.4 × 10^4), 270 (ε 6.5 × 10^3), 245 (ε 9.2 × 10^3); fluorescence (EtOH) excitation 331 nm and emission 378 nm; mass spectrum, *m/z* (relative intensity) 496 (M$^+$, 1.4), 494 (M$^+$, 3.5), 259 (sugar$^+$, 22.6), 239 (3.0), 238 (3.8), 237 (7.5), 236 (Pur$^+$, 8.4), 203 (2.0), 202 (1.7), 201 (Pur$^+$ – Cl, 8.9), 199 (1.3), 157 (14.3), 139 (100.0).

2-Pyridyl-6-chloro-9β-(2,3,5-tri-*O*-acetyl-D-ribofuranosyl)purine (9). A solution consisting of 0.127 g (0.236 mmol) of 1 and 50 mL of dry pyridine was photolyzed for 24 h as described for 4 to give 0.016 g (0.033 mmol, 14.0%) of the mixture 9 as a brown oil: UV (EtOH) λ_{max} 232 nm, 288, 321; mass spectrum, *m/z* (relative intensity) 491 (M$^+$, 0.9), 489 (M$^+$, 2.1), 259 (46.8), 234 (14.0), 233 (8.2), 232 (44.0), 231 (8.7), 230 (Pur$^+$, 0.7), 199 (1.3), 157 (14.1), 139 (100.0).

2-(5-Methylfur-2-yl)-6-(*N*-methylpyrr-2-yl)-9β-(2,3,5-tri-*O*-acetyl-D-ribofuranosyl)purine (10). A solution consisting of 0.087 g (0.177 mmol) of 6 and 50 mL of dry *N*-methylpyrrole was photolyzed for 3 h as described for 2. Separation afforded 0.052 g (0.097 mmol, 54.8%) of 10 as a light brown low melting glassy solid: ^{13}C NMR ($CDCl_3$) δ 14.0, 20.4, 20.5, 20.7, 38.4, 63.1,

70.5, 73.5, 85.2, 86.9, 108.4, 109.0, 113.8, 119.6, 127.0, 127.6, 130.0, 141.4, 150.0, 151.2, 151.3, 151.8, 154.7, 169.4, 170.4; ^{1}H NMR ($CDCl_3$) δ 2.03 (s, 3 H), 2.10 (s, 3 H), 2.15 (s, 3 H), 2.44 (s, 3 H), 4.27 (s, 3 H), 4.48 (m, 3 H), 6.33–6.01 (m, 5 H), 6.88 (dd, 1 H), 7.22 (d, 1 H, J = 2.9 Hz), 7.82 (dd, 1 H, J = 3.9, 1.8 Hz), 8.08 (s, 1 H); UV (EtOH) λ_{max} 310 nm (ϵ 3.0 × 10^4), 326 (ϵ 2.8 × 10^4), 336 (2.7 × 10^4), 352 (ϵ 2.4 × 10^4), 250 (ϵ 8.3 × 10^3); fluorescence (EtOH) excitation 350 nm and emission 400 nm; mass spectrum, m/z (relative intensity) 537 (M^+, 1.7), 308 ($Pur^+ + CH_2O$, 3.4), 280 (14.1), 279 (39.9), 278 (Pur^+, 100.0), 259 ($sugar^+$, 1.5), 199 (1.7), 157 (9.1), 139 (68.3).

2-Phenylinosine (11). To 50 mL of dry ethanol saturated with ammonia gas at ice–salt bath temperatures was added 0.267 g (0.546 mmol) of **2**. The solution was stirred at ice–salt bath temperatures for 1 h and at 25 °C for 23 h. The solvent was removed under reduced pressure and the residue was lyophilized. The deprotected nucleoside (0.186 g) in 400 mL of water was photolyzed as described for **2** for 34 h. The solvent was removed under reduced pressure and the residue chromatographed on silica gel plates that were developed in 4:1 ethyl acetate:methanol. The band at R_f 0.22 gave 0.157 g (0.455 mmol, overall yield = 83.4%) of **11** as a light yellow crystalline compound: mp 98–101 °C; ^{13}C NMR ($CDCl_3$) δ 61.2, 70.3, 73.9, 85.5, 87.2, 122.8, 127.8, 128.5, 131.2, 132.0, 139.2, 148.5, 153.3, 157.2; ^{1}H NMR ($CDCl_3$) δ 3.72–3.54 (m, 2 H), 4.20 (m, 1 H), 4.58 (m, 1 H), 5.14 (m, 1 H), 5.97 (d, 1 H, J = 5.9 Hz), 7.60 (m, 3 H), 8.12 (m, 2 H), 8.37 (s, 1 H), 11.2 (s, 1 H); UV (EtOH) λ_{max} 290 nm (ϵ 5.8 × 10^3), 260 (4.9 × 10^3); fluorescence (EtOH) excitation 366 nm and emission 456 nm; mass spectrum, m/z (relative intensity) 254 ($Pur^+ + C_3H_2O$, 10.9), 240 ($Pur^+ + CHO$, 20.0), 225 ($Pur^+ + CH_2$, 22.7), 213 (20.0), 212 (96.4), 211 (Pur^+, 42.7).

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(2 × 50 ml). The organic extract is dried with sodium sulfate and evaporated under reduced pressure with mild heating. In general, the crude reaction products **3** are purified by distillation or recrystallization from methanol or benzene. In some cases, preliminary purification by column chromatography on silica gel may be necessary (elution with benzene or cyclohexane/benzene mixtures).

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Carbon Elongations with 1,5-Diazapentadienium Salts: Reaction with Aralkyl Ketones

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Carbonyl compounds play a central role in carbon-carbon bond elaboration. For this reason, reaction sequences that result in carbon chain elongation are especially significant. Although numerous methods are available for the conversion of ketones into higher homologs involving unsaturation attendant with one and two carbon chain extension, the methodology for such three carbon elaborations of these structures is very limited[1]. We have discovered recently[2] that 1,5-diazapentadienium or vinamidinium salts react selectively with enolates of alicyclic ketones, esters, lactones, and lactams to give products with conjugated three-carbon elongation at the α-position of the original compound.

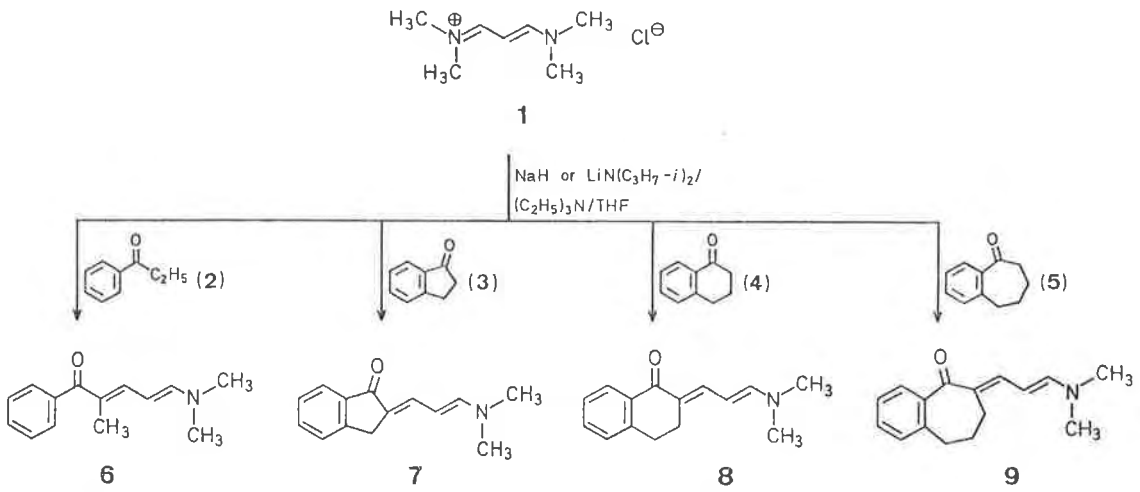
This paper reports on the utilization of the vinamidinium salt **1** for the carbon elongation of aralkyl ketones (**2**–**5**). The multifunctional dienaminones (**6**–**9**) produced can be regarded as potential synthetic intermediates for a number of natural products including maytansinoids[3,4], gibberelins[5,6], steroids[7,8], and colchicines[9,10]. The vinamidinium salt **1** was prepared from β-(dimethylamino)-acrolein[11] as previously described[2]. When the salt **1** was treated with enolates generated *in situ* by reaction of sodium hydride or lithium diisopropylamide with the aralkyl ketones **2**–**5** in triethylamine, multifunctional dienaminones **6**–**9** were isolated in good to excellent yields (Table).

Method A involves the reaction with enolates generated *in situ* by reaction with sodium hydride and Method B used lithium diisopropylamide as base. There was no significant difference in yields between the two methods. However, dramatic reductions in reaction times were observed in all cases when lithium diisopropylamide was used. Determination of reaction times was carried out by U.V.-visible spectral methods. The substrates absorb at wavelengths shorter than that for **1** (which has a λ_{max} at 309 nm), and the products absorb at wavelengths longer than that for **1** (in the range of 360–440 nm). Optimization of the transformation required that a 2 : 1 ratio of **1** and ketone be used.

The stereochemistry of the dienaminones were determined by high-field ^{1}H-N.M.R. data which showed that these compounds were exclusively the (*E,E*)-(*s-trans*)-geometric isomers. The stereospecificity of the reaction was further confirmed by ^{13}C-N.M.R. data. ^{13}C-Chemical shift assignments were aided by delayed decoupling experiments. The mass spectra of all of the dienaminones gave parent ions and fragmentation patterns consistent with their structures.

Carbon Elongation Reactions; General Procedures:

Method A: The carbonyl compound (2.0 mmol) in tetrahydrofuran (3 ml) is added dropwise with stirring to 1,1,5,5-tetramethyl-1,5-diazapentadienium chloride (**1**; 4.0 mmol) and sodium hydride (3.0 mmol) in triethylamine (7 ml). The mixture is stirred over 4-A molecular sieves (0.3 g) at 0 °C for 30 min. It is then warmed to room temperature and progress of reaction is monitored by ultraviolet-visible spectroscopy. On completion, the mixture is worked up by carefully pouring it into saturated sodium chloride solution (40 ml) followed by extraction with dichloromethane (4 × 40 ml). The combined organic layers are dried with sodium sulfate. After removal of



1 6 7 8 9

Table. Products from the Reaction of Vinamidinium Salt **1** with Aralkyl Ketones

Product	Yield [%][a]	m.p. [°C]	Molecular Formula[b]	M.S. *m/e* (rel. intens. %)	U.V.(C_2H_5OH) λ_{max} (ε)	^{1}H-N.M.R.($CDCl_3$) δ [ppm]	^{13}C-N.M.R. ($CDCl_3$) δ [ppm]
6	52[c]	100–102°	$C_{14}H_{17}NO$ (215.3)	215 (M^+, 45.4); 200 (7.2); 186 (1.3); 171 (100); 157 (9.5)	249 (9104); 387 (42166)	1.98 (s, 3H); 2.89 (s, 3H); 5.17 (t, 1H, J = 11.9 Hz); 6.54 (d, 1H, J = 11.9 Hz); 6.90 (d, 1H, J = 11.9 Hz); 7.40 (m, 5H)	11.7; 40.6; 95.3; 123.6; 127.8; 128.6; 129.5; 141.2; 148.9; 150.7; 197.4
7	67	153–155°	$C_{14}H_{15}NO$ (213.3)	213 (M^+, 100); 198 (10.5); 184 (22.8); 169 (71.3); 156 (7.4)	273 (18954); 438 (69804)	2.85 (s, 6H); 3.54 (s, 2H); 5.06 (t, 1H, J = 12.3 Hz); 6.74 (d, 1H, J = 12.3 Hz); 7.35 (d, 1H, J = 12.3 Hz); 7.43 (m, 3H); 7.79 (d, 1H, J = 6.6 Hz)	30.8; 40.6; 95.5; 123.2; 123.8; 125.8; 126.8; 132.3; 138.0; 141.2; 148.3; 152.6; 192.0
8	93	145–147°	$C_{15}H_{17}NO$ (227.3)	227 (M^+, 24.6); 212 (1.9); 198 (1.9); 183 (100); 170 (1.7)	274 (23374); 438 (48823)	2.76 (m, 2H); 2.91 (m, 2H); 2.94 (s, 6H); 5.20 (t, 1H, J = 12.3 Hz); 6.84 (d, 1H, J = 12.3 Hz); 7.29 (m, 3H); 7.62 (d, 1H, J = 12.3 Hz); 8.07 (d, 1H, J = 7.7 Hz)	24.8; 28.7; 40.7; 95.1; 121,3; 126.5; 127.5; 127.6; 131.6; 135.1; 140.8; 142.9; 152.2; 185.8
9	65	90–93°	$C_{16}H_{19}NO \cdot H_2O$ (259.3)	241 (M^+, 32.7); 226 (2.0); 212 (3.2); 197 (100); 184 (5.6)	267 (11285); 418 (38546)	1.90 (m, 2H); 2.32 (m, 2H); 2.71 (m, 2H); 2.83 (s, 6H); 5.18 (t, 1H, J = 12.3 Hz; 6.78 (d, 1H, J = 12.3 Hz); 7.31 (m, 4H); 7.40 (d, 1H, J = 12.3 Hz)	24.2; 27.0; 31.6; 40.6; 94.5; 124.6; 126.5; 128.2; 128.4; 130.7; 139.1; 141.2; 141.3; 152.2; 196.1

[a] The yields quoted are averaged yields from small and large scale experiments. Only small variations were found between these runs.
[b] Satisfactory microanalyses obtained: C ±0.20, H ±0.38, N ±0.44; exception: **9**, C +0.7.
[c] Yield based on recovered starting material; the yield of isolated product is 27%.

the solvent, the products are purified by preparative layer chromatography on E. Merck aluminum oxide 60-PF-254 plates with chloroform/ethyl acetate as the eluting solvent. Crystallizations are performed from hexane.

Method B: The carbonyl compound (2.0 mmol) in dry triethylamine (5 ml) is added dropwise to a stirred solution of lithium diisopropylamide [generated by adding 1.5 molar *n*-butyllithium solution (2.0 ml) to dry diisopropylamine (3.0 mmol) in dry tetrahydrofuran (7 ml) at 0 °C and stirring the mixture at 0 °C for 20 min after the addition is complete]. To this is added 1,1,5,5-tetramethyl-1,5-diazapentadienium chloride (**1**; 2.0 mmol). The mixture is stirred at 0 °C for 30 min and then at room temperature for 2 h. At this stage, another portion of (2.0 mmol) is added. Progress of reaction is monitored by ultraviolet-visible spectroscopy. The mixture is worked up by carefully pouring it into saturated sodium chloride solution (40 ml) followed by extraction with dichloromethane (4 × 40 ml). The combined organic layers are dried with sodium sulfate. After removal of the solvent, the products are purified as described in Method A.

Method C (Large Scale Procedure): The carbonyl compound (10.0 mmol) in dry triethylamine (10 ml) is added dropwise to a stirred solution of lithium diisopropylamide [generated by adding 1.5 molar *n*-butyllithium solution (10.0 ml) to dry diisopropylamine (15 mmol) in dry tetrahydrofuran (30 ml) at 0 °C and stirring the mixture at 0 °C for 20 min after the addition is complete]. To this is added 1,1,5,5-tetramethyl-1,5-diazapentadienium chloride (**1**; 10.0 mmol). The mixture is stirred at 0 °C for 30 min and then at room temperature for 2 h. At this stage, another portion of **1**

(10.0 mmol) is added. Progress of reaction is monitored by ultraviolet-visible spectroscopy. The mixture is worked up by carefully pouring it into saturated sodium chloride solution (100 ml) followed by extraction with dichloromethane (4 × 100 ml). The combined organic layers are dried with sodium sulfate. After removal of the solvent, the products are purified by column chromatography on activated alumina (80–325 mesh) with mixtures of chloroform and ethyl acetate as the eluting solvent. They are crystallized from hexane.

Note: The lithium diisopropylamide method is much more convenient for larger scale reactions than the procedure using sodium hydride.

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6-(Alkyldimethylammonio)-hexanoates

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The 6-ammoniohexanoate functional group is of particular interest to surfactant science[1]. The zwitterionic subclass of hydrophilic groups to which it belongs is the most hydrophilic of the nonionic subclasses, and the ammoniohexanoates are among the most hydrophilic members of this subclass. The synthesis of this functional group by quaternizing tertiary amines with salts of 6-halohexanoic acid[2], or by hydrolyzing ammonium ester halides[3], is chemically straightforward. The difficult aspect of these syntheses is the separation of the zwitterionic product from the physically similar salt by-products. When the zwitterionic material is an association colloid as well, purification is further complicated by the natural tendencies of the material to foam and to solubilize or emulsify non-polar impurities. The emphasis in this report is upon resolution of these problems, which are exaggerated in the case of ammoniohexanoates by their unusually high water solubility.

The 6-ammoniohexanoates (**3**) are best synthesized by base hydrolysis, at or near room temperature, of an ammonium ester halide (**2**). More vigorous hydrolysis (e.g., refluxing ethanol) was shown to cause Hofmann degradation at the ammonium center. The ammonium esters (**2**) were easily prepared by alkylation of 6-dimethylaminohexanoic esters (**1**) with alkyl bromides or iodides; the isolation of the ammonium ester intermediate (**2**) is unnecessary.

Separation of by-product salt was effected in either of two ways. In one, stoichiometric quantities of an anion exchange resin (in the hydroxide form) were used as the hydrolysis reagent[4]. In the other, an alkali metal hydroxide in an alcohol was used to hydrolyze the ester. The precipitated metal halide was filtered off, and the remainder removed using mixed-bed ion-exchange resin. The yields of isolated products **3** were in the 60–90% range. Based on T.L.C., these yields are governed entirely by losses during isolation, provided the temperature is kept below ~ 45° during the hydrolysis step. The 6-ammoniohexanoate hydrates do not melt reversibly but decompose at temperatures around 150 °C.

The simplicity of the anion-exchange method is it's main advantage. Ion exchange is faster than ester hydrolysis, as evidenced by a sharp rise in pH (followed by a slower decay) during the process. Alcoholic media may be used for the ion exchange, but significantly retard the exchange process[5].

The base hydrolysis of the ammonium esters proceeds smoothly in the lower alcohols, and up to 95% of the metal halide precipitates in a readily filterable form. The metal halide and excess metal hydroxide remaining in solution are effectively removed by stirring with mixed-bed resin in a ~ 1:1 aqueous alcohol medium. The nature of the medium in this step is critical; sufficient water must be present to swell the resin and permit sufficiently fast ion exchange. The level of removal is easily monitored by conductivity, recognizing that a low residual conductivity is to be expected because of the basicity of the ammoniohexanoates[7]. The pH must also be monitored and kept high during mixed-bed resin treatment. If allowed to drop, the product is lost into the resin (presumably by ion exchange as the protonated species). Once lost it is very difficult to recover.

Residual lipophilic impurities (alkyl halide, alcohol, etc.) can be removed by liquid-liquid extraction with lower alkane solvents from the 1:1 water:lower alcohol solvents.

Uncontrollable foaming during distillation or vacuum evaporation precludes the use of these methods for recovery of 6-ammoniohexanoates from water, but azeotropic distillation with acetonitrile is effective for this purpose. Acetonitrile forms an azeotrope rich in water (14.2%) and forms ternary azeotropes with water and lower alcohols as well[8]. The process can be monitored via the boiling point, and when the free water has been removed the 6-ammoniohexanoates actually precipitate from the boiling solvent. This process does not remove lipophilic impurities nor does it remove tightly bound water but it effectively removes loosely bound water and facilitates the formation of crystals, even from hygroscopic homologs. The principle involved is probably widely applicable.

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NOVEL INTERMEDIATES FOR THE SYNTHESIS OF CARBOCYCLIC SPIRO COMPOUNDS

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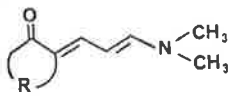
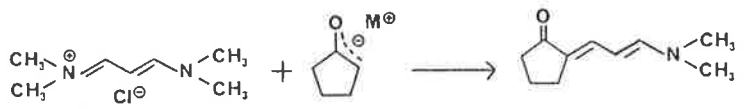
Abstract: Cyclic dienaminones, synthesized from the α-carbon elongation reaction of cyclic ketones with vinamidinium salts, are useful synthetic intermediates to carbocyclic spiro compounds.

The list of naturally occurring carbocyclic spiro compounds continues to grow, and as it does, the need for useful intermediates for their syntheses also grows.[1] This report will show that dienaminones of cyclic ketones 1 may act as synthetic precursors to highly-functionalized spiro compounds via thermal or photochemical [4+2] cycloadditions.



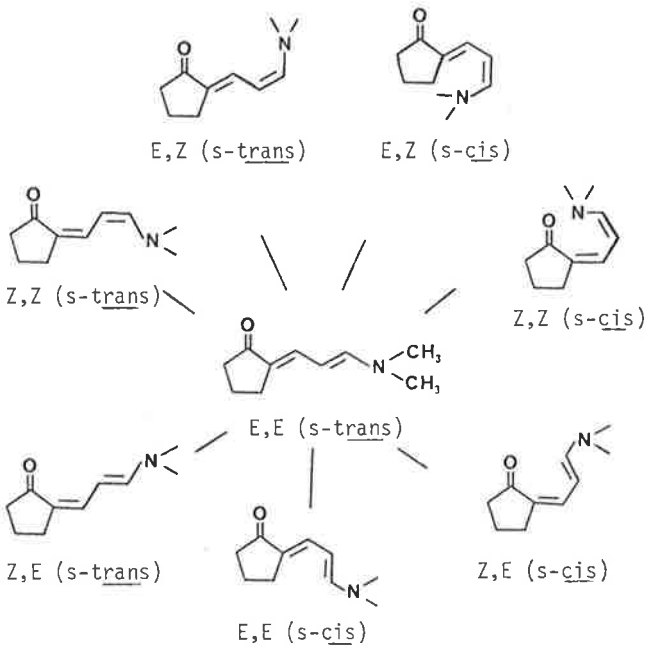
1

The dienaminones were synthesized by the reaction of 1,1,5,5-tetramethyl-1,5-diazapentadienium chloride 2 with a metal enolate. A wide variety of such dienaminones have been synthesized in our Laboratory.[3-5] An example is the reaction of the metal enolate of cyclopentanone with 2 which gave the dienaminone 3 in ~ 90% isolated yield. The conversion is stereospecific and the sole product is the E,E (s-trans) isomer 3 as evidenced by ^{1}H and ^{13}C NMR data. This was found to be true for all dienaminones formed from cyclic ketones.



2 3

Dienaminone 3 is a push-pull heterosubstituted diene.[6-8] In order for the diene moiety to participate as a 4π-component in Diels-Alder reactions, it is necessary for it to acquire a cisoid conformation. There are eight possible stereoisomers for the dienaminone from cyclopentanone, four of which are cisoid (Scheme 1). ^{1}H NMR evidence from the thermal interconversion of dienaminone 3 at 110°C in DMF-d_7 indicates the presence of another stereoisomer

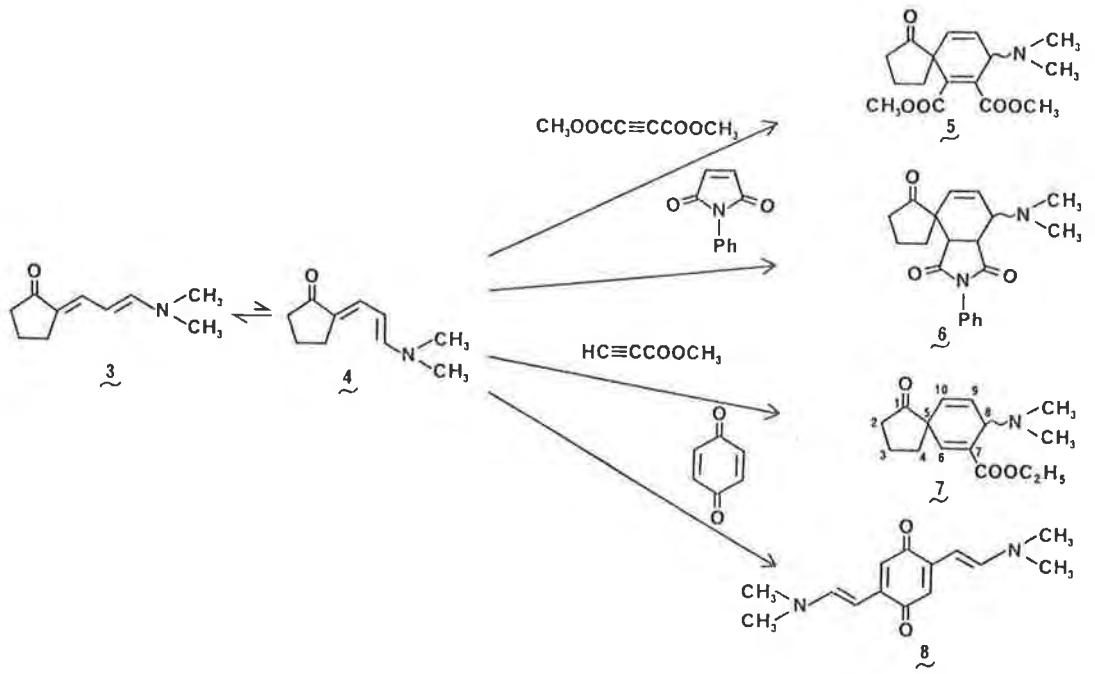


Scheme 1

exhibiting a trans coupling and a cis coupling for the three protons of the diene moiety.[9] This rules out two of the possible isomerization products, i.e. E,Z (s-cis) and Z,Z (s-cis). Of the two possibilities remaining [i.e. Z,E (s-cis) and E,E (s-cis)], differentiation can be made on the basis of the chemical shift of the proton γ to the nitrogen. This proton in the E,E (s-cis) isomer is deshielded by the carbonyl group and, based on related simpler systems,[10,11] is expected to show resonance at ~ δ 7.0 ppm. The same proton in the Z,E (s-cis) isomer is expected to occur at ~ δ 5.8.[10,11] Observation of a doublet at δ 7.0 (J = 2.4 Hz) for this proton suggested the presence of the E,E (s-cis) isomer. The equilibrium favors largely the E,E, (s-trans) isomer although an exact ratio could not be calculated from the ^{1}H NMR spectrum due to the presence of overlapping peaks. This isomerization can also be induced photochemically.

When the dienaminone 3 in toluene was treated with dimethylacetylene dicarboxylate under thermal[12] or photochemical conditions,[13] a 90% conversion to the spiro[4,5]deca-6,9-dien-1-one 5, m.p. 52-53°C, occurred (Scheme 2). The structure of 5 was established by its mass spectrum (307, M^+), its UV spectrum in ethanol [λ_{max} 412 nm (ε 2870), 283 nm (ε 33650)], and its high-field ^{1}H and ^{13}C NMR data including delayed-decoupling experiments. Similarly, formation of the spiro compound 6, m.p. 261-262°C, from the cycloaddition of dienaminone 3 with N-phenylmaleimide was also realized.

The presence of an electron-donating dimethylamino group at one terminus of the dienaminone and an electron-attracting carbonyl group at the other should confer orientational selectivity in its reactions with electron-poor dienophiles. This regiochemistry was established by examination of the thermal cycloaddition with ethyl propiolate. The direction of addition was determined by examination of the coupled and homonuclear decoupled 360 MHz ^1H NMR spectra in $CDCl_3$ of the adduct 7 (25% yield).[14] The NMR data showed H_9 as a doublet of doublets at δ 7.36 ($J_{9,8}$ = 7.7, $J_{9,10}$ = 14.5 Hz), H_8 as a doublet of triplets at δ 7.39 ($J_{8,9}$ = 7.7, $J_{8,10}$ = 2.1, $J_{8,6}$ = 2.1 Hz), and H_6 and H_{10} as an overlapping multiplet. Irradiation of H_8 and H_9 reduced the latter multiplet to a singlet at δ 7.89. The regiospecificity observed in the formation of the spiroadduct 7 is consistent with the polarization in the dienaminone discerned from its ^{13}C NMR data.



Scheme 2

When the dienaminone (3) was treated with p-benzoquinone under reflux in toluene for 12 h, a red crystalline product 8, m.p. 98-100°C, (25% yield) was isolated after preparative layer chromatography and recrystallization from hexane. A plausible mechanism for the formation of this "2:1" adduct involves two sequential nucleophilic attacks of dienaminone on benzoquinone followed in each case by elimination of α-methylenecyclopentanone. The para substitution pattern of the two vinyl dialkylamino moieties in 7 could be deduced unambiguously from the high-field ^{13}C NMR data.

Finally, as dienaminones may be formed from a great many cyclic ketones, the methodology discussed in this paper may be applicable for the synthesis of a variety of carbocyclic spiro compounds.

Acknowledgments. Support of these investigations by a grant (CHE-8200818) from the National Science Foundation is gratefully acknowledged. The high-field NMR spectrometer (Bruker WM-360) used in this work was purchased in part from funds (CHE-8201836) provided by the National Science Foundation.

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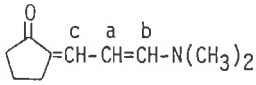
9.  ^{1}H NMR (DMF-d_7) for new isomer: 5.01 (d,d, J = 12.1, 2.4 Hz, H_a); 6.93 (d, J = 12.1 Hz, H_b); 7.00 (d, J = 2.4 Hz, H_c).
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12. The dienaminone (2 mmol) and dienophile (20 mmol) in dry toluene (5 mL) were heated to 110°C under N_2 for 28 h. The reaction mixture was taken to dryness under reduced pressure, and the residue was chromatographed on silica gel plates (ethyl acetate).
13. The photochemical cycloaddition was carried out as described in (12) but in a Rayonet photochemical reactor fitted with 3500 Å lamps for 12 h.
14. This isolated yield is probably much lower than the actual yield in this reaction because of considerable decomposition of the product 7 during chromatographic separation.

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The Thiobarbituric Acid Test for Lipid Peroxidation: Structure of the Adduct with Malondialdehyde

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ABSTRACT

The complete structure of the red crystalline 2:1 adduct from thiobarbituric acid and malondialdehyde has been unambiguously determined by FTIR and high-field ^1H and ^{13}C NMR studies. *Lipids* 19:804-805, 1984.

INTRODUCTION

The thiobarbituric acid (TBA) is a commonly used method for the detection of peroxidation of unsaturated fatty acids and lipids (1,2). It is dependent on the development of a red pigment resulting from the reaction of TBA with oxidized lipids. It originally was suggested by Sinnhuber, et al. (3) that the red compound formed in the TBA test probably was a 2:1 adduct of TBA and malondialdehyde (MDA). However, several types of compounds other than MDA give positive TBA tests (4-7). Also, the presence of metal ions strongly influences the results (8).

Despite these limitations, the TBA test continues to be useful when used judiciously in studies of lipid peroxidation. Although the early proposed structural nature of the adduct of TBA and MDA is still cited, complete establishment of the structure of this red pigment is lacking. We now wish to report on a detailed and unambiguous assignment of the structure of this adduct.

EXPERIMENTAL PROCEDURES

The adduct of TBA and MDA was prepared and purified as described previously (3), except that pure sodium malondialdehyde (9) instead of 1,1,3,3-tetraethoxypropane was used in the preparation. The dark red needles that formed melted above 350 C. The high-field ^1H and ^{13}C NMR data, including delayed decoupling experiments, were determined on a Bruker WM-360 pulse Fourier transform instrument. The UV-visible spectra were recorded on a Varian-Cary Model 219 spectrophotometer. Fourier transform IR measurements were made on an IBM Model 98 instrument.

RESULTS AND DISCUSSION

The crystalline TBA-MDA adduct correctly analyzed for $C_{11}H_8N_4O_4S_2$. Its UV-visible spectrum in H_2O showed absorptions at λ_{max} 532 nm (ϵ = 159,200), 305 nm (ϵ = 11,250), and 243 nm (ϵ = 23,000), indicative of a highly conjugated system. The FTIR spectrum (KBr) was very diagnostic and exhibited bands characteristic of OH and NH stretching (broad peaks at 3490, 3200 cm^{-1}), amide carbonyl stretching (1633, 1670(sh) cm^{-1}), C-N vibration of S ‖ -C-NH- (1494 cm^{-1}), OH bending (1361 cm^{-1}), C-O stretching (1210, 1177 cm^{-1}), and thiomide C=S stretching (1127 cm^{-1}). The S-H stretching vibration which normally occurs at about 2500 cm^{-1} was not observed.

The high-field 360 MHz ^1H NMR spectrum of the adduct in DMSO-d_6 showed only 4 types of resonances. There were 5 exchangeable hydrogens, 3 appearing as a broad peak ($W_{1/2}$ = 41.7 Hz) at δ 6.11 and 2 appearing as a broad peak ($W_{1/2}$ = 10.8 Hz) at δ 11.52. The presence of vinyl protons of the MDA derived component easily could be discerned as a doublet (J = 13.9 Hz) integrating for 2 hydrogens and a triplet (J = 13.9 Hz) integrating for 1 hydrogen. No -SH resonance was observed (Table 1). The 90.56 MHz ^{13}C NMR data in DMSO-d_6 suggested that the adduct had considerable symmetry within its structure. The 11 carbons of the molecule showed only 5 resonances. Delayed decoupled high-field ^{13}C NMR experiments revealed the presence in the adduct of 3 different quaternary carbons and 2 types of tertiary carbons. A broadened peak at δ 161.9 was interpreted as being due to the presence of C=S of a thioamide. The carbons of the MDA moiety were assigned with the aid of the delayed-decoupling data to resonances at δ 117.5 and δ 157.4. The absorption at δ 176.3 was assigned as being due to the presence of amide carbons and that at δ 101.3 to the remaining two equivalent ring carbons (Table 2). The assignments are consistent with those expected on the basis of electronic, tautomeric

and multiplicity considerations, and also on the basis of comparisons with ^{13}C NMR spectra of some compounds with related moieties (10-12).

In conclusion, the combined spectroscopic data are totally consistent with two spectrally equivalent tautomeric structures 1 and 2. It should be mentioned, however, that variation in concentration of solution and the presence of trace contaminants may cause prototropic shifts to favor equilibrating structures similar to 1 and 2 but bearing 3 hydroxyl and 2 amide hydrogens. Formation of the 2:1 adduct of TBA and MDA probably is initiated by nucleophilic attack involving carbon-5 of TBA onto carbon-1 of MDA followed by dehydration and similar subsequent reaction of the intermediate 1:1 adduct with a second molecule of TBA.

TABLE 1

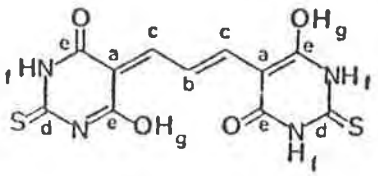
360 MHz ^1H NMR Data (in DMSO-d_6)

δ (ppm), TMS	Assignment
6.11 (s, br, 3H) (variable)	f
7.72 (d, J = 13.9 Hz, 2H)	c
8.56 (t, J = 13.9 Hz, 1H)	b
11.52 (s, br, 2H)	g

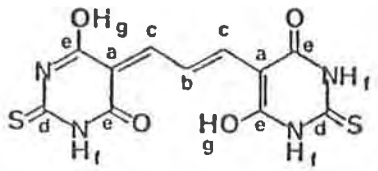
TABLE 2

90.56 MHz ^{13}C NMR Data (in DMSO-d_6)

δ (ppm), TMS	Delayed Decoupling	Assignment
101.3	C	a
117.5	CH	b
157.4	CH	c
161.9	C	d
176.3	C	e



STRUCTURE 1



STRUCTURE 2

ACKNOWLEDGMENTS

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A New Synthesis of Isoguanosine

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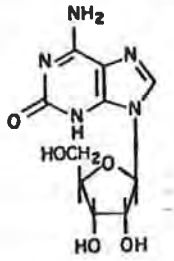
Isoguanosine (1) (crotonoside or 2-hydroxyadenosine) is one of only a few naturally occurring nucleoside analogues of guanosine.[1] It was first isolated from *Croton*



1

tiglium L. by Cherbuliez and Bernhard.[2] More recently, Pettit and his co-workers isolated isoguanine from butterfly wings of *Prioneris thestylis*.[3] Isoguanosine is incorporated in mammalian but not bacterial nucleic acids.[4,5] It stim-

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(2) Cherbuliez, E.; Bernhard, K. *Helv. Chim. Acta* **1932**, *15*, 464, 978.
(3) Pettit, G. R.; Ode, R. H.; Coomes, R. M.; Ode, S. L. *Lloydia* **1976**, *39*, 363.

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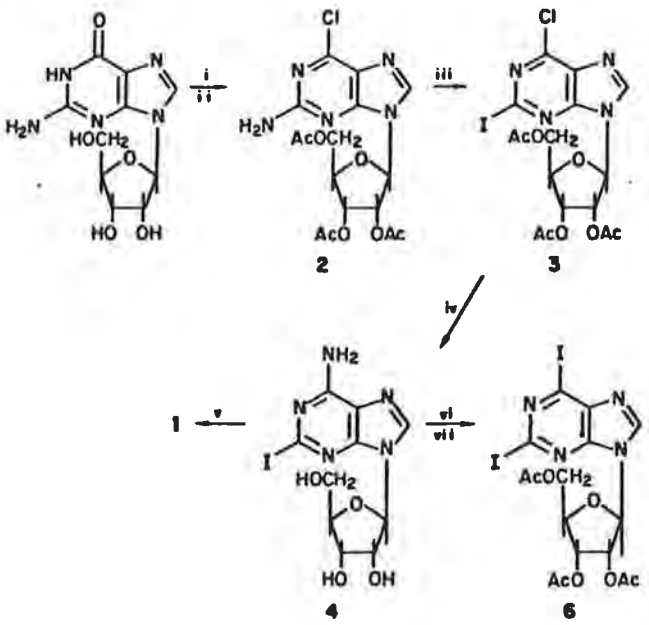
Scheme I[a]



[a] (i) Acetic anhydride, DMF, pyridine; (ii) $POCl_3$, *N,N*-dimethylaniline, Δ; (iii) *n*-$C_5H_{11}ONO$, CH_2I_2, Δ; (iv) NH_3, C_2H_5OH; (v) H_2O, *hν*; (vi) acetic anhydride, pyridine; (vii) *n*-$C_5H_{11}ONO$, CH_2I_2, Δ.

ulates the accumulation of cyclic AMP in the brain.[6] It is an inhibitor of IMP:pyrophosphorylase.[7] Isoguanosine 5′-di- and 5′-triphosphates bind strongly and inhibit glutamic acid dehydrogenase.[8]

The synthesis of isoguanosine was initially achieved by the selective deamination of 2,6-diamino-9β-(D-ribofuranosyl)purine with nitrous acid.[9] However, the overall yield from 2,6-diaminopurine was low and the procedure used undesirable heavy metal salts (e.g., Hg, Pb) in two of the steps. Isoguanosine has also been prepared in low yields from a 4,5-dicyanoimidazole nucleoside precursor.[10] In the synthesis of 2-fluoroadenosine from 2,6-diaminopurine nucleoside, isoguanosine was reported as a side product.[11] A photochemical preparation of isoguanosine from adenosine *N*′-oxide has been reported,[12] but this procedure gives variable results. We report a new, reproducible, and efficient synthesis of isoguanosine.

Guanosine served as the starting point for this synthesis. It was converted first to 2-amino-6-chloro-9β-(2,3,5-tri-*O*-acetyl-D-ribofuranosyl)purine (2) by selective acetylation followed by reaction with phosphorus oxychloride and *N,N*-dimethylaniline[13] (Scheme I). Treatment of 2 with *n*-pentyl nitrite and diiodomethane at 80 °C for 2 h gave pure protected 2-iodo-6-chloropurine nucleoside (3) in 83% yield (66% overall yield for three steps from guanosine).[14-16] When 3 was allowed to react with ethanolic ammonia at ice-bath temperatures, 2-iodoadenosine (4) was produced in 93% isolated yield. The ease of displacement of the 6-chloro group in compound 3 by ammonia is in sharp contrast to the high temperatures and pressures or very long reaction times required for similar nucleophilic substitution of 6-chloropurines.[17,18] Although 2-iodoadenosine has been cited previously, its method of synthesis and physical and spectral data have not been reported.[19] The key step in the conversion of 4 to 1 is an interesting photoinduced hydration reaction. Photolysis of 4 in water was carried out in a Rayonet photochemical reactor with UV irradiation from mercury lamps with the principal wavelength of 2537 Å. The isoguanosine formed was isolated by reverse-phase HPLC on Amberlite XAD-4 resin and crystallized from water to give a 55% yield of pure product.

An interesting sidelight of this work was the synthesis of the novel nucleoside 2,6-diiodonebularine (6) from 2-iodoadenosine (4) through a halogenative deamination reaction. This compound (mp 160–162 °C) was characterized by its mass spectrum [*m/z* 630 (M^+)], its UV spectrum in ethanol [λ_{max} 290 (ε 8.17 × 10^3), 252 (ε 8.76 × 10^3), 226 (ε 1.70 × 10^4) nm], and its high-field ^1H and ^{13}C NMR data. Further synthetic utilization of the iodinated nucleosides described here are currently under investigation in our laboratory.

Experimental Section

Melting points are uncorrected. Preparative-layer chromatography employed EM silica gel PF_{254} plates activated for 3 h at 135 °C.

2-Amino-6-chloro-9β-(2,3,5-tri-*O*-acetyl-D-ribofuranosyl)purine (2) was prepared from guanosine in 75% yield by established literature procedures.[13]

2-Iodo-6-chloro-9β-(2,3,5-tri-*O*-acetyl-D-ribofuranosyl)purine (3) was prepared in 83% yield by treatment of 2 thermally with *n*-pentyl nitrite and diiodomethane by using a procedure previously described by us.[14]

2-Iodoadenosine (4). To 125 mL of dry ethanol saturated with ammonia gas at ice–salt bath temperatures was added 0.401 g (0.744 mmol) of 3. The solution was stirred at this temperature for 1 h and then at 25 °C for 23 h. The solvent was removed under reduced pressure and the residue was purified by reverse-phase HPLC on Amberlite XAD-4 resin using 75:25 H_2O:ethanol as the eluting solvent. 2-Iodoadenosine (4) crystallized from H_2O as white crystals (0.272 g, 0.692 mmol, 93%): mp 142–144 °C; ^{13}C NMR (D_2O, pH 4) δ 61.1, 70.1, 73.5, 85.4, 88.6, 116.4, 117.8, 140.4, 147.7, 147.8; ^1H NMR (Me_2SO-d_6) δ 3.65 (m, 2 H), 3.92 (m, 1 H), 4.07, (m, 1 H), 4.56 (m, 1 H), 5.62 (d, 1 H, *J* = 6.4 Hz), 7.45 (br s, 2 H), 7.89 (s, 1 H); UV (H_2O) λ_{max} 264.5 nm (ε 1.31 × 10^4); mass spectrum, *m/z* (relative intensity) 393 (M^+, 0.2), 262 (6.4), 261 (Pur^+ + H, 33.3), 135 (18.4), 134 [(Pur^+ − I) + H, sugar + H, 100.0].

Isoguanosine (1). A solution of 0.056 g (0.142 mmol) of 4 in 75 mL of water was placed in a quartz reaction vessel and photolyzed for 7.5 h with a Rayonet photochemical reactor using light with the principal wavelength of 2537 Å. The solvent was then removed under reduced pressure, and the residue was purified by reverse-phase HPLC on a column of Amberlite XAD-4 using 90:10 H_2O:ethanol as the solvent. The separated product was lyophilized and the residue crystallized from H_2O to give 0.022 g (0.078 mmol, 55%) of 1 as white crystals: mp 237–241 °C (lit.[12] mp 237–241 °C); ^{13}C NMR (D_2O, pH 4) δ 60.6, 70.5, 73.7, 85.9, 89.5, 110.5, 139.0, 141.6, 148.7, 152.1; ^1H NMR (Me_2SO-d_6) δ 3.94 (m, 2 H), 4.12 (m, 1 H), 4.52 (m, 1 H), 5.19 (m, 1 H), 5.46 (br s, 2 H), 5.81 (d, 1 H, *J* = 6.0 Hz), 8.38 (s, 1 H); UV (H_2O) λ_{max} 292 nm (ε 1.10 × 10^4), 248 (ε 9.02 × 10^3).

2-Iodo-6-amino-9β-(2,3,5-tri-*O*-acetyl-D-ribofuranosyl)purine (5). A mixture of 25 mL of pyridine and 20 mL of acetic anhydride was cooled to ice-bath temperatures and treated with 0.470 g (1.200 mmol) of 4. The solution was stirred at ice bath

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temperatures for 1 h and at 25 °C for 2 h. The solvent was removed under reduced pressure followed by coevaporation (4×) with 95% ethanol. The residue was purified by using silica gel chromatography with 9:1 chloroform:methanol as the developing solvent. The band at R_f 0.65 upon elution yielded 0.522 g (1.01 mmol, 84%) of **5** as off-white crystals: mp 78–80 °C; ^{13}C NMR ($CDCl_3$) δ 20.5, 20.6, 20.9, 63.1, 70.6, 73.4, 80.5, 86.1, 119.7, 120.1, 138.33, 149.9, 155.4, 169.4, 169.5, 170.3; ^{1}H NMR ($CDCl_3$) δ 2.10 (s, 3 H), 2.13 (s, 3 H), 2.16 (s, 3 H), 4.41 (m, 3 H), 5.30 (t, 1 H), 5.79 (t, 1 H), 6.13 (d, 1 H), 6.40 (br s, 2 H), 7.87 (s, 1 H); UV (EtOH) λ_{max} 222 nm (ϵ 1.97 × 10^4), 264.5 (ϵ 1.32 × 10^4); mass spectrum, m/z (relative intensity) 519 (M^+, 2.1), 262 (15.3), 261 (4.6), 260 (Pur^+, 4.3), 259 ($sugar^+$, 30.5), 157 (11.8), 139 (100), 135 (6.4), 134 (12.8), 133 ($Pur^+ - I$, 1.4).

2,6-Diiodo-9β-(2,3,5-tri-*O*-acetyl-D-ribofuranosyl)purine (6). A mixture of 0.320 g (0.616 mmol) of **5**, 5.4 mL (40 mmol) of *n*-pentyl nitrite, and 16 mL of diiodomethane was protected from moisture and stirred for 7 h and 80 °C. The solvent was then removed under reduced pressure and the residue was chromatographed on silica gel plates. After elution with 20:1 chloroform:methanol, the band at R_f 0.68 afforded 0.198 g (0.314 mmol, 51%) of **6** as light yellow crystals: mp 160–162 °C; ^{13}C NMR ($CDCl_3$) δ 20.4, 20.5, 20.8, 62.9, 70.6, 73.3, 80.8, 86.6, 117.1, 122.2, 139.3, 142.5, 148.2, 169.3, 169.5, 170.1; ^{1}H NMR ($CDCl_3$) δ 2.10 (s, 3 H), 2.14 (s, 3 H), 2.17 (s, 3 H), 4.42 (m, 3 H), 5.60 (t, 1 H), 5.80 (t, 1 H), 6.19 (d, 1 H), 8.24 (s, 1 H); UV (EtOH) λ_{max} 290 nm (ϵ 8.17 × 10^3), 252 (ϵ 8.76 × 10^3), 226 (ϵ 1.70 × 10^4); mass spectrum, m/z (relative intensity) 630 (M^+, 0.8), 415 (4.4), 401 (1.2), 373 (12.4), 372 ($Pur^+ + H$, 1.3), 259 ($sugar^+$, 40.4), 246 (1.4), 245 [($Pur^+ - I$) + H, 4.1], 157 (12.6), 139 (100.0).

Acknowledgment is made to the NSF for support of our investigations.

Registry No. **1**, 1818-71-9; **2**, 16321-99-6; **3**, 5987-76-8; **4**, 35109-88-7; **5**, 94042-04-3; **6**, 94042-05-4; guanosine, 118-00-3.

Novel Photoinduced Carbon–Carbon Bond Formation in Purines[1]

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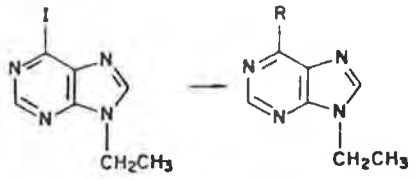
Recently much attention has been focused on C-alkylated purines.[2-8] The reported antitumor activity of these compounds and the limited synthetic methodology available to attain them prompted us to consider alternate synthetic approaches to this class of compounds. This paper reports on the successful development of a new synthetically useful method of carbon–carbon bond formation in purines through a photochemical $S_{RN}1$ reaction

(1) Presented at the 188th National Meeting of the American Chemical Society, Philadelphia, PA, Aug 1984.
(2) Yamane, A.; Matsuda, A.; Ueda, T. *Chem. Pharm. Bull.* **1980**, *28*, 150.
(3) Bergstrom, D. E.; Reddy, P. A. *Tetrahedron Lett.* **1982**, *23*, 4191.
(4) Leonard, N. J.; Bryant, J. D. *J. Org. Chem.* **1979**, *44*, 4612.
(5) Taylor, E. C.; Martin, S. F. *J. Am. Chem. Soc.* **1974**, *96*, 8095.
(6) Yamane, A.; Inoue, H.; Ueda, T. *Chem. Pharm. Bull.* **1980**, *28*, 157.
(7) McGarrity, G. J.; Carson, D. A. *Exp. Cell Res.* **1982**, *139*, 199.
(8) Nair, V.; Richardson, S. G.; Coffman, R. E. *J. Org. Chem.* **1982**, *47*, 4520.

Table I. Products and Yields for the $S_{RN}1$ Reaction of Halopurines[13]



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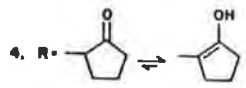
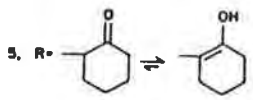
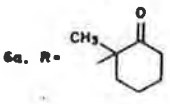
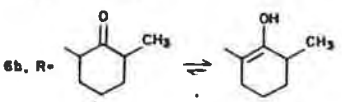
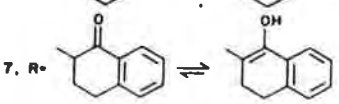
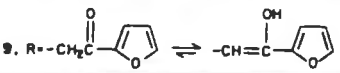
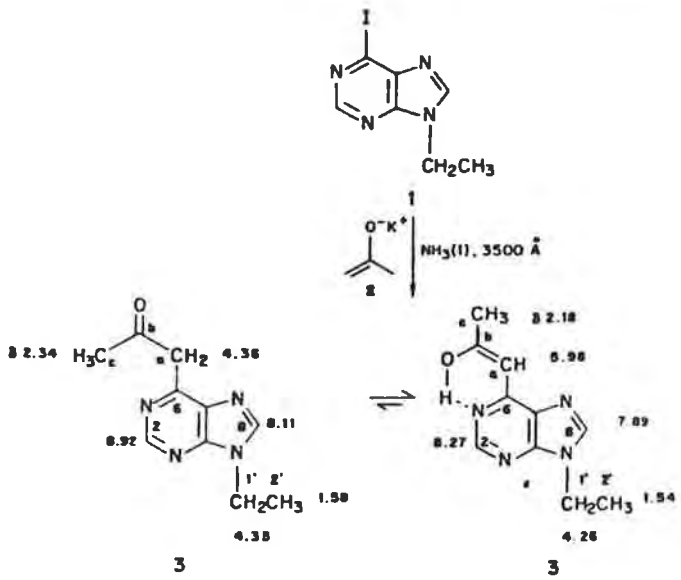
reactn of 1 with	prod(s)	% purified yield	keto:enol (%) in $CDCl_3$ (25 °C)	mp, °C
acetone	3, R = $-CH_2-C(=O)CH_3 \rightleftharpoons -CH=C(OH)CH_3$	70	20:80	148–149
acetone and DNB (quenching expt)	3	6		
cyclopentanone		65	20:80	162–164
cyclohexanone		50	25:75	132–134
2-methylcyclohexanone		30	100:0	76–78
		7	~50:50	
α-tetralone		80	15:85	191–193
acetophenone	8, R = $-CH_2C(=O)Ph \rightleftharpoons -CH=C(OH)Ph$	70	5:95	153–154
2-acetylfuran		67	15:85	146–148

(substitution, radical, nucleophilic, unimolecular).[9,10] The synthetic approach discussed has wide applicability. In addition, the products of these photoinduced reactions have remarkable versatility in terms of conversion to other biologically interesting purine systems.

When the potassium enolate of acetone **2** was photolyzed in a Rayonet photochemical reactor (3500 Å) in the presence of 6-iodo-9-ethylpurine (**1**)[11] in anhydrous liquid ammonia for $^1/_2$ h, 6-acetonyl-9-ethylpurine (**3**), mp 148–149 °C, was isolated in 70% yield after separation on preparative silica gel plates (Scheme I). The product was identified by its mass spectrum (*m/z*, 204, M^+), by its UV data in ethanol [λ_{max} 362 (ε 23 300), 345 (ε 18 450), 330 sh (ε 13 650), 266 nm (ε 3600)], by its high-field 360-MHz ^1H (Scheme I) and 90.6 MHz ^{13}C (ref 12) NMR data in $CDCl_3$, and by its FTIR data. The data were also consistent with a keto–enol equilibrium (in $CDCl_3$) with preponderance of the enol isomer probably because of added stabilization due to increased conjugation and hydrogen bonding (Table I). The keto and enol forms could be discerned, not only by the marked difference in the chemical shifts of H_a but also from the downfield shift of H_2 observed in each case for the keto form (Scheme I). Further support for the existence of these two forms comes from the expected direction of shift in the keto–enol equilibrium observed with variation in solvent and temperature. At 25 °C, the keto:enol ratio in $CDCl_3$ for **3** is 20:80 but in D_2O this ratio is

Scheme I



3 3

close to 50:50. Variable-temperature ^1H NMR data of **3** in Me_2SO-d_6 show an increase in the keto form from 18% at 15 °C to 46% at 100 °C.

Support for the $S_{RN}1$ mechanism came from several observations. The short reaction time and the mild reaction conditions are not consistent with a simple displacement reaction of a 6-halopurine. Also, when the photolysis was carried out in the presence of a known radical anion inhibitor (e.g., *p*-dinitrobenzene),[14] the yield of the reaction dropped to about 6%. In

(9) Kim, J. K.; Bunnett, J. F. *J. Am. Chem. Soc.* **1970**, *92*, 7463, 7464
(10) Bunnett, J. F. *Acc. Chem. Res.* **1978**, *11*, 413 and references therein.
(11) Nair, V.; Richardson, S. G. *J. Org. Chem.* **1980** *45*, 3969
(12) ^{13}C NMR ($CDCl_3$, K = keto, E = enol) δ 15.3 ($C_{2'}$, K), 15.6 ($C_{2'}$, E), 26.3 (C_c, E), 30.4 (C_c, K), 38.9 ($C_{1'}$, E), 39.0 ($C_{1'}$, K), 48.1 (C_a, K), 88.6 (C_a, E), 124.8 (C_4, E), 133 4 (C_4, K), 140.7, 144.1, 145.5, 146.5, 151.3, 152.3 (C_2, C_4, C_8), 151.0 (C_6, K), 154.8 (C_6, E), 185.9 (C_b, E), 203.2 (C_b, K)
(13) Reaction conditions: KO-*t*-Bu, NH_3(l), *hν* (3500 Å), $^1/_2$ h, −33 °C. Reaction rates and yields for 6-halogenated purines were in the order 6-I > 6-Br > 6-Cl

(14) Hoffman, A. K.; Feldman, A. M.; Gelblum, E.; Hodgson, W. G. *J. Am. Chem. Soc.* **1964**, *86*, 639

addition, the iodopurine is capable of a slow, dark substitution reaction[15] of low yield (22%) which apparently is of the $S_{RN}1$ type as evidenced by radical anion inhibition.

We have extended these investigations to a variety of other ketone enolates (Table I). For example, cyclopentanone enolate reacts with **1** to give crystalline 6-(2-cyclopentanoyl)-9-ethylpurine (**4**) (65% yield) which exists largely (80%) in the enolic form. Cyclohexanone behaves similarly. When 2-methylcyclohexanone was treated with **1** under the same conditions, both the thermodynamic (major) and kinetic (minor) products **6a** and **6b** were formed. The thermodynamic product **6a** exists exclusively in the keto form as evidenced by ^1H and ^{13}C NMR and FTIR data. The lower yield of the products in this case results apparently from a significant (30%) competing side reaction, i.e., formation of 9-ethylpurine through hydrogen abstraction. Photolysis of the enolate of α-tetralone with **1** gave an excellent yield of the aralicylic substituted product **7**. The aralkyl ketone acetophenone also underwent a smooth photochemical $S_{RN}1$ reaction with the iodopurine **1**. The conversion product **8** exists almost exclusively in the enol form. We have discovered that purines can be modified at the 6-position with acylated heteroaromatic systems. Of particular interest to us was the furan derivative **9** because of the close structural resemblance to plant growth regulators called cytokinins.[16] We are currently extending this methodology to the synthesis of some biologically active highly functionalized nucleosides.

Acknowledgment. We thank the American Cancer Society for partial support of this research. The high-field NMR spectrometer (Bruker WM-360) and the FTIR instrument (IBM Model 98) used in this work were purchased in part from funds (CHE-8201836, CHE-8310536) provided by the National Science Foundation.

Supplementary Material Available: NMR (^1H and ^{13}C), UV, and mass spectral data for all adducts (8 pages). Ordering information is given on any current masthead page.

Novel Photoinduced Functionalized C-Alkylations in Purine Systems[1]

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Received April 16, 1985

The photoinduced reactions of metal enolates with 6-halopurine derivatives result in the formation, in high yields, of a variety of novel functionalized 6-alkylated purines that exist preferentially in the hydrogen-bonded enolic form in nonpolar solvents. High-field ^1H and ^{13}C NMR data provide unambiguous support for the structures proposed. An $S_{RN}1$ mechanism is implicated in these photochemical transformations. The synthetic versatility of the photoproducts is illustrated by their conversion to other functionalized 6-substituted purines such as those bearing alkenyl, epoxy, and diol groups. Extension of the photochemical functionalized C-alkylation to purine nucleosides is described.

Carbon–carbon bond forming reactions are potentially very effective approaches to the synthesis of a wide variety of interesting functionalized nucleosides. However, this methodology has been of limited synthetic utility in the chemistry of purine nucleosides. For example, direct displacement of leaving groups such as methyl sulfone and halide by nucleophiles is limited to nucleophiles derived from carbons bearing one or two strong electron-withdrawing groups.[2] Purines can be converted to nucleophiles by a metal–halogen exchange reaction, and the resulting intermediate may participate in alkylation reactions.[3] However, these reactions must be conducted at very low temperatues (−130 °C) to avoid nucleophilic attack by the butyllithium in the first step and to minimize the relocation of the carbanion to the C-8 position in the lithiopurine intermediate. Although metal-catalyzed reactions have been used to synthesize C-6-alkylated purines, these re-

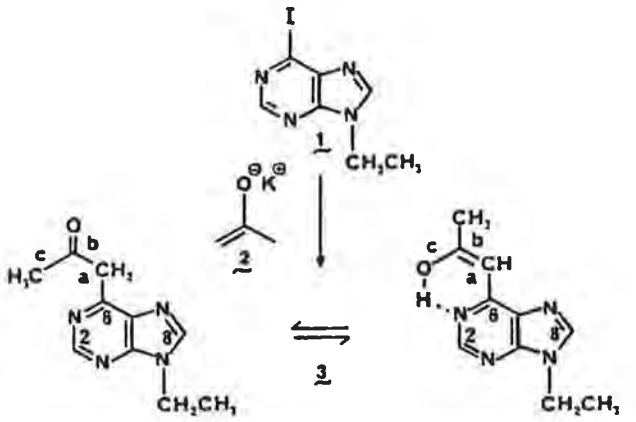
(1) Presented in part at the 188th National Meeting of the American Chemical Society, Philadelphia, PA, Aug 1984.

(2) Yamane, A.; Matsuda, A.; Ueda, T. *Chem. Pharm. Bull.* 1980, *28*, 150.

(3) Leonard, N. J.; Bryant, J. D. *J. Org. Chem.* 1979, *44*, 4612.

0022-3263/85/1950-5069$01.50/0

Scheme I



actions have been restricted to some nonfunctionalized alkylpurines.[4]

The Eschenmoser sulfide contraction has been used for C-6 alkylation of purine nucleosides.[5] However, this procedure gives variable results for functionalized C-alkylation.[6] The ylide reaction of 6-chloropurines has been used for the synthesis of some aralkenylpurines,[7] although the application to nucleoside synthesis remains largely unexplored.

Recognition of the potential biological activity of C-6-alkylated purine nucleosides is slowly emerging. The reported antitumor activity of some of these compounds,[6,8-13] and the limitations in generalized synthetic methodology available to attain them, prompted us to consider alternate synthetic approaches to this class of compounds. This paper[14] reports on the successful development of a new, synthetically useful method of carbon–carbon bond formation in purines through a photochemical $S_{RN}1$ reaction (substitution, radical, nucleophilic, unimolecular).[15,16] The synthetic approach has wide applicability. In addition, the products of these photoinduced reactions have remarkable versatility in terms of conversion to other biologically interesting purine systems, and this is exemplified.

Results and Discussion

Photochemically generated transient purinyl radicals or their equivalent provide an excellent approach to a variety of specific arylated or heteroarylated purines.[17,18] However, the approach used in the aforementioned studies of arylations by us is not directly applicable to the synthesis of functionalized C-alkylated purine nucleosides. It was envisaged that functionalized carbon–carbon bond formation could be possible through a reaction involving an excited metal enolate and a 9-substituted 6-iodopurine. Thus, when the potassium enolate of acetone was photolyzed in an ammonia solution in the presence of 6-iodo-9-ethylpurine (1) in a Rayonet photochemical reactor for $^1/_2$ h, 6-acetonyl-9-ethylpurine (3) was isolated in 70% yield after chromatographic separation (Scheme I). Its UV data in ethanol showed a bathochromically shifted spectrum [λ_{max} 362 nm (ϵ 23 300), 345 (18 450), 330 (13 650), 266 (3600)] compared to the starting material [λ_{max} 276.5 nm (ϵ 12 000)]. This was taken as evidence that the acetonyl product could exist in the enol form. The 360-MHz ^{1}H NMR spectrum (in $CDCl_3$) at 25 °C provided substantiating evidence for a keto–enol equilibrium with preponderance of the enol isomer probably because of added stabilization from increased conjugation and hydrogen bonding. The keto and enol forms could be discerned, not only by the marked difference in the chemical shifts of H_a but also from the downfield shift of H_2 in each case for the keto form. Solvent and variable-temperature ^{1}H NMR studies showed the expected direction of shift in the keto–enol equilibrium. The keto to enol ratio in $CDCl_3$ for 3 at 25 °C is 20:80, but in D_2O this ratio is close to 50:50. Variable-temperature ^{1}H NMR data of 3 in Me_2SO-d_6 shows an increase in the keto form from 18% at 15 °C to 46% at 100 °C. Further support for the structures for 3 came from the 90.6-MHz ^{13}C NMR data in $CDCl_3$ that showed two resonances for each of the carbons in 3. As expected, there was a major difference in the chemical shift for C_a in the keto form (48.1 ppm) compared to the enolic form (88.6 ppm). The carbonyl carbon appeared at δ 203.2, and this carbon in the enolic form was at δ 185.9. The mass spectrum showed a parent ion peak at m/z 204 and major fragmentation peaks at m/z 189 (loss of methyl) and 162 (loss of CH_3CHO).

The acetonylation of 1 probably occurs via an $S_{RN}1$ (substitution, radical, nucleophilic, unimolecular) mechanism.[19] Support for the $S_{RN}1$ mechanism came from several observations. Although the molecularity of this reaction is identical with that of an S_NAr reaction, if a photostimulative effect can be demonstrated along with an inhibitory effect by a radical scavenger, an $S_{RN}1$ mechanism is implied. When 6-iodo-9-ethylpurine (1) was treated with acetone enolate anion in the dark for $^1/_2$ h, the yield of the reaction dropped to 22%. Longer reaction times resulted in higher yields of 3 but with an abundance of side products. When the photostimulated reaction was carried out in the presence of *p*-dinitrobenzene (DNB),[20] product 3 was isolated in 6% yield while 65% of the starting material was recovered. Additional support for the involvement of an $S_{RN}1$ mechanism can be inferred from the mild conditions and the very short reaction times necessary for our reactions. The normal nucleophilic displacement of groups from the 6-position of purines requires elevated temperatures and extended reaction times.[2] It should also be mentioned that the much slower dark reaction of acetone enolate with 6-iodo-9-ethylpurine (1) in liquid ammonia appears to be of the $S_{RN}1$ type as evidenced by the almost complete quenching of this reaction by DNB. The mechanistic implication is that, in

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(5) Vorbrüggen, H.; Krolikiewicz, K. *Angew. Chem., Int. Ed. Engl.* 1976, *15*, 689.
(6) Yamane, A.; Inoue, H.; Ueda, T. *Chem. Pharm. Bull.* 1980, *28*, 167.
(7) Taylor, E. C.; Martin, S. F. *J. Am. Chem. Soc.* 1974, *96*, 8095.
(8) Miyasaka, T.; Suemune, H. Yamasa Shoyu Co., Ltd. *Chem. Abstr.* 1979, *92*, P198713.
(9) Miyasaka, T.; Sue, M. Yamasa Shoyu, Co., Ltd. *Chem. Abstr.* 1979, *92*, P198714.
(10) Yamasa Shoyu Co., Ltd. *Chem. Abstr.* 1983, *99*, P122839.
(11) Yamasa Shoyu Co., Ltd. *Chem. Abstr.* 1983, *99*, P140311.
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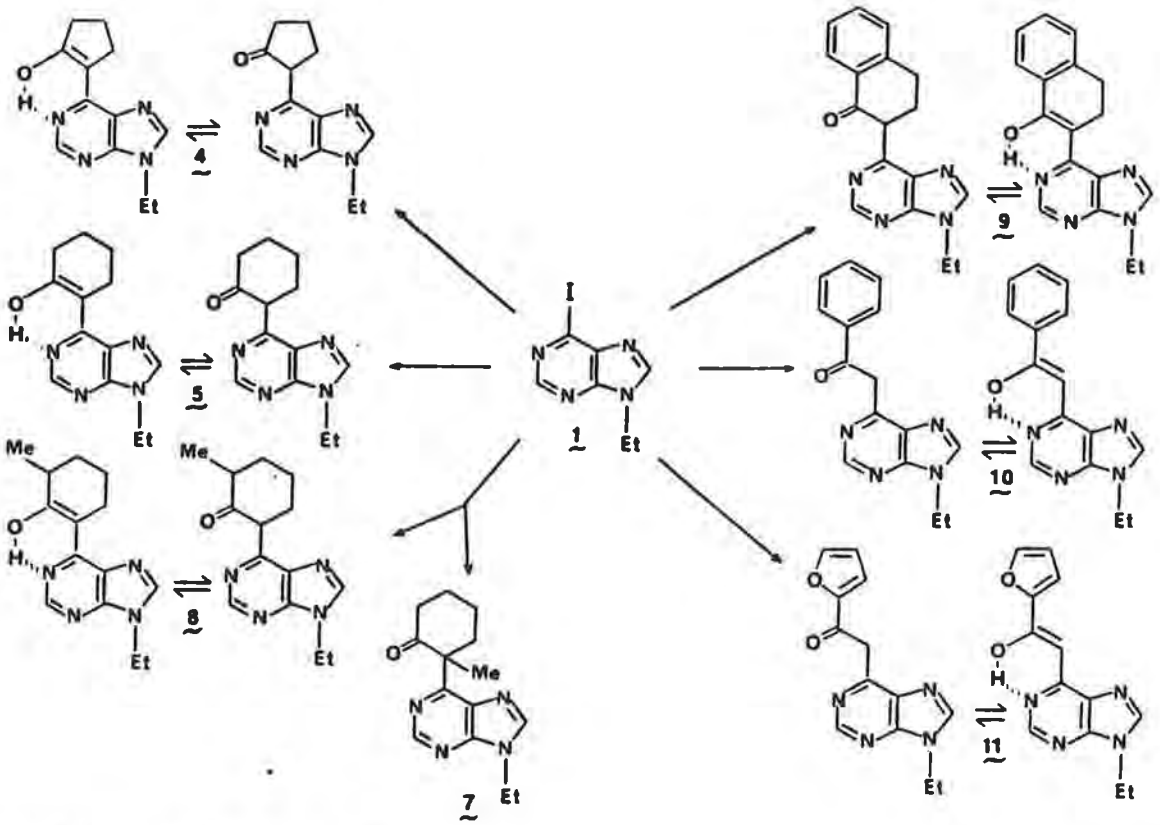
(13) Ueda, M.; Inoue, H.; Akira, Y. Yamasa Shoyu Co., Ltd. *Chem. Abstr.* 1979, *91*, P91929.
(14) A preliminary account of a part of this work appeared as a communication in: *J. Am. Chem. Soc.* 1985, *107*, 2183.
(15) Kim, J. K.; Bunnett, J. F. *J. Am. Chem. Soc.* 1970, *92*, 7463, 7464.
(16) Bunnett, J. F. *Acc. Chem. Res.* 1978, *11*, 413 and references herein.
(17) Nair, V.; Richardson, S. G.; Coffman, R. E. *J. Org. Chem.* 1982, *47*, 4520.
(18) Nair, V.; Young, D. A. *J. Org. Chem.* 1984, *49*, 4340.
(19) Rossi, R. A.; de Rossi, R. H. In "Aromatic Substitution by the $S_{RN}1$ Mechanism"; American Chemical Society: Washington, DC, 1983; ACS Monogr. No. 178.
(20) Hoffman, A. K.; Feldman, A. M.; Gelblum, E.; Hodgson, W. G. *J. Am. Chem. Soc.* 1964, *86*, 639.

Scheme II



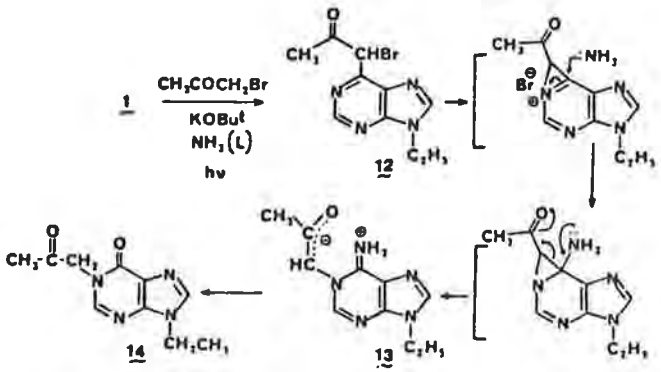
the photostimulated reaction, although photoinduced carbon–iodine bond homolysis may be possible, a much more facile redox initiation pathway may be preferred as the entry point to the $S_{RN}1$ mechanism. It is this redox initiation step that is perhaps stimulated by light,[21] resulting in the highly efficient transformation observed.

We have extended these studies to include other ketone enolates. For example, the photoinduced reaction of the enolate of cyclopentanone with **1** results in the formation of 6-cyclopentanon-2-yl-9-ethylpurine (**4**), a light yellow solid, in 65% yield. The high-field ^1H NMR data in $CDCl_3$ at 25 °C again showed the existence of a keto–enol equilibrium, with the enol form predominating (90%). Using the enolate of cyclohexanone, 6-cyclohexanon-2-yl-9-ethylpurine (**5**) was synthesized in 48% yield as a light yellow solid (Scheme II).

The reaction of an unsymmetrical ketone, 2-methylcyclohexanone, capable of forming a kinetic or thermodynamic enolate anion was also examined. The thermodynamic product, 6-(2-methylcyclohexanon-2-yl)-9-ethylpurine (**7**), was formed in a 30% yield. Compound **7** exists entirely in the keto form as it is not readily enolized. This was confirmed by high-field NMR and UV data. The UV absorption maximum at λ_{max} 266 nm (ethanol) is expected for a nonconjugated C-6-alkylated purine. The kinetic product of this reaction, **8**, was obtained in 7% yield and existed in both the keto and enol forms (Scheme II). The relatively low yield of products in this case is the result of a competing side reaction, i.e. the formation of 9-ethylpurine (**6**) through hydrogen abstraction. Anions generated from ketones with β-hydrogens often lead to substantial amounts of reduction products in $S_{RN}1$ reactions.[16,19]

Enolate anions derived from aryl alkyl ketones are known generally to be poor $S_{RN}1$ nucleophiles.[22] However, the enolate anions derived from acetophenone and α-tetralone reacted readily with **1** to give high yields of **10** and **9**, respectively (Scheme II). This may be due to the ease of electron transfer to the electron-deficient 6-iodo-9-ethylpurine (**1**) system.[23] We were also successful in functionalizing the purine C-6 position with 2-acetylfuran in a reaction that appears to have generality for acylated heteroaromatics. The product of this reaction, **11**, has a close structural resemblance to plant growth regulators called cytokinins.[24]

Scheme III



Interestingly, the photoinduced reaction of the enolate of bromoacetone with **1** results in the formation of *N*-1-acetonyl-9-ethylhypoxanthine (**14**). A plausible mechanism for the formation of **14** involves an initial $S_{RN}1$ reaction of **1** to produce the 6-alkylated purine **12**. Intramolecular displacement of bromine, followed by nucleophilic attack on the azirinium system[25] by the solvent and ring opening,

(21) Julliard, M.; Chanon, M. *Chem. Rev.* **1983**, *83*, 425.
(22) Bunnett, J. F.; Sundberg, J. E. *J. Org. Chem.* **1976**, *41*, 1702.
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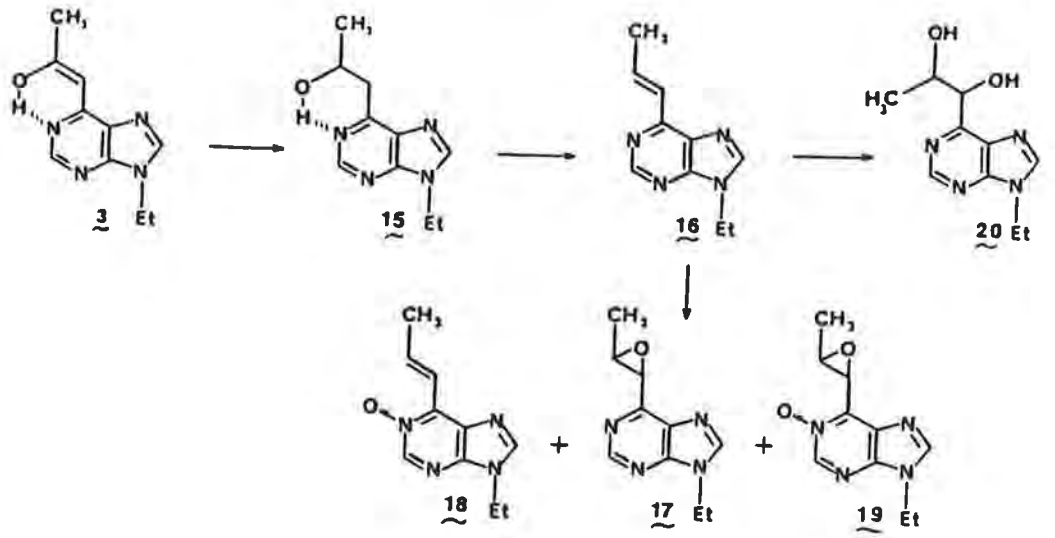
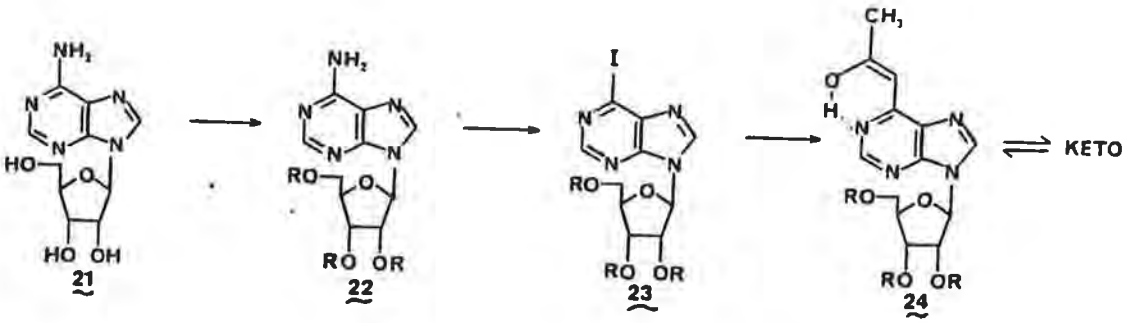
(24) Hecht, S. M.; Leonard, N. J.; Schmitz, R. Y.; Skoog, F. *Phytochemistry* **1970**, *9*, 1173.

Scheme IV



Scheme V



R= $SiMe_2Bu^t$

ould give the zwitterion 13, which undergoes rapid hy-olysis during workup and separation to give 14 (Scheme I).

A major advantage of the C-6-alkylated purines syn-esized by this methodology is that the products have rsatility in terms of further synthetic elaborations. Thus, ıen the acetonyl product 3 was reduced with sodium rohydride, 6-(2-hydroxypropyl)-9-ethylpurine (15) was)lated as a white solid in 72% purified yield. Dehy-ation of 15 with potassium bisulfate in toluene results stereospecific formation of the alkenylpurine 16 in 84%)lated yield. The trans *E* geometry of the double bond uld be easily discerned from the vicinal coupling constant = 15.7 Hz) of the alkenyl group. Epoxidation of alkene was accomplished with *m*-chloroperbenzoic acid to give *trans*-epoxide 17 in 16% yield. Several side products luding the alkene *N*-1-oxide 18 and the epoxy *N*-1-oxide were also produced. Treatment of the alkene 16 with nium tetroxide for 24 h followed by workup with sodium ulfite produced 6-(1,2-dihydroxypropyl)-9-ethylpurine) in 76% isolated yield (Scheme IV).

The long-term goal of this investigation is to develop a thodology for the synthesis of biologically active func-nalized C-alkylated nucleosides. Therefore, extension the photoinduced C-alkylation reaction to purine nu-osides was undertaken. To accomplish this, readily ilable adenosine (21) would be iodinated and subse-ntly subjected to the $S_{RN}1$ reaction. However, pro-ion of hydroxyl groups would be required in both steps. tate and related protecting groups would be cleaved in ammonia solution, and so we chose the *tert*-butyldimethylsilyl group. This blocking group could be readily introduced with high yields and selectivity for the ribose hydroxyl groups and not the exocyclic amino group of adenine.[26] However, preliminary experiments with 6-iodo-9-[2,3,5-tri-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]purine (23) gave low yields of product due to limited solubility in liquid ammonia and desilylation. To circumvent these problems, we examined other solvents for this reaction. Using the model compound, 6-iodo-9-ethylpurine (1), it was discovered that tetrahydrofuran was an excellent solvent for this reaction (yield 87%), when it was conducted at low temperatures (e.g. −44 °C) and with potassium hydride as base. Application of this modified procedure to nucleosides was carried out as follows. Trisilylated adenosine 22 was synthesized by the procedure of Ogilvie and co-workers[26] and purified by flash chromatography on silica gel. The 6-halogenated nucleoside 23 was synthesized from 22 by treatment with trimethylsilyl iodide, diiodomethane, and *n*-pentyl nitrite in hexane at 60 °C, which is a modification of a procedure previously reported by us.[27] Application of the aforementioned photoinduced reaction of 23 with the potassium enolate of acetone in tetrahydrofuran resulted in the formation of 24 in a 51% isolated purified yield (Scheme V).

In summary, novel functionalized 6-alkylated purines can be synthesized from the 6-iodinated purine precursors through an efficient photoinduced reaction with metal enolates. The C-alkylated products may be converted to

5) Nair, V. In "Heterocyclic Compounds", Part 1, Hassner, A., Ed.; y: New York, 1983; Vol. 42, p 215.

(26) Ogilvie, K. K.; Beaucage, S. L.; Schifman, A. L.; Theriault, N. Y.; Sadana, K. L. *Can. J. Chem.* 1978, *56*, 2768.
(27) Nair, V.; Richardson, S. G. *J. Org. Chem.* 1980, *45*, 3969.

other functionalized purine systems.

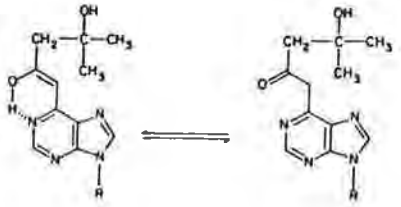
Experimental Section

A Rayonet photochemical reactor, fitted with 3500-Å bulbs, was used in the photolysis experiments. The melting points provided are uncorrected and were taken on a Thomas-Hoover melting point apparatus fitted with a microscope. Nuclear magnetic resonance spectra using tetramethylsilane as an internal standard were recorded on JEOL Model FX90Q and Bruker Model WM360 pulse Fourier transform spectrometers. A Hewlett-Packard 5985 GC/MS system was used for the mass spectra. The ultraviolet spectra were recorded on a Varian Cary Model 219 spectrophotometer. Infrared spectra were recorded on an IBM Model 98 Fourier transform instrument. Microanalytical data were determined on a Perkin-Elmer 240A CHN analyzer. Anhydrous ammonia (Matheson) was used without distillation. Potassium *tert*-butoxide, and potassium hydride (Aldrich) were used directly. Acetone (MCB Omnisolve), cyclopentanone, cyclohexanone, 2-methylcyclohexanone (Aldrich), and tetrahydrofuran (MCB, Omnisolve) were distilled prior to use. Acetophenone, α-tetralone, and acetylfuran (Aldrich) were used without further purification. Preparative layer chromatography employed EM silica gel plates, activated for 3 h at 135 °C.

General Procedure for Photoinduced $S_{RN}1$ Reactions (Procedure A). A 250-mL three-neck RBF fitted with a stir bar, cold finger, and constant-addition funnel was assembled hot and purged with nitrogen. After cooling, potassium *tert*-butoxide (3 equiv) was added and anhydrous ammonia was condensed into the flask with dry ice and acetone in the cold finger. The ketone (3 equiv) was dissolved in freshly distilled tetrahydrofuran (5 mL) and added over 15 min to the ammonia solution. The solution was stirred for 45 min, and 6-iodo-9-ethylpurine (1 equiv) dissolved in a minimum amount of tetrahydrofuran was added. The constant-addition funnel was removed, and the apparatus was placed in the Rayonet photochemical reactor fitted with 3500-Å lamps. Photolysis was carried out for 30 min, with the condensate being washed off the reaction vessel with acetone every 10 min. The reaction mixture was poured into a beaker and quenched with excess ammonium chloride. The ammonia was allowed to evaporate and was replaced with dichloromethane. The solvent was decanted, and the residual salts were washed with more dichloromethane. After drying with anhydrous sodium sulfate, the solution was rotoevaporated and the residue was separated on silica gel plates with 1:15 methanol/dichloromethane. The solid products were crystallized from ether/hexane.

6-Acetonyl-9-ethylpurine (3) by Procedure A. 6-Iodo-9-ethyl-purine (1)[27] (0.500 mmol) was treated with the enolate of acetone (1.600 mmol) as described above. The residue from the workup was eluted twice on silica gel plates using 1:15 methanol/dichloromethane. The major band with R_F 0.32 afforded 0.072 g (0.353 mmol, 70%)[28] of 3 as a light yellow solid: mp 148–149 °C; ^{13}C NMR ($CDCl_3$) δ 15.3, 15.6, 26.3, 30.4, 38.9, 39.0, 48.1, 88.6, 124.8, 133.4, 140.7, 144.1, 145.5, 146.5, 151.0, 151.3, 152.3, 154.8, 185.9, 203.2; ^{1}H NMR ($CDCl_3$) δ 8.92 (s), 8.27 (s), 8.11 (s), 7.89 (s), 5.98 (s), 4.36 (s), 4.35 (q, J = 7.3 Hz), 4.26 (q, J = 7.3 Hz), 2.34 (s), 2.18 (s), 1.58 (t, J = 7.3 Hz), 1.54 (t, J = 7.3 Hz); UV (EtOH) λ_{max} 362 nm (ε 2.3 × 10^4), 345 (1.8 × 10^4), 330 (1.4 × 10^4), 264 (3.6 × 10^3); FTIR (KBr) 3093, 2983, 1636, 1567, 1557 cm^{-1}; mass spectrum, m/z (relative intensity) (30 eV) 204 (M$^+$, 50.8), 189 (85.9), 175 (3.4), 162 (100), 147 (9.3), 134 (72.3). Anal. Calcd for $C_{10}H_{12}N_4O$: C, 58.41; H, 6.18; N, 27.70. Found: C, 58.81; H, 5.92; N, 27.43.

(28) An additional lower R_f side product is obtained in both procedures A and B. This product has been tentatively assigned the structure:



The yields of this material are as follows: procedure A (R = Et), 15%; procedure B (R = Et), 6%, and (R = protected ribose), 3%.

Dark $S_{RN}1$ Reaction. The procedure for the formation of 6-acetonyl-9-ethylpurine (3) (0.500 mmol) was followed except that the reaction flask was protected from light. Workup and chromatography provided 6-acetonyl-9-ethylpurine (3) (22%) as the major product together with 6-iodo-9-ethylpurine (1) (55%).

Inhibition of Photochemical $S_{RN}1$ Reaction. Following procedure A, 6-iodo-9-ethylpurine (1) (0.500 mmol) and acetone enolate (1.600 mmol) were mixed together, and *p*-dinitrobenzene (0.110 mmol) was added to the reaction mixture. Photolysis for 30 min, followed by workup and purification on silica gel plates, produced 6-acetonyl-9-ethylpurine (3) (0.006 g, 0.030 mmol, 6%) and recovered starting material (0.091 g, 0.333 mmol, 65%).

Inhibition of Dark $S_{RN}1$ Reaction. The reaction mixture was prepared as described in the foregoing experiment. This solution was stirred in the dark for 30 min. It was quenched, worked up, and purified to give 6-acetonyl-9-ethylpurine (3) (3%) and 6-iodo-9-ethylpurine (1) (49%).

6-Acetonyl-9-ethylpurine (3) (Procedure B). A low-temperature Hanovia photolysis apparatus with a quartz, vacuum-jacketed inner core and Pyrex filter sleeve was assembled hot and flushed with nitrogen. Dry 6-iodo-9-ethylpurine (1) (0.953 mmol) was added directly, followed by the double-tipped needle transfer of freshly distilled tetrahydrofuran (30 mL) that had been purged with nitrogen for 1 h. This solution was cooled to −44 °C in an acetonitrile–dry ice bath. In a separate flask, fitted with a septum, a dispersion of 35% potassium hydride (3.320 mmol) in mineral oil was weighed out and washed twice with dry hexane (5 mL) in a glovebox. Tetrahydrofuran (15 mL) was added via a double-tipped needle. This solution was magnetically stirred at room temperature, and acetone (3.000 mmol) was added directly via a gas-tight syringe. The acetone enolate solution was transferred rapidly to the Hanovia apparatus in the cold bath using a double-tipped needle and washed in with tetrahydrofuran (5 mL). The solution was immediately photolyzed with a 450-W mercury lamp for 20 min. Excess ammonium chloride was added to quench the reaction. The reaction mixture was passed through a short silica gel (60–200 mesh) scrubber column using 1:9 methanol/dichloromethane. The solvent was rotoevaporated to produce a yellow solid that was purified as described in procedure A, providing 6-acetonyl-9-ethylpurine (3, 0.169 g, 0.828 mmol, 87%).[28]

6-Cyclopentanon-2-yl-9-ethylpurine (4). A solution of 1 (0.520 mmol) and the enolate of cyclopentanone (1.6 mmol) was prepared and reacted as described in procedure A to give 6-cyclopentanon-2-yl-9-ethylpurine (4) (0.078 g, 0.340 mmol, 65%) as a yellow solid: mp 162–164 °C; ^{13}C NMR ($CDCl_3$) δ 15.4, 15.7, 20.8, 24.3, 24.4, 35.6, 37.9, 38.6, 38.9, 39.0, 55.4, 98.9, 123.8, 139.7, 140.1, 143.5, 143.9, 146.1, 147.2, 151.8, 152.3, 159.3, 175.9, 216.2; ^{1}H NMR ($CDCl_3$) δ 1.52 (t, J = 7.3 Hz), 1.60 (t, J = 7.3 Hz), 1.99 (m), 2.49 (m), 3.09 (m), 4.22 (q, J = 7.3 Hz), 7.82 (s), 7.96 (s), 8.05 (s), 8.89 (s); UV (EtOH) λ_{max} 383 nm (ε 2.1 × 10^4), 366 (1.8 × 10^4), 349 (1.1 × 10^4), 266 (2.5 × 10^3), 235 (3.8 × 10^3); mass spectrum, m/z (relative intensity) (30 eV) 230 (M$^+$, 68.6), 229 (M$^+$–H, 43.4), 201 (42.8), 187 (26.6), 175 (100), 158 (17.2), 147 (28.8), 119 (15.1). Anal. Calcd for $C_{12}H_{14}N_4O$: C, 62.59; H, 6.13; N, 24.33; Found: C, 62.97; H, 6.42; N, 24.42.

6-Cyclohexanon-2-yl-9-ethylpurine (5) was prepared by procedure A in 48% yield: mp 132–134 °C; ^{13}C NMR ($CDCl_3$) δ 15.3, 15.4, 21.4, 22.0, 23.2, 25.6, 25.9, 26.0, 32.5, 38.7, 38.9, 42.1, 53.2, 101.0, 126.9, 140.6, 141.0, 143.6, 144.5, 147.2, 149.7, 152.0, 155.0, 158.8, 176.2, 208.0; ^{1}H NMR ($CDCl_3$) δ 1.54 (t, J = 7.3 Hz), 1.58 (t, J = 7.3 Hz), 1.79 (m), 2.47 (m), 3.09 (m), 4.29 (q, J = 7.3 Hz), 4.33 (q, J = 7.3 Hz), 7.95 (s), 8.04 (s), 8.47 (s), 8.94 (s); UV (EtOH) λ_{max} 384 nm (ε 1.1 × 10^4), 368 (9.7 × 10^3), 348 (9.9 × 10^3), 266 (2.5 × 10^3), 238 (4.3 × 10^3); mass spectrum, m/z (relative intensity) (30 eV) 244 (M$^+$, 43.3), 243 (M$^+$–H, 18.6), 215 (29.6), 201 (8.5), 188 (100), 175 (52.5), 162 (29.1), 147 (19.5). Anal. Calcd for $C_{13}H_{16}N_4O$: C, 63.92; H, 6.60; N, 22.93. Found: C, 63.97; H, 6.34; N, 22.68.

6-(2-Methylcyclohexanon-2-yl)-9-ethylpurine (7) and 6-(6-Methylcyclohexanon-2-yl)-9-ethylpurine (8). Following procedure A, 6-iodo-9-ethylpurine (I) (0.910 mmol) and the enolate of 2-methylcyclohexanone (2.700 mmol) were photolyzed. The residue from the workup was eluted twice on silica gel plates using 1:20 methanol/dichloromethane as the eluting solvent. The major band with R_f 0.43 afforded 0.062 g (0.240 mmol, 26%) of 7 as a white solid: mp 76–78 °C; ^{13}C NMR ($CDCl_3$) δ 15.4, 22.6, 24.2,

28.3, 38.9, 41.1, 56.7, 131.6, 143.3, 151.6, 151.9, 163.3, 211.6; ^{1}H NMR ($CDCl_3$) δ 1.53 (s, 3 H), 1.59 (t, 3 H, J = 7.3 Hz), 1.50–1.85 (m, 5 H), 2.53 (d, 2 H, J = 5.5 Hz), 3.45 (d, 1 H, J = 11.7 Hz), 4.35 (q, 2 H, J = 7.3 Hz), 8.06 (s, 1 H), 8.91 (s, 1 H); UV (EtOH) λ_{max} 266 nm (ϵ 8.6 × 10^3); mass spectrum, m/z (relative intensity) (30 eV) 258 (M^+, 8.5), 243 (2.1), 230 (26.9), 215 (100), 201 (27.0), 189 (39.7), 175 (16.9), 162 (20.1), 147 (9.2), 112 (8.9). Anal. Calcd for $C_{14}H_{18}N_4O$: C, 65.09; H, 7.02; N, 21.69. Found: C, 65.17; H, 7.11; N, 21.63.

The minor band with R_f 0.32 afforded 0.019 g (0.073 mmol, 8%) of **8** as a golden oil: ^{13}C NMR ($CDCl_3$) δ 14.6, 15.3, 15.4, 18.8, 21.0, 25.2, 26.6, 30.6, 32.3, 36.4, 36.5, 38.8, 38.9, 45.9, 55.1, 100.6, 127.1, 133.3, 140.9, 143.4, 147.2, 152.2, 155.3, 157.5, 158.9, 163.6, 179.9, 209.0; ^{1}H NMR ($CDCl_3$) δ 1.08 (d, J = 6.8 Hz), 1.28 (d, J = 6.8 Hz), 1.53 (m), 1.84 (m), 2.60 (m), 3.09 (m), 4.24–4.37 (m), 7.95 (s), 8.02 (s), 8.45 (s), 8.94 (s); UV (EtOH) λ_{max} 383 nm (ϵ 3.1 × 10^3), 344 (5.5 × 10^3), 266 (5.4 × 10^3), 248 (5.8 × 10^3); FTIR (neat) 3100, 3060, 2970, 2930, 2830, 1715, 1580, 1570 cm^{-1}; mass spectrum, m/z (relative intensity) (30 eV) 258 (M^+, 27.6), 257 (34.0), 243 (6.2), 229 (100), 215 (41.8), 201 (29.7), 189 (60.2), 175 (56.5), 162 (41.7), 147 (22.3). Anal. Calcd for $C_{14}H_{18}N_4O$: C, 65.09; H, 7.02; N, 21.69. Found: C, 65.24; H, 7.17; N, 21.48.

6-(1-Tetralon-2-yl)-9-ethylpurine (9) was prepared in 79% yield by procedure A: mp 191–193 °C; ^{13}C NMR ($CDCl_3$) δ 15.4, 15.5, 23.0, 28.5, 28.6, 28.9, 38.8, 38.9, 52.6, 100.5, 125.1, 126.5, 126.6, 126.7, 127.2, 127.3, 127.8, 128.7, 130.4, 133.2, 133.6, 140.2, 140.4, 143.7, 144.1, 146.4, 148.9, 151.0, 152.3, 152.8, 159.9, 171.9, 195.9; ^{1}H NMR ($CDCl_3$) δ 1.54 (m), 2.94 (m), 3.53 (m), 4.28 (m), 7.26–7.33 (m), 7.93 (s), 7.98 (s), 8.42 (s), 8.91 (s); UV (EtOH) λ_{max} 403 nm (ϵ 2.0 × 10^4), 250 (9.6 × 10^3); mass spectrum, m/z (relative intensity) (30 eV) 292 (M^+, 91.9) 291 (100), 263 (67.5), 235 (20.7), 162 (38.6), 149 (28.2), 118 (25.6), 90 (42.0). Anal. Calcd for $C_{17}H_{16}N_4O$: C, 68.55; H, 5.75; N, 19.99. Found: C, 68.82; H, 5.66; N, 20.31.

6-Acetophenon-2-yl-9-ethylpurine (10) was prepared in 70% yield by procedure A: mp 153–154 °C; ^{13}C NMR ($CDCl_3$) δ 15.4, 15.6, 38.9, 43.5, 86.4, 125.9, 126.6, 128.3, 128.6, 130.7, 133.4, 137.4, 141.1, 143.9, 145.9, 147.0, 152.7, 178.4, 194.9; ^{1}H NMR ($CDCl_3$) δ 8.95 (s), 8.39 (s), 8.04–7.94 (m), 7.93 (s), 7.43–7.38 (m), 6.75 (s), 4.89 (s), 4.29 (q, J = 7.3 Hz), 1.56 (t, J = 7.3 Hz); UV (EtOH) λ_{max} 379 nm (ϵ 3.1 × 10^4), 243 (1.0 × 10^4); mass spectrum, m/z (relative intensity) (30 eV) 266 (M^+, 39.2), 265 (35.2), 238 (26.2), 189 (31.0), 161 (9.2), 133 (11.4). Anal. Calcd for $C_{15}H_{14}N_4O$: C, 67.65; H, 5.30; N, 21.04. Found: C, 67.84; H, 5.37; N, 20.87.

6-[(Furylcarbonyl)methyl]-9-ethylpurine (11) was prepared in 67% yield by procedure A: mp 146–148 °C; ^{13}C NMR ($CDCl_3$) δ 15.4, 15.6, 39.1, 43.1, 65.9, 84.6, 111.9, 112.5, 112.7, 118.4, 124.9, 140.6, 144.2, 144.6, 145.7, 146.8, 150.6, 152.3, 152.9, 173.1, 183.7; ^{1}H NMR ($CDCl_3$) δ 1.54 (t, J = 7.3 Hz), 4.26 (q, J = 7.3 Hz), 4.76 (s), 6.51 (m), 6.59 (s), 7.07 (d, J = 3.3 Hz), 7.52 (d, J = 1.5 Hz), 7.88 (s), 8.08 (s), 8.21 (s), 8.93 (s); UV (EtOH) λ_{max} 404 nm (ϵ 3.8 × 10^4), 386 (3.7 × 10^4), 281 (7.1 × 10^3); mass spectrum, m/z (relative intensity) (30 eV) 256 (M^+, 48.1), 228 (71.9), 202 (76.3), 199 (85.4) 171 (51.6), 160 (35.3), 149 (20.5), 129 (20.1), 111 (14.0), 95 (100). Anal. Calcd for $C_{13}H_{12}N_4O_2$: C, 60.93; H, 4.72; N, 21.86. Found: C, 60.93; H, 4.72; N, 21.61.

Photolysis of 6-Iodo-9-ethylpurine (1) with Bromoacetone. Following procedure A the enolate anion of bromoacetone was photolyzed with **1** for 1 h. Purification on silica gel plates provided *N*-acetonyl-6-oxo-9-ethylpurine (**14**), 50%, R_f 0.25, as a white solid: mp 102–104 °C; ^{13}C NMR ($CDCl_3$) δ 15.1, 25.9, 38.8, 70.1, 120.8, 142.0, 151.0, 152.1, 159.0, 202.6; ^{1}H NMR ($CDCl_3$) δ 1.56 (t, 3 H, J = 7.3 Hz), 2.27 (s, 3 H), 4.32 (q, 2 H, J = 7.3), 5.13 (s, 2 H), 7.97 (s, 1 H), 8.48 (s, 1 H); UV (EtOH) λ_{max} 250 nm (ϵ 1.2 × 10^4); FTIR (KBr) 3100, 2879, 1734, 1607, 1573, 1477 cm^{-1}; mass spectrum (relative intensity) (30 eV) 220 (M^+, 7.4), 205 (74.4), 192 (3.2), 177 (100), 165 (6.0), 149 (93.7), 121 (32.2). Anal. Calcd for $C_{10}H_{12}N_4O_2$: C, 54.54; H, 5.49; N, 25.44. Found: C, 54.17; H, 5.58; N, 25.35.

6-(2-Hydroxypropyl)-9-ethylpurine (15). To a solution of 6-acetonyl-9-ethylpurine (**3**) (0.101 g, 0.495 mmol) in absolute ethanol (10 mL) was added sodium borohydride (0.113 g, 3.040 mmol). The solution was stirred for 3 h at room temperature. Acidification of the cooled reaction mixture with 3 N HCl (4 equiv) was followed by neutralization with saturated aqueous sodium bicarbonate. The ethanol was removed by rotoevaporation, and the remaining aqueous solution was extracted with dichloromethane (3 × 30 mL). The combined extracts were dried (Na_2SO_4), and the solvent was removed. The residue was purified on silica gel with 1:9 methanol/dichloromethane as the eluting solvent. The major band at R_f 0.5 was extracted to produce 0.073 g (0.355 mmol, 72%) of a white solid: mp 81–83 °C; ^{13}C NMR ($CDCl_3$) δ 15.4, 23.2, 39.0, 41.2, 66.3, 132.6, 143.5, 150.6, 151.8, 160.5; ^{1}H NMR ($CDCl_3$) δ 1.37, (d, 3 H, J = 6.2 Hz), 1.59 (t, 3 H, J = 7.3 Hz), 3.08–3.59 (m, 2 H), 4.04–4.47 (m, 3 H), 4.0–5.0 (br s, 1 H), 8.08 (s, 1 H), 8.89 (s, 1 H); UV (EtOH) λ_{max} 263.5 nm (ϵ 9.4 × 10^3); mass spectrum, m/z (relative intensity) (30 eV) 206 (M^+, 0.7), 205 (M^+–H, 0.6), 191 (5.7), 189 (1.1), 162 (100), 134 (48.3), 107 (12.1). Anal. Calcd for $C_{10}H_{14}N_4O$: C, 58.23; H, 6.84; N, 27.16. Found: C, 57.74; H, 7.16; N, 27.34.

6-Prop-1-enyl-9-ethylpurine (16). To a solution of 6-(2-hydroxypropyl)-9-ethylpurine (**15**) (1.14 mmol) in toluene (20 mL) was added acetic anhydride (1.1 equiv) and excess potassium bisulfate. The solution was stirred at 80 °C for 12 h and cooled in an ice bath. Saturated sodium bicarbonate (5 mL) and water (5 mL) were used to wash the toluene phase. The combined aqueous solutions were washed with toluene (2 × 15 mL). After the toluene solutions were dried (Na_2SO_4) and the solvent was removed, the residue was purified on silica gel plates using 1:9 methanol/dichloromethane. The main band at R_f 0.52 provided 0.180 g (0.960 mmol, 84%) of **16** as a white solid: mp 51–53 °C; ^{13}C NMR ($CDCl_3$) δ 15.4, 19.2, 38.8, 126.7, 130.6, 140.0, 143.3, 151.5, 152.2. 154.1; ^{1}H NMR ($CDCl_3$) δ 1.57 (t, 3 H, J = 7.3 Hz), 2.08 (dd, 3 H, J = 6.6 Hz, J = 1.47 Hz), 4.33 (q, 2 H, J = 7.3 Hz), 7.01 (dd, 1 H, J = 15.8 Hz, J = 1.47 Hz), 7.66 (d of quartets, 1 H, J = 15.8 Hz, J = 6.6 Hz), 8.04 (s, 1 H), 8.86 (s, 1 H); UV (EtOH) λ_{max} 287.5 nm (ϵ 1.4 × 10^4); FTIR (KBr) 3095, 3058, 2918, 1653, 1577 cm^{-1}; mass spectrum, m/z (relative intensity) (30 eV) 189 (M^+ + H, 11.8), 188 (M^+, 94.1), 187 (77.0), 173 (5.7), 162 (6.8), 159 (91.4). Anal. Calcd for $C_{10}H_{12}N_4$: C, 63.81; H, 6.43; N, 29.76. Found: C, 63.57; H, 6.42; N, 29.53.

6-[(*E*)-1,2-Oxiranylpropyl]-9-ethylpurine (17). To a solution of 6-prop-1-enyl-9-ethylpurine (**16**) (0.094 g, 0.497 mmol) in dry dichloromethane (10 mL) at 0 °C was added *m*-chloroperbenzoic acid (0.780 mmol) dissolved in dichloromethane (20 mL). The solution was stirred for 12 h at room temperature under N_2. The reaction mixture was subsequently washed with saturated sodium bisulfite (5 mL) and 5% sodium bicarbonate (5 mL). The combined aqueous phases were extracted with dichloromethane (60 mL). The solvent was removed from the combined and dried (Na_2SO_4) extracts under reduced pressure. The residue was purified on silica gel plates developed with 1:20 methanol/dichloromethane. The band at R_f 0.5 provided 0.016 g (0.079 mmol, 16%) of **17** as a clear oil: ^{13}C NMR ($CDCl_3$) δ 15.3, 17.7, 40.0, 56.1, 57.6, 132.5, 144.3, 151.3, 152.4, 156.1; ^{1}H NMR ($CDCl_3$) δ 1.56 (d, 3 H, J = 5.1 Hz), 1.57 (t, 3 H, J = 7.3 Hz), 3.84 (dq, 1 H, J = 5.1 Hz, J = 2.2 Hz), 4.27 (d, 1 H, J = 1.8 Hz), 4.35 (q, 2 H, J = 7.3 Hz), 8.10 (s, 1 H), 8.91 (s, 1 H); UV (EtOH) λ_{max} 268 nm (ϵ 9.5 × 10^3); FTIR (KBr) 3103, 3077, 2963, 1513, 1217, 886 cm^{-1}; mass spectrum, m/z (relative intensity) (30 eV) 204 (M^+, 10.5), 189 (28.4), 175 (9.0), 159 (19.4), 148 (60.3), 133 (12.9), 120 (100), 106 (22.5). Anal. Calcd for $C_{10}H_{12}N_4O$: C, 58.81; H, 5.92; N, 27.43. Found: C, 58.50; H, 6.21; N, 27.14.

6-(1,2-Dihydroxypropyl)-9-ethylpurine (20). To a solution of 6-prop-1-enyl-9-ethylpurine (**16**) 0.433 g (2.300 mmol) in dry pyridine (5 mL) was added osmium tetroxide 0.500 g (1.91 mmol) dissolved in pyridine (5 mL). The solution was stirred for 24 h at room temperature. Sodium bisulfite (0.90 g), pyridine (10 mL), and water (15 mL) were added to the reaction mixture. After stirring for 1.5 h, the solution was extracted with dichloromethane (225 mL). The organic phase was dried (Na_2SO_4) and the solvent removed under vacuum. The residue was purified on silica gel plates developed with 1:9 methanol/dichloromethane. The band at R_f 0.28 provided 0.333 g (1.50 mmol, 78%) of **20** as a white solid: mp 98–100 °C; ^{13}C NMR ($CDCl_3$) δ 15.3, 19.3, 39.2, 70.0, 74.3, 130.8, 143.9, 151.2, 151.6, 159.3; ^{1}H NMR ($CDCl_3$) δ 1.33 (d, 3 H, J = 6.6 Hz), 1.59 (t, 3 H, J = 7.3 Hz), 3.40 (d, 1 H, J = 5.8 Hz), 4.37 (q, 3 H, J = 7.3 Hz), 4.78 (d, 1 H, J = 5.9 Hz), 5.11 (m, 1 H), 8.09 (s, 1 H), 8.94 (s, 1 H); UV (EtOH) λ_{max} 265 nm (ϵ 9.9 × 10^3); mass spectrum, m/z (relative intensity) 223 (M^+ + H, 2.3), 179 (12.0), 178 (95.8), 149 (100), 121 (58.8). Anal. Calcd for $C_{10}H_{14}N_4O_2$: C, 54.04; H, 6.35; N, 25.21. Found: C, 53.77; H, 6.55; N, 25.16.

6-Amino-9-[2,3,5-tri-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]purine (22).[26] Adenosine (21) (0.259 g, 0.972 mmol) and imidazole (0.536 g, 7.89 mmol) were dissolved in dry dimethylformamide (1 mL), followed by *tert*-butyldimethylsilyl chloride (0.638 g, 4.2 mmol). The solution was stirred under N_2 for 2 h until homogeneous and then allowed to sit for 24 h. The solution was pumped to dryness, taken up in ethyl acetate, and eluted through a short silica gel scrubber column with 1:1 hexane/ethyl ether. Final purification by flash chromatography on silica gel (1:1 hexane/ethyl ether) provided 0.444 g (0.728 mmol, 75%) of a white solid: mp 142–144 °C (lit.[26] mp 142–144 °C); ^{1}H NMR ($CDCl_3$) δ −0.04 (s), 0.11 (s), 0.13 (s), 0.80 (s), 0.94 (s), 0.96 (s), 3.72–4.04 (m, 2 H), 4.14 (m, 1 H), 4.37 (m, 1 H), 4.75 (m, 1 H), 5.90 (br s, 1 H), 6.06 (d, 1 H, J = 5.1 Hz), 8.18 (s, 1 H), 8.37 (s, 1 H).

6-Iodo-9-[2,3,5-tri-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]purine (23). To a solution of 6-amino-9-[2,3,5-tri-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]purine (22) (0.316 g, 0.519 mmol) in dry nitrogen purged hexane (30 mL) was added trimethylsilyl iodide (0.125 mL, 0.880 mmol) followed by diiodomethane (0.90 mL, 11.2 mmol) and *n*-pentyl nitrite (1.50 mL, 11.1 mmol). The mixture was stirred at 60 °C for 24 h under N_2. Upon cooling, hexane (20 mL) was added and the reaction mixture was extracted with saturated sodium sulfite (5 mL) and water (5 mL). The combined aqueous phases were extracted with ethyl ether (60 mL). The combined extracts were dried (Na_2SO_4), and the solvent was removed under reduced pressure. The residue was purified on silica gel plates developed with 1:1 hexane/ethyl ether. The band at R_f 0.80 provided 0.261 g (0.363 mmol, 70%) of 23 as a clear oil: ^{1}H NMR ($CDCl_3$) δ −0.04 (s), 0.10 (s), 0.13 (s), 0.78 (s), 0.93 (s), 0.95 (s), 3.71–4.19 (m, 3 H), 4.31 (t, 1 H, J = 3.8 Hz), 4.63 (t, 1 H, J = 4.6 Hz), 6.08 (d, 1 H, J = 5.1 Hz), 8.48 (s, 1 H), 8.61 (s, 1 H); UV (EtOH) λ_{max} 276 nm (ε 1.1 × 10^4); mass spectrum m/z (relative intensity) (30 eV) 705 (M^+–CH_3, 0.4), 663 (M^+–C_4H_9, 12.6), 417 (2.3), 403 (5.7), 261 (6.7), 231 (4.5), 149 (4.9), 147 (10.5), 133 (3.9). Anal. Calcd for $C_{28}H_{53}N_4IO_4Si_3$: C, 46.65; H, 7.41; N, 7.77. Found: C, 46.30; H, 7.38; M, 7.64.

6-Acetonyl-9-[2,3,5-tri-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]purine (24). Following procedure B the enolate anion of acetone was photolyzed with 6-iodo-9-[2,3,5-tri-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]purine (23) (0.551 mmol). Purification on silica gel plates using 1:1 hexane/ethyl ether as the developer produced 24, 0.183 g (0.281 mmol, 51%),[28] R_f 0.53, as a yellow solid: mp 108–110 °C; ^{1}H NMR ($CDCl_3$) δ −0.04 (s), 0.11 (s), 0.14 (s), 0.79 (s), 0.94 (s), 0.96 (s), 2.17 (s), 2.29 (s), 3.72–4.75 (m), 5.81–6.14 (m), 8.22 (s), 8.26 (s), 8.41 (s), 8.89 (s); UV (EtOH) λ_{max} 362 nm (ε 3.0 × 10^4), 345 (2.5 × 10^4), 330 sh (1.8 × 10^4), 264 (3.8 × 10^3); mass spectrum, m/z (relative intensity) (30 eV) 650 (M^+, 0.8), 635 (M^+–CH_3, 4.1), 593 (M^+–C_6H_9, 100), 447 (13.2), 417 (12.5), 343 (18.0), 333 (83.0), 301 (17.7), 285 (18.0), 275 (16.0), 261 (44.1), 231 (35.9), 211 (30.1), 177 (31.0), 147 (66.3), 133 (9.6), 115 (32.5). Anal. Calcd for $C_{31}H_{58}N_4O_5Si_3$: C, 57.19; H, 8.98; N, 8.60. Found: C, 57.09; H, 8.60; N, 8.45.

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Ring-Extended Products from the Reaction of Epoxy Carbonyl Compounds and Nucleic Acid Bases

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Purine and pyrimidine bases react with epoxy carbonyl compounds in aqueous solution to yield ring-extended adducts. These products include etheno-modified bases as well as adducts in which the modification involves the formation of an additional six-membered ring. The latter examples are among the first known cases of this type of modification of pyrimidine bases. Plausible mechanisms for the formation of these adducts are discussed.

Epoxides occur widely in nature and have been identified in compounds from microorganisms and plants.[1-6] They are produced also in mammalian systems in the oxidation of polyunsaturated lipids.[7-9] The deleterious effects of some epoxy compounds are well documented. For example, aflatoxin B_1, sterigmatocystin, and the polycyclic aromatic hydrocarbons such as benzo[*a*]pyrene are known to be toxic and carcinogenic. Their detrimental effects are thought to be mediated by their conversion in vivo to their epoxides and subsequent modification of nucleic acid bases by these epoxides.[10-18] Simpler monofunctional epoxides have been known to modify nucleic acid bases.[19,20] In addition, the mode of formation and the detailed structures of adducts between carbonyl compounds and nucleic acid bases have been of considerable interest in studies of the constitution and mechanism of action of nucleic acids. Our interest in the modification of nucleic acid bases by malonaldehyde and related systems,[21,22] and in the synthesis of compounds related to the "Y" bases,[23] led us to examine such reactions with epoxy carbonyl compounds, the results of which are reported in this paper.

Results and Discussion

Very few studies have been undertaken to determine the detailed structures of adducts arising from the reaction of

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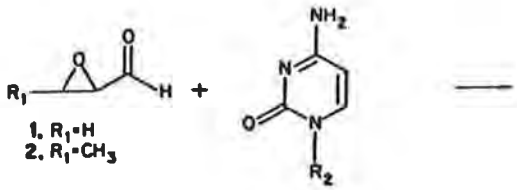
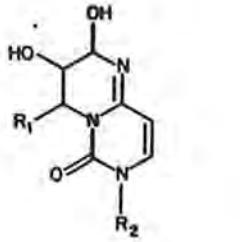
Scheme I



1. R_1=H
2. R_1=CH_3

3. R_2=CH_3
4. R_2=D-ribofuranosyl



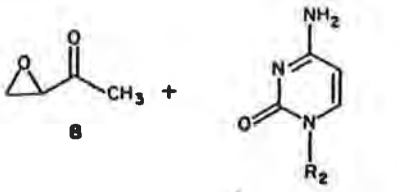
5. R_1=H; R_2=CH_3
6. R_1=H; R_2=D-ribofuranosyl
7. R_1=CH_3; R_2=D-ribofuranosyl

epoxides with nucleic acid bases. Almost all of the reported work in this area deals with polycyclic aromatic epoxides or the aflatoxins,[11,13,15,18] where modification involves a monofunctional epoxide moiety. Little, however, is known about the reactivity of multifunctional epoxides toward nucleic acid bases. We have found that epoxy carbonyl compounds readily modify pyrimidine and purine bases to give interesting ring-extended adducts. These modifications were carried out by treating the appropriate nucleoside or alkylated base in aqueous media at defined pHs with the epoxy carbonyl compounds. The latter were prepared from the corresponding enals by a modification of the method of White and co-workers.[24] The modifying reactions were monitored by UV spectral methods and terminated when product formation had maximized. The ribosyl-containing adducts were purified by reversed-phase HPLC on Amberlite XAD-4 resin, while the corresponding alkylated bases were separated on preparative silica gel plates.

The reaction of glycidaldehyde (1) with cytidine (4) at pH 10 yielded a white crystalline adduct (mp 111–113 °C) in 41% yield. To facilitate interpretation of the spectral data and assignment of structure for this product, the related adduct from 1-methylcytosine (3) was also prepared. The mass spectrum of the latter showed a molecular ion at m/z 197 and a more intense peak at m/z 179, indicating facile loss of H_2O from the adduct. The UV spectrum exhibited absorbance maxima at 223 (ϵ 10400) and 286 nm (ϵ 9700), suggesting the absence of extended conjugation. The 360-MHz ^1H NMR spectrum in Me_2SO–d_6 showed doublets at δ 7.26 and 5.65 integrating for one proton each and with coupling constant of 7.8 Hz (cytosine moiety). Two singlets at δ 4.83 and 5.70 which underwent exchange with D_2O were attributed to the presence of hydroxyl groups. A doublet at δ 5.27 (1 H, J = 2.7 Hz) and multiplets at δ 3.72 (1 H) and 3.62 (2 H) were assigned as the remaining protons of a newly formed ring. The methyl group appeared as a singlet at δ 3.17. In the ^{13}C NMR spectrum (in Me_2SO–d_6), three additional carbon resonances, apart from those of the cytosine ring and the methyl group, occurred at δ 58.4, 65.4, and 90.7 and were indicative of the presence of a saturated three-carbon moiety. Taken collectively, the data suggested that

(24) Wellman, G. R.; Lam, B.; Anderson, E. L.; White, E. *Synthesis* 1976, 547.

Scheme II



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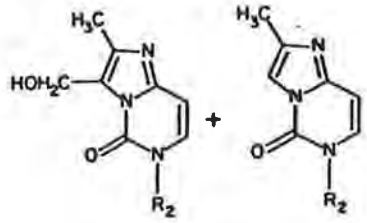
4. R_2=D-ribofuranosyl



9 10

the new compound was 7-methyl-3,4-dihydro-2,3-dihydroxy-2*H*-pyrimido[1,6-*a*]pyrimidin-6(7*H*)-one (5) (Scheme I). The spectral data for the cytidine adduct 6 were more complex because of the presence of the D-ribofuranosyl moiety; however, excellent correlation was clearly evident between 5 and 6 for the modified base moiety. In both neutral and acidic (pH 5) media, cytidine was converted to adduct 6 in 38% and 40% yields, respectively. No ethenocytidine derivative was isolated in any of these cases.[22] It should be mentioned that formation of six-membered rings in the modification of pyrimidine nucleosides is rare.

The formation of six-membered rings was also seen in the reaction of 2,3-epoxybutanal (2). For example, in aqueous solutions at pH 10, cytidine (4) was transformed into 7 in 30% yield, while in neutral and acidic (pH 5) media, conversion to 7 occurred in 45% and 36% yields, respectively.

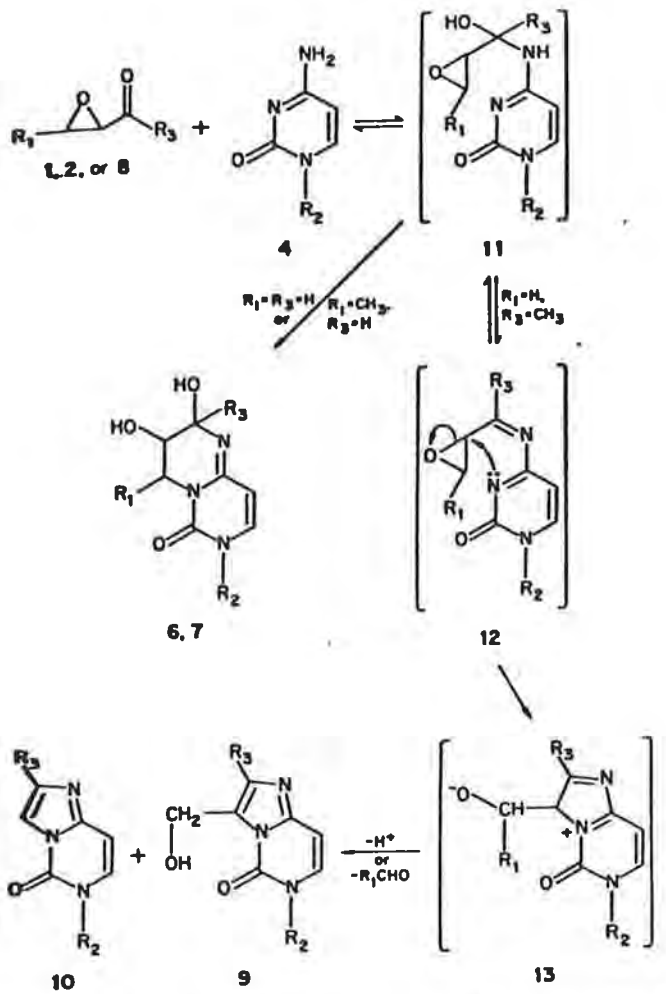
When the less reactive epoxy carbonyl compound, 3,4-epoxybutanone (8), was employed, modification of cytidine was still observed but the transformation occurred in low yield (about 13%) under neutral, acidic, or basic conditions. The products of these reactions were the ethenocytidine derivatives 9 and 10 (Scheme II). These products were identified by their mass spectral, UV, and NMR data.[22]

A plausible and generalized mechanism for the formation of these adducts is shown in Scheme III. Attack by the amino group of cytidine on the carbonyl carbon of the epoxy carbonyl compound produces the amino alcohol intermediate 11. Ring opening of the epoxide moiety in 11 may occur in two ways. Direct nucleophilic attack by N-3 on the terminal position of the epoxide results in the formation of the six-membered ring products 6 and 7, which are observed for the epoxy aldehydes 1 and 2. In the case of the epoxy ketone 8, the initially formed intermediate 11 has a tertiary alcohol group which will dehydrate rapidly to form the imine 12. If ring opening in 12 involves the internal carbon of the epoxide (i.e., the allylic and now more electrophilic carbon) intermediate 13 is generated. This species can eliminate a proton to give 9 or it can eliminate $H_2C{=}O$ to give 10 (Scheme III). Differentiation between the two pathways therefore resides on the ability of intermediate 11 to eliminate water to produce an α,β-unsaturated epoxide.

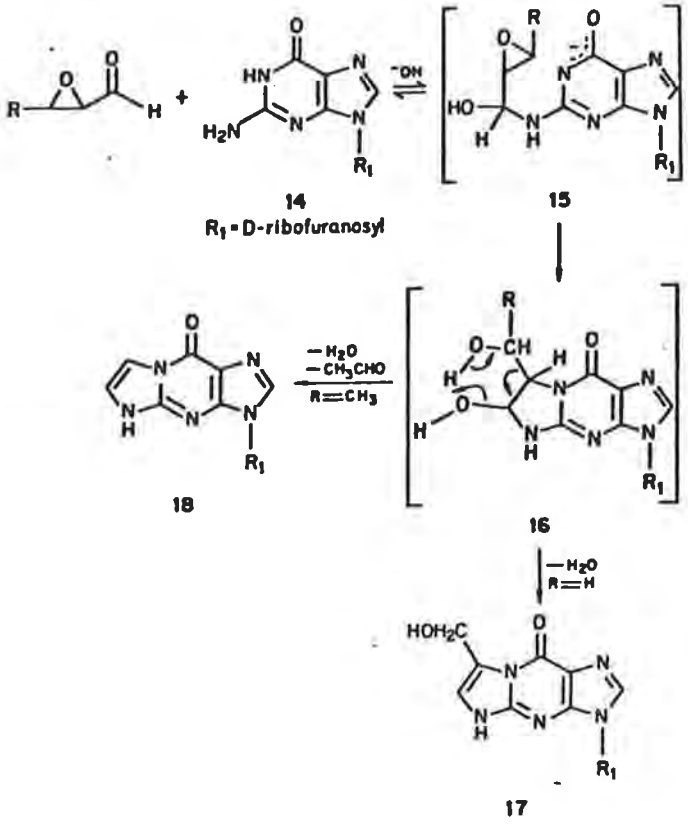
Glycidaldehyde has been reported to show high specificity toward guanine components in its modification of nucleic acids.[25] Despite its importance as a probe in the

(25) Van Duuren, B. L.; Loewengart, G. *J. Biol. Chem.* 1977, 252, 5370.

Scheme III

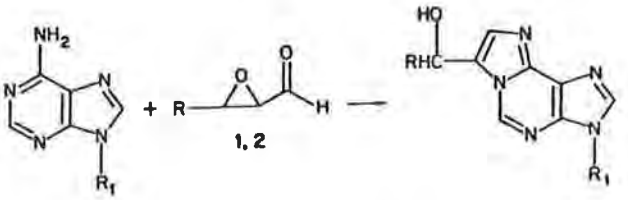


Scheme IV



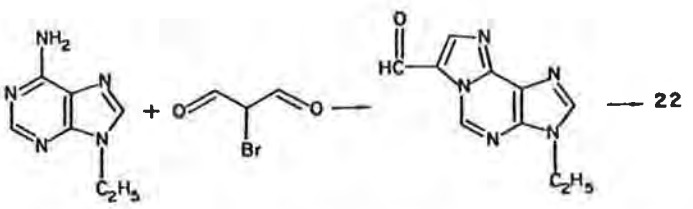
mode of action of alkylating carcinogens,[26] the structural details of its modification of guanine residues had not been fully determined.[27] Since publication of our original communication on the structure of the adduct from the reaction of guanosine and glycidaldehyde at pH 10,[28,29] we have performed extensive delayed decoupling and NOE experiments which unequivocally established the correct structure as the regioisomer 17. However, adduct 17 was not isolated when the reaction was conducted under neutral or acidic conditions. 2,3-Epoxybutanal also failed to provide adducts with guanosine in neutral or acidic media. In basic media, however, 1,N^6-ethenoguanosine (18) was isolated in 41% yield. The formation of adducts 17 and 18 can best be appreciated by considering the regiochemistry and a plausible mechanism for the transformations. Attack of the exocyclic amino group on the carbonyl carbon of the epoxy aldehyde would generate intermediate 15. Ring opening involving the internal carbon of the epoxide by the N-1 anion of guanosine (pK_a = 9.2), under the basic conditions (pH 10), would result in the formation of 16. Intermediate 16 can eliminate water to form 17 or it can undergo a double elimination through a cyclic transition state as shown to give 18. Both pathways are observed depending on the structure of the epoxide (Scheme IV). The synthesis of 1,N^6-ethenoguanosine (18) has been reported previously[30] from the reaction of chloroacetaldehyde and guanosine in about an 8% yield. The procedure using the epoxybutanal is a more efficient way to produce this compound.

Adenine bases have also been found to be modified by functionalized epoxides. For example, glycidaldehyde and 2,3-epoxybutanal convert adenosine to the etheno-adenosine derivatives 20 and 21 in low yields under acidic conditions. Unambiguous proof of the structure of these



19

20, R=H; R_1=D-ribofuranosyl
21, R=CH_3; R_1=D-ribofuranosyl
22, R=H; R_1=C_2H_5



23

adducts came from an alternate synthesis of the ethyl analogue 22 from the reduction of 9-ethyl-1,N^6-etheno-adenine-10-carboxaldehyde prepared by the reaction of 9-ethyladenine and bromomalonaldehyde.[22]

In summary, functionalized epoxides are ubiquitous in nature, but few studies have been reported on the reactivity of such epoxides with nucleic acid bases. We have found that epoxy carbonyl compounds are able to modify

(26) Singer, B.; Kusmierek, J. T. *Ann. Rev. Biochem.* 1982, *51*, 655.
(27) Goldschmidt, B. M.; Blazej, T. P.; Van Duuren, B. L. *Tetrahedron Lett.* 1968, 1583.
(28) Nair, V.; Turner, G. A. *Tetrahedron Lett.* 1984, *25*, 247.
(29) Fribart, C. R.; Feinberg, A. M.; Nakanishi, K. *J. Org. Chem.* 1978, *43*, 1644.
(30) Sattsangi, P. D.; Leonard, N. J.; Fribart, C. R. *J. Org. Chem.* 1977, *42*, 3292.

both purine and pyrimidine bases to give extended ring systems some of which have been rarely encountered.

Experimental Section

The melting points reported are uncorrected and were taken on a Thomas-Hoover melting point apparatus fitted with a microscope. The ^1H NMR and ^{13}C NMR spectra were recorded on a JEOL FX90Q pulse Fourier transform NMR spectrometer or on a Bruker WM 360 pulse Fourier transform NMR spectrometer. Tetramethylsilane was the internal reference. Mass spectra at 30 eV were obtained on a Hewlett-Packard 5985 GC/MS system. The ultraviolet data were taken with a Cary Model 219 ultraviolet–visible spectrophotometer. HPLC separations were done at low pressure utilizing a column of Amberlite XAD-4 resin (230–400 mesh). Preparative layer chromatography was done on E. Merck silica gel PF-254.

Preparation of 1-Methylcytosine (3). This compound was prepared by the method of Hosmane and Leonard[31] in a 63% yield.

Preparation of Epoxy Carbonyl Compounds. The epoxy carbonyl compounds were prepared by the method of White and co-workers.[24] Glycidaldehyde (1) was obtained in a 22% yield: bp 48–52 °C (75 torr) [lit.[32] bp 57–58 °C (100 torr)]; IR (neat) 1700 (C=O), 1200, 970, and 820 cm^{-1} (epoxide ring); ^1H NMR ($CDCl_3$) δ 3.43–2.05 (m, 3 H), 8.96 (d, 1 H, J = 6.4 Hz). 2,3-Epoxybutanal (2) was obtained in a 61% yield: bp 51–56 °C (40 torr); IR (neat) 1730 (C=O), 1270 and 970 cm^{-1} (epoxide ring); ^1H NMR ($CDCl_3$) δ 1.50 (d, 3 H, J = 5.1 Hz), 3.08–3.14 (m, 1 H), 3.29–3.35 (m, 1 H), 9.01 (d, 1 H, J = 5.9 Hz). 3,4-Epoxybutanone (8) was obtained in a 60% yield: bp 66–80 °C (60 torr) [lit.[24] bp 60–80 °C (60 torr)]; IR (neat 1700 (C=O), 1240 and 825 cm^{-1} (epoxide ring); ^1H NMR ($CDCl_3$) δ 2.06 (s, 3 H), 2.83–3.07 (m, 2 H), 3.36–3.42 (m, 1 H).

General Procedure for the Reaction of Epoxy Carbonyl Compounds with Nucleosides or Alkylated Nucleic Acid Bases. One of three buffers was employed: (i) basic medium (pH 10), $NaOH/NaHCO_3$; (ii) acidic medium (pH 5), acetic acid/sodium acetate; (iii) neutral medium (pH 7.4), potassium phosphate.

The nucleoside or alkylated base was dissolved in the appropriate buffer and stirred to allow dissolution. The epoxy carbonyl compound was added, and the pH was adjusted accordingly. The reaction mixture was then stoppered and allowed to stir at room temperature. In studies with epoxybutanone, the reaction mixtures were heated to facilitate conversions. In all cases, when product formation had reached a maximum as evidenced by UV spectroscopy or TLC, the reaction mixtures were neutralized and the solvent removed in vacuo. The methylated adducts were purified on silica gel preparative layer plates using 5–20% $MeOH/CHCl_3$ as the eluent, while the ribosyl adducts were separated by HPLC on a column of Amberlite XAD-4 resin (230–400 mesh) using 2–20% $EtOH/H_2O$ as the eluent.

Reaction of 1-Methylcytosine (3) with Glycidaldehyde (1) at pH 10. 1-Methylcytosine (3) (0.138 g, 1.1 mmol) and glycidaldehyde (1) (0.108 g, 1.5 mmol) were allowed to react for 6 h at room temperature at pH 10. Separation yielded 0.074 g (0.37 mmol, 34%) of 5 in the band with R_f 0.30. Product 5 crystallized from MeOH/ether as white crystals: mp 159–162 °C; UV (H_2O) λ_{max} 223 (ϵ 1.04 × 10^4), 286 nm (9.7 × 10^3); mass spectrum, m/z (relative intensity) 197 (M^+, 5.1), 179 ($M^+ - H_2O$, 74.0), 162 ($M^+ - H_2O - OH$, 100), 150 (94.0); ^1H NMR (Me_2SO-d_6) δ 3.17 (s, 3 H), 3.62 (m, 2 H), 3.72 (m, 1 H), 4.83 (brs, 1 H), 5.27 (d, 1 H, J = 2.7 Hz), 5.70 (brs, 1 H), 5.65 (d, 1 H, J = 7.8 Hz), 7.26 (d, 1 H, J = 7.8 Hz); ^{13}C NMR (Me_2SO-d_6) δ 35.0, 58.4, 65.4, 90.7, 94.8, 143.5, 148.7, 155.1.

Anal. Calcd for $C_6H_{11}N_3O_3 \cdot {}^1/_2H_2O$: C, 46.60; H, 5.87; N, 20.38. Found: C, 46.47; H, 5.53; N, 20.87.

Reaction of Cytidine (4) with Glycidaldehyde (1) at pH 10. Cytidine (4) (0.244 g, 1.0 mmol) and glycidaldehyde (1) (0.097 g, 1.3 mmol) were allowed to react for 6 h at room temperature in basic medium. After purification the product was crystallized from MeOH, giving 0.130 g (0.41 mmol, 41%) of 6 as white needles: mp 111–113 °C; UV (H_2O) λ_{max} 223 nm (ϵ 9.4 × 10^3), 279 (7.9 × 10^3); mass spectrum, m/z (relative intensity) 297 ($M^+ - H_2O$, 3.9), 165 ("base" + H − H_2O, 75.5), 135 (100.0); ^1H NMR (Me_2SO-d_6) δ 3.51–3.67 (m, 4 H), 3.75–3.81 (m, 2 H), 3.94–4.04 (m, 2 H), 4.84–5.19 (m, 4 H), 5.29 (br s, 1 H), 5.74–5.81 (m, 3 H), 7.50 (d, 1 H, J = 8.1 Hz); ^{13}C NMR (Me_2SO-d_6) δ 58.2, 61.1, 65.1, 70.0, 72.9, 84.6, 87.2, 90.9, 96.4, 137.2, 148.1, 153.7.

Anal. Calcd for $C_{12}H_{17}N_3O_7$: C, 45.71; H, 5.43; N, 13.33. Found: C, 45.71; H, 5.53; N, 13.59.

Reaction of Cytidine (4) with Glycidaldehyde (1) at pH 7.4. Compound 6 was obtained in 38% yield from this reaction after 3.5 h.

Reaction of Cytidine (4) with Glycidaldehyde (1) at pH 5. A 40% yield of 6 was obtained from the reaction of cytidine (4) with glycidaldehyde (1) at pH 5 for 6 h.

Reaction of Cytidine (4) with 2,3-Epoxybutanal (2) at pH 10. Cytidine (4) (0.277 g, 1.1 mmol) was stirred with 2,3-epoxybutanal (2) (0.123 g, 1.4 mmol) at room temperature for 18 h in basic medium. Purification yielded 0.110 g (0.33 mmol, 30%) of 7 as hygroscopic white crystals: mp 119–121 °C; UV (H_2O) λ_{max} 222 nm (ϵ 7.8 × 10^3), 280 (6.3 × 10^3); mass spectrum, m/z (relative intensity) 179 ("base" − OH, 12.1), 164 (19.4), 135 (49.0); ^1H NMR (Me_2SO-d_6) δ 1.10 (d, 3 H, J = 6.5 Hz), 3.44–3.60 (m, 3 H), 3.80 (m, 1 H), 4.04–4.32 (m, 2 H), 4.32–4.34 (m, 1 H), 5.03–5.21 (m, 3 H), 5.36 (brs, 1 H), 5.75–5.90 (m, 3 H), 7.50 (d, 1 H, J = 8.1 Hz); ^{13}C NMR (Me_2SO-d_6) δ 18.6, 61.3, 62.4, 69.3, 70.2, 73.3, 84.7, 87.1, 88.7, 96.6, 137.2, 148.4, 153.8.

Anal. Calcd for $C_{13}H_{19}N_3O_7 \cdot {}^1/_2H_2O$: C, 46.15; H, 5.96; N, 12.42. Found: C, 45.79; H, 6.39; N, 12.17.

Reaction of Cytidine (4) with 2,3-Epoxybutanal (2) at pH 5. A 36% yield of adduct 7 was obtained when cytidine (4) was allowed to react with 2,3-epoxybutanal (2) for 16 h in acidic medium.

Reaction of Cytidine (4) with 2,3-Epoxybutanal (2) at pH 7.4. The reaction of cytidine (4) with 2,3-epoxybutanal (2) provided a 45% yield of 7 when the reaction was allowed to proceed for 23 h at pH 7.4.

Reaction of Cytidine (4) with 3,4-Epoxybutanone (8) at pH 10. Cytidine (4) (0.245 g, 1.0 mmol) and 3,4-epoxybutanone (8) (0.122 g, 1.4 mmol) were allowed to react at 50 °C for 48 h in basic medium. Separation of reaction mixture yielded two products. The first product was identified as 2-methyl-3-(hydroxymethyl)-6-β-D-ribofuranosylimidazo[1,2-c]pyrimidin-5-(6H)-one (9) and was isolated in 5% yield, 7% conversion (0.015 g, 0.05 mmol) as white needles after crystallization from MeOH/ether: mp 189–191 °C; UV (H_2O) λ_{max} 283 nm (ϵ 1.2 × 10^4); mass spectrum, m/z (relative intensity) 311 (M^+, 7.0), 179 ("base" + H, 100.0), 162 (57.6); ^1H NMR (Me_2SO-d_6) δ 2.23 (s, 3 H), 3.70–5.50 (m, 11 H), 6.05 (d, 1 H, J = 4.4 Hz), 6.57 (d, 1 H, J = 7.8 Hz), 7.71 (d, 1 H, J = 7.8 Hz).

Anal. Calcd for $C_{13}H_{17}N_3O_6 \cdot {}^1/_2H_2O$: C, 48.74; H, 5.66; N, 13.12. Found: C, 48.72; H, 5.12; N, 12.67.

The second product was identified as 2-methyl-6-β-D-ribofuranosylimidazo[1,2-c]pyrimidin-5(6H)-one (10) and was isolated in a 7% yield, 10% conversion (0.019 g, 0.07 mmol) as off-white blunt crystals: mp 110–112 °C; UV (H_2O) λ_{max} 278 nm (ϵ 8.9 × 10^3); mass spectrum, m/z (relative intensity) 281 (M^+, 2.1), 150 (15.9), 149 ("base" + H, 100.0); ^1H NMR (Me_2SO-d_6) δ 2.24 (s, 3 H), 3.70–5.50 (m, 8 H) 6.05 (d, 1 H, J = 4.9 Hz), 6.62 (d, 1 H, J = 7.8 Hz), 7.51 (s, 1 H), 7.72 (d, 1 H, J = 7.8 Hz).

Anal. Calcd for $C_{12}H_{15}N_3O_5$: C, 51.24; H, 5.38; N, 14.94. Found: C, 50.93; H, 5.56; N, 14.99.

Reaction of Cytidine (4) with 3,4-Epoxybutanone (8) at pH 5. Compounds 9 and 10 were obtained in 9% yield (12% conversion) and 4% yield (6% conversion), respectively, when 4 was allowed to react with 8 in acidic medium at 50 °C for 48 h.

Reaction of Cytidine (4) with 3,4-Epoxybutanone (8) at pH 7.4. Cytidine (4) was allowed to react with epoxybutanone (8) at 50 °C for 20 h at pH 7.4. Adducts 9 and 10 were produced in 8% and 2% yields, respectively.

Reaction of Guanosine (14) with 2,3-Epoxybutanal (2). Guanosine (14) (0.735 g, 2.6 mmol) was added to 180 mL of H_2O, and the pH was adjusted to 10. Epoxybutanal (2) (0.300 g, 3.5 mmol) was added, and the reaction was stirred at room tem-

(31) Hosmane, R. S.; Leonard, N. J. *Synthesis* 1981, 118.
(32) Payne, G. B. *J. Am. Chem. Soc.* 1959, *81*, 4901.

perature for 3.5 h. The reaction mixture was neutralized and cooled overnight at 5 °C to allow precipitation of product. The product was collected by vacuum filtration and then lyophilized to yield 0.330 g (1.1 mmol, 41%) of ethenoguanosine (18). The spectroscopic data of 18 were consistent with the literature values.[30]

Reaction of Guanosine (14) with Glycidaldehyde (1). Guanosine (1.942 g, 6.9 mmol) was placed in H_2O (200 mL), and the solution was basified to pH 10 with warming to aid dissolution. Glycidaldehyde (1) 0.57 g (7.9 mmol) was added, and the reaction mixture was stirred at room temperature for 1 h. The reaction mixture was neutralized and cooled to allow precipitation of white crystals. This material was suspended in water and lyophilized to give 1.364 g (4.0 mmol) of 5,9-dihydro-7-(hydroxymethyl)-9-oxo-3-β-D-ribofuranosyl-3*H*-imidazo[1,2-*a*]purine (17) as white crystals in 59% yield: mp >300 °C dec; UV (0.1 N HCl) λ_{max} 300 nm (ϵ 7.7×10^3), 276 (9.9×10^3), 226 (2.54×10^4), (pH 7 buffer) 285 (9.9×10^3), 228 (2.63×10^4), (0.1N NaOH) 310 (7.4×10^3), 285 (6.6×10^3), 238 (2.85×10^4); mass spectrum, *m/z* (relative intensity) 207 (2.6), 189 (75.9, "base" + 2 H − OH), 188 (36.5), 133 (7.1); ^{1}H NMR (Me_2SO-d_6) δ 3.53–3.65 (m, 2 H), 3.91 (m, 1 H), 4.12 (m, 1 H), 4.45 (m, 1 H), 4.84 (d, 2 H, *J* = 6.0 Hz), 4.98 (t, 1 H, *J* = 6.0 Hz), 5.05 (t, 1 H, *J* = 5.3 Hz), 5.16 (d, 1 H, *J* = 4.7 Hz), 5.42 (d, 1 H, *J* = 6.0 Hz), 5.81 (d, 1 H, *J* = 5.9 Hz), 7.23 (s, 1 H), 8.14 (s, 1 H), 12.36 (s, 1 H); ^{13}C NMR (Me_2SO-d_6) δ 55.0, 61.2, 70.2, 73.6, 85.1, 86.8, 113.7, 115.9, 124.6, 137.3, 146.7, 150.2, 153.6.

Anal. Calcd for $C_{13}H_{15}N_5O_6$·H_2O: C, 43.95; H, 4.82; N, 19.71. Found: C, 44.37; H, 4.48; N, 20.20.

Reaction of Adenosine (19) with Glycidaldehyde (1) at pH 5. Adenosine (19) (0.280 g, 1.0 mmol) was allowed to react with glycidaldehyde (1) (0.122 g, 1.7 mmol) for 36 h at pH 5. Separation yielded 0.090 g (0.3 mmol, 30%) of 3-β-D-ribofuranosyl-7-(hydroxymethyl)-3*H*-imidazo[2,1-*i*]purine (20) as blunt transparent crystals: mp 214–216 °C; UV (H_2O) λ_{max} 231 nm (ϵ 2.9×10^4), 268 (6.7×10^3), 279 (6.4×10^3), 300 (3.0×10^3); mass spectrum, *m/z* (relative intensity) 321 (M^+, 4.2), 189 ("base" + H, 100.0), 188 ("base", 22.9), 172 ("base" + H − OH, 91.8), 135 (50.7), 133 (14.7); ^{1}H NMR (Me_2SO-d_6) δ 3.67 (m 2 H), 4.00 (m, 1 H), 4.22 (t, 1 H, *J* = 4.4 Hz), 4.61 (t, 1 H, *J* = 4.9 Hz), 4.91 (s, 2 H), 5.1–5.5 (brs, 4 H), 6.08 (d, 1 H, *J* = 5.4 Hz), 7.48 (s, 1 H), 8.59 (s, 1 H), 9.15 (s, 1 H).

Anal. Calcd for $C_{13}H_{15}N_5O_5$: C, 48.60; H, 4.71; N, 21.80. Found: C, 48.70; H, 4.83; N, 21.69.

Reaction of Adenosine (19) with 2,3-Epoxybutanal (2) at pH 5. Adenosine (19) (0.282 g, 1.1 mmol) was stirred for 48 h with 0.094 g (1.1 mmol) of 2,3-epoxybutanal (2). Separation yielded 0.022 g (0.07 mmol, 6% yield, 7% conversion based on unreacted adenosine) of 21 as fluffy white crystals: mp 219–221 °C; UV (H_2O) λ_{max} 231 nm (ϵ 3.00×10^4), 268 (5.9×10^3), 279 (5.9×10^3), 300 (sh, 3.1×10^3); mass spectrum, *m/z* (relative intensity) 203 ("base" + H, 14.2), 188 ("base" + H − CH_3, 25.0), 159 ("base" − C_2H_4O, 100.0); ^{1}H NMR (Me_2SO-d_6) δ 1.64 (d, 3 H, *J* = 6.4 Hz), 3.68 (m, 2 H), 4.00 (d, 1 H, *J* = 3.4 Hz), 4.19 (m, 1 H), 4.60 (m, 1 H), 5.24–5.05 (m, 3 H), 5.51 (m, 2 H), 6.07 (d, 1 H, *J* = 5.9 Hz), 7.44 (s, 1 H), 8.57 (s, 1 H), 9.19 (s, 1 H).

Anal. Calcd for $C_{14}H_{17}N_5O_5$·H_2O: C, 47.59; H, 5.42; N, 19.82. Found: C, 48.02; H, 5.43; N, 19.25.

Preparation of 9-Ethyl-1,N^6-ethenoadenine-10-carboxaldehyde (23). This compound was prepared as described previously[22] and was obtained in 38% yield as white crystals: mp 223–225 °C; UV (95% ethanol) λ_{max} 230 nm (ϵ 2.06×10^4), 328 (1.51×10^4), 339 (1.50×10^4); mass spectrum, *m/z* (relative intensity) 216 (M^+ + 1, 12.2), 215 (M^+, 100.0), 187 (M^+ − CO, 34.4), 186 (M^+ − C_2H_5, 34.4); ^{1}H NMR ($CDCl_3$) δ 1.62 (t, 3 H), 4.44 (q, 2 H), 8.13 (s, 1 H), 8.37 (s, 1 H), 10.02 (s, 1 H), 10.08 (s, 1 H).

Reduction of 9-Ethyl-1,N^6-ethenoadenine-10-carboxaldehyde (23). To a solution of 0.042 g (1.1 mmol) of $NaBH_4$ in 20 mL of cold ethanol was added 0.052 g (0.24 mmol) of 9-ethyl-1,N^6-ethenoadenine-10-carboxaldehyde (23). The reaction was stirred for $^1/_2$ h at room temperature and then the solvent removed in vacuo. Separation on silica gel preparative layer plates with 13% MeOH/$CHCl_3$ yielded 0.023 g (0.11 mmol, 46%) of 3-ethyl-7-(hydroxymethyl)-3*H*-imidazo[2,1-*i*]purine (22) as off-white crystals: mp 195 °C dec; UV (H_2O) λ_{max} 233 nm (ϵ 2.7×10^4), 269 (6.5×10^3), 279 (9.2×10^3), 300 (3.9×10^3); mass spectrum *m/z* (relative intensity) 218 (M^+ + 1, 6.7), 217 (M^+, 50.4), 200 (M^+ − OH, 100.0), 172 (39.4); ^{1}H NMR (Me_2SO-d_6) δ 1.48 (t, 3 H, *J* = 7.3 Hz), 4.35 (q, 2 H, *J* = 7.3 Hz), 4.91 (m, 2 H), 5.34 (m, 1 H), 7.44 (s, 1 H), 8.33 (s, 1 H), 9.11 (s, 1 H).

Anal. Calcd for $C_{10}H_{11}N_5O$·H_2O: C, 51.05; H, 5.57; N, 29.77. Found: C, 51.22; H, 5.70; N, 29.13.

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Registry No. 1, 765-34-4; 2, 3209-33-4; 3, 1122-47-0; 4, 65-46-3; 5, 99310-29-9; 6, 99310-30-2; 7, 99310-31-3; 8, 4401-11-0; 9, 99310-32-4; 10, 90754-37-3; 14, 118-00-3; 17, 89647-27-8; 18, 62462-38-8; 19, 58-61-7; 20, 99310-33-5; 21, 99310-34-6; 22, 99310-35-7; 23, 91898-80-5.

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PAPERS

Photoinduced Alkylthiolation of Halogenated Purine Nucleosides

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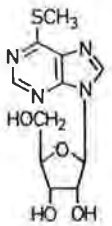
A new and highly efficient methodology for the synthesis of biologically important methylmercaptopurine nucleosides is described. The approach represents a substantial improvement over earlier reported methods for this class of compounds.

A number of thioalkylated purine nucleosides have been found to have interesting biological activity. For example, 6-methylmercapto-9β-(D-ribofuranosyl)-purine (**1**) is one of the most potent inhibitors of *de novo* purine synthesis[1]. It is readily phosphorylated by adenosine kinase and incorporated into RNA and DNA[2]. The 5'-monophosphate of **1** specifically inhibits amidophosphoribosyltransferase[3]. Both compound **1** and its 2-amino analogue exhibit antitumor activity[4,5]. 2-Methylmercaptoadenosine analogues are excellent aggregators of mammalian blood platelets[6].



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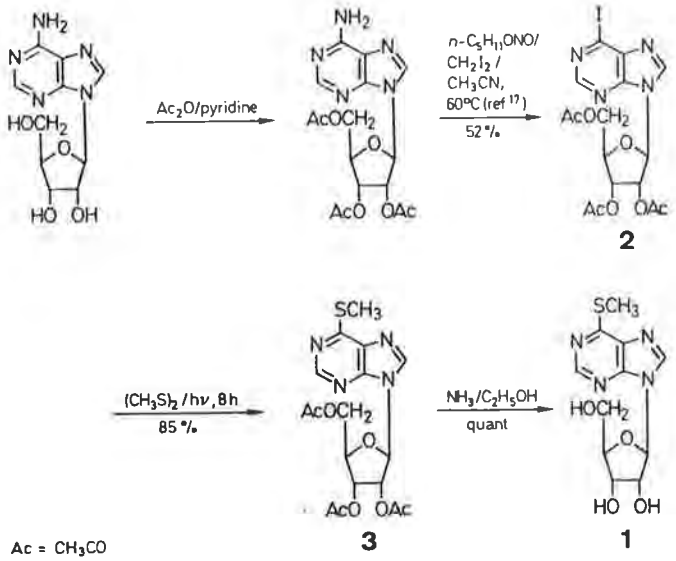
Previous syntheses of 6-methylmercaptopurine nucleosides involved conversion of protected inosine and guanosine to the 6-thio compound. Deprotection followed by alkylation with methyl iodide and base gave the desired product (e.g. **1** and **5b**) generally in low overall yields[5,7-11]. The syntheses of 2-methylmercapto- and 2,6-dimethylmercaptopurine nucleosides have been achieved previously but in very low yields (< 5%) from ring closure of appropriate imidazole derivatives with carbon disulfide, followed by alkylation with methyl iodide[12-16]. We report a new, highly efficient and reproducible approach to the synthesis of thioalkylated purine nucleosides.

In the synthesis of 6-methylmercapto-9β-(D-ribofuranosyl)-purine (**1**), adenosine served as the starting material. It was selectively acetylated with acetic anhydride and pyridine and then converted to the 6-iodo compound **2** by reaction with *n*-pentyl nitrite and diiodomethane in acetonitrile at 60 °C. This deamination-halogenation procedure is a modification of one reported previously by us[17]. Photolysis of a nitrogen-purged solution of **2** in dimethyl disulfide (or in dimethyl disulfide dissolved in acetonitrile) in a Hanovia photochemical apparatus with irradiation from a Vycor-sleeved 450 W mercury lamp for 8 h, resulted in a clean conversion to **3** (85% yield of pure product, Table). Deprotection of **3** with ethanolic ammonia proceeded smoothly and almost quantitatively (Scheme A). Compound **1** was purified by reversed-phase H.P.L.C. on Amberlite XAD-4 resin. The overall yield from adenosine was about 40% (Table).



Scheme A

Guanosine served as the starting material for the synthesis of 2,6-disubstituted purine nucleosides in which at least one position contained a methylmercapto functionality. It was converted to **4** by selective acetylation followed by reaction with phosphoryl chloride and *N,N*-dimethylaniline[18] (Scheme B). Photolysis of **4** in dimethyl disulfide or in dimethyl disulfide dissolved in acetonitrile gave **5a** in 57% yield after purification. The overall yield from guanosine after deprotection (33%) is a significant improvement over previously reported procedures (Table)[5,9,11].

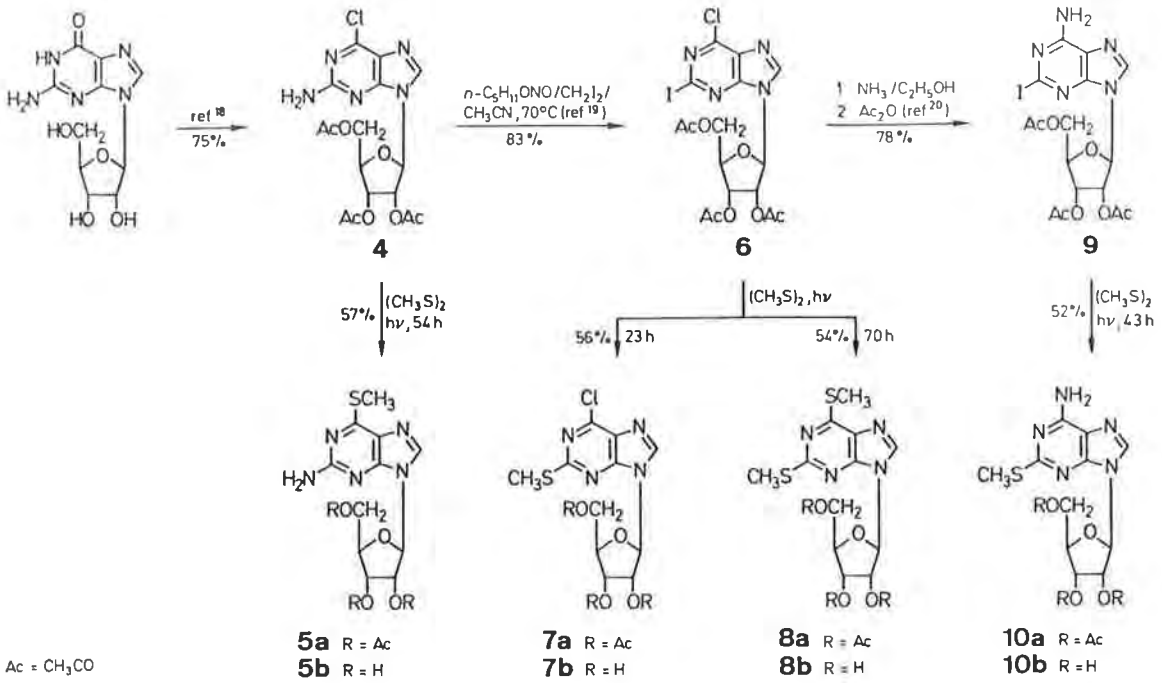
Treatment of **4** with n-pentyl nitrite, diiodomethane, and acetonitrile at 70 °C for 3 h gave the protected dihalogenated nucleoside **6**[19]. Controlled photolysis of **6** in the presence of dimethyl disulfide gave **7a**. On prolonged photolysis, both halogens in the dihalogenated nucleoside **6** were replaced and 2,6-dimethylmercaptopurine nucleoside **8a** was produced in good yields. These alkylthiolations were monitored by mass spectrometry.

The importance of 2-alkylmercaptoadenosine analogues in blood platelet studies and the cumbersome synthetic methods available for these analogues[6,12-16], led us to investigate a possible approach to these compounds through

Table. Experimental and Physical Data for Methylmercaptopurine Nucleosides

Starting Material	Procedure (Time)	Product	Yield [%]	R_f*	M.S. *m/e* (rel. int. %)	U.V. (C_2H_5OH) λ_{max} (ε)	^{1}H-N.M.R. ($CDCl_3$) δ [ppm]	^{13}C-N.M.R. ($CDCl_3$) δ [ppm]	Deprotection Product	m.p. [°C] found	m.p. [°C] reported	Overall Yield [%]
2	A (8 h)	3	85	0.66 (70)	424 (M^+, 2.3); 259 ($Sugar^+$, 25.2); 167 (30.4); 166 (17.2); 165 (Pur^+, 5.4); 157 (13.7); 139 (100.0)	290 (1.5×10^4); 282 (1.5×10^4); 215 (1.0×10^4)	2.08 (s, 3H); 2.12 (s, 3H); 2.15 (s, 3H); 2.72 (s, 3H); 4.78–4.40 (m, 3H); 5.70 (t, 1H); 6.00 (t, 1H); 6.25 (d, 1H); 8.20 (s, 1H); 8.73 (s, 1H)	11.7; 20.3; 20.5; 20.7; 63.0; 70.6; 73.0; 80.3; 86.4; 131.8; 141.1; 147.8; 152.1; 162.1; 169.2; 169.5; 170.2	**1**	160–162°	163–164°[7]	40
4	A (54 h)	5a	57	0.68 (100)	440 (3.6); 439 (M^+, 13.8); 259 ($Sugar^+$, 20.3); 210 ($Pur^+ + CH_2O$, 3.0); 183 3.0); 182 (15.8); 181 (36.4); 180 (Pur^+, 6.2); 157 (14.7); 139 (100.0)	308 (1.0×10^4); 244 (1.4×10^4); 216 (1.4×10^4)	2.08 (s, 3H); 2.12 (s, 3H); 2.13 (s, 3H); 2.62 (s, 3H); 4.40 (m, 3H); 5.03 (s, 2H); 5.78 (t, 1H); 5.91 (t, 1H); 6.01 (d, 1H); 7.78 (s, 1H)	11.6; 20.4; 20.6; 20.7; 63.0; 70.5; 72.8; 79.8; 86.2; 125.9; 138.3; 149.8; 159.0; 162.6; 169.3; 169.6; 170.5	**5b**	184–185°	183°[5]	33
6	B (23 h)	7a	27 (56 net)	0.45 (70)	460 (M^+, 1.3); 458 (M^+, 3.1); 259 ($Sugar^+$, 35.1); 203 (3.0); 202 (2.5); 201 (9.3); 200 (4.8); 199 (Pur^+, 2.8); 157 (10.7); 139 (100.0)	304.5 (7.1×10^3); 263 (1.1×10^4); 234.5 (1.5×10^4)	2.09 (s, 3H); 2.10 (s, 3H); 2.15 (s, 3H); 2.65 (s, 3H); 4.39 (m, 3H); 5.65 (t, 1H); 5.99 (t, 1H); 6.08 (d, 1H); 8.13 (s, 1H)	14.8; 20.4; 20.5; 20.7; 62.7; 70.1; 72.9; 80.0; 87.1; 129.0; 142.3; 151.2; 151.9; 167.3; 169.2; 169.4; 170.2	**7b**	179–181°	181°[21]	13 (28 net)
6	A (70 h)	8a	54	0.50 (100)	470 (M^+, 10.4); 259 ($Sugar^+$, 20.6); 241 ($Pur^+ + CH_2O$, 2.2); 213 (15.6); 212 (20.0); 211 (Pur^+, 2.6); 199 (1.3); 197 (3.6); 165 ($Pur^+ - SCH_2$, 3.0); 157 (11.3); 139 (100.0)	306 (7.2×10^3); 260 (1.5×10^4); 226 (8.0×10^3)	2.09 (s, 3H); 2.14 (s, 6H); 2.64 (s, 3H); 2.69 (s, 3H); 4.38 (m, 3H); 5.68 (t, 1H); 6.00 (t, 1H); 6.11 (d, 1H); 7.95 (s, 1H)	14.1; 14.7; 20.4; 20.5; 20.7; 62.9; 70.3; 73.0; 80.0; 86.7; 129.1; 139.9; 148.7; 162.0; 166.1; 169.2; 169.4; 170.3	**8b**	148–151°	150–155°[15]	28
9	A (43 h)	10a	52	0.56 (100)	441 (0.8); 440 (2.1); 439 (M^+, 8.5); 259 ($Sugar^+$, 11.2); 210 ($Pur^+ + CH_2O$, 5.7); 183 (4.1); 182 (22.4); 181 (43.8); 180 (Pur^+, 7.1); 157 (10.7); 139 (100.0)	273 (1.1×10^4); 236 (1.9×10^4)	2.07 (s, 3H); 2.10 (s, 3H); 2.13 (s, 3H); 2.57 (s, 3H); 4.36 (m, 3H); 5.74 (t, 1H); 6.15–5.85 (m, 4H); 7.79 (s, 1H)	14.5; 20.4; 20.5; 20.7; 62.9; 70.2; 73.0; 79.6; 87.1; 117.6; 138.1; 150.2; 155.0; 166.7; 169.3; 169.4; 170.4	**10b**	222–224°	222–223°[22]	20

* On silica gel; value in brackets is the % of ethyl acetate in hexane.



Scheme **B**

a photoinduced alkylthiolation. The dihalogenated nucleoside **6** was converted to protected 2-iodoadenosine **9** by treatment with ethanolic ammonia at ice/salt bath temperatures followed by reacetylation of the product with acetic anhydride and pyridine[20]. Photolysis of **9** in the presence of dimethyl disulfide gave **10a** (52%) which was easily deprotected and purified by H.P.L.C. on Amberlite XAD-4 resin. This procedure is far superior (five to ten times in overall yields) to previously used methods involving ring closure of imidazole derivatives.

In summary, we have developed a highly efficient methodology for the synthesis of methylmercaptopurine nucleosides. The utility of the procedure was demonstrated by the syntheses of five known methylmercaptopurine compounds. In each case the overall yield was significantly improved. The methodology has generality and can be extended to include a wide variety of thioalkylated heterocyclic systems.

Melting points are uncorrected and were determined on a Thomas-Hoover melting point apparatus fitted with a microscope. Nuclear magnetic resonance spectra were recorded on JEOL Model FX90Q and Bruker Model WM360 Pulse Fourier transform spectrometers. Mass spectra at 30 eV were obtained on a Hewlett-Packard 5985 G.C.-mass spectrometer. Preparative layer chromatography employed EM silica gel PF254 plates activated for 3 h at 135°C.

Photoinduced Thioalkylation; Procedure A:
A solution of the halogenated nucleoside (0.4 mmol) in dry dimethyl disulfide (60 ml) [or in dry dimethyl disulfide (2 ml) and acetonitrile (40 ml)] is transferred to the pyrex immersion well of a quartz photochemical reactor. The system is purged with nitrogen, and photolysis is carried out using a 450 W mercury U.V. source with a Vycor glass filter. When the photolysis is completed, the solvent is removed under reduced pressure (50°C bath temperature). The residue is purified by preparative layer chromatography with ethyl acetate/hexane as the developing solvent.

Photoinduced Thioalkylation; Procedure B:
A solution of the halogenated nucleoside (0.4 mmol) in dry dimethyl disulfide (60 ml) [or in dry dimethyl disulfide (2 ml) and acetonitrile (40 ml)] is transferred to a quartz tube, and purged with nitrogen. Photolysis is carried out in a Rayonet photochemical reactor using light with the principal wavelength of 253.7 nm. The reaction is worked up and purified as described in Procedure A.

6-Iodo-9β-(2′,3′,5′-tri-*O*-acetyl-D-ribofuranosyl)-purine (2):
This product is prepared from adenosine in 52% yield using a modification of a procedure previously described by us[17].

2-Amino-6-chloro-9β-(2′,3′,5′-tri-*O*-acetyl-D-ribofuranosyl)-purine (4):
This compound is prepared from guanosine in 75% yield by established literature procedures[18].

2-Iodo-6-chloro-9β-(2′,3′,5′-tri-*O*-acetyl-D-ribofuranosyl)-purine (6):
This dihalogenated nucleoside is prepared in 83% yield by treatment of **4** thermally with *n*-pentyl nitrite, diiodomethane, and acetonitrile[19].

2-Iodo-6-amino-9β-(2′,3′,5′-tri-*O*-acetyl-D-ribofuranosyl)-purine (9):
This compound is prepared in 78% yield by treatment of **6** with enthanolic ammonia followed by reacetylation[20].

Deprotection of Alkylthiolated Nucleosides; General Procedure:
To dry ethanol (50 ml) saturated with ammonia gas at ice/salt bath temperatures is added the protected nucleoside (0.2 mmol). The solution is stirred at this temperature for 1 h and then at 25°C for 23 h. The solvent is removed under reduced pressure and the residue is purified by reversed-phase H.P.L.C. on Amberlite XAD-4 resin using 60% water/ethanol as the eluting solvent. The deprotected nucleosides are crystallized from water to give the following known nucleosides in ~80–90% yields: 6-methylmercapto-9β-(D-ribofuranosyl)-purine **(1)**[7], 2-amino-6-methylmercapto-9β-(D-ribofuranosyl)-purine **(5b)**[5], 2-methylmercapto-6-chloro-9β-(D-ribofuranosyl)-purine **(7b)**[21], 2,6-dimethylmercapto-9β-(D-ribofuranosyl)-purine **(8b)**[15], and 2-methylmercapto-6-amino-9β-(D-ribofuranosyl)-purine **(10b)**[22].

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Stereocontrolled Total Synthesis of Lipoxins B

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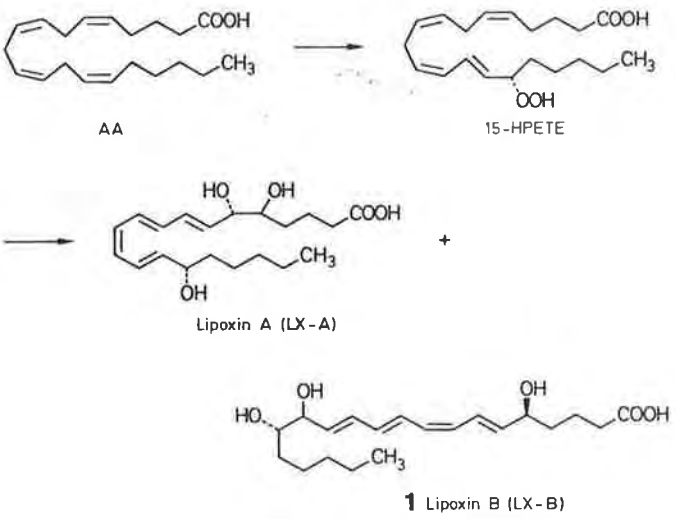
A stereocontrolled total synthesis of six lipoxin B isomers are described. The flexible and stereoselective strategy involves Sharpless asymmetic epoxidation and pinylborane asymmetic reduction to secure the three hydroxyl-bearing stereocenters and a Wittig-type as well as palladium(0)-copper(I) coupling reactions to construct the carbon skeleton of the target molecules.

Introduction

Recently, a new class of linear trihydroxy eicosanoids with a conjugated tetraene system in their structure, was reported by Samuelsson's group[1,2]. This novel family of oxygenated compounds were formed in human leukocytes from arachidonic acid (AA) via 15*S*-hydroperoxy-5,8,11,13-eicosatetraenoic acid (15-HPETE)[1,2]. The two major compounds of this pathway were proposed as 5,6,15*S*-trihydroxy-7,9,11,13-eicosatetraenoic acid and 5*S*,14,15*S*-trihydroxy-6,8,10,12-eicosatetraenoic acid and named lipoxin A (LX-A) and lipoxin B (LX-B), respectively (Scheme A)[1,2]. These partial structural assignments were based on biosynthetic considerations and degradation experiments and left open the question of configuration at C-5 and C-6 for LX-A and C-14 for LX-B. Furthermore, the geometry of the double bonds, particularly those indicated as *Z* was tentative. In view of these remaining uncertainties, the interesting biological properties attributed to lipoxins, and their extreme scarcity from biosynthetic sources their total synthesis was undertaken. The total synthesis of the lipoxin A family has recently been completed in these[3] and other laboratories[4,5]. Furthermore, preliminary communications on the synthesis[6,7] and structural assignment[7] of lipoxin B have also appeared. In this article we describe a total and stereocontrolled total synthesis of a series of lipoxins B including those isomers identified by Leblanc et al.[7] as composing the naturally derived material.



Scheme A: Biosynthesis of lipoxins A (LX-A) and B (LX-B)

Strategy

Since biogenetic considerations and Samuelsson's structural studies left the questions of geometry at Δ^8 and of configuration at C-14 of lipoxin B as the main stereochemical ambiguities, we designed our strategy with flexibility to vary these parameters. The expectation of ready isomerization of an 8*Z* to an 8*E* double bond led us to target the 8*Z* isomers of these compounds as the initial goals. Furthermore, utilization of the Sharpless asymmetric epoxidation reaction was expected to provide the necessary flexibility for producing the requisite hydroxy compounds as well as other desired

The Chemistry of Lipid Peroxidation Metabolites: Crosslinking Reactions of Malondialdehyde

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Malondialdehyde reacts readily with amino acids to form adducts containing vinylogous amidine linkages. Crosslinking reactions between nucleic acid bases and amino acids induced by malondialdehyde also have been investigated. The physical data obtained for the adducts provide structural information on the possible mode of crosslinking of proteins and nucleic acids induced by this lipid metabolite.
Lipids 21, 6-10 (1986).

The ubiquitous natural compound malondialdehyde (MDA) is produced in substantial quantity in mammalian tissues both as an end product of unsaturated lipid peroxidation and as a side product of prostaglandin and thromboxane biosynthesis (1-4). It also is produced in the γ-irradiation of carbohydrates (5). The measurement of MDA by the thiobarbituric acid test has been used commonly as a method for the detection of peroxidation of unsaturated fatty acids and in the estimation of oxidative rancidity in foods (2,3,6). We have reported recently on a detailed and unambiguous assignment of the structure of this adduct (7). MDA has been reported to be toxic (8,9), carcinogenic (8,10) and mutagenic (10-12). This reported degenerative chemistry of MDA may be the result of its ability to covalently bond and to crosslink a variety of biological macromolecules. For example, MDA is reactive towards nucleic acids, resulting in the loss of their template activity (13,14). Also, it has been suggested that MDA-induced modification of lipoproteins may play a role in atherosclerosis (15-17). Valuable information on both the reactive sites and the structural nature of modification can be obtained through investigation of model systems that represent the vulnerable components. We have shown previously that MDA reacts rapidly at the α-amino group of amino acids to form 1:1 adducts (18), and that reaction occurs with adenine and cytosine bases to form hypermodified products (19). This paper reports some model crosslinking reactions of MDA.

MATERIALS AND METHODS

Melting points are uncorrected and were determined on a Thomas-Hoover melting point apparatus fitted with a microscope. The ^1H and ^{13}C NMR spectra were recorded on a Bruker WM-360 or on a JEOL FX-90Q pulse Fourier transform nuclear magnetic resonance (NMR) spectrometer. Mass spectra were determined on a Hewlett-Packard 5985 gas chromatography/mass spectroscopy (GC/MS) instrument. Ultraviolet data were obtained on a Varian-Cary Model 219 UV-Visible spectrophotometer. Fluorescence spectra were recorded on an SLM-Aminco SPF-500C instrument. Elemental analyses were performed by Galbraith Laboratories. Amino acid derivatives and adenine were purchased from Sigma Chemical Co. (St. Louis, Missouri). MDA *bis*-dimethylacetal was purchased from Aldrich Chemical Co. (Milwaukee, Wisconsin) and 2-methyl-3-ethoxyprop-2-enal from Fluka Chemical Co. (Hauppauge, New York). 9-Ethyladenine was prepared as described previously (20).

Sodium MDA was prepared from MDA *bis*-dimethylacetal as described previously (18), except that after hydrolysis the reaction mixture was basified to pH 10 before work up.

Sodium methylmalondialdehyde (sodium MMDA) was prepared from commercial 2-methyl-3-ethoxyprop-2-enal (18).

General procedure for formation of 2:1 adducts from amino acids and MDA and MMDA. In an oven-dried flask with a condenser and a nitrogen bubbler were placed the amino acid methyl ester hydrochloride (2.0 mmol) and sodium MDA or sodium MMDA (1.0 mmol) in dry methanol (10 ml). The reaction mixture was heated under reflux in a nitrogen atmosphere and the progress of reaction was followed by UV spectroscopy. The solvent was removed under reduced pressure and the residue was triturated with 20 ml of 20% CH_3OH/CH_2Cl_2. The precipitated NaCl was removed by filtration and the filtrate was concentrated. The resulting residue was crystallized from CH_3OH/CH_2Cl_2. All adducts gave satisfactory elemental analyses.

Preparation of 9-ethyladenine enaminal 6. 9-Ethyladenine hydrochloride (1.170 g, 5.85 mmol) in dry methanol (65 ml), was treated with sodium MDA (0.657 g, 5.87 mmol). The mixture was heated (60 C under N_2) and progress of reaction was monitored by UV spectroscopy (19). The reaction was terminated when the 322 nm to 260 nm peak ratio had maximized (about 15 hr). The solvent was removed under reduced pressure and the residue was chromatographed on silica gel plates using 8% CH_3OH/CH_2Cl_2 as the eluent. The band at R_f 0.48 afforded 0.162 g (13%) of 6 as yellow crystals: mp 148-149 C; UV (EtOH) λ max 322 nm (ϵ 42114), 243 nm (ϵ 8810), 223 nm (ϵ 10934); ^1H NMR (Me_2SO-d_6) δ 11.30 (d, 1H, J = 11.5 Hz), 9.41 (d, 1H, J = 8.7 Hz), 8.77 (dd, 1H, J = 11.5, 13.4 Hz), 8.55 (s, 1H), 8.52 (s, 1H), 5.98 (dd, 1H, J = 8.7, 13.4 Hz), 4.28 (q, 2H), 1.45 (t, 3H); ^{13}C NMR (Me_2SO-d_6) δ 191.2, 151.6, 151.3, 149.1, 148.9, 143.6, 120.2, 38.4, 15.0; mass spectrum, m/z (relative intensity) 217 (M^+, 11.3), 189 (M^+ - CHO, 20.6), 188 (100), 160 (29.4), 148 (4.8), 135 (10.3), 120 (purine, 4.2), 119 (10.7). Anal. Calcd. for $C_{10}H_{11}N_5O$: C, 55.29; H, 5.10; N, 32.24. Found: C, 55.64; H, 5.27; N, 31.97.

Reaction of 9-ethyladenine enaminal 6 *with glycine methyl ester hydrochloride.* In an oven-dried flask fitted with a condenser and nitrogen bubbler was placed glycine methyl ester hydrochloride (0.093 g, 0.74 mmol) in dry methanol (22 ml). To this was added 9-ethyladenine enaminal (0.160 g, 0.74 mmol) and 3Å molecular sieves. The reaction mixture was heated under reflux with stirring and under a nitrogen atmosphere. Aliquots were removed

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periodically for kinetic analysis by UV spectral methods. Isolation of 7 in a completely pure form was difficult because of its instability, but strong evidence for its formation came from UV, mass spectral and high-field ^1H NMR data.

RESULTS AND DISCUSSION

Crosslinking of proteins by MDA has been reported to occur within 24 hr of reaction (21-23). However, characterization of the products of such modification remains incomplete. The investigation of the structural details of crosslinking of amino acid residues induced by MDA therefore was undertaken as models of protein modification by this natural multifunctional compound. The choice of amino acids for this study was based on several considerations. Initially, the reaction of glycine methyl ester was examined, as its structural simplicity facilitated isolation and identification of the adduct. Lysine, tyrosine, histidine and arginine were chosen because they represent amino acids that were most consistently altered by MDA in studies involving enzymes and other proteins (21-23). These amino acids also have reactive sites at positions other than the α-amino group. Studies with lysine were particularly important as the only primary amino group in protein structures apart from the N-terminal α-amino groups is the ϵ-amino group of lysine. For purposes of comparison, a substituted MDA, MMDA, also was utilized in these studies.

The reactions were carried out by combining solutions of the amino acid methyl ester hydrochloride (two molar equivalents [meq]) and the dialdehyde enolic sodium salt (one meq) in dry methanol (Scheme I). The reactions were monitored by UV spectroscopy. The disappearance of MDA and the appearance of the vinylogous amidine were monitored at their absorption maxima of ca. 247 and 300 nm, respectively. MMDA reactions were monitored at ca. 252 and 310 nm. The yields, physical properties and UV data of the products are shown in Table 1.

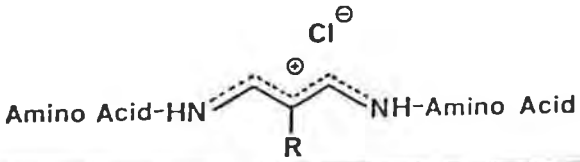
Use of methanol as solvent under acid catalysis provided optimum conditions for conversion to crosslinked adducts in terms of reaction times, product yields and ease of purification. The rates of formation of adducts containing vinylogous amidine linkages were much slower in acetate buffer at pH 4.2, the reported optimum conditions for the reaction of MDA with proteins (18). Considerably faster protein modification by MDA in aqueous buffered systems at pH 4.2 than under the same conditions in these model studies may be due to the presence of a more favorable environment in proteins, not only for crosslinking, but also for survival of the vinylogous amidine linkage. With respect to the latter, our model studies suggest that, once formed, the 1:2 adducts of MDA and amino acids are relatively stable even in aqueous acidic solutions.

Unambiguous evidence for the formation of the adducts 1-5 came from spectral data and elemental analysis. The mass spectral data showed parent ions (minus HCl) for each product. The ^1H and ^{13}C NMR spectra of the products provided insight into their stereochemistry and are summarized in Tables 2 and 3. In all cases for the MDA adducts, the coupling constants ($J \geq 11.0$ Hz) for the vinylogous amidine moiety and the single resonance for H_α and C_α in the ^1H and ^{13}C NMR spectra suggests an all-*trans* or W form for the stereochemistry of these crosslinked adducts. Although unequivocal assignment with respect to the stereochemistry of the vinylogous amidine moiety cannot be made for the MMDA adducts, it is reasonable to assume that the *trans* form predominates for these compounds as well (24).

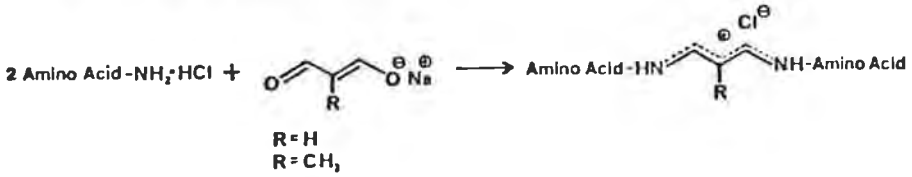
Tappel and coworkers have reported that UV absorp-

TABLE 1

Yields and Physical and Spectral Properties of Vinylogous Amidinium Salts from the Reaction of MDA and MMDA with Amino Acid Derivatives



Compound (amino acid, R)	Yield, %	mp, °C	$UV_{max}(H_2O)(\log \epsilon)$
1a Glycine methyl ester R=H	47	160	298 nm (4.55)
1b Glycine methyl ester R=CH_3	60	98-100	307 nm (4.54)
2a α-N-Acetyllysine, methyl ester R=H	58	Low melting solid	300 nm (4.52)
2b α-N-Acetyllysine, methyl ester R=CH_3	65	Low melting solid	307 nm (4.53)
3a Tyrosine methyl ester R=H	76	92-94	300 nm (4.58)
3b Tyrosine methyl ester R=CH_3	67	93-95	310 nm (4.55)
4a Histidine methyl ester (2HCl) R=H	54	113-115	301 nm (4.55)
4b Histidine methyl ester (2HCl) R=CH_3	60	133-135	310 nm (4.58)
5a Arginine methyl ester (2HCl) R=H	76	85-88	302 nm (4.54)
5b Arginine methyl ester (2HCl) R=CH_3	51	96-98	306 nm (4.57)



SCHEME 1

TABLE 2

H NMR Data for 2:1 Adducts

Compound	Solvent[a]	H_α	H_β	$-CH_3$	$-\overset{O}{\overset{\|}{C}}OCH_3$	H (others)
1a	Me_2SO-d_6	7.90 d, J = 11.7	5.75 t, J = 11.7	—	3.71	4.29 (s, 4H); 9.55 (bs, 2H)
1b	Me_2SO-d_6	7.70 s	—	1.82	3.71	4.32 (s, 4H); 9.10 (bs, 2H)
2a	CD_3OD	7.74 d, J = 11.5	5.64 t, J = 11.5	—	3.71	1.40-1.75 (m, 12H); 2.00 (s, 6H); 2.84 (t, 4H); 4.38 (t, 2H)
2b	CD_3OD	7.62 s	—	2.00	3.71	1.40-1.75 (m, 12H); 2.00 (s, 9H); 2.83 (t, 4H); 4.38 (t, 2H)
3a	CD_3OD	7.45 d, J = 11.3	5.49 t, J = 11.3	—	3.77	3.29 (m, 4H); 4.12 (t, 2H); 6.75 (m, 4H); 6.95 (m, 4H)
3b	CD_3OD	7.23 s	—	1.61	3.82	3.10 (d, 4H); 4.10 (m, 2H); 6.82 (d, 4H); 6.85 (d, 4H)
4a	D_2O*	7.47 d, J = 11.0	6.10 t, J = 11.0	—	3.43	3.51 (m, 4H); 4.52 (m, 2H); 7.38 (s, 2H); 8.68 (m, 2H)
4b	CD_3OD	7.07 s	—	1.50	3.45	3.40 (m, 4H); 4.30 (t, 2H); 7.37 (s, 2H); 8.30 (s, 2H)
5a	D_2O*	7.93 d, J = 11.0	6.20 t, J = 11.0	—	3.92	1.85-2.06 (m, 12H); 3.35 (m, 2H)
5b	CD_3OD	7.50 s	—	1.57	3.72	1.75-2.90 (m, 12H); 3.10 (m, 2H)
7	Me_2SO-d_6	8.69 bd, J = 11.5	6.70 bt, J = 11.5	—	3.72	1.45 (m); 4.24 (m); 4.36 (m); 8.35 (s); 8.41 (s)

Chemical shifts given are with Me_4Si as internal standard (δ=0), except for *, where external Me_4Si was used.

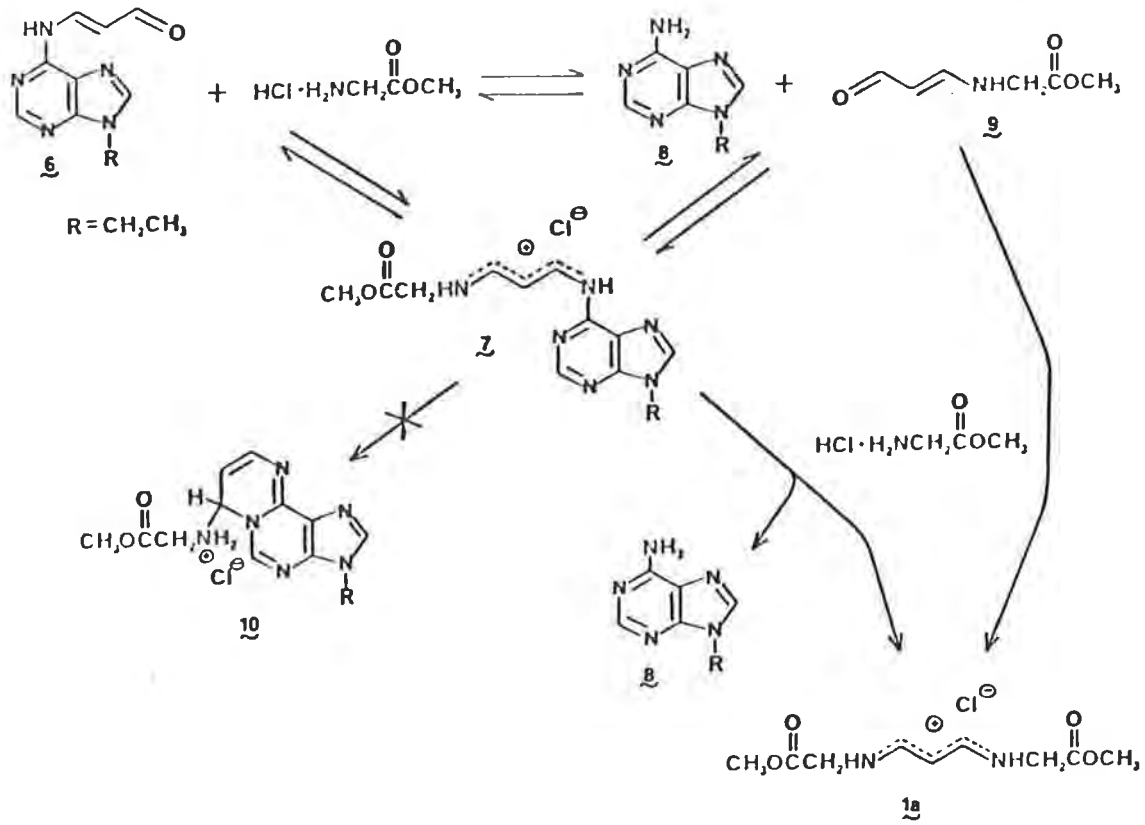
TABLE 3

^{13}C NMR Data for 2:1 Adducts

Compound	Solvent[a]	C_α	C_β	$-CH_3$	$-\overset{O}{\overset{\|}{C}}OCH_3$	C (others)
1a	Me_2SO-d_6	163.3	90.7	—	52.2	44.7; 168.1
1b	Me_2SO-d_6	164.9	101.5	8.3	52.3	48.6; 169.3
2a	CD_3OD	163.4	90.0	—	53.7	22.5; 23.9; 29.9; 32.0; 41.1; 52.6; 173.1; 174.0
2b	CD_3OD	164.3	101.8	7.9	53.7	22.4; 24.0; 29.9; 32.1; 41.2; 52.7; 173.3; 174.1
3a	CD_3OD	158.1	95.5	—	53.1	29.3; 61.7; 115.5; 127.3; 131.2; 157.6; 173.2
3b	CD_3OD	157.6	116.1	7.0	52.6	28.2; 61.0; 116.4; 127.8; 131.2; 157.4; 171.1
4a	D_2O*	166.5	93.4	—	54.5	28.2; 61.8; 118.6; 128.5; 134.8; 171.3
4b	CD_3OD	165.6	104.2	8.3	54.5	27.9; 62.0; 118.6; 129.0; 134.8; 171.4
5a	D_2O*	166.1	93.1	—	54.2	24.9; 28.7; 41.2; 62.3; 157.6; 173.1
5b	CD_3OD	165.1	103.5	8.3	54.2	25.2; 29.1; 41.2; 62.4; 157.5; 173.1

[a]Chemical shifts given are with Me_4Si as internal standard (δ=0), except for *, where internal dioxane (δ=67.4) was used.

ɔn maxima at approximately 256, 285, 370 and 435 nm sult from the products of MDA interaction with proins (21,25). The model crosslinked compounds isolated our work gave UV data inconsistent with these observaɔns. The UV data reported previously were very likely r a mixture of products including those containing vinylɡous amidine linkages. It also should be explained that DA generated in situ from the acid-catalyzed hydrolysis the corresponding *bis*-acetals is contaminated significantly by reactive side products such as β-methoxyacrolein and 3,3-dimethoxypropionaldehyde. We have employed highly purified sodium MDA in this work. Fluorescence spectra of MDA-modified proteins exhibit emission at 440-470 nm with excitation at 370-400 nm (22,25), also indicative of the formation of vinylogous amidines and other linkages. The lysine crosslinked compound 2a showed a fluorescence emission maximum at 440 nm on excitation at 360 nm, with the other adducts giving similar



SCHEME 2

spectra. These data lend further support for the formation of vinylogous amidine linkages in the modification of proteins by MDA.

MDA is reactive towards nucleic acids, resulting in the loss of their template activity (13,14). The modification of nucleic acids may involve direct modification of the bases, crosslinking between the bases or crosslinking between the bases and proteins (26,27). The direct modification of nucleic acid bases by MDA has been reported by us (19). To provide further basic chemical information on the nature and stability of MDA-induced protein-nucleic acid crosslinks, we studied the reactivity of a 9-substituted adenine with MDA in the presence of glycine methyl ester. No adenine-amino acid crosslinking was observed under these conditions and the only product formed was the glycine adduct **1a**. However, in the absence of the amino acid, 9-ethyladenine was converted slowly to its enaminal **6** by reaction with MDA. No crosslinked base pairs could be detected under a wide variety of conditions. However, if the enaminal were allowed to react with an equimolar amount of glycine methyl ester hydrochloride in scrupulously dry methanol, formation of the base-amino acid crosslinked adduct **7** could be detected at 348 nm in the UV spectrum. Quantitative monitoring of the reaction by UV and high-field ^1H NMR spectroscopy showed an initial buildup of **7** to a maximum value of 21% after about one hr. At this point no enaminal **6** remained, and the reaction mixture also contained the glycine adduct **1a** (26.5%) and 9-ethyladenine, **8** (52.5%). After three hr, however, the level of **7** had fallen to 3%, the amount of **1a** had maximized at 35.5% and that of **8** had stabilized at 61.5%. The 2:1 adduct **1a** very likely is formed through the intermediacy of **9**, the initial product of a reaction involving the transfer of the MDA moiety from **6** to glycine. Adduct **1a** also may be produced from the reaction of glycine with **7**, followed by elimination of 9-ethyladenine (Scheme 2).

The crosslinked adduct **7** is much less stable in aqueous solutions than the corresponding amino acid adducts containing vinylogous amidine linkages. However, although adduct **7** could not be isolated in a pure state because of its instability, its structure could be established unambiguously by its UV, high-field NMR and mass spectral data. The mass spectrum showed a parent ion at m/z 288 (M^+-HCl) and appropriate peaks for product fragmentation. The 360 MHz ^1H NMR data showed a doublet at δ8.69 (J = 11.5 Hz) and a triplet at δ6.70 (J = 11.5 Hz) corresponding to H_α and H_β of the diazapentadienium moiety. Resonances due to the amino acid components also were observed (see Table 2). The possibility of cyclization of the crosslinked adduct **7** to give **10** (cf ref. 19) may be ruled out by the UV and NMR data.

In summary, we conclude that MDA is capable of crosslinking amino acids through vinylogous amidine linkages. Additionally, MDA may crosslink amino acids with nucleic acid bases or may transfer the MDA moiety from one to the other. The detrimental biological effects of MDA may be mediated by such modifications.

ACKNOWLEDGMENTS

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Novel Fluorescent 1,4-Dihydropyridines[1]

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Malondialdehyde (MDA) is produced in mammalian tissues as a side product of prostaglandin and thromboxane biosynthesis and, along with other aldehydes, as an end product of unsaturated lipid peroxidation.[2,3] Aldehydes have been implicated in degenerative processes in vivo,[4] and MDA particularly may be of considerable importance physiologically because of its ability to modify and cross-link biological macromolecules.[5-8] Although vinylogous amidine linkages have been suggested as being formed in lipofuscins, a seemingly ubiquitous group of fluorescent pigments which have been linked to aging,[9] the chromophoric component responsible for the fluorescence of lipofuscins or other cross-linked biomolecules[10,11] remains unknown. UV–visible and fluorescence data[12,13] appear to be consistent with the formation of vinylogous amidines as well as highly fluorescent heterocyclic systems of unknown structure. This paper reports on model studies with MDA that involve the isolation and structural characterization of novel heterocyclic adducts of similar UV and fluorescence data as those reported in the aforementioned biological studies.

We have discovered that when MDA (**1**) was allowed to react with amino acids (e.g., glycine methyl ester) under aqueous acidic conditions for prolonged periods (72 h), the UV spectrum shifted gradually from a single absorption at about 250 nm to absorptions

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(1) Dedicated to Professor Nelson J. Leonard on the occasion of his 70th birthday.

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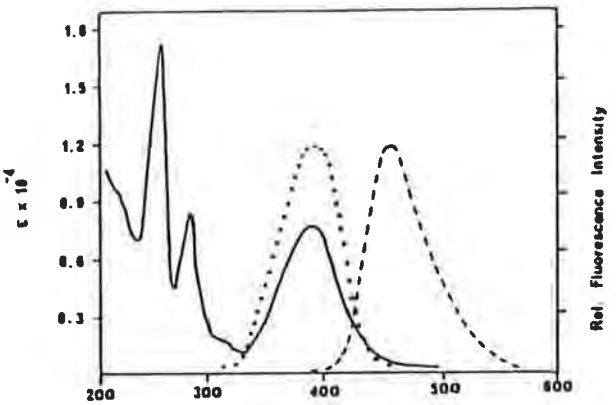
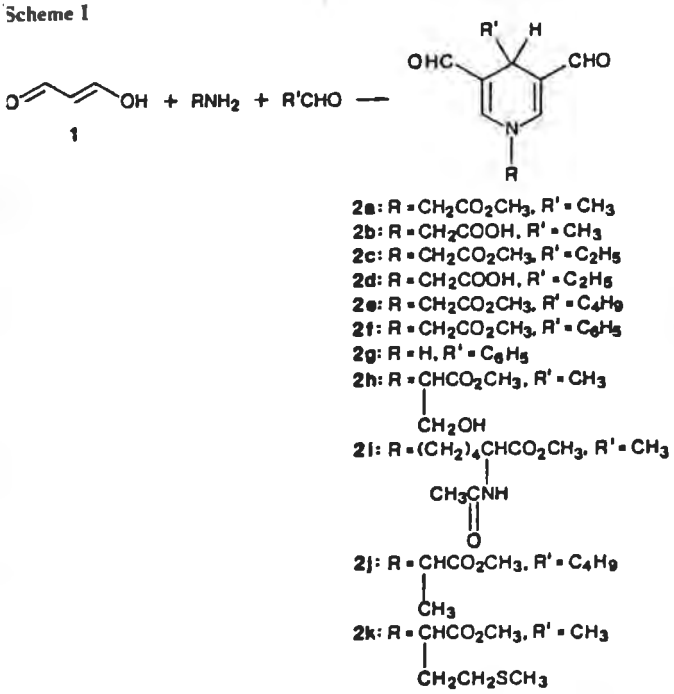


Figure 1. Ultraviolet absorption (—), fluorescence excitation (···), and corrected fluorescence emission spectra (---) of 1,4-dihydropyridine **2a** in water, pH 7.0

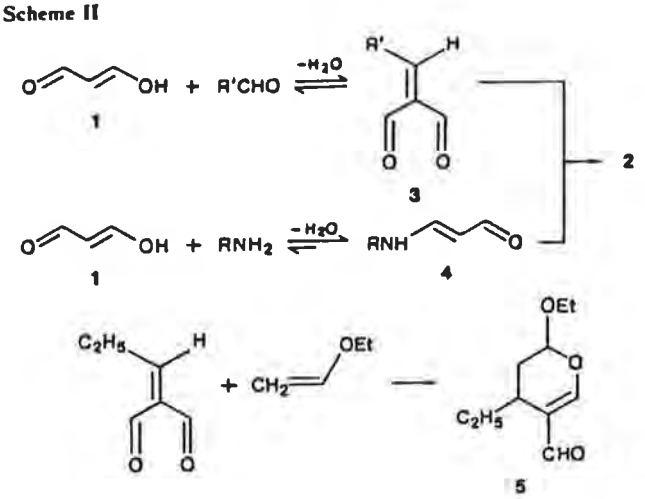
Scheme I



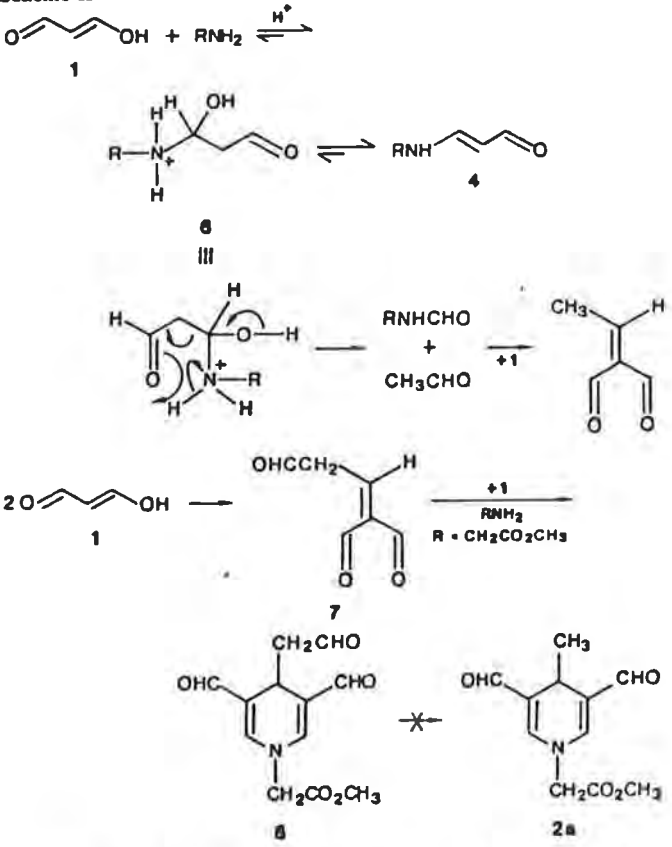
it about 240, 265, and 390 nm. Workup and purification gave ow yields of a product with UV absorptions (in H_2O) at 236 (ϵ = 18 900), 262 (ϵ = 7900), and 384 nm (ϵ = 8800). The compound was highly fluorescent, emitting at 454 nm upon excitation it 386 nm with a relative quantum efficiency (Φ) of 0.36 (Figure 1).[14] The mass spectrum showed a molecular ion at m/z 223. The high-field ^{1}H NMR spectrum (in $CDCl_3$) showed two equivalent aldehyde protons as a singlet at δ 9.30, two equivalent protons as a singlet at δ 6.64, and a multiplet at δ 3.84 integrating for four protons. Additional doublet and singlet resonances integrating for three and two protons at δ 1.14 and 4.20, respectively, were also observed. Taken collectively, the data suggested that the product was the 4-methyl-1,4-dihydropyridine-3,5-dicarboxaldehyde **2a** (Scheme I). Further support for the structures came from the delayed-decoupled 90.6-MHz ^{13}C NMR spectral data, which showed eight distinct resonances with multiplicities consistent with the assigned structure.

The identical product **2a** was obtained in about 50% yield when MDA was allowed to react with glycine methyl ester in the presence of acetaldehyde at pH 4.3[15] for 7 h. The generality of the reaction of MDA with amino acids in the presence of additional aldehydes was established by isolation of related 1,4-dihydropyridines in similar yields from alanine, serine, methionine, and lysine methyl esters with acetaldehyde, propanal, pentanal, and benzaldehyde (Scheme I). Unprotected amino acids can also be used in these reactions (e.g., glycine), but use of the esters makes isolation of products somewhat easier.

Scheme II



Scheme III



It is very probable that alkylidenemalondialdehydes **3** are the intermediates in the mechanism of these transformations (Scheme II). These reactive species[16] are apparently produced in situ from the condensation of MDA with the additional aldehyde and behave as Michael acceptors for the enaminals **4**. The latter are 1:1 adducts formed rapidly from the condensation of MDA with the amino acids.[7] Evidence for the formation and consumption of the enaminals came from UV data (~280 nm) obtained during the course of the reaction and from the formation of the same dihydropyridines from the reaction of isolated enaminals. The intermediacy of the alkylidene MDA was inferred by a Diels–

(14) Relative quantum yield vs. quinine sulfate (0.70) in 0.1 N H_2SO_4. Scott, T. G.; Spencer, R. D.; Leonard, N. J.; Weber, G. *J. Am. Chem. Soc.* 1970, *92*, 687.

(15) The pH was adjusted to just below the pK_a of MDA (4.46).

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Alder trapping experiment[17] using ethyl vinyl ether to yield **5** (Scheme II).

The formation of the 1,4-dihydropyridine **2a** from the reaction of MDA with glycine methyl ester in the absence of added second aldehyde may be rationalized by the mechanism shown in Scheme III, where the very slow formation of acetaldehyde from the thermal cleavage of the amino alcohol (hydrated enaminal) **6** results in the eventual formation of an alkylidene MDA which can be trapped by a second molecule of enaminal. Malondialdehyde itself could behave as the "second aldehyde" in this reaction forming the alkylidene MDA **7** which would then result in the dihydropyridine **8**. However, **8** was not isolated in this reaction. In order to prove that dihydropyridine **2a** was not produced by the in situ decarbonylation of **8**, an authentic sample of **8** was prepared by an alternative route. It was found to be thermally stable under the conditions used to produce the 1,4-dihydropyridines.

We conclude from these initial studies that MDA is able to modify amino acid residues to fluorescent 1,4-dihydropyridines. These findings may be of significance in understanding the biological chemistry of MDA in vivo and may explain some of the spectral disparities reported earlier on the interaction of MDA with amino acids and proteins.[12,13] In addition, some of the dihydropyridines produced in this study may be of interest as fluorescent biological probes of the calcium channel in living systems.[18] Further studies on the dihydropyridines as well as the alkylidene malondialdehydes are in progress.

Acknowledgment. We thank the National Science Foundation for support of this research.

Supplementary Material Available: Tables of NMR (^{1}H and ^{13}C), UV, fluorescence, and mass spectral data for adducts (10 pages). Ordering information is given on any current masthead page.

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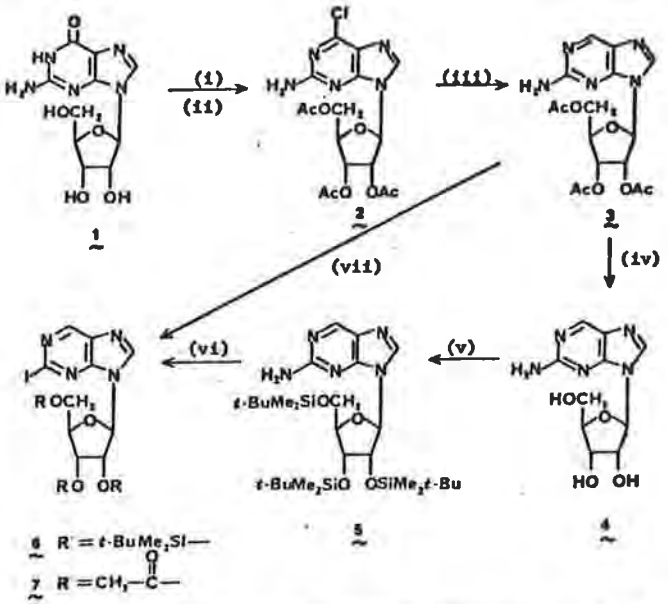
(18) *Calcium Regulation by Calcium Antagonists*; Rahwan, R. G., Witiak, D. T., Eds.; American Chemical Society: Washington, DC, 1982.

Scheme I[a]



[a] (i) Ac_2O, $(C_2H_5)_3N$, *N,N*-(dimethylamino)pyridine; (ii) $POCl_3$, *N,N*-diethylaniline, Δ; (iii) $(C_2H_5)_3N$, THF, *hν*; (iv) NH_3, C_2H_5OH; (v) *t*-Bu$(CH_3)_2$SiCl, imidazole, DMF, Δ; (vi) *n*-$C_5H_{11}ONO$, CH_2I_2, $(CH_3)_3SiI$, hexane, Δ; (vii) *n*-$C_5H_{11}ONO$, CH_2I_2, CH_3CN.

2-Halogenated Purine Nucleosides: Synthesis and Reactivity[1]

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Although considerable attention has been devoted to the synthesis and reactions of 6-halogenated purine nucleosides,[3-7] the same cannot be said for the corresponding 2-halogenated compounds.[8,9] This is in part due to limitations in synthetic accessibility to this class of nucleosides. 2-Halogenated purines are potentially key synthetic intermediates to a variety of novel 2-substituted purine nucleosides. A logical approach to 2-halogenated purines may be via the corresponding 2-amino compound. 2-Amino-9-β-D-ribofuranosylpurine is an important biologically active nucleoside. It is a potent inhibitor of a number of purine metabolizing enzymes including adenosine deaminase,[10] purine nucleoside phosphorylase,[11] and adenosine kinase.[12] It is incorporated in *E. coli* and phage T4 DNA.[13,14] However, an efficient general method for the preparation of this compound is not currently available. Previous syntheses involved, as the key step, the hydrogenolysis of the corresponding 6-chloro compound (protected) using Pd/C and hydrogen, treatment of the protected 6-thio compound with Raney nickel in water, and coupling of a halogenated sugar with protected 2-aminopurine in the presence of mercuric chloride.[9,13,15,16] Photochemical methods are rarely used in nucleoside synthesis. We wish to report a high yielding and reproducible photochemical synthesis of 2-aminopurine nucleoside, its conversion to the corresponding novel 2-halogenated compound, and the synthetic utilization of the latter.

The starting material for the synthesis was guanosine (1) which was selectively acetylated in 93% yield by using acetic anhydride, triethylamine, and 4-(dimethylamino)-pyridine in acetonitrile.[17] Treatment of the triacetylated guanosine with phosphorus oxychloride and *N,N*-diethylaniline at 70 °C for 1 h gave the 6-chloro compound

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(2) Deceased, May 22, 1986.
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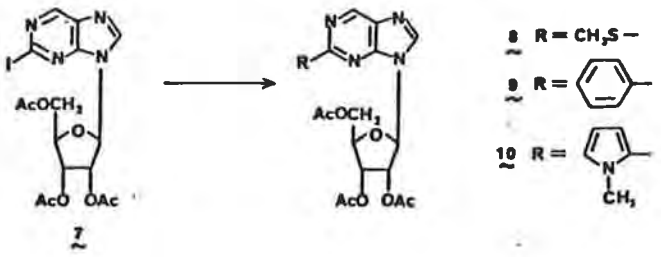
0022-3263/87/1952-1344$01.50/0

Scheme II

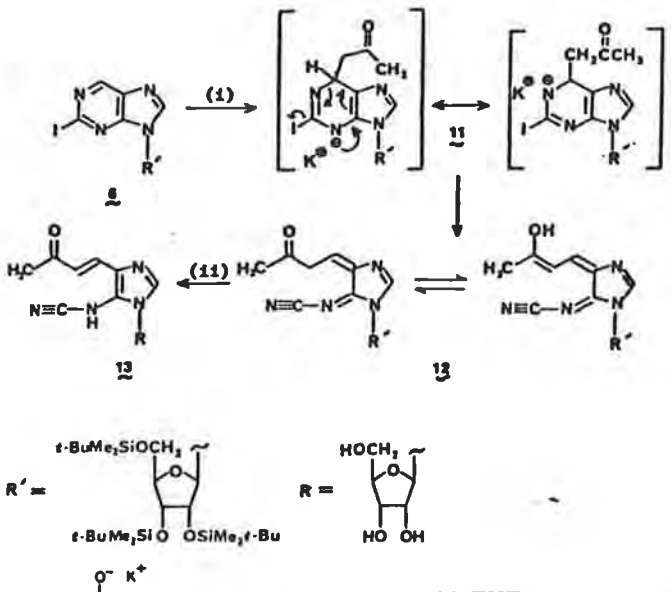


2 in about 89% yield. Photolysis of 2 in dry, nitrogen-purged THF containing 10% triethylamine in a Rayonet photochemical reactor (2537 Å) produced the 2-aminopurine nucleoside 3 in 84% isolated yield (Scheme I). This reaction is a photoinduced reductive dehalogenation and has not been reported previously in purine nucleoside chemistry. A plausible mechanistic interpretation of the conversion is that an exciplex is formed between 2 and triethylamine[18] and that this is followed by electron transfer, carbon–chlorine bond cleavage, and hydrogen atom abstraction from the amine. Support for this mechanism comes from several observations. First, in the absence of triethylamine, 2 is not converted to 3. Second, the reaction does not proceed in the absence of light and is slow at longer wavelengths (e.g., 3500 Å, compound 2 absorbs at 220, 249, and 310 nm). Third, in competition experiments with triethylamine and excess benzene, the only product isolated was 3.

2-Aminopurine nucleosides are excellent potential precursors for a variety of new 2-substituted purines via their 2-halogenated derivatives. Development of a procedure for the conversion of an amino group to a halogen in the purine system has been reported previously by us.[5,19] A modification of this procedure was used for the halogenations described here. Thus, nucleoside 5 (i.e., the silylated derivative of 4) can be converted to the new 2-iodopurine 6 in 67% yield by a deamination–halogenation reaction using *n*-pentyl nitrite, diiodomethane, and trimethylsilyl iodide in hexane. The acetylated 2-aminopurine nucleoside 3 may be converted by a similar procedure (but without trimethylsilyl iodide) to the corresponding 2-iodo compound 7 (Scheme I).

When compound 7 was photolyzed in dry nitrogen-purged dimethyl disulfide in acetonitrile, the novel 2-(methylthio)purine nucleoside 8 was isolated in 61% yield (Scheme II). Nucleoside 7 can participate in photoinduced arylation reactions. For example, photolysis in benzene results in the formation of the protected 2-phenylpurine 9 in 47% yield. Extension to photoinduced heteroarylations is also possible. Thus, photolysis of 6 in the presence of *N*-methylpyrrole for 1 h resulted in the formation of 10 in 75% yield. High-field ^{1}H NMR data (in $CDCl_3$) was used to confirm the regiochemistry of the heteroarylation reaction. The chemical shift of the H-3 proton of the pyrrole ring (δ 7.22) and the coupling constants ($J_{3,4}$ = 3.9 Hz and $J_{3,5}$ = 1.8 Hz) provide strong evidence for reaction at the α-position of the pyrrole ring.[20] The heteroarylated nucleoside 10 shows a bathochromically shifted UV spectrum compared to nebularine with absorption maxima at 332 (ϵ 10 000), 296 (ϵ 10 000), and 242 nm (ϵ 8000). It is a highly fluorescent compound with emission at 444 nm when excited at 339 nm.

Scheme III[a]



[a] (i) $CH_3C(O^-K^+)=CH_2$, THF, −48 °C, *hν*; (ii) Bu_4NF, THF.

Carbon–carbon bond-forming reactions of 2-iodinated purines are also potentially feasible through the $S_{RN}1$ reaction.[21,22] However, when the 2-iodinated purine 6 was photolyzed in the presence of the potassium enolate of acetone in anhydrous THF at −48 °C for 20 min, the expected 2-acetonylpurine derivative was not isolated. Instead, a highly functionalized imidazole 13 was obtained after deprotection (Scheme III). Any plausible mechanism for this transformation would require, as the initial step, the addition of potassium acetone enolate to the 1,6-π-bond of 6 in a reaction related (but not mechanistically similar) to the photoinduced addition of methanol to nebularine.[23] Ring opening[24] and concomitant ejection of iodide in 11 would give 12, which can be isolated and characterized. However, during further manipulations (e.g., deprotection and purification), the latter apparently undergoes a 1,5-sigmatropic hydrogen shift to give the thermodynamically more stable 13. The structure of 13 was deduced from its mass spectrum (M^+ = 308), UV data (309 nm), FTIR spectrum (2125, 1675, 1625 cm^{-1}) and high-field NMR data including delayed decoupling. In the 90.6-MHz ^{13}C NMR spectrum, the C=O (197.1 ppm), C=C (123.1, 137.7 ppm), C≡N (129.8 ppm), and CH_3 (27.3 ppm) groups could be easily discerned. The remaining carbon resonances were those expected for the imidazole and ribose portions of the molecule. The 360-MHz ^{1}H NMR data complimented the ^{13}C NMR information and also established the trans stereochemistry about the C=C bond (J = 15.6 Hz).

In summary, a highly efficient methodology to the 2-aminopurine nucleoside 4 is described. This compound can be easily transformed into the corresponding 2-halogenated purine system which can be converted to thioalkylated, arylated, and heteroarylated purines and to a highly functionalized imidazole nucleoside.

Experimental Section

Irradiations were accomplished in a Hanovia 450-W mercury photolysis apparatus or in a Rayonet photochemical reactor. The melting points provided are uncorrected and were taken on a Thomas-Hoover melting point apparatus fitted with a microscope. Nuclear magnetic resonance spectra using tetramethylsilane as

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an internal standard were recorded on JEOL Model FX90Q and Bruker Model WM360 pulse Fourier transform spectrometers. A Hewlett-Packard 5985 GC/MS system was used for the mass spectra. The ultraviolet spectra were recorded on a Varian Cary Model 219 spectrophotometer. Infrared spectra were recorded on an IBM Model 98 Fourier transform instrument. Satisfactory elemental analyses could not be obtained for the new compounds described because of their instability.

2-Amino-6-chloro-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purine (2)[25] was prepared from guanosine in 83% overall yield by selective acetylation with acetic anhydride, triethylamine, and 4-(dimethylamino)pyridine in acetonitrile,[17] followed by reactio of the product with $POCl_3$ and *N,N*-diethylaniline at 70 °C for 1 h.

2-Amino-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purine (3). A solution of 2 (0.760 g, 1.78 mmol) in triethylamine (60 mL) and tetrahydrofuran (240 mL) was purged with nitrogen and photolyzed in a Rayonet photochemical reactor (2537 Å) for 17 h. The solvent was then removed, and the residue was chromatographed on preparative silica gel plates with ethyl acetate/methanol (19:1) as the eluting solvent. 2-Amino-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purine (3) (0.586 g, 1.5 mmol, 84%) was obtained as tan crystals: mp 142 °C (lit.[16] mp 142–143 °C).

2-Amino-9-β-D-ribofuranosylpurine (4). To 220 mL of dry ethanol saturated with ammonia gas at ice–salt bath temperatures was added 1.817 g (4.62 mmol) of 3. The solution was stirred at this temperature for 1 h and then at 25 °C for 23 h. The solvent was removed under reduced pressure, and the residue was purified by crystallization from H_2O/ethanol to give 1.184 g (4.43 mmol, 96%) of 4: mp 164–166 °C (lit.[15] mp ~165 °C); ^{13}C NMR (Me_2SO-d_6) δ 61.7, 70.7, 73.7, 85.6, 86.6, 127.3, 141.1, 149.6, 153.2, 160.6; ^1H NMR (Me_2SO-d_6) δ 3.59 (m, 2 H), 3.91 (m, 1 H), 4.13 (m, 1 H), 4.51 (m, 1 H), 4.99 (d, 1 H), 5.12 (d, 1 H), 5.41 (d, 1 H), 5.85 (d, 1 H), 6.51 (s, 2 H), 8.29 (s, 1 H), 8.60 (s, 1 H); UV (EtOH) λ_{max} 244 nm (ε 6.6 × 10^3), 308 (ε 7.7 × 10^3).

2-Iodo-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purine (7). To a solution consisting of 15 mL of acetonitrile, 2 mL of diiodomethane, and 4 mL of *n*-pentyl nitrite was added 0.300 g (0.76 mmol) of 3. The solution was protected from moisture and heated under nitrogen at 50 °C for 7 h. The solvent was removed under reduced pressure and the residue was chromatographed on silica gel plates (4:1 ethyl acetate/hexane). The only major band (R_f 0.68) gave 0.133 g (0.26 mmol, 35%) of 7 as a low-melting solid: ^{13}C NMR ($CDCl_3$) δ 20.4, 20.7, 20.8, 63.0, 70.6, 73.3, 80.7, 86.2, 119.5, 134.4, 150.0, 152.0, 169.4, 169.5, 170.2; ^1H NMR ($CDCl_3$) δ 2.10 (s, 3 H), 2.13 (s, 3 H), 2.17 (s, 3 H), 4.43 (m, 3 H), 5.64 (t, 1 H), 5.83 (t, 1 H), 6.24 (d, 1 H), 8.22 (s, 1 H), 8.90 (s, 1 H); ^{15}N NMR (Me_2SO-d_6) δ 177.8 (d, J = 8.2 Hz, N-9), 256.0 (d, J = 12.8 Hz, N-7), 284.3 (s, N-3), 313.5 (d, J = 11.6 Hz, N-1); UV (EtOH) λ_{max} 219 nm (ε 2.0 × 10^4), 247 (ε 7.6 × 10^3), 278 (ε 9.2 × 10^3); mass spectrum, *m/z* (relative intensity) 504 (M^+, 4.0), 259 (sugar$^+$, 52.9), 247 (base + 2 H, 71.9), 246 (Base + H, 6.4).

2-(Methylthio)-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-purine (8). To 10 mL of dry dimethyl disulfide in 40 mL of acetonitrile was added 1.32 g (2.62 mmol) of 7. The solution was purged with nitrogen and photolyzed for 27 h in a quartz Hanovia reactor with a Vycor filter. The solvent was removed under reduced pressure, and the residue was chromatographed on silica gel plates (ethyl acetate). The band at R_f 0.5 gave 0.68 g (1.60 mmol, 61%) of 8 as a yellow oil: ^{13}C NMR ($CDCl_3$) δ 14.7, 20.4, 20.5, 20.7, 62.8, 70.2, 72.9, 79.9, 86.8, 131.6, 142.5, 149.2, 151.7, 167.2, 169.3, 169.4, 170.3; ^1H NMR ($CDCl_3$) δ 2.08 (s, 3 H), 2.10 (s, 3 H), 2.15 (s, 3 H), 2.65 (s, 3 H), 4.39 (m, 3 H), 5.70 (t, 1 H), 6.05 (t, 1 H), 6.16 (d, 1 H), 8.09 (s, 1 H), 8.94 (s, 1 H); UV (EtOH) λ_{max} 231 nm (ε 1.1 × 10^4), 261 (ε 8.6 × 10^3), 305 (ε 5.5 × 10^3); mass spectrum, *m/z* (relative intensity) 424 (M^+, 17.7), 259 (sugar$^+$, 49.1), 167 (base + 2H, 32.6), 166 (base + H, 28.1), 165 (base, 8.5), 139 (100.0).

2-Phenyl-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purine (9). To 70 mL of dry benzene was added 0.081 g (0.16 mmol) of 7. The solution was photolyzed for 6 h as described for 8. Chromatography on silica gel plates (ethyl acetate) afforded (R_f 0.48) 0.034 g (0.077 mmol, 47%) of 9 as a pale yellow oil: ^{13}C NMR ($CDCl_3$) δ 20.4, 20.5, 20.7, 62.6, 70.1, 73.1, 79.9, 86.9, 128.4, 128.6, 128.7, 130.4, 133.1, 137.5, 144.0, 149.1, 151.6, 159.9, 169.3, 169.4, 170.3; ^1H NMR ($CDCl_3$) δ 1.96 (s, 3 H), 2.11 (s, 3 H), 2.19 (s, 3 H), 4.43 (m, 3 H), 5.91 (t, 1 H), 6.17 (t, 1 H), 6.25 (d, 1 H), 7.54–7.47 (m, 3 H), 8.22 (s, 1 H), 8.59–8.48 (m, 2 H), 9.23 (s, 1 H); UV (EtOH) λ_{max} 233 nm (ε 1.2 × 10^4), 272 (ε 1.0 × 10^4), 282 (ε 1.2 × 10^4); mass spectrum, *m/z* (relative intensity) 454 (M^+, 0.8), 259 (sugar$^+$, 19.5), 198 (4.0), 197 (base + 2H, 8.3), 196 (base + 1H, 1.6), 195 (base, 0.4), 139 (100.0).

2-(*N*-Methylpyrr-2-yl)-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purine (10). A solution consisting of 0.078 g (0.16 mmol) of 7 and 70 mL of dry *N*-methylpyrrole was photolyzed for 1 h as described for 8. Chromatography (silica gel plates, ethyl acetate) afforded (R_f 0.70) 0.056 g (0.12 mmol, 75%) of 10 as a light brown low-melting solid: ^{13}C NMR ($CDCl_3$) δ 20.4, 20.5, 20.6, 38.0, 62.7, 70.1, 73.0, 79.9, 86.6, 108.2, 115.3, 128.2, 131.2, 131.5, 142.8, 148.7, 151.2, 155.7, 169.3, 169.4, 170.3; ^1H NMR ($CDCl_3$) δ 2.03 (s, 3 H), 2.10 (s, 3 H), 2.15 (s, 3 H), 4.11 (s, 3 H), 4.40 (m, 3 H), 5.72 (t, 1 H), 6.21–6.04 (m, 3 H), 6.77 (dd, 1 H), 7.21 (dd, 1 H), 8.14 (s, 1 H), 9.07 (s, 1 H); UV (EtOH) λ_{max} 242 nm (ε 8.0 × 10^3), 296 (ε 1.0 × 10^4), 332 (ε 1.1 × 10^4); fluorescence (EtOH) excitation 339 nm and emission 444 nm; mass spectrum, *m/z* (relative intensity) 457 (M^+, 3.6), 259 (sugar$^+$, 2.9), 200 (base + 2H 18.8), 199 (base + H 84.9), 198 (base, 45.5), 45.5), 139 (100.0).

2-Iodo-9-(2,3,5-tris-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl)purine (6). A solution consisting of 2.047 g (7.66 mmol) of 4, 3.843 g (25.50 mmol) *tert*-butyldimethylsilyl chloride, 2.967 g (43.59 mmol) imidazole, and 4 mL of dry DMF was stirred under N_6 at 60 °C for 11 h. The solvent was removed (1 torr), and the residue was dissolved in 75 mL of chloroform and washed with 5 × 100 mL of H_2O. The organic layer was dried (Na_2SO_4), concentrated, and purified by flash chromatography (1:1 ether/hexane) to give 2.911 g (4.77 mmol, 62%) of 5 as a tan oil: mass spectrum, *m/z* (relative intensity) 610 (M^+, 14.6) 580 (96.5), 553 (M^+ – *t*-Bu, 43.4), 552 (100.0).

A solution consisting of 0.274 g (0.45 mmol) of 5, 2.0 mL of diiodomethane, 20 mL of nitrogen-purged hexane, 0.4 mL of trimethylsilyl iodide, and 1.0 mL of *n*-pentyl nitrite was heated under nitrogen at 60 °C for 4 h. The solution was cooled to ambient temperature, diluted with hexane (25 mL), and washed with saturated aqueous sodium sulfite (5 mL). The aqueous layer was extracted with hexane (2 × 20 mL), and the organic layers were combined and evaporated under reduced pressure. Purification by flash chromatography on silica gel (1:3 ether/hexane) gave 0.214 g (0.30 mmol, 67%) of 6 as a yellow oil: ^{13}C NMR ($CDCl_3$) δ –5.7, –5.6, –5.3, –5.1, –5.0, –4.6, 17.5, 17.7, 18.1, 25.4, 25.5, 25.7, 61.6, 70.9, 75.2, 84.8, 88.7, 118.6, 134.3, 144.0, 149.3, 151.5; ^1H NMR ($CDCl_3$) δ 0.17 to –0.13 (m, 18 H), 0.92 (m, 27 H), 4.04–3.80 (m, 2 H), 4.16 (m, 1 H), 4.32 (m, 1 H), 4.61 (m, 1 H), 6.03 (d, 1 H), 8.47 (s, 1 H), 8.87 (s, 1 H); UV (EtOH) λ_{max} 222 nm (ε 2.2 × 10^4), 247 (ε 8.2 × 10^3), 278 (ε 1.0 × 10^4); mass spectrum, *m/z* (relative intensity) 664 (20.1), 663 (M^+ – *t*-Bu, 47.9).

Photoinduced Reaction of 6 with Potassium Acetone Enolate. Nucleoside 6 (0.140 g, 0.19 mmol) was dissolved in dry nitrogen-purged THF and transferred via double-tipped needle to a low-temperature Hanovia photochemical apparatus with Pyrex filter. The solution was cooled to –48 °C. The potassium enolate (1.70 mmol) in THF (15 mL) was transferred via double-tipped needle to the photochemical reactor. The cooled solution was immediately photolyzed with a 450-W mercury lamp for 0.5 h. Excess NH_4Cl in methanol (5 mL) was added to quench the reaction. The mixture was filtered, and the solvent was removed under reduced pressure. Purification on silica gel plates (19:1 $CHCl_3/CH_3OH$) gave 0.09 g (0.14 mmol, 74%) of 12, which was deprotected with tetrabutylammonium fluoride in THF. Compound 13 was obtained as a low-melting solid (0.032 g, 76%) after HPLC on Amberlite XAD-4 resin (4:1 H_2O/C_2H_5OH) and ion-exchange chromatography on Dowex 50-W resin: ^{13}C NMR (Me_2SO-d_6) δ 27.2, 60.7, 69.6, 74.7, 84.6, 87.5, 123.1, 129.8, 129.9, 133.7, 134.5, 156.2, 197.1; ^1H NMR (Me_2SO-d_6) δ 2.21 (s, 3 H), 3.50–4.20 (m, 5 H), 5.42 (d, 1 H), 6.16 (s, 1 H, D_2O exchangeable) 6.59 (d, 1 H, J = 15.6 Hz), 7.29 (d, 1 H, J = 15.6 Hz), 7.96 (s, 1 H); UV (H_2O) λ_{max} 309 nm (ε 8250); FTIR (KBr) 2125, 1675, 1625 cm^{-1}; mass spectrum, *m/z* (relative intensity) 308 (M^+, 3.0), 177 (base$^+$ + 2H, 18.1), 176 (base$^+$ + H, 10.0), 175 (base$^+$, 8.5), 149 (base$^+$ – CN, 100), 133 (sugar$^+$, 21.5).

(25) Gerster, J. F.; Jones, J. W.; Robins, R. K. *J. Org. Chem.* **1963**, *28*, 945.

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MODEL MULTIFUNCTIONAL EPOXIDES RELATED TO HEPOXILIN A[1]

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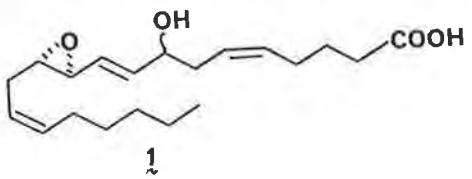
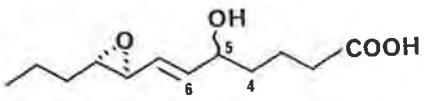
Abstract: An enolic epoxy fatty acid, 8,9-(S,S)-epoxy-5-hydroxy-6-dodecenoic acid, has been synthesized. This compound is a model system for the biologically important compound, hepoxilin A. The model epoxy fatty acid cyclized readily to form an epoxy lactone. Acid-catalyzed ring opening resulted in the formation of isomeric oxadienes which were structurally differentiated by comparison of their 2-D COSY NMR spectra. Thiols cleaved the epoxide ring rapidly and quantitatively.

One of the most abundant polyunsaturated, long chain fatty acids in humans and other mammals is arachidonic acid, a major component of cellular phospholipids. Arachidonic acid is released from membrane stores by calcium-dependent phospholipases and it may then undergo autoxidation[2] or it may be enzymatically oxygenated.[3,4] The 12-lipoxygenase pathway of arachidonic acid oxidation eventually results in the formation of two multifunctional epoxy fatty acids, hepoxilin A(1) and hepoxilin B (2). These compounds have been isolated from rat lung and rat pancreatic islet cells by Pace-Asciak and coworkers and have been shown to potentiate the glucose-dependent release of insulin.[5] It has been suggested that they may also be involved in the mobilization of calcium ions.[6-10]



1



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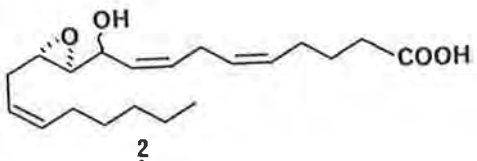
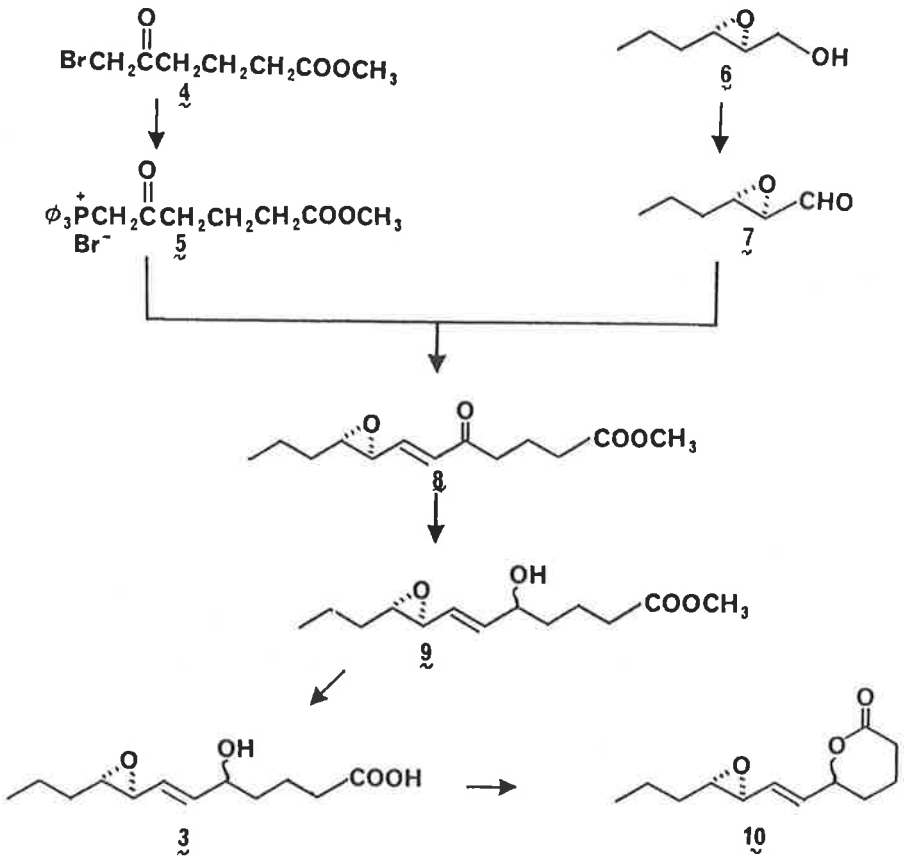


2

The multifunctional epoxide moieties present in the structures of the hepoxilins have been suggested as playing very important roles in their biological activities. In order to contribute to the understanding of the chemistry of these functionalized unsaturated epoxides of lipid origin, we have been investigating the stereospecific synthesis and reactivity of simpler model systems with the same multifunctional nature and stereochemistry. We wish to present the synthesis and cleavage reactions of a simple analogue of hepoxilin A, 8,9-(S,S)-epoxy-5-hydroxy-6-dodecenoic acid (3).

The starting material for the synthesis was methyl-6-bromo-5-oxohexanoate (4), which was prepared from methyl-4-(chloroformyl)butyrate by reaction with diazomethane followed by gaseous HBr.[11] Reaction of the α-bromoketone with triphenylphosphine in toluene produced the phosphonium salt 5 which was properly constituted to couple as its ylide with an aldehyde. The latter, 2,3-(R,S)-epoxyhexanal (7), was easily prepared in two steps from trans- 2-hexen-1-ol. The first step was a Sharpless asymmetric epoxidation to the 2,3-(S,S)-epoxyhexan-1-ol (6).[12] The second step was an oxidation of the epoxy alcohol 6 with pyridinium dichromate[13] to give 2,3-(R,S)-epoxyhexanal (7) in 76% yield. Carbon elongation of the epoxyaldehyde 7 was achieved in 57% yield by reaction with the ylide of the phosphonium salt 5.[14] The carbon elongated product 8 was optically active and showed a UV absorbance at 234 nm (ε 11500). The ^{13}C NMR spectrum showed an expected single resonance for each of the carbons. Reduction of 8 with $NaBH_4$[15] under carefully controlled conditions resulted in the two diastereoisomers 9. This reaction could be conveniently monitored by the disappearance of the UV absorbance at λ_{max} 234 nm.

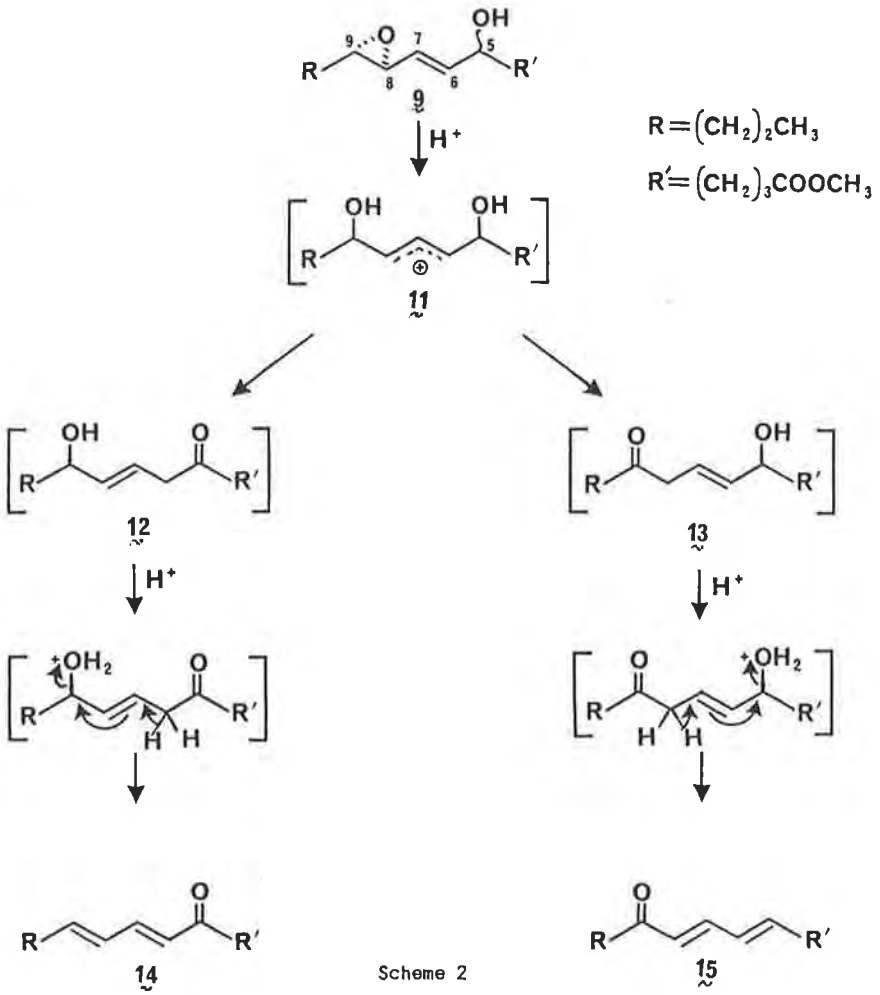
The diastereoisomers of compound 9 could not be separated on silica gel or alumina, due in part to the instability of 9 on these adsorbents. However, evidence for the presence of two stereoisomers in approximately equal amounts was clearly seen in the ^{13}C NMR data. Carbons 4, 5, and 6 in each case exhibited two resonances. The ^{1}H NMR spectrum showed that the trans epoxide stereochemistry was maintained ($J_{8,9}$ = 2.0 Hz) and that the geometry about the double bond was trans ($J_{6,7}$ = 15.6 Hz).



Scheme 1

Compound 9 is the appropriate model multifunctional epoxy fatty acid for reactivity studies. However, its hydrolysis to the deprotected compound was also examined. Conditions were carefully chosen so that the epoxide moiety would remain intact. This could be achieved by warming 9 with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in toluene[16] followed by an appropriate aqueous work-up. Purification by preparative layer chromatography on silica gel gave a compound whose spectral data were consistent with deprotection but also with a structure 10 containing a lactone rather than a carboxylic acid. The ^{1}H NMR data showed that the epoxide moiety was intact in the final product with a multiplet at δ 2.82 and the doublet of doublets at δ 3.10. Loss of the methyl ester was shown in the ^{1}H NMR data by the disappearance of the sharp singlet at δ 3.67 ppm observed for compound 9. Missing from the ^{1}H NMR data, however, was a peak representing the acid proton. The ^{13}C NMR spectrum showed a carbonyl peak at 171.4 ppm indicative of a lactone rather than a carboxylic acid.

Treatment of the multifunctional epoxide 9 in diethyl ether with aqueous acid resulted in the formation of isomeric oxodienes 14 and 15. A plausible mechanism for the formation of the oxodienes is shown in Scheme 2. The acid-catalyzed cleavage of the epoxide ring would result initially in the formation of a regioequivalent carbocation intermediate 11. Loss of a proton from the 5- or 9-carbon yield dienols which are capable of tautomerizing in each case to the δ-ketol intermediates 12 and 13. Acid-catalyzed dehydration of the latter forms the fully conjugated oxodienes.



Scheme 2

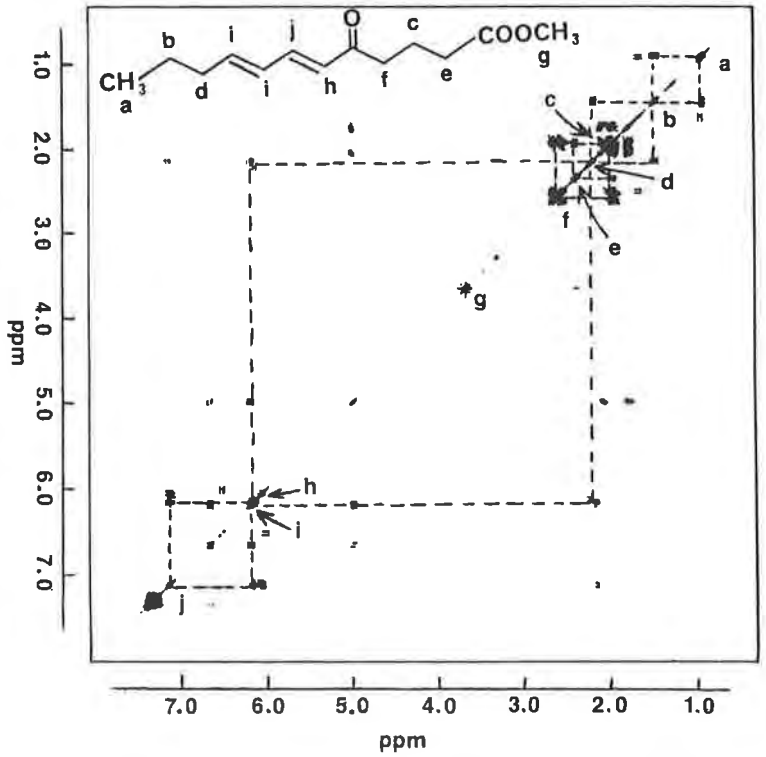


Fig. 1 COSY spectrum of oxodiene 14

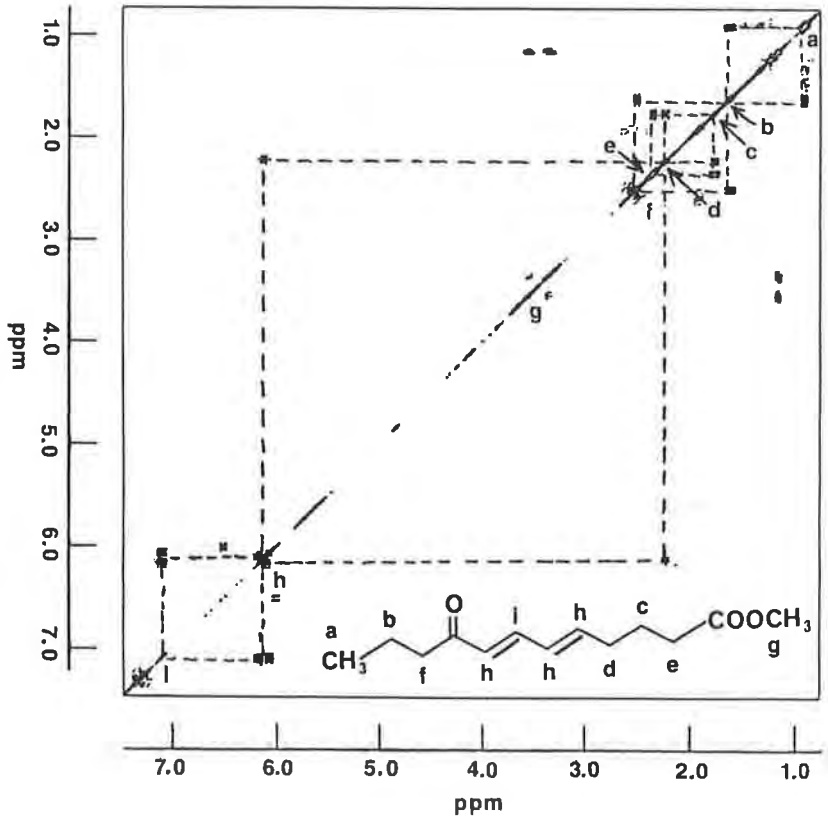
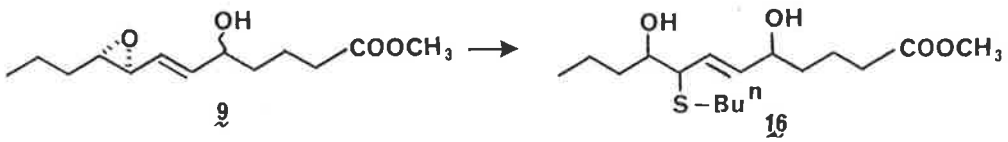


Fig. 2 COSY spectrum of oxodiene 15

The oxodienes 14 and 15 are very similar structurally and spectroscopically. From the spectral data obtained it was obvious that two isomeric oxodienes had been isolated. However, the ^{1}H NMR and ^{13}C NMR were so similar that it was impossible to differentiate between the two isomers from routine high-field NMR spectra. However, the use of 2-D NMR with correlated spectroscopy (COSY) allowed differentiation between the two structures. The ^{1}H COSY spectrum for compounds 14 and 15 including assignments are shown in Figures 1 and 2.

Although oxodienes have not been isolated yet from the various lipid peroxidation pathways in mammalian systems, this does not preclude their existence in natural systems as some products isolated from model studies have correlated well with those obtained from mammalian systems.[17,18] Very recently a novel natural eicosanoid, having an ultraviolet absorbance similar to the oxodienes has been isolated.[19] The identification of this compound has not yet been made.

A model reaction of the synthetic multifunctional epoxide 9 with a thiol was also carried out. This reaction was of interest because of the analogy to leukotriene C_4 (LTC_4) which is enzymatically formed from LTA_4 by the addition of glutathione to the epoxide function of LTA_4.[3] The reaction of 9 with n-butanethiol produced, in quantitative yield, the epoxide ring opened product 16 (Scheme 3). This compound was identified by NMR and mass spectral data which showed loss of the epoxide ring and addition of a butane thiol moiety.



9 16

Scheme 3

In summary, a model multifunctional epoxide related to hepoxilin A has been synthesized. It is reactive and relatively unstable. The free acid prefers to exist in the corresponding lactone form. Under acid catalyzed conditions, it is readily cleaved to oxodienes in high yields. It is quantitatively converted to its ring opened thiol derivative by reaction with thiols.

EXPERIMENTAL SECTION

The infrared spectra were recorded on a Beckman 20A and IBM Model 98 FTIR. The ^{1}H NMR, ^{13}C NMR, and COSY spectra were recorded on a Bruker WM-360 pulse Fourier transform NMR spectrometer. The mass spectrometers employed were a Hewlett-Packard 5985 GC/MS system and an AEI MS-30 high resolution instrument. The ultraviolet data were recorded with a Cary Model 219 ultraviolet-visible spectrophotometer. Optical rotations were recorded on a Perkin-Elmer 141 Automated Polarimeter at 25 °C. Toluene and dichloromethane were dried and distilled over CaH_2 prior to use. Methyl-4-(chloroformyl)butyrate (Aldrich) and trans-2-hexen-1-ol (Aldrich) were used without further purification. Triphenylphosphine was recrystallized from hexane and dried in vacuo (50 °C). Preparative layer chromatography employed EM silica gel PF254 plates, activated for 3 h at 135 °C. Column chromatography employed powder silica gel (60-200 mesh).

Methyl 6-bromo-5-oxo-hexanoate 4. Methyl-4-(chloroformyl)butyrate (3.4 g, 25.2 mmol) was added to a solution of diazomethane (121.6 mmol) in diethylether (400 mL) at room temperature. After 2 h,

N_2 was bubbled through the solution to remove excess CH_2N_2. Then gaseous HBr was bubbled through the solution until the yellow color of the diazoketone was dissipated. The solution was washed with sodium bicarbonate solution (2 x 50 mL) and water (2 x 50 mL). The organic layer was dried (Na_2SO_4), filtered and the solvent removed in vacuo to yield 4.5 g (72%) of product. The product is contaminated with approximately 10% of the α-chloroketone analogue. ^1H NMR ($CDCl_3$) δ 1.94 (tt, J = 7.0 Hz, 7.0 Hz, 2H), 2.38 (t, J = 7.0 Hz, 2H), 2.76 (t, J = 7.0 Hz, 2H), 3.67 (s, 3H), 3.90 (s, 2H); mass spectrum m/z (relative intensity) 193 (M^+ - OCH_3, 32.9), 191 (33.6), 165 (M^+ - $COOCH_3$, 27.0), 163 (27.0), 129 (M^+ - CH_2Br, 100.0), 123 (25.2), 121 (23.4), 101 (36.1), 95 (19.9), 93 (20.2), 59 (12.1).

Phosphonium salt 5. Triphenylphosphine (2.6 g, 10 mmol) was added to a stirred solution of methyl-6-bromo-5-oxo-hexanoate 4 (1.89 g, 7.2 mmol) in toluene (50 mL). The mixture was allowed to stir at room temperature with protection from moisture for 28 h. The reaction mixture was filtered and the filtrate was dried in vacuo (50 °C) to yield the phosphonium salt 5 as an off white solid (1.7 g, 50%): ^1H NMR ($CDCl_4$) δ 1.89 (m, 2H), 2.33 (m, 2H), 3.01 (m, 2H), 3.63 (s, 3H), 5.88 (d, J=14.8 Hz, 2H), 7.5 (m, 15H); mass spectrum, m/z(relative intensity) 405 (M^+-Br, 1.2), 404 (M^+-HBr, 4.2), 373 (5.2), 318 (15.8), 303 (100.0), 262 (6.9).

2,3-(S,S)-Epoxyhexan-1-ol 6. This compound was prepared from trans-2-hexen-1-ol (5.9 mL, 50.0 mmol) as previously described[12] to give 3.94 g (68%) of product: bp 65-66 °C/1 torr (lit.[11] bp 31-33 C/0.30-0.40 torr); $[\alpha]_D$ = -44.7 ; ^1H NMR ($CDCl_3$) δ 0.96 (m, 3H), 1.52 (m, 4H), 2.90 (m, 2H), 3.63 (m, 3H).

2,3-(R,S)-Epoxyhexan-1-al 7. 2,3-(S,S)-Epoxyhexan-1-ol 6 (0.4g, 3.4 mmol) and pyridinium dichromate (1.9 g, 5.0 mmol) in dry dichloromethane (50 mL) was stirred at room temperature for 14 h. Then 50 mL Et_2O was added to the reaction mixture and this was filtered and dried over Na_2SO_4. The solvent was removed and the residue was purified by column chromatography (Et_2O) to yield 0.3 g (76%) of 7: ^1H NMR ($CDCl_3$) δ 0.98 (m, 3H), 1.55 (m, 4H), 3.17 (m, 2H), 9.01 (d, J=6.2 Hz, 1H); ^{13}C NMR ($CDCl_3$) 13.7, 19.2, 33.3, 56.6, 59.1, 198.1; IR (neat) 2900 cm^{-1} (C-H), 1725 cm^{-1} (C=O), 1205 cm^{-1}, 920 cm^{-1}, 870 cm^{-1} (epoxide ring); mass spectrum, m/z (relative intensity) 114 (M^+, 0.5), 113 (M^+-H, 3.4), 85 (0.7), 71 (100.0); HRMS (EI) calcd for $C_6H_{10}O_2$ 114.0681, found 114.0670; optical rotation $[\alpha]_D$ +2.55 (c 3.84, $CHCl_3$).

Methyl-8,9-(S,S)-epoxy-5-oxo-6-dodecenoate 8. A solution of the phosphonium ylide 5 (1.5 g, 3.0 mmol) in 50 mL $CHCl_3$ was shaken with 1 N NaOH (20 mL) for 5 minutes. The organic layer was saved and the aqueous layer was extracted with $CHCl_3$ (2 x 20 mL). The organic layers were combined, dried over Na_2SO_4, filtered, and condensed. The residue, without further purification, was dissolved in toluene (50 mL) and 2,3-(R,S)-epoxyhexanal (0.34 g, 3.0 mmol) was added. The reaction was allowed to stir at room temperature under a N_2 atmosphere for 36 h. The solvent was removed under reduced pressure and then the residue was washed with Et_2O (3 x 50 mL) and filtered. The filtrate was dried over Na_2SO_4, filtered, and condensed to yield 1.4 g of residue. Purification by silica gel column chromatography with ether as the eluent yielded 0.4 g (57%) product as an oil: UV λ_{max} (EtOH) 234 nm (ε 11500); ^1H NMR ($CDCl_3$) δ 0.97 (m, 3H), 1.55 (m, 4H), 1.95 (m, 2H), 2.36 (t, J=7.0 Hz, 2H), 2.63 (t, J=6.8 Hz, 2H), 2.88 (m, 1H), 3.20 (m, 1H), 3.65 (s, 3H), 6.47 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 13.8, 19.1(2C), 33.0, 33.9, 39.3, 51.4, 56.4, 61.4, 131.1, 142.9, 173.4, 198.3. IR (neat) 2980 cm^{-1} (C-H), 1730 cm^{-1} (C=O), 1675 cm^{-1} (C=C), 1630 cm^{-1}, 1200 cm^{-1}, 970 cm^{-1}, 890 cm^{-1} (epoxide ring); mass spectrum m/z (relative intensity) 240 (M^+, 7.3), 222 (M^+ - H_2O, 10.8), 209 (4.0), 197 (30.3), 181 (M^+ - $COOCH_3$, 1.3), 139 (22.0), 129 (18.8), 101 (13.2), 84 (100.0); HRMS (EI) calcd for $C_{13}H_{20}O_4$ 240.1362, found 240.1360; optical rotation $[\alpha]_D$ -19.3 (c 0.68, $CHCl_3$).

Methyl-8,9-(S,S)-epoxy-5-hydroxy-6-dodecenoate 9. A solution of $NaBH_4$ (0.038 g, 1.0 mmol) in MeOH (5 mL) was added to a solution of methyl-8,9-(S,S)-epoxy-5-oxo-6-dodecenoate 8 (0.34 g, 1.4 mmol) in MeOH (10 mL) which had been cooled to 0 C. The mixture was allowed to stir at 0° C for 30 minutes and then allowed to warm to room temperature for 30 minutes. The reaction mixture was poured into a 20 mL brine solution, which was extracted with Et_2O (4 x 20 mL). The combined organic layers were dried over Na_2SO_4, filtered and condensed. The residue was purified by preparative layer chromatography on silica gel using 5% MeOH/CH_2Cl_2 as the eluent. The band at R_f 0.3 yielded 0.19 g (80%) of 9: ^1H NMR ($CDCl_3$) δ 0.96 (m, 3H), 1.52 (m, 6H), 2.35 (m, 5H), 2.83 (m, 1H), 3.09 (dd, J=7.5, 2.0 Hz, 1H), 3.67 (s, 3H), 4.15 (m, 1H), 5.42 (dd, J=15.6, 7.5 Hz, 1H), 5.93 (dd, J=15.6, 6.0 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 13.9, 19.2, 20.7, 33.8, 34.0, 36.36, 36.41, 51.5, 57.8, 60.5, 71.40, 71.50, 128.39, 128.45, 137.5, 174.0; IR (neat) 3437 cm^{-1} (O-H), 2909 cm^{-1} (C-H), 1735 cm^{-1} (C=O), 1200 cm^{-1}, 901 cm^{-1} (epoxide ring); mass spectrum m/z (relative intensity) (TMS derivative) 314 (M^+, 5.6), 313 (M^+ -H, 12.0), 299 (M^+ -CH_3, 6.8), 285 (3.3), 225 (5.2), 213 (20.9), 203 (18.2), 145 (42.9), 117 (77.8), 93 (100.0), 73 (68.5); HRMS (EI) calcd for $C_{13}H_{22}O_4$ 242.1518, found 224.1412 (M^+ - H_2O).

Lactone Product 10. Methyl-8,9-(S,S)-epoxy-5-hydroxy-6-dodecenoate 9 (0.25 g, 1.0 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (1.5 mL, 10 mmol) in toluene (100 mL) were heated to 110°C for 48 h. The solvent was removed and the residue was dissolved in ether (100 mL). This solution was washed with 5% HCl (2 x 50 mL) and $NaHCO_3$ (1 x 20 mL). The organic layer was dried over Na_2SO_4. The solvent was removed and the residue was purified by silica gel preparative layer chromatography using 1:1 ether/ethyl acetate as the eluting agent to give 0.11 g (51%) of 10 as an oil: ^1H NMR

($CDCl_3$) δ 0.96 (m, 3H), 1.53 (m, 4H), 1.95 (m, 4H), 2.51 (m, 2H), 2.82 (m, 1H), 3.10 (dd, J = 2.0, 6.8 Hz, 1H), 4.84 (m, 1H), 5.65 (ddd, J = 1.8, 6.8, 15.8 Hz, 1H), 5.93 (dd, J = 5.1, 15.8 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 14.5, 18.7, 18.8, 19.8, 28.7, 30.2, 34.5, 57.93, 57.99, 61.20, 61.30, 74.7, 130.8, 131.5, 132.5, 132.7, 171.4; IR (neat) 2960 cm^{-1} (C-H), 1735 cm^{-1} (C=O), 1240 cm^{-1} (C-O), 1039 cm^{-1}, 969 cm^{-1}; mass spectrum m/z (relative intensity) 210 (M^+, 0.3), 193 (M^+-OH, 1.1), 181 (M^+-C_2H_5, 1.7), 174 (5.5), 167 (1.7), 156 (11.0), 138 (12.6), 111 (28.2), 95 (45.2), 81 (100.0), 43 (15.3); HRMS (EI) calcd for $C_{12}H_{18}O_3$ 210.1256, found 210.1258.

Methyl 5-oxo-6(E),8(E)-dodecadienoate 14 and Methyl 9-oxo-5(E),7(E)-dodecadienoate 15. A solution of methyl-8,9-(S,S)-epoxy-5-hydroxy-6-dodecenoate 9 (0.086 g, 0.34 mmol) in diethyl ether (10 mL) was cooled to 0°C with an ice bath and then a 30% $HClO_4$ solution (1 mL) was added. The reaction was allowed to warm to room temperature with vigorous stirring. After 72 h, 5 mL H_2O was added to it. The organic layer was saved and the aqueous layer was extracted with CH_2Cl_2 (3 x 20 mL). The organic layers were combined, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by preparative layer chromatography on silica gel with 5% MeOH/CH_2Cl_2 as the eluent. Bands at R_f 0.8 and 0.5 yielded 36.0 mg (44%) of 14 and 38.0 mg (46%) of 15, respectively. For the oxodiene 14: UV (EtOH) λ_{max} 268 (ε 10750); ^1H NMR ($CDCl_3$) δ 0.92 (t, J = 7.4 Hz, 3H), 1.46 (m, 2H), 1.95 (m, 2H), 2.16 (m, 2H), 2.37 (m, 2H), 2.62 (t, J = 7.2 Hz, 2H), 3.67 (s, 3H), 6.07 (d, J = 15.6 Hz, 1H), 6.20 (m, 2H), 7.15 (dd, J = 6.0, 15.6 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 13.6, 19.5, 21.9, 33.2, 35.1, 38.8, 51.5, 127.7, 128.8, 143.2, 145.6, 173.6, 199.8; IR (neat) 2957 cm^{-1} (C-H), 1738 cm^{-1} (C=O, ester), 1653 cm^{-1} (C=O, ketone), 1507 cm^{-1} (C=C), 950 cm^{-1}; mass spectrum m/z (relative intensity) 224 (M^+, 2.4), 193 (M^+ -OCH_3, 16.5), 181 (M^+ -$CH_2CH_2CH_3$, 84.1), 165 (M^+ -$COOCH_3$, 5.1), 123 (100.0), 107 (28.2). For the oxodiene 15: UV (EtOH) λ_{max} 273 (ε 11320); ^1H NMR ($CDCl_3$) δ 0.92 (t, J = 7.4 Hz, 3H), 1.63 (q, J = 7.4 Hz, 2H), 1.77 (m, 2H), 2.23 (q, J = 7.0 Hz, 2H), 2.35 (m, 2H), 2.51 (t, J = 7.3 Hz, 2H), 3.29 (s, 3H), 6.13 (m, 3H), 7.10 (dd, J = 9.9, 15.6 Hz, 1H); ^{13}C NMR ($CDCl_3$)δ13.9, 17.8, 23.9, 29.6, 32.2, 35.2, 42.4, 128.3, 129.7, 142.4, 143.4, 174.6, 200.9; IR (neat) 2957 cm^{-1} (C-H), 1735 cm^{-1} (C=O, ester), 1635 cm^{-1} (C=O, ketone), 1550 cm^{-1} (C=C), 950 cm^{-1}; mass spectrum m/z (relative intensity) 225 (M^+ +H, 5.2), 224 (M^+, 0.5), 209 (M^+ -CH_3, 3.7), 193 (M^+ -OCH_3, 10.7), 165 (3.4), 129 (69.8), 115 (60.4), 71 (100.0); HRMS (EI) calcd for $C_{13}H_{20}O_3$ 224.1413, found 224.1414.

Methyl-5,9-dihydroxy-8-butanethiol-6-dodecenoate 16. Methyl-8,9-(S,S)-epoxy-5-hydroxy-6-dodecenoate 9 (0.05 g, 0.21 mmol), triethylamine (0.14 mL, 0.8 mmol), and n-butanethiol (0.09 mL, 0.8 mmol) in MeOH (6 mL) were allowed to stir at room temperature for 45 h. The reaction mixture was streaked on a silica gel preparative layer plate. The plate was eluted with 5% MeOH/$CHCl_3$. The band at R_f = 0.4 was cut out and eluted with 5% MeOH/$CHCl_3$ to give 59.6 mg of (100%) of 16 as an oil: ^1H NMR ($CDCl_3$) δ 0.91 (m, 6H), 1.49 (m, 12H), 2.37 (m, 4H), 3.09 (m, 4H), 3.66 (s, 3H), 4.01 (m, 1H), 5.59 (m, 2H); mass spectrum m/z (relative intensity) 315 (M^+-OH, 1.0), 301 (M^+-OCH_3, 1.6), 283 (0.7), 277 (1.7), 242 (M^+-BuSH, 75.7), 229 (12.9), 211 (1.3), 185 (29.6), 153 (100.0); HRMS (EI) calcd for $C_{17}H_{32}O_4S$ 332.2022, found 332.2012.

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Conformational Correlation of Purine Nucleosides by High-Field Carbon-13 NMR Data

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Correlation of the nucleic acid base conformation in 43 purine nucleosides with high-field ^{13}C NMR data is described. A key to the correlation is the chemical shift difference between C-2′ and C-3′.

KEY WORDS: ^{13}C NMR Purine nucleosides Conformational correlation.

INTRODUCTION

Practical methods for determining the conformation of the glycosidic bond of nucleosides in solution are very important goals in nucleoside chemistry, because the relative orientations of the heterocyclic base and sugar moiety about the glycosidic bond are often a critical factor in the biological activity (e.g. antiviral) of these compounds. The *syn* and *anti* conformations of some natural nucleosides and nucleotides in solution have been determined by pD studies,[1,2] by fast FT ^1H and ^1H-{^{31}P} NMR methods[3] and by circular dichroism.[4] However, these methods are not practical for routine conformational studies of synthetic nucleosides in solution. We report here a practical and effective method for the determination of the glycosidic bond conformation of purine nucleosides in solution by the utilization of high-field ^{13}C NMR data.

RESULTS AND DISCUSSION

Proton and ^{13}C NMR data reveal that there are diagnostic differences in chemical shifts in the carbohydrate portion of purine nucleosides in the *syn* compared with the *anti* conformations.[5,6] In particular, the chemical shift of C-2′ of the D-ribofuranosyl moiety appears to be sensitive to changes in the glycosidic bond conformation. The only other carbon resonance that changes significantly in the carbohydrate portion of the molecule is C-1′, and this appears to be largely dependent on the modification of the base rather than the conformation. C-2′ experiences an upfield shift in the *syn* conformation in comparison with the *anti* conformation. The effect may be due to the proximity of the lone pair of electrons of N-3 to the D-ribofuranosyl C-2′—H-2′ bond in the *syn* conformation, and may be easily appreciated by examining the chemical shift difference between C-2′ and C-3′ (see Table 1). For example, 8-bromoadenosine (11) is known from x-ray crystallographic data[7] to exist in the *syn* conformation in the solid state. Its ^{13}C NMR spectrum shows that the chemical shift of C-2′ is at 71.1 ppm, only 0.3 ppm downfield from that of C-3′. In contrast, in adenosine (**1**), where it is known that the *anti* conformation is preferred,[8] the chemical shift of C-2′ is at 73.6 ppm, about 2.8 ppm downfield from that of C-3′. This is also the case for other natural nucleosides which are known to prefer high populations in the *anti* conformation in solution. For example, in guanosine

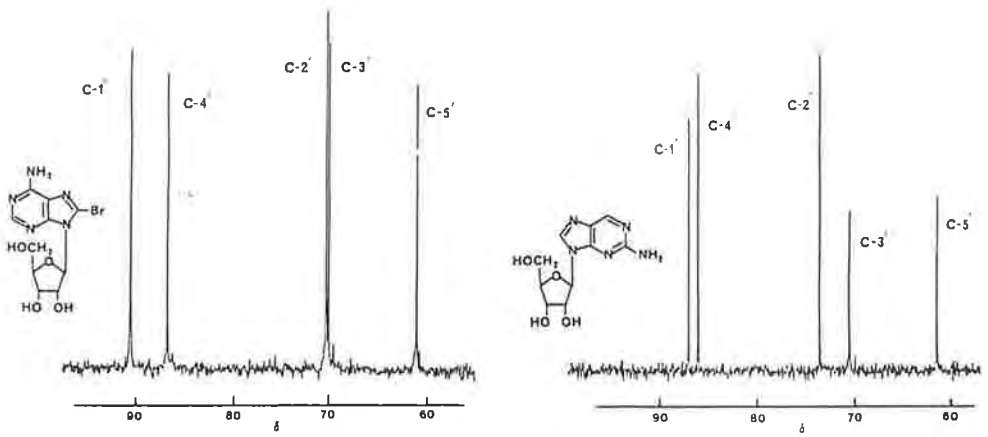

Figure 1. The Carbohydrate Regions of the 90.57 MHz ^{13}C NMR Spectra of 8-Bromoadenosine and 2-Aminonebularine in Me_2SO-d_6.

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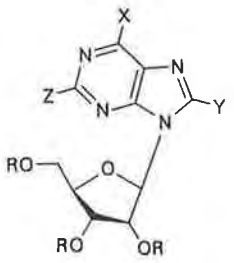
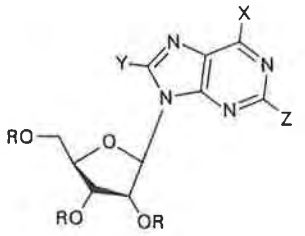
Table 1. Correlation of conformation of purine and nucleosides with carbon chemical shifts (ppm)



syn



anti

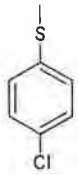
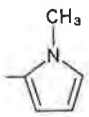
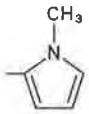
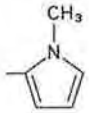
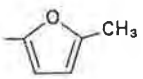
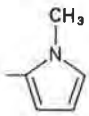
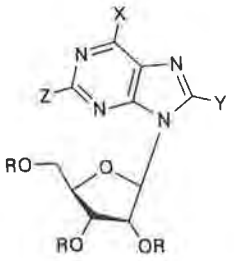
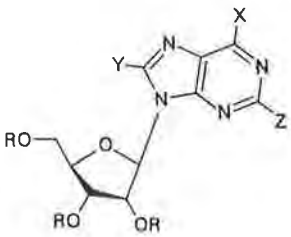
Compound	X	Y	Z	R	Solvent	C-1′	C-2′	C-3′	C-4′	C-5′	Δ(C-2′ – C-3′)	Conformation
1	NH_2	H	H	H	Me_2SO-d_6	88.1	73.6	70.8	86.0	61.9	2.8	*anti*
2	OH	H	NH_2	H	Me_2SO-d_6	87.3	74.9	71.6	86.3	62.2	3.3	*anti*
3	OH	H	H	H	Me_2SO-d_6	87.6	74.2	70.4	85.7	61.4	3.8	*anti*
4	NH_2	H	I	H	D_2O	88.6	73.5	70.1	85.4	61.1	3.4	*anti*
5	NH_2	H	OH	H	D_2O	89.5	73.7	70.5	85.9	60.6	3.2	*anti*
6	OCH_3	H	I	H	Me_2SO-d_6	87.7	74.0	70.5	86.1	64.1	3.5	*anti*
7	OH	H	Ph	H	Me_2SO-d_6	87.2	73.9	70.3	85.5	61.2	3.6	*anti*
8	H	H	SCH_3	H	Me_2SO-d_6	87.1	73.3	70.2	85.5	61.2	3.1	*anti*
9	H	H	NH_2	H	Me_2SO-d_6	86.4	73.6	70.6	85.5	61.5	3.0	*anti*
10	H	H	CH_2COCH_3	H	Me_2SO-d_6	87.1	74.0	70.6	85.8	61.5	3.4	*anti*
11	NH_2	Br	H	H	Me_2SO-d_6	90.3	71.1	70.8	86.6	62.0	0.3	*syn*
12	NH_2		H	H	Me_2SO-d_6	89.8	71.7	71.2	86.9	62.4	0.5	*syn*
13	NH_2	OCH_3	H	H	Me_2SO-d_6	86.8	71.2	71.0	86.1	62.3	0.2	*syn*
14	OH	Br	H	H	Me_2SO-d_6	90.5	71.2	70.5	86.3	61.9	0.7	*syn*
15	Cl	H	I	Ac	$CDCl_3$	86.7	73.3	70.5	80.8	62.9	2.8	*anti*
16	NH_2	H	SCH_3	Ac	$CDCl_3$	87.1	73.0	70.2	79.6	62.9	2.8	*anti*
17	H	H	NH_2	Ac	$CDCl_3$	86.2	72.8	70.5	79.9	63.0	2.3	*anti*
18	Cl	H	Ph	Ac	$CDCl_3$	82.3	73.2	70.1	80.1	62.6	3.1	*anti*
19	Ph	H	Ph	Ac	$CDCl_3$	87.0	73.2	70.2	80.0	62.7	3.0	*anti*
20	Cl	H		Ac	$CDCl_3$	87.0	73.1	70.0	80.8	62.6	3.1	*anti*
21		H		Ac	$CDCl_3$	86.5	73.1	70.2	79.8	62.7	2.9	*anti*
22	Cl	H		Ac	$CDCl_3$	87.5	73.5	70.3	80.4	62.8	3.2	*anti*
23	Cl	H		Ac	$CDCl_3$	87.7	73.4	70.3	80.2	62.8	3.1	*anti*
24		H		Ac	$CDCl_3$	86.9	73.5	70.5	80.2	63.1	3.0	*anti*
25	NH_2	H	I	Ac	$CDCl_3$	86.1	73.4	70.6	80.5	63.1	2.8	*anti*
26	I	H	I	Ac	$CDCl_3$	86.6	73.3	70.6	80.8	62.9	2.7	*anti*
27	OH	H	I	Ac	$CDCl_3$	86.3	73.3	70.4	80.3	62.9	2.9	*anti*
28	$CH(CO_2Et)_2$	H	I	Ac	$CDCl_3$	86.1	73.2	70.6	80.7	63.1	2.6	*anti*
29	$CH(CO_2Et)_2$	H	H	Ac	$CDCl_3$	86.4	73.0	70.6	80.5	63.0	2.4	*anti*

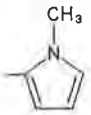
Table 1. Continued.



syn



anti

Compound	X	Y	Z	R	Solvent	C-1′	C-2′	C-3′	C-4′	C-5′	Δ(C-2′ − C-3′)	Conformation
30	OCH_3	H	H	Ac	$CDCl_3$	86.5	73.1	70.6	80.3	63.0	2.5	*anti*
31	I	H	Ph	Ac	$CDCl_3$	87.0	73.2	70.2	80.0	62.7	3.0	*anti*
32	SCH_3	H	H	Ac	$CDCl_3$	86.4	73.0	70.6	80.3	63.0	2.4	*anti*
33	SCH_3	H	NH_2	Ac	$CDCl_3$	86.2	72.8	70.5	79.8	63.0	2.3	*anti*
34	Cl	H	SCH_3	Ac	$CDCl_3$	87.1	72.9	70.1	80.0	62.7	2.8	*anti*
35	SCH_3	H	SCH_3	Ac	$CDCl_3$	86.7	73.0	70.3	80.0	62.9	2.7	*anti*
36	H	H	I	Ac	$CDCl_3$	86.2	73.3	70.6	80.7	63.0	2.7	*anti*
37	H	H	SCH_3	Ac	$CDCl_3$	86.8	72.9	70.2	79.9	62.8	2.7	*anti*
38	H	H	Ph	Ac	$CDCl_3$	86.9	73.1	70.1	79.9	62.6	3.0	*anti*
39	H	H		Ac	$CDCl_3$	86.6	73.0	70.1	79.9	62.7	3.0	*anti*
40	NH_2	Br	H	Ac	$CDCl_3$	88.9	71.8	70.4	79.8	62.8	1.4	*syn*
41	H	H	NH_2	$BuMe_2Si$	$CDCl_3$	87.5	76.0	71.8	85.1	62.4	4.2	*anti*
42	H	H	I	$BuMe_2Si$	$CDCl_3$	88.7	75.2	70.9	84.8	61.6	4.3	*anti*
43	NH_2	Br	H	$BuMe_2Si$	$CDCl_3$	90.6	72.2	71.8	85.6	62.3	0.4	*syn*

and inosine, the chemical shift differences between C-2′ and C-3′ are 3.3 and 3.8 ppm, respectively.

We have correlated the NMR spectral data of a diverse group of synthetic and natural purine nucleosides in order to establish the generality and applicability of the observations discussed above (Table 1). For the unprotected nucleosides, the trend is clear. Compounds in the *syn* conformation show Δ(C-2′ − C-3′) of less than 0.5 ppm. The unprotected nucleosides in the *anti* conformation exhibit Δ(C-2′ − C-3′) of greater than 2.8 ppm. This trend is also seen for the silylated compounds. For most of the acetylated nucleosides in the *anti* conformation, this chemical shift difference is close to or greater than 2.8 ppm. However, in some cases (**17**, **33**, etc.) values closer to 2.0 ppm may be indicative of a shift to a slightly different population of rotamers or to a slightly different preferred glycosidic torsional angle within the *anti* range. Also, for nucleoside **40**, Δ(C-2′ − C-3′) = 1.4 ppm, and the higher value for a *syn* conformation in this case may reflect a shift (towards the *anti* direction) of the preferred glycosidic torsional angle. An example of the dramatic change in the chemical shift of C-2′ in going from the *syn* to the *anti* conformation is shown in Fig. 1.

In summary, the glycosidic bond conformation of purine nucleosides in solution expressed in terms of preferred *syn* or *anti* conformations can be rapidly and qualitatively determined by the magnitude of the ^{13}C NMR chemical shift difference between C-2′ and C-3′.

EXPERIMENTAL

The natural abundance ^{13}C NMR spectra were measured at 90.57 MHz on a Bruker WM-360 high-field spectrometer interfaced to an Aspect 2000 computer system. A switchable 5 mm $^1H/^{13}C$ probe was used. The spectra were recorded using a deuterium lock at 25 °C. Typical parameters were pulse width 4.1 μs (30°), spectral width 23 809 Hz, acquisition time 0.7 s, delay time 2.3 s, data points (FID) 32K and, sample concentration 0.1 M. Chemical shifts (δ) are given with respect to TMS = 0. In some representative examples, the chemical shift assignments were confirmed by a combination of correlation spectroscopy (COSY)[9] and a carbon–hydrogen correlation (CHORTLE) technique.[10]

The few natural nucleosides used were purchased from Sigma Chemical Co. The synthetic purine nucleosides were prepared in our laboratory by previously reported procedures.[11–13]

Acknowledgements

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Novel Approaches to Functionalized Nucleosides via Palladium-Catalyzed Cross Coupling with Organostannanes

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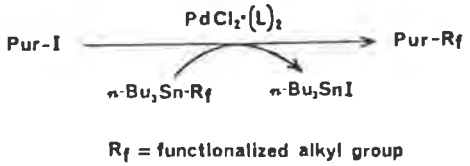
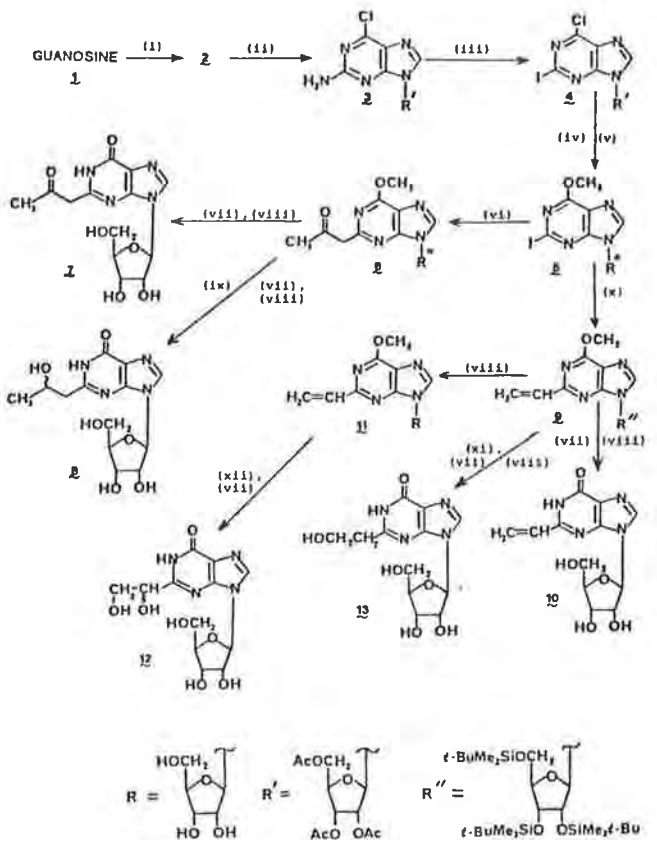
Purine nucleosides and related systems are currently receiving a considerable amount of renewed interest because of the remarkable biological activity of some of these compounds as antiviral agents.[1-4] Although a wide variety of C-6 substituted purine nucleosides bearing functionalized alkyl groups are known, the same cannot be said for the C-2 position.[5] While a number of simple C-2 alkylated compounds have been synthesized,[6-9] very few functionalized alkyl derivatives have been reported.[10] The single general method known for obtaining 2-alkylated purine nucleosides involves ring closure from the appropriately substituted ribofuranosyl imidazole.[6-8] A few other methods are known but are of limited scope.[11,12] Functionalized C-2 alkylated inosine analogues are not only of considerable potential interest as antiviral agents[4] but also there is enzymological interest in these novel compounds as potential inhibitors of a key purine metabolizing enzyme, inosine monophosphate dehydrogenase.[13] This communication reports on the development and application of a general methodology (Scheme I) for the introduction of functionalized carbon–carbon bonding at the 2-position of the hypoxanthine ring.

A logical approach to the synthesis of these rare nucleosides would be through the corresponding 2-halogenated precursor. Thus, protected 2-iodo-6-methoxypurine **5** was the key precursor for all of the target molecules described in this communication. This precursor can be prepared from the 6-chloro-2-aminopurine **3**[14,15] in three steps. The first step (i.e., **3** → **4**) involved a radical deamination–halogenation procedure developed and previously reported by us.[16,17] Nucleophilic displacement of the 6-chloro group in **4** with methoxide was accompanied by the desired deprotection of the acetate groups (96% yield). Subsequent protection of the carbohydrate moiety with *tert*-butyldimethylsilyl chloride and imidazole in DMF[18] gave **5** in 96% yield. The key step in the synthesis of the target molecule **7** was the conversion of **5** to **6** in 70% yield by a palladium-catalyzed cross-coupling reaction (Schemes I and II). This conversion presumably involves

Scheme I



Scheme II



[a] (i) Ac_2O, $(C_2H_5)_3N$, *N,N*-(dimethylamino)pyridine; (ii) $POCl_3$, *N,N*-diethylaniline, Δ; (iii) *n*-$C_5H_{11}ONO$, CH_2I_2, CH_3CN; (iv) $NaOCH_3$, MeOH; (v) *t*-Bu$(CH_3)_2SiCl$, imidazole, DMF; (vi) $Pd(OAc)_2$, (*o*-Tolyl)$_3$P, *n*-Bu_3SnOMe, $CH_2{=}C(CH_3)OAc$, toluene, Δ; (vii) $(CH_3)_3SiI$, CH_3CN; (viii) Et_4NF, CH_3CN; (ix) $NaBH_4$, THF; (x) *n*-$Bu_3SnCH{=}CH_2$, $PdCl_2(CH_3CN)_2$, toluene, Δ; (xi) 9-BBN, THF, Δ; (xii) OsO_4, pyridine.

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oxidative insertion of palladium into the carbon–iodine bond of the iodopurine followed by coupling of the derived Pd(II) complex with the tin enolate of acetone (formed in situ from isopropenyl acetate and tri-*n*-butyltin methoxide), trans–cis isomerization, and reductive elimination to give the product with concomitant regeneration of the Pd(0) catalyst.[19] It is the first example of the use of an organotin reagent in a palladium-catalyzed cross-coupling reaction involving nucleosides. Other methods attempted, such as the photoinduced $S_{RN}1$ reaction,[5,20] the Eschenmoser sulfide contraction,[21] and Meerwein-type reactions[22] were all unsuccessful.

The aforementioned protected acetonylated nucleoside **6** was converted to the target molecule **7** in two steps by reaction first with trimethylsilyl iodide (64%) and subsequently with tetraethylammonium fluoride (93%). The overall yield of **7** starting from guanosine was 18%. Masking of the amide carbonyl oxygen at the 6-position as a methoxy group is an effective way of protecting the inosine system as this group is relatively stable and can be easily removed at the conclusion of a reaction sequence. Compound **7** (a solid, mp 114–116 °C) was purified by reversed-phase HPLC on Amberlite XAD-4 resin and was characterized by UV, FTIR, and high field NMR spectroscopy. Only the keto tautomer of the compound was present, this form being stabilized by intramolecular hydrogen bonding. The 2-acetonyl compound **6** could be reduced readily by sodium borohydride to give, after deprotection, the diastereoisomeric alcohols **8**.

The scope of this palladium-catalyzed cross-coupling reaction can be extended to include other activated organostannanes. For example, reaction of **5** with tri-*n*-butyl(cyanomethyl)stannane[23] under palladium catalysis resulted in the formation of the 2-cyanomethylinosine in 55% yield. 2-Vinylinosine **10** (or **9**), potentially a key precursor for the synthesis of a variety of functionalized alkylated purine nucleosides, is also readily available with use of the aforementioned methodology. Thus, the thermal reaction of **5** with tri-*n*-butyl(vinyl)stannane in the presence of palladium chloride afforded **9** in excellent yields (>90%). Compound **11** (the partially deprotected form of **9**), can be hydroxylated with osmium tetroxide (65%) and then deprotected to give the highly hydroxylated compound **12**. Additionally, reaction of the vinyl compound **9** with 9-BBN followed by oxidative workup resulted in the regiospecific formation (52% yield, 65% conversion) of the terminal alcohol which was deprotected to afford **13**. Hydroboration reactions have rarely been used previously to elaborate structures in purine nucleoside chemistry.

In summary, palladium-catalyzed cross-coupling reaction of 2-iodinated purines with organostannanes is a highly efficient approach to the synthesis of new and rare functionalized purine nucleosides. This approach may find wide application in purine and related heterocyclic chemistry. Biological studies assessing the antiviral activities of the target molecules against RNA viruses are currently under investigation.[24]

Acknowledgment. We thank the U.S. Army Medical Research and Development Command for support of this research. The high field NMR spectrometer and the FTIR instrument used in this work were purchased in part from funds provided by the National Science Foundation.

Supplementary Material Available: NMR (^1H and ^{13}C), UV, FTIR, and FAB (HRMS) spectral data for target molecules (5 pages). Ordering information is given on any current masthead page.

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Fluorescent 1,4-Dihydropyridines: The Malondialdehyde Connection

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Abstract: Under suitable conditions, malondialdehyde is capable of modifying amino acid residues to novel, highly fluorescent 1,4-dihydropyridines. The structures assigned to these compounds are supported by UV, HRMS, high-field NMR, and X-ray crystallographic data. The mechanism of these transformations, which is fully discussed, involves the Michael reaction of alkylidene malondialdehydes with enaminals, both of which are produced as detectable intermediates. These findings may be of significance in explaining some of the biological chemistry of malondialdehyde. The transformation also provides a new approach to the synthesis of a wide range of light stable 4-arylated-1,4-dihydropyridines of potential interest as calcium channel antagonists.

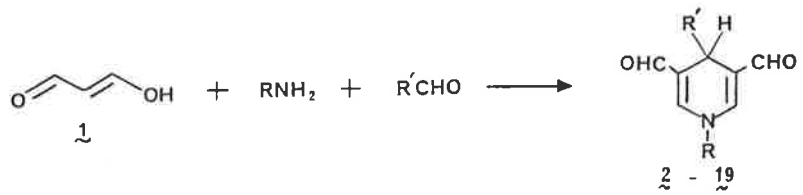
The ubiquitous natural metabolite, malondialdehyde (MDA), is an important carbonyl product of polyunsaturated lipid oxidation.[1-3] The radiolysis of carbohydrates and certain amino acids also produces this dialdehyde.[4,5] Malondialdehyde has long been of interest in food chemistry and its detection by the thiobarbituric acid (TBA) test has been used for the estimation of oxidative rancidity in foods.[1,2,6,7] The chemistry of MDA may be of considerable importance in degenerative processes *in vivo*,[8,9] because of its ability to interact with biological macromolecules.[10-13] For example, MDA is able to modify nucleic acids[14-17] and this is consistent with its observed mutagenicity.[9,10,18] The reactivity of MDA towards proteins to produce fluorescent crosslinked adducts has also been known for some time.[19,20]

Malondialdehyde is readily formed in blood plasma in response to thrombin and other substances that cause blood platelet aggregation.[21,22] It has been shown that hemoglobin A is modified by MDA and that this modified hemoglobin exhibited fluorescence spectra similar to that seen in the overall erythrocytic modification.[23] UV-Visible and fluorescence data on the modified proteins appear to be consistent with the formation of vinylogous amidines,[19,20,24] as well as highly fluorescent heterocyclic systems of unknown structure. This paper reports on model studies of MDA with amino acids and peptides that involve the detection, isolation, and complete characterization of heterocyclic systems of similar UV and fluorescence data as those reported in the aforementioned biological studies.[25] In addition, synthetic ramifications of these model studies are also reported.

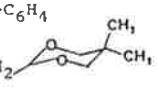
When MDA (1, 3 equiv) was allowed to react with amino acids (e.g. glycine methyl ester, 1 equiv) under aqueous acidic conditions for prolonged periods (> 40 h), the UV spectrum gradually underwent a bathochromic shift. Work-up and chromatographic purification gave low yields of a product which showed a HRMS molecular mass ion at m/z 223.0870. Its UV and

high-field ^{1}H and ^{13}C NMR data (including delayed decoupling) when taken collectively, suggested that the product was the 4-methyl-1,4-dihydropyridine-3,5-dicarboxaldehyde **2**. The compound was highly fluorescent, emitting at 454 nm upon excitation at 386 nm with a relative quantum efficiency (Φ) of 0.36.[26] Interestingly, the glycine adduct **3** (i.e. the unprotected form of **2**) has a Φ of 0.47 which makes it one of the most fluorescent dihydropyridine systems known. The efficiency of this fluorescence emission is particularly remarkable when compared to the well-known natural 1,4-dihydropyridine system, NADH (Φ= 0.02).

On further investigation, it was discovered that dihydropyridine **2** could be obtained in about 50% yield when MDA (2 equiv) was allowed to react with glycine methyl ester (1 equiv) in the presence of acetaldehyde (1 equiv) at pH 4.3[27] for 7 h. This transformation involving MDA was found to be general and related 1,4-dihydropyridines in about the same yields could be isolated from alanine, serine, methionine, and lysine methyl esters with acetaldehyde, propanal, pentanal, and benzaldehyde (Scheme 1). Studies with lysine were particularly important as a model study for protein modification as the only primary amino group in protein structures apart from the N-terminal α-amino groups is the ε-amino group of lysine. The UV and fluorescence spectra for **2** (see exptl) are in general typical for dihydropyridines of all of the representative amino acids studied. They can be used for the detection of the formation of these 1,4-dihydropyridines from the modification of peptides by MDA. Two representative examples studied include glutathione and GlyHisLys.



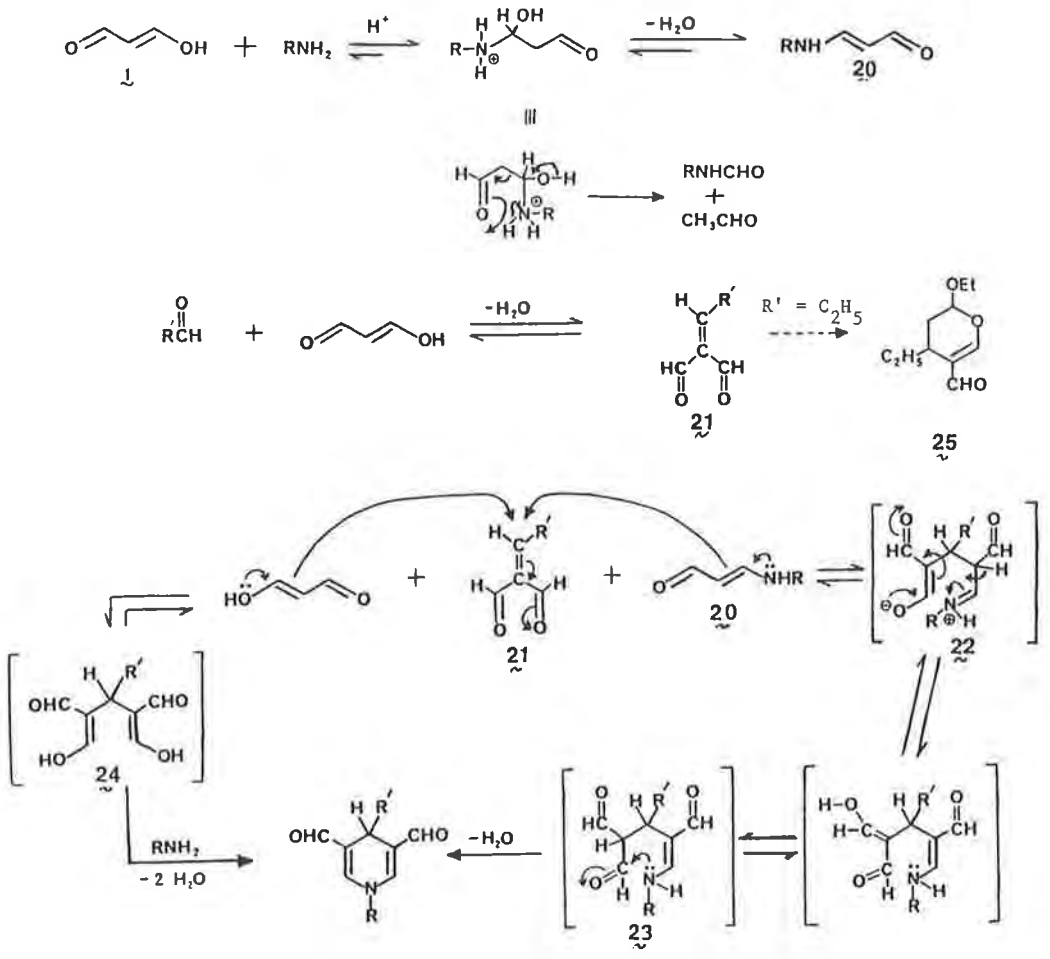
1 **2** - **19**

2	R = $CH_2CO_2CH_3$,	R' = CH_3	**11**	R = $CHCO_2CH_3$, $CH_2CH_2SCH_3$	R' = CH_3
3	R = CH_2CO_2H,	R' = CH_3	**12**	R = Glu·Cys·Gly Side Chain,	R' = CH_3
4	R = $CH_2CO_2CH_3$,	R' = C_2H_5	**13**	R = Gly·His·Lys Side Chain,	R' = CH_3
5	R = CH_2CO_2H,	R' = C_2H_5	**14**	R = H, R' = C_2H_5	R' = C_2H_5
6	R = $CH_2CO_2CH_3$,	R' = C_4H_9	**15**	R = H,	R'= C_6H_5
7	R = $CH_2CO_2CH_3$,	R' = C_6H_5	**16**	R = H,	R' = 2Me-C_6H_4
8	R = $CHCO_2CH_3$, CH_2OH	R' = CH_3	**17**	R = H,	R' = 2F-C_6H_4
9	R = $-(CH_2)_4CHCO_2CH_3$, $NHCOCH_3$	R' = CH_3	**18**	R = $CH_2CO_2CH_3$	R' = 
10	R = $CHCO_2CH_3$, CH_3	R' = CH_4H_9	**19**	R = $CH_2CO_2CH_3$,	R' = CH_2CHO

Scheme 1

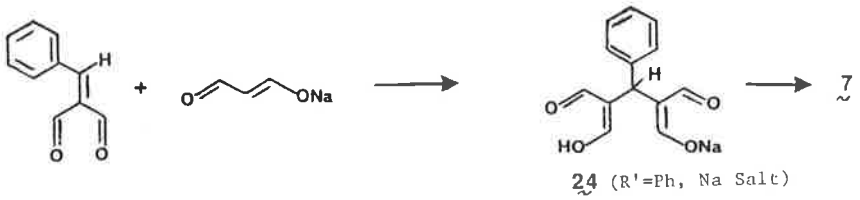
The aforementioned transformation to the dihydropyridines provides a new approach to the synthesis of a variety of N-unsubstituted dihydropyridines by replacement of the amino acid in these reactions with ammonia. For example, when MDA was treated with benzaldehyde and ammonium hydroxide at pH 4.2 at 60 °C for 3 h, 4-phenyl-1,4-dihydropyridine-3,5-dicarboxaldehyde (15) was isolated in 26% yield after chromatographic separation and crystallization. The synthesis has generality and may be used for the preparation of a wide variety of such N-unsubstituted compounds.

A plausible mechanism for the formation of the dihydropyridines derived from the amino acids (or ammonia) and MDA is shown in Scheme 2. The reaction apparently proceeds *via* the enaminal **20** and the alkylidene malondialdehyde **21**. The formation of the isolable intermediate **20**, which occurs relatively rapidly, can be clearly seen in the UV spectrum at 280 nm. Intermediate **21** (which can be trapped as the dihydropyran cycloadduct **25**[29]) is the result of an aldol condensation of MDA and an additional aldehyde (explained later), followed by dehydration.[28] This α,β-unsaturated dialdehyde then serves as a Michael acceptor for enaminal **20** to form **22** which can undergo cyclization *via* **23** followed by dehydration to give the 1,4-dihydropyridines.



Scheme 2

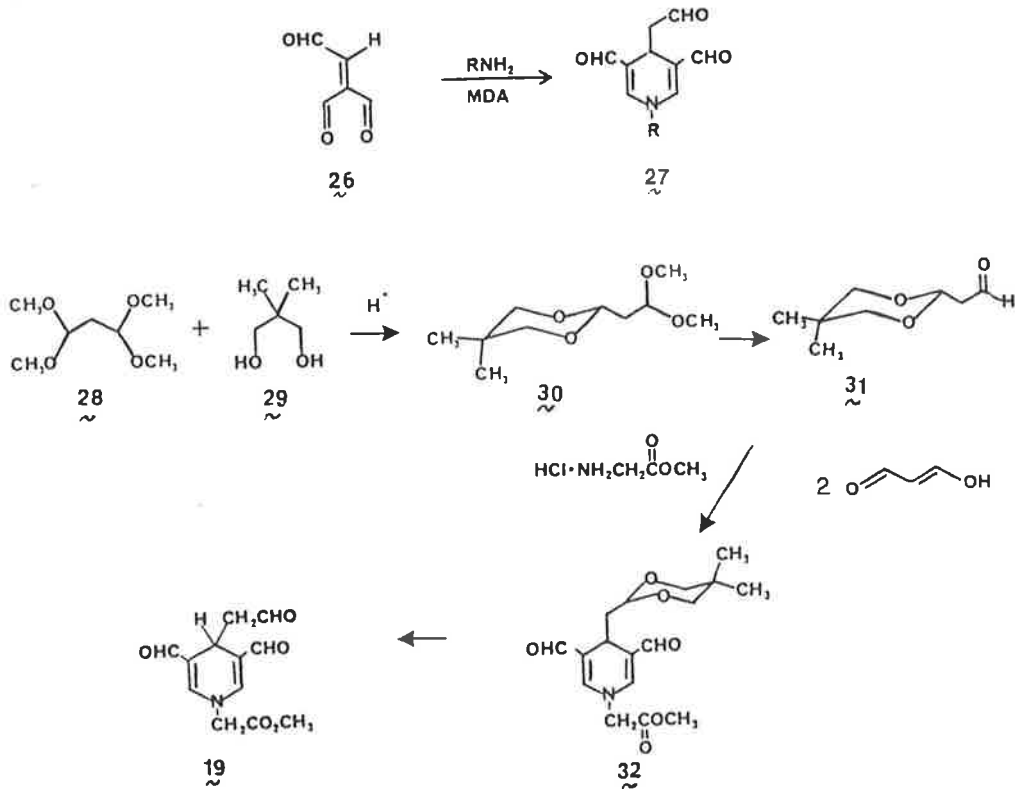
The formation of 1,4-dihydropyridines through the intermediacy of the "bis-MDA" derivative **24** was also examined. For example, benzyl "bis-MDA" (**24**, R'=Ph), a stable compound, can be easily prepared from benzylidene MDA and MDA (Scheme 3). It is smoothly converted to the 1,4-dihydropyridine **7** by reaction with glycine methyl ester. Propyl "bis-MDA" (**24**, R'=Et) gave similar results.



24 (R'=Ph, Na Salt)

Scheme 3

Malondialdehyde interacts with amino acids in the absence of added second aldehyde to give 1,4-dihydropyridines (e.g. **2**, **3**, **9**, etc) and this requires explanation. It is very likely, that in these reactions, the second aldehyde (acetaldehyde) is produced slowly from the thermal cleavage of the amino alcohol (hydrated enaminal) formed from the initial reaction of amino acid and malondialdehyde (Scheme 2). The requirement of the second aldehyde in the formation of 1,4-dihydropyridines raises the question as to why MDA does not itself serve in this role. If MDA behaved as the second aldehyde in this reaction, the alkylidene MDA **26** would form which would result in the 1,4-dihydropyridine system **27**. However, no dihydropyridines with this structure were isolated from any of the reactions studied.



26 **27** **28** **29** **30** **31** **32** **19**

Scheme 4

The possibility that dihydropyridine **2** (and others) were derived by the *in situ* decarbonylation of **27** was also investigated through the unambiguous preparation of an authentic sample of **27** ($R=CH_2CO_2CH_3$, i.e. **19**) (Scheme 4). Treatment of 1,1,3,3-tetramethoxypropane **28** with 2,2-dimethyl-1,3-propanediol **29** afforded the mixed *bis*-acetal of MDA **30** in 40% yield. Selective hydrolysis of **30** with oxalic acid and silica gel in a tetrahydrofuran/dichloroethane/water solution gave the monoacetal of MDA **31** in 79% yield. Utilization of **31** under the standard reaction conditions with MDA and glycine methyl ester afforded the dihydropyridine **32** in 20% yield. Careful hydrolysis of the cyclic acetal molety was accomplished with pyridinium tosylate/p-toluenesulfonic acid in acetone which gave **19** in 38% yield. However, compound **19** was found to be thermally stable under the conditions used to produce the 1,4-dihydropyridines and even at much higher temperatures.

Finally, it should be mentioned that a number of 4-arylated 1,4-dihydropyridines related to some of the compounds synthesized in this paper are of considerable interest as calcium channel antagonists.[30,31] Some of these compounds are being used clinically in the treatment of various disorders of the cardiovascular system.[32] Two conformational requirements that appear to be important for the biological activity of known 4-arylated 1,4-dihydropyridines are the orthogonal orientation of the phenyl ring and the planarity of the dihydropyridine ring.[33,34] In order to confirm the structures of the dihydropyridines produced in these reactions and to examine the conformational properties of the 4-arylated compounds, we carried out a single crystal X-ray study on compound **15**. The results are presented in Fig. 1 and show that the plane of the phenyl ring bisects approximately the dihydropyridine ring even though an ortho substituent is not present (cf. refs. 33,34). In addition, the dihydropyridine ring shows a relatively small deviation from planarity. Calcium channel antagonist activities for this and other dihydropyridines are currently being investigated.

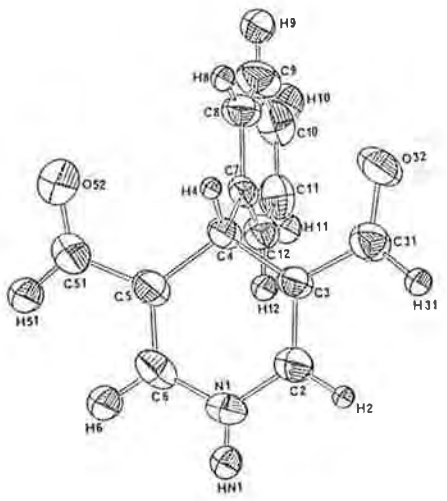


Figure 1. ORTEP plot showing the conformation of **15** determined from single crystal X-ray data.

In summary, it can be stated that MDA is able to modify amino acid residues to highly fluorescent 1,4-dihydropyridines. A mechanistic interpretation of these results has been suggested. These findings may be of significance in understanding the biological chemistry of MDA. Synthetic ramifications of this work includes a new approach to the synthesis of a variety of N-unsubstituted 1,4-dihydropyridines. In contrast to many known 1,4-dihydropyridines, the compounds produced in this study are remarkably light stable. Some of the dihydropyridines synthesized may be useful as fluorescent biological probes of the calcium channel in living systems and the X-ray crystallographic data provide strong structure-activity prediction for this activity.

Experimental Section

Melting points reported are uncorrected and were determined on a Thomas-Hoover melting point apparatus fitted with a microscope. The ^1H and ^{13}C NMR spectra were recorded on either a Bruker WM-360 or a JEOL FX-90Q Fourier transform NMR spectrometer. Mass spectra were obtained on a Hewlett-Packard 5985 GC/MS system or a VG Analytical Model ZAB-HF instrument. Ultraviolet spectra were obtained on a Varian-Cary Model 219 ultraviolet-visible spectrophotometer. Fourier transform IR measurements were recorded on an IBM Model 98 instrument. Corrected fluorescence spectra were obtained on an SLM-Aminco SPF-500C spectrofluorimeter interfaced with an IBM personal computer. Relative quantum yields were determined by the method of Guilbault[26] using quinine sulfate (quantum yield 0.70) in 0.1N sulfuric acid as a reference. The X-ray structure was determined on an Enraf-Nonius CAD-4 diffractometer. Lyophilizations were performed on a Virtis Freezemobile 3. Air and moisture sensitive reagents and/or reaction products were handled and transferred, when necessary, in a Labconco glove box under a dry nitrogen atmosphere. Preparative layer chromatography plates for separating reaction mixtures were prepared by coating 20 x 20 cm glass plates with 65 ml each of a slurry prepared from 150 g of E. Merck PF-254 silica gel in 400 ml of water. The plates were air dried for two days, then activated at 135 °C for 4 hours prior to use.

General Procedure for the Formation of 1,4-Dihydropyridines.

(Procedure A). To a 50 ml RBF equipped with a stir bar was added acetate buffer (pH 4.2), sodium MDA (3 equiv),[35] and an amine or amino acid (1 equiv). The pH of this mixture was adjusted to 4.2 with 2M HCl or 2M NaOH. The reaction flask was sealed and stirred in a pre-heated oil bath for several hours. The solution was neutralized with 2M NaOH and the solvent was removed under reduced pressure. The residue was dissolved in a methanol-chloroform mixture and chromatographed on a silica gel (60-230 mesh) column. Fractions with the expected dihydropyridine UV spectrum were collected, pooled, and concentrated. For further purification, the material was chromatographed on silica gel (PF_{254}) preparative layer plates with a specific combination of methanol and chloroform as the developing solvent.

Procedure B. The amine or amino acid (1 equiv), aldehyde (1-2 equiv), and sodium MDA (2 equiv) were dissolved in acetate buffer (pH 4.2) in a 50 ml RBF equipped with a stir bar. The mixture was stirred for several minutes and then the pH was adjusted to 4.2. The reaction flask was sealed and heated in an oil bath for several hours. The reaction mixture was then neutralized with 2M NaOH, and the solvent was removed *in vacuo*. The residue was taken up in a methanol and chloroform mixture and run through a short silica scrubber column to remove NaCl and other polar impurities. Fractions with the appropriate dihydropyridine UV pattern were collected and pooled. The solvent was evaporated and the residue was chromatographed on silica gel plates with methanol-chloroform as the developing solvent.

4-Methyl-1,4-dihydropyridine-3,5-dicarboxaldehyde 2 from MDA and Glycine Methyl Ester by Procedure A. Glycine methyl ester hydrochloride (130 mg, 1.04 mmol) and sodium MDA (335 mg, 2.98 mmol) were stirred at 60 °C in 20 ml of water at pH 4.2 for 45 h. The reaction mixture was worked up and chromatographed as described above. The band with R_f 0.30 (5% CH_3OH-$CHCl_3$, 2 elutions) afforded 18 mg (8%) of 2 as a solid, mp 153 °C: UV (H_2O) λ_{max} 236 nm (ϵ 18,900), 262 nm (ϵ 7900), 384 nm (ϵ 8800); Fluorescence data (H_2O) excitation 386 nm, emission 454 nm (Φ = 0.36); ^1H NMR ($CDCl_3$) δ 1.14 (d, 3H), 3.84 (m, 4H), 4.20 (s, 2H), 6.64 (s, 2H), 9.30 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 22.1, 23.0, 53.0, 54.9, 124.4, 146.1, 168.1, 188.7; mass spectrum, m/z (relative intensity) (30 eV) 224 (M^++1, 1.5), 223 (M^+, 6.4), 208 (100), 149 (15.9); HRMS (EI) calcd for $C_{11}H_{13}O_4N$; 223.0845, found 223.0870.

4-Methyl-1,4-dihydropyridine-3,5-dicarboxaldehyde 2 by Procedure B. Using this procedure with acetaldehyde (2 equiv) as the additional aldehyde, the reaction time was 3 h at 60 °C. Compound **2** was produced in 50% yield.

4-Ethyl-1,4-dihydropyridine-3,5-dicarboxaldehyde 4 from Glycine Methyl Ester by Procedure B. Glycine methyl ester hydrochloride (136 mg, 1.08 mmol), sodium MDA (247 mg, 2.20 mmol) and propionaldehyde (0.08 ml, 1.10 mmol) were stirred in 20 ml of water at pH 4.2 at 55 °C for 4 h. The reaction mixture was worked up and chromatographed to give **4** as a yellow oil, 138 mg (54%). UV (H_2O) λ_{max} 238 nm (ε 19480), 262 nm (ε 8640), 385 nm (ε 8680); Fluorescence data (H_2O) excitation 390 nm, emission 455 nm (Φ = 0.36); ^1H NMR ($(CD_3)_2SO$) δ 0.66 (t, 3 H), 1.33 (m, 2 H), 3.71 (m, 4 H), 4.53 (s, 2 H), 7.35 (s, 2 H), 9.24 (s, 2 H); ^{13}C NMR ($(CD_3)_2SO$) δ 8.6, 25.7, 27.4, 52.2, 53.9, 119.7, 149.5, 169.1, 189.0; mass spectrum, m/z (relative intensity) (30 eV) 237 (M^+, 1.7), 222 (0.6), 208 (100), 179 (5.5), 178 (12.6), 176 (8.6), 164 (1.9), 150 (10.3), 149 (31.1); HRMS (EI) calcd for $C_{10}H_{10}O_4N(M^+-CH_2CH_3)$ 208.0609, found 208.0599.

4-Ethyl-1,4-dihydropyridine-3,5-dicarboxaldehyde 5 from Glycine by Procedure B. Glycine (116 mg, 1.55 mmol), sodium MDA (354 mg, 3.16 mmol) and propionaldehyde (0.15 ml, 2.08 mmol) were stirred in 20 ml of water at pH 4.2 at 50 °C for 3 h. Compound **5** (139 mg, 40%) was obtained as yellow crystals: mp 185-186 °C; UV (H_2O) λ_{max} 238 nm (ε 21,819), 265 nm (ε 8423), 393 nm (ε 9792); Fluorescence data (H_2O) excitation 397 nm, emission 462 nm (Φ = 0.47). ^1H NMR ($(CD_3)_2SO$) δ 0.66 (t, 3 H), 1.34 (m, 2 H), 3.74 (t, 1 H), 4.41 (s, 2 H), 7.35 (s, 2 H), 9.24 (s, 2 H); ^{13}C NMR ($(CD_3)_2SO$) δ 8.7, 25.9, 27.4, 54.1, 119.7, 149.8, 170.0, 189.2; mass spectrum, m/z (relative intensity) (30 eV) 223 (M^+, 2.0), 208 (10.0), 194 (100), 178 (3.8), 176 (14.9), 165 (10.4), 149 (33.3), 136 (33.7), 92 (71.7); HRMS (EI) calcd for $C_{11}H_{13}O_4N$ 223.0845, found 223.0856.

4-Butyl-1,4-dihydropyridine-3,5-dicarboxaldehyde 6 from Glycine Methyl Ester by Procedure B. Glycine methyl ester·HCl (252 mg, 2.01 mmol), sodium MDA (495 mg, 4.40 mmol), and pentanal (333 mg, 3.86 mmol) were stirred in 30 ml of pH 4.2 acetate buffer at 60 °C for 3 h. The reaction mixture was worked up and chromatographed to give **6** as an oil in 43% yield. UV (H_2O) λ_{max} 237 (ε 20,870), 264 (ε 8700), 385 (ε 9500); Fluorescence data (H_2O) excitation 389 nm, emission 464 nm (Φ = 0.28); ^1H NMR ($CDCl_3$) δ 1.56-0.75 (m, 9 H), 3.83 (s, 3 H), 3.97 (t, J = 4.4 Hz, 1 H), 4.33 (s, 2 H), 6.64 (s, 2 H), 9.30 (s, 2 H); ^{13}C NMR ($CDCl_3$) δ 13.7, 22.4, 26.6, 27.1, 33.6, 52.5, 54.5, 121.7, 147.4, 168.1, 188.7; mass spectrum, m/z (relative intensity) (30 eV) 265 (M^+, 0.7), 209 (12.0), 208 ($M^+-C_4H_9$, 100.0) 149 (9.9); HRMS (EI) calcd for $C_{14}H_{19}O_4N$ 265.1314, found 265.1290.

4-Methyl-1,4-dihydropyridine 3,5-dicarboxaldehyde 9 from α-N-Acetyllysine Methyl Ester (Procedure A). Sodium MDA (371 mg, 3.31 mmol) and α-N-acetyllysine-HCl (232 mg, 0.97 mmol) were dissolved in 20 ml of pH 4.2 acetate buffer. The reaction flask was stoppered and heated for 18 h at 60 °C. Work up and chromatography gave **9** as a yellow oil in 11% yield. UV (H_2O) λ_{max} 239 (ε 7,900), 267 (ε 8540), 399 nm (ε 7260); Fluorescence data (H_2O) excitation maxima 398 nm, emission maxima 464 nm (Φ = 0.35); ^1H NMR ($CDCl_3$) δ 1.08 (d, J = 6.8 Hz, 3 H), 1.95-1.34 (m, 6H), 2.02 (s, 3 H), 3.59-3.41 (m, 2 H), 3.74 (s, 3 H), 3.89 (q, 1 H), 4.75-4.50 (m, 1 H), 6.75 (s, 2 H), 9.27 (s, 2 H); ^{13}C NMR ($CDCl_3$) δ 21.9, 22.0, 22.7, 22.8, 29.1, 31.6, 51.6, 52.1, 54.4, 123.4, 146.3, 170.1, 172.4, 188.7; mass spectrum, m/z (relative intensity) (30 eV) 337 (M^++1, 6.4), 336 (M^+, 28.3), 322 (M^+-CH_2, 11.8), 321 (M^+-CH_3, 79.2), 186 (18.5), 149 (26.0), 144 (63.5), 126 (100.0); HRMS (EI) calcd for $C_{17}H_{24}O_5N_2$ 336.1685, found 336.1696.

Adduct **9** was obtained in 25% yield from the reaction of α-N-acetyllysine methyl ester with acetaldehyde and MDA by Procedure B.

4-Phenyl-1,4-dihydropyridine-3,5-dicarboxaldehyde 15 by Procedure B. Ammonium hydroxide (262 mg, 2.16 mmol), sodium MDA (452 mg, 4.03 mmol), and benzaldehyde (437 mg, 4.12 mmol) were dissolved in 25 ml of pH 4.2 acetate buffer. The reaction mixture was sealed and stirred in an oil bath for 3 h at 60 °C. The reaction mixture was worked up and the product was isolated by preparative layer chromatography to give **15** as long needles (26%): mp 240 °C; UV (95% EtOH) λ_{max} 228 (ε 12920), 246 (ε 6640), 276 (sh) (ε 2520), 373 nm (ε 6850); Fluorescence data (H_2O) excitation 383 nm, emission 445 nm (Φ = 0.32); ^1H NMR ($(CD_3)_2SO$) δ 4.76 (s, 1 H), 7.17 (m, 5 H), 7.47 (s, 2 H), 9.25 (s, 2 H), 10.02 (brs, 1 H); ^{13}C NMR ($(CD_3)_2SO$) δ 32.8, 119.8, 125.9, 127.4, 127.8, 144.0, 145.5, 189.0; mass spectrum, m/z (relative intensity) (30 eV) 214 (M^++1, 2.9), 213 (M^+, 16.5), 154 (10.1), 136 ($M^+-C_6H_5$, 100.0) 128 (13.7); HRMS (EI) calcd for $C_{13}H_{11}O_2N$ 213.0790, found 213.0815.

4-(2-Methylphenyl)-1,4-dihydropyridine-3,5-dicarboxaldehyde (16) was prepared by Procedure B as described for 15. Compound **16** was obtained as light yellow crystals in 25% yield: mp 223 °C; UV (95% EtOH) λ_{max} 376 nm (ε 7530), 281.5 nm (ε 2140), 247 nm (ε 2530), 229.5 nm (ε 13120); Fluorescence data (95% EtOH) excitation 385 nm, emission 435 nm (Φ = 0.20); ^{13}C NMR ($(CD_3)_2SO$) δ 19.5, 29.2, 121.8, 125.7, 126.0, 128.3, 128.8, 134.9, 143.9, 145.8, 189.2; ^1H NMR ($(CD_3)_2SO$) δ 2.64 (s, 3 H), 4.83 (s, 1 H), 6.99 (m, 4 H), 7.46 (d, 2 H), 9.16 (s, 2 H), 9.93 (s, 1 H); mass spectrum, m/z (relative intensity) (30 eV) 228 (M^++1,

9.2), 227 (M^+, 45.4), 154 (16.6), 136 (100), 128 (16.6); HRMS (EI) calcd for $C_{14}H_{13}O_2N$ 227.0946, found 227.0952.

4-(2-Fluorophenyl)-1,4-dihydropyridine-3,5-dicarboxaldehyde (17) was prepared by Procedure B as described for 15. Compound 17 was obtained as yellow crystals (22%); mp 220 °C; UV (95% EtOH) λ_{max} 376 nm (ε 3710), 270 (ε 4960), 228 (ε 6870); Fluorescence data (95% EtOH) excitation 385 nm, emission 435 nm (Φ= 0.25); ^{13}C NMR ($(CD_3)_2SO$) δ 27.7, 119.2, 123.8, 127.7, 127.9, 130.5, 132.7, 144.2, 144.4, 188.9; ^{1}H NMR ($(CD_3)_2SO$) δ 4.94 (s, 1 H), 7.09 (m, 4 H), 7.47 (d, 2 H), 9.18 (s, 2 H), 10.04 (s, 1 H); mass spectrum m/z (relative intensity) (30 eV) 232 (M^++1, 9.2), 231 (M^+, 53.2), 154 (8.9), 136 (100); HRMS (EI) calcd for $C_{13}H_{10}O_2NF$ 231.0696, found 231.0718.

1,4-Dihydropyridine Trialdehyde 19. Sodium MDA (568 mg, 5.07 mmol) was treated with MDA-acetal 31 (398 mg, 2.52 mmol) and glycine methyl ester·HCl in 40 ml of water at pH 4.2 and 50 °C for 6 h (Procedure B). Work up and purification afforded 318 mg (38%) of 32 as yellow crystals: mp 182-184 °C; UV (H_2O) λ_{max} 235 nm (ε20,375), 259 nm (ε 9247), 373 nm (ε 9564); Fluorescence data (H_2O) excitation 385 nm, emission 455 nm (Φ = 0.21); ^{1}H NMR ($(CD_3)_2SO$) δ 0.64 (s, 3 H), 1.02 (s, 3 H), 1.53 (m, 2 H), 3.26 (d, 2 H, J = 11 Hz), 3.42 (d, 2 H, J = 11 Hz), 3.72 (s, 3 H), 3.76 (m, 1 H), 4.32 (m, 1 H), 4.56 (s, 2 H), 7.32 (s, 2 H), 9.21 (s, 2 H); ^{13}C NMR ($(CD_3)_2SO$) δ 21.4, 22.8, 23.2, 29.4 (two carbons), 52.2, 53.8, 76.0, 100.0, 120.0, 149.1, 169.2, 188.8; mass spectrum, m/z (relative intensity) (30 eV) 337 (M^+, 6.2), 308 (0.1), 279 (0.1), 278 (0.7), 264 (0.1), 235 (1.1), 234 (5.6), 208 (100), 195 (0.7), 179 (2.3), 150 (2.1); HRMS (EI) calcd for $C_{17}H_{23}O_6N$ 337.1525, found 337.1556.

1,4-Dihydropyridine 32 (133 mg, 0.39 mmol) was dissolved in 1:4 water:acetone (15 ml) and treated with pyridinium tosylate (102 mg, 0.41 mmol) and p-toluenesulfonic acid (18 mg, 0.10 mmol) and heated under reflux for 30 h. The reaction mixture was neutralized with a small amount of $NaHCO_3$ and the solvent removed <u>in vacuo</u>. The residue was chromatographed on a silica preparative layer plate with 4% methanol/dichloromethane. The band with R_f 0.49 afforded 38 mg (38% yield, 58% conversion) of 19 as a yellow oil: UV (H_2O) λ_{max} 234 nm (ε 18335), 259 nm (ε 7543), 380 nm (ε8108); Fluorescence data (H_2O) excitation 390 nm, emission 455 nm (Φ = 0.32); ^{1}H NMR ($CDCl_3$) δ 2.65 (dd, 2 H), 3.48 (t, 1 H), 3.85 (s, 3 H), 4.24, (s, 2 H), 6.76 (s, 2 H), 9.29 (s, 2 H), 9.73 (t, 1 H); ^{13}C NMR ($CDCl_3$) δ 23.8, 49.0, 53.1, 54.9, 120.9, 147.7, 168.1, 188.6, 201.2; mass spectrum, m/z (relative intensity) (30 eV) 251 (M^+, 3.2), 222 (3.3), 208 (100), 192 (1.5), 180 (5.8), 179 (4.9), 150 (4.3), 136 (4.8); HRMS (EI) calcd for $C_{12}H_{13}O_5N$ 251.0794, found 251.0774.

Propylidene-MDA and Formation of Diels-Alder Adduct 25 with Ethyl Vinyl Ether. Sodium MDA (231 mg, 2.12 mmol) and propionaldehyde (0.3 ml, 2.08 mmol) were dissolved in 6 ml of deoxygenated water, and allowed to stand at 5 °C under a nitrogen atmosphere for 12 hr. The reaction mixture was warmed to room temperature and 660 mg of $NaH_2PO_4 \cdot H_2O$ (5.16 mmol) was added followed by ethyl vinyl ether (0.6 ml, 6.27 mmol) in 2 ml of chloroform. The biphasic reaction solution was stirred vigorously for 3 h, after which $NaHCO_3$ (500 mg) in 5 ml of water was added. The aqueous layer was separated and extracted with chloroform (3 x 20 ml). The organic layer was dried over Na_2SO_4 and chromatographed on a silica gel preparative plate with 8% methanol/chloroform as the solvent. The band with R_f 0.65 afforded 85 mg (22%) of 26 as a pale yellow oil. ^{1}H NMR ($CDCl_3$) δ 0.90 (t, 3 H), 1.23 (m, 4 H), 1.87 (m, 3 H), 2.49 (br m, 1 H), 3.75 (br m, 2 H), 5.14 (m, 1 H), 7.17 (s, 1 H), 9.22 (s, 1 H); mass spectrum, m/z (relative intensity) (70 eV) 184 (M^+, 2.1) 155 (5.6), 139 (4.6), 138 (9.8), 122 (15.4), 109 (100); HRMS (EI) calcd for $C_{10}H_{16}O_3$ 184.1099, found 184.1098.

Benzyl "bis-MDA" and Its Conversion to 1,4-Dihydropyridine-3,5-dicarboxaldehyde 15. Sodium MDA (210 mg, 1.87 mmol) in 2 ml of water was added to benzylidene MDA[36] (303 mg, 1.89 mmol) in 7 ml of acetone. The reaction mixture was stirred for 2.5 h at room temperature. The solvent was evaporated under reduced pressure and the residue was crystallized (EtOH) to give the benzyl "bis MDA" (24, R'=Ph, Na salt) as yellow crystals (440 mg, 93%): UV (0.1N HCl)λ_{max} 249 nm (ε 13849); UV (0.1N NaOH) λ_{max} 272 nm (ε29277). ^{1}H NMR (D_2O) δ 5.50 (s, 1 H), 7.24 (m, 5 H), 8.45 (br s, 4 H).

Benzyl "bis-MDA" (1 equiv) was converted to dihydropyridine 7 (35%) by reaction with ammonium acetate (1 equiv) at 60 °C and pH 4.2 for 3 h.

Propyl "bis-MDA" and Its Conversion to 1,4-Dihydropyridine-3,5-dicarboxaldehyde 4. Sodium MDA (187 mg, 1.67 mmol) was dissolved in 1 ml of water to which was added propionaldehyde (0.06 ml, 0.85 mmol) in 7 ml of acetone. The reaction mixture was stirred at room temperature for 12 h. The solvent was removed under reduced pressure and the residue was crystallized from ethanol-ether to give the propyl "bis-MDA" (Na salt) (91 mg, 47%) as off-white crystals: UV (0.1N HCl)λ_{max} 248 nm (ε13334); UV (0.1N NaOH) λ_{max} 270 nm (ε 30322); ^{1}H NMR (D_2O) δ 0.78 (m, 3 H), 1.74 (m, 2 H), 4.47 (t, 1 H), 8.40 (br s, 4 H).

Propyl "bis-MDA" (1 equiv) was converted to the 1,4-dihydropyridine 4 (50%) by heating with glycine methyl ester (1 equiv) at 55 °C and pH 4.2 for 3 h.

Single-Crystal X-ray Structure Determination of 1,4-Dihydropyridine 15. A colorless needle-like crystal, .05 mm(0,1,0)x .10 mm (0,1,-1) x .62 mm (1,0,0) mounted on a glass fiber with [1,0,0] roughly parallel phi rotation axis of Enraf-Nonius CAD-4 diffractometer; graphite monochromator, MoKalpha radiation, alpha(aver)=.71073 A; 295K data collection; omega/two theta scan, 0.6 + .35 tan(theta); background counts, 25% below and above range; peak counting time/background counting time= 2/1; horizontal aperture, 2.4 to 3.0 mm depending on angle; scan speed, 0.5 - 2.5 deg/min depending on intensity; reflections collected to 2 theta(max) = 40. Lorentz and polarization corrections were made but absorption corrections were not (mu= 0.59 cm^{-1}). The three standard reflections used to monitor decay showed a decrease of only 2.1% so reflections were not corrected for decay. A total of 7995 reflections were measured of which 3000 were classed as absent. Net averaged reflections = 1212, of which 661 exceeded 3 sigma. Agreement among equivalent reflections observed is 3.2% based on F, 2.7% based on F*F. Cell dimensions were obtained from 25 reflections used to determine the orientation matrix, a = 7.524(6), b = 14.009(7), c = 20.236(11) A. The cell volume is 2132.95 A^3. For Z = 4, F.W. = 213.25, the calculated density is 1.328 g/cm^3.

The structure was solved by direct methods and refined by full matrix least squares. All hydrogen atoms were located from difference maps, and refined. Anisotropic refinement on all non-hydrogen atoms, but not including hydrogen atom positions = 146 parameters, 661 reflections, gave R = .081, Rw = .120. Anisotropic refinement on all non-hydrogen atoms and isotropic refinement on hydrogen atoms gave R(1) = .022, R(2) = R(w) = .026. The standard deviation of an observation of unit weight = 1.074. Weights used in the refinement are those of Killean and Laurence[37] with P = .01, Q = 0.0. The last parameter shift/error was less than 0.03. The final difference map has a maximum residual electron density of 0.08(2) el/A^3. The rather small ratio of reflections/parameter is justified by (1) the use of averaged data from a full sphere, (2) by the large decrease in the agreement factor on addition of H atoms to the calculation, and (3) the subsequent refinement of H atom positions to reasonable values. All crystallographic calculations were made using the SDP set of programs of Enraf-Nonius Corp. Atom parameters and bond distances and angles are summarized in Tables 1 and 2.

Table 1. Atom parameters for 4-phenyl-1,4-dihydropyridine-3,5-dicarboxaldehyde (15).

Atom	x	y	z	B
	x10000	x10000	x100000	
N1	1954(2)	1301(2)	51913(9)	3.86(5)
C2	3549(3)	1380(2)	48798(11)	3.27(5)
C3	5096(3)	1237(2)	51968(9)	2.59(5)
C4	5214(3)	1033(1)	59251(9)	2.57(5)
C5	3369(3)	831(2)	61889(10)	3.01(5)
C6	1904(3)	987(2)	58264(11)	3.67(6)
C31	6679(3)	1278(2)	48049(11)	3.54(5)
C51	3161(3)	460(2)	68457(11)	3.92(6)
C7	6076(2)	1850(2)	62981(9)	2.52(5)
C8	7626(3)	1710(2)	66555(10)	3.44(5)
C9	8418(3)	2458(2)	69848(11)	4.65(6)
C10	7684(3)	3348(2)	69711(11)	4.73(6)
C11	6136(3)	3502(2)	66269(12)	4.19(6)
C12	5340(3)	2754(2)	62939(10)	3.22(5)
O32	8175(2)	1137(1)	50181(8)	4.42(4)
O52	4390(2)	232(1)	71992(7)	4.83(4)
Atom	x	y	z	B
	x1000	x1000	x10000	
HN1	600(2)	368(1)	5033(10)	2.0(5)*
H2	649(2)	657(1)	609(9)	1.0**
H4	599(2)	458(1)	1006(8)	1.0**
H6	571(3)	413(1)	3974(9)	2.3(5)*
H8	810(2)	395(1)	1693(10)	1.6(5)*
H9	548(3)	765(2)	2235(11)	3.4(6)*
H10	176(3)	610(2)	2802(12)	4.2(7)*
H11	444(3)	585(2)	3390(10)	2.7(5)*
H12	574(3)	713(1)	3965(9)	1.5(5)*
H31	351(2)	639(1)	714(9)	1.4(5)*
H51	186(3)	463(2)	1990(9)	2.3(5)*

Starred atoms were refined isotropically.
Anisotropically refined atoms are given in the form of the isotropic equivalent displacement parameter defined as:
(4/3)*[a2*B(1,1)+b2*B(2,2)+c2*B(3,3)+ab(cos gamma)*B(1,2)+ac(cos beta)*B(1,3)+bc(cos alpha)*B(2,3)]

Double starred atoms had the B value fixed, because on refinement the B value became negative.

Table 2. Bond distances and angles for 4-phenyl-1,4-dihydropyridine-3,5-dicarboxaldehyde (15).

N1-C2	1.360(3)	C6-N1-C2	119.3(2)
N1-C6	1.360(3)	N1-C2-C3	122.1(3)
C2-C3	1.344(3)	N1-C6-C5	123.1(2)
C6-C5	1.342(3)	C2-C3-C4	123.2(2)
C3-C4	1.504(3)	C6-C5-C4	122.0(2)
C5-C4	1.514(3)	C2-C3-C31	116.8(2)
C3-C31	1.432(3)	C6-C5-C51	118.4(2)
C5-C51	1.436(4)	C4-C3-C31	120.1(2)
C31-032	1.221(3)	C4-C5-C51	119.6(2)
C51-052	1.212(3)	C3-C4-C5	109.1(2)
C4-C7	1.516(3)	C3-C4-C7	111.7(2)
C7-C8	1.386(3)	C5-C4-C7	110.9(2)
C7-C12	1.383(3)	C3-C31-032	124.4(2)
C8-C9	1.378(4)	C5-C51-052	124.0(3)
C12-C11	1.382(4)	C4-C7-C8	120.9(2)
C9-C10	1.364(4)	C4-C7-C12	121.1(2)
C11-C10	1.374(4)	C8-C7-C12	118.0(2)
C8-C81		C7-C8-C9	120.6(3)
C81-F82A		C7-C12-C11	121.2(3)
C81-F82B		C8-C9-C10	120.7(3)
C81-F82C		C12-C11-C10	119.7(3)
		C9-C10-C11	119.8(3)
C7-C8-C81		C8-C81-F81A	
C9-C8-C81		C8-C81-F81B	
F81A-C81-F81B		C8-C81-F81C	
F81A-C81-F81C		F81B-C81-F81C	
N1-H1	.85(3)	HN1-N1-C2	119(2)
C2-H2	1.02(2)	HN1-N1-C6	120(2)
C6-H6	1.00(3)	H2-C2-N1	116(1)
C31-H31	1.07(3)	H2-C2-C3	121(1)
C51-H51	1.03(3)	H6-C6-N1	118(1)
C4-H4	1.05(3)	H6-C6-C5	119(1)
C8-H8	1.00(3)	H31-C31-C3	116(1)
C12-H12	.98(2)	H51-C51-C5	114(1)
C9-H9	.98(3)	H31-C31-032	119(1)
C11-H11	1.01(3)	H51-C51-052	122(1)
C10-H10	.99(3)	C3-C4-H4	110(1)
		C5-C4-H4	107(1)
		C7-C4-H4	108(1)
C7-C8-H8	118(1)	C8-C9-H9	120(2)
C9-C8-H8	121(1)	C10-C9-H9	121(2)
C9-C10-H10	122(2)	C10-C11-H11	121(2)
C11-C10-H10	118(2)	C12-C11-H11	119(2)
C7-C12-H12	119(1)		
C11-C12-H12	120(1)		

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New Methodologies for the Synthesis of C-2 Functionalized Hypoxanthine Nucleosides

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Under palladium-catalyzed conditions, newly synthesized 2-iodohypoxanthine systems undergo efficient cross-coupling with organostannanes to furnish rare C-2 functionalized congeners of inosine. This represents the first examples of the use of organotin reagents in palladium-catalyzed cross-coupling involving nucleosides. 2-Acetonyl-, 2-(cyanomethyl)-, and 2-vinylinosines not only are readily accessible by this approach but can also be elaborated further through regiospecific hydroboration, osmylation, and reduction reactions. The reaction with enol acetates has considerable generality. In addition, this methodology for functionalized carbon–carbon bond formation can be extended to include 2-iodoadenosine. The products of the latter modification are exceedingly poor substrates for the enzyme adenosine deaminase.

The recent surge of interest in the synthesis of rare and previously inaccessible purine nucleosides is the result of observations that some compounds belonging to this family have potent antiviral therapeutic activity as well as being of enzymological usefulness as biological probes for the study of key viral-encoded enzymes.[1-6] Our interest in new antiviral nucleosides has focused attention on functionalized C-2 alkylated hypoxanthine systems. Although several C-2 substituted hypoxanthine nucleosides are known,[7-9] very few functionalized alkylated derivatives have been reported.[10] This is largely because of limitations in synthetic methodologies that allow access to this class of compounds. Virtually all of the 2-substituted inosines known have been synthesized from imidazole nucleosides through ring-closure reactions.[7,8] Other methods known for entry into this general class of compounds appear to be of more limited scope.[11,12] This paper reports on the development of a general methodology for the introduction of functionalized carbon–carbon bonding at the 2-position of the hypoxanthine ring and elaboration of the synthons introduced for the preparation of new congeners of inosine.[13] Also reported are the application of this methodology to an adenine nucleoside and the behavior of the resulting system toward the enzyme adenosine deaminase.

The key intermediate for the synthesis of these rare nucleosides was protected 2-iodo-6-methoxypurine 5 (Scheme I). This precursor can be prepared from guanosine in five steps. The first two steps are well-known and involve selective acetylation in 93% yield with acetic anhydride, triethylamine, and 4-(dimethylamino)pyridine in acetonitrile,[14] followed by treatment of the triacetylated guanosine with phosphorus oxychloride and *N,N*-diethylaniline at 70 °C for 1 h to give the 6-chloro compound 2 in about 89% yield.[15,16] It should be emphasized that consistently high yields in the conversion of the triacetylated guanosine to 2 were obtained when the reaction time was kept to 1 h under these reaction conditions. Considerable decomposition of both starting material and product occurred when longer reaction times were used. Preparation of the dihalogenated compound 3 from 2 involved a radical deamination–halogenation reaction.[16-18] The presence of an electron-withdrawing group at the 2-position in 3 renders the 6-position extremely susceptible to nucleophilic attack. Thus, nucleophilic displacement of the 6-chloro group in 3 with methoxide ion not only occurred with considerable ease but was accompanied by the desired deprotection of the acetate groups (96%). The carbohydrate moiety was then protected with *tert*-butyldimethylsilyl groups to give the key precursor 5 (96%). The silyl protecting groups[19] were deemed synthetically more appropriate for subsequent further elaboration of the base moiety (Scheme I). Also, when the 6-methoxy-2-iodopurine nucleoside formed in the treatment of 3 with sodium methoxide was demethylated with trimethylsilyl iodide, 2-iodoinosine was isolated. 2-Iodoinosine (4) has not been previously reported.

Synthesis of the novel C-2 functionalized hypoxanthine nucleosides described in this paper required, as the crucial step, a palladium-catalyzed cross-coupling reaction of a masked 2-iodoinosine system with an organostannane containing the desired synthon. Transfer of the synthon to the hypoxanthine ring, as for example in the conversion of 5 to 6 (70% yield), presumably involves oxidative insertion of palladium into the carbon–iodine bond of the masked iodohypoxanthine followed by cross-coupling of the derived Pd(II) complex with the tin enolate of acetone and reductive elimination (via the cis intermediate) to give the desired product (Scheme II).[20] The tributyltin enolate

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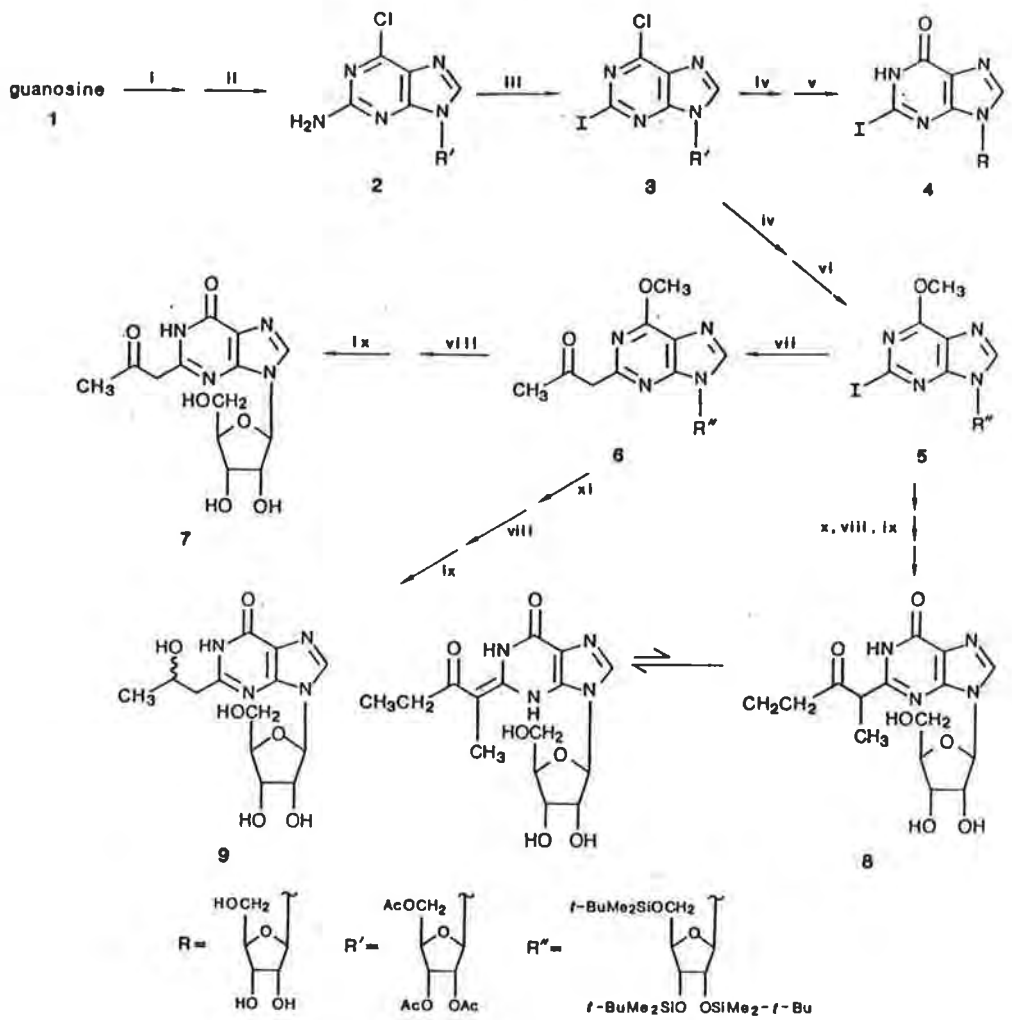
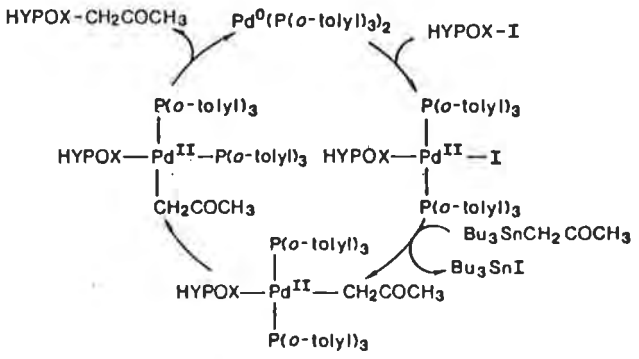
Scheme I[a]



[a] Conformation about glycosidic bond not implied by these structural representations. (i) Ac_2O, $(C_2H_5)_3N$, 4-(dimethylamino)pyridine, CH_3CN; (ii) $POCl_3$, *N,N*-diethylaniline, Δ; (iii) *n*-$C_6H_{11}ONO$, CH_2I_2, CH_3CN, Δ; (iv) $NaOCH_3$, CH_3OH; (v) $(CH_3)_3SiI$, CH_3CN, DMF; (vi) *t*-$Bu(CH_3)_2SiCl$, imidazole, DMF; (vii) $Pd(OAc)_2$, (*o*-tolyl)$_3$P, *n*-Bu_3SnOMe, $CH_2{=}C(CH_3)OAc$, toluene, Δ; (viii) $(CH_3)_3SiI$, CH_3CN; (ix) Et_4NF, CH_3CN; (x) $Pd(OAc)_2$, (*o*-tolyl)$_3$P, *n*-Bu_3SnOMe, $CH_3CH{=}C(C_2H_5)OAc$, toluene, Δ; (xi) $NaBH_4$, THF.

Scheme II[a]



[a] HYPOX-I = 2-iodohypoxanthine system.

of acetone, required for the cross-coupling step, is generated in situ from isopropenyl acetate and tributyltin methoxide. The palladium catalyst appears to be regenerated in this reaction as only very small amounts are needed. This work represents the first examples of the use of an organostannane in palladium-catalyzed cross-coupling involving nucleosides.

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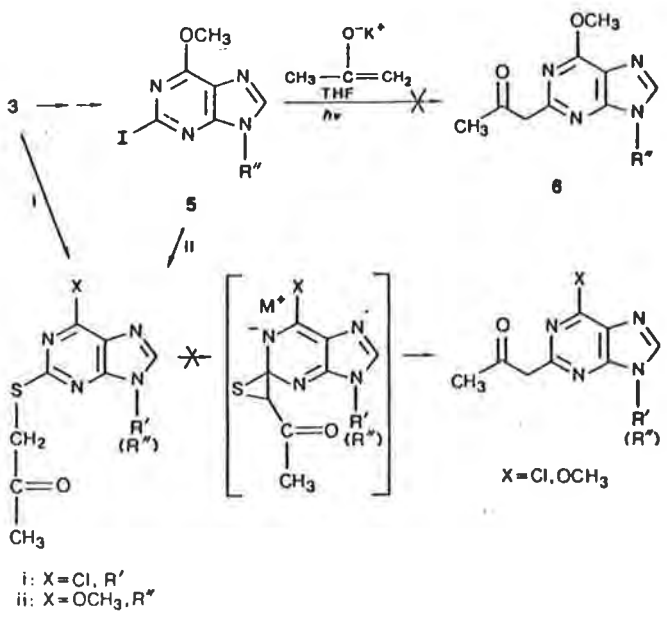
Scheme III



i: X=Cl, R'
ii: X=OCH_3, R''

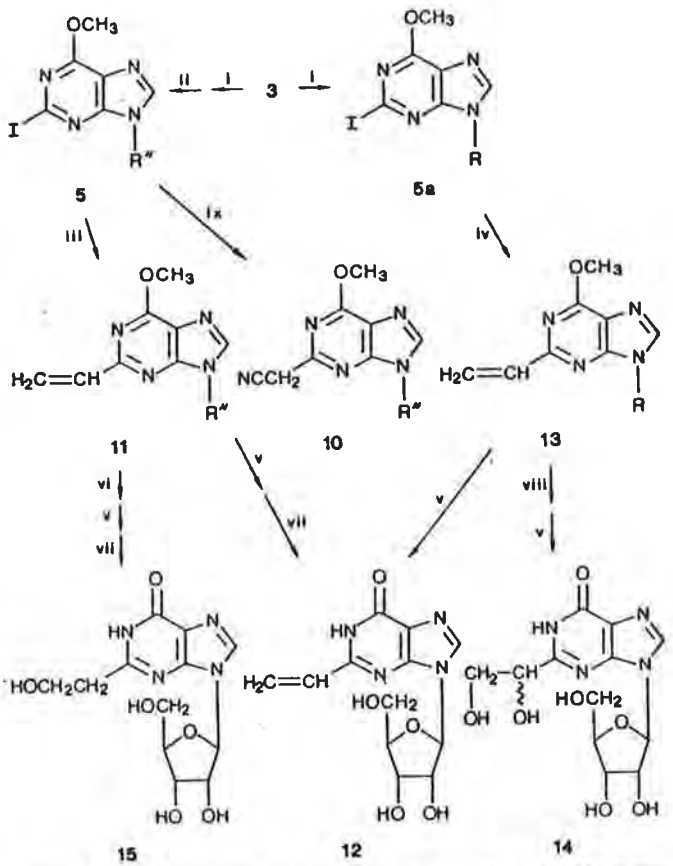
Other methods for the introduction of functionalized alkyl groups at the 2-position of the hypoxanthine ring are also possible. For example, the $S_{RN}1$ reaction[21] has been

used successfully by us for the modification of the purine ring at the C-6 position.[22] However, the photoinduced $S_{RN}1$ reaction of 5 with the potassium enolate of acetone was unsuccessful (Scheme III). Purine nucleosides bearing a thioketone group at the 6-position are known to undergo the Eschenmoser sulfide contraction reaction.[23] In theory, such a reaction should also be possible with a thioacetonyl group at the 2-position. Compounds 2 and 5 can be converted to their 2-thioacetonyl derivatives through a thermal deamination–thiolation (*n*-pentyl nitrite, diacetonyl disulfide) and a photochemical reaction (diacetonyl disulfide), respectively. However, when the sulfur extrusion reaction was carried out on these compounds under a variety of conditions that involved changes in solvent, base, and phosphine, no 2-acetonyl product was isolated (Scheme III). This is consistent with the lack of any precedence in the literature for the success of the sulfide contraction at the C-2 position of the purine ring. Other approaches attempted, such as the Meerwein reaction[24] on the 2-amino compound 2, were also unsuccessful.

The acetonylated nucleoside 6 is the protected form of 2-acetonylinosine (7). The methoxy group is an effective way of protecting the amide carbonyl group in the hypoxanthine system. This protective group is relatively stable and can be easily removed at the end of a reaction sequence. Accordingly, in the case of 6, conversion to the target molecule 7 was achieved in two steps by reaction first with trimethylsilyl iodide (64%), and subsequently with tetrabutylammonium fluoride (93%). The overall yield of 7 starting from guanosine was 18% (Scheme I). Purification of compound 7 and all other free nucleosides described in this paper was carried out by reversed-phase HPLC on Amberlite XAD-4 resin (styrene–divinylbenzene copolymer) with ethanol/water as the eluting solvent. Characterizations were carried out by UV, FTIR, and high-field NMR spectroscopy. 2-Acetonylinosine exists in a single tautomeric form (i.e., the keto isomer), which is apparently stabilized by intramolecular hydrogen bonding. The spectral data (particularly the UV and FTIR) supported the presence of the keto tautomer. The high-field carbon-13 NMR data also provided qualitative information on the preferred glycosidic bond conformation in solution through the magnitude of the ^{13}C NMR chemical shift difference between C-2′ and C-3′. A small difference (<0.5 ppm) is correlated with a preferred syn conformation in solution whereas a larger difference (>3.0 ppm) suggests a preferred anti conformation.[25] The high-field carbon spectrum of 7 shows this chemical shift difference to be 3.4 ppm, which suggests a preferred anti conformation about the glycosidic bond. This was found to be the case for all of the C-2 substituted nucleosides described in this paper.

The palladium-catalyzed cross-coupling methodology with enol acetates described above appears to have some generality. For example, the 2-iodopurine 5 undergoes coupling under these conditions with 3-acetoxypent-2-ene[26] in the presence of tri-*n*-butyltin methoxide to give the expected keto product, which can be deprotected to 8. Other enol acetates, both symmetrical and unsymmetrical (except the parent vinyl acetate) are also reactive under

Scheme IV[a]



[a] (i) $NaOCH_3$, CH_3OH; (ii) *t*-Bu$(CH_3)_2SiCl$, imidazole, DMF; (iii) *n*-$Bu_3SnCH{=}CH_2$, $PdCl_2(CH_3CN)_2$, toluene, Δ; (iv) $PdCl_2(CH_3CN)_2$, *n*-$Bu_3SnCH{=}CH_2$, DMF, Δ; (v) $(CH_3)_3SiI$, CH_3CN; (vi) 9-BBN, THF, Δ; (vii) Et_4NF, CH_3CN; (viii) OsO_4, pyridine; (ix) $Pd(OAc)_2$, (*o*-tolyl)$_3$P, *n*-Bu_3SnCH_2CN, toluene, Δ.

the conditions of cross-coupling. Interestingly, compound 8 appears to prefer the tautomeric structure shown with an exocyclic double bond ("xanthosine-like" arrangement) as evidenced from ^1H NMR data (singlet methyl adjacent to keto group), ^{13}C NMR data (carbon adjacent to carbonyl at 79.8 ppm, ketone carbonyl at 209.2 ppm), and the FTIR spectrum (overlapping ketone and lactam carbonyls at 1685 cm^{-1}). The difference in the preferred tautomeric form in the case of 8 compared with 7 is not entirely clear.

The protected 2-acetonyl compound 6 could be reduced readily by sodium borohydride to give, after deprotection, the new nucleoside 9 (Scheme I).

The palladium-catalyzed cross-coupling reaction described above can be extended further to include other functionalized organostannanes. For example, tri-*n*-butyl(cyanomethyl)stannane[27] can be prepared from the reaction of tributyltin methoxide and (trimethylsilyl)acetonitrile. Reaction of this reagent with 5 under our cross-coupling procedure gave the 2-(cyanomethyl)inosine 10 in 55% yield (Scheme IV). 2-Vinylinosine (12) (or 11), potentially a key precursor for the synthesis of a variety of functionalized alkylated purine nucleosides, is also readily available via the aforementioned methodology. Thus, the thermal reaction of 5 with tri-*n*-butylvinylstannane in the presence of palladium chloride afforded 11 in excellent yields (>90%). *Interestingly, the vinylation reaction can be carried out in almost quantitative yields with the 2-*

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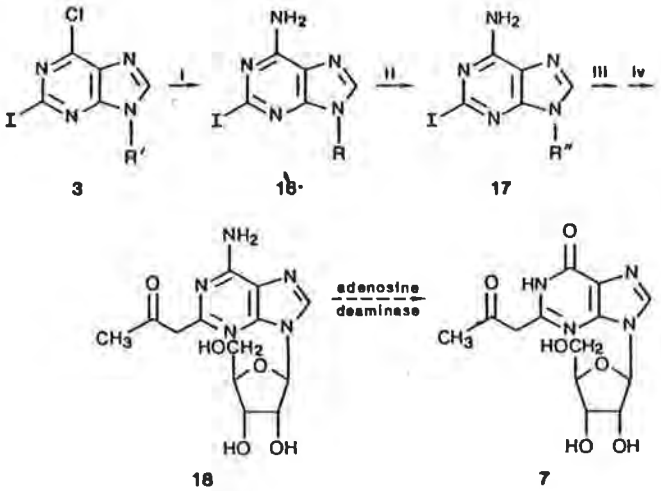
Scheme V[a]



3 16 17 18 7

[a] (i) NH_3, C_2H_5OH; (ii) t-$Bu(CH_3)_2SiCl$, imidazole, DMF; (iii) $Pd(OAc)_2$, (o-tolyl)$_3$P, n-Bu_3SnOMe, CH_2=$C(CH_3)OAc$, toluene, Δ; (iv) Et_4NF, CH_3CN.

iodo nucleoside 5a, where the carbohydrate moiety is unprotected!

Oxidative elaboration of the vinyl group in the aforementioned 2-vinyl nucleoside was carried out by two methods. The first one involved reaction of compound 13 (the partially deprotected form of 11) with osmium tetraoxide (65% yield). Demethylation of the product with trimethylsilyl iodide gave the highly hydroxylated compound 14. The second method of elaboration was hydroboration of the vinyl compound 11 with 9-borabicyclo-[3.3.1]nonane (9-BBN). Oxidative workup of the organoborane intermediate resulted in the regiospecific formation (52% yield, 65% conversion) of the terminal alcohol. Deprotection of this compound afforded 15 (Scheme IV). It is of interest to mention that hydroboration reactions have rarely been used previously to elaborate structures in purine nucleoside chemistry.

Finally, it should be mentioned that we have applied the synthetic chemistry developed above for the preparation of C-2 functionalized adenosines. The starting material for this synthesis was the dihalogenated nucleoside 3. Treatment of 3 with ethanolic ammonia resulted in both the displacement of the 6-chloro group and deprotection of the acetate protecting groups to give 2-iodoadenosine (16) (90%). Silylation of 16 with *tert*-butyldimethylsilyl chloride and imidazole afforded 17, the immediate precursor for the functionalization reaction, in 94% yield. When compound 17 was treated with palladium acetate, tri-*o*-tolylphosphine, tributyltin methoxide, and isopropenyl acetate in toluene at 100 °C, smooth conversion to the acetonyl compound occurred. Deprotection gave the target molecule 18, which exists almost entirely in the keto form depicted as evidenced from spectral data. The overall yield of purified product 18 for the two steps was 40% (Scheme V).

A fundamental reason for the synthesis of the 2-acetonyladenosine system was to examine if compounds bearing functionalized alkylation at this position would be deaminated by the ubiquitous mammalian enzyme adenosine deaminase. This enzyme normally catalyzes the hydrolytic deamination of adenosine to inosine.[28-31] Synthetically, this information would be useful because of the possibility of entering both the adenine and hypoxanthine series of nucleosides by initially synthesizing the adenosine analogues and subsequently deaminating these to the corresponding inosine compounds with adenosine deaminase. Biologically, the results would contribute to the design of prodrugs or of molecules that would be resistant to deamination. The deamination studies with adenosine deaminase were followed by UV spectral methods with adenosine as the standard, using procedures previously described by us.[29] The target molecule, 2-acetonyladenosine (18), was found to be resistant to deamination by this enzyme. Deamination does occur eventually but only after prolonged periods (about 24 h) and with large excesses of enzyme. This provides experimental evidence that substrate binding and significant substrate activity involving this enzyme requires the adenine ring system to be relatively unhindered at N-1, apparently one of the binding sites for the enzyme.

In summary, the new halogenated nucleoside 2-iodoinosine has been synthesized. The palladium-catalyzed cross-coupling reactions of protected or partially protected 2-iodoinosine with organostannanes provide highly efficient approaches to the synthesis of new and rare functionalized congeners of the natural nucleoside inosine. These studies were extended to the corresponding adenosine system. The cross-coupling methodology appears to have generality with respect to both the organostannane reagent and the halogenated nucleoside. Biological studies assessing the antiviral activities of the target molecules against RNA viruses are currently under investigation, and preliminary in vitro results suggest that some of the compounds described are active. The complete biological studies will be reported elsewhere.

Experimental Section

Melting points reported are uncorrected and were determined on a Thomas-Hoover melting point apparatus fitted with a microscope. Nuclear magnetic resonance spectra were recorded on JEOL Model FX90Q and Bruker Model WM360 pulse Fourier transform spectrometers. Mass spectra were determined on a Hewlett-Packard 5985 GC/MS system of a VG Analytical Model ZAB-HF instrument with high-resolution FAB capability. Ultraviolet spectra were recorded on a Varian Cary Model 219 or a Gilford Response spectrophotometer. Infrared spectra were recorded on an IBM Model 98 Fourier transform instrument. Lyophilizations were performed with a Virtis Freezemobile 3 unit. Preparative layer chromatography plates were prepared by coating six 20 cm × 20 cm plates with a slurry made from 150 g of E. Merck PF_{254} silica gel in 400 mL of water. The silica gel plates were allowed to dry slowly and were then activated for 3 h at 135 °C. Flash chromatography was carried out by using glass columns packed with 230–400-mesh silica gel. High-performance liquid chromatography was done by using Altex columns packed with Amberlite XAD-4 resin (Rohm and Haas) which was ground and sieved to 40–60 μm. Samples were injected with a gas-tight syringe through an Altex four-way slide valve. Separations were carried out at 20–80 psi by using an FMI RRPSY-SS $^1/_4$ in. piston pump. Fractions were monitored by a Pharmacia UV-2 ultraviolet monitor, and products were collected on a Gilson FC-100 fraction collector. Satisfactory elemental analyses were obtained for all final products. They were determined by Galbraith Laboratories, Inc., Knoxville, TN.

6-Chloro-2-iodo-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-purine (3). The protected nucleoside 2 (5.549 g, 12.97 mmol), prepared as described previously,[15,16] was taken up in acetonitrile (40 mL). Diiodomethane (5 mL) and n-pentyl nitrite (12 mL) were added. The solution was purged with nitrogen for 15 min and heated to a light reflux for 20 h under N_2. Concentration

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of the solution in vacuo was followed by purification on a silica gel flash column, first using hexane and then a 2:1 mixture of hexane and ethyl acetate as the eluting solvents. The product 3 was isolated as a light orange glass (3.852 g, 7.15 mmol, 55% yield). Crystallization from ethanol provided white needles: mp 181–183 °C (lit.[15] mp 181–183 °C); ^{1}H NMR ($CDCl_3$) δ 2.11 (s, 3 H), 2.13 (s, 3 H), 2.18 (s, 3 H), 4.43 (m, 3 H), 5.65 (t, 1 H), 5.81 (t, 1 H), 6.23 (d, 1 H), 8.27 (s, 1 H); UV (EtOH) λ_{max} 222.5 (ϵ 2.1 × 10^4), 258 (6.6 × 10^3), 281 nm (9.3 × 10^3).

2-Iodo-6-methoxy-9-[2,3,5-tri-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]purine (5). Dry 6-chloro-2-iodo triacetylated nebularine 3 (1.780 g, 3.30 mmol) was taken up in dry (Omnisolve) methanol (50 mL) to which sodium methoxide (1.045 g, 19.35 mmol) was subsequently added. The solution was fitted with a septum and stirred at 25 °C for 12 h. The reaction was quenched by heating (50 °C) with 1 g of NH_4Cl. After filtration, the solution was concentrated in vacuo. The residue was adsorbed on 5 g of 230–400-mesh silica gel and added to the top of a 40-mm silica gel flash chromatography column. The product was eluted off with 1:10 methanol/chloroform. The eluant was concentrated to give 1.059 g (2.60 mmol, 78.6%) of product, which was silylated with *tert*-butyldimethylsilyl chloride (1.56 g, 10.40 mmol) and imidazole (1.414 g, 20.8 mmol) in dimethylformamide (20 mL). The product 5 was isolated as a low-melting gum (1.560 g, 80% yield) after flash chromatography on silica gel: ^{1}H NMR ($CDCl_3$) δ −0.02–0.13 (m, 18 H), 0.90 (m, 27 H), 3.71–4.63 (m, 5 H), 4.13 (s, 3 H), 5.93 (d, 1 H), 8.20 (s, 1 H); UV (EtOH) λ_{max} 262 nm (ϵ 7.8 × 10^3); mass spectrum, *m/z* (relative intensity) 751 (M^+, 1.2), 693 ($M^+ - t$-Bu, 100), 433 (26.8), 285 (5.7), 261 (27.7), 231 (19.0), 211 (18.0), 175 (4.1), 155 (7.0), 147 (47.5), 129 (21.9), 115 (19.4).

2-Iodoinosine (4). 2-Iodo-6-methoxy-9-(β-D-ribofuranosyl)-purine (1.560 g, 3.800 mmol), prepared from the reaction of 3 with sodium methoxide as described above, was dissolved in a mixture of dry DMF (4.0 mL) and dry acetonitrile (6.5 mL), and to this was added potassium iodide (2.770 g, 16.7 mmol). This mixture was purged with nitrogen, treated with trimethylsilyl chloride (1.75 mL, 15.8 mmol), and then stirred at 25 °C under nitrogen for 24 h. Dilute aqueous NaOH was then added to the reaction mixture, and the pH was adjusted to 7.0. The solvents and water were removed under reduced pressure, and the residue was flash chromatographed on silica gel with 1:4 methanol/dichloromethane as the eluting solvent. The crude product was purified by HPLC on Amberlite XAD-4 resin with ethanol/water as the eluting solvent. Pure 2-iodoinosine (4) was obtained as off-white crystals (0.221 g, 0.561 mmol, 15%). The low yield is the result of considerable decomposition of product under the reaction conditions. Compound 4 gave the following data: mp 255 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 3.58 (m, 2 H), 3.93 (m, 1 H), 4.13 (m, 1 H), 4.46 (m, 1 H), 5.00 (t, 1 H), 5.17 (d, 1 H), 5.46 (d, 1 H), 5.80 (d, 1 H), 8.24 (s, 1 H); ^{13}C NMR (Me_2SO-d_6) δ 61.1, 70.2, 73.9, 85.7, 87.0, 109.6, 123.8, 138.3, 148.2, 156.8; UV (EtOH) λ_{max} 253 (ϵ 14 900), 272 nm (sh, 11 900); FAB HRMS obsd (M^+ + H) 394.9874, calcd for $C_{10}H_{11}N_4O_5I$ 394.9852.

2-Acetonyl-6-methoxy-9-[2,3,5-tri-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]purine (6). Silylated 2-iodo-6-methoxynebularine (5) (1.208 g, 1.611 mmol) was placed in a 100-mL round-bottomed flask already containing palladium acetate (0.036 g, 0.161 mmol) and tri-*o*-tolylphosphine (0.098 g, 0.322 mmol), and the flask was placed on the vacuum pump to remove residual oxygen and moisture. Toluene (25 mL), freshly distilled from sodium hydride, was added under nitrogen. In a separate flask, toluene (10 mL), isopropenyl acetate (0.28 mL, 2.42 mmol), and tributyltin methoxide (0.70 mL, 2.42 mmol) were combined. This solution was kept at 40 °C for 30 min. The nucleoside solution was then transferred by double-tipped needle to the flask containing the tin reagent. After heating of this solution under N_2 for 6 h at 95 °C, no starting material remained (TLC, 2:1 hexane/ether). The solution was cooled to room temperature and partitioned between ether (70 mL) and 5% aqueous disodium ethylenediaminetetraacetic acid (Na_2EDTA). The ether layer was then extracted with water (30 mL). The aqueous phases were back extracted with ether (30 mL), and the combined ether layers were dried (Na_2SO_4) and concentrated. The crude product was purified by flash chromatography, using 2:1 hexane/ether as the eluting solvent, to give the protected acetonylated product 6 (0.7952 g, 1.17 mmol, 73%) as a low-melting solid: ^{13}C NMR ($CDCl_3$) δ −5.3, −4.9, −4.7, −4.3, 17.9, 18.1, 18.6, 25.7, 25.8, 26.1, 29.8, 54.1, 54.3, 62.2, 71.3, 76.1, 84.9, 88.6, 120.4, 141.0, 152.3, 158.4, 160.7, 204.3; ^{1}H NMR ($CDCl_3$) δ −0.02–0.13 (m, 18 H), 0.92 (m, 27 H), 2.23 (s, 3 H), 3.78–4.05 (m, 3 H), 3.98 (s, 2 H), 4.13 (s, 3 H), 4.30 (t, 1 H), 4.52 (t, 1 H), 6.03 (d, 1 H), 8.27 (s, 1 H); UV (EtOH) λ_{max} 250 (ϵ 9.0 × 10^3), 290 nm (ϵ 2.7 × 10^3). (Note: All experimental work with organostannanes must be carried out in efficient fume hoods.)

2-Acetonyl-9-(β-D-ribofuranosyl)hypoxanthine (7). The protected acetonylated product 6 (0.850 g, 1.25 mmol) was taken up in 25 mL of acetonitrile, which had been freshly distilled from calcium hydride. Potassium iodide (0.293 g, 1.75 mmol), which had been dried on the vacuum pump at 50 °C, was added, followed by trimethylsilyl chloride (0.223 mL, 1.75 mmol) via a gas-tight syringe. The solution was stirred for 8 h at room temperature and then filtered, and the precipitate was washed with ether. The filtrate and ether washings were concentrated and purified by flash chromatography using ether as the eluting solvent to give the demethylated product (0.5305 g, 0.7965 mmol, 64% yield). This compound was dissolved in dry tetrahydrofuran (50 mL) and treated with 3.186 mmol of tetrabutylammonium fluoride (TBAF) in tetrahydrofuran (1 M solution). The solution was stirred until no starting material remained (2 h). An aqueous solution of a 10-fold excess of ammonium chloride was added, and the tetrahydrofuran was removed in vacuo. The remaining aqueous solution was heated at 50–60 °C for 45 min. The aqueous phase was then extracted with chloroform (2 × 25 mL) and then with ether (2 × 25 mL). The aqueous phase was then concentrated under reduced pressure, and the residue was purified by reversed-phase HPLC on Amberlite XAD-4 resin (40–60 μm) using ethanol/water as the mobile phase. The combined fractions containing the product were lyophilized, and compound 7 was obtained as white crystals (0.180 g, 0.557 mmol, 70%): mp 114–116 °C; ^{13}C NMR (Me_2SO-d_6) δ 30.0, 49.2, 61.5, 70.6, 74.0, 85.8, 87.1, 122.8, 138.7, 148.5, 152.7, 157.0, 202.9; ^{1}H NMR (Me_2SO-d_6) δ 2.23 (s, 3 H), 3.59 (m, 2 H), 3.92 (br s, 3 H), 4.11 (m, 1 H), 4.47 (m, 1 H), 5.11 (t, 1 H), 5.23 (d, 1 H), 5.50 (d, 1 H), 5.84 (d, 1 H), 8.32 (s, 1 H); UV (H_2O) λ_{max} 250 (ϵ 1.2 × 10^4), 266 nm (6.8 × 10^3); FTIR (KBr) 3317, 2961, 2934, 1701, 1692, 1687, 1585, 1558 cm^{-1}; FAB HRMS obsd (M^+ + H) 325.1171, calcd for $C_{13}H_{16}N_4O_6$ 325.1148.

2-(1-Methyl-2-oxobutyl)-9-(β-D-ribofuranosyl)hypoxanthine (8). Palladium acetate (0.178 g, 0.793 mmol) and tri-*o*-tolylphosphine (0.483 g, 1.587 mmol) were added to a flask containing 3.774 g (5.026 mmol) of 5. Freshly distilled toluene (80 mL) was added under N_2. In a separate flask, 2.20 mL (7.64 mmol) of tributyltin methoxide and 2.25 g (17.55 mmol) of 3-acetoxypent-2-ene[26] were warmed at 45 °C for 40 min. This solution was then transferred via double-tipped needle to the flask containing 5. The solution was stirred for 9 h at toluene reflux under N_2. Upon cooling, the reaction mixture was filtered and diluted with 75 mL of ethyl ether. The organic solution was washed with 10% Na_2EDTA (3 × 30 mL) and water (50 mL) and was then dried (Na_2SO_4). The solvents were removed under reduced pressure. The residue was flash chromatographed on silica gel by using hexane followed by 3:1 hexane/ether to provide 2.763 g (3.896 mmol, 77.5%) of product as an oil: ^{1}H NMR ($CDCl_3$) δ −0.21 to −0.05 (m, 18 H), 0.62–0.76 (m, 27 H), 0.82 (t, 3 H, *J* = 7.3 Hz), 1.32 (d, 3 H, *J* = 7.3 Hz), 2.25 (q, 2 H, *J* = 7.3 Hz), 3.64 (m, 2 H), 3.82 (m, 1 H), 3.93 (m, 4 H), 4.14 (m, 1 H), 4.37 (m, 1 H), 5.85 (d, 1 H, *J* = 3.9 Hz), 8.12 (s, 1 H); UV (EtOH) λ_{max} 250, 295 nm; mass spectrum, *m/z* (relative intensity) (30 eV) 708 (M^+, 0.6), 651 (M − *tert*-butyl, 57.8), 391 (28.5), 261 (22.5), 235 (59.4).

The two-step deprotection of the above-mentioned product (2.564 g, 3.615 mmol) was carried out as described for the synthesis of 7 except that tetraethylammonium fluoride (TEAF) was used for the desilylation step instead of TBAF. (Fewer difficulties were encountered with replacement of the tetraethylammonium counterion by a proton than the tetrabutylammonium ion.) The final product was purified as described for 7 to give 8 (0.440 g, 1.249 mmol, 34.6%) as a white solid: mp 104–106 °C; ^{13}C NMR (Me_2SO-d_6) δ 8.0, 23.6, 29.6, 61.7, 70.7, 74.0, 79.8, 85.8, 87.5, 123.3, 139.5, 148.0, 156.8, 158.4, 209.2; ^{1}H NMR (Me_2SO-d_6) δ 0.95 (t, 3 H, *J* = 7.33 Hz), 1.63 (s, 3 H), 2.65 (q, 2 H, *J* = 7.32 Hz), 3.58 (m, 2 H), 3.94 (m, 1 H), 4.14 (m, 1 H), 4.51 (m, 1 H), 4.99 (m, 1 H), 5.19 (m, 1 H), 5.45 (m, 1 H), 5.84 (d, 1 H, *J* = 5.86 Hz), 6.55

(br s, 1 H), 8.32 (s, 1 H); UV (H_2O) λ_{max} 251.6 (ϵ 10.5 × 10^4), 272 nm (sh, 5.6 × 10^3); FTIR (KBr) 3200–3500, 1695, 1685, 1554 cm^{-1}; FAB HRMS obsd (M^+ + H) 353.1987, calcd for $C_{15}H_{21}N_4O_6$ 353.1466.

2-(2-Hydroxypropyl)-9-(β-D-ribofuranosyl)hypoxanthine (9). In a 100-mL round-bottomed flask, the protected 2-acetonylinosine (6) (0.790 g, 1.166 mmol) was dissolved in dry tetrahydrofuran (30 mL). Sodium borohydride (0.208 g, 5.520 mmol) was added, and the reaction mixture was stirred at 25 °C for 4 h. The reaction was worked up by addition of 0.02 M HCl followed by extraction with dichloromethane (3 × 30 mL). The combined organic layers were dried (Na_2SO_4), concentrated, and purified on silica gel plates to give the protected reduced compound (496 mg, 62%). The latter was deprotected in two steps and purified as described for compound 7. The target compound 9 was obtained as a white crystalline solid: mp 115–117 °C (sealed cap); ^{13}C NMR (Me_2SO-d_6) δ 23.3, 43.7, 61.4, 65.0, 70.5, 73.8, 85.7, 87.2, 122.5, 138.4, 148.5, 156.9, 157.0; ^1H NMR (Me_2SO-d_6) δ 1.12 (d, 3 H), 2.68 (d, 2 H), 3.59 (m, 2 H), 3.93 (m, 1 H), 4.13 (m, 2 H), 4.35 (m, 1 H), 4.51 (m, 1 H), 5.11 (m, 1 H), 5.21 (m, 1 H), 5.45 (m, 1 H), 5.84 (d, 1 H), 8.26 (s, 1 H), 12.10 (s, 1 H); UV (H_2O) λ_{max} 267 (ϵ 5994), 250 nm (11 420); FAB HRMS obsd (M^+ + H) 327.1332, calcd for $C_{13}H_{18}N_4O_6$ 327.1304.

2-Vinyl-9-(β-D-ribofuranosyl)hypoxanthine (12). The palladium catalyzed coupling reaction of 5 to give 11 was carried out as described for 6 but with the following modifications: (i) bis(acetonitrile)palladium chloride replaced palladium acetate, (ii) vinyltri-*n*-butyltin replaced the mixture of tributyltin methoxide and isopropenyl acetate, and (iii) the reaction time was reduced to 3 h. The yield of 11 from this reaction was 90%. The two-step deprotection of 11 and purification of the resulting product were executed as described for 7. Compound 12 was obtained in pure form as white crystals (50% yield, from 11) after HPLC separations: mp 225–230 °C dec; ^{13}C NMR (Me_2SO-d_6) δ 61.3, 70.4, 73.6, 85.6, 87.2, 123.2, 125.2, 129.5, 139.3, 148.5, 151.7, 156.8; ^1H NMR (Me_2SO-d_6) δ 3.62 (m, 2 H), 3.96 (m, 1 H), 4.17 (m, 1 H), 4.56 (m, 1 H), 5.05 (t, 1 H), 5.25 (d, 1 H), 5.50 (d, 1 H), 5.83 (dd, 1 H), 5.91 (d, 1 H), 6.50 (m, 2 H), 8.35 (s, 1 H), 12.41 (s, 1 H); UV (H_2O) λ_{max} 292 (ϵ 6486), 260 (7850), 207 nm (19 891); FTIR (KBr) 3300, 3080, 2900, 1686, 1640, 1554 cm^{-1}; FAB HRMS obsd (M^+ + H) 295.1034, calcd for $C_{12}H_{14}N_4O_5$ 295.1042.

2-Vinyl-9-(β-D-ribofuranosyl)hypoxanthine (12) from Unprotected Nucleoside 5a. To a solution of 5a (9.000 g, 22.050 mmol) and bis(acetonitrile)palladium chloride (0.249 g, 0.96 mmol) in DMF (200 mL) was added vinyltri-*n*-butyltin (7.09 mL, 24.260 mmol). The reaction mixture was stirred at 90 °C for 3 h under N_2, then cooled to ambient temperature, and filtered. The filtrate was concentrated, and the resulting residue was flash chromatographed on silica gel with 3% followed by 10% methanol/chloroform as the eluting solvent. The product 13 was obtained as a tan solid (6.530 g, 21.180 mmol, 96%). It was deprotected with trimethylsilyl chloride and KI as described for 7. Compound 12 was characterized as shown above.

2-(2-Hydroxyethyl)-9-(β-D-ribofuranosyl)hypoxanthine (15). In a 125-mL three-neck round-bottomed flask was placed 7.750 g (11.90 mmol) of the protected vinyl compound 11 in dry tetrahydrofuran (80 mL). The flask was fitted with septa and a nitrogen bubbler. 9-Borabicyclo[3.3.1]nonane (26.2 mL of a 0.5 M solution in tetrahydrofuran, 13.10 mmol) was added via syringe and needle. The reaction mixture was heated under nitrogen at 60 °C for 72 h, during which time three additional portions of the hydroborating agent (3 × 15 mL) were added. The reaction mixture was then cooled to 0 °C and treated with 3 M sodium hydroxide (10 mL, 30 mmol) followed by 30% hydrogen peroxide (10 mL, 98 mmol). The reaction mixture was then stirred at room temperature for 2 h. Water (20 mL) was added, and the solution was extracted with ether (3 × 100 mL). The organic extract was washed with a 20% solution of ferrous sulfate (25 mL), then dried (Na_2SO_4), and concentrated. Flash chromatography on silica gel gave the product as an oil (4.128 g, 52%). This compound was deprotected and purified as described for 7. Compound 15 was obtained in 60% yield as a white crystals: mp 153–155 °C; ^{13}C NMR (Me_2SO-d_6) δ 37.7, 58.8, 61.4, 70.5, 73.8, 85.7, 87.7, 122.5, 138.4, 148.5, 157.0; ^1H NMR (Me_2SO-d_6) δ 2.78 (t, 2 H), 3.59 (m, 2 H), 3.92 (m, 2 H), 4.09 (m, 1 H), 4.47 (m, 1 H), 4.73 (m, 2 H), 5.12 (m, 2 H), 5.38 (m, 1 H), 5.84 (d, 1 H), 8.23 (s, 1 H), 12.19 (s, 1 H); UV (H_2O) λ_{max} 250 nm (ϵ 11 124); FAB HRMS obsd (M^+ + H) 313.1149, calcd for $C_{12}H_{16}N_4O_6$ 313.1148.

2-(1,2-Dihydroxyethyl)-9-(β-D-ribofuranosyl)hypoxanthine (14). Compound 13 (the desilylated form of 11) served as the starting material for the preparation of 14. Compound 13 (0.163 g, 0.535 mmol) was placed in a 50-mL round-bottomed flask and dissolved in pyridine (4 mL). Osmium tetraoxide (0.123 g, 0.484 mmol) in pyridine (3 mL) was then added to the solution. The reaction mixture was stirred at room temperature for 4 h and then treated with sodium bisulfite (0.5 g in 10 mL of H_2O) for 45 min. The solution was concentrated and flash chromatographed on silica gel to give the partially protected hydroxylated product (0.119 g, 65%). Demethylation of this product was carried out as described for 7. Purification was done by HPLC as described for 7. Compound 14 was obtained in 83% yield as white crystals: mp 133–135 °C; ^{13}C NMR (Me_2SO-d_6) δ 61.3, 64.1, 70.4, 72.0, 73.8, 85.6, 87.0, 122.9, 138.7, 148.3, 156.5, 158.8; ^1H NMR (Me_2SO-d_6) δ 3.37 (m, 1 H), 3.67 (m, 2 H), 3.79 (m, 1 H), 3.96 (m, 1 H), 4.17 (m, 1 H), 4.62 (m, 2 H), 5.05 (m, 1 H), 5.12 (m, 1 H), 5.20 (m, 1 H), 5.28 (m, 1 H), 5.55 (m, 1 H), 5.86 (d, 1 H), 8.32 (s, 1 H), 11.74 (s, 1 H); UV (H_2O) λ_{max} 250 nm (ϵ 11 194); FAB HRMS obsd (M^+ + H) 329.1132, calcd for $C_{12}H_{16}N_4O_7$ 329.1097.

Preparation of Tri-*n*-butyl(cyanomethyl)stannane. A solution of 2.80 mL (20.450 mmol) of (trimethylsilyl)acetonitrile and 3 mL (10.420 mmol) of tributyltin methoxide in 20 mL of DMF was heated under reflux under N_2 for 15 h. Fractional distillation (130–148 °C, 1.5 Torr)[27] gave 2.854 g (83%) of the tin reagent.

2-(Cyanomethyl)-9-[2,3,5-tri-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]hypoxanthine (10). 2-Iodo-6-methoxy-9-[2,3,5-tri-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-purine (5) (0.864 g, 1.15 mmol), palladium acetate (0.040 g, 0.178 mmol), and tri-*o*-tolylphosphine (0.115 g, 0.378 mmol) were dried in vacuo overnight. Toluene (20 mL) and tri-*n*-butyl(cyanomethyl)stannane (0.295 mL, 1.32 mmol) were added, and the reaction mixture was stirred under N_2 and at toluene reflux for 6 h. Upon cooling, ethyl ether (30 mL) was added and the reaction mixture was extracted with 10% Na_2EDTA solution (10 mL). The solvent was removed under reduced pressure. The residue was purified on silica gel plates with 11:9 ethyl ether/hexane as the developing solvent. The band at R_f 0.53 provided 0.351 g (0.526 mmol, 46%) of 10 as an orange oil: ^{13}C NMR ($CDCl_3$) δ 27.9, 54.3, 62.0, 71.3, 75.9, 84.9, 88.6, 115.9, 120.8, 141.6, 151.9, 153.8, 160.9; ^1H NMR ($CDCl_3$) δ −0.02 to 0.12, 0.80 (s), 0.91 (s), 0.94 (s), 3.82 (m, 1 H) 3.98 (s, 3 H), 4.17 (s, 4 H), 4.28 (m, 1 H), 4.56 (t, 1 H), 6.02 (d, 1 H), 8.42 (s, 1 H); UV (ethyl ether) λ_{max} 252, 287 nm; FTIR (KBr) 2290 cm^{-1}.

6-Amino-2-iodo-9-(β-D-ribofuranosyl)purine (16). Compound 3 (0.390 g, 0.724 mmol) was taken up in absolute ethanol (50 mL) and saturated with ammonia for 45 min at 0 °C. The solution was stirred overnight at room temperature and was then concentrated in vacuo. The residue was azeotroped with *o*-xylene (40 mL) to remove the byproduct, acetamide. The crude product was then adsorbed onto silica gel and purified by flash chromatography starting with 1:10 methanol/chloroform and increasing the polarity of the eluant up to 1.5:10. The fractions from the column were concentrated to give 0.269 g (0.652 mmol, 90%) of compound 16 as a white crystalline product: mp 142–145 °C (lit.[32] mp 142–144 °C); ^1H NMR (Me_2SO-d_6) δ 3.65 (m, 2 H), 3.92 (m, 1 H), 4.07 (m, 1 H), 4.56 (m, 1 H) 5.62 (d, 1 H), 7.45 (s, 2 H), 7.89 (s, 1 H); UV (H_2O) λ_{max} 264.5 nm (ϵ 1.2 × 10^4).

2-Acetonyl-6-amino-9-(β-D-ribofuranosyl)purine (18). 2-Iodoadenosine (16) (0.125 g, 0.318 mmol) was silylated by using the general silylating procedure as described above for the preparation of 5. Flash chromatography using 1:1 ether/hexane as the eluant gave 0.219 g (0.299 mmol, 94%) of product 17 as a low-melting solid: ^1H NMR ($CDCl_3$) δ −0.14 to −0.12 (m, 18 H), 0.90 (m, 27 H), 3.70–4.09 (m, 3 H), 4.28 (t, 1 H), 4.66 (t, 1 H), 5.87 (d, 1 H), 6.33 (s, 2 H), 8.03 (s, 1 H); UV (EtOH) λ_{max} 222 (ϵ 1.9 × 10^4), 265 nm (1.3 × 10^4).

Silylated 2-iodoadenosine 17 (0.733 g, 0.998 mmol) was added to a flask charged with palladium acetate (0.336 g, 0.150 mmol) and tri-*o*-tolylphosphine (0.091 g, 0.300 mmol). The flask was

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fitted with a septum, and toluene (25 mL, freshly distilled from sodium hydride and purged with nitrogen) was added via a Leur needle. Isopropenyl acetate (2 mL) and 1 equiv (0.290 mL, 1.00 mmol) of tributyltin methoxide were added via a gas-tight syringe. The solution was stirred in an oil bath under N_2 and at 100 °C. After 4 h and 7 h, additional aliquots (1 mL and 0.15 mL, respectively) of isopropenyl acetate and tributyltin methoxide were added. After 10 h, the solution was cooled, taken up in hexane (50 mL), and extracted first with 5% aqueous solution of Na_2EDTA (15 mL) and then with H_2O (10 mL). The aqueous layers were back extracted with hexane (20 mL). The combined organic extracts were dried (Na_2SO_4), concentrated, and purified by flash chromatography with 1:1 ether/hexane as the eluting solvent. The desired acetonylated product was isolated as a low-melting solid (0.354 g, 0.532 mmol, 53%): ^{13}C NMR ($CDCl_3$) δ −5.4, −4.9, −4.7, −4.3, 17.9, 18.1, 18.5, 25.7, 25.8, 26.1, 29.5, 54.6, 62.3, 71.6, 75.6, 84.8, 88.6, 118.6, 139.6, 150.4, 155.5, 159.0, 204.3; ^1H NMR ($CDCl_3$) δ −0.3 to 0.12 (m, 18 H), 0.91 (m, 27 H), 2.19 (s, 3 H), 3.90–4.09 (m, 3 H), 3.85 (s, 2 H), 4.29 (t, 1 H), 4.61 (t, 1 H), 5.64 (br s, 2 H), 5.95 (d, 1 H), 8.13 (s, 1 H); UV (EtOH) λ_{max} 262 (ϵ 1.3×10^3), 300 nm (sh, 2.5×10^2); mass spectrum, m/z (relative intensity) 666 (M^+, 2.0), 665 (2.3), 650 (4.0), 608 (100), 552 (3.1), 476 (7.2), 462 (12.3), 436 (3.1), 417 (1.7), 348 (21.8), 306 (3.3), 285 (3.5), 261 (9.6), 248 (8.1), 231 (11.0), 220 (10.3), 211 (9.9), 192 (30.1).

The desilylation procedure of the protected 2-acetonyladenosine (0.514 g, 0.773 mmol) was carried out as described for 7. 2-Acetonyladenosine (18) was obtained as a white crystalline hygroscopic compound in 75% yield (0.187 g, 0.580 mmol) after purification by HPLC: mp 113–115 °C; ^{13}C NMR (Me_2SO-d_6) δ 29.1, 57.7, 61.4, 70.5, 73.2, 85.7, 88.4, 117.3, 140.5, 148.3, 154.9, 157.8, 209.8; ^1H NMR (Me_2SO-d_6) δ 2.15 (s, 3 H), 3.61 (br s, 1 H), 3.77 (s, 2 H), 3.97 (m, 1 H), 4.15 (m, 1 H), 4.60 (m, 1 H), 5.23 (s), 5.43 (br s, 3 H), 5.78 (d), 5.84 (d, 1 H), 8.31 (s, 1 H); UV (EtOH) λ_{max} 262.5 (ϵ 12050), 300 nm (4650); FAB HRMS obsd ($M^+ + H$) 324.1299, calcd for $C_{13}H_{17}N_5O_5$ 324.1308.

Enzymatic Deamination Studies. All assays with adenosine deaminase (Type I from calf intestinal mucosa, Sigma) were followed spectrophotometrically at 25 °C by using a Gilford Response UV–visible spectrometer. Adenosine was used as the standard. Solutions of substrates of appropriate concentrations in 0.05 M phosphate buffer (pH 7.40) were used, and deamination reactions were initiated by addition of the enzyme. For example, the conversion of 18 to 7 could be monitored quantitatively by the change in the major absorption band in the UV spectrum (263 nm → 250 nm). Details of the procedure for the assays have been previously described by us.[29]

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Rare Purine Nucleosides: Congeners of the Antibiotic, Nebularine[1]

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Novel functionalized analogues of the biologically active nucleoside, nebularine, have been synthesized. The key step of the syntheses involved palladium-catalyzed cross-coupling reactions between 2-iodonebularine and organostannanes. This synthetic approach has considerable potential generality, and the only limitation appears to be the availability of the appropriate organostannane coupling reagent.

Nebularine or 9-(β-D-ribofuranosyl)purine is a natural nucleoside which has been isolated from both microorganisms (*S. yokosukanensis*)[2] and fungi (*Clitocybe nebularis batsch*).[3] It is an antibiotic,[4] which is a strong competitive inhibitor of the purine metabolizing enzyme, adenosine deaminase.[5-7] It has been studied as an antileukemic agent in combination chemotherapy.[8] Congeners of nebularine are of considerable potential biological interest. For example, 2-aminonebularine [i.e. 2-amino-9-(β-D-ribofuranosyl)purine] is a potent inhibitor of a number of enzymes including adenosine deaminase,[9] purine nucleoside phosphorylase,[10] and adenosine kinase.[11] It is incorporated in *E. Coli* and phage T4 DNA.[12,13] However, very few other congeners of nebularine involving functionalization at the C-2 position are known.[14-16] Our interest in the synthesis of rare purine nucleosides with antiviral therapeutic potential led us to explore new approaches to congeners of nebularine. This paper reports on the use of both photochemical and organometallic methodologies for the synthesis of novel C-2 functionalized analogues of nebularine.

2-Halogenated purine sytems were the key intermediates in this investigation. It was envisioned that they in turn would be accessible through the 2-aminopurine ribonucleoside if a facile approach could be found for the gram scale preparation of this compound. The approach used in this work, which differed from previous syntheses of 2-aminonebularine,[17-19] involved a high yielding photochemical transformation as the key step.[15,20] The starting material for the synthesis was guanosine **1** which was converted to the 2-iodo derivative **2** in several steps as reported in the literature.[15,21,22]

Conversion of the 2-iodopurine **2** into the 2-vinyl compound **3** was achieved in 90% yield by reaction with tri-*n*-butyl(vinyl)stannane in the presence of palladium chloride. This palladium-catalyzed cross-coupling reaction is thought to involve oxidative insertion of palladium into the carbon–iodine bond, coupling of the Pd(II) complex with the organostannane, and reductive elimination (via the *cis* isomer) of the product with regeneration of the Pd(0) catalytic species.[23,24] Deprotection of the coupled product **3** with tetrabutylammonium fluoride (TBAF) in tetrahydrofuran gave 2-vinylnebularine **4** in 71% yield. Nucleoside **4** was purified by reversed-phase HPLC on Amberlite XAD-4 resin and fully characterized by FTIR, UV, and high-field NMR spectroscopy (Table). Further elaboration of **4** is also possible. For example, direct hydroxylation of **4** with osmium tetroxide in pyridine gave the highly hydroxylated diastereoisomeric compound **5** in 34% yield after purification.

The generality of this palladium-catalyzed reaction for the regiospecific functionalization of the nebularine system can be demonstrated by the synthesis of other analogues of this

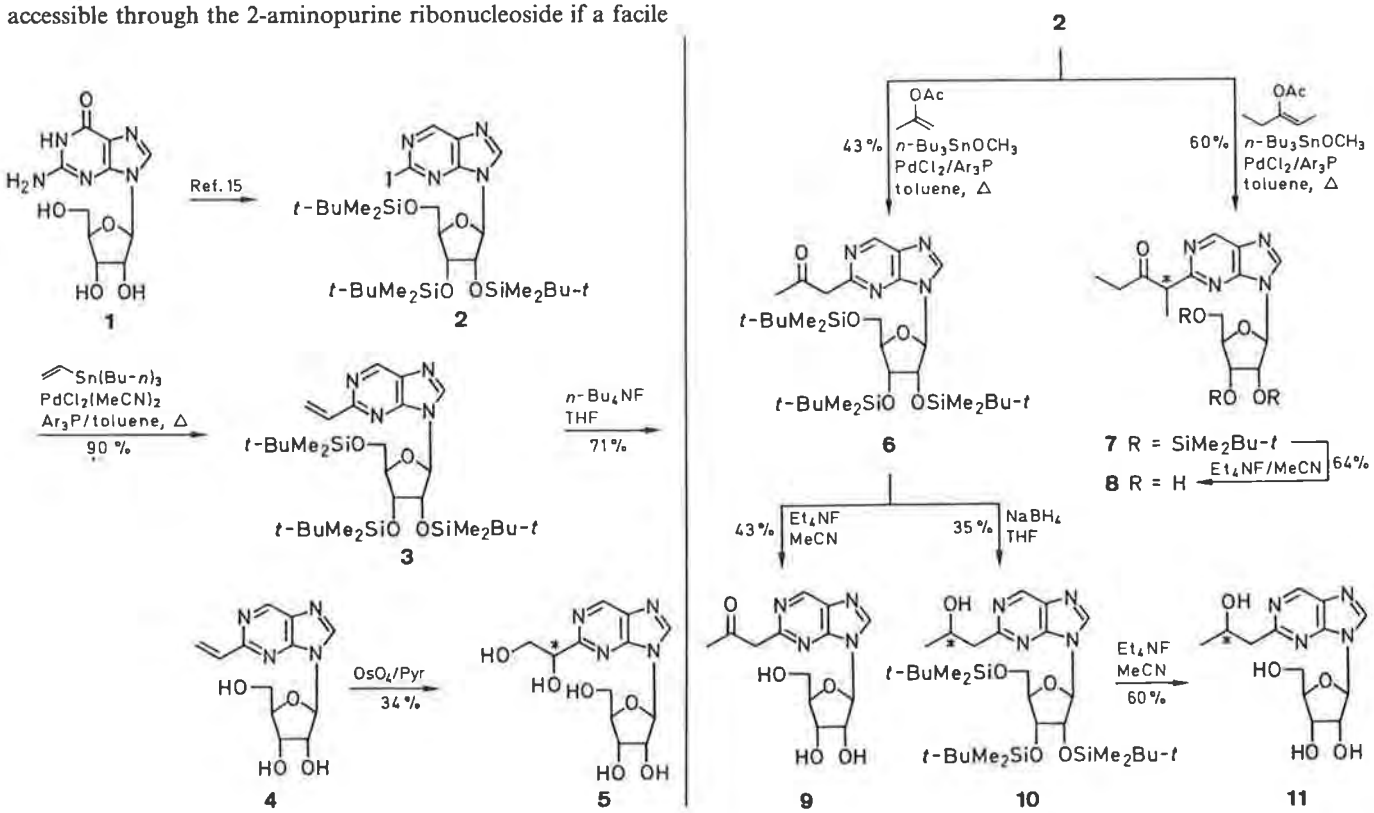

Table. Physical and Spectral Data for 2-Substituted Purine Nucleosides

Product	Yield[a] (%)	mp[b] (°C)	Molecular Formula[c]	UV (H_2O) λ_{max} (ε)	IR (KBr)[d] ν (cm^{-1})	^{1}H-NMR[e] δ, J (Hz)	^{13}C-NMR[e] δ
4	64	158–161	$C_{12}H_{14}N_4O_4$ (278.3)	266 (7400)	3351; 2927; 1600; 1576; 1423	3.62 (m, 2H); 3.99 (m, 1H); 4.24 (m, 1H); 4.71 (m, 1H), 5.07 (m, 1H); 5.25 (d, 1H, J = 4.9); 5.52 (d, 1H, J = 5.9); 5.73 (dd, 1H, J = 10.0, 2.4); 6.05 (d, 1H, J = 6.4); 6.54 (dd, 1H, J = 17.3, 2.4); 6.93 (dd, 1H, J = 17.3, 10.0); 8.81 (s, 1H); 9.17 (s. 1H)	61.5; 70.5; 73.6; 85.9; 87.4; 122.7; 132.9; 136.6; 145.8; 148.2; 151.5; 157.8
5	22	75–78	$C_{12}H_{16}N_4O_6$ (312.3)	266 (7200)		3.70 (m, 4H); 3.98 (m, 1H); 4.21 (m, 1H); 4.69 (m, 3H); 5.09 (m, 1H); 5.27 (m, 2H); 5.54 (d, 1H, J = 5.9); 6.08 (d, 1H, J = 5.9); 8.82 (s, 1H); 9.17 (s. 1H)	61.4; 65.4; 70.5; 73.5; 75.3; 85.8; 87.2; 132.8; 145.3; 147.7; 151.3; 163.9
8	38	46–48	$C_{15}H_{20}N_4O_5$ (336.3)	267 (7200), 246 (4000)	3356; 2933; 1707; 1581; 1388	0.90 (t, 3H, J = 7.3); 1.44 (d, 3H, J = 6.8); 2.45 (q, 2H, J = 7.3); 3.59 (m, 2H); 3.99 (m, 1H); 4.20 (m, 2H); 4.68 (m, 1H); 5.02 (m, 1H); 5.23 (m, 1H); 5.49 (m, 1H); 6.03 (d, 1H, J = 5.9); 8.80 (s, 1H); 9.14 (s, 1H)	7.9; 15.6; 34.0; 55.0; 61.6; 70.6; 73.5; 85.9; 87.4; 132.6; 145.6; 148.5; 151.7; 163.2; 208.9
9	18	71–74	$C_{13}H_{16}N_4O_5$ (308.3)	267 (5900)	3370; 2924; 1716; 1603; 1582;1420	2.21 (s, 3H); 3.63 (m, 2H); 3.98 (m, 1H); 5.15 (m, 3H); 4.62 (m, 1H); 5.05 (t, 1H, J = 5.4); 5.19 (d, 1H, J = 4.9); 5.47 (m, 1H); 6.03 (d, 1H, J = 5.9); 8.79 (s, 1H); 9.13 (s, 1H)	29.9; 53.5; 61.3; 70.4; 73.6; 85.8; 87.0; 132.3; 145.1; 148.1; 151.5; 158.6; 204.4
11	9	70–72	$C_{13}H_{18}N_4O_5$ (310.3)	268 (5300), 240 (3500)		1.12 (d, 3H, J = 6.4); 3.04 (m, 2H); 3.63 (m, 2H); 3.99 (m, 1H); 4.18 (m, 2H); 4.65 (m, 2H); 5.05–5.25 (m, 2H); 5.48 (d, 1H, J = 6.4); 6.03 (d, 1H, J = 5.9); 8.74 (s, 1H); 9.10 (s, 1H)	23.2; 48.5; 61.4; 66.0; 70.5; 73.4; 85.8; 87.2; 132.2; 144.3; 147.8; 151.3; 162.5

[a] Overall yield of isolated product involving several steps.
[b] Uncorrected.
[c] Satisfactory microanalyses obtained: C ±0.37, H ±0.13, N ±0.31 (Exceptions: **4**; N–0.66; **11**; N–0.47).
[d] Recorded on an IBM Model 98 FTIR spectrophotometer.
[e] In DMSO-d_6, with TMS as internal reference.

antibiotic. For example, reaction of **2** with the tin enolate of acetone (generated from isopropenyl acetate and tri-*n*-butyltin methoxide) in the presence of palladium chloride, gave the 2-acetonyl compound **6** which could be easily deprotected with tetraethylammonium fluoride (TEAF) in acetonitrile to give 2-acetonylnebularine (**9**). This compound exists in only the keto form (cf. reference 25) as evidenced from the high-field ^{13}C-NMR data (carbonyl carbon at δ = 204.4, methyl and methylene carbons of acetonyl group at δ = 29.9 and 53.5, respectively, with no evidence for carbons of the enolized acetonyl group), and the FTIR data (ketone carbonyl stretch at ν = 1716 cm^{-1}) (also see the Table). Reduction of the protected ketone **6** with sodium borohydride followed by desilylation with TEAF gave the diastereoisomeric alcohols **11**.

Other enol acetates may also be used in the aforementioned cross-coupling reactions. For example, when 2-pentene-3-acetate was substituted for isopropenyl acetate in the reaction with **2**, smooth conversion to the ketopurine nucleoside **7** occurred. The only exception that we have found is vinyl acetate which tends to polymerize under the reaction conditions.

In summary, the palladium-catalyzed cross-coupling reaction of 2-iodonebularine with organostannanes results in the formation of novel C-2 functionalized nebularines. This methodological approach may be used for the synthesis of a wide variety of new analogues of nebularine with the only limiting factor being the availability of the appropriate tin reagent.

Melting points reported are uncorrected and were determined on a Thomas-Hoover melting point apparatus fitted with a microscope. Nuclear magnetic resonance spectra were recorded on JEOL Model FX90Q and Bruker Model WM360 instruments. Mass spectra were obtained on a Hewlett-Packard 5985 GC/MS system or a VG Analytical Model ZAB-HF instrument with FAB-HRMS capability. IR spectra were recorded on an IBM Model 98 FT-IR instrument. UV data were determined using a Varian Cary Model 219 or a Gilford Response spectrophotometer.

2-Iodo-9-[2,3,5-tri-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-purine (**2**) was prepared from guanosine **1** as reported by us.[15,20–22]

2-Vinyl-9-β-D-ribofuranosylpurine (4):

Preparation of **3**: Bis(acetonitrile)palladium(II) chloride (0.049 g, 0.189 mmol) and tri-*o*-tolylphosphine (0.117 g, 0.384 mmol) are added to a flask containing **2** (2.316 g, 3.213 mmol). The flask is placed on a vacuum pump to remove residual oxygen. Toluene (40 mL) is added under nitrogen followed by vinyltributyltin (1.03 mL, 3.52 mmol). The solution is stirred at toluene reflux for 3 h under N_2. Upon cooling, ether (50 mL) is added, and the mixture is extracted with 10% ethylenediaminetetraacetic acid disodium salt (2 × 15 mL) and water (20 mL). The organic layer is dried (Na_2SO_4), and the solvents are removed under reduced pressure. The residue is dissolved in hexanes and eluted through a short silica gel scrubber column with 1:1 hexanes/ether. Final purification by flash chromatography on silica gel (hexanes/ether 3:1) provides **3** as a tan oil; yield: 1.79 g (90%).

^{1}H-NMR ($CDCl_3$) δ = −0.16–0.13 (m, 18H); 0.80–0.95 (m, 27H); 3.84–4.14 (m, 3H); 4.33 (m, 1H); 4.61 (m, 1H); 5.66 (dd, 1H, J = 10.3, 2.4 Hz); 6.05 (d, 1H, J = 3.9 Hz); 6.56 (dd, 1H, J = 17.6, 2.4 Hz); 6.96 (dd, 1H, J = 17.6, 10.3 Hz); 8.49 (s, 1H); 9.07 (s, 1H).

UV (EtOH): λ_{max} = 268 nm.

MS (30 eV): m/z = 605 (M^+–CH_3); 563 (M^+–*t*-butyl); 303; 261; 147 (base$^+$ + 2H).

Desilylation of **3** *to* **4**: To a solution consisting of **3** (2.603 g, 4.235 mmol) in THF is added a 1 M THF solution of TBAF (16.95 mL). The mixture is stirred under nitrogen for 35 min, then reduced to one-half its volume, and water (40 mL) and NH_4Cl (1.813 g, 33.89 mmol) are added. Stirring is maintained at 60 °C for 4 h. THF is removed and the aqueous portion extracted with $CHCl_3$ (25 mL) followed by ether (25 mL). The $CHCl_3$

portion is back extracted with water (20 mL). The aqueous portions are combined and evaporated under reduced pressure. The resulting residue is dissolved in water and purified by reversed-phase HPLC on Amberlite XAD-4 resin using 15% EtOH/water as the eluting solvent to give **4** as a white solid; yield: 0.838 g (71%).

2-(1,2-Dihydroxyethyl)-9-β-D-ribofuranosylpurine (5):
To a solution of **4** (0.274 g, 0.985 mmol) in dry pyridine (20 mL) is added OsO_4 (0.250 g, 0.983 mmol) dissolved in pyridine (2.5 mL). The solution is stirred for 4 h at room temperature. $NaHSO_3$ (0.95 g) and water (10 mL) are added to the reaction mixture. After stirring for 1.5 h, the solvents are removed under vacuum. The residue is dissolved in MeOH and eluted through a short silica gel scrubber column with 5–20% MeOH/$CHCl_3$. Final purification by reversed-phase HPLC on Amberlite XAD-4 using 5% EtOH/water as the eluting solvent gives **5** as a white solid; yield: 0.104 g (34%).

2-Acetonyl-9-β-D-ribofuranosylpurine (9):

Preparation of **6**: $PdCl_2$ (0.177 g, 0.998 mmol) and tri-*o*-tolylphosphine (0.607 g, 1.994 mmol) are added to a flask containing **2** (4.789 g, 6.643 mmol). The flask is connected to a vacuum pump to remove residual oxygen. Freshly distilled toluene (100 mL) is added under nitrogen. In a separate flask, $(n\text{-Bu})_3SnOCH_3$ (2.86 mL, 9.93 mmol) and isopropenyl acetate (2.92 mL, 26.54 mmol) are warmed at 40 °C for 35 min. This solution is transferred by double tipped needle to the flask containing **2**. After stirring the solution at toluene reflux for 4 h, additional $(n\text{-Bu})_3SnOCH_3$ (1.2 mL, 4.17 mmol) and isopropenyl acetate (3 mL, 27.3 mmol) are added. The solution is stirred for an additional 20 h at toluene reflux under nitrogen. Work-up and purification are as for compound **3** to afford **6** as a low-melting solid; yield: 1.864 g (43%).

^{1}H-NMR ($CDCl_3$): $\delta = -0.18$–0.13 (m, 18 H); 0.80–0.95 (m, 27 H); 2.23 (s, 3 H); 3.82 (m, 2 H); 4.00 (m, 1 H); 4.12 (m, 2 H); 4.31 (m, 2 H); 4.55 (m, 1 H); 6.07 (d, 1 H, $J = 3.9$ Hz); 8.50 (s, 1 H); 9.07 (s, 1 H).

UV (EtOH): $\lambda_{max} = 270$, 245 nm.

MS (30 eV): $m/z = 650$ (M^+), 593 (M^+-*t*-bu), 333; 291; 261; 177 (base$^+$ + 2 H), 175 (base$^+$).

Desilylation of **6** *to* **9**: The desilylation of **6** (1.999 g, 3.070 mmol) is similar to that of **3** except that TEAF and MeCN are used in place of TBAF and THF, respectively. Final purification by reversed-phase HPLC on Amberlite XAD-4 using 5% EtOH/water gives **9** as a pale yellow solid; yield: 0.408 g (43%).

2-(2-Hydroxypropyl)-9-β-D-ribofuranosylpurine (11):

Preparation of **10**: To a cooled (ice bath) solution consisting of **6** (3.157 g, 4.849 mmol) in THF (65 mL) is added $NaBH_4$ (0.220 g, 5.82 mmol). The solution is stirred at 0 °C under nitrogen. After 3.5 h HCl (25 mL) is added till pH becomes 2 and the mixture quickly extracted with CH_2Cl_2 (3 × 75 mL). The organic portions are combined, washed with ice-cold water (20 mL), dried (Na_2SO_4), and evaporated under vacuum. The residue is purified by flash chromatography on silica gel (3:1 ether/hexanes) to give **10** as a brown oil; yield: 1.101 g (35%).

^{1}H-NMR ($CDCl_3$): $\delta = -0.25$–0.13 (m, 18 H); 0.78–0.95 (m, 27 H); 1.29 (d, 3 H, $J = 6.4$ Hz); 3.15 (m, 2 H); 3.74–4.31 (m, 6 H); 6.07 (d, 1 H, $J = 4.9$ Hz); 8.45 (s, 1 H); 9.02 (s, 1 H).

UV (EtOH): $\lambda_{max} = 269$ nm.

Desilylation of **10** *to* **11**: Desilylation of **10** (0.414 g, 0.634 mmol) is carried out as for compound **6**. Purification of the residue by reversed-phase HPLC on Amberlite XAD-4 resin using 5% ethanol/water affords **11** as a white solid; yield: 0.118 g (60%).

2-(1-Methyl-2-oxobutyl)-9-β-D-ribofuranosylpurine (8):
The preparation of **7** (from 5.985 g, 8.302 mmol of **2**) follows the procedure used for **6** but with one exception. 3-Acetoxypent-2-ene[26] is used in place of isopropenyl acetate. Purification by flash chromatography on silica gel using 1:5 ether/hexanes and then 1:1 ether/hexanes gives **7** as an oil; yield: 3.364 g (60%).

^{1}H-NMR ($CDCl_3$): $\delta = -0.19$–0.13 (m, 18 H); 0.78–0.95 (m, 27 H); 0.99 (t, 3 H, $J = 7.3$ Hz); 1.53 (d, 3 H, $J = 7.3$ Hz); 2.41 (q, 2 H, $J = 7.3$ Hz); 3.82–4.56 (m, 6 H); 6.07 (d, 1 H, $J = 4.4$ Hz); 8.50 (s, 1 H); 9.06 (s, 1 H).

UV (EtOH): $\lambda_{max} = 268$ nm.

Desilylation of **7** *to* **8**: The deprotection of **7** (3.364 g, 4.953 mmol) is as for compound **6**. Final purification by reversed-phase HPLC on Amberlite XAD-4 resin using 20% ethanol/water gives **8** as a white solid; yield: 1.066 g (64%).

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Sulfone of the Antibiotic, Nebularine: Synthesis and Conversion to Novel Analogues of Nebularine

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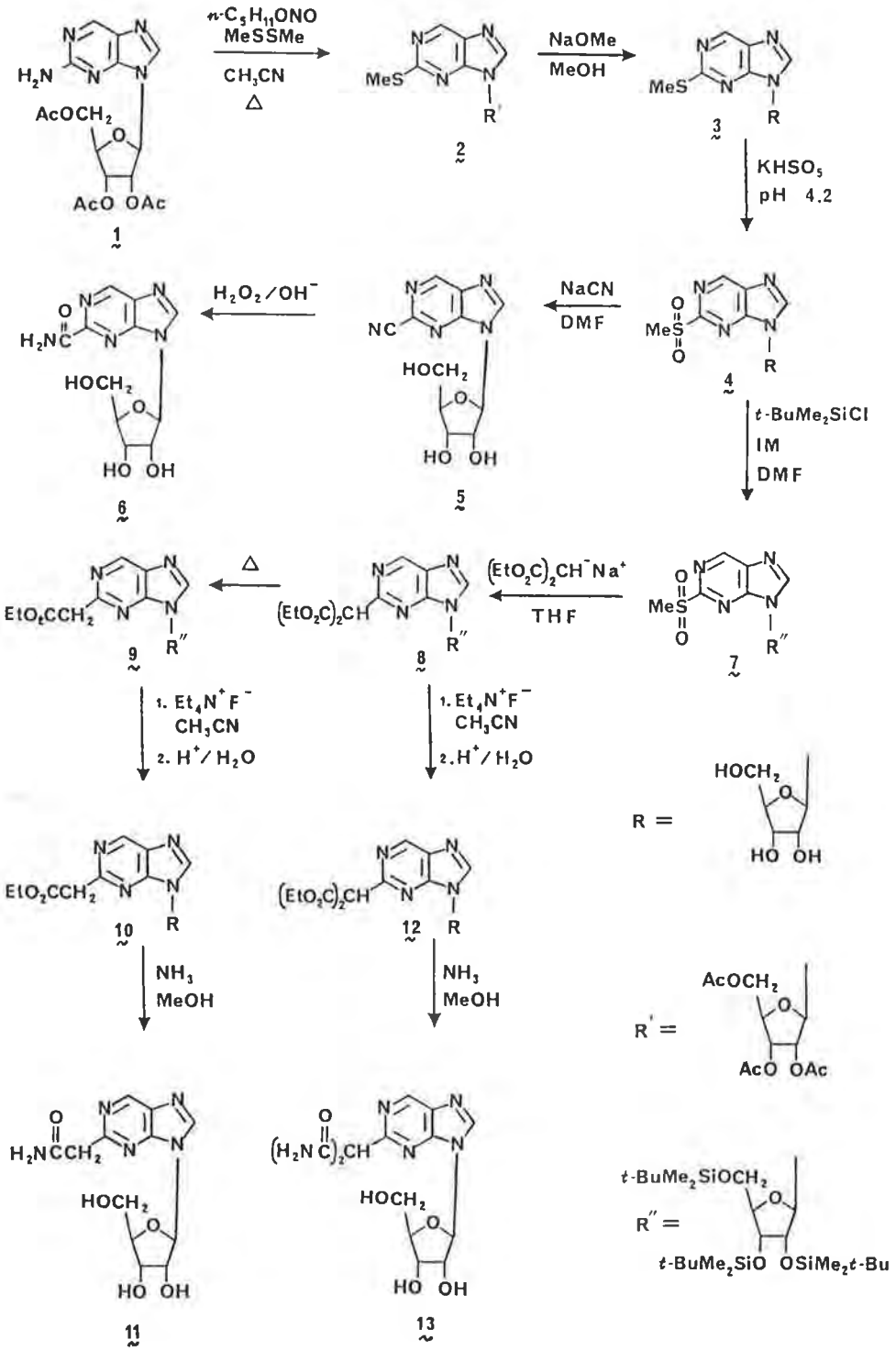
Abstract: 2-Methylsulfonylnebularine has been synthesized from 2-aminonebularine (isoadenosine or 2-aminopurine ribonucleoside) by a radical deamination-thioalkylation followed by sulfur oxidation. This sulfone is potentially an intermediate for the synthesis of many new 2-substituted analogues of nebularine as the nucleofugic methylsulfonyl group can be displaced by a variety of nucleophiles. Representative examples include 2-cyano-, 2-carboxamido-, 2-acetamido-, and 2-bis (carboxamido)methyl- nebularine. Structures of the intermediate and products were confirmed by spectroscopic data, particularly high-field NMR and FAB HRMS data.

While many syntheses have been reported for analogues of the natural purine nucleosides, adenosine and guanosine (for a few representative examples see references 1-10), relatively few compounds related to nebularine are known.[11-13] Nebularine [9-(β -D-ribofuranosyl)purine] has been isolated from microorganisms *S. yokosukanensis*[14], fungi *Clitocybe nebularis batsch*[15], and *Microbispora sp. SCC 1779*.[16] It is an antibiotic with strong competitive inhibitory properties for the enzyme, adenosine deaminase.[17] Additionally, it has been studied as an anticancer agent.[15,18] Analogues of nebularine could potentially be of considerable biological importance. For example, 2-aminonebularine has been found to be an inhibitor of a number of purine metabolizing enzymes.[19-21] Also, 2-methylnebularine has shown activity as a presynaptic inhibitor of acetylcholine release,[22] and the corresponding 2-trifluoromethylpurine nucleoside has proven to be active against sarcomas and neoplasts.[23,24] Our interest in the synthesis of unusual purine nucleosides as potential antiviral agents has led us to explore general approaches to functionalized nebularine systems. This paper reports on the preparation of the new intermediate 4, a purine sulfone system, and its conversion to some novel congeners of nebularine.

Synthesis of the purine 2-sulfone 4, required the development of a procedure that would allow gram scale preparation of this intermediate. The starting compound was protected 2-aminopurine nucleoside 1, which, when treated with n-pentyl nitrite (or t-butyl nitrite) and dimethyl disulfide in refluxing acetonitrile under nitrogen, gave a 37 % yield of 2-methylmercaptonebularine (2). The reaction appears to be a radical deamination-thioalkylation and is inhibited by molecular oxygen. Deprotection of 2 followed by selective oxidation of the sulfur with potassium hydrogen persulfate (oxone) in buffered aqueous

solution,[25] gave the sulfone **4** in about 80% yield after chromatographic purification (Scheme 1). The pH of this reaction must be maintained between about 4 and 6. Below this pH, the nucleoside decomposes slowly *via* glycosidic bond cleavage, while at higher pHs, decomposition of the oxone reagent impairs the oxidation reaction. It should be mentioned that oxone exhibits much greater selectivity and efficiency in this reaction than potassium permanganate.



Scheme 1

The nucleofugic sulfonyl group in **4** is susceptible to displacement by selected nucleophiles. Thus, reaction of **4** with sodium cyanide in DMF at 0 °C for 15 h afforded the novel 2-cyanonebularine **5** in 40% yield after purification. If this reaction is viewed as a dark $S_{RN}1$ reaction [26,27], then the failure of previous displacements of halogens at this position [12] may be explained by a more favorable reduction potential for the sulfone compared to the halogenated systems. The cyano functionality at the 2-position in **5** can be hydrolyzed to give the carboxamide **6**, another new nucleoside. The structures of both the cyanide and the carboxamide were established by UV, FTIR, FAB HRMS, and high-field NMR data.

The generality of the utility of the sulfone as a precursor can be illustrated by the synthesis of other analogues of nebularine. For example, when the silyl protected sulfone **7** was treated with the anion of diethylmalonate, the corresponding malonate derivative **8** was isolated. If the reaction is run at room temperature, it could be stopped at the malonate stage. However, raising the temperature to that of refluxing tetrahydrofuran, results in decarboxylation to produce the ethylacetate derivative **9**. The conversion of **8** to **9** may be mechanistically explained through a base-catalyzed retro-Claisen reaction.

Compound **9** was elaborated by reaction of its deprotected form (i.e. compound **10**) with methanolic ammonia to give the novel carboxamide **11**. Similarly, the diethylmalonyl derivative **8** was converted through its deprotected form to the bis-carboxamide **13**.

In summary, the novel 2-methylsulfonylnebularine, synthesized from 2-aminonebularine by a radical alkylthiolation followed by oxidation, is an important intermediate for the synthesis of some new analogues of nebularine. This approach to novel congeners of nebularine may have considerable generality as a wide variety of nucleophiles could conceivably be utilized to displace the nucleofugic group of the nebularine sulfone intermediate.

Experimental Section

Irradiations were accomplished using a Rayonet photochemical reactor. The melting points provided are uncorrected and were taken on a Thomas-Hoover melting point apparatus fitted with a microscope. Nuclear magnetic resonance spectra using tetramethylsilane or chloroform as internal standards were recorded on JEOL Model FX90Q and Bruker Model WM360 pulse Fourier transform spectrometers. Mass spectra were obtained on a Hewlett-Packard 5985 GC/MS system or a VG Analytical Model ZAB-HF instrument with high-resolution FAB capability. The ultraviolet spectra were recorded on Varian Cary Model 219 or Gilford Response spectrophotometers. Infrared spectra were recorded on a Mattson FTIR instrument.

2-Amino-9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)purine (1) was prepared from guanosine in 50% overall yield as previously described.[12]

2-Methylmercapto-9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)purine (2). A solution consisting of 1.630 g (4.14 mmol) of **1**, 50 mL of acetonitrile, 1.1 mL (12.3 mmol) of dimethylsulfide, and 2.6 mL (22 mmol) of n-pentyl nitrite was purged with nitrogen. The solution was then heated to reflux under N_2 with protection from light for 12 h. The solvent was removed under reduced pressure, and the residue was purified by flash chromatography on silica gel (elution with ether) to give 0.663 g (1.56 mmol, 37%) of **2** as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 2.15-2.04 (m, 9 H), 2.65 (s, 3 H), 4.39 (m, 5 H), 6.14 (d, 1 H), 8.93 (s, 1 H),

9.25 (s, 1 H); UV(EtOH) λ_{max} 258, 295nm; mass spectrum m/z (relative intensity) 424 (M^+, 7.4), 166 (M^+-sugar, 15.9). The physical data for **2** was identical to that reported previously for the same compound prepared by a different method.[28]

2-Methylsulfonyl-9-β-D-ribofuranosylpurine (4). A solution consisting of 1.080 g (2.54 mmol) of **2**, 0.480 g (8.90 mmol) of sodium methoxide, and 40 mL of methanol was stirred for 1 h. Ammonium chloride (0.600 g, 10.9 mmol) was then added, and the solution stirred at room temperature for an additional hour. The solvent was removed under reduced pressure, and the residue was purified by flash chromatography on silica gel (9:1 chloroform/methanol) to give 0.674 g (2.22 mmol, 81%) of **3** as a yellow oil: ^{1}H NMR (Me_2SO-d_6) δ 2.59 (s, 3 H), 3.62 (m, 2 H), 3.93 (m, 1 H), 4.16 (m, 1 H), 4.37 (m, 1 H), 4.52 (m, 1 H), 5.23 (m, 1 H), 5.55 (m, 1 H), 5.97 (d, 1 H), 8.68 (s, 1 H), 9.01 (s, 1 H); UV(EtOH) λ_{max} 258, 295nm.

A solution consisting of 0.798 g (2.63 mmol) of **3** and 2.420 g (3.94 mmol) of oxone in 30 mL of acetate buffer (pH 4.2) was stirred for 12 h at 0 °C. After adjusting the pH to 7.0 with 2 N NaOH, the solvent was removed under reduced pressure. Methanol (50 mL) was then added and the insoluble material was filtered. The filtrate was dried ($MgSO_4$), and the solvent was removed under reduced pressure. The residue was purified by flash chromatography on silica gel (2% triethylamine/chloroform followed by 9:1 chloroform/methanol) to give 0.655 g (1.95 mmol, 74%) of **4** as a hygroscopic white solid: ^{13}C NMR (Me_2SO-d_6) δ 57.9, 67.1, 70.6, 76.0, 82.8, 84.6, 132.3, 145.3, 145.6, 148.0, 155.7; ^{1}H NMR (Me_2SO-d_6) δ 3.28 (s, 3 H), 3.63 (m, 2 H), 3.90 (m, 1 H), 4.18 (m, 1 H), 4.35 (m, 1 H), 4.52 (m, 1 H), 5.25 (m, 1 H), 5.62 (m, 1 H), 6.03 (d, 1 H), 8.75 (s, 1 H), 9.11 (s, 1 H); UV(EtOH) λ_{max} 265 nm; FAB HRMS 331.0719 (M^++H), calculated for $C_{11}H_{14}N_4O_6S$ 331.0712 (M^++H).

2-Cyano-9-β-D-ribofuranosylpurine (5). A solution consisting of **4** (0.271 g, 0.807 mmol), sodium cyanide (0.060 g, 1.2 mmol), and DMF (10 mL) was stirred at 0°C for 15 h. The solvent was removed under reduced pressure, and the residue purified by flash chromatography on silica gel (9:1 chloroform/methanol) to give 0.084 g (0.16 mmol, 40%) of **5** as a white hygroscopic solid: ^{1}H NMR (Me_2SO-d_6) δ 3.68 (m, 2 H), 3.99 (m, 1 H), 4.03 (m, 1 H), 4.55 (m, 1 H), 5.08 (t, 1 H), 5.25 (d, 1 H), 5.59 (d, 1 H), 6.08 (d, 1 H), 9.15 (s, 1 H), 9.38 (s, 1 H); ^{13}C NMR (Me_2SO-d_6) δ 61.1, 70.2, 74.3, 86.0, 88.3, 116.9, 136.0, 136.3, 148.6, 149.4, 151.1, UV(EtOH) λ_{max} 269nm (ε = 9,550); FTIR(KBr) 2248 cm^{-1}; FAB HRMS 278.0900 (M^++H), calculated for $C_{11}H_{11}N_5O_4$ 278.0889 (M^++H).

2-Carboxamido-9-β-D-ribofuranosylpurine (6). To a solution consisting of 0.285 g (1.09 mmol) of **5**, 30 mL of ethanol, and 1 mL of 2N NaOH was added 0.4 mL of 30% aqueous hydrogen peroxide. After t.l.c. showed no evidence of starting material (1 h), a small amount of $FeSO_4$ was added until no evidence of peroxides was observed. The insoluble material was filtered and the filtrate removed under reduced pressure. The residue was purified by reversed-phase HPLC on Amberlite XAD-4 resin (2.5% ethanol/water) to give 0.111 g (0.370 mmol, 34%) of **6** as white crystals: mp 152°C (decomp.); ^{1}H NMR (Me_2SO-d_6) δ 3.67 (m, 2 H), 4.00 (m, 1 H), 4.24 (m, 1 H), 4.70 (m, 1 H), 5.05 (t, 1 H), 5.21 (d, 1 H), 5.51 (d, 1 H), 6.13 (d, 1 H), 7.71 (bs, 1 H), 8.21 (bs, 1 H), 8.97 (s, 1 H), 9.27 (s, 1 H); ^{13}C NMR (Me_2SO-d_6) δ 61.3, 70.4, 74.0, 86.1, 87.7, 134.8, 147.4, 147.9, 151.7, 152.3, 164.5; UV(EtOH) λ_{max} 267 (ε = 11,000); FTIR(KBr) 1601, 1688 cm^{-1}; FAB HRMS 296.1018 (M^++H), calculated for $C_{11}H_{13}N_5O_5$ 296.0995 (M^++H).

2-Bis(carboxamido)methyl-9-β-D-ribofuranosylpurine (13). A solution consisting of 0.958 g (2.85 mmol) of **4**, 1.72 g (11.4 mmol) of t-butyldimethylsilyl chloride, 1.55 g (22.8 mmol) of imidazole, and 10 mL of DMF was stirred at 40°C for 24h. The reaction mixture was partitioned between water (100 mL) and ether (40 mL). The organic layer was collected and dried ($MgSO_4$), and the solvent was removed under reduced pressure. The residue was purified by flash chromatography on silica gel (1:1 ether/hexanes) to give 1.37 g (2.04 mmol, 72%) of **7** as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 0.117 to -0.238 (m, 18H), 0.931-0.736 (m, 27 H), 2.90 (s, 3 H), 3.95 (m, 2 H), 4.07 (m, 1 H), 4.30 (m, 1 H), 4.55 (m, 1 H), 6.08 (d, 1 H), 8.63 (s, 1 H), 9.17 (s, 1 H); UV(EtOH) λ_{max} 265.5 nm; mass spectrum m/z (relative intensity) 616 (22.6), 615 (M^+-t-Bu, 48.9).

A solution of 0.650 g (16.3 mmol) of sodium hydride (60%), 3.1 mL (20 mmol) of diethylmalonate, and 10 mL of THF was added dropwise to 90 mL of a THF solution containing 1.37 g (2.04 mmol) of **7**. The resulting solution was purged with nitrogen, and stirred for 29 h. After mass spectral evidence indicated the absence of the starting material, 1.300 g (25.00 mmol) of ammonium chloride was added. The solution was stirred for an additional 2 h and the solvent was removed under reduced pressure. The residue was purified by flash chromatography (hexanes followed by 1:1 ether/hexanes) to give a mixture of 0.763 g (1.06 mmol, 50%) of **8** and 0.344 g (0.505 mmol, 25%) of **9**. Data for **8**: ^{1}H NMR ($CDCl_3$) δ 0.39 to -0.29 (m, 18 H), 0.85-0.69 (m, 27 H), 1.15 (t, 6 H), 3.95 (m, 2 H), 4.27-3.95 (m, 6 H), 4.45 (m, 1 H), 5.02 (s, 1 H), 5.98 (d, 1 H), 8.47 (s, 1 H), 8.99 (s, 1 H); UV(EtOH) λ_{max} 274.5 nm; mass spectrum m/z (relative intensity) 754 (2.7), 696 (21.9), 695 (M^+-t-Bu, 40.9). Data for **9**: ^{1}H NMR ($CDCl_3$) δ 0.07 to -0.23 (m, 18 H), 0.88-0.74 (m, 27 H), 1.17 (t, 3 H), 3.65 (m, 2 H), 3.99 (s, 2 H), 4.23-4.15 (m, 4 H), 4.52 (m, 1 H), 6.01 (d, 1 H), 8.44 (s, 1 H), 8.99 (s, 1 H); UV(EtOH) λ_{max} 272.5 nm; mass spectrum m/z (relative intensity) 651 (M^+- CO), 624 (45.8), 623 (M^+ - t-Bu, 100.0).

A solution consisting of 1.34 g (2.01 mmol) of **8**, 50 mL of acetonitrile, and 12 mL of 0.5 M tetraethylammonium fluoride (acetonitrile) was stirred for 2 h after which time 0.540 g (10.0 mmol) ammonium chloride was added. The resulting solution was stirred for an additional 2 h. The solvent was removed under reduced pressure and the residue purified by flash chromatography on silica gel (9:1 chloroform/methanol) to give 0.586 g (1.73 mmol, 86%) of **12** as a yellow oil: ^{1}H NMR (Me_2SO-d_6) δ1.20 (t, 6 H), 3.62 (m, 2 H), 4.24-4.04 (m, 6 H), 4.32 (m, 1 H), 5.18 (t, 1 H), 5.26 (m, 2 H), 5.51 (d, 1 H), 5.97 (d, 1 H), 8.87 (s, 1 H), 9.19 (s, 1 H); UV(EtOH) λ_{max} 274.0 nm. A solution containing 0.379 g (1.12 mmol) of **12** in 50 mL of methanol was purged with ammonia at 0°C and stored in the freezer for 6 days. The solvent was then removed under reduced pressure and the residue purified by reversed-phase HPLC on Amberlite XAD-4 resin (2.5% ethanol/water) to give 229 mg (0.651 mmol, 58%) of **13** as a pale yellow hygroscopic solid: ^{1}H NMR (Me_2SO-d_6) δ 3.43 (m, 2 H), 3.51 (m, 1 H), 4.38 (m, 1 H), 4.68 (m, 1 H), 4.78 (s, 1 H), 4.98 (t, 1 H), 5.23 (d, 1 H), 5.50 (d, 1 H), 6.03 (d, 1 H), 7.29 (bs, 2 H), 7.59 (bs, 2 H), 8.82 (s, 1 H), 9.15 (s, 1 H); ^{13}C NMR (Me_2SO-d_6) δ 62.2, 63.7, 71.3, 74.4, 86.6, 87.6, 133.4, 146.2, 148.8, 152.4, 159.6, 169.4; UV(EtOH) λ_{max} 266.5 nm (ε = 7090); IR (KBr pellet) 1687 cm^{-1}; FAB HRMS 353.1217 (M^++H), calculated for $C_{13}H_{16}N_6O_6$ 353.1209 (M^++H).

2-Acetamido-9-(β-D-ribofuranosyl)purine (11). A solution consisting of 0.560 g (14.0 mmol) of sodium hydride (60%), 2.50 mL (16.4 mmol) of diethylmalonate, and 15 mL of THF was added dropwise to 40 mL of a THF solution containing 1.100 g (1.64 mmol) of **7**. The resultant solution was purged with nitrogen and stirred for 48 h at reflux. After nmr evidence indicated the absence of intermediate **8**, ammonium chloride (0.810 g, 15.0 mmol) was added. The solution was stirred for an additional 2 h and the solvent was removed under reduced pressure. The residue was purified by flash chromatography (hexanes followed by 1:1 ether/hexanes) to give 0.803 g (1.18 mmol, 72%) of **9** as a light yellow oil.

Deprotection of **9** (0.803 g, 1.18 mmol) was carried out as described previously for the deprotection of **8** to give 0.301 g (0.956 mmol, 81%) of **10** as a light-yellow oil: ^{1}H NMR (Me_2SO-d_6) δ 1.25 (t, 3 H), 3.62 (m, 2 H), 4.28-4.10 (m, 6 H), 4.35 (m, 1 H), 5.15 (t, 1 H), 5.32 (d, 1 H), 5.55 (d, 1 H), 6.05 (d, 1 H), 8.71 (s, 1 H), 9.02 (s, 1 H); UV(EtOH) λ_{max} 272.0 nm. The formation of **11** from **10** (0.204 g, 0.603 mmol) was carried out as described previously for the formation of **13** to afford 0.160 g (0.514 mmol, 85%) of **11** as white crystals: mp. 175-176°C; ^{1}H NMR (Me_2SO-d_6) δ 3.65 (m, 2 H), 3.81 (s, 2 H), 3.95 (m, 1 H), 4.16 (m, 1 H), 4.60 (m, 1 H), 5.08 (t, 1 H), 5.21 (d, 1 H), 5.49 (d, 1 H), 6.03 (d, 1 H), 7.41 (bs, 1 H), 7.48 (bs, 1 H), 8.78 (s, 1 H), 9.11 (s, 1 H); ^{13}C NMR (Me_2SO-d_6) δ 46.2, 61.5, 70.6, 73.7, 87.1, 89.0, 132.5, 145.1, 148.2, 151.6, 159.7, 170.5; UV(EtOH) λ_{max} 266.5 (ε = 7730); IR (KBr pellet) 1664 cm^{-1}; FAB HRMS 310.1132 (M^++H), calculated for $C_{12}H_{15}N_5O_5$ 310.1151 (M^++H).

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NOVEL UNSATURATED PURINE NUCLEOSIDES[1]

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Abstract: The synthesis of a number of novel purine ribonucleosides bearing one degree of unsaturation at the 2-position is described. The two key reactions used in the synthesis of these compounds is a palladium-catalyzed cross-coupling reaction and an ozonolysis. These synthetic transformations have rarely been used in nucleoside chemistry. Isomerization of an allylpurine system to a vinylpurine system under the conditions of the cross-coupling reaction and also under fluoride ion catalysis is discussed. The novel 2-formylpurine nucleosides described appear to be easily hydrated.

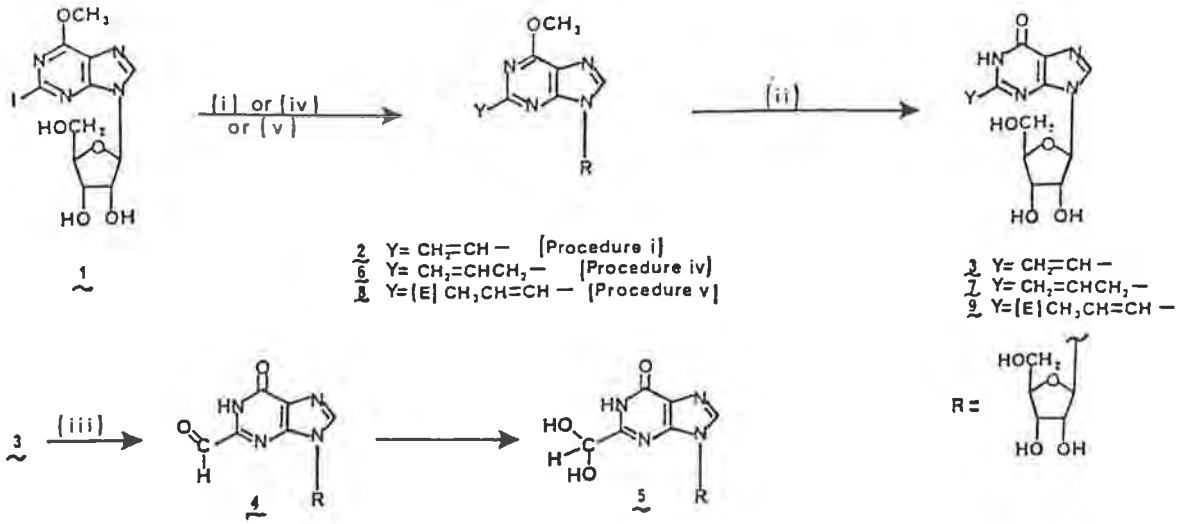
The design and synthesis of strategically modified nucleosides are of interest because of the potential biological value of these compounds as anticancer and antiviral agents.[2-8] One class of nucleosides currently being studied in our laboratory are purine ribonucleosides that contain modification at the 2-position of the base moiety.[9,10] In this paper, we describe the synthesis of novel purine and hypoxanthine nucleosides with one degree of unsaturation in the substitution at the 2-position. These compounds are not only of potential interest as antiviral and anticancer agents, but also as inhibitors of some key enzymes in purine metabolism.[11-14] Some of the steps utilized in the synthesis of these novel nucleosides have rarely been used in nucleoside chemistry.

For compounds belonging to the hypoxanthine series, the immediate precursor was 2-iodo-6-methoxypurine nucleoside (1).[10] Replacement of the 2-iodo group with a vinyl group at this position was carried out by a palladium-catalyzed cross-coupling reaction reported previously by us.[10] Deprotection of the resulting 6-methoxy-2-vinyl compound 2 with trimethylsilyl iodide (TMSI) gave the α,β-unsaturated inosine 3. Ozonolysis of 3 in methanol at -65 °C followed by rapid reductive work-up with dimethyl sulfide, furnished the novel inosine carboxaldehyde 4 (Scheme 1). Rearrangement reactions of the intermediate secondary ozonide

may be minimized by addition of dimethyl sulfide prior to warm up to room temperature. Compound 4 was purified by reversed-phase HPLC on Amberlite XAD-4 resin with ethanol-water as the eluting solvent to give 4 as a white solid in 55% yield. Interestingly, the high-field ^{13}C NMR spectrum of 4 showed that it existed in both the carbonyl form (C=O carbon at 185.5 ppm) and the hydrated carbonyl structure 5 (geminal diol carbon at 88.1 ppm). The resonance of the formyl carbon suggests that it may have partial characteristics of the carbonyl of an iminamidyl type linkage. It should be mentioned that ozonolysis reactions have rarely been in nucleoside synthesis.[15]

The palladium-catalyzed cross-coupling procedure was also used to synthesize the novel unsaturated nucleosides, 2-allylinosine [2-(2-propenyl)inosine] 7 and 2-(1-propenyl)inosine 9 (Scheme 1). Thus, treatment of the 2-iodo compound 1 with bis(acetonitrile)palladium II chloride with allyl tri-n-butylstannane in DMF at 100 °C for 6 h afforded a mixture of the 2-allyl-6-methoxypurine 6 and the rearranged product, the 2-(1-propenyl)-6-methoxypurine 8 in a ratio of 2.5 to 1 and a combined yield of 92%. Temperature and reaction time appear to be very important factors in these palladium-catalyzed reactions. Between 90 and 95 °C, selectivity of formation of the allyl product 6 is optimum (i.e. formation of the rearranged product 8 can be almost fully suppressed by lowering the reaction temperature). Alternatively, raising the reaction temperature to about 105 °C and increasing the reaction time result in high selectivity in the formation of 8. Finally, below 90 °C, the reaction is extremely sluggish. Our experimental data do not provide an unambiguous mechanistic interpretation of the pathway for the isomerization of the allyl group.[16] Deprotection of the allyl and vinyl nucleosides, 6 and 8, with TMSI in acetonitrile gave the target compounds 7 and 9, respectively, in about 64% yield. The latter products were purified by reversed-phase HPLC and characterized by their spectral data: UV, FTIR, FAB HRMS, and high-field NMR. In the case of compound 9, the high-field ^{1}H NMR spectrum (in DMSO-d_6) gave unequivocal evidence for the E-stereochemistry of the exocyclic double bond (J = 17.1 Hz).

For the novel compounds of the nebularine series, 2-aminonebularine (isoadenosine) 10[9] served as the starting material (Scheme 2). Compound 10 was converted to 11 by silylation followed by deamination-halogenation. Palladium-catalyzed cross-coupling of 2-iodopurine ribonucleoside 11 with vinyl tri-n-butylstannane followed by deprotection with fluoride ions



1

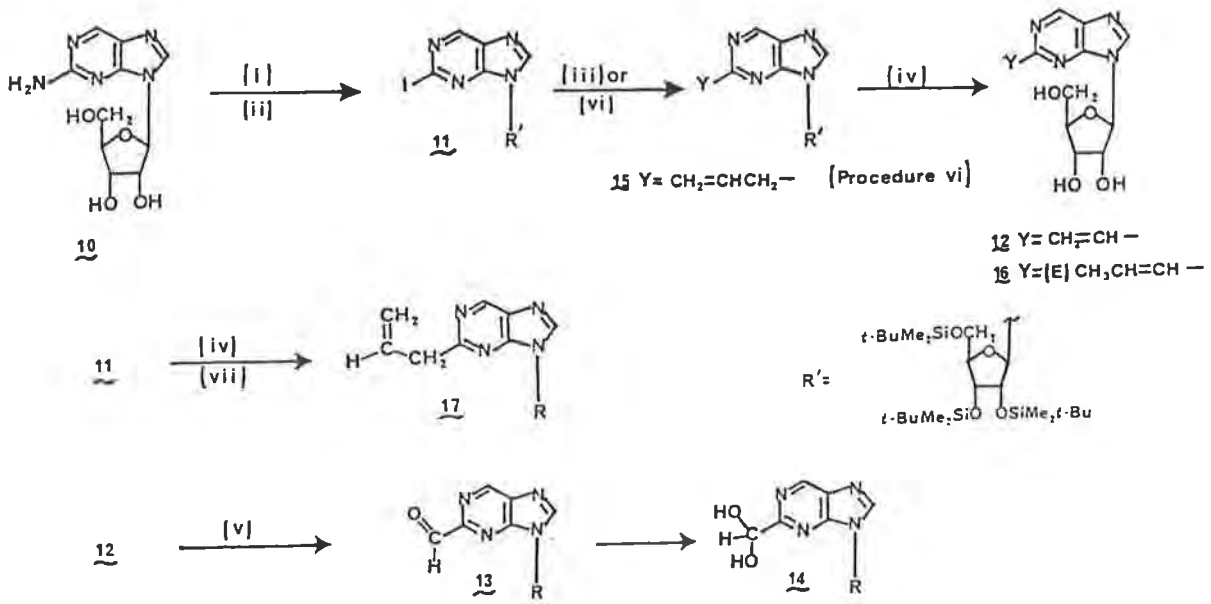
2 Y= CH₂=CH— [Procedure i]
6 Y= CH₂=CHCH₂— [Procedure iv]
8 Y=(E) CH₃CH=CH — [Procedure v]

3 Y= CH₂=CH —
7 Y= CH₂=CHCH₂—
9 Y=(E) CH₃CH=CH —

R =

(i) n-Bu₃Sn CH=CH₂, Pd Cl₂(CH₃CN)₂, DMF, Δ (ii) (CH₃)₃SiI, CH₃CN/DMF
(iii) O₃, MeOH, −65°C, Me₂S (iv) n-Bu₃SnCH₂CH=CH₂, PdCl₂(CH₃CN)₂, DMF, 90°C
(v) n-Bu₃SnCH₂CH=CH₂, PdCl₂(CH₃CN)₂, DMF, 105°C

Scheme 1



10

11

15 Y= CH₂=CHCH₂— [Procedure vi]

12 Y= CH₂=CH —
16 Y=(E) CH₃CH=CH —

R'=

(i) t-BuMe₂SiCl, imidazole, DMF; (ii) t-BuONO, CH_2I_2, $(CH_3)_3SiI$, hexane, Δ; (iii) n-Bu₃SnCH=CH₂, $PdCl_2(CH_3CN)_2$, P(o tolyl)₃, toluene, Δ; (iv) Et_4NF, CH_3CN; (v) O_3, 15% EtOH/H_2O, 4°C, Me_2S; (vi) n-Bu₃SnCH₂CH=CH₂, $PdCl_2(CH_3CN)_2$, P(o tolyl)₃, toluene, Δ; (vii) n-Bu₃SnCH₂CH=CH₂ $Pd(PPh_3)_4$, DMF, Δ

Scheme 2

furnished 2-vinylnebularine 12 (63% yield for 2 steps). Cleavage of the vinyl group with ozone at 4 °C in ethanol-water gave the novel 2-formyl compound 13 in 61% yield after reductive work-up and purification by HPLC. Compound 13 was characterized spectroscopically as described for 4. It appears to be readily hydrated and both the aldehyde 13 and geminal diol 14 were clearly discerned in the high-field ^{1}H and ^{13}C NMR spectra.

Two approaches were examined for the synthesis of the new nucleoside, 2-allylnebularine (17). In the first approach, the silyl protected 2-iodo compound 11 was treated with allyl tri-n-butylstannane, and bis(acetonitrile) palladium II chloride in the presence of tri-o-tolylphosphine in refluxing toluene for 36 h to give the protected allyl compound 15 in 44% yield. However, when compound 15 was deprotected with tetraethylammonium fluoride and the reaction mixture worked up with ammonium chloride, the isomerized 2-(1-propenyl)purine nucleoside 16 was isolated in 81% yield. The persistence of this double bond isomerization even under neutral work-up conditions suggested that it was probably occurring through the involvement of fluoride ions used for the removal of the silyl protecting groups. This unexpected facile double bond isomerization in the deprotection step prompted examination of an alternative method of arriving at compound 17. This approach involved initial deprotection of 11 followed by cross-coupling with allyl tri-n-butylstannane and tetrakis(triphenylphosphine)palladium (0) in DMF at 100 °C for 18 h. Allylnebularine 17 was obtained in 50% yield after reversed-phase HPLC and was fully characterized by its physical data. As in the case of the aforementioned inosine series, raising the temperature of the cross-coupling reaction resulted in increased amounts of the isomerized product.

In summary, novel unsaturated nucleosides of the inosine and nebularine families have been prepared by two useful synthetic approaches that have seen little previous utilization in purine nucleoside chemistry. The aldehydes synthesized represent new congeners of hypoxanthine and purine nucleosides and no compounds related to these interesting systems have previously been reported. In terms of reactivity as it relates to carbon-carbon bond formation, the purine system is much less reactive than the hypoxanthine system. A temperature-dependent isomerization of an allyl nucleoside to a vinyl nucleoside was also observed under the conditions of cross-coupling. Biological studies assessing the RNA antiviral activities of these compounds are currently under investigation.

EXPERIMENTAL SECTION

Melting points provided are uncorrected, and were taken on a Thomas-Hoover melting point apparatus fitted with a microscope. Nuclear magnetic resonance spectra using tetramethylsilane as the internal standard were recorded on JOEL Model FX-90Q and Bruker Model WM-360 pulse Fourier transform spectrometers. A VG Analytical Model VG-II-250 MS system was used for the FAB-HRMS data. The ultraviolet spectra were recorded on a Varian Cary Model 219 spectrophotometer. Infrared spectra were recorded on a Mattson Cygnus Model 25 Fourier transform instrument. All solvents were distilled over appropriate drying agents before use. Flash chromatography was carried out on 230-400 mesh silica gel with methanol-dichloromethane as the eluting solvent. HPLC was performed on Amberlite XAD-4 resin (40-60 μm) with ethanol-water as the mobile phase.

2-Vinyl-9-(β-D-ribofuranosyl)hypoxanthine (3) was synthesized from 2-iodo-6-methoxy-9-(β-D-ribofuranosyl)purine (1) as previously described.[10]

2-Formyl-9-(β-D-ribofuranosyl)hypoxanthine (4). A solution consisting of 1.000 g (3.40 mmol) of 2-vinyl-9-(β-D-ribofuranosyl)hypoxanthine (3) and 500 mL of dry (Omnisolve) methanol was cooled to -65 °C in an isopropanol/dry ice bath. This mixture was ozonized for 15 min and then purged with nitrogen while slowly letting it warm to room temperature. Dimethyl sulfide (3 mL) was added and the solution was allowed to stir at room temperature for 12 h. The solvent was then removed under reduced pressure, and the residue was purified by · HPLC. Compound 4 was obtained as a crystalline white solid in 55% yield (0.554 g, 1.87 mmol). Mp 161 °C; ^{13}C NMR (DMSO-d_6) δ (61.1, 61.2), (70.2, 70.3), (73.9, 74.1), (85.6, 85.7), (86.8, 87.2), 88.1, (123.2, 126.6), (138.9, 141.1), (147.0, 147.6), (147.9, 155.8), (156.4, 157.4), 185.5. ^1H NMR (Me_2SO-d_6) δ 3.60 (m, 2H), 3.93 (m, 1H), 4.13 (m, 1H), 4.50 (m, 1H), 5.10 (m, 2H), 5.50 (m, 2H), 5.89 (d, J = 5.86 Hz, 1H), 6.82 (d, J = 6.84 Hz, 1.5 H), 8.32 (s, 1H), 9.55 (s, 1H), 11.7 (s, 1H); UV (H_2O) λ_{max} 249, 270 nm (ε 4965); FTIR (KBr) 1692, 3300 cm^{-1}; FAB (HRMS) Calcd for $C_{11}H_{12}N_4O_5$ 297.0835 (M^++H); Found: 297.0831 (M^++H).

2-Allyl-9-(β-D-ribofuranosyl)hypoxanthine (7). A solution consisting of 1.090 g (2.67 mmol) of compound 1, 0.035 g (0.134 mmol) of $PdCl_2$ $(MeCN)_2$, and 5.00 mL of dry DMF was

purged with N_2 (1/2 h). Allyltributyltin (0.911 mL, 2.94 mmol) was added dropwise via gas-tight syringe. The reaction mixture was then stirred under N_2 at 90 °C for 6 h. It was cooled to ambient temperature, and the solvent removed under vacuum. The residue was flash chromatographed. Compound **6** was obtained as a low melting tan solid in 76% yield (0.654 g, 2.03 mmol): ^{1}H NMR (Me_2SO-d_6) δ 3.18 (d, 2H), 3.63 (m, 2H), 3.97 (m, 1H), 4.09 (s, 3H), 4.11 (m, 1H), 4.51 (m, 1H), 5.17 (m, 5H), 5.93 (d, 1H), 6.05 (m, 1H), 8.56 (s, 1H); UV (EtOH) λ_{max} 252 nm.

A solution consisting of 1.000 g of **6** (3.11 mmol), 2.450 g (14.8 mmol) of KI and 3.50 mL (67.1 mmol) of acetonitrile in dry DMF (5.0 mL) was purged with nitrogen for 30 min. Trimethylsilyl chloride (1.83 mL, 14.4 mmol) was added dropwise to the solution, and the mixture was stirred for 3 h at room temperature. The reaction was quenched by the addition of 3.0 mL of 3M NaOH in 7.0 mL of H_2O to bring the pH of the reaction to 7. The solvents were removed in vacuo and the residue was purified by HPLC to give 2-allylinosine (**7**) as a white solid (0.613 g, 1.99 mmol, 64%): mp 137-139 °C; ^{13}C NMR (Me_2SO-d_6) δ 30.6, 61.4, 70.5, 73.7, 85.7, 87.0, 118.1, 122.4, 132.6, 138.5, 148.5, 156.5, 157.0; ^{1}H NMR (Me_2SO-d_6) δ 3.42, (d, 2H), 3.60 (m, 2H), 3.97 (m, 1H), 4.12 (m, 1H), 4.52 (m, 1H), 5.20 (m, 5H), 5.84 (d, 1H), 6.07 (m, 1H), 8.25 (s, 1H), 12.04 (bs, 1H); UV (H_2O) λ_{max} 249 nm (ϵ 11102); FAB (HRMS) Calcd for $C_{13}H_{16}N_4O_5$: 309.1199 (M^++H). Found: 309.1162 (M^++H).

E-2-(1-Propenyl)-9-(β-D-ribofuranosyl)hypoxanthine (9). A solution consisting of 1.000 g (2.45 mmol) of compound **1**, and 0.270 g (0.105 mmol) of $PdCl_2(MeCN)_2$ in dry DMF (5 mL) was purged with N_2 (1/2 h) and allyltributyltin (0.836 mL, 2.70 mmol) was added and the reaction was carried out as described for synthesis of **6** except for reaction temperature and time (105 °C, 24 h). Compound **8** was obtained as a low-melting solid in 63% yield (0.474 g, 1.54 mmol); ^{1}H NMR (Me_2SO-d_6) δ 1.93 (dd, 3H, J = 1.46, 8.3 Hz), 3.62 (m, 2H), 3.95 (m, 1H), 4.08 (m, 4H), 4.61 (m, 1H), 5.07 (m, 2H), 5.46 (m, 1H), 5.93 (d, 1H), 6.45 (dd, 1H, J = 1.46, 16.6 Hz), 7.11 (m, 1H), 8.50 (s, 1H); UV (H_2O) λ_{max} 261 nm.

A solution consisting of 1.000 g (3.11 mmol) of compound **8** was deprotected as described for **7** to give E-2-(1-propenyl)-9-(β-D-ribofuranosyl)hypoxanthine **9** as a white solid (0.613 g, 1.99 mmol, 64%): mp 221-223 °C; ^{13}C NMR (Me_2SO-d_6) δ 18.1, 61.3, 70.4, 73.8, 85.5, 87.1, 122.6, 123.5, 138.4, 138.8, 148.7, 152.0, 156.8; ^{1}H NMR (Me_2SO-d_6) δ 1.92 (dd, 3H, J = 6.84,

small), 3.58 (m, 2H), 3.94 (m, 1H), 4.13 (m, 1H), 4.53 (m, 1H), 5.18 (m, 1H), 5.38 (m, 1H), 5.44 (d, 1H), 5.87 (d, 1H), 6.30 (dd, 1H, J = 17.1, 1.5 Hz), 7.10 (m, 1H), 8.26 (s, 1H), 12.17 (s, 1H); UV (H_2O) λ_{max} 260 (ϵ 6123), 290 (ϵ 6290) nm; FAB (HRMS) Calcd for $C_{13}H_{16}N_4O_5$: 309.1199 (M^++H). Found: 309.1210 (M^++H).

2-Iodo-9-[2,3,5-tri-O-(tert-butyldimethylsilyl)-β-D-ribofuranosyl]purine (11). This precursor was prepared from isoadenosine 10 by silylation followed by radical halogenation.[9] The overall yield of 11 from guanosine (6 steps) was 34%.

2-Formyl-9-(β-D-ribofuranosyl)purine (13). A solution of 0.238 g (0.858 mmol) of 2-vinyl-9-(β-D-ribofuranosyl)purine (12), prepared as described for 3, in 270 mL of 15% ethanol/water was cooled to 4 °C and then ozonized for 10 min. The reaction mixture was worked up as described for ozonolysis of 3. This procedure furnished 0.147 g (0.525 mmol, 61%) of compound 13 as a white solid: mp 115-117 °C; ^{13}C NMR (Me_2SO-d_6) δ (61.1, 61.4), (70.3, 70.8), (73.6, 73.8), (85.8, 86.9), (87.0, 87.4), 90.8, (133.1, 133.3), (134.8, 135.1), (147.8, 148.0), (151.1, 151.4), (153.2, 162.7), 190.8; ^1H NMR (Me_2SO-d_6) δ 3.62 (m, 4H), 4.02 (m, 2H), 4.22 (m, 3H), 4.55 (m, 2H), 4.95 (m, 3H), 5.52 (m, 2H), 6.22 (m, 2H), 6.56 (d, 1H), 9.01 (s, 1H), 9.09 (s, 1H), 9.39 (s, 1H), 9.43 (s, 1H), 10.07 (s, 1H); UV (EtOH) λ_{max} 276 nm; FTIR (KBr) 1718 cm^{-1} FAB (HRMS) Calcd for $C_{11}H_{12}N_4O_5$: 281.0886 (M^++H); Found: 281.0887 (M^++H).

E-2-(1-Propenyl)-9-(β-D-ribofuranosyl)purine (16). Bis(acetonitrile) palladium II chloride (0.021 g, 0.080 mmol) and tri-O-tolylphosphine (0.049 g, 0.160 mmol) were added to a 100 mL RBF containing 0.963 g (1.33 mmol) of compound 11. The flask was placed on the vacuum line to remove residual oxygen. Freshly distilled toluene (50 mL) was added to the flask followed by 0.46 mL (1.46 mmol) of allyltributyltin. The mixture was purged with N_2 (1/2 h) and then heated under toluene reflux (under N_2) for 16 h. At this time, an additional 0.2 mL (0.645 mmol) of allyltributyltin was added and the reaction allowed to proceed for an additional 10 h. Upon cooling, ethyl ether (40 mL) was added and the reaction mixture extracted with 10% Na_2EDTA (2x20 mL) and water (20 mL). The organic layer was dried (Na_2SO_4), and the solvents were removed under reduced pressure. The residue was taken up in hexanes and eluted through a short silica gel scrubber column with 1:1 hexanes/ethyl ether.

Final purification by flash chromatography on silica gel (1:1 hexanes/ethyl ether) provided 0.322 g of starting material, and 0.213 g (0.337 mmol, 44%) of the protected allyl compound **15** as a tan oil: ^{1}H NMR ($CDCl_3$) δ -0.16-0.13 (m, 18H), 0.80-0.95 (m, 27H), 3.79 (d, 2H), 4.01 (m, 2H), 4.13 (m, 1H), 4.32 (m, 1H), 4.64 (t, 1H), 5.10 (m, 2H), 6.05 (d, 1H), 6.20 (m, 1H), 8.44 (s, 1H), 9.05 (s, 1H); UV (EtOH) λ_{max} 267 nm.

To a solution consisting of 0.311 g (0.491 mmol) of compound **15** and 10 mL of dry acetonitrile was added 3.93 mL (1.96 mmol) of tetraethylammonium fluoride (0.5 M solution in acetonitrile). The resulting solution was allowed to stir at room temperature for 3 h under nitrogen. The reaction was worked up by the addition of 0.276 g (5.16 mmol) of ammonium chloride and 10 mL of water. This solution was allowed to stir for 12 h at room temperature. The acetonitrile was then removed and the aqueous portion extracted with chloroform (15 mL) and ethyl ether (15 mL). The chloroform portion was then back extracted with water (20 mL). The aqueous portions were combined and concentrated. The residue was taken up in methanol and purified on preparative silica gel plates using 17% methanol/chloroform for development. Recrystallization from water gave 0.116 g (3.98 mmol, 81%) of the isomerized compound **16** as a white solid: mp 86-88 °C ; ^{13}C NMR (Me_2SO-d_6) δ 18.3, 61.7, 70.8, 73.8, 86.0, 87.6, 131.0, 132.7, 135.7, 145.5, 148.4, 151.7, 158.4; ^{1}H NMR (Me_2SO-d_6) δ 1.94 (dd, 3H, J = 8.30, 1.46 Hz), 3.63 (m, 2H), 3.98 (q, 1H), 4.20 (q, 1H), 4.67 (q, 1H), 5.09 (t, 1H), 5.24 (d, 1H), 5.50 (d, 1H), 6.03 (d, 1H), 6.56 (dd, 1H, J = 17.1, 1.5 Hz), 7.10 (m, 1H), 8.73 (s, 1H), 9.08 (s, 1H); UV (H_2O) λ_{max} 231 nm (ϵ 17441); 268 nm (ϵ 11290); 287 nm (ϵ 9660); FAB (HRMS) Calcd for $C_{13}H_{16}N_4O_4$: 293.1250 (M^++H). Found: 293.1270 (M^++H).

2-Allyl-9-(β-D-ribofuranosyl)purine (17). To a solution consisting of 0.909 g (1.26 mmol) of compound **11** in acetonitrile (20 mL) was added 10.0 mL (5.04 mmol) of tetraethylammonium fluoride (0.5 M solution in acetonitrile). The solution was stirred under N_2 for 1 h, at which time 0.710 g of ammonium chloride (13.24 mmol) and 10 mL of water was added and stirring was continued for an additional 12 h. The reaction was worked up as described for the deprotection of **15** to give deprotected **11** as a white solid (0.364 g, 0.960 mmol, 76%): mp 163-165 °C; ^{1}H NMR (Me_2SO-d_6) δ 3.61 (m, 2H), 3.95 (m, 1H), 4.18 (m, 1H), 4.56 (m, 1H), 5.04 (t, 1H), 5.27 (d, 1H), 5.55 (d, 1H), 5.96 (d, 1H), 8.78 (s, 1H), 8.98 (s, 1H); UV (EtOH) λ_{max} 278.5 nm.

Tetrakis (triphenylphosphine)palladium (0) (0.0162 g, 0.014 mmol) was added to a 100 mL RB flask containing 0.1060 g (0.280 mmol) of deprotected 11 in a glove box. The flask was then placed on a vacuum line to remove residual oxygen. Dry DMF (20 mL) was added to the reaction flask via double-tipped needle followed by 0.095 mL (0.308 mmol) of allyltributyltin. The reaction mixture was purged with N_2 (0.5 h) and then heated at 100 °C under N_2 for 18 h. The solution was filtered and the DMF was then removed under reduced pressure. The residue was purified by HPLC to give 17 as a highly hygroscopic white solid (0.062 g, 0.214 mmol, 76%): ^{1}H NMR (Me_2SO-d_6) δ 3.67 (m, 4H), 3.97 (m, 1H), 4.18 (m, 1H), 4.65 (m, 1H), 5.15 (m, 4H), 5.49 (d, 1H), 6.02 (d, 1H), 6.18 (m, 1H), 8.76 (s, 1H), 9.11 (s, 1H); ^{13}C NMR (Me_2SO-d_6) δ 43.1, 61.3, 70.4, 73.4, 85.8, 87.0, 116.6, 132.2, 135.1, 144.9, 148.1, 151.4, 162.3; UV (H_2O) λ_{max} 267 nm, 247 nm (sh); FAB (HRMS) Calcd for $C_{13}H_{16}N_4O_4$: 293.1250 (M^++H). Found: 293.1230 (M^++H).

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Novel, Stable Congeners of the Antiretroviral Compound 2′,3′-Dideoxyadenosine

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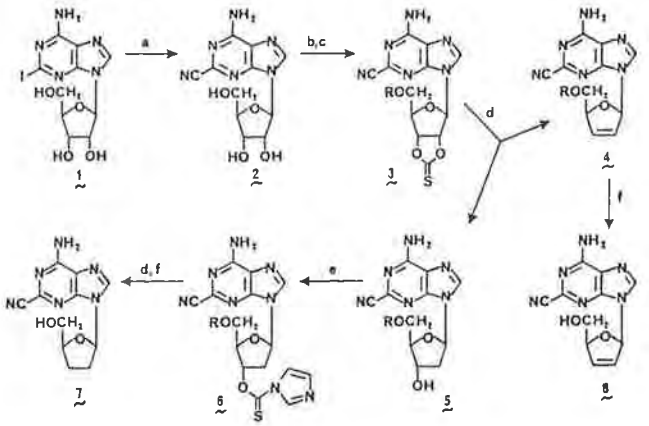
Abstract: Novel congeners of the antiretroviral compound 2′,3′-dideoxyadenosine (ddA) have been synthesized through metal-mediated and photochemical conversions as the key steps. These compounds are inherently more stable than ddA with respect to both glycosidic bond cleavage and deamination by adenosine deaminase.

2′,3′-Dideoxygenated analogues of the natural ribonucleosides have elicited considerable antiviral interest recently because of their ability to inhibit the cytopathic effect of the human immunodeficiency virus (HIV), the etiologic agent of acquired immunodeficiency syndrome (AIDS).[1-5] For example, 2′,3′-dideoxyadenosine (ddA), as its cellularly produced 5′-triphosphate (ddATP), is an inhibitor of HIV reverse transcriptase, an enzyme which plays a vital role in the life cycle of this virus.[5-8] However, ddA is rapidly deaminated by the ubiquitous enzyme adenosine deaminase to 2′,3′-dideoxyinosine (ddI),[7] which can return to the ddA nucleotide pool via ddIMP or be catabolized by purine-nucleoside phosphorylase.[9] In addition to this enzymatic instability, ddA is also unstable with respect to hydrolytic cleavage of the glycosidic bond.[10] Both of these factors limit the usefulness of ddA both as a biological probe and as an antiviral agent. The design of congeners of ddA that would be hydrolytically and enzymatically stable would be of considerable significance in this area. This paper reports on the synthesis and stability studies of such novel congeners of ddA. The rationale for the choice of functionalization at the 2-position was 2-fold. First, initial phosphorylation of ddA by mammalian deoxycytidine kinase[11] appears to be significant for its subsequent conversion to its triphosphate and its eventual biological activity.[5-9] Enzymatic data for 2′-deoxyadenosines suggest that judicious 2-substitution in general does not eliminate substrate activity by deoxycytidine kinase.[12] Second, greater stabilization of the glycosidic bond may also be possible with appropriate substitution at this position.

The syntheses are exemplified by the case of 2-cyano-2′,3′-dideoxyadenosine (**7**) where 2-iodoadenosine (**1**)[13] served as the key precursor (Scheme I). Palladium-catalyzed cross-coupling of *unprotected* 2-iodoadenosine with tri-*n*-butylcyanostannane in DMF resulted in regiospecific formation of 2-cyanoadenosine (**2**)[14] in 86% yield. This conversion represents another new type of application of the palladium-catalyzed methodology in nucleoside chemistry. Regiospecific 5′-silylation (70%) followed by treatment of the resulting silylated compound with 1,1′-thiocarbonyldiimidazole in DMF gave the cyclic thiocarbonate **3** (87%). Re-

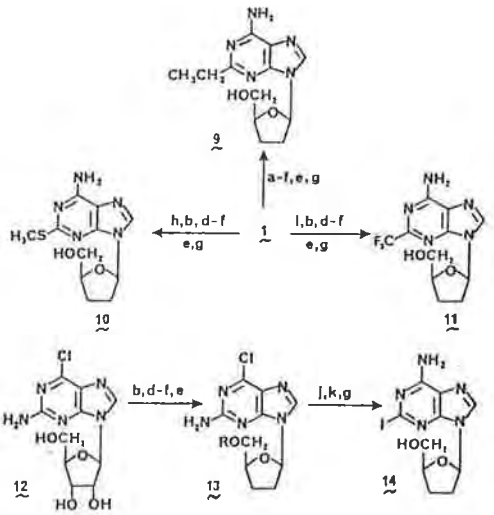
Scheme I[a]



R = *t*-BuMe$_2$Si—

[a] (a) Bu_3SnCN, $[P(C_6H_5)_3]_4Pd$, DMF, Δ; (b) *t*-$Bu(CH_3)_2SiCl$, 4-(dimethylamino)pyridine, $(C_2H_5)_3N$, DMF, CH_2Cl_2; (c) 1,1′-thiocarbonyldiimidazole, DMF, 25 °C; (d) Bu_3SnH, AIBN, toluene, 110 °C; (e) 1,1′-thiocarbonyldiimidazole, DMF, 90 °C; (f) Et_4NF, CH_3CN.

Scheme II[a]



[a] (a) $Bu_3SnCH{=}CH_2$, $PdCl_2(CH_3CN)_2$, DMF, Δ; (b) *t*-$Bu(CH_3)_2SiCl$, 4-(dimethylamino)pyridine, $(C_2H_5)_3N$, DMF, CH_2Cl_2; (c) H_2, 10% Pd/C, C_2H_5OH; (d) 1,1′-thiocarbonyldiimidazole, DMF, 25 °C; (e) Bu_3SnH, AIBN, toluene, 110 °C; (f) 1,1′-thiocarbonyldiimidazole, DMF, 90 °C; (g) Et_4NF, CH_3CN; (h) $(CH_3S)_2$, CH_3CN, *hν*; (i) CF_3ZnBr, CuBr, DMF, HMPA; (j) *t*-BuONO, CH_2I_2, hexane, 70 °C; (k) NH_3, C_2H_5OH.

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ductive cleavage of **3** with *n*-Bu_3SnH in the presence of AIBN[15] accomplished two things. First, 2′-deoxygenation occurred regiospecifically to give the deoxynucleoside **5** in 57% yield. This regiospecific cleavage at the 2′-position of nucleoside cyclic thiocarbonates has not been observed previously (cf. ref 15), but it is mechanistically consistent with the preferred mode of cleavage of the intermediate radical formed from **3** and *n*-Bu_3SnH and AIBN. Second, and interestingly, complete elimination of the thiocarbonate group produced the unsaturated nucleoside **4** in 30% yield. This is the first example of substantial amounts of such a product being produced from a purine nucleoside cyclic thiocarbonate under this radical deoxygenation procedure.[16]

The 2′-deoxygenated compound **5** was converted to the novel 2-cyano-2′,3′-dideoxyadenosine (**7**) through its 3′-imidazolide by treatment with *n*-Bu_3SnH and AIBN,[17] followed by deprotection of the silyl group with tetraethylammonium fluoride. Alternatively, compound **7** may be synthesized more directly through the catalytic hydrogenation of **4** followed by deprotection. Compound **7** was characterized by UV data (λ_{max} 260, 266, 297 nm), FTIR (2200 cm^{-1}), FAB HRMS ($M^+ + H$ ion at 261.1069), and high-field ^1H and ^{13}C NMR data. Deprotection of **4** gave the novel didehydrodideoxynucleoside **8**. Thus, the deoxygenation reaction described can be used to synthesize both dideoxynucleosides and dideoxydidehydronucleosides through a single route.

The key precursor for the synthesis of 2-ethyl-2′,3′-dideoxyadenosine was also 2-iodoadenosine (**1**) (Scheme II). Palladium-catalyzed cross-coupling of **1** with vinyltri-*n*-butylstannane,[18] resulted in regiospecific introduction of the vinyl group at the 2-position in almost quantitative yield. Subsequent selective 5′-silylation followed by catalytic hydrogenation of the 2-vinyl group and dideoxygenation and deprotection gave the target molecule **9**. 2-(Methylthio)-2′,3′-dideoxyadenosine (**10**) was of interest because of the known contribution of the thiomethyl group to the biological activity of some related ribonucleosides.[19-21] The immediate precursor of **10** was 2-(methylthio)adenosine, which was prepared from 2-iodoadenosine (**1**) by photochemical alkylthiolation.[22] Application of the dideoxygenation procedure to this precursor gave **10**.

2-(Trifluoromethyl)adenosine was the immediate precursor for the synthesis of **11** through the previously described dideoxygenation sequence. This precursor was prepared directly from **1** in 70% yield by reaction with "CF_3Cu" (cf. ref 23). The copper reagent was generated in situ from CF_3ZnBr and CuBr.[24] Another 2-halogenated congener, 2-iodo-2′,3′-dideoxyadenosine (**14**), was also prepared. 2-Amino-6-chloropurine ribonucleoside (**12**)[25] was dideoxygenated to provide the new dideoxy compound **13**. Halogen–amino group interchange followed by deprotection gave the novel dideoxynucleoside **14**.

The glycosidic bond stabilities of these dideoxynucleosides were investigated by differential UV spectroscopy at pH 3 where easily observable rates could be obtained.[26] The relative rate data can be summarized as follows: ddA (100), 2-cyano-ddA (47), 2-ethyl-ddA (75), 2-(methylthio)-ddA (64), 2-(trifluoromethyl)-ddA (81), and 2-iodo-ddA (55). It is immediately apparent that all of the dideoxynucleosides synthesized in this work are more stable with respect to glycosidic bond cleavage than 2′,3′-dideoxyadenosine. Both electronic and conformational effects may be contributing to this increased stability. Additionally, all of the novel dideoxynucleosides synthesized were totally resistant to deamination by mammalian adenosine deaminase! Inhibition studies with this enzyme are currently being investigated.

The preferred glycosidic bond conformations of these dideoxypurine nucleosides in solution were qualitatively determined through correlation of their high-field ^{13}C NMR data.[27] Although such correlations for dideoxynucleosides have not been studied previously, it appears from the data of these and other dideoxynucleosides synthesized in our laboratory that in the preferred anti conformation Δ(C-2′ minus C-3′) is generally greater than 6 ppm and in the syn conformation this difference is less than 3 ppm.

In summary, novel congeners of the antiretroviral compound 2′,3′-dideoxyadenosine, with high potential for antiretroviral activity, have been synthesized through photochemical and metal-mediated conversions as the key steps. These congeners are inherently more stable with respect to glycosidic bond cleavage than the parent ddA, and they are totally resistant to hydrolytic deamination by mammalian adenosine deaminase. High-field ^{13}C NMR data suggest that these functionalized congeners of ddA prefer the anti conformation in solution.

Experimental Section

The reported melting points are uncorrected and were determined on a Thomas-Hoover melting point apparatus fitted with a microscope. Nuclear magnetic resonance spectra were recorded on JEOL Model FX90Q and Bruker Model WM360 and MSL 300 pulse Fourier transform spectrometers. Mass spectra were determined on a Hewlett-Packard 5985 GC/MS system or a VG Analytical Model ZAB-HF instrument with high-resolution FAB capability. Ultraviolet spectra were recorded on a Varian Cary Model 219 or a Gilford Response spectrophotometer. Infrared spectra were recorded on a Mattson Cygnus 25 fourier transform instrument. Preparative layer chromatography plates were prepared by coating six 20 cm × 20 cm plates with a slurry made from 150 g of E. Merck PF_{254} silica gel in 400 mL of water. The silica gel plates were allowed to dry slowly and were then activated for 3 h at 135 °C. Flash chromatography was carried out in glass columns packed with 230–400-mesh silica gel.

General Synthetic Procedures (A–F). Procedure A: Preparation of 5′-*O*-(*tert*-Butyldimethylsilyl) Nucleosides. A mixture of the nucleoside (2 mmol), *tert*-butyldimethylsilyl chloride (2.2 mmol), triethylamine (2 mmol), and *N*,*N*-dimethylaminopyridine (0.3 mmol) in dimethylformamide (10 mL) and dichloromethane (5 mL) was stirred at room temperature under nitrogen for 20 h. The solvents were evaporated and the residue was chromatographed on silica gel with 5% methanol/chloroform.

Procedure B: Preparation of 2′,3′-*O*-(Cyclic thiocarbonate). To a solution of the 5′-silylated nucleoside (3 mmol) in dry dimethylformamide (30 mL) was added 1,1′-thiocarbonyldiimidazole (5.25 mmol), and the resulting mixture was stirred at room temperature under nitrogen for 24 h. The solvent was evaporated, and the residue was dissolved in dichloromethane (50 mL) and extracted with water (3 × 20 mL). The organic layer was dried (Na_2SO_4) and evaporated. The residue was purified by flash chromatography on silica gel with chloroform.

Procedure C: Deoxygenation of 2′,3′-*O*-(Cyclic thiocarbonate). A nitrogen-purged solution of tri-*n*-butyltin hydride (10.4 mmol) and azoisobutyronitrile (AIBN) (1.8 mmol) in anhydrous toluene (30 mL) was added dropwise to a refluxing solution of the cyclic thiocarbonate (2.6 mmol) in toluene (60 mL). The mixture was heated to 110 °C for 4 h, and the solvent was evaporated. The residue was purified on silica gel with chloroform followed by 5% methanol/chloroform.

Procedure D: Preparation of 2′-Deoxy-3′-*O*-(1-imidazolylthiocarbonyl)-5′-*O*-(*tert*-butyldimethylsilyl) Nucleosides. To a solution of the 2′-deoxynucleoside (3 mmol) in dry dimethylformamide (25 mL) was added 1,1′-thiocarbonyldiimidazole (4.5 mmol), and the mixture was

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stirred at 90 °C for 4 h with protection from moisture. The solvent was removed under reduced pressure, and the residue was purified on silica gel with 5% methanol/chloroform.

Procedure E: Deoxygenation of 3′-*O*-(1-Imidazolylthiocarbonyl) Nucleosides. To a refluxing solution of the 3′-*O*-(imidazolylthiocarbonyl) nucleoside (1 mmol) in dry toluene (25 mL) was added a solution of tri-*n*-butyltin hydride (3.5 mmol) and AIBN (0.8 mmol) in toluene (25 mL). The mixture was refluxed for 2 h, the solvent was evaporated, and the residue was purified by preparative TLC with 10% methanol/chloroform as the eluting solvent.

Procedure F: Desilylation. The 5′-silylated 2′,3′-dideoxynucleoside (1.5 mmol) was dissolved in acetonitrile (40 mL). Tetraethylammonium fluoride (4.5 mmol) was added, and the mixture was stirred at room temperature for 2 h. Water (10 mL) was added and stirring continued for 20 min. The solvents were evaporated and the residue was purified by preparative TLC (10% methanol/chloroform) to provide the dideoxynucleoside.

2-Cyano-2′,3′-dideoxyadenosine (7). To a solution of 2-iodoadenosine (**1**) (0.500 g, 1.27 mmol) in DMF (70 mL) were added tetrakis(triphenylphosphine)palladium(0) (0.220 g, 0.19 mmol) and tri-*n*-butyltin cyanide (0.442 g, 1.39 mmol). The mixture was stirred at 120 °C for 20 h under nitrogen. The solvent was then evaporated, and the residue was purified on silica gel to give 2-cyanoadenosine (**2**)[14] in 86% yield. 2-Cyanoadenosine (**2**) was converted to **3** by procedure A (60% yield) and procedure B (89% yield). Deoxygenation of the cyclic thiocarbonate with procedure C gave the 2′-deoxy and the 2′,3′-dideoxy-2′,3′-didehydro compounds **5** and **4** in 57% and 30% yields, respectively. Compound **5**: ^{1}H NMR (Me_2SO-d_6) δ 0.00 (s, 6 H), 0.83 (s, 9 H), 2.67 (m, 2 H), 3.77 (m, 3 H), 4.42 (m, 1 H), 5.37 (m, 1 H), 6.33 (m, 1 H), 7.95 (br s, 2 H), 8.49 (s, 1 H); UV (EtOH) λ_{max} 296, 266, 260 nm. Compound **4**: ^{1}H NMR (Me_2SO-d_6) δ −0.04 (s, 6 H), 0.81 (s, 9 H), 3.81 (m, 2 H), 4.95 (m, 1 H), 6.20 (m, 1 H), 6.50 (m, 1 H), 6.94 (s, 1 H), 7.96 (br s, 2 H), 8.33 (s, 1 H); UV (EtOH) λ_{max} 296, 266, 260 nm.

Desilylation of **4** with procedure F provided 2-cyano-2′,3′-didehydro-2′,3′-dideoxyadenosine (**8**) in 45% yield: mp >250 °C dec ^{1}H NMR (Me_2SO-d_6) δ 3.51 (m, 2 H), 4.90 (m, 2 H), 6.16 (m, 1 H), 6.51 (m, 1 H), 6.94 (m, 1 H), 7.94 (br s, 2 H), 8.39 (s, 1 H); UV (H_2O) λ_{max} 296 (ϵ 6770), 265.5 (ϵ 10710), 260 nm (ϵ 10050); FAB HRMS obsd (M^+ + H) 259.0970, calcd for $C_{11}H_{10}N_6O_2$ 259.0943.

Compound **5** was converted to 2-cyano-2′,3′-dideoxyadenosine (**7**) by in sequence procedure D (63% yield), procedure E (70% yield), and procedure F (70% yield): mp 195–197 °C; ^{13}C NMR (Me_2SO-d_6) δ 25.4, 32.0, 62.6, 82.3, 84.7, 117.0, 120.7, 136.6, 141.5, 147.9, 156.2; ^{1}H NMR (Me_2SO-d_6) δ 2.06 (m, 2 H), 2.39 (m, 2 H), 3.56 (m, 2 H), 4.13 (m, 1 H), 4.92 (m, 1 H), 6.24 (m, 1 H), 7.90 (br s, 2 H), 8.59 (s, 1 H); UV (H_2O) λ_{max} 297 (ϵ 6470), 266 (ϵ 9980), 260 nm (ϵ 9270); FAB HRMS obsd (M^+ + H) 261.1069, calcd for $C_{11}H_{12}N_6O_2$ 261.1099.

2′,3′-Dideoxy-2-ethyladenosine (9). To a solution of 2-iodoadenosine (**1**) (1.585 g, 4.03 mmol) and bis(acetonitrile)palladium chloride (0.053 g, 0.20 mmol) in DMF (20 mL) was added vinyltributyltin (1.24 mL, 4.23 mmol), and the mixture was stirred at 100 °C for 1 h. The reaction mixture was cooled and filtered. The solvent was evaporated, and the residue was purified on silica gel with chloroform and 10% methanol/chloroform to give 1.099 g (92%) of 2-vinyladenosine: ^{1}H NMR (Me_2SO-d_6) δ 3.66 (m, 2 H), 3.98 (m, 1 H), 4.16 (m, 1 H), 4.65 (m, 1 H), 5.18 (d, 1 H), 5.38–5.62 (m, 3 H), 5.89 (d, 1 H), 6.41 (dd, 1 H), 6.59 (dd, 1 H), 7.27 (br s, 2 H), 8.32 (s, 1 H); UV (EtOH) λ_{max} 293, 271, 265 nm.

2-Vinyladenosine was silylated with procedure A (54% yield). To a solution of 5′-silylated 2-vinyladenosine (0.925 g, 2.27 mmol) in absolute ethanol (110 mL) was added 5% palladium/charcoal (0.220 g). This mixture was shaken under 33 psi of hydrogen for 2 h and was filtered through cotton. The solvent was evaporated, and the residue was purified on silica gel (5% methanol/chloroform) to give 0.670 g (72%) of 2-ethyl-5′-*O*-(*tert*-butyldimethylsilyl)adenosine: ^{1}H NMR (Me_2SO-d_6) δ 0.04 (s, 6 H), 0.87 (s, 9 H), 1.23 (t, 3 H, J = 7.3 Hz), 2.67 (q, 2 H, J = 7.3 Hz), 3.80 (m, 3 H), 4.17 (m, 1 H), 4.60 (m, 1 H), 5.40 (br s, 2 H), 5.88 (d, 1 H, J = 5.4 Hz), 7.11 (br s, 2 H), 8.18 (s, 1 H); UV (EtOH) λ_{max} 262 nm.

2-Ethyl-5′-*O*-(*tert*-butyldimethylsilyl)adenosine was dideoxygenated with in sequence procedure B (82% yield), procedure C (53% yield), procedure D (76%), and procedure E (80%) to give 2′,3′-dideoxy-2-ethyl-5′-*O*-(*tert*-butyldimethylsilyl)adenosine. Deprotection of the latter compound by procedure F provided **9** in 79% yield: mp 205–207 °C; ^{13}C NMR (Me_2SO-d_6) δ 13.2, 26.0, 31.6, 31.9, 63.3, 81.5, 84.5, 117.5, 138.8, 149.6, 155.8, 165.4; ^{1}H NMR (Me_2SO-d_6) δ 1.23 (t, 3 H, J = 7.3 Hz), 2.11 (m, 2 H), 2.38 (m, 2 H), 2.66 (q, 2 H, J = 7.3 Hz), 3.51 (m, 2 H), 4.11 (m, 1 H), 5.11 (m, 1 H), 6.19 (t, 1 H, J = 5.41 Hz), 7.08 (br s, 2 H), 8.33 (s, 1 H); UV (H_2O) λ_{max} 262.5 nm (12630); FAB HRMS obsd (M^+ + H) 264.1482, calcd for $C_{12}H_{17}N_5O_2$ 264.1461.

2′,3′-Dideoxy-2-(methylthio)adenosine (10). 2-(Methylthio)-adenosine[22] was converted to **10** by in sequence procedures A (70% yield), B (75% yield), C (59% yield), and D, E, and F (49% overall yield): mp 200–203 °C; ^{13}C NMR (Me_2SO-d_6) δ 13.7, 26.0, 31.5, 63.1, 81.7, 84.2, 116.8, 138.2, 149.7, 155.4, 164.0; ^{1}H NMR (Me_2SO-d_6) δ 2.10 (m, 2 H), 2.48 (m, 5 H), 3.55 (m, 2 H), 4.17 (m, 1 H), 4.88 (t, 1 H, J = 5.4 Hz), 6.18 (m, 1 H), 7.28 (br s, 2 H), 8.21 (s, 1 H); UV (H_2O) λ_{max} 274.5 nm (ϵ 13150); FAB HRMS obsd (M^+ + H) 282.1003, calcd for $C_{11}H_{15}N_5O_2S$ 282.1025.

2′,3′-Dideoxy-2-(trifluoromethyl)adenosine (11). A solution of (trifluoromethyl)zinc bromide (1.308 g, 6.10 mmol) in DMF (25 mL) and HMPA (10 mL) was added to copper bromide (0.438 g, 3.05 mmol), and the resulting mixture was stirred for 30 min.[24] 2-Iodoadenosine (0.800 g, 2.03 mmol) was added, and the solution was warmed at 70 °C for 4 h. The solvents were evaporated and the residue was purified on silica gel with 10% methanol/chloroform to give 2-(trifluoromethyl)adenosine[23] in 63% yield. 2-(Trifluoromethyl)adenosine was converted to **11** by in sequence procedure A (58% yield), procedure B (84% yield), procedure C (51% yield), procedure D (73% yield), procedure E (75% yield), and procedure F (53% yield): mp 173–175 °C; ^{13}C NMR (Me_2SO-d_6) δ 25.7, 31.8, 62.8, 82.1, 84.5, 119.8, 141.1, 148.3, 156.2; ^{1}H NMR (Me_2SO-d_6) δ 2.10 (m, 2 H), 2.40 (m, 2 H), 3.51 (m, 2 H), 4.13 (m, 1 H), 4.88 (m, 1 H), 6.25 (m, 1 H), 7.85 (br s, 2 H), 8.54 (s, 1 H); UV (H_2O) λ_{max} 259.5 nm (ϵ 11300); FAB HRMS obsd (M^+ + H) 304.0996, calcd for $C_{11}H_{12}F_3N_5O_2$ 304.1021.

2′,3′-Dideoxy-2-iodoadenosine (14). 2-Amino-6-chloronebularine (**12**) was converted to 2-amino-6-chloro-2′,3′-dideoxy-5′-*O*-(*tert*-butyldimethylsilyl)nebularine by in sequence procedures A (82%), B (73%), C (62%), D (75%), and E (83%): ^{1}H NMR (Me_2SO-d_6) δ 0.00 (s, 6 H), 0.84 (s, 9 H), 2.07 (m, 2 H), 2.35 (m, 2 H), 3.74 (m, 2 H), 4.13 (m, 1 H), 6.11 (m, 1 H), 6.88 (br s, 2 H) , 8.27 (s, 1 H); UV (EtOH) λ_{max} 310, 247, 222 nm.

Desilylation of 2-amino-6-chloro-2′,3′-dideoxy-5′-*O*-(*tert*-butyldimethylsilyl)nebularine by procedure F gave 2-amino-6-chloro-2′,3′-dideoxynebularine in 69% yield: mp 139–141 °C; ^{1}H NMR (Me_2SO-d_6) δ 2.05 (m, 2 H), 2.38 (m, 2 H), 3.55 (m, 2 H), 4.10 (m, 1 H), 4.91 (m, 1 H), 6.10 (t, 1 H, J = 4.9 Hz), 6.88 (br s, 2 H), 8.36 (s, 1 H); UV (H_2O) λ_{max} 307, 248 nm.

To a nitrogen-purged solution of 2-amino-6-chloro-2′,3′-dideoxy-5′-*O*-(*tert*-butyldimethylsilyl)nebularine (0.232 g, 0.604 mmol) and diiodomethane (0.20 mL, 2.483 mmol) in hexane (50 mL) was added *tert*-butyl nitrite (0.32 mL, 2.690 mmol). The reaction mixture was stirred at 70 °C for 3 h under N_2. The solvents were evaporated, and the residue was purified on silica gel with 5% methanol/chloroform to provide 0.087 g (29%) of 6-chloro-2-iodo-2′,3′-dideoxy-5′-*O*-(*tert*-butyldimethylsilyl)nebularine: ^{1}H NMR (Me_2SO-d_6) δ −0.03 (s, 6 H), 0.81 (s, 9 H), 2.10 (m, 2 H), 2.40 (m, 2 H), 3.75 (m, 2 H), 4.13 (m, 1 H), 6.30 (m, 1 H), 8.74 (s, 1 H); UV (EtOH) λ_{max} 281, 255, 220 nm. This 6-chloro-2-iodo compound (0.087 g, 0.175 mmol) was dissolved in 50 mL of absolute ethanol saturated with ammonia. This solution was allowed to stand at room temperature for 7 h. The solvent was evaporated and the residue purified on silica gel with 5% methanol/chloroform to give 0.043 g (52%) of 2-iodo-2′,3′-dideoxy-5′-*O*-(*tert*-butyldimethylsilyl)-adenosine, which was desilylated with procedure F to give 2-iodo-2′,3′-dideoxyadenosine (**14**) in 83% yield: mp >220 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 2.07 (m, 2 H), 2.36 (m, 2 H), 3.55 (m, 2 H), 4.10 (m, 1 h), 4.89 (m, 1 H), 6.14 (m, 1 H), 7.63 (br s, 2 H), 8.28 (s, 1 H); UV (H_2O) λ_{max} 266.5 nm (ϵ 13250); FAB HRMS obsd (M^+ + H) 362.0088, calcd for $C_{10}H_{12}IN_5O_2$ 362.0114.

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Synthesis of Congeners of Adenosine Resistant to Deamination by Adenosine Deaminase

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Synthesis of Congeners of Adenosine Resistant to Deamination by Adenosine Deaminase

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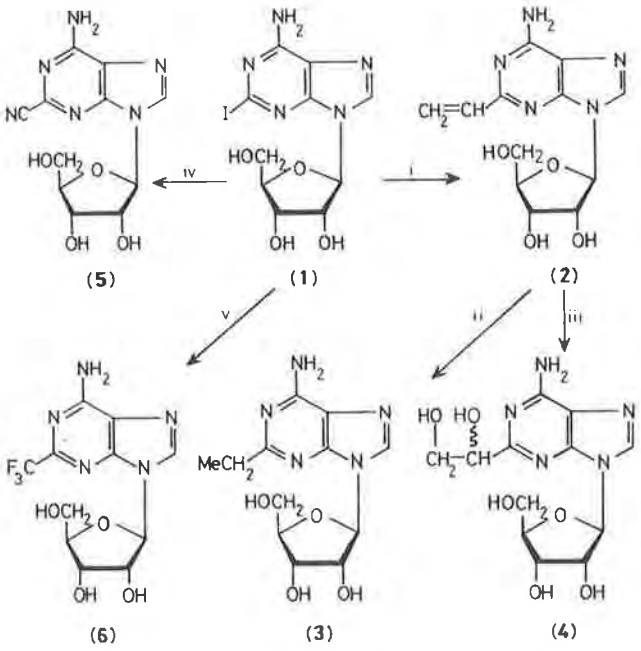
The novel metal-mediated syntheses of adenosine deaminase resistant congeners of adenosine are described.

Development of methodologies for the efficient and regiospecific functionalization of the adenine ring is of fundamental importance in the design and synthesis of therapeutically useful nucleosides of this family. Of particular interest are strategic functionalizations that result in congeners that are totally resistant to hydrolytic deamination by the ubiquitous mammalian enzyme, adenosine deaminase (ADA). This enzyme limits the therapeutic efficacy of adenosine analogues, including those such as 2′,3′-dideoxyadenosine (ddA), which exhibit significant inhibition of the cytopathic effect of the human immunodeficiency virus (HIV-1).[1,2] We report on the novel, metal-mediated syntheses of some analogues of adenosine specifically functionalized at the 2-position and the behaviour of these nucleosides towards mammalian adenosine deaminase.

2-Iodoadenosine (1)[3] served as the precursor for the



Scheme 1. *Reagents and conditions:* i, $PdCl_2(MeCN)_2$, $Bu_3SnCH{=}CH_2$, DMF, heat; ii, Pd/C, H_2, EtOH; iii, OsO_4, pyridine; iv, Pd^0 $(Ph_3P)_4$, Bu_3SnCN, DMF, heat; v, CF_3ZnBr, CuBr, hexamethylphosphoric triamide, DMF, heat.

synthesis of the target compounds. One approach for the regiospecific functionalization at the 2-position using this compound was a palladium-catalysed cross-coupling reaction with organostannanes.[4] Thus, when *unprotected* 2-iodoadenosine (**1**) was treated with $PdCl_2(MeCN)_2$ in the presence of $Bu^n_3SnCH{=}CH_2$ in dimethylformamide (DMF) with heating, the novel compound 2-vinyladenosine (**2**) was isolated in 84% yield. Reduction of (**2**) with H_2–5% Pd/C gave (**3**) (76%). Hydroxylation of (**2**) with osmium tetroxide in pyridine gave the novel diastereoisomeric diols (**4**) in 67% yield. Other regiospecific modifications involving 2-iodoadenosine and the palladium-catalysed cross-coupling approach are also possible. Thus, treatment of (**1**) with $Pd^0(Ph_3P)_4$ and Bu^n_3SnCN in DMF gave, on heating, 2-cyanoadenosine (**5**) in 86% yield.

Other metal-mediated reactions also allow direct functionalization of halogenated purine nucleosides. For example, compound (**1**) is easily converted to the trifluoromethyl compound (**6**) (70%) by treatment with 'CF_3Cu', generated *in situ* from CF_3ZnBr and CuBr.[5] This reaction presumably involves insertion of copper into the C–I bond of the iodopurine moiety followed by transfer of the trifluoromethyl group. These direct organometallic approaches to compounds (**5**) and (**6**) are superior to the previously reported syntheses of these compounds.[6,7]

Substrate activity studies of compounds (**1**)—(**6**) with mammalian adenosine deaminase were followed spectrophotometrically.[8] All these compounds were found to be totally resistant to deamination by this enzyme.[9] It is likely that substitution at the 2-position interferes with the normal substrate binding of this enzyme at N-1 of adenine nucleosides. Studies on the extension of these metal-mediated methodologies utilizing unprotected nucleosides are in progress.

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NUCLEOSIDES & NUCLEOTIDES, 8(5&6), 699-708 (1989)

DEVELOPMENT OF METHODOLOGIES FOR THE STRATEGIC MODIFICATION OF PURINE RIBONUCLEOSIDE SYSTEMS

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Abstract:

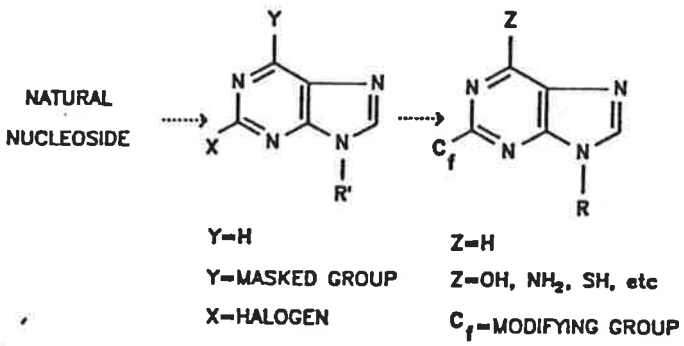
Development and application of new methodologies for the synthesis of novel functionalized analogues of nebularine, inosine, and adenosine of antiviral interest are described.

Synthesis of rare and previously inaccessible purine nucleosides has elicited considerable attention recently because of observations that some compounds belonging to this family have potent antiviral therapeutic activity as well as being of enzymological usefulness as biological probes for the study of key mammalian and viral-encoded enzymes.[1-10] In an ongoing program in our laboratory directed at the synthesis of new antiviral nucleosides, we have developed methods for the specific modification of both the base and the carbohydrate moieties of purine nucleosides.

Our research efforts in the area of base-modified purine nucleosides have focused attention on regiospecific functionalization involving the 2-position of nebularine, inosine, and adenosine.[11-13]. The natural nucleoside, nebularine [9-(β-D-ribofuranosyl)purine], is an antibiotic with strong competitive inhibitory properties for the enzyme, adenosine deaminase.[14] Additionally, it has been studied as an anticancer agent.[15]

Although analogues of nebularine, such as the 2-amino, 2-methyl-, and 2-trifluoromethyl- systems have shown some very interesting biological activities,[16-21] relatively few congeners of this interesting natural nucleoside involving functionalization at the 2-position are known.[11,22,23] While the synthesis of a number of C-2 substituted hypoxanthine nucleosides are known,[24-26] very few functionalized alkylated derivatives have been reported.[27] Functionalized C-2 alkylated inosine analogues are not only of potential interest as antiviral agents, but there is enzymological interest in these novel compounds as potential inhibitors of a key purine metabolizing enzyme, inosine monophosphate dehydrogenase.[28] However, limitations in synthetic methodology restrict accessibility to a variety of novel C-2 functionalized hypoxanthine nucleosides. The ring closure reaction of appropriately constructed imidazole nucleosides has provided the major approach to the synthesis of most 2-substituted inosines.[24,25] Other methods known for entry into this general class of compounds appear to be of more limited scope.[29] The aforementioned comments pertaining to limitations in synthetic methodology for C-2 functionalization, are also applicable to analogues of adenosine.[24-6,30] Such analogues and their deoxygenated congeners are of interest as potential anticancer and antiviral agents and they are also of enzymological interest with respect to purine metabolizing enzymes such as adenosine deaminase and nucleoside kinases.[2,9,31-39]

The general synthetic approach to the aforementioned 2-substituted nucleosides is shown in Scheme 1. The starting compound was a natural or readily available synthetic nucleoside which was converted in a series of steps to a key intermediate where both the carbohydrate and base moieties were protected (if needed). The 2-position was appropriately substituted with a halogen (commonly iodine). This key intermediate was converted through a key transformation to an immediate product which was either deprotected or elaborated further to the final target molecule. The major synthetic emphasis of this paper will be on the key step of the synthesis.

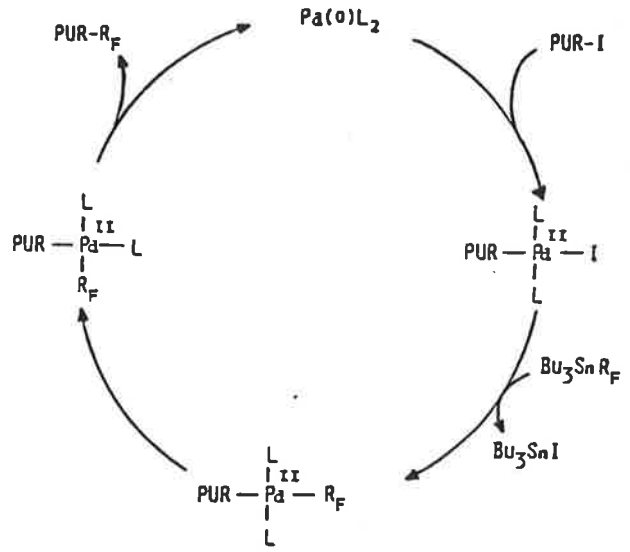


Scheme 1

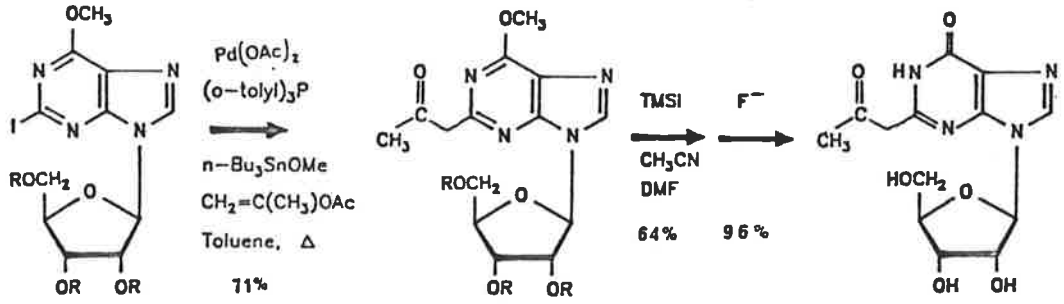
In previous work from our laboratory involving structural modification at the 6-position of the purine ring, reductive deaminations, deamination-halogenations, thermal and photochemical alkylthiolations, and photoinduced $S_{RN}1$ reactions were developed for the synthesis of specific target nucleosides. Unfortunately, the photochemical $S_{RN}1$ reaction,[40,41] one of the key functionalizing steps used in these syntheses, failed when applied to the 2-position. Various other approaches including the Eschenmoser sulfide contraction and Meerwein-type reactions were all unsuccessful.

We have now developed a general methodology for the regiospecific functionalization of the 2-position of purine nucleosides. This methodology involves a palladium-catalyzed cross-coupling reaction between an organostannane containing the desired synthon to be introduced and a 2-iodinated purine system.[12] A plausible mechanistic explanation of this reaction is summarized in Scheme 2.

The methodology can be illustrated with the synthesis of 2-acetonylinosine from protected 2-iodo-6-methoxypurine ribonucleoside as summarized in Scheme 3.[13] The final target nucleoside was purified by reversed-phase HPLC on Amberlite XAD-4 resin with ethanol/water as the eluting solvent and characterized by FAB HRMS, high-field multinuclear NMR data, and by UV and FTIR spectroscopy. It exhibits high <u>in vitro</u> antiviral



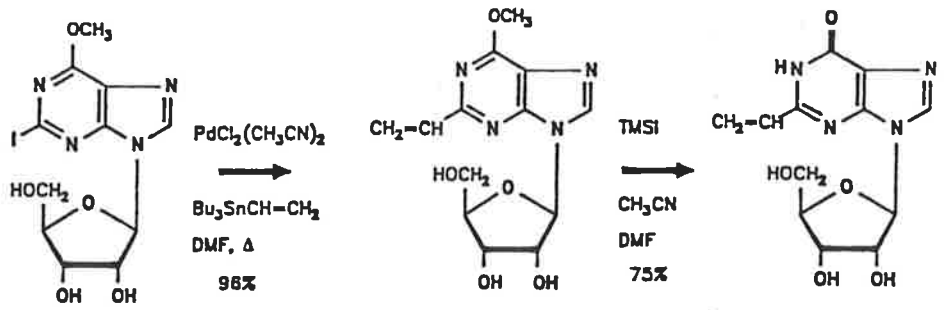
Scheme 2



Scheme 3

activity (TI = MTC/ID_{50} = >1000) against the Sandfly Fever Virus (an RNA virus of the Phlebovirus family).

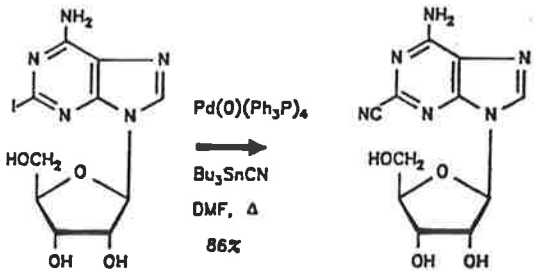
This palladium-catalyzed cross-coupling reaction with enol acetates appears to have considerable generality. In addition, these reactions can be extended to include other functionalized organostannanes. For example, 2-vinylinosine, a novel hypoxanthine nucleoside analogue which is



Scheme 4

potentially a precursor to a variety of new inosine compounds (*via* ozonolysis, hydroboration, osmylation, etc.), is also readily available using this methodology (Scheme 4). Allylation reactions are also possible. Interestingly, 2-vinylinosine exhibits low but broad-spectrum *in vitro* activity against a number of RNA viruses of the Flavi, Bunya, Pox, and Arena families.

The aforementioned and other reactions involving organostannanes can be extended to the nebularine series. The starting nucleoside for these syntheses was isoadenosine, synthesized by a photochemical reductive dehalogenation previously described by us.[11] Application to adenine nucleosides is also possible with 2-iodoadenosine as the tailored precursor.[42] A series of C-2 functionalized adenosine analogues have been synthesized using this and related organozinc coupling reactions. An example of the key transformation is illustrated in Scheme 5 for the direct synthesis of 2-cyanoadenosine (cf. reference 43). Interestingly, the series of C-2 functionalized adenosines synthesized are either very poor substrates (<< 1% compared to adenosine) or in most cases, totally resistant, to hydrolytic deamination by the ubiquitous enzyme, adenosine deaminase.

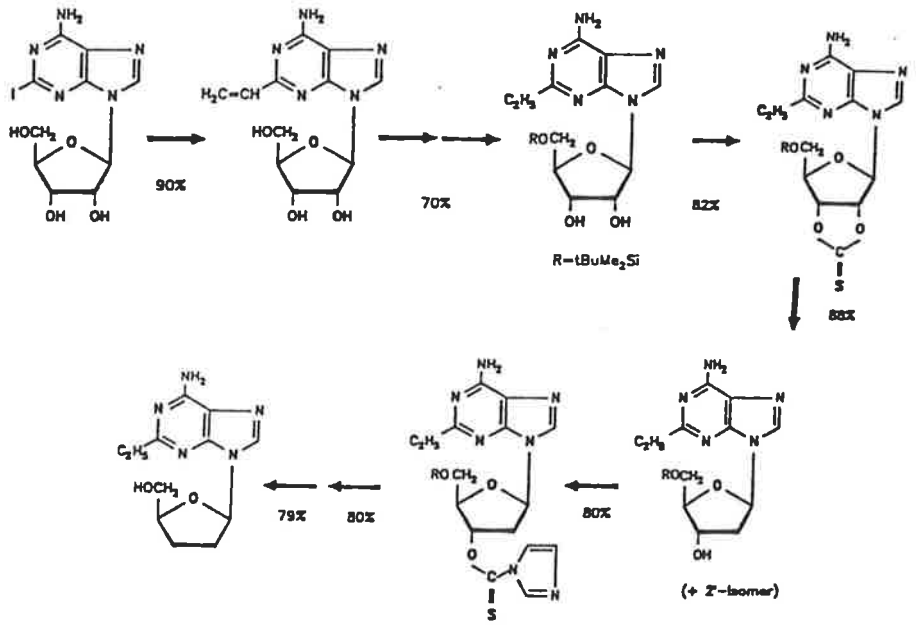


Scheme 5

In our work directed at novel analogues of the anti-AIDS compound, 2',3'-dideoxyadenosine[2,8], we have synthesized several congeners of this dideoxygenated that have greater stability than the parent compound with respect to deamination by adenosine deaminase and hydrolytic cleavage of the glycosidic bond. One of our approaches is shown in Scheme 6 with the synthesis of 2-ethyl-2',3'-dideoxyadenosine from 2-iodoadenosine.

Glycosidic bond stabilities were determined by differential UV spectroscopy.[44] Results for some representative cases are shown in Table 1. The analogues were resistant to deamination by adenosine deaminase.

Finally, it should be mentioned that the glycosidic bond conformation of purine nucleosides may play a critical role in determining their ability to be substrates for key purine metabolizing enzymes and also subsequently in their antiviral and related biological activities. We have previously reported that the preferred conformation of purine ribonucleosides in solution may be qualitatively determined through correlation of their high-field ^{13}C NMR data.[45] A key factor in this determination is the magnitude of the chemical shift difference between C-2' and C-3'. This correlation can be extended to dideoxypurine nucleosides as illustrated in Table 2. It appears that for the dideoxynucleosides in the preferred <u>syn</u> conformation the magnitude of the chemical shift difference between C-2'



Scheme 6

Table 1. Relative Rates of Hydrolysis of Dideoxyadenosines (25° C, pH 3)

2',3'-Dideoxyadenosine	100
2',3'-Dideoxynebularine	177
8-Methoxy-2',3'-dideoxyadenosine	61
2-Ethyl-2',3'-dideoxyadenosine	75

Table 2. Preferred Conformation of Purine Dideoxynucleosides Discerned from High-Field ^{13}C NMR Data

90.6 MHz Carbon Data (ppm)	ddAdo	ddNeb	2-Et ddAdo	8-OCH_3 ddAdo
C-2'	31.72	31.74	31.87	28.88
C-3'	25.62	25.36	26.00	26.96
(C2' - C3')	6.10	6.38	5.87	1.92

and C-3' is < 2 ppm whereas in the preferred anti conformation, this difference is > 5 ppm.

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ORGANIC PREPARATIONS AND PROCEDURES INT. 22(1), 57-61 (1990)

REGIOSPECIFIC 5'-SILYLATION OF NUCLEOSIDES

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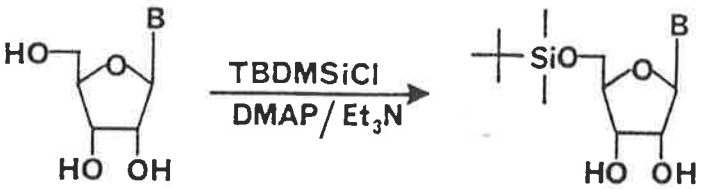
There has been considerable interest in recent years in the synthesis of carbohydrate-modified nucleoside analogues because of the potential of these compounds to exhibit antiviral activities. Some examples of such antiviral nucleosides include 3'-azidothymidine (AZT),[1] 2',3'-dideoxynucleosides,[2,3] 2',3'-didehydro-2',3'-dideoxynucleosides,[4] and 2'- or 3'-substituted 2',3'-dideoxynucleosides.[5-9] Synthetic access to these compounds and other novel carbohydrate modified nucleosides require the regiospecific protection of the 5'-hydroxyl group prior to chemical modification at the 2'- and/or 3'-position. Stable silyl protecting groups are becoming increasingly important in nucleoside synthesis. This paper reports on the utilization of N,N-dimethylaminopyridine (DMAP) as a catalyst for the regiospecific silylation of the 5'-hydroxyl group of natural and synthetic purine and pyrimidine nucleosides. The 5'-silylated compounds are potentially useful intermediates for the synthesis of sugar-modified nucleosides. The procedure described complements previously



1a-g **2a-g**

a) B = adenine b) B = guanine c) B = thymine d) B = cytosine
e) B = purine f) B = 2-aminopurine g) B = 8-bromoadenine

reported methods.[10,11] However, in terms of generality of application to both natural and non-natural nucleosides, we believe that its simplicity makes it superior to these methods.

The stable silyl protecting system chosen for this study was the t-butyldimethylsilyl group. A number of natural and synthetic nucleosides (**1a–g**) were chosen as representative examples for this study. For example, when the synthetic nucleoside, 8-bromoadenosine (**1g**), was treated with t-butyldimethylsilyl chloride (TBDMSICl), triethylamine, and DMAP in N,N-dimethylformamide (DMF)-dichloromethane at room temperature for 4 h, 8-bromo-5'-t-butyldimethylsilyl adenosine was produced in 76 % yield (Table 1). Small amounts of unreacted 8-bromoadenosine and disilylated nucleosides were also isolated. Under the same conditions with the literature procedure,[11] we obtained only a 43% yield of product. Similarly, much higher yields were also obtained with our procedure in the case of 2-aminonebularine (**2f**). In order to establish the generality of this experimental approach, five natural nucleosides (**1a–e**) were also converted with complete reproducibility to their corresponding 5'-silyl ethers **2a–e** in excellent yields (Table 1).

All of the silylations were done using catalytic amounts of DMAP which was constantly regenerated in the reaction mixture by triethylamine. The use of DMAP permits greater selectivity for silylation of the primary alcohol (5'-CH_2OH)) versus the secondary alcohols (2'-OH and 3'-OH) of the carbohydrate moiety.[12] Also, the best results were obtained when slightly more than one equivalent of TBDMSiCl was used in the silylation reaction. Increasing the amount of silylating agent in excess of this amount resulted in an increase in the formation of multiply silylated nucleoside. It should be noted that the base moieties of the nucleosides do not need to be protected in these conversions. The 5'-silylated nucleosides were

identified by UV, mass spectral and ^{1}H nmr data (Table 2) and by comparison of the physical data available in the literature for the known compounds.

TABLE 1. Yields, mps and UV data for Compounds 2a-g.

Starting Compound	Reaction Time (hr)	Product	Yield (%)	mp. (solvent)[b] (lit. mp. [°C])	UV (EtOH) λ_{max} (ε)
Adenosine	6	2a	85	179-181° (178-180°)[10]	259.5 (10400)
Guanosine	6	2b	70	dec. 200° (dec. 205°)[13]	254.5 (5915) 275.0 sh(3775)
Thymidine	9	2c	70	197-198°[c] (198-199°)[14]	208.5 (9090) 267.0 (9115)
Cytidine	9	2d	80	182-185°[c] (160-163°)[13]	235.0 (5150) 274.0 (6300)
Nebularine	12	2e	67 [83][a]	113-115°	262.0 (6190)
2-Amino-nebularine	5	2f	70	164-167°	309.0 (6900) 246.0 (5980)
8-Bromo-adenosine	4	2g	76	189-192°	264.0 (14840)

a) Conversion after recovery of starting material b) Water unless otherwise noted c) EtOH-H_2O

EXPERIMENTAL SECTION

Mps were determined on a Thomas-Hoover melting point apparatus fitted with a microscope and are uncorrected. Nmr spectra were recorded on Bruker WM 360 and MSL 300 instruments. Ultraviolet data were taken on a Gilford Response spectrophotometer. Mass spectra at 30 eV were obtained on a Hewlett Packard 5985 GC/MS system. All new compounds gave satisfactory elemental analyses.

General Procedure for Regiospecific Silylation.- A mixture of nucleoside (2 mmol), t-butyldimethylsilyl chloride (2.2 mmol), triethylamine (2 mmol), N,N-dimethylaminopyridine (0.3 mmol), N,N-dimethylformamide (10 ml), and dichloromethane (5 ml) was stirred at room temperature under nitrogen

for 4-12 h. The solvents were evaporated and the residue was taken up in 5% methanol/chloroform and purified by flash chromatography on silica gel (230-400 mesh) with 5-10 % methanol/chloroform as the eluting solvent. The products were crystallized from ethanol/water or water.

TABLE 2. Mass Spectra and ^{1}H nmr data for Compounds 2a-g.

Product	MS m/e (rel. int. %)	^{1}H-nmr (DMSO-d_6) δ [ppm]
2a	366 (1.2); 324 (53.5); 178 (100); 164 (30.7); 136 (56.2); 135 (11.1)	0.04 (s, 6H); 0.87 (s, 9H); 3.82 (m, 3H); 4.17 (m, 1H); 4.55 (m, 1H); 5.21 (d, 1H); 5.53 (d, 1H); 5.92 (d, 1H); 7.26 (br.s, 2H); 8.15 (s, 1H); 8.28 (s, 1H)
2b	397 (3.4); 340 (29.9); 308 (19.5); 194 (80.8); 152 (49.4); 151 (79.3)	0.04 (s, 6H); 0.87 (s, 9H); 3.76 (m, 2H); 3.87 (m, 1H); 4.08 (m, 1H); 4.32 (m, 1H); 5.17 (d, 1H); 5.47 (d, 1H); 5.69 (d, 1H); 6.71 (br.s, 2H); 7.82 (s, 1H); 10.8 (s, 1H)
2c	299 (24.9); 281 (100); 263 (29.9); 213 (18.6); 183 (53.4); 165 (30.9); 127 (44.3)	0.07 (s, 6H); 0.88 (s, 9H); 1.77 (s, 3H); 2.06 (m, 2H); 3.77 (m, 3H); 4.20 (m, 1H); 5.27 (m, 1H); 6.17 (t, 1H); 7.46 (s, 1H); 11.2 (s, 1H)
2d	300 (7.0); 282 (10.8); 186 (22.3); 168 (34.8); 140 (27.3); 112 (100)	0.08 (s, 6H); 0.89 (s, 9H); 3.89 (br.m, 5H); 5.00 (br.s, 1H); 5.35 (br.s, 1H); 5.76 (m, 2H); 7.24 (br.s, 2H); 7.81 (d, 1H)
2e	351 (0.6); 309 (26.2); 163 (100); 149 (21.1); 121 (63.4); 120 (2.9)	0.03 (s, 6H); 0.85 (s, 9H); 3.84 (m, 2H); 3.99 (m, 1H); 4.21 (m, 1H); 4.66 (m, 1H); 5.24 (d, 1H); 5.59 (d, 1H); 6.06 (d, 1H), 8.76 (s, 1H); 8.96 (s, 1H); 9.19 (s, 1H)
2f	381 (0.5); 366 (1.5); 324 (71.5); 178 (100); 136 (34.0); 135 (17.2)	0.04 (s, 6H); 0.87 (s, 9H); 3.79 (m, 2H); 3.92 (m, 1H); 4.12 (m, 1H); 4.47 (m, 1H); 5.22 (d, 1H); 5.53 (d, 1H); 5.85 (d, 1H); 6.53 (br.s, 2H); 8.21 (s, 1H); 8.59 (s, 1H)
2g	404 (34.2); 402 (34.6); 258 (72.2); 256 (75.3); 216 (59.8); 214 (69.3)	-0.06 (s, 6H); 0.79 (s, 9H); 3.85 (m, 3H); 4.34 (m, 1H); 5.16-5.48 (m, 3H); 5.81 (d, 1H); 7.41 (br.s, 2H); 8.11 (s, 1H)

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Hydrolysis of Dideoxygenated Purine Nucleosides: Effect of Modification of the Base Moiety[1]

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Dideoxynucleosides are receiving a considerable amount of interest currently because of their ability to inhibit the cytopathic effect of the human immunodeficiency virus (HIV-1), the etiologic agent of AIDS.[2-5] Dideoxyadenosine (ddA) and dideoxyinosine (ddI), members of this class of nucleosides, have potent activity against the AIDS virus and are currently undergoing extensive biological and clinical studies.[5-9] However, both ddA and ddI are unstable with respect to hydrolytic cleavage of the glycosidic bond.[10] This inherent factor limits considerably the usefulness of these compounds as biological probes and antiviral agents. The design of congeners that would be more stable hydrolytically than the parent compounds would be of considerable significance in this area. However, the rational design of such new analogues requires some information on the effect of structural modification on hydrolytic stabilities. Although the hydrolytic stabilities of ribonucleosides have received considerable attention,[11-15]

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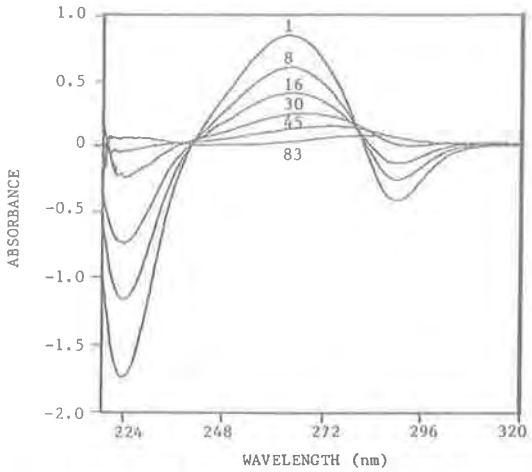


Figure 1. Differential UV spectra for the glycosidic bond hydrolysis of a representative compound, **12**, in 1×10^{-3} M HCl at 22 °C. Curves are labeled with respect to minutes of reaction.

the same cannot be said for dideoxynucleosides. We wish to report on the correlation of base structure and hydrolytic stability of some novel congeners of ddA.

Results and Discussion

Studies of the glycosidic bond hydrolysis of the dideoxynucleosides were carried out at pH 3 and 22 °C, where easily observable rates could be obtained. The reactions were followed by differential UV spectroscopy (Figure 1). Samples were monitored at appropriate time intervals by adjusting the pH to 13, thus separating the absorption of the unchanged nucleoside from that of the bathochromically shifted hydrolyzed base anion. For example, at pH 13, adenosine absorbs at 260 nm and adenine (as its anion) at 268 nm. The reference bases were synthesized in each case where they were unknown or not commercially available. The apparent first-order rate constants were calculated from the slopes of the log plots of eq 1 as described by Garrett and Mehta.[16] In this

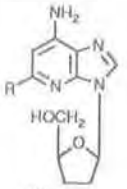
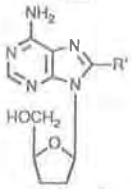
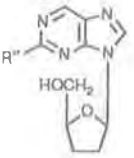
$$\log (A - A_\infty) = \log (A_0 - A_\infty) - \frac{kt}{2.303} \qquad (1)$$

equation A = absorbance at time t, A_0 = initial absorbance, A_∞ = final absorbance, t = time, and k = apparent first-order rate constant. The relative rates of hydrolysis compared to ddA are summarized in Table I.

The acid-catalyzed glycosidic bond hydrolysis of purine nucleosides is believed to proceed by an A1 mechanism, the rate being dependent on the concentration of the protonated nucleoside.[12,14] Removal of the 2′-hydroxyl group and the 2′,3′-hydroxyl groups of adenosine dramatically enhances the rate of hydrolysis of the modified nucleosides,[10,16] presumably because of an increased tendency for the formation of an incipient carbonium ion at C-1′ because of the absence of the $-I$ effect of the hydroxyl group(s). Much less is known about the effect of modification of the base moiety.

Our data show that substitution at the 2-position, in general, results in decreased rates of hydrolysis. Thus, 2-amino, 2-cyano, 2-iodo, 2-(methylthio), 2-ethyl, and 2-(trifluoromethyl) analogues (compounds **2**–**7**) are all more

Table I. pK_{a_1} Data and Relative Rates of Hydrolysis of 2′,3′-Dideoxyadenosine Analogues at pH 3 and 22 °C

	compound	pK_a[a]	rel rate at pH 3[b]	λ (nm)[c]
	**1**, R = H	3.7	100	254.5
	2, R = NH_2	4.3	20	256.5
	3, R = CN	0.6	47	259
	4, R = I	1.4	55	259
	5, R = SCH_3	3.3	64	264
	6, R = CH_2CH_3	4.2	75	259
	7, R = CF_3	0.7	79	255
	**8**, R′ = OH	3.2	0[d]	
	9, R′ = OCH_2Ph	3.8	39	254.5
	10, R′ = SCH_3	3.6	40	269
	11, R′ = OCH_3	3.9	61	253
	12, R′ = NH_2	*e*	2050	263
	**13**, R″ = H	2.1	177	244.5
	14, R″ = NH_2	3.2	110	244.5

[a] Determined by UV spectrophotometric methods. [b] Rates of hydrolysis are relative to dideoxyadenosine (rate = 100). The apparent first-order rate constant for the hydrolysis of ddA at pH 3 is 8.23×10^{-4} min^{-1}. [c] Rate of change in absorbance monitored at this wavelength by differential UV spectroscopy. The monitoring wavelength represents the wavelength of maximum difference at pH 13 between the intact dideoxynucleoside and its cleaved heterocyclic base (see discussion above). [d] No detectable hydrolysis even at pH 1. [e] The pK_{a_1} of **12** could not be reliably determined because of its rapid breakdown under acidic conditions.

stable than ddA. While the transposition of the amino group from the 6-position to the 2-position (compounds **1** and **14**) resulted in little change in the hydrolysis rate, replacement of the amino group with hydrogen (compound **13**) resulted in almost a 2-fold increase in the rate of hydrolysis. Although the pK_a data for protonation of N-1 varies considerably in the aforementioned compounds (see Table I), there appears to be no recognizable correlation between these pK_a values and the rates of hydrolysis.

The most dramatic effects of modification on hydrolysis rates occurred with the 8-position. While *O*-aralkyl, *S*-alkyl, and *O*-alkyl groups at this position increased the stabilities of these molecules (compounds **9**, **10**, and **11**), introduction of an NH_2 group at this position (compound **12**) decreased stability by a factor of 20. In stark contrast to this, when a hydroxyl group was introduced at the 8-position, the resulting compound (**8**, pK_a 3.2) was totally resistant to hydrolysis even at pH 1. As the rate of acid-catalyzed glycosidic bond hydrolysis is dependent to some extent on the concentration of the protonated nucleoside, the increased instability of the 8-amino compound **12** may be attributed to the marked resonance stabilization of the intermediate amidinium cation, which shifts the equilibrium of the initial protonation step toward this intermediate. Thus, it is possible that pK_{a_1} of this particular compound involves N-7 rather than N-1. The results with **8** are much more difficult to explain. Structurally this compound is different from the other dideoxynucleosides examined in that it exists almost entirely in the lactam form as evidenced from FTIR and high-field NMR data. A plausible explanation for the remarkable stability of **8** may be that facile hydrolysis requires the protonation of N-7, which is difficult in the inherent lactam form of **8** because of the weakly basic nature of this nitrogen (pK_a ~0, N-7). In contrast, and as supporting evidence for this

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explanation, protonation occurs readily for N-7 of **12**. Further substantiating evidence for this comes from the observation that 7-deaza-2′,3′-dideoxyguanosine is much more stable than 2′,3′-dideoxyguanosine,[17] because of the absence of N-7 in the deaza compound.

In summary, while modifications at the 2- and 6-positions result in small but nevertheless significant effects on the rates of glycosidic bond cleavage of 2′,3′-dideoxyadenosine analogues, the most dramatic effect is seen with appropriate substitution at the 8-position. These findings may be of significance in the design of stable biologically active dideoxynucleosides. They also contribute to further understanding of the mechanism of glycosidic bond hydrolysis of nucleosides.

Experimental Section

Synthesis. The compounds described in this project were synthesized by the dideoxygenation of the corresponding ribonucleosides using published procedures.[18,19] Functionalization of the ribonucleosides was carried out by thermal, photochemical, and metal-catalyzed methodologies previously described by us.[19-21]

Procedure for Kinetic Studies. Differential UV spectroscopy was used to observe the acid-catalyzed hydrolysis of the dideoxynucleosides.[16] Briefly, the dideoxynucleoside was dissolved in nitrogen-purged aqueous hydrochloric acid (pH 3) to give a 2.5×10^{-4} solution of the substrate. The solution was maintained at 22 °C and aliquots were removed periodically and adjusted to pH 13 with 0.25 M sodium hydroxide solution and monitored by UV spectroscopy. The blank was the appropriate base solution in each case of the same molarity as the initial dideoxynucleoside solution. The bases were prepared by the complete hydrolysis of the dideoxynucleosides. The differential UV spectra for the rate studies were recorded at periodic intervals between 200 and 320 nm on a Gilford Response spectrophotometer. The apparent first-order rate constants were determined from the slopes of the plots of absorbance versus time. These plots were generated by using TELEGRAF on a Prime 9950 computer.

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COPPER MEDIATED REACTIONS IN NUCLEOSIDE SYNTHESIS

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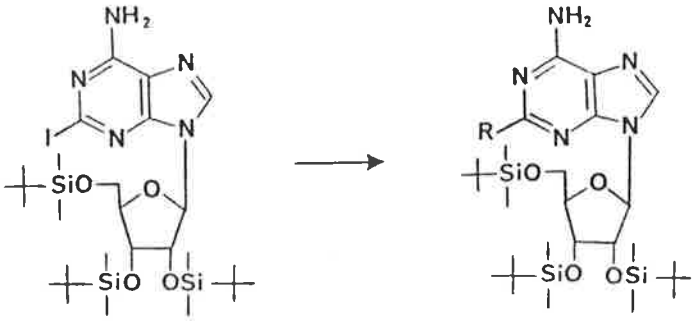
Summary: The regiospecific functionalization of the base moiety of purine nucleosides through copper-mediated nucleophilic reactions is described.

Although copper mediated reactions have played a significant role in aromatic nucleophilic displacements,[1] such transformations are nearly non-existent in synthesis involving nucleosides. Cuprous iodide has been used to prepare alkynyl copper reagents[2] and these have been used in palladium-catalyzed cross-coupling reactions to furnish alkyn-1-yl pyrimidine and purine nucleosides.[3,4] However, due to the limited availability of useful Cu(I) organocopper reagents, transformations involving these have been of limited scope.[5] The reaction of a halogenated nucleoside with Cu(I)X and an appropriate nucleophile potentially allows for the introduction of a wide range of interesting functional groups or synthons into specific positions of nucleosides. This communication reports on the development of copper-mediated reactions leading to interesting functionalized analogues of adenosine. The work complements our previous reports on palladium-catalyzed cross-coupling with organostannanes.[6,7]

The requisite precursor, the silylated 2-iodoadenosine, 1, was prepared as described previously by us.[8] Treatment of 1 with sodium cyanide and cuprous bromide in DMF at 120° C for 1 h gave the 2-cyano compound 2 in 64% yield after work-up and purification (Table 1, Entry 1). Similar results were obtained with cuprous cyanide (Entry 2). In order to circumvent complexation of the nucleoside with copper ions,[9] the work-up included neutralization of the basic reaction mixture and subsequent treatment with gaseous hydrogen sulfide. Line broadening of the H-8 resonance in the high-field ^{1}H NMR spectrum was evidence of copper complexation.[10] Although 2-halogenated adenine nucleosides are

susceptible to nucleophilic displacements, the reactions proceed in low yields and for a very limited number of nucleophiles.[11,12] Thus, for example, the conversion of 1 to 2 did not proceed with sodium cyanide in the absence of CuBr.



1

2 R = CN
3 R = SCN
4 R = NH_2
5 R = N_3
6 R = NHOH,
7 R = $C{\equiv}CC_4H_9$
8 R = $CH(CO_2Et)_2$

When the reaction was carried out with copper thiocyanide, clean conversion to 2-thiocyanoadenosine 3 occurred (Entry 3). In contrast, when compound 1 was treated with NaSCN and CuBr, much longer reaction times were required, and the reaction proceeded less cleanly and a mixture of 2-thiocyanide 3 and the corresponding 2-bromide was produced (Entry 4). The latter compound, the product of a copper-mediated halogen exchange reaction,[13] is of interest, because, in the absence of nucleophiles, 1 can be converted easily to the corresponding chloro and bromo compounds with appropriate cuprous salts. Copper mediated reactions involving other nucleophiles may also be affected. For example, exposure of 1 to ammonia in the presence of CuBr gave a 75% yield of 4 which could be readily deprotected to 2,6-diaminoadenosine (Entry 5). This new synthesis of 2,6-diaminoadenosine avoids the use of the high temperatures and pressures of previous preparations.[14]

2-Azidoadenosine, a biologically active nucleoside,[12,15,16] can be easily prepared in its protected form (5) through the reaction of 1 with CuBr and NaN_3 at room temperature (Entry 6). A side product of this transformation was the 2-N-hydroxyaminoadenosine derivative 6 which apparently results from the copper catalyzed decomposition of the 2-azido compound (Entry 6). The yield of this side product can be maximized to about 72% by

raising the reaction temperature (Entry 7). Other N-hydroxyamino nucleosides have been evaluated for anticancer activity and for use as biological probes,[17] and have been found to be mutagenic through covalent modification of guanine residues in DNA.[18] Monosubstituted alkynes may also be introduced at the 2-position of adenosine (Entry 8). The copper salt of diethylmalonate reacts cleanly with 1 to furnish 8, a potential precursor to a number of other 2-substituted adenosines.

Table 1. Copper Catalyzed Functionalization of Purine Nucleosides

Entry	Reagents	Conditions[a]	Product[b]	Functionality (R)	% Yield
1	NaCN, CuBr	120 °C, 1 h	2	CN	64
2	CuCN	120 °C, 1 h	2	CN	68
3	CuSCN	120 °C, 8 h	3	SCN	64
4	NaSCN, CuBr	120 °C, 18 h	3	SCN	55[c]
5	NH_3, CuBr	R.T., 24 h	4	NH_2	75
6	NaN_3, CuBr	R.T., 24 h	5 6	N_3 NHOH	66 27
7	NaN_3, CuBr	120 °C, 1 h	6	NHOH	72
8	$C_4H_9C{\equiv}CH$, NaH, CuI	120 °C, 1 h	7	$C{\equiv}CC_4H_9$	61 [72][d]
9	$CH_2(CO_2Et)_2$, NaH, CuI	120 °C, 1 h	8	$CH(CO_2Et)_2$	71 [85][d]

a. DMF was the solvent of choice.

b. These products were purified by preparative TLC on silica gel. They were converted to the deprotected nucleosides by reaction with tetraethylammonium fluoride. The deprotected functionalized nucleosides were purified by reversed-phase HPLC on Amberlite XAD-4 resin with ethanol/water as the eluting solvent. The yields of purified deprotected compounds were in the range of 65-70%. The silylated and deprotected products were characterized by high-field ^1H and ^{13}C NMR, UV, FTIR, and mass spectral (including FAB HRMS) data.

c. 2-Bromo-9-(2,3,5-tri-O-t-butyldimethylsilyl-β-D-ribofuranosyl)adenine was produced in 24% yield.

d. % Conversion.

In summary, cuprous ion mediated reactions provide a facile approach to the regiospecific functionalization of the base moiety of purine nucleosides. This methodology, although known in aromatic chemistry, has seen little utilization previously

in nucleoside systems. In addition to the examples presented, a wide variety of other nucleophiles may potentially be used in these reactions.

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SYNTHETIC APPROACHES TO NEW DOUBLY MODIFIED NUCLEOSIDES: CONGENERS OF CORDYCEPIN AND RELATED 2′-DEOXYADENOSINE

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Abstract: The syntheses of novel analogues of cordycepin and 2′-deoxyadenosine are reported. In order to obtain entry into both the 3′-deoxy and 2′-deoxy isomeric series from a common starting compound, 2-amino-6-chloropurine ribonucleoside, this precursor was protected by conversion to a mixture of 2′,5′- and 3′,5′- bis-silyl compounds prior to modifications at the 2′- or 3′- positions of the carbohydrate moiety and the 2-position of the base component. Observation of silyl group isomerization is discussed. The other key transformations in the syntheses were radical deoxygenations (carbohydrate moiety), radical iodinations (tailoring of base for modification), and metal- mediated functionalization reactions (regiospecific modifications of base component). Structures and purities of the final target molecules were established by UV, high-field ^{1}H and ^{13}C NMR, and FAB HRMS data. The synthetic approaches presented have generality and provide entry into a variety of doubly modified nucleosides.

Cordycepin (3′-deoxyadenosine) was originally isolated from Cordyceps militaris and Aspergillus nidulans.[1] Cordycepin is known to have antiviral activity against a number of RNA viruses.[2] The biochemical basis for this mechanism of action is thought to be the inhibition of viral RNA polymerase activity by cordycepin 5′-triphosphate.[3,4] The polynucleotide chain appears to be terminated at the point at which the cordycepin component is attached because of the absence of the 3′-hydroxyl group for further chain elongation. Biological activity has also been attributed to several 2′-

deoxyadenosines, particularly the 2-halo compounds.[5] Analogues of cordycepin and the related 2′-deoxyadenosine would therefore be of considerable potential antiviral and antitumor interest. However, very few examples of such compounds have been described.[6-8] This is in part due to limitations in methodologies that provide facile access to strategically modified analogues of these nucleosides. We report in this paper the development of approaches to the synthesis of novel 3′-deoxy and 2′-deoxy isomeric series of doubly modified nucleosides related to cordycepin.

The strategy for the synthesis of the novel deoxynucleosides was to start with readily available natural guanosine and to modify first the carbohydrate and then the base moiety in this compound. Modification of the carbohydrate moiety would involve regiospecific deoxygenation. Earlier methods of synthesis of deoxy nucleosides involved reaction of arabino halo sugar moieties with tin hydride.[9-11] Nucleoside 2′,3′-epoxides may be ring opened by hydride to form deoxy nucleosides.[12] Deoxygenation may also be carried out <u>via</u> a cyclic thiocarbonate by reaction with tributyltin hydride and AIBN according to the methodology originally developed by Barton and Subramanian.[13] However, reductive cleavage of the cyclic thiocarbonate gives mixtures of 2′- and 3′-deoxy nucleosides with the 2′-deoxy compound being the major product.[14] A more regiospecific deoxygenation, however, would be possible through the 3′-imidazolide.[14,15] If the imidazolides could be specifically prepared, entry to both the 3′-deoxy and the 2′-deoxy series could be achieved through related pathways but with the same initial precursor molecules.

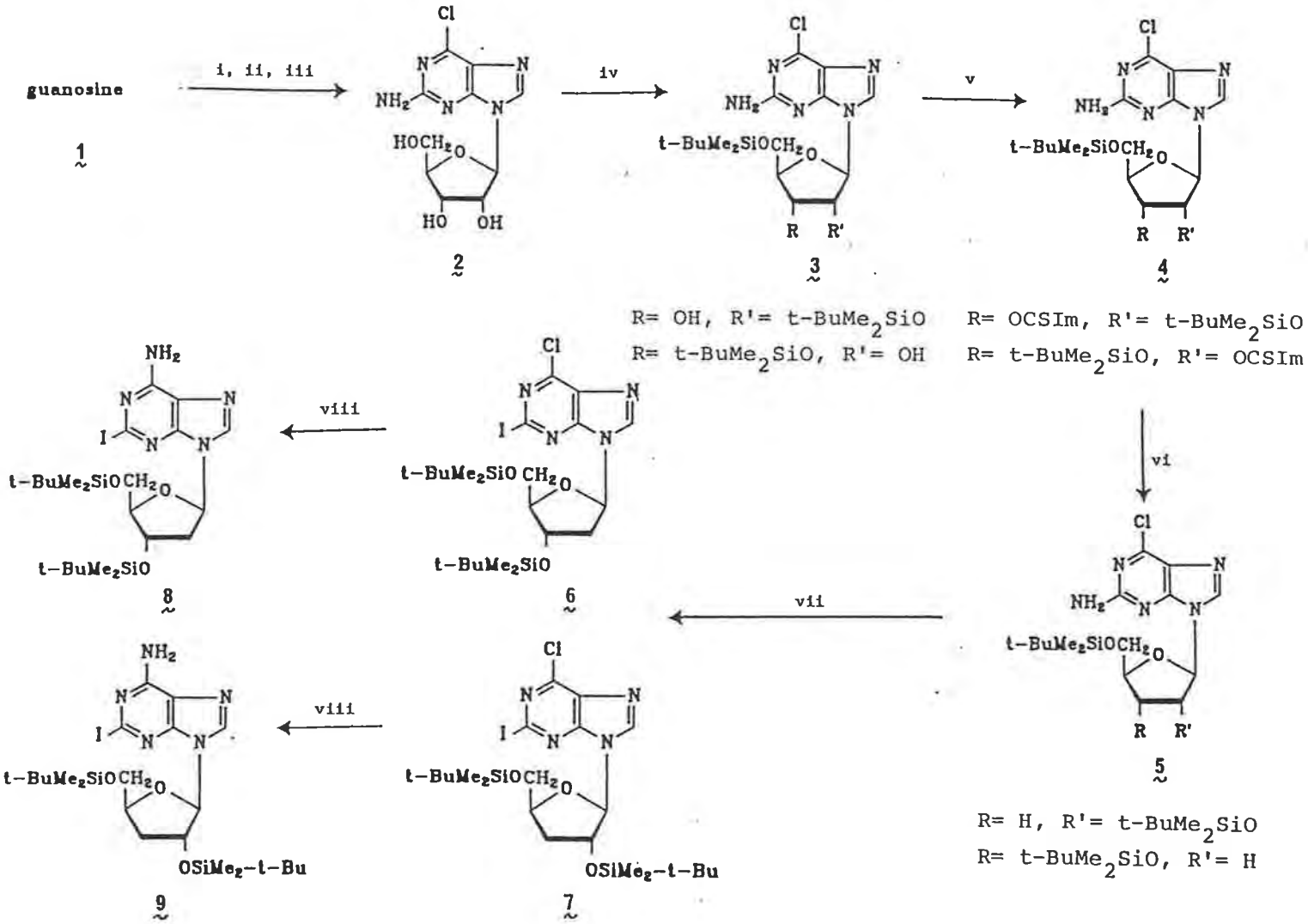
Guanosine **1** was converted in three known steps to 6-chloro-2-aminopurine ribonucleoside, **2**, in high yields.[16,17] When compound **2** was treated with 2.2 equivalents of t-butyldimethylsilyl chloride and 4.4 equivalents of imidazole in DMF at room temperature for 2 h, a mixture of 2′,5′- and 3′,5′-disilylated product **3** was obtained in 62% yield after

purification (Scheme I). The isolation of these compounds required chromatographic separation from two side products: the 5′-monosilylated compound (19 %) and the trisilylated material (5 %). All four compounds could be easily identified by mass spectrometry. The use of pyridine as base (cf. ref. 18) in this silylation reaction with this substrate is problematic in terms of yield, work up and purification. The isomer ratio also changes when pyridine is used in this reaction.[18] The mixture of the 2′,5′- and 3′,5′-disilylated compounds (regioisomer ratio approximately 1:1 by ^{1}H NMR data, anomeric H integration) may be separated into the individual isomers but with difficulty at this juncture or the mixture may be separated at a later stage in the synthetic pathway. We chose the latter as the more efficient approach.

Treatment of compound **3** with 1,1′-thiocarbonyldiimidazole in refluxing dichloroethane for about 4h afforded the thiocarbonyl ester mixture **4** in 87% yield. Longer reaction times led to decomposition products and lower yields. If DMAP is used in this reaction,[19] the yields drop to about 20% because of extensive decomposition of starting material. Interestingly, thermal isomerization of the silyl protecting groups occurred in the reaction, most likely prior to imidazolide formation, to produce a mixture of the 3′,5′- and 2′,5′-imidazolides in a 3:2 ratio, different from the original 1:1 ratio. In controlled thermal experiments monitored by ^{1}H NMR spectroscopy, we have shown that pure 2′,5′-disilyl compound in $CDCl_3$ containing imidazole rearranges slowly to a mixture containing both the 2′,5′- and the 3′,5′-isomer (cf. refs 20, 21). The rate of rearrangement increases with the addition of a small amount of water.

Deoxygenation of the chromatographically purified imidazolides was carried out with tributyltin hydride and AIBN in refluxing toluene for 1 h to give the deoxygenated products **5** in 86% yield (Scheme I). Iodination of the deoxygenated compounds with t-butyl nitrite, methylene diiodide, and

Scheme I[a]



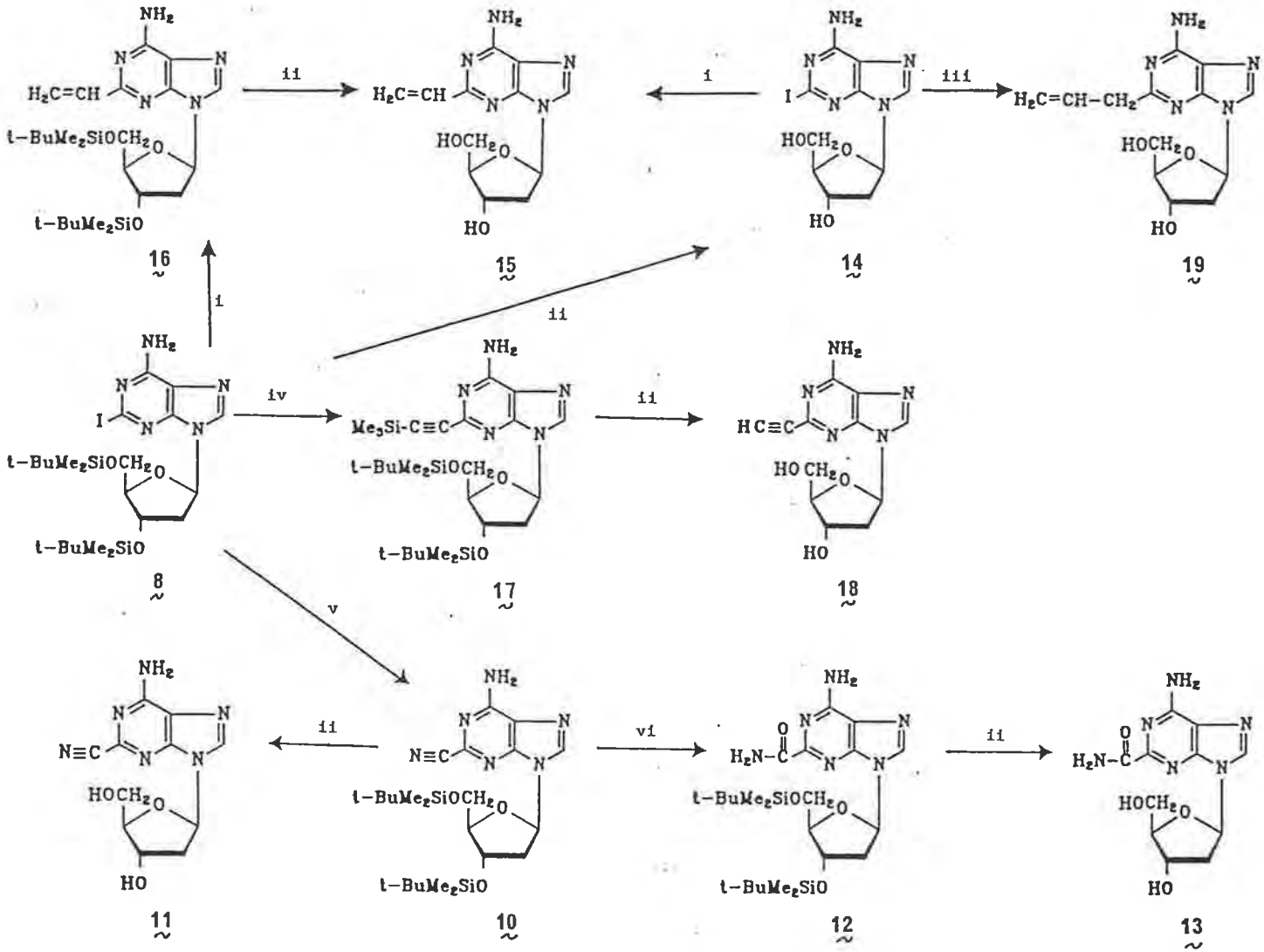
[a](i) Ac_2O, $(C_2H_5)_3N$, 4-(dimethylamino)pyridine, CH_3CN; (ii) $POCl_3$, N,N-diethylaniline, Δ; (iii) NH_3, MeOH; (iv) t-Bu$(CH_3)_2SiCl$, imidazole, DMF; (v) Im_2CS, $(CH_2Cl)_2$, Δ; (vi) n-Bu_3SnH, AIBN, toluene, Δ; (vii) n-$C_5H_{11}ONO$, CH_2I_2, $(CH_3)_3SiI$, hexane, Δ; (viii) NH_3, EtOH.

trimethylsilyl iodide in hexane gave the 2-iodo-6-chloro compounds **6** and **7** in a combined yield of 58 %. The iodination reaction of these substrates has to be conducted under carefully controlled conditions (dried redistilled reagents and solvents, N_2 atmosphere, optimum reaction time and temperature, catalytic amounts of TMSI, etc.) in order to obtain synthetically acceptable yields. Complete separation of the regioisomers was easily achieved at this stage of the synthesis by column chromatography on silica gel with hexane/ ethyl acetate (5:1) as the eluting solvent. The ratio of the two separated isomers was still 3:2 [2'-deoxy (**6**) : 3'-deoxy (**7**)] as shown by high-field ^1H NMR data (integration for H-1', triplet for 2'-deoxy and doublet for 3'-deoxy).

Two modifications of the purine ring were planned for the next stages of the syntheses; first, the conversion of the 6-chloro group to the 6-amino group, and second, the elaboration of the 2-position utilizing the carbon-iodine bond at this position. Transformation of the 2-iodo-6-chloropurine moiety in **6** and **7** to the 2-iodoadenine moiety could be easily brought about because of the nucleophilic lability of the 6-chloro group. Thus, treatment of the 2-iodo-6-chlorodeoxynucleosides **6** and **7** with ethanolic ammonia resulted in displacement of the 6-chloro group to furnish the deoxygenated adenine nucleosides **8** and **9**, respectively (Scheme I).

Carbon-carbon bond forming reactions leading to functionalization at the 2-position of the adenine moiety were carried out by palladium-catalyzed cross-coupling reactions with synthon bearing organostannanes. Thus, with the 2'-deoxy series, treatment of **8** with tri-n-butylcyanostannane and tetrakis(triphenylphosphine) palladium(0) in DMF at 120 ^oC gave the 2-cyanoadenine product **10** in 91% yield (Scheme II). Compound **10** can be converted to the deprotected novel target molecule **11** by treatment with tetraethylammonium fluoride. The structure of the 2-cyano product **11** was confirmed by spectral data: high-field ^1H and ^{13}C NMR, FTIR,

Scheme II[a]



[a](i) n-$Bu_3SnCH{=}CH_2$, $PdCl_2(CH_3CN)_2$, DMF, Δ; (ii) Et_4NF, CH_3CN; (iii) n-$Bu_3SnCH_2{-}CH{=}CH_2$, $Pd^{\circ}(Ph_3P)_4$, DMF, Δ; (iv) $(CH_3)_3SiC{\equiv}CH$, Et_3N, CuI, $Pd^{\circ}(Ph_3P)_4$, DMF, Δ; (v) n-Bu_3SnCN, $Pd^{\circ}(Ph_3P)_4$, DMF, Δ; (vi) Bu_4NHSO_4, CH_2Cl_2, NaOH, H_2O_2.

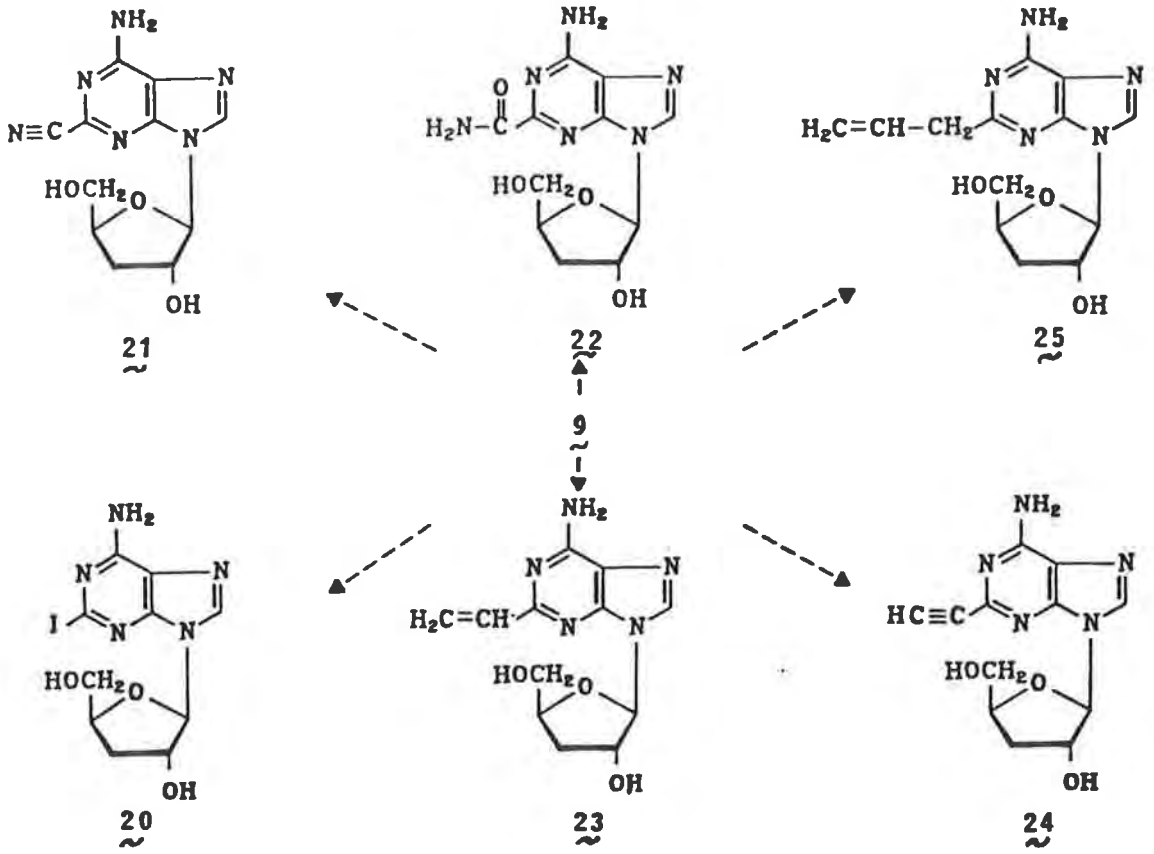
UV, and FAB HRMS. 2-Carboxamido-2′-deoxyadenosine **13**, another new nucleoside, can be synthesized from **10** through hydrolysis and deprotection. The well established methods for effecting this functional group transformation involve hydrolysis under acidic or basic conditions or under basic conditions with hydrogen peroxide.[22] These methods appeared not to be useful for deoxy nucleosides because of the lability of their glycosidic bond under these conditions. However, the hydrolysis could be effectively carried out using a modification of the phase transfer conditions of Cacchi, Misiti and LaTorre [23] with aqueous sodium hydroxide and hydrogen peroxide to furnish the protected carboxamide **12** (89% yield). The novel target carboxamide **13** was obtained from **12** by deprotection (85%).

2-Iodo-2′-deoxyadenosine **14** has been reported recently to have antitumor activity.[24] Precursor **8** could be deprotected to this compound with fluoride ion in 97% yield. In this unprotected form, compound **14** can be converted to the new 2-vinyl compound **15** in 80% isolated yield by palladium-mediated cross-coupling with vinyl tri-n-butylstannane with heating in DMF.[25,26] Product **15** was also prepared from **8** by the palladium-catalyzed methodology followed by deprotection. Allylation reactions are also possible. Thus, unprotected **14** was converted to 2-allyl-2′-deoxyadenosine **19** by reaction with allyl tri-n-butylstannane and Pd(0) in DMF with heating. The temperature of this reaction was kept at 95 ^{o}C which is sufficient for the palladium-catalyzed carbon-carbon bond formation but not high enough for the isomerization of the allyl group on the base moiety to the thermodynamically more favorable 2-methylvinyl system. Introduction of the ethynyl group at the 2-position was also carried out but using a slightly different approach.[27] Treatment of **8** with trimethylsilyl acetylene in the presence of cuprous iodide, tetrakis(triphenylphosphine)palladium (0), and triethylamine in DMF gave **17** (> 90%) which could be deprotected directly to 2-ethynyl-2′-deoxyadenosine (**18**) (Scheme II). In contrast, palladium-catalyzed cross-coupling of **8** or

14 with ethynyl tri-n-butylstannane gave only low yields of the ethynyl products.

Using a related sequence of reactions, the 2-iodo-3′-deoxynucleoside 9 was converted to the following functionalized nucleosides: 2-iodo 20, 2-cyano 21, 2-carboxamide 22, 2-vinyl 23, 2-ethynyl 24 and 2-allyl 25 (Scheme III).

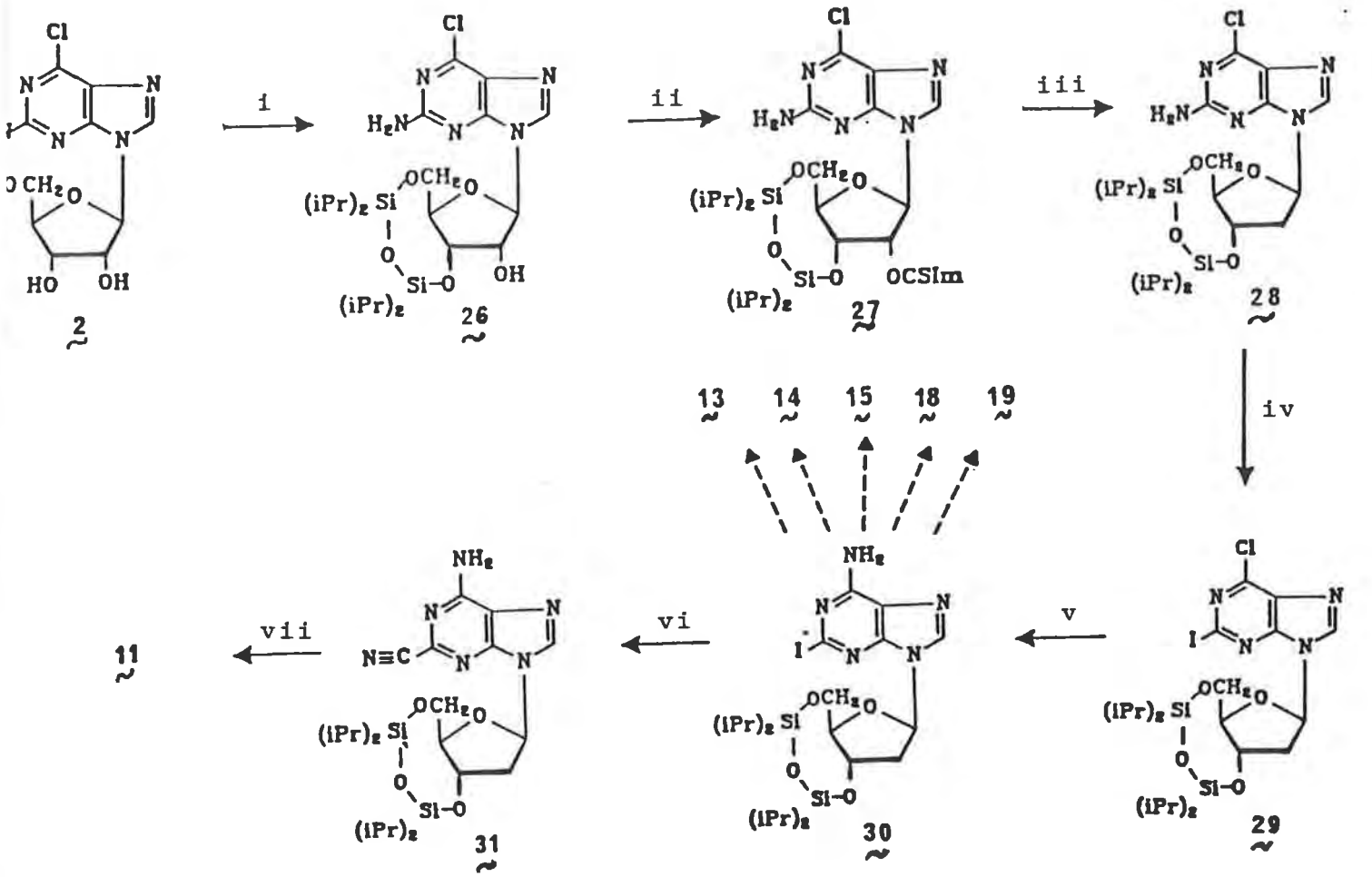
Scheme III



Finally, a regiospecific approach to functionalized 2′-deoxyadenosines may be realized through protection involving a 3′,5′-cyclic silyl group. Thus, nucleoside 2 can be converted to regiospecifically protected 26 in 73% yield by reaction with 1,3-dichloro-1,1,3,3-tetraisopropyl-1,3-disiloxane[28,29] and imidazole in DMF at room temperature (Scheme IV). Compound 26 was transformed into the key precursor for this series, the protected 2-iodo-2′-deoxyadenosine, 30, through a sequence of reactions similar to that described for the conversion of 3 to 8 and 9. Conversion

of **30** to the 2-cyano-2′-deoxyadenosine **11** (Scheme IV) is related to the conversion of **8** to **11** (see experimental description). Intermediate **30** may also be converted to the target molecules **13**, **14**, **15**, **18**, and **19** through the functionalization methodologies previously described (see Scheme II).

Scheme IV[a]



[a](i) TPDS-Cl_2, imidazole, DMF; (ii) Im_2CS, $(CH_2Cl)_2$, Δ; (iii) n-Bu_3SnH, AIBN, toluene, Δ; (iv) n-$C_5H_{11}ONO$, CH_2I_2, $(CH_3)_3SiI$, hexane, Δ; (v) NH_3, EtOH; (vi) Pd°$(Ph_3P)_4$, n-Bu_3SnCN, DMF, Δ; (vii) Et_4NF, CH_3CN.

In summary, the synthesis of a series of doubly modified nucleosides, 2′- and 3′-isomeric analogues of the nucleoside antibiotic, cordycepin, is described. All of the target compounds are new and contain functionality at the 2-position that can be exploited for further elaboration of these deoxygenated nucleosides. The key transformations used in these syntheses are regioselective bis-silylation, thermal radical deoxygenation and halogenation, and regiospecific metal-mediated carbon-carbon bond

formation. The approaches presented have generality and can be extended for the synthesis of other new deoxygenated nucleosides functionalized in the base moiety.

Experimental Section

Melting points reported are uncorrected and were determined on a Thomas-Hoover melting point apparatus fitted with a microscope. Nuclear magnetic resonance spectra were recorded on Bruker Models WM360, MSL300, and AC300 pulse Fourier transform spectrometers. Mass spectra were determined on a VG ZAB-HF high resolution mass spectrometer with FAB capability or a VG TRIO single quadrupole GC/MS system. Ultraviolet spectra were recorded on a Varian Cary Model 219 or a Gilford Response spectrophotometer. Infrared spectra were recorded on a Mattson Cygnus 25 Fourier transform instrument. Lyophilizations were performed with a Virtis freezemobile 3 unit. Preparative layer chromatography plates were prepared by coating six 20 cm x 20 cm plates with a slurry made from 150 g of E. Merck PF_{254} silica gel in 400 mL of water. The silica gel plates were allowed to dry slowly and were then activated for 3 h at 135°C. Flash chromatography was carried out using glass columns packed with 230-400 mesh silica gel. High performance liquid chromatography was done at 80 psi using Altex columns packed with 40-60 μm Amberlite XAD-4 resin (Rohm and Haas). Fractions were monitored by a Pharmacia UV-2 ultraviolet monitor and products were collected on a Gilson FC-100 fraction collector. Preparative HPLC separations were also carried out with a Waters automated 600E system with photodiode array detector and FOXY fraction collector.

2-Amino-6-chloro-9-[3,5 and 2,5- bis-O-(tert-butyldimethylsilyl)-β-D-ribofuranosyl]purine (3). A mixture of the nucleoside 2[16,17] (2.217 g, 7.35 mmol) , tert-butyldimethylsilyl chloride (2.436 g, 16.16 mmol), and

imidazole (2.199 g, 32.33 mmol) in DMF (8 mL) was stirred at room temperature for 1.5 h. The solvent was removed under reduced pressure, the residue was partitioned between ethyl acetate (40 mL) and H_2O (40 mL), and the organic layer was re-extracted with H_2O (2 X 30 mL). The organic layer was dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by flash chromatography using $CHCl_3$ followed by 2-5% $CH_3OH/CHCl_3$ to give the 2',3',5'-tri-O-(tert-butyldimethylsilyl) derivative (0.250 g, 0.39 mmol, 5%), followed by **3** (2.428 g, 4.58 mmol, 62%). The mono-silylated derivative was removed from the column with 5% $CH_3OH/CHCl_3$ (0.591 g, 1.42 mmol, 19%). Data for **3a**: ^1H NMR ($CDCl_3$) δ 0.05-0.13 (m, 12H), 0.88-0.91 (m, 18H), 2.66 (d, 1H), 3.82 (dd, 1H), 3.95 (dd, 1H), 4.18 (m, 1H), 4.23 (q, 1H), 4.51 (t, 1H), 5.04 (s, 2H), 5.96 (d, 1H, J=5.2 Hz), 8.16 (s, 1H); UV (EtOH) λ_{max} 247, 308 nm. Data for **3b**: ^1H NMR ($CDCl_3$) δ 0.05-0.13 (m, 12H), 0.88-0.91 (m, 18H), 3.05 (d, 1H), 3.73 (dd, 1H), 3.90 (dd, 1H), 4.04 (m, 1H), 4.39 (q, 1H), 4.49 (t, 1H), 5.40 (s, 2H), 5.90 (d, 1H, J=4.3 Hz), 8.05 (s, 1H); UV (EtOH) λ_{max} 247, 308 nm.

2-Amino-6-chloro-9[3-O-(1-imidazolyl)thiocarbonyl-2,5-bis-O-(tert-butyl-dimethylsilyl)- β -D-ribofuranosyl]purine 4a and Its Isomer 4b. A mixture of the nucleosides **3** (2.312 g, 4.36 mmol) and 1,1'-thiocarbonyldiimidazole (1.17 g, 6.54 mmol) in dry 1,2-dichloroethane was refluxed under a N_2 atmosphere with stirring for 2.5 h. The solvent was removed under reduced pressure, the residue partitioned between ethyl acetate (40 mL) and H_2O (40 mL) and the organic layer was re-extracted with saturated aqueous NaCl (30 mL). The organic layer was dried (Na_2SO_4), concentrated in vacuo, and purified by flash chromatography with $CHCl_3$ followed by 2% $CH_3OH/CHCl_3$ to afford **4** (2.419 g, 3.78 mmol, 87%) as an off-white foam. Data for **4a**: ^1H NMR ($CDCl_3$) δ 0.05-0.13 (m, 12H), 0.81-0.93 (m, 18H), 3.77 (dd, 1H), 3.95 (dd, 1H), 4.21 (m, 1H), 4.88 (m, 1H), 5.11 (s, 2H), 5.93 (m, 1H), 6.05 (d, 1H), 7.08 (s, 1H), 7.69 (s, 1H), 8.12 (s, 1H), 8.42 (s, 1H); UV (EtOH)λ_{max} 251, 281, 305 nm. Data for **4b**: ^1H NMR ($CDCl_3$) δ 0.05-0.13 (m, 12H),

0.81-0.93 (m, 18H), 3.77 (dd, 1H), 3.94 (dd, 1H), 4.20 (m, 1H), 4.85 (m, 1H), 5.24 (s, 2H), 5.95 (t, 1H), 6.37 (d, 1H), 6.99 (s, 1H), 7.59 (s, 1H), 8.08 (s, 1H), 8.33 (s, 1H); UV (EtOH) λ_{max} 251, 281, 305 nm; mass spectrum (**4**), m/z (relative intensity) 582 (M^+-tBu-H, 0.5), 454 (M^+-tBu-OCSIm-2H, 0.8), 169 (B^++H, 23.9).

2-Amino-6-chloro-9-[3-deoxy-2,5-bis-O-(tert-butyldimethylsilyl)- β **-D-ribofuranosyl]purine 5a and Its Isomer 5b.** To a refluxing solution of the nucleosides **4** (2.445 g, 3.82 mmol) in toluene (40 mL) under N_2 was added a mixture of tributyltin hydride (1.08 mL, 4.01 mmol) and AIBN (0.385 g, 1.91 mmol) in N_2 purged toluene (40 mL) over a 40 min period. The reaction was allowed to reflux for an additional 5 min and then concentrated <u>in vacuo</u>. The residue was dissolved in chloroform/ hexanes and eluted through a short scrubber column with hexanes followed by chloroform. Further purification by flash chromatography with chloroform gave **5** as a white foam (1.682 g, 3.27 mmol, 86%). Data for **5a**: ^{1}H NMR ($CDCl_3$) δ 0.05-0.13 (m, 12H), 0.81-0.94 (m, 18H), 1.83 (m, 1H), 2.19 (m, 1H), 3.82 (dd, 1H), 1H), 4.06 (dd, 1H), 4.49 (m, 2H), 5.06 (s, 2H), 5.85 (d, 1H), 8.25 (s, 1H); UV (EtOH) λ_{max} 247, 308 nm. Data for **5b**: ^{1}H NMR ($CDCl_3$) δ 0.05-0.13 (m, 12H), 0.81-0.94 (m, 18H), 2.38 (m, 1H), 2.53 (m, 1H), 3.73 (dd, 1H), 3.79 (dd, 1H), 3.96 (m, 1H), 4.56 (m, 1H), 5.14 (s, 2H), 6.29 (t, 1H), 8.05 (s, 1H); UV (EtOH) λ_{max} 247, 308 nm; mass spectrum (**5**), m/z (relative intensity) 457 (M^+-tBu, 0.4), 169 (B^++H, 21.2), 171 ($B^+(^{37}Cl)$+H, 10.5).

2-Iodo-6-chloro-9-[2-deoxy-3,5-O-bis-(tert-butyldimethylsilyl)- β **-D-ribofuranosyl]purine 6 and Its 3'-Deoxy Isomer 7.** To a chilled (0°C) suspension of nucleoside **5** (0.685 g, 1.33 mmol) in N_2 purged hexane (30 mL) was added diiodomethane (0.43 mL, 5.33 mmol), tert-butyl nitrite (0.63 ml, 5.33 mmol), followed by **SLOW** addition of TMSI (0.04 mL, 0.26 mmol) <u>via</u> syringe. The reaction was stirred at 55°C under a N_2 atmosphere for 1.5 h

and worked up by the addition of saturated aqueous Na_2SO_3 (5 mL). After the mixture was stirred for 5 min, the organic layer was separated and concentrated. The residue was purified by flash chromatography with 5:1 hexanes/ethyl acetate and afforded 0.193 g (0.31 mmol, 23%) of the 3'-deoxy regioisomer **7**, followed by 0.295 g (0.35 mmol, 35%) of the 2'-deoxy isomer **6**, both as viscous oils. Data for **7**: ^1H NMR ($CDCl_3$) δ 0.05-0.13 (m, 12H), 0.81-0.92 (m, 18H), 1.82 (m, 1H), 2.16 (m, 1H), 3.75 (dd, 1H), 4.17 (dd, 1H), 4.59 (m, 2H), 5.97 (d, 1H), 8.63 (s, 1H); UV (EtOH) λ_{max} 254, 280nm; mass spectrum, m/z (relative intensity) 568 (M^+-tBu, 0.6), 280 (B^++H, 0.3). Data for **6**: ^1H NMR ($CDCl_3$) δ 0.05-0.13 (m, 12H), 0.81-0.92 (m, 18H), 2.46 (m, 1H), 2.57 (m, 1H), 3.74 (dd, 1H), 3.86 (dd, 1H), 4.00 (m, 1H), 4.60 (m, 1H), 6.41 (t, 1H), 8.36 (s, 1H); UV (EtOH) λ_{max} 254, 282 nm; mass spectrum, m/z (relative intensity) 568 (M^+-tBu, not observed), 280 (B^++H, 4.7).

2-Iodo-9-[2-deoxy-3,5-O-bis-(tert-butyldimethylsilyl)-β-D-ribofuranosyl] adenine (8). A chilled (0°C) solution of the deoxynucleoside **6** (0.429 g, 0.68 mmol) in absolute ethanol (40 mL) was saturated with NH_3 gas. The reaction mixture was allowed to proceed for 12 h with stirring at room temperature. The solvent was removed and the residue was dissolved in ethyl acetate (15 mL) and washed with H_2O (15 mL). The organic layer was dried (Na_2SO_4), concentrated <u>in vacuo</u>, and the residue was purified by flash chromatography with $CHCl_3$ followed by 5% $CH_3OH/CHCl_3$ to afford 0.358 g (0.59 mmol, 86%) of **8** as a white foam: ^1H NMR ($CDCl_3$) δ 0.07-0.14 (m, 12H), 0.81-0.91 (m, 18H), 2.40 (m, 1H), 2.63 (m, 1H), 3.74 (dd, 1H), 3.85 (dd, 1H), 3.97 (m, 1H), 4.60 (m, 1H), 5.93 (s, 2H), 6.33 (t, 1H), 7.99 (s, 1H); UV (EtOH) λ_{max} 266 nm; mass spectrum, m/z (relative intensity) 548 (M^+-tBu, 1.7), 261 (B^++H, 42.6).

2-Iodo-9-[3-deoxy-2,5-bis-O-(tert-butyldimethylsilyl)-β-D-ribofuranosyl] adenine (9) was prepared from **7** as described above for the preparation of

8. The product was obtained as a white foam (86% yield): ^{1}H NMR ($CDCl_3$) δ 0.07-0.14 (m, 12H), 0.81-0.91 (m, 18H), 1.76 (m, 1H), 2.13 (m, 1H), 3.73 (dd, 1H), 4.10 (dd, 1H), 4.57 (m, 2H), 5.88 (d, 1H), 6.38 (s, 2H), 8.22 (s, 1H); UV (EtOH) λ_{max} 266 nm; mass spectrum, m/z (relative intensity) 548 (M^+-tBu, 30.1), 262 (B^++2H, 6.8).

2-Cyano-9-[2-deoxy-3,5-O-bis-(tert-butyldimethylsilyl)-β-D-ribofuranosyl] adenine (10). A mixture of the 2-iodo deoxynucleoside **8** (0.351 g, 0.58 mmol), tetrakis(triphenylphosphine)palladium(0) (0.080 g, 0.07 mmol), and tributyltin cyanide (0.202 g, 0.64 mmol) in DMF (10 mL) was stirred at 120°C for 40 min under a N_2 atmosphere. The solvent was removed under reduced pressure and the residue initially purified on a short silica gel scrubber column with hexanes followed by ethyl acetate. Final purification by flash chromatography with chloroform afforded 0.267 g (0.53 mmol, 91%) of **10** as an off-white foam: ^{1}H NMR ($CDCl_3$) δ 0.06-0.13 (m, 12H), 0.85-0.94 (m, 18H), 2.43 (m, 1H), 2.63 (m, 1H), 3.74 (dd, 1H), 3.88 (dd, 1H), 3.99 (m, 1H), 4.07 (m, 1H), 6.36 (t, 1H), 6.49 (s, 2H), 8.27 (s, 1H); UV (EtOH) λ_{max} 259, 264, 296 nm; mass spectrum, m/z (relative intensity) 447 (M^+-tBu, 0.5), 160 (B^++H, 41.4).

2-Cyano-9-(2-deoxy-β-D-ribofuranosyl)adenine (11). To a solution of **10** (0.156 g, 0.31 mmol) in dry CH_3CN (7 mL) was added tetraethylammonium fluoride in acetonitrile (0.5M, 1.86 mL, 0.93 mmol) via syringe. The reaction was stirred at room temperature for 1.5 h. The solvent was removed under reduced pressure and the residue was purified on silica gel plates with 12% $CH_3OH/CHCl_3$ as the mobile phase. The band at R_f 0.47 afforded 0.079 g (0.28 mmol, 92%) of almost pure product **11**. Further purification was carried out by reversed-phase HPLC on Amberlite XAD-4 resin employing 12% ethanol/water as the mobile phase: mp 205-207 °C; ^{13}C NMR (Me_2SO-d_6) δ 38.6, 61.4, 70.4, 83.6, 87.9, 116.7, 120.5, 136.5, 141.6, 148.0, 156.0; ^{1}H NMR (Me_2SO-d_6) δ 2.34 (m, 1H), 2.65 (m, 1H), 3.51 (m,

1H), 3.61 (m, 1H), 3.87 (m, 1H), 4.41 (m, 1H), 4.96 (t, 1H), 5.36 (m, 1H), 6.33 (t, 1H), 7.97 (s, 2H), 8.58 (s, 1H); UV (H_2O) λ_{max} 261 (ε 9000), 265.5 (9600), 296 nm (6300); FAB HRMS obsd (M^++H) 277.1076, calcd for $C_{11}H_{13}N_6O_3$ 277.1049.

2-Carboxamido-9-[2-deoxy-3,5-O-bis-(tert-butyldimethylsilyl)-β-D-ribofuranosyl]adenine (12). To a solution of **10** (0.216 g, 0.43 mmol) in CH_2Cl_2 (20mL) was added 30% hydrogen peroxide (0.21 mL, 1.71 mmol), tetrabutylammonium hydrogen sulfate (0.029 g, 0.085 mmol), and 0.5M aqueous sodium hydroxide (1.03 mL, 0.51 mmol). The reaction mixture was stirred at room temperature for 3 h. Water (20 mL) and CH_2Cl_2 (10 mL) were then added. The organic layer was separated, washed with saturated aqueous NaCl (10 mL), and dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue was purified on preparative silica gel plates with 8% $CH_3OH/CHCl_3$ as the mobile phase. The band at R_f 0.46 afforded 0.201 g (0.38 mmol, 89%) of **12** as a white foam: ^1H NMR ($CDCl_3$) δ 0.04-0.13 (m, 12H), 0.85-0.94 (m, 18H), 2.42 (m, 1H), 2.65 (m, 1H), 3.77 (dd, 1H), 3.85 (dd, 1H), 3.98 (m, 1H), 4.57 (m, 1H), 6.38 (s, 2H), 6.52 (t, 1H), 6.62 (s, 1H), 7.77 (s, 1H), 8.27 (s, 1H); UV (EtOH) λ_{max} 261, 265, 284 nm; mass spectrum, m/z (relative intensity) 523 (M^+, 0.9), 465 (M^+-tBu, 4.8), 178 (B^++H, 11.6).

2-Carboxamido-9-(2-deoxy-β-D-ribofuranosyl)adenine (13). Deprotection of **12** was carried out as described for the conversion of **10** to **11**. The product was obtained as a solid after preparative thin layer chromatography on silica gel and was recrystallized from methanol/water (85% yield): mp > 240 °C (decomp); ^{13}C NMR (Me_2SO-d_6) δ 40.9, 61.5, 70.6, 83.2, 87.7, 119.3, 140.7, 149.1, 152.7, 155.5, 164.9; ^1H NMR (Me_2SO-d_6) δ 2.30 (m, 1H), 2.71 (m, 1H), 3.57 (m, 2H), 3.87 (m, 1H), 4.43 (m, 1H), 4.93 (t, 1H), 5.33 (d, 1H), 6.42 (t, 1H), 7.50 (s, 2H), 7.54 (s, 1H), 7.81 (s, 1H), 8.47 (s, 1H); UV (H_2O) λ max 259.0 (ε 8600), 264.3 (8900), 291.5 (4900); FTIR

(KBr) 1685 cm^{-1}; FAB HRMS obsd (M^++H) 295.1147, calcd for $C_{11}H_{14}N_6O_4$ 295.1154.

2-Iodo-9-(2-deoxy-β-D-ribofuranosyl)adenine (14). The silylated 2-iodo deoxynucleoside **8** (0.173 g, 0.28 mmol) was dissolved in dry CH_3CN (7 mL) and deprotected with fluoride as described for **10** -> **11** to afford 0.105 g (0.28 mmol, 97%) of **14** as a white solid: mp >215 °C (decomp); ^1H NMR (Me_2SO-d_6) δ 2.27 (m, 1H), 2.63 (m, 1H), 3.52 (m, 1H), 3.57 (m, 1H), 3.85 (m, 1H), 4.38 (m, 1H), 4.94 (t, 1H), 5.31 (d, 1H), 6.24 (t, 1H), 7.70 (s, 2H), 8.27 (s, 1H); UV (EtOH) λ_{max} 266 nm.

2-Vinyl-9-[2-deoxy-3,5-O-bis-(tert-butyldimethylsilyl)-β-D-ribofuranosyl] adenine (16). To a mixture of **8** (0.312 g, 0.52 mmol), and bis(acetonitrile)palladium (II) chloride (0.007g, 0.025 mmol) in DMF (4 mL) was added vinyltributyltin (0.16 mL, 0.57 mmol). The reaction was allowed to proceed for 45 min at 90°C under N_2 and then cooled, concentrated under reduced pressure, and purified on silica gel plates with 5% $CH_3OH/CHCl_3$ as the developing solvent. The band at R_f 0.42 afforded 0.179 g (0.35 mmol, 69%) of **16** as a yellow oil: ^1H NMR ($CDCl_3$) δ 0.05-0.13 (m, 12H), 0.82-0.93 (m, 18H), 2.38 (m, 1H), 2.69 (m, 1H), 3.74 (dd, 1H), 3.85 (dd, 1H), 3.96 (m, 1H), 4.59 (m, 1H), 5.54 (dd, 1H), 5.98 (s, 2H), 6.43 (m, 2H), 6.71 (dd, 1H), 8.04 (s, 1H); UV (EtOH) λ_{max} 265, 272, 295(s) nm; mass spectrum, m/z (relative intensity) 448 (M^+-tBu, 0.15), 161 (B^++H, 29.7).

2-Vinyl-9-(2-deoxy-β-D-ribofuranosyl)adenine (15). Deprotection of **16** was carried out as described for the conversion of **10** -> **11**. The product **15** was obtained as white crystals (98% yield). (Characterization described below).

2-Vinyl-9-(2-deoxy-β-D-ribofuranosyl)adenine (15). Unprotected **14** was converted to **15** as described for the conversion of **8** -> **16** to give **15** as a white solid (80% yield): mp 181-183°C; ^{13}C NMR (Me_2SO-d_6) δ 39.8, 61.8,

70.9, 83.7, 87.9, 118.3, 120.9, 137.0, 134.8, 149.3, 155.5, 157.6; ^{1}H NMR (Me_2SO-d_6) δ 2.26 (m, 1H), 2.73 (m, 1H), 3.53 (m, 1H), 3.61 (m, 1H), 3.88 (m, 1H), 4.41 (m, 1H), 5.21 (t, 1H), 5.31 (d, 1H, 5.54 (dd, 1H, 2.3, 10.4 Hz), 6.34 (m, 2H), 6.60 (dd, 1H, J=10.4, 17.2 Hz), 7.26 (s, 2H), 8.31 (s, 1H); UV (H_2O) λ_{max} 265.4 (ε10990), 270.2 (10820), 292(s) nm (5500); FAB HRMS obsd (M^++H) 278.1266, calcd for $C_{12}H_{16}N_5O_3$ 278.1253.

2-Ethynyl-9-(2-deoxy-β-D-ribofuranosyl)adenine (18). A mixture of the nucleoside **8** (0.102 g, 0.169 mmol), tetrakis(triphenylphosphine) palladium (0) (0.010 g, 0.008 mmol) and cuprous iodide (0.002 g, 0.013 mmol) was dissolved in DMF (5 mL) and triethylamine (1 mL). Trimethylsilylacetylene (0.05 ml, 0.338 mmol) was subsequently added dropwise <u>via</u> syringe to the reaction mixture, which was then stirred under nitrogen at 80° C for 70 min. Upon removal of the solvents under reduced pressure, the residue was taken up in ethyl acetate (20 mL) and extracted with 10% Na_2EDTA (2 X 15 mL). The organic layer was washed with aqueous NaCl (15 mL), dried (Na_2SO_4), and concentrated. The residue was dissolved in $CHCl_3$ and eluted through a short silica gel scrubber column with chloroform followed by ethyl acetate to afford **17** as a brown oil. The crude product in dry CH_3CN (6 mL) was treated with 0.646 mmol of tetraethylammonium fluoride (TEAF) in CH_3CN (0.5 M solution) and stirred at room temperature for 2 h. The solvent was removed under reduced pressure and the residue purified on silica gel plates with 14 % MeOH/$CHCl_3$ as the developing solvent. The band at R_f 0.50 gave 0.036 g (0.130 mmol, 77%) as a clear glass which was crystallized from CH_3CN to give the title nucleoside **18** as white needles: mp 187-189 °C; ^{1}H NMR (Me_2SO-d_6) δ 2.29 (m, 1H), 2.68 (m, 1H), 3.54 (m, 1H), 3.59 (m, 1H), 3.88 (m, 1H), 3.96 (s, 1H), 4.40 (m, 1H), 4.99 (s, 1H), 5.27 (t, 1H), 6.31 (dd, 1H), 7.42 (s, 2H), 8.39 (s, 1H); UV (H_2O) λ_{max} 263 (ε 10300), 268 (10800), 287 nm (6600); FAB HRMS obsd (M^++H) 276.1122, calcd for $C_{12}H_{14}N_5O_3$ 276.1096.

2-Allyl-9-(2-deoxy- β -D-ribofuranosyl)adenine (19). To a solution containing the unprotected nucleoside **14** (0.044 g, 0.118 mmol) and tetrakis(triphenylphosphine)palladium (0) (0.010 g, 0.009 mmol) in DMF (4 mL) was added allyltributyltin (0.04mL, 0.130 mmol) dropwise <u>via</u> syringe. The reaction mixture was stirred under nitrogen for 30h at 95°C and the solvent was removed under reduced pressure and the residue was purified on silica gel plates with ether followed by 14 % MeOH/ $CHCl_3$ as the developing solvent. The band at R_f 0.43 gave 0.028g (0.096 mmol, 82%) of the title nucleoside as a white solid: mp 75-77°C; ^1H NMR (Me_2SO-d_6) δ 2.25 (m, 1H), 2.71 (m, 1H), 3.42 (dm, 2H), 3.53 (m, 1H), 3.63 (m, 1H), 3.89 (m, 1H), 4.40 (m, 1H), 5.07 (ddm, 2H), 5.25 (m, 2H), 6.10 (m, 1H), 6.32 (dd, 1H), 7.17 (s, 2H), 8.24 (s, 1H); UV (H_2O) λ_{max} 262 nm (ε 11000); FAB HRMS obsd (M^++H) 292.1415, calcd for $C_{13}H_{18}N_5O_3$ 292.1409.

2-Iodo-9-(3-deoxy-β-D-ribofuranosyl)adenine (20). The silylated nucleoside **9** (0.173 g, 0.28 mmol) was dissolved in dry CH_3CN (7 mL) and treated with a 0.5 M TEAF solution (1.00 mL, 0.497 mmol) in CH_3CN. The reaction proceeded at room temp for 2 h. The solvent was removed under reduced pressure and the residue purified on a silica gel plate with 12% methanol/chloroform as the developing solvent. The band at R_f 0.44 afforded 0.057 g (0.152 mmol, 92%) of product which was recrystallized from acetonitrile to afford the title nucleoside **20**[30] as a white solid: mp 207-208 °C; ^{13}C NMR (Me_2SO-d_6) δ 33.9, 62.3, 74.6, 80.7, 90.4, 118.7, 120.6, 138.6, 149.1, 155.7; ^1H NMR (Me_2SO-d_6) δ 1.94 (m, 1H), 2.24 (m, 1H), 3.52 (m, 1H), 3.68 (m, 1H), 4.34 (m, 1H), 4.53 (m, 1H), 4.94 (m, 1H), 5.62 (s, 1H), 5.79 (d, 1H, J=2.25 Hz), 7.62 (s, 2H), 8.27 (s, 1H); UV (H_2O) λ_{max} 262 nm (ε 12700).

2-Cyano-9-(3-deoxy-β-D-ribofuranosyl)adenine (21) was synthesized from the protected 2-iodo-3'-deoxyadenosine (**9**) by the palladium catalyzed procedure followed by deprotection described for the conversion of **8** to **11**. Product **21** crystallized from CH_3CN as white needles (87% yield): mp 189-191 °C;

^{13}C NMR (Me_2SO-d_6) δ 33.7, 62.1, 75.0, 81.3, 90.9, 116.9, 120.6, 136.7, 141.3, 149.9, 156.1; ^{1}H NMR (Me_2SO-d_6) δ 1.91 (m, 1H), 2.21 (m, 1H), 3.53 (m, 1H), 3.71 (m, 1H), 4.38 (m, 1H), 4.53 (m, 1H), 5.04 (t, 1H), 5.73 (s, 1H), 5.89 (d, 1H), 7.96 (s, 2H), 8.61 (s, 1H); UV (H_2O)λ_{max} 261 (ε 8740), 265.5 (9200), 296 nm (6000); FAB HRMS obsd (M^++H) 277.1059, calcd for $C_{11}H_{13}N_6O_3$ 277.1049.

2-Carboxamido-9-(3-deoxy-β-D-ribofuranosyl)adenine (22) was synthesized by the phase transfer hydrolysis of the silylated 2-cyano compound synthesized from **9** followed by deprotection. The procedure is similar to the preparation of **13** from **8**. Crystallization of **22** from CH_3OH/H_2O afforded white needles (49% yield for 3 steps): mp 227-229°C; ^{13}C NMR (Me_2SO-d_6) δ 33.6, 62.1, 74.8, 80.8, 90.5, 119.2, 140.2, 148.8, 152.2, 155.5, 164.9; ^{1}H NMR (Me_2SO-d_6) δ 1.91 (m, 1H), 2.26 (m, 1H), 3.52 (m, 1H), 3.72 (m, 1H), 4.36 (m, 1H), 4.57 (m, 1H), 5.03 (t, 1H), 5.72 (s, 1H), 5.95 (d, 1H), 7.49 (s, 2H), 7.57 (s, 1H), 7.86 (s, 1H), 8.48 (s, 1H); UV (H_2O) λ_{max} 259 (ε 8000), 264 (8300), 291.5 nm (4500); FAB HRMS obsd (M^++H) 295.1118, calcd for $C_{11}H_{14}N_6O_4$ 295.1154.

2-Vinyl-9-(3-deoxy-β-D-ribofuranosyl)adenine (23) was synthesized from **9** in a procedure similar to the conversion of **8** to **15**. Purification by reverse-phase HPLC with 11% ethanol/water followed by 15% ethanol/water afforded 0.039g (0.14 mmol, 72%) of the title nucleoside **23** as a white solid (63% yield for 2 steps): mp 86-88°C; ^{13}C NMR (Me_2SO-d_6) δ 34.2, 40.1, 62.7, 74.2, 80.3, 90.5, 118.1, 120.8, 137.1, 139.5, 149.3, 155.5, 157.6; ^{1}H NMR (Me_2SO-d_6) δ 1.95 (m, 1H), 2.30 (m, 1H), 3.52 (m, 1H), 3.65 (m, 1H), 4.34 (m, 1H), 4.61 (m, 1H), 5.14 (t, 1H), 5.54 (dd, 1H), 5.64 (s, 1H), 5.86 (d, 1H), 6.36 (dd, 1H), 6.61 (dd, 1H), 7.23 (s, 2H), 8.32 (s, 1H); UV (H_2O) λ_{max} 265.5 (ε 11300), 270 (11200), 292(s) nm (6300); FAB HRMS obsd (M^++H) 278.1278, calcd for $C_{12}H_{16}N_5O_3$ 278.1253.

2-Ethynyl-9-(3-deoxy-β-D-ribofuranosyl)adenine (24) was prepared in 72%

yield from **9** as described above for the conversion of **8** to **18**: mp 122-124°C; ^{1}H NMR (Me_2SO-d_6) δ 1.92 (m, 1H), 2.23 (m, 1H), 3.41 (m, 1H), 3.53 (m, 1H), 3.96 (s, 1H), 4.37 (m, 1H), 4.54 (m, 1H), 5.01 (t, 1H), 5.63 (s, 1H), 5.85 (d, 1H, J=2.1 Hz), 7.42 (s, 2H), 8.42 (s, 1H); UV (H_2O) λ_{max} 263 (9600), 268 (10 000), 287 nm (6300); FAB HRMS obsd (M^++H) 276.1128, calcd for $C_{12}H_{14}N_5O_3$ 276.1096.

2-Allyl-9-(3-deoxy-β-D-ribofuranosyl)adenine (25) was prepared in 79% yield from **20** as described for the conversion of **14** to **19**: mp 73-75°C; ^{1}H NMR (Me_2SO-d_6) δ 1.97 (m, 1H), 2.30 (m, 1H), 3.43 (dm, 2H), 3.67 (m, 1H), 3.79 (m, 1H), 4.32 (m, 1H), 4.60 (m, 1H), 5.11 (ddm, 2H), 5.22 (t, 1H), 5.61 (d, 1H), 5.82 (d, 1H, J=2.85 Hz), 6.11 (m, 1H), 7.22 (s, 2H), 8.26 (s, 1H); UV (H_2O) λ_{max} 262 nm (ε 11000); FAB HRMS obsd (M^++H) 292.1422, calcd for $C_{13}H_{18}N_5O_3$ 292.1409.

2-Amino-6-chloro-9-(3,5-O-tetraisopropyldisiloxane-β-D-ribofuranosyl) purine (26). To a stirred mixture of the nucleoside **2** (1.934 g, 6.47 mmol), imidazole (0.990 g, 14.56 mmol) in dry DMF was added 2.14 ml (2.14 g, 6.80 mmol) of TPDS-Cl_2. The mixture was stirred at room temp for 45 min. The solvent was removed under reduced pressure, the residue dissolved in ether (40 mL) and extracted with H_2O (2 X 40 mL). The organic layer was dried (Na_2SO_4), concentrated <u>in vacuo</u>, and purified by flash chromatography with $CHCl_3$ followed by 2% MeOH / $CHCl_3$ to afford 2.162 g (3.97 mmol, 62%) of product as a white foam: ^{1}H NMR ($CDCl_3$) δ 1.07 (m, 28H), 3.03 (s, 1H), 4.06 (m, 3H), 4.48 (m, 1H), 4.75 (m, 1H), 5.11 (s, 2H), 5.89 (d, 1H, J=1.6 Hz), 7.92 (s, 1H); UV (EtOH) λ_{max} 247, 308 nm; mass spectrum, m/z (relative intensity) 544 (M^+, 5.3), 501 (M^+-iPr, 64.4).

2-Iodo-9-(2-deoxy-3,5-O-tetraisopropyldisiloxane-β-D-ribofuranosyl) adenine (30). Nucleoside **26** was deoxygenated, iodinated, and aminated as described for the conversion of **4** to **8**. The overall yield from **26** to **30**

was 25%: ^{1}H NMR ($CDCl_3$) δ 1.07 (m, 28H), 2.65 (m, 2H), 3.85 (m, 1H), 4.01 (d, 2H, J=4.0 Hz), 4.85 (q, 1H), 5.68 (s, 2H), 6.20 (dd, 1H, 7.89 (s, 1H); UV (EtOH) λ_{max} 266 nm.

2-Cyano-9-(2-deoxy-β-D-ribofuranosyl)adenine (11) was prepared from **30** as described for the conversion of **8** to **11**.

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Dideoxygenated Purine Nucleosides Substituted at the 8-Position: Chemical Synthesis and Stability[1]

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Synthesis of novel analogues of the largely unknown family of 8-substituted 2',3'-dideoxyadenosines is described. Hydrolytic deamination of two of these analogues by mammalian adenosine deaminase was utilized for an enzymatic synthesis of 2',3'-dideoxyinosine compounds. The glycosidic bond of 8-hydroxy-2',3'-dideoxyadenosine is remarkably stable with respect to hydrolytic cleavage which is very unusual for a dideoxynucleoside.

2',3'-Dideoxygenated analogues of the natural purine and pyrimidine nucleosides are known to be inhibitors of HIV reverse transcriptase, a key enzyme in the replication of the human immunodeficiency virus (HIV-1).[2,3] Recently, efforts have been made to increase the activity of dideoxynucleosides through selective modification of either the carbohydrate or base moieties. For example, dideoxypyrimidine analogues modified in both the ribose and pyrimidine base have been synthesized.[4-7] However, much more emphasis has been placed on carbohydrate modification due to the anti-HIV activity of 3'-azido-3'-deoxythymidine (AZT).[3] Dideoxypurine nucleosides are also of interest as 2',3'-dideoxyadenosine (ddA) has a high anti-HIV selectivity index.[8] Modified dideoxyadenosines have been prepared in the quest for compounds with greater activity. Substituents introduced into the dideoxyribose moiety have generally resulted in decreased selectivity of the dideoxypurine analogues.[9] Dideoxyadenosine analogues with small group modifications at the 2-

and 6-positions were found to retain anti-HIV activity.[8,10,11] Thus, in general, it appears that base modifications are better tolerated than changes in the dideoxyribose moiety. However, very little is known about dideoxynucleosides with specific modifications at the 8-position. With the exception of 8-bromo-2',3'-dideoxyadenosine,[6] no 8-substituted dideoxyadenosine nucleoside is known. This paper reports on a generalized approach to novel dideoxyadenosine congeners with modifications at the 8-position. In addition, the enzymatic synthesis of two 8-substituted 2',3'-dideoxyinosines is also described.

The key precursor for the syntheses was 8-bromoadenosine (**1**), prepared in high yields from adenosine.[12] The halogen atom in **1** is susceptible to displacement by appropriately chosen nucleophiles to provide 8-substituted adenosines. For example, 8-bromoadenosine

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underwent a very facile substitution when treated with sodium thiomethoxide in DMF to give 8-methylthioadenosine (**2a**).[13] Compound **2a** was then dideoxygenated utilizing an approach recently described by us.[14] Thus, the 5'-hydroxyl group of 8-methylthioadenosine was selectively silylated and the hydroxyl groups at the 2' and 3' positions converted to the cyclic thionocarbonate **4a**. Radical deoxygenation of the thionocarbonate with tributyltin hydride and AIBN[15] in refluxing toluene provided the 2'-deoxygenated compound **5a** as the major product. The 3'-hydroxyl group was then deoxygenated via the imidazolide **6a** to provide the protected dideoxynucleoside **7a**. Desilylation of **7a** with tetraethylammonium fluoride in acetonitrile gave 8-methylthio-2',3'-dideoxyadenosine (**8a**). The dideoxynucleoside

8a and the other dideoxynucleosides synthesized in this paper were fully characterized by FTIR, UV, and high-field NMR spectroscopy (see Table).

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Oxygen nucleophiles also displace the halogen of **1**. 8-Methoxyadenosine (**2b**) was prepared by heating **1** with sodium methoxide in methanol.[16] Dideoxygenation of **2b** provided 8-methoxy-2',3'-dideoxyadenosine (**8b**) in 15% overall yield. The benzyloxy group was introduced at the 8-position by heating **1** with the sodium salt of benzyl alcohol to give 8-benzyloxyadenosine (**2c**) in good yield after purification by silica gel chromatography. Compound **2c** was dideoxygenated to the silylated dideoxynucleoside **7c** using the procedure outlined previously. Desilylation of **7c** gave 8-benzyloxy-2',3'-dideoxyadenosine (**8c**).

The benzyloxy group can be considered as the protected form of the corresponding alcohol. Thus, catalytic hydrogenation (10% Pd/C, H_2, EtOH) of **7c** cleanly removed the benzyl group to produce the 8-hydroxy nucleoside **7d**. This compound was desilylated to the novel 8-hydroxy-2',3'-dideoxyadenosine (**8d**). The FTIR (1711 cm^{-1}, lactam carbonyl) and NMR data (Table) suggest that 8-hydroxy-2',3'-dideoxyadenosine exists entirely in the 8-carbonyl form. It should be mentioned that hydrogenation of the ribonucleoside, 8-benzyloxyadenosine (**2c**), provides an alternative route for the synthesis of 8-hydroxyadenosine.[16,17]

8-Bromo-2'-deoxyadenosine (**9a**)[12] served as the key starting material for the preparation of 8-amino-2',3'-dideoxyadenosine (**8e**).

Compound **9a** was heated with sodium azide in DMF to give the 8-azido nucleoside **9b** in 91% yield.[16,18] Silylation of the 5′-hydroxyl was followed by formation of the imidazolide **6d** as previously described. This nucleoside was subjected to the radical deoxygenation reaction which accomplished two things: firstly, the 3′-position was deoxygenated; and secondly, the azido functional group was reduced.[19] Desilylation of the resulting product, **7e**, provided the target molecule, 8-amino-2′,3′-dideoxyadenosine, **8e**, in good yield.

The stability of these new dideoxynucleosides with respect to deamination by the mammalian enzyme, adenosine deaminase, was investigated. This enzyme normally catalyzes the hydrolytic deamination of adenosine to inosine.[20,21] Adenosine deaminase is known to tolerate some substitution at the 8-position of adenosine with retention of minimal substrate activity.[22,23] It was found that 8-hydroxy ddA (**8d**) and 8-amino ddA (**8e**) were deaminated by adenosine deaminase to the corresponding 8-substituted dideoxyinosine analogues **10a** and **10b**. The 8-substituent slows the rate of deamination to about 2% of the rate for the deamination of the natural substrate, adenosine. The methylthio, methoxy, and benzyloxy groups at the 8-position gave ddA analogues which were totally resistant to deamination by adenosine deaminase. Finally, it should be mentioned that the glycosidic bond of 8-hydroxy-2′,3′-dideoxyadenosine (**8d**) is stable towards hydrolytic cleavage even at pH 1 which is very unusual for a dideoxynucleoside. This remarkable stability of **8d** may be a result of the preferred lactam structure of the imidazole ring.

Melting points reported are uncorrected and were determined on a Thomas-Hoover apparatus fitted with a microscope. Nuclear magnetic

resonance spectra were recorded on JEOL Model FX 90Q and Bruker Model WM 360 and MSL 300 instruments. FTIR spectra were recorded on a Mattson Cygnus 25 Fourier transform instrument. UV data were determined using a Gilford Response spectrophotometer. Preparative layer chromatography plates were prepared by coating six 20 cm X 20 cm plates with a slurry made from 140 g of E. Merck PF_{254} silica gel in 400 mL of water. The silica gel plates were allowed to dry slowly and were then activated for 3 h at 135 °C. Flash chromatography was carried out in glass columns packed with 230-400 mesh silica gel. Elemental analyses were determined by Galbraith Laboratories, Inc., Knoxville, TN.

2',3'-Dideoxy-8-methylthioadenosine (8a):

Preparation of **2a**[13]: To a solution of 8-bromoadenosine (**1**)[12] (0.502g, 1.45 mmol) in dry DMF (10 ml) is added sodium thiomethoxide (0.203 g, 2.90 mmol) and the solution is stirred for 2 h at room temperature. The mixture is neutralized with 1 N hydrochloric acid and the solvent evaporated under reduced pressure. The residue is purified by preparative layer chromatography using 10% methanol/chloroform as the eluting solvent to give **2a**; yield: 0.417 g (92%); mp 235-237 °C (lit.[13] mp 230-233 °C).

^{1}H-NMR (Me_2SO-d_6) δ = 2.72 (s, 3H); 3.55 (m, 2H); 3.96 (m, 1H); 4.10 (m, 1H); 4.95-5.14 (m, 2H); 5.35-5.69 (m, 3H); 7.23 (br. s, 2H); 8.05 (s, 1H).

UV (EtOH) λ_{max} = 279 nm.

Preparation of **3a**: To a flask containing **2a** (0.809 g, 2.58 mmol) is

added t-butyldimethylsilyl chloride (0.467 g, 3.09 mmol), N,N-dimethylaminopyridine (0.0407 g, 0.39 mmol), triethylamine (0.37 mL, 2.66 mmol), dichloromethane (10 mL), and DMF (20 mL), and the resulting mixture stirred for 12 h at room temperature. The solvents are evaporated and the residue is purified by flash chromatography (5% methanol/chloroform) to give **3a** as a low melting solid; yield: 0.863 g (78%).

^{1}H-NMR (Me_2SO-d_6) δ= -0.03 (s, 6H); 0.81 (s, 9H); 2.71 (s, 3H); 3.85 (m, 3H); 4.31 (m, 1H); 5.13-5.35 (m, 3H); 5.73 (d, 1H); 7.09 (br. s, 2H); 8.05 (s, 1H).

Preparation of **4a**: A solution of **3a** (0.863 g, 2.02 mmol) and 1,1'-thiocarbonyldiimidazole (0.680 g, 3.43 mmol) in dry DMF (15 mL) is stirred for 6 h at room temperature. The solvent is evaporated and the residue is purified by flash chromatography using 0-5% methanol/chloroform to provide **4a** as a low melting solid; yield: 0.821 g (87%).

^{1}H-NMR (Me_2SO-d_6) δ = -0.11 (s, 6H); 0.78 (s, 9H); 2.72 (s, 3H); 3.64 (m, 2H); 4.48 (m, 1H); 5.85 (m, 1H); 6.27 (m, 1H); 6.53 (m, 1H); 7.27 (br. s, 2H); 8.07 (s, 1H).

UV (EtOH) λ_{max} = 279 nm.

Preparation of **5a**: A nitrogen-purged solution of tributyltin hydride (1.64 mL, 6.11 mmol) and AIBN (0.229 g, 1.39 mmol) in toluene (30 mL) is added dropwise over 40 minutes to a refluxing solution of **4a** (0.820 g, 1.75 mmol) in toluene (40 mL). The reaction mixture is stirred at reflux for 2.5 h. The solvent is then evaporated under reduced pressure. The residue is purified by flash chromatography using hexanes followed by 0-5% methanol/chloroform to give **5a** as an oil;

yield : 0.442 g (61%).

^{1}H-NMR (Me_2SO-d_6) δ= -0.06 (s, 6H); 0.81 (s, 9H); 2.25 (m, 2H); 2.71 (s, 3H); 3.74 (m, 3H); 4.36 (m, 1H); 5.30 (m, 1H); 6.21 (m, 1H); 7.06 (br. s, 2H); 8.05 (s, 1H).

Preparation of **6a**: A solution of **5a** (0.442 g, 1.07 mmol) and 1,1'-thiocarbonyldiimidazole (0.361g, 1.82 mmol) in dry DMF (10 mL) is stirred for 5 h at 90 °C. Evaporation of the solvent gives a residue which is purified on silica gel with 0-5% methanol/chloroform to give **6a** as a low melting solid; yield: 0.420 g (76%).

^{1}H-NMR (Me_2SO-d_6) δ= -0.05 (s, 6H); 0.79 (s, 9H); 2.73 (s, 3H); 3.87 (m, 2H); 4.25 (m, 1H); 6.37 (m, 1H); 7.12 (br. s, 3H); 7.89 (s, 1H); 8.08 (s, 1H); 8.58 (s, 1H).

Preparation of **7a**: To a refluxing solution of **6a** (0.420 g, 0.81 mmol) in toluene (40 mL) is added dropwise over 30 minutes a solution of tributyltin hydride (0.65 mL, 2.42 mmol) and AIBN (0.105 g, 0.64 mmol) in toluene (30 mL). The reaction mixture is stirred for 1.5 h at toluene reflux. The solvent is evaporated and the residue purified by preparative layer chromatography (5% methanol/chloroform) to give **7a** as a low melting solid; yield: 0.151 g (47%).

^{1}H-NMR (Me_2SO-d_6) δ = -0.10 (s, 6H); 0.78 (s, 9H); 2.20 (m, 4H); 2.70 (s, 3H); 3.70 (m, 2H); 4.01 (m, 1H); 6.08 (m, 1H); 7.02 (br. s, 2H); 8.05 (s, 1H).

UV λ_{max} = 278 nm.

Preparation of **8a**: To a solution of **7a** (0.150 g, 0.38 mmol) in acetonitrile (15 mL) is added tetraethylammonium fluoride (0.5 M, 2.30 mL) and the resulting solution is stirred for 3 h at room temperature.

Evaporation of the solvent gives a residue which is purified by preparative layer chromatography (10% methanol/chloroform) to give **8a**; yield: 0.080 g (74%).

$C_{11}H_{15}N_5O_2S$ (281.3) calc. C 46.93 H 5.37 N 24.89
found C 46.31 H 5.16 N 24.54

2',3'-Dideoxy-8-methoxyadenosine (8b):

Preparation of **2b**[16]: A solution of 8-bromoadenosine (1.679 g, 4.85 mmol) and sodium methoxide (0.688 g, 12.73 mmol) in methanol (60 mL) is stirred at reflux for 23 h. The solvent is evaporated and the residue is purified by flash chromatography using 10% methanol/chloroform to give 8-methoxyadenosine (**2b**); yield: 1.145 g (79%); mp 205-207 °C (lit.[16] mp 206-208 °C).

^{1}H-NMR (Me_2SO-d_6) δ = 3.59 (m, 2H); 3.92 (m, 1H); 4.11 (m, 4H); 5.10 (d, 1H); 5.30-5.48 (m, 2H); 5.71 (d, 1H); 6.95 (br. s, 2H); 8.03 (s, 1H).

UV (EtOH) λ_{max} = 261 nm.

Preparation of **4b**: Using the procedures described for the preparation of **4a** from **2a**, 8-methoxyadenosine (1.132 g, 3.81 mmol) is silylated and converted to the thionocarbonate **4b** as a low melting solid; yield: 0.832 g (48%, 2 steps).

^{1}H-NMR (Me_2SO-d_6) δ = -0.09 (s, 6H); 0.79 (s, 9H); 3.63 (d, 2H, J = 6.8 Hz); 4.12 (s, 3H); 4.42 (m, 1H); 5.80 (dd, 1H, J = 2.9, 7.8 Hz); 6.21 (d, 1H, J = 1.5 Hz); 6.45 (dd, 1H, J = 1.5, 7.3 Hz); 7.02 (br. s, 2H); 8.04 (s, 1H).

Preparation of **7b**: Compound **4b** (0.830 g, 1.83 mmol) is dideoxygenated as previously described to give **7b** as a low melting solid; yield: 0.358 g (51.5%, 3 steps).

^{1}H-NMR (Me_2SO-d_6) δ = -0.07 (s, 6H); 0.79 (s, 9H); 2.21-2.77 (m, 4H); 3.64 (m, 2H); 4.09 (m, 4H); 6.10 (m, 1H); 6.79 (br. s, 2H); 8.03 (s, 1H).

UV (EtOH) λ_{max} = 260 nm.

Preparation of **8b**: Compound **7b** (0.152 g, 0.40 mmol) is desilylated as described for the preparation of **8a** to give **8b**; yield: 0.0825 g (79%).

$C_{11}H_{15}N_5O_3$	calc.	C 49.81	H 5.70	N 26.40
(265.3)	found	C 49.52	H 5.81	N 26.19

2′,3′-Dideoxy-8-benzyloxyadenosine (8c):

Preparation of **4c**: 8-Bromoadenosine (**1**) (2.122 g, 6.13 mmol) is added to a mixture of sodium (0.500 g, 21.7 mmol) in benzyl alcohol (12.5 ml) and DMF (40 ml) and the resulting mixture stirred for 1.5 h at 80 °C. The reaction mixture is neutralized with glacial acetic acid and the solvents evaporated. The residue is purified on silica gel with 5-10% methanol/chloroform to provide **2c**; yield: 1.40 g (61%). Compound **2c** (1.333 g, 3.57 mmol) is then silylated and converted to the thionocarbonate as previously described to give **4c** as a low melting solid; yield: 1.225 g (65%).

^{1}H-NMR (Me_2SO-d_6) δ = -0.13 (s, 6H); 0.77 (s, 9H); 3.60 (d, 2H); 4.36 (m, 1H); 5.55 (s, 2H); 5.71 (dd, 1H); 6.25 (m, 1H); 6.48 (m, 1H); 7.02 (br. s, 2H); 7.45 (m, 5H); 8.05 (s, 1H).

Preparation of **7c**: Compound **4c** (1.225 g, 2.31 mmol) is dideoxygenated as described previously to give **7c** as a low melting solid; yield:

0.534 g (51%).

^{1}H-NMR (Me_2SO-d_6) δ = -0.10 (s, 6H); 0.78 (s, 9H); 2.12 (m, 4H); 3.55 (d, 2H); 4.00 (m, 1H); 5.54 (s, 2H); 6.14 (m, 1H); 6.79 (br. s, 2H); 7.44 (m, 5H); 8.05 (s, 1H).

UV (EtOH) λ_{max} = 259 nm.

Preparation of **8c**: Compound **7c** (0.099 g, 0.22 mmol) is desilylated following the procedure for preparing **8a** to give **8c**; yield: 0.061 g (82%).

$C_{17}H_{19}N_5O_3 \cdot 1/2H_2O$	calc.	C 58.28	H 5.75	N 19.99
(341.4)	found	C 58.30	H 5.60	N 19.58

2',3'-Dideoxy-8-hydroxyadenosine (8d):

Preparation of **7d**: To a solution of **7c** (0.429 g, 0.94 mmol) in absolute ethanol (75 mL) is added 10% palladium on charcoal (0.290 g) and the resulting mixture shaken under hydrogen (36 psi) for 17 h. The catalyst is removed by filtration and the solvent evaporated. Purification of the residue by preparative layer chromatography (7% methanol/chloroform) provides **7d** as a low melting solid; yield: 0.226 g (66%).

^{1}H-NMR (Me_2SO-d_6) δ = -0.04 (s, 6H); 0.82 (s, 9H); 2.22 (m, 4H); 3.70 (m, 2H); 3.99 (m, 1H); 6.01 (m, 1H); 6.43 (br. s, 2H); 8.01 (s, 1H).

UV (EtOH) λ_{max} = 270 nm, 256 nm.

Preparation of **8d**: Compound **7d** (0.223 g, 0.61 mmol) is desilylated via the procedure previously described for **8a** to give **8d**; yield: 0.0922 g (60%).

$C_{10}H_{13}N_5O_3$	calc.	C 47.81	H 5.22	N 27.88

(251.2) found C 47.33 H 5.16 N 26.77

FAB HRMS calc. (M^+ + H) 252.1097, found 252.1071.

2′,3′-Dideoxy-8-aminoadenosine (8e):

Preparation of **9b**: To a solution of 8-bromo-2′-deoxyadenosine (**9a**)[12] (0.403 g, 1.22 mmol) in DMF (25 mL) is added sodium azide (0.278 g, 4.27 mmol) and the mixture stirred for 1.5 h at 90 °C. The solvent is removed and the residue purified by flash chromatography (10% methanol/chloroform) to give **9b**; yield: 0.325 g (91%); mp dec. >185 °C.

UV (EtOH) λ_{max} = 282.5 nm.

FTIR (KBr) ν = 2155 cm^{-1}.

Preparation of **6d**: Compound **6d** (low melting solid) is formed in 61% from **9b** via silylation of the 5′-hydroxyl group and formation of the 3′-imidazolide as described for the preparation of **6a**.

UV λ_{max} = 278 nm.

FTIR (KBr) ν = 2145 cm^{-1}.

Preparation of **7e**: Compound **6d** (0.193 g, 0.37 mmol) is deoxygenated as previously described to give **7e** as a low melting solid; yield: 0.1056 g (77%).

^{1}H-NMR (Me_2SO-d_6) δ = 0.00 (s, 6H); 0.84 (s, 9H); 2.16 (m, 4H); 3.73 (m, 2H); 4.09 (m, 2H); 6.06 (m, 1H); 6.33 (br. s, 4H); 7.89 (s, 1H).

UV (EtOH) λ_{max} = 273.5 nm.

Preparation of **8e**: Desilylation of **7e** (0.1014 g, 0.28 mmol) provides **8e**; yield: 0.064 g (92%).

$C_{10}H_{14}N_4O_2 \cdot 1/2H_2O$ calc. C 46.33 H 5.83 N 32.41

(251.2) found C 46.56 H 5.48 N 31.98

2′,3′-Dideoxy-8-hydroxyinosine (10a):

Compound **8d** (0.021 g, 0.08 mmol) is dissolved in water and excess adenosine deaminase (Type 1 from calf intestinal mucosa, Sigma) is added. The reaction is allowed to stand at room temperature and is monitored by UV spectroscopy.[24] After 12 h the water is evaporated and the residue is purified by preparative layer chromatography (10% methanol/chloroform) to give **10a**; yield: 0.0166 g (79%); mp 181-183 °C.

^{1}H-NMR (Me_2SO-d_6) δ = 2.00 (m, 1H); 2.19 (m, 2H); 2.59 (m, 1H); 3.51 (m, 2H); 3.98 (m, 1H); 4.80 (br. s, 1H); 5.96 (m, 1H); 7.96 (s, 1H).

UV (H_2O) λ_{max} = 256 nm.

FTIR (KBr) ν = 1716, 1680 cm^{-1}.

2′,3′-Dideoxy-8-aminoinosine (10b):

Compound **8e** (0.0598 g, 0.24 mmol) is dissolved in water and excess adenosine deaminase is added. The reaction mixture is allowed to stand at room temperature for 4 h. The water is evaporated and the residue purified by preparative layer chromatography (10% methanol/chloroform) to give **10b**; yield: 0.044 g (74%); mp dec >230 °C.

^{1}H-NMR (Me_2SO-d_6) δ = 2.22 (m, 4H); 3.73 (m, 2H); 4.10 (m, 1H); 5.30 (m, 1H); 6.10 (m, 1H); 6.44 (br. s, 2H); 7.78 (s, 1H).

UV (H_2O) λ_{max} = 266.5 nm.

FTIR (KBr) ν = 1684 cm^{-1}.

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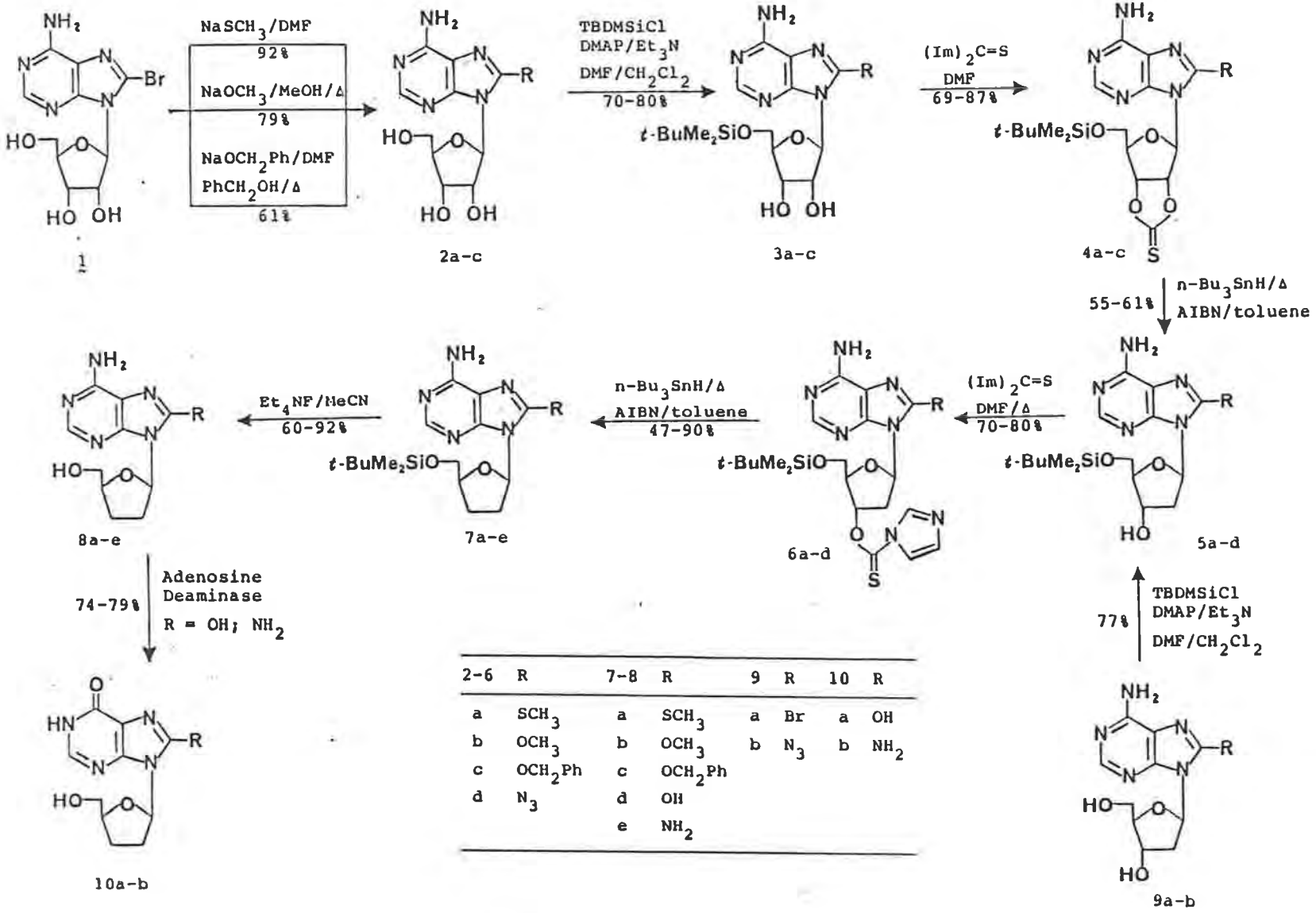
Table. Physical and Spectral Data for 8-Substituted 2',3'-Dideoxyadenosines

Product	Yield[a] (%)	mp[c] (°C)	Molecular Formula[d]	UV (H_2O) λ_{max} (ϵ)	IR (KBr)[e] ν(cm^{-1})	^{1}H-NMR[f] δ(ppm)	^{13}C-NMR[f] δ(ppm)
8a	10	197-199	$C_{11}H_{15}N_5O_2S$ (281.3)	277 (15300)	1651, 1600 1292, 1052	2.20 (m, 4H); 2.71 (s, 3H); 3.51 (m, 2H); 4.09 (m, 1H); 5.11 (m, 1H); 6.09 (m, 1H); 7.12 (br.s, 2H); 8.06 (s, 1H)	14.5; 26.9; 29.1; 63.9; 81.1; 85.3; 119.4; 149.2; 150.8; 151.3; 154.3
8b	15	197-199	$C_{11}H_{15}N_5O_3$ (265.3)	260 (11040)	1643, 1610 1393, 1055	2.16 (m, 4H); 3.51 (m, 2H); 4.10 (m, 4H); 5.01 (m, 1H); 6.08 (m, 1H); 6.85 (br.s, 2H); 8.03 (s, 1H)	27.0; 28.9; 57.2; 64.0; 81.1; 83.3; 114.8; 148.8; 150.7; 153.9; 154.3
8c	16	149-151	$C_{17}H_{19}N_5O_3$ (341.4)	261 (13900)	1655, 1612 1556, 1354	2.08 (m, 4H); 3.43 (m, 2H); 4.05 (m, 1H); 4.94 (m, 1H); 5.54 (s, 2H); 6.10 (m, 1H); 6.84 (br.s, 2H); 7.44 (m, 5H); 8.04 (s, 1H)	26.9; 28.8; 63.9; 71.2; 81.0; 83.1; 114.7; 128.4; 128.5; 135.4; 148.7; 150.7; 153.5; 153.9
8d	8	204-206	$C_{10}H_{13}N_5O_3$ (251.2)	265 (9500)	1711, 1653	2.10 (m, 4H); 3.51 (m, 2H); 4.01 (m, 1H); 4.90 (m, 1H); 5.99 (m, 1H); 6.48 (br.s, 2H); 7.24 (br.s, 1H); 8.02 (s, 1H)	27.0; 28.0; 64.1; 80.6; 82.3; 103.5; 146.6; 147.1; 150.7; 151.4
8e	39[b]	177-179	$C_{10}H_{14}N_6O_2$ (251.2)	273.5 (13900)	3329, 3184 1643	2.16 (m, 4H); 3.61 (m, 2H); 4.11 (m, 1H); 5.54 (m, 1H); 6.10 (m, 1H); 6.40 (br.s, 2H); 6.52 (br.s, 2H); 7.89 (s, 1H)	26.0; 29.0; 63.1; 79.5; 83.9; 117.1; 148.4; 148.9; 151.4; 152.3

a) Overall yield of isolated product from 1.
b) Overall yield of isolated product from 9a.
c) Uncorrected.
d) Satisfactory microanalyses obtained.
e) Recorded on a Mattson Cygnus 25 FTIR spectrometer.
f) In DMSO-d_6, with TMS as internal reference.



2-6	R	7-8	R	9	R	10	R
a	SCH_3	a	SCH_3	a	Br	a	OH
b	OCH_3	b	OCH_3	b	N_3	b	NH_2
c	OCH_2Ph	c	OCH_2Ph				
d	N_3	d	OH				
		e	NH_2				

HYPOXANTHINE NUCLEOSIDE COUNTERPARTS OF THE ANTIBIOTIC, CORDYCEPIN

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Abstract: Analogues of 3′-deoxyinosine, although of potential RNA antiviral interest, are virtually unknown. This paper reports on approaches to the synthesis of base-modified hypoxanthine 3′-deoxynucleosides. All of the target compounds are new and contain functionality at the 2-position that can be further elaborated for the synthesis of a variety of other novel analogues of 3′-deoxyinosine. Intact natural guanosine was used as the precursor and the key transformations utilized were regioselective bis-silylation, thermal radical deoxygenation, regiospecific radical halogenation, metal-mediated functionalization, and selective ozonolysis. The synthetic approaches described have considerable generality in terms of entry to novel analogues of 3′-deoxyinosine.

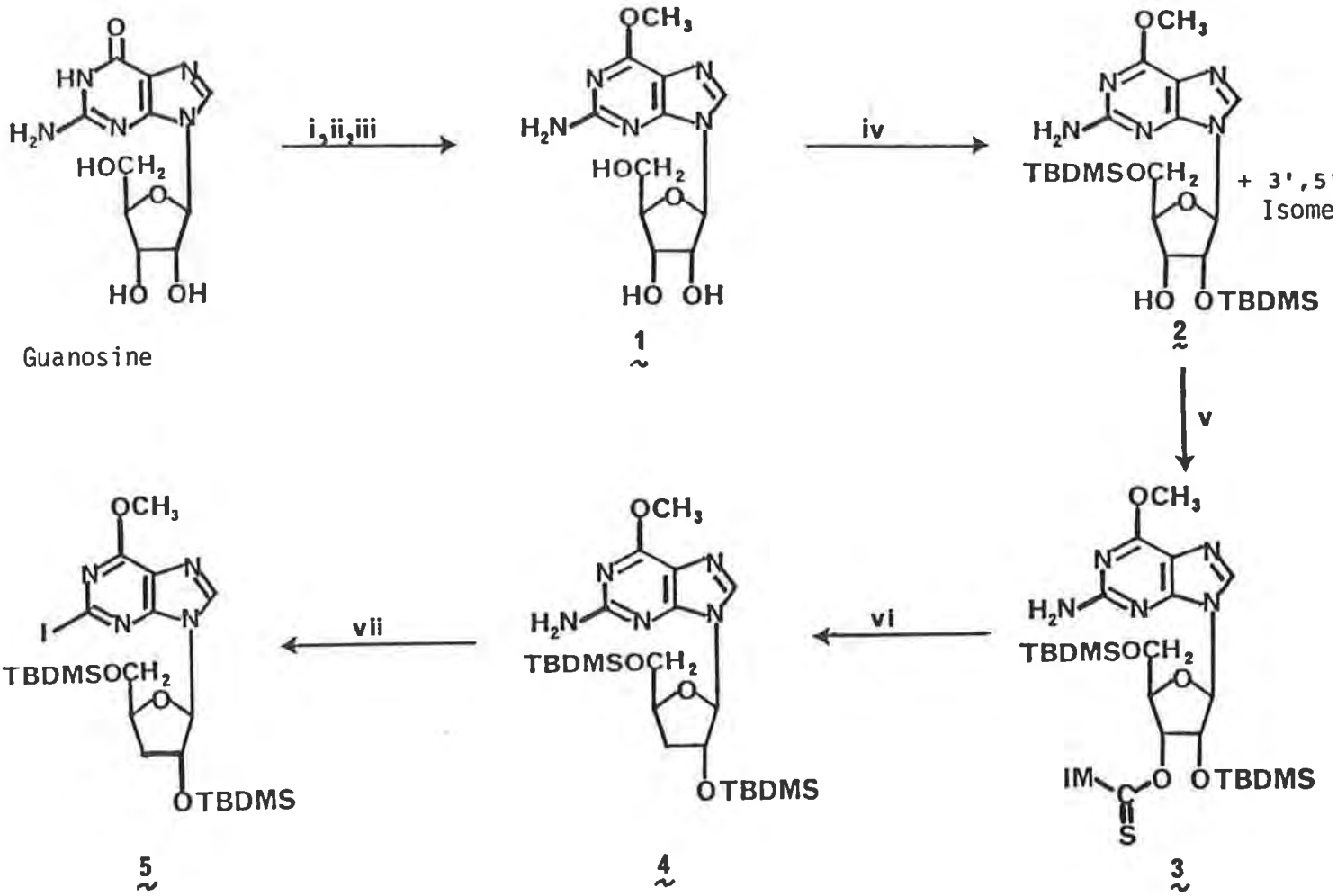
While numerous investigations have been carried out on the synthesis and modifications of purine ribonucleosides, the same cannot be said for their deoxygenated counterparts. This is particularly true for purine 3′-deoxynucleosides which are of potential biological interest as antiviral agents and as inhibitors of key purine metabolizing enzymes. For example, very little is known about hypoxanthine or guanine 3′-deoxynucleosides. The corresponding 2′-deoxynucleosides of the guanine family have received somewhat more attention.[2-5] 2′-Deoxyguanosine (presumably as its triphosphate) inhibits the growth of mouse lymphoma T-cell lines and is inhibitory to a number of viruses.[5-7] A derivative of this compound, N^2-phenyl-2′-deoxyguanosine, shows significant antiviral activity against the Herpes Simplex Virus (Type I).[8] 2′-Deoxyguanosine

and 2′-deoxyinosine are toxic to a number of microorganisms.[9-11] The triphosphate of 2′-deoxyguanosine is a potent inhibitor of mammalian ribonucleotide reductase.[12] In the adenine family of 3′-deoxynucleosides, the most studied of the few compounds known is the nucleoside antibiotic, cordycepin (3′-deoxyadenosine). This compound has RNA antiviral activity and is an inhibitor of viral RNA polymerase in its phosphorylated form.[13] This paper reports on the synthesis of analogues of the hypoxanthine counterpart of the antiviral compound, cordycepin. The doubly modified nucleosides have not been described before and are of interest, not only as potential antiviral agents (in their triphosphate forms), but also as potential inhibitors (in their monophosphate forms) of a key enzyme in purine metabolism, inosine monophosphate dehydrogenase.[14]

Two rational approaches can be envisaged for the synthesis of these compounds from intact ribonucleosides through modifications involving both the carbohydrate and aglycon moieties. Modifications may be carried out on the carbohydrate component first followed by alterations in the base moiety or this order may be reversed. We chose to use the first approach as several of the functional groups to be placed at the 2-position were expected to be sensitive to the reactions to be used for the alteration of the carbohydrate portion.

An important precursor for the carbohydrate modification was the O^6-methylated guanosine **1**.[15] This compound can be prepared in three steps from guanosine with an overall yield of 80%.[15,16] The hydroxyl groups on the ribose were partially protected with tert-butyldimethylsilyl groups to give compound **2** and its 3′,5′-isomer in a combined yield of 53%. An additional 31% of the total product was due to the 5′-monosilylated compound. In practice, this compound was recycled using 1.1 eq. of TBDMSCl, and 2.2 eq. of imidazole to produce additional disilyl compounds. Total conversions were over 80%. The desired 2′,5′-disilylated compound **2**

was separated from its 3′,5′-isomer by careful column chromatography on silica gel using a gradient of 20-50% ether/ hexanes as the eluting solvent. Compound **2** was deoxygenated <u>via</u> its imidazolides **3**. The latter could be synthesized in almost quantitative yield by reaction of **3** with thiocarbonyldiimidazole and DMAP in dry DMF at room temperature for 12 h. Treatment of compound **3** with tri-n-butyltin hydride and AIBN in refluxing toluene[17] furnished the deoxygenated, protected guanosine intermediate **4** in 98% yield (Scheme 1).



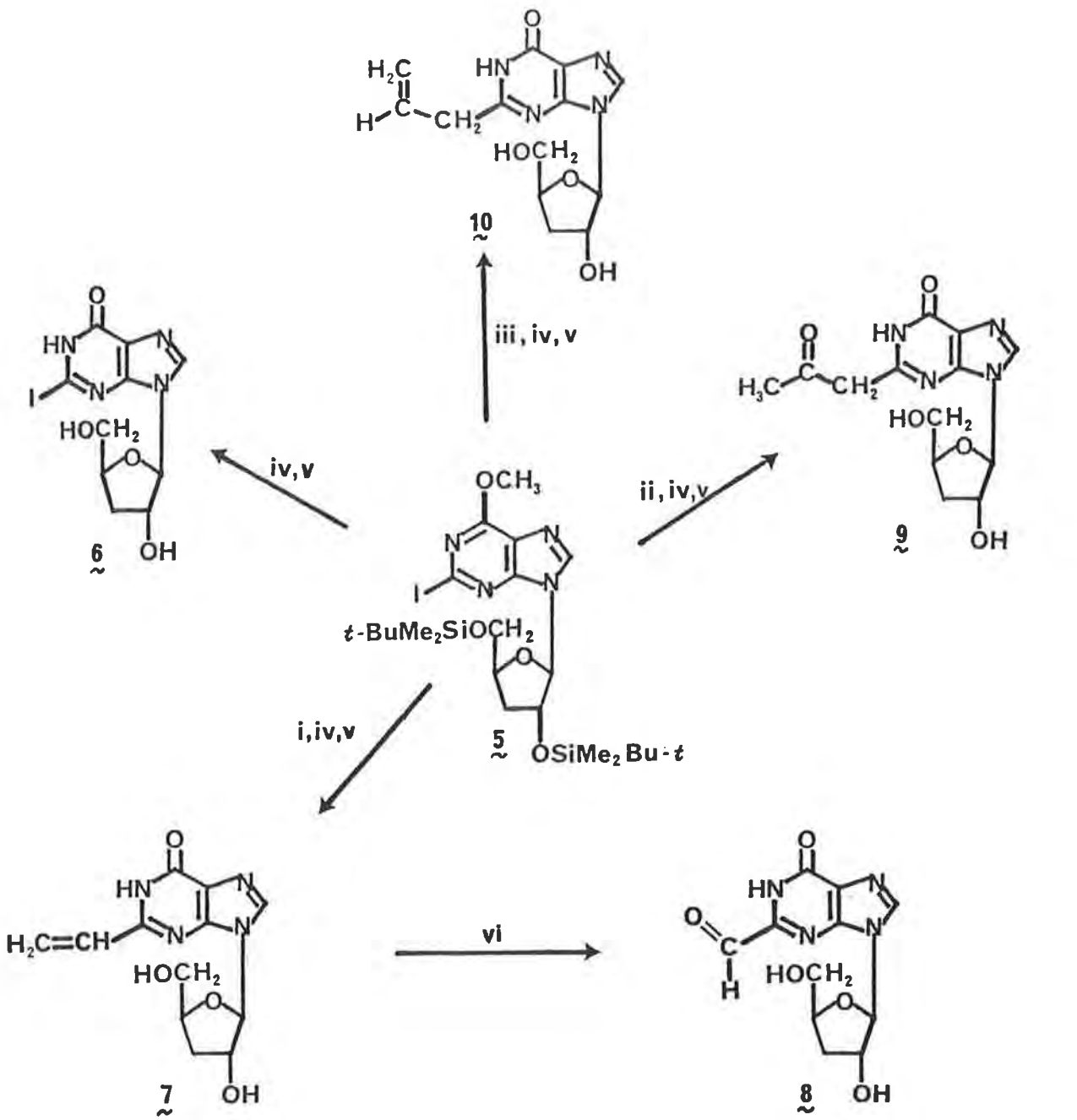
Scheme 1

The carbohydrate moiety having being modified, the next stage of the synthesis was to tailor the base moiety for alteration. This was done through the 2-iodo compound **5** which was prepared by treatment of **4** with diiodomethane, tert-butylnitrite, and trimethylsilyl iodide in dry hexane at 50 ^{o}C[18]. The 2-iodo intermediate **5** was the immediate precursor for the synthesis of the doubly modified hypoxanthine nucleosides. For example, the novel 2-iodo-3'-deoxyinosine (**6**) was synthesized from **5** by a two-step deprotection process: first, treatment of compound **5** with TMSCl and KI in acetonitrile resulted in demethylation at the O^6 position; second, treatment of the resulting product with tetraethylammonium fluoride in acetonitrile deprotected the silyl groups on the carbohydrate moiety. Compound **6** was isolated in a 39% overall yield from **5**.

Modification of the base moiety was carried out through palladium-catalyzed cross-coupling reactions on **5** with a variety of organostannanes.[19] Thus, treatment of compound **5** with $PdCl_2(CH_3CN)_2$ and n-$Bu_3SnCH{=}CH_2$ in dry toluene at 90 ^{o}C furnished the fully protected 2-vinyl compound in 97% yield. The two step deprotection gave 2-vinyl-2'-deoxyinosine **7** (Scheme 2).

Modified nucleosides bearing formyl groups on the base moiety have scarcely been investigated. 2-Formyl-3'-deoxyinosine (**8**), was easily synthesized from the 2-vinyl compound **7** in 77% yield by oxidation of the exocyclic ethylenic moiety with ozone. The high-field carbon-13 NMR data of **8** showed that it existed in two forms in equilibrium, the carbonyl and its hydrated (geminal diol) form. Interestingly, ozonolysis reactions have rarely been used for the elaboration of nucleosides.

Another novel deoxyinosine bearing a carbonyl group at the 2-position was also synthesized. This was 2-acetonyl-2'-deoxyinosine (**9**) which, like the 2-vinyl compound, was also prepared by metal-catalyzed methods (88% yield). In this case, the cross-coupling organostannane reagent, tri-n-



(i) n-$Bu_3SnCH{=}CH_2$, $PdCl_2(CH_3CN)_2$, $P(o\text{-tolyl})_3$, Toluene, Δ; (ii) n-Bu_3SnOCH_3, $P(o\text{-tolyl})_3$, $CH_3C(OAc){=}CH_2$, $PdCl_2(CH_3CN)_2$, Toluene, Δ; (iii) $PdCl_2(CH_3CN)_2$, $P(o\text{-tolyl})_3$, n-$Bu_3SnCH_2CH{=}CH_2$, Toluene, Δ; (iv) TMSCl, KI, CH_3CN; (v) $Et_4N^+F^-$, CH_3CN; (vi) O_3, H_2O

Scheme 2

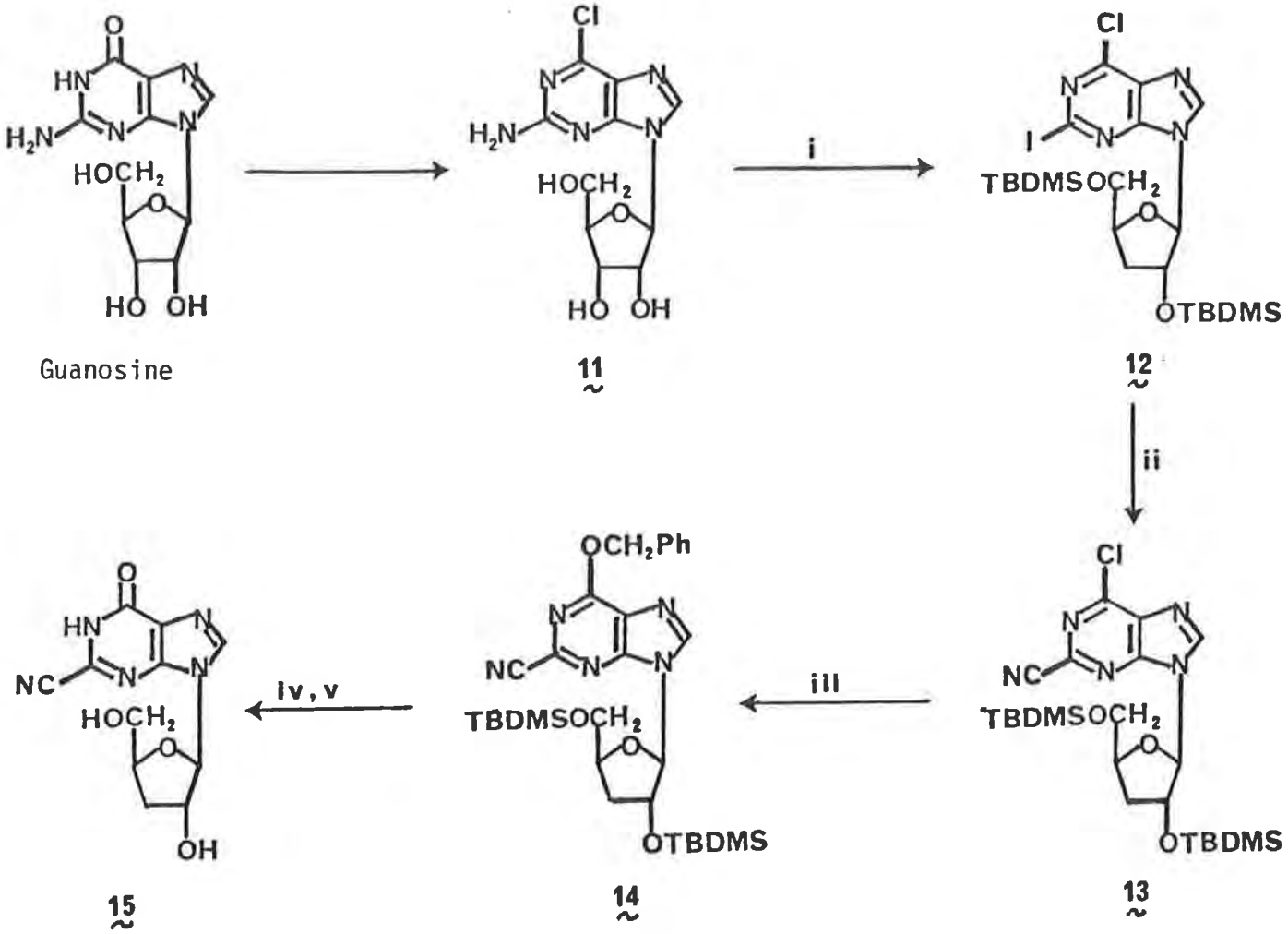
butylacetonylstannane, was generated <u>in situ</u> by reaction of tri-n-butyltin methoxide with isopropenyl acetate. Double deprotection gave the 2-acetonyl-3'-deoxyinosine **9**. The 2-allyl compound **10** was produced by using

allyltri-n-butylstannane as the transmetalation reagent in the palladium reaction followed by deprotection of the product. It is important in this reaction to maintain the temperature at about 95 °C, i.e. sufficient to surmount the barrier for the metal-mediated reaction, but not high enough for the isomerization of the allyl group in the product to the thermodynamically more stable methylvinyl system.

Introduction of a cyano group at the 2-position of 3'-deoxyinosine was of interest because of the potential of this group to be a precursor for the corresponding carboxylic acid, amide, amine, amidine, and other functionalities. Initially, we had attempted to use compound **5** as an immediate precursor for the introduction of the cyano group. While this reaction was successful, considerable decomposition of product occurred at the demethylation step with trimethylsilyl iodide. An alternative masking group for the lactam carbonyl, the benzyl group, was then chosen. This group can be removed by catalytic hydrogenation using Pd/C thus eliminating the aforementioned complications. The 2-iodo-6-chloro-3'-deoxy precursor **12** was prepared in four steps from 2-amino-6-chloropurine ribonucleoside (**11**),[15] using the series of reactions described for the conversion of **1** to **5**. Treatment of **12** with n-Bu_3SnCN, $Pd(PPh_3)_4$ in toluene at 100 °C produced the 2-cyano-6-chloro compound **13** in 77% yield (Scheme 3). For the introduction of oxygen at the 6-position, compound **13** was treated with the sodium salt of benzyl alcohol which furnished the 6-benzyl derivative **14** in 85% yield. Catalytic hydrogenation of **14** with 10% Pd/C and H_2 (65% yield) followed by desilylation of the deoxyribose moiety with fluoride ions (76% yield) furnished the novel 2-cyano-3'-deoxyinosine.[15]

In summary, the synthesis of some novel analogues of 3'-deoxyinosine is described. The approaches presented have sufficient generality that they can be applied to the synthesis of many other deoxygenated hypoxanthine nucleosides functionalized in the base moiety. Biological

studies pertaining to the RNA antiviral activities of these compounds will be reported elsewhere.



(i) see 1→5; (ii) *n*-Bu_3SnCN, $Pd(PPh_3)_4$, Toluene, Δ ; (iii) $PhCH_2OH$, Na, DMF ; (iv) H_2, Pd/C, 10%; (v) $Et_4N^+F^-$, CH_3CN

Scheme 3

EXPERIMENTAL

Melting points reported are uncorrected and were determined on a Thomas-Hoover melting point apparatus fitted with a microscope. Nuclear magnetic resonance spectra were recorded on Bruker Models WM360, MSL300, and AC300 pulse Fourier transform spectrometers. Mass spectra were determined on a VG ZAB-HF high resolution mass spectrometer with FAB

capability or a VG TRIO single quadrupole GC/MS system. Ultraviolet spectra were recorded on a Varian Cary Model 219 or a Gilford Response spectrophotometer. Infrared spectra were recorded on a Mattson Cygnus 25 Fourier transform instrument. Lyophilizations were performed with a Virtis freezemobile 3 unit. Preparative layer chromatography plates were prepared by coating six 20 cm x 20 cm plates with a slurry made from 150 g of E. Merck PF_{254} silica gel in 400 mL of water. The silica gel plates were allowed to dry slowly and were then activated for 3 h at 135°C. Flash chromatography was carried out using glass columns packed with 230-400 mesh silica gel. High performance liquid chromatography was done at 80 psi using Altex columns packed with 40-60 μm Amberlite XAD-4 resin (Rohm and Haas). Fractions were monitored by a Pharmacia UV-2 ultraviolet monitor and products were collected on a Gilson FC-100 fraction collector.

2-Amino-6-methoxy-9-(2',5'-di-O-tert-butyldimethylsilyl-β-D-ribofuranosyl) purine (2). Guanosine was converted to 6-chloro-2-amino purine nucleoside as previously described[15,16]. To a solution of 12.200 g (28.5 mmol) of this product in 100 mL of dry methanol, was added 7.700 g (142.6 mmol) of sodium methoxide. The solution was stirred at room temp. for 15 h., when it was shown to be complete by UV spectral examination. The solvents were removed in vacuo, and the residue was adsorbed on 5 g of silica gel to form a plug for column chromatography. The residue was purified on silica gel using 15% methanol/chloroform as the eluent to give 8.085 g (27.2 mmol, 96%) of compound **1** as an off-white solid: m.p. 131-133°C (lit[15] m.p.133-135°C); UV λ_{max}(EtOH) 247, 280 nm; ^{1}H NMR (DMSO-d_6) δ : 8.08 (s, 1H), 6.41 (brs, 2H), 5.75 (d, 1H), 5.35 (d, 1H), 5.08 (m, 2H), 4.45 (m, 1H), 4.08 (m, 1H), 3.96 (s, 3H), 3.90 (m, 1H), 3.55 (m, 2H).

To a solution of 2.490g (8.39 mmol) of **1** in dry DMF (5 mL) was added 2.790 g (18.45 mmol) of TBDMSCl and 2.510 g (36.9 mmol) of imidazole. The reaction flask was sealed under N_2 and allowed to stand at room temperature

for 15 h. The solvent was removed in vacuo, and the residue was partitioned between ether and water. The organic layer was separated and the aqueous layer was washed with ether (2 x 50 mL). The combined organic layers were dried (Na_2SO_4), concentrated, purified and separated on silica gel using gradient elution with 20-50% ether/hexanes as solvent. The bis-silyl compound **2** was obtained as a clear glass (0.930 g, 1.77 mmol, 21%). The 3',5'-di-O-silyl isomer of **2** was the major product (1.400 g, 2.66 mmol, 32%). In addition, 1.080 g (2.64 mmol, 31%) of the 5'-silyl derivative was obtained. This material was recycled through the reaction using 1.1 equivalents of TBDMSCl and 2.2 equivalents of imidazole. Spectral data for **2**: UV λ_{max}(EtOH) 247, 280 nm; mass spectrum, m/z (rel. intensity) 468 [$(M-tBu)^+$,16.92]; ^1H NMR (DMSO-d_6) δ : 8.31 (s, 1H), 8.24 (s, 1H), 6.92 (m, 2H), 5.87 (d, 1H), 5.81 (d, 1H), 5.43 (d, 1H), 5.10 (d, 1H), 4.55 (m, 1H), 4.27 (m, 1H), 4.03 (m, 1H), 3.80 (m, 2H), 0.90, 0.06, (m, 30H).

2-Iodo-6-methoxy-9-[3'-deoxy-2',5'-di-O-tert-butyldimethylsilyl- β -D-ribo-furanosyl]purine (5). To a solution of **2** (0.580g, 1.11mmol) in dry DMF (5 mL) was added, under N_2, thiocarbonyldiimidazole (0.297g , 1.67mmol), and DMAP (0.339 g, 2.78 mmol). The reaction was sealed under N_2, and allowed to stand at room temp. for 24 h. The volatiles were removed in vacuo, and the residue was purified on silica gel using 2% methanol/chloroform. Compound **3** was obtained as an oil (0.697g, 1.097mmol, 99%): UV λ_{max}(EtOH) 248, 280 nm; mass spectrum, m/z (rel. intensity) 635 (M^+,0.15), 578 [$(M-tBu)^+$, 9.04], 152 ($Base^+$, 0.29), 153 [$(Base+H)^+$, 2.86]; ^1H NMR (DMSO-d_6) δ : 8.56 (s, 1H), 8.05 (s, 1H), 7.87 (s, 1H), 7.11 (s, 1H), 6.51 (d, 1H), 6.27 (brs, 2H), 5.02 (t, 1H), 3.98 (m, 6H), 1.03, 0.84 (m, 30H).

A solution consisting of 6.89 mL (25.71 mmol) of n-Bu_3SnH, 0.675 g (4.11 mmol) of AIBN, and 25 mL of dry toluene was purged (N_2 ,30 min), then added dropwise to a boiling solution of 3.260 g (5.15 mmol) of **3** in 100 mL of toluene. The reaction was stirred under N_2 at 110°C for 2 h. The

reaction mixture was concentrated, and the resulting oil was chromatographed on silica gel using hexanes followed by 70% ether/hexanes as the eluent. Compound **4** was obtained as a clear glass (2.550 g, 5.02mmol, 98%); UV λ_{max}(EtOH) 247, 280 nm; mass spectrum, m/z (rel. intensity) 452 [$(M-tBu)^+$, 12.85], 287 [$(Sugar-tBu)^+$, 5.05], 165 [$(Base+H)^+$, 9.60], 194 [$(Base+CHO)^+$, 4.17]; ^1H NMR (DMSO-d_6) δ: 8.25 (s, 1H), 8.05 (s, 1H), 6.30 (t, 1H), 5.84 (d, 1H), 5.07 (m, 1H), 4.57 (m, 1H), 4.00 (m, 1H), 3.80 (m, 2H), 2.40-1.80 (m, 4H), 0.90, 0.09 (m, 30H).

To an ice-cold, N_2 purged solution of **4** (1.592g, 3.13 mmol) in 100 mL of hexane (distilled from $LiAlH_4$) was added 2.53 mL (31.3 mmol) of diiodomethane, 3.72 mL (31.3 mmol) of tert-butylnitrite, and 0.89 mL (6.26 mmol) of iodotrimethylsilane. The reaction was stirred at 60°C under a nitrogen atmosphere for 2 h and concentrated. The resulting residue was partitioned between ether and saturated aqueous Na_2SO_3. The organic layer was set aside, and the aqueous layer was extracted with ether (3 x 50mL). The combined organic layers were dried (Na_2SO_4), concentrated, and chromatographed on silica gel using hexanes followed by chloroform as the eluent. Compound **5** (1.017 g, 1.640 mmol, 53%) was obtained as an oil: UV $_{max}$(EtOH) 258 nm; mass spectrum, m/z (rel. intensity) 563 [$(M-tBu)^+$, 15.95], 287 [$(Sugar-tBu)^+$,13.49], 277 [$(Base+2H)^+$, 5.26), 304 [$(Base+CHO)^+$, 0.07]; ^1H NMR (DMSO-d_6) δ: 8.44 (s, 1H), 5.89 (d, 1H), 4.71 (m, 1H), 4.42 (m, 1H), 4.07 (s, 3H), 3.91 (dd, 2H), 2.10 (m, 2H), 0.87, 0.06 (m, 30H).

2-Iodo-9-(3'-deoxy- β -D-ribofuranosyl)hypoxanthine (6). To a solution consisting of 0.090 g (0.145 mmol) of compound **5**, and 0.029 g (0.174 mmol) of dry KI in dry acetonitrile (20 mL) was added chlorotrimethylsilane (0.020 mL, 0.174 mmol). The solution was stirred under N_2 for 3 hr. The solvents were removed and the residue was dried overnight on the vacuum pump. This residue was then dissolved in dry acetonitrile (10mL), purged (N_2, 10 min), and treated with 0.87 mL (0.435 mmol) of tetraethylammonium fluoride/acetonitrile solution. The reaction mixture was stirred under N_2

for 4 hr. Upon completion of the reaction (TLC), the solvents were removed and the residue was partitioned between water and chloroform. The aqueous layer was extracted with ether, the combined organic layers back-extracted with 10 mL of water. The aqueous layers were combined and concentrated. The residue was purified by HPLC with 2% ethanol/water as the eluting solvent. 2-Iodo-3'-deoxyinosine **6** was recovered as a clear solid (0.021g, 0.056 mmol, 39%): m.p. >200°C dec; UV $\lambda_{max}(H_2O)$ 253 (ε=15341), 272 nm (sh, ε=11900); ^1H NMR (Me_2SO-d_6) δ : 2.1 - 2.4 (m, 2H), 3.58 (m, 2H), 3.93 (m, 2H), 4.46 (m, 1H), 5.00 (t, 1H), 5.50 (d, 1H), 5.92 (d, 1H), 8.24 (s, 1H); ^{13}C NMR (DMSO-d_6) δ : 34.3, 62.7, 74.1, 80.0, 90.1, 122.6, 124.4, 135.3, 149.4, 164.6; FAB(HRMS) calcd for $C_{10}H_{11}N_4O_4I$: 378.9903 (M^++H), found: 378.9900 (M^++H).

2-Vinyl-9-(3'-deoxy-β-D-ribofuranosyl)hypoxanthine (7). A solution consisting of 0.180g (0.29 mmol) of **5**, 0.005 g (0.02 mmol) of $PdCl_2(MeCN)_2$, 0.018 g (0.06 mmol) of P(o-Tolyl)$_3$, and 0.10 mL (0.319 mmol) of n-$Bu_3SnCH=CH_2$ in dry toluene (50 mL) was purged (N_2, 30 min.) and then heated at 100 °C for 1 hr. The solvent was removed under vacuum and the residue remaining was purified on silica gel with 30% ether/hexanes to give the protected vinyl compound as an oil (0.146g, 0.281 mmol, 97%). This compound was subjucted to the two-step deprotection as described for the conversion of **5** to **6** to give 0.026 g. (0.093 mmol, 33%) of **7** as a white solid: m.p. 115-117 °C; UV $\lambda_{max}(H_2O)$ 207 (ε=22724), 260 (ε=9088), 290 (ε=7669); ^1H NMR (DMSO-d_6) δ : 12.3 (brs, 1H), 8.32 (s, 1H), 6.60 (m, 2H), 5.89 (d, 1H), 5.80 (dd, 1H), 5.70 (s, 1H), 5.00 (s, 1H), 4.56 (m, 1H), 4.35 (m, 1H), 3.58 (m, 2H), 2.27, 1.92 (m, 2H); ^{13}C NMR (DMSO-d_6) δ: 157.0, 151.8, 148.1, 138.9, 129.7, 125.3, 123.3, 90.7, 81.1, 75.2, 62.6, 34.3; FAB (HRMS) calcd for $C_{12}H_{15}N_4O_4$: 279.1093 (M^++H), found: 279.1112 (M^++H);

2-Formyl-9-(3'-deoxy-β-D-ribofuranosyl)hypoxanthine (8). A solution consisting of 0.015g (0.054 mmol) of compound **8** in distilled water (350 mL)

was cooled down to ice-bath temp. and ozonized for 1 min. Air was then bubbled through the solution for 12 h, while slowly allowing the solution to reach room temp. The solvent was removed in vacuo and the residue purified by HPLC column using 9% EtOH/H_2O as the eluent. The title compound **8** was obtained (0.0117 g, 0.042 mmol, 77%) as a white crystalline solid: m.p. 162-165 ^oC; UV $\lambda_{max}(H_2O)$ 249 (ε=9991), 272 nm (ε=4823); ^1H NMR (DMSO-D_6) δ : 9.56 (s,1H), 8.53 (s,1H), 5.93 (d,1H), 5.67 (m,1H), 5.00 (m,1H), 4.54 (m,1H), 4.38 (m,1H), 3.59 (m,2H), 2.27, 1.97 (m,2H). ^{13}C NMR (DMSO-d_6)δ : (33.9, 34.1), (62.4, 62.7), (75.0, 75.5), (81.4, 81.5), (91.0, 91.2), 97.5, (123.2, 124.4), (139.6, 139.9), (146.4, 147.2), (149.2, 151.8), (153.3, 155.0), 186.1; FAB(HRMS) calcd for $C_{11}H_{12}N_4O_5$: 281.0886 (M^++H), found: 281.0851 (M^++H).

2-Acetonyl-9-(3'-deoxy-β-D-ribofuranosyl)hypoxanthine (9). In a 50 mL RBF were mixed 5 mL of dry toluene (from NaH), 0.050 mL (0.425 mmol) of isopropenyl acetate, and 0.122 mL (0.425 mmol) of tri-butyltin methoxide. This flask was sealed under N_2 and stirred for 1 h at 50^{o}C. The iodo compound **5** (0.211 g, 0.340 mmol), 0.006g (0.024 mmol) of $PdCl_2(MeCN)_2$, and 0.021g (0.068 mmol) of P(o-tolyl)$_3$ were combined in a 100 mL RBF and evacuated briefly on the vacuum line. The 100 mL RBF was then sealed under N_2, and the contents dissolved in toluene (25 mL). This solution was purged (N_2, 30 min), and the preformed acetonyl tin reagent was added to the 100 mL RBF via double-tipped needle. The reaction mixture was stirred for 2 h under N_2 at 90^{o}C and then concentrated. The residue was purified on silica gel using hexanes followed by 30% ether/hexanes as the eluent. The protected 2-acetonyl compound (0.165 g, 0.300 mmol, 88%) was isolated and then subjected to the two-step deprotection reaction described for the conversion of **5** to **6** to give **9** (0.105 g, 0.341 mmol, 40%) as a white solid: m.p. 112-114 ^oC; UV $\lambda_{max}(H_2O)$ 249 (ε=10900), 266 (ε=6440); FTIR (KBr) 3400, 1699, 1550 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ :12.4 (s, 1H), 8.28 (s, 1H), 5.80

(m, 1H), 4.57 (m, 1H), 4.00 (m, 1H), 3.80 (m, 2H), 2.40-1.80 (m, 4H), 0.90, 0.09 (m, t-BuSi); mass spectrum, m/z (rel. intensity) 456 [$(M\text{-}t\text{-}Bu)^+$, 1.98], 169 ($Base^+$, 25.6), 134 ($Base^+$ -Cl, 23.1).

Compound **12** was prepared from the 2-amino-6-chloro-3'-deoxy compound by radical deamination-halogenation as described previously for the conversion of **4** to **5**. Data for **12**: UV λ_{max}(EtOH) 248, 281 nm; ^1H NMR (DMSO-d_6) δ: 8.36 (s, 1H), 8.62 (s, 1H), 6.42 (t, 1H), 5.97 (d, 1H), 4.60 (m, 2H), 4.00 (m, 1H), 3.82 (m, 2H), 2.60-1.80 (m, 4H), 0.90, 0.10 (m, 30H); mass spectrum, m/z (rel.intensity) 567 [$(M\text{-}t\text{-}Bu)^+$, 2.3].

2-Cyano-9-(3'-deoxy- β -D-ribofuranosyl)purine (15). Compound **12** (0.238g, 0.381 mmol), 0.031g (0.027 mmol) of $Pd(PPh_3)_4$, 0.132 g (0.419 mmol) of n-Bu_3SnCN, and 0.023g (0.076 mmol) of P(o-tolyl)$_3$ were mixed in a 50 mL RBF in a glove box under a N_2 atmosphere.. The contents were dissolved in toluene (15 ml, dried from NaH). The solution was purged (N_2, 30 min), and the reaction mixture then allowed to stir under N_2 at 90°C for 8 h and then concentrated. The residue was purified on silica gel using 1:1 ether/hexanes as the eluent. The protected 2-cyano compound **13** was isolated as an oil (0.154 g, 0.294 mmol, 77%): UV λ_{max}(EtOH) 273 nm; ^1H NMR (DMSO-d_6) δ : 9.08 (s, 1H), 6.47 (t, 1H), 4.72 (m, 1H), 3.80 (m, 3H), 2,50 (m, 2H), 0.85, 0.13 (m, 30H); mass spectrum m/z (rel. intensity) 466 [$(M\text{-}t\text{-}Bu)^+$, 7.42], 468 [$[M(^{37}Cl)\text{-}t\text{-}Bu]^+$, 3.30], 179 [$(Base+H)^+$, 41.02], 181 [$[Base(^{37}Cl)+H]^+$, 13.77].

To 0.5 mL of benzyl alcohol was added 0.007 g (0.281 mmol) of metallic sodium under a N_2 atmosphere. When all the sodium had been consumed, a solution of the nucleoside **13** (0.113 g, 0.216 mmol) in dry DMF (5 mL) was added under N_2 via a double-tipped needle. The nucleoside flask was rinsed out with DMF (2 mL) and this was added to the reaction flask. The resulting solution was stirred at room temperature for 6 h, concentrated, and the residue was purified on a silica gel column using 30% ether/ hexanes as the eluting solvent. The 6-benzyloxy compound **15** was isolated

as an oil (0.086 g, 0.145 mmol, 85%): UV λ_{max}(EtOH) 260, 272 nm; mass spectrum: m/z (rel. intensity) 538 [$(M-t-Bu)^+$, 9.84].

A solution of 0.130 g (0.220 mmol) of compound **14** in absolute ethanol (200 mL) was purged (N_2, 20 min). Approximately 0.065 g of 10% Pd/C was added to the reaction vessel. This mixture was hydrogenated on a Parr apparatus for 12 h, using 35 psi of H_2. The palladium was then filtered off, and the filtrate was concentrated in vacuo. This residue was dried on the vacuum line overnight and was then treated with 1.32 mL (0.660 mmol) of a 0.5 M tetraethylammonium fluoride/acetonitrile solution to deprotect the silyl groups. The title compound **15** was purified by HPLC to give 0.015g (0.054 mmol, 25% for the two steps) of purified product as a solid: m.p. 170 °C (dec); UV λ_{max}(H_2O) 296 (ε=5299), 257 (ε=6206), 252 nm (ε=6214); FTIR (KBr): 2260 cm^{-1} (CN stretch); ^{1}NMR (DMSO-d_6) δ: 1.91, 2.21 (m, 2H), 3.65 (m, 2H), 4.33 (m, 1H), 4.50 (m, 1H), 5.12 (m, 1H), 5.75 (d, 1H), 8.11 (s, 1H); FAB(HRMS) calcd for $C_{11}H_{12}N_5O_4$: 278.1789 (M^++H), found: 278.1759 (M^++H).

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NOVEL 2,6-DISUBSTITUTED ADENOSINE ANALOGUES: POTENTIAL AGONISTS FOR ADENOSINE RECEPTORS

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Abstract. Synthesis of novel 2-iodo- and 2-oxo- N^6-cyclosubstituted adenosine analogues with potential adenosine A_1 or A_2 agonist activity is described. The procedure for the 2-oxo compounds represents a new and general synthetic approach to isoguanosine analogues.

INTRODUCTION

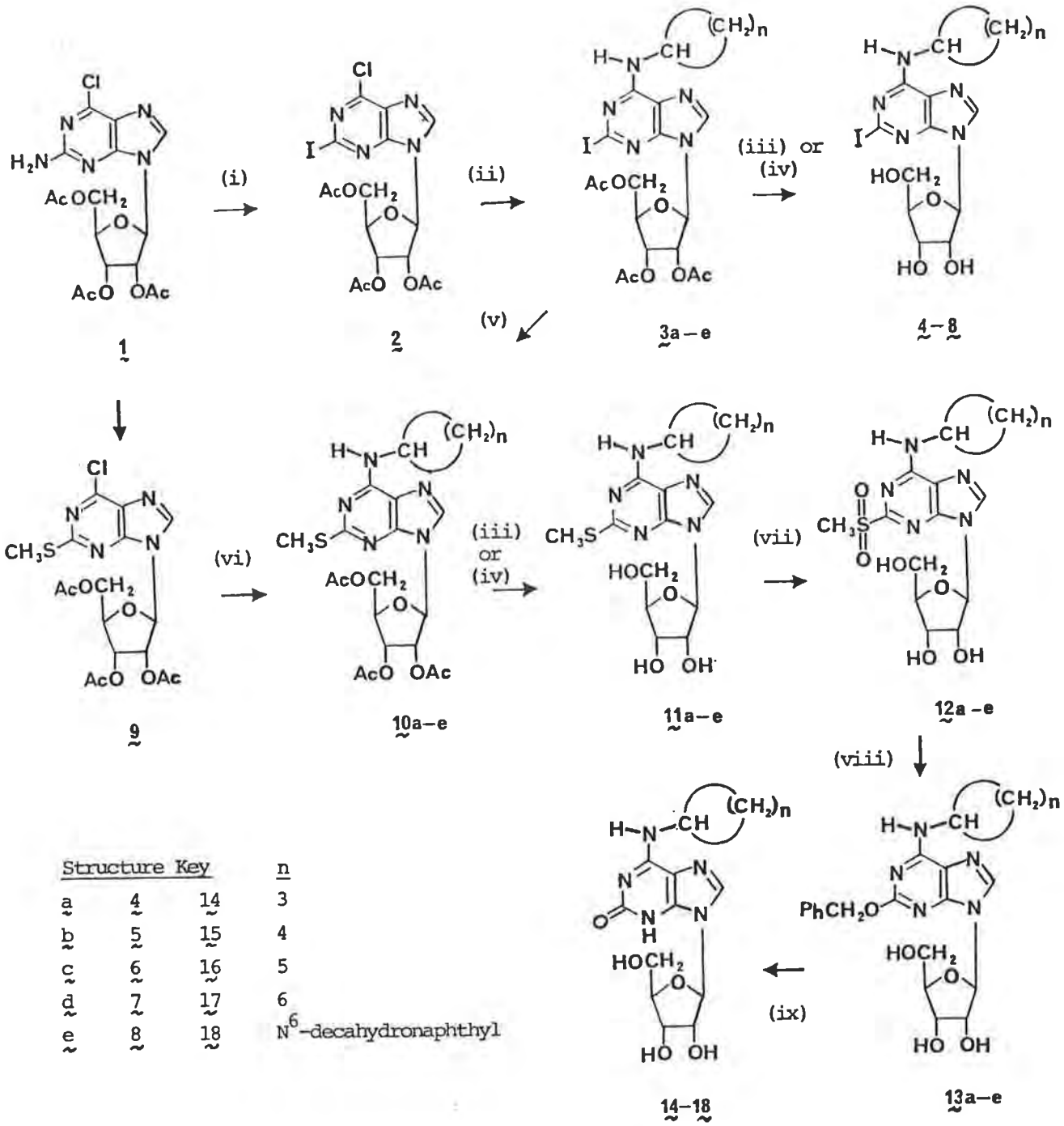
Although the cardiovascular effects of adenosine were first described by Drury and Szent-Gyorgyi in 1929,[1] it was not until much later and particularly in the last decade that the biochemical basis for the physiological effects of adenosine began to be understood.[2-6] Adenosine exerts its effects *via* the extracellular receptors, A_1 and A_2, distributed throughout a wide variety of tissues in the mammalian body. The chemistry and biology of the adenosinergic system and the physiological effects mediated by adenosine have been reviewed.[2,3,5,7,8] Adenosine appears to act as a local hormone, produced on demand in response to a pathological stimulus such as ischemia or in response to increases in cellular electrical activity.[7] Adenosine has recently been approved clinically for the treatment of acute supraventricular tachycardia and, while effective, suffers from a half-life in the bloodstream of less than ten seconds. Thus, there has been considerable interest in developing adenosine analogs that mimic the pharmacological properties of adenosine but with high A_1 or

A_2 receptor specificity and with resistance to rapid metabolic degradation.[3] Several recent reports of highly selective and potent analogs for both the A_1 and A_2 receptors,[9-19] have focused attention on 2- and/or N^6-modified adenosines. The natural minor nucleoside, isoguanosine (2-hydroxyadenosine) has been reported to have vasodepressor activity greatly exceeding that of adenosine.[18,20] However, very few examples of isoguanosine analogs of related interest are known.[21] This paper reports on the synthesis of novel 2-iodo- and 2-oxo- N^6-cyclosubstituted adenosines with potential A_1 or A_2 agonist activity and cellular stability.

RESULTS AND DISCUSSION

Synthesis of 2-iodo-6-cyclosubstituted adenosines were achieved by taking advantage of the known nucleophilic lability of the 6-chloro group in 2-iodo-6-chloro purine nucleoside.[22] This precursor, in its protected form (i.e. **2**), can be prepared by the deamination-iodination of 2-amino-6-chloropurine riboside **1**.[22] Treatment of **2** with the primary amine to be substituted at the 6-position in chloroform at 60 °C gave **3a-e** in excellent yields. Deprotections of compounds **3** were carried out with sodium methoxide in methanol or with ethanolic ammonia to give the target compounds **4 - 8**. Overall yields from guanosine were of the order of 40%. The structures of these compounds were confirmed by ^1H NMR and UV data and elemental analyses.

The strategy for the synthesis of the novel isoguanosine analogues was more complicated. Although a number of approaches are available for the synthesis of isoguanosine, these methodologies were not easily applicable



Structure Key			n
a	4	14	3
b	5	15	4
c	6	16	5
d	7	17	6
e	8	18	N^6-decahydronaphthyl

(i) CH_2I_2, $n\text{-}C_5H_{11}ONO$, CH_3CN; (ii) RNH_2, TEA, $CHCl_3$; (iii) $NaOCH_3$, MeOH; (iv) NH_3, EtOH; (v) CH_3SSCH_3, CH_3CN, hv; (vi) RNH_2, TEA, DMF; (vii) oxone, acetate buffer; (viii) sodium benzyloxide, DMF; (ix) H_2, Pd/C 10%, EtOH.

to the synthesis of the 2-oxo-N^6-cycloalkylated adenosines. These include the selective deamination of 2,6-diaminopurine nucleoside with nitrous acid,[23,24] cyclization of a 4,5-dicyanoimidazole nucleoside precursor,[25] photolysis of adenosine N^1-oxide,[26] or synthesis from AICA-riboside.[27] In 1985, Nair and Young reported a photochemical synthesis of isoguanosine.[22] However, this procedure, while easily applicable for the photohydration of 2-iodoadenosine to natural isoguanosine, was unsuccessful for the photohydration reaction of the 2-iodo-N^6-cyclosubstituted compounds **4-8**.

We now report a new approach to the preparation of isoguanosine and related N^6-substituted analogs of isoguanosine. The precursor for this synthesis was the 2-amino-6-chloro compound **1** which was converted to **9** through a thermal radical deamination-thioalkylation with n-pentyl nitrite and dimethyl disulfide in acetonitrile.[28,29] The thioalkylated compound **9** was converted to compounds **10** by displacement of the 6-chloro group with the primary cyclic amines. These intermediates (i.e. **10a-e**) were also prepared from compounds **3** by photochemical thioalkylation.[28] Formation of the thiomethyl group at the 2-position was to convert it eventually to a good leaving group for introduction of the oxo group. Thus, compounds **10** were converted to the sulfones **12** by deprotection followed by oxidation with oxone in acetate buffer. Treatment of the sulfones **12** with the sodium salt of benzyl alcohol followed by hydrogenolysis of the resulting 2-benzyloxy compounds **13** with 10% Pd/C and 30 p.s.i. of H_2, gave the target compounds **14-18**. Overall yields starting from guanosine <u>via</u> the photochemical thioalkylation pathway were of the order of 9% whereas the yields were slightly higher (~ 12 %) for the pathway involving thermal alkylation. The structures of the target novel isoguanosine analogues were

confirmed by ^{1}H NMR, FTIR and UV data and by elemental analysis.

In summary, N^6-cycloalkylated analogues of 2-iodo and 2-oxo adenosines have been synthesized by highly efficient methods. The procedure for the 2-oxo compounds represents a new and general approach to isoguanosine analogues. The target compounds described are of potential interest as A_1 or A_2 agonists with respect to these adenosine receptors. They are not substrates for mammalian adenosine deaminase.

EXPERIMENTAL

The reported melting points are uncorrected and were determined on a Thomas-Hoover melting point apparatus fitted with a microscope. Nuclear magnetic resonance spectra were recorded on JEOL Model FX90Q and Bruker MSL 300 pulse Fourier transform spectrometers. Ultraviolet spectra were recorded on a Varian Cary Model 219 or a Gilford Response spectrophotometer. Infrared spectra were recorded on a Mattson Cygnus 25 Fourier transform instrument. Elemental analyses were carried out by Galbraith Laboratories Inc., Knoxville, TN. Preparative layer chromatography plates were prepared by coating six 20cm x 20cm plates with a slurry made from 150g of E. Merck PF_{254} silica gel in 400 mL of water. The silica gel plates were allowed to dry slowly and were then activated for 3 h at 150 °C. Flash chromatography was carried out in glass columns packed with 230-400 mesh silica gel.

General Synthetic Procedures (A - F). Procedure A: Preparation of N^6-substituted-2-iodo-9-(2',3',5'-tri-acetyl- β-D-ribofuranosyl)purines. A solution containing 6-chloro-2-iodo-9-(2',3',5'-tri-O-acetyl- β -D-

ribofuranosyl)purine (6.6 mmol) 2,[30] triethylamine (7.9 mmol), and the amine to be substituted in the 6-position (7.9 mmol) was stirred in chloroform (30 mL) and heated at 60 °C for 2 h. The solvent was evaporated and the residue was purified by flash chromatography eluting with 1% methanol/ chloroform. Alternatively, the N^6-substituted-2-iodo compound could be prepared by doubling the equivalents of the amine to be substituted and omitting the triethylamine.

Procedures B-1 and B-2: Deprotection of triacetylated purine ribofuranosides. Procedure B-1: To a solution of triacetylated nucleoside (7.4 mmol) in methanol (50 mL) was added sodium methoxide (25.7 mmol). The solution was stirred for 1 h at 25 °C at which time NH_4Cl (31.7 mmol) was added. Stirring was continued for 1 h. The solvent was removed and the residue triturated with 9:1 chloroform/methanol and filtered. The filtrate was concentrated and the residue was purified on silica gel plates with 10% methanol/chloroform in the case of the 2-iodo analogues or by flash chromatography eluting with 2% methanol/chloroform in the case of the 2-methylthio analogues.

Deprotection of triacetylated purine ribofuranosides. Procedure B-2: Alternatively, the triacetylated nucleoside was dissolved in absolute ethanol (200 mL) and the solution was saturated with anhydrous ammonia at 0 °C. The solution was allowed to stand at room temperature for 24 h. The solvent was then removed and the residue triturated with o-xylene and the acetamide/o-xylene azeotrope and excess o-xylene were distilled off and the residue was purified as described in B-1.

Procedure C: Photochemical preparation of 2-methylthio-N^6-substituted-9-(2',3',5'-tri-O-acetyl-β-D-ribofuranosyl)purines. A nitrogen-purged solution of **3b** or **3c** (1.8 mmol) containing dimethyl disulfide (25 mmol) in acetonitrile (100 mL) was irradiated under N_2 for 40 h with ultraviolet light of principle wavelength 254 nm using a Rayonet Photochemical Reactor. The solvent was then removed and the residue was incorporated into a silica gel plug and purified by flash chromatography eluting with ethyl acetate/hexanes.

Procedure D: Preparation of 2-methylsulfonyl-N^6-substituted-9-(β -D-ribofuranosyl)purines (12a-12e). Compounds **11a-11e** (6.4 mmol) were dissolved in each case in methanol (20 mL) and cooled to 0 ^oC in an ice bath. Oxone (10.2 mmol) was dissolved in acetate buffer at pH 4.2 and added slowly to the nucleoside. The reaction mixture was allowed to attain room temperature and was stirred for 4 h and then neutralized with NaOH. The solvent was removed and the residue was triturated with 9:1 chloroform/methanol and filtered. The filtrate was incorporated into a silica gel plug and purified by flash chromatography eluting with 2% methanol/chloroform.

Procedure E: Preparation of 2-benzyloxy-N^6-substituted-9-(β-D-ribofuranosyl)purines (13a-13e). To a solution of the 2-methylsulfonyl-N^6-substituted ribonucleoside (2.9 mmol) in DMF (30 mL) was added sodium benzyloxide (0.389 g Na in excess benzyl alcohol, 5 mL). The solution was heated to 60 ^oC for 2 h with stirring at which time the reaction mixture was cooled to room temp and NH_4Cl (19.2 mmol) was added. Stirring was continued for an additional 1 h. The DMF was removed under reduced

pressure (50 °C) and the resulting syrup incorporated into a silica gel plug and purified by flash chromatography eluting initially with chloroform to remove benzyl alcohol and then with 6% methanol/chloroform to elute the product.

Procedure F: Catalytic reduction of 2-benzyloxy-N^6-substituted-9-(β-D-ribofuranosyl)purines. A solution of each of the compounds **13a-13e** (0.4 mmol) in absolute ethanol (150 mL) was purged with N_2 and to it was added one fourth the mass of 10% Pd/C. The suspension was then hydrogenated at 30 p.s.i. of H_2 for 18 h on a Parr Hydrogenation Apparatus. The suspension was then suction filtered through a fritted glass filter and the solvent was removed. The residue was purified on silica gel plates with 10% methanol/chloroform. The product was subsequently crystallized from isopropanol/diethyl ether.

N^6-Cyclobutyl-2-iodo-9-(β-D-ribofuranosyl)purine (4). Compound **3a** was prepared from **2**[30] using Procedure A (88% yield) and deprotected to give **4** using Procedure B-1 (92% yield). The product obtained by preparative layer chromatography was crystallized from ethanol/diethyl ether/hexanes to give **4** as white crystals: m.p. 109-112 °C dec.; ^{1}H NMR (Me_2SO-d_6) δ 1.65-2.20 (m, 7H), 3.61 (m, 2H), 3.96 (m, 1H), 4.09 (m, 1H), 4.52 (m, 1H), 5.00 (t, 1H), 5.17 (m, 1H), 5.44 (d, 1H), 5.83 (d, 1H), 8.33 (s, 1H), 8.47 (d, 1H); UV (Ethanol) λ_{max} 274 nm (ε 15,080).

Anal. Calcd. for $C_{14}H_{18}IN_5O_4$: C, 37.60; H, 4.06; N, 15.66. Found: C, 36.91; H, 4.53; N, 14.75

N^6-Cyclopentyl-2-iodo-9-(β-D-ribofuranosyl)purine (5) was

prepared from **2** using in sequence Procedure A (93% yield) and Procedure B-2 (88%) to give **5** which crystallized from ethanol/diethyl ether/hexanes as white crystals: m.p. 173-175 °C; ^{1}H NMR (Me_2SO-d_6) δ 1.61 (m, 9H), 3.63 (m, 2H), 3.93 (m, 1H), 4.13 (m, 1H), 4.52 (m, 1H), 5.01 (m, 1H), 5.21 (m, 1H), 5.43 (m, 1H), 5.79 (d, 1H), 8.15 (d, 1H), 8.28 (s, 1H); UV (Ethanol) λ_{max} 274.5 nm (ε 14,980).

Anal. Calcd. for $C_{15}H_{20}IN_5O_4$: C, 39.06; H, 4.37; N, 15.18. Found: C, 39.12; H, 4.42; N, 14.60

N^6-Cyclohexyl-2-iodo-9-(β-D-ribofuranosyl)purine (6) was prepared from **2** by using Procedure A (80% yield) followed by Procedure B-2 (73% yield). Compound **6** crystallized from ethanol/diethyl ether/hexanes to provide white crystals: m.p. 110-115 °C dec.; ^{1}H NMR (Me_2SO-d_6) δ 1.23-1.75 (m, 11H), 3.64 (m, 2H), 3.95 (m, 1H), 4.13 (m, 1H), 4.50 (m, 1H), 5.02 (m, 1H), 5.19 (m, 1H), 5.46 (m, 1H), 5.80 (d, 1H), 8.03 (d, 1H), 8.28 (s, 1H); UV (Ethanol) λ_{max} 273.5 nm (ε 10,400).

Anal. Calcd. for $C_{16}H_{22}IN_5O_4$: C, 40.43; H, 4.66; N, 14.73. Found: C, 40.69; H, 4.87; N, 14.64.

N^6-Cycloheptyl-2-iodo-9-(β -D-ribofuranosyl)purine (7) was prepared from **2** using in sequence Procedure A (90% yield) and Procedure B-1 (91% yield). Compound **7** crystallized from ethanol/ diethyl ether/hexanes as white crystals: m.p. 115-118 °C; ^{1}H NMR (Me_2SO-d_6) δ 1.55 (m, 13H), 3.61 (m, 2H), 3.95 (m, 1H), 4.15 (m, 1H), 4.53 (m, 1H), 5.00 (m, 1H), 5.17 (m, 1H), 5.41 (m, 1H), 5.83 (d, 1H), 8.07 (d, 1H), 8.32 (s, 1H); UV (Ethanol) λ_{max} 273.5 nm (ε16,070).

Anal. Calcd. for $C_{17}H_{24}IN_5O_4$: C, 41.73; H, 4.94; N, 14.31. Found: C, 42.32; H, 5.21; N, 13.90.

N^6-(2-Decahydronaphthyl)-2-Iodo-9-(β-D-ribofuranosyl)purine (8) was prepared from **2** by Procedure A (60% yield) using DMF as the solvent at 90 °C for 5 h to give **3e** which was deprotected using Procedure B-1 (90% yield) to give **8**. Compound **8** was crystallized from ethanol/diethyl ether/hexanes as white crystals: m.p. 132-134 °C; ^{1}H NMR (Me_2SO-d_6) δ 1.56 (m, 17H), 3.60 (m, 2H), 3.95 (m, 1H), 4.09 (m, 1H), 4.52 (m, 1H), 5.02 (m, 1H), 5.21 (m, 1H), 5.44 (m, 1H), 5.83 (d, 1H), 8.04 (m, 1H), 8.32 (s, 1H); UV (Ethanol) λ_{max} 273.5 nm (ε 17,600).

Anal. Calcd. for $C_{20}H_{28}IN_5O_4$: C, 45.38; H, 5.33; N, 13.23. Found: C, 45.15; H, 6.00; N, 12.94.

6-Chloro-2-methylthio-9-(2',3',5'-tri-O-acetyl-β-D-ribofuranosyl) purine (9).[28,29] To a solution of **1** (14.7 mmol) in acetonitrile (60 mL) at 0 °C was added dimethyl disulfide (147.0 mmol), and n-pentyl nitrite (88.0 mmol). The solution was purged with N_2 for 30 min, and was heated at 60 °C under N_2 for 16 h. The solvent was removed under reduced pressure and the residue incorporated into a silica gel plug and purified by flash chromatography eluting with 1% methanol/ chloroform. The product was isolated as a yellow oil in 85% yield. ^{1}H NMR (Me_2SO-d_6) δ 1.97 (s, 3H), 2.08 (s, 3H), 2.12 (s, 3H), 2.64 (s, 3H), 4.40 (m, 3H), 5.71 (m, 1H), 6.06 (m, 1H), 6.32 (d, 1H), 8.71 (s, 1H); UV (Ethanol) λ_{max} 264, 304.5 nm.

N^6-Cyclobutyl-2-oxo-9-(β-D-ribofuranosyl)purine (14). Compound **10a** was prepared from **9** by Procedure A except in DMF at 90 °C for 5 h (80% yield).

It was then converted to **11a** using Procedure B-1 (81% yield). **11a:** m.p. 96-100 °C dec.; ^{1}H NMR (Me_2SO-d_6) δ 2.24-2.77 (m, 7H), 2.59 (s, 3H), 3.67 (m, 2H), 4.01 (m, 1H), 4.24 (m, 1H), 4.62 (m, 1H), 5.08-5.38 (m, 3H), 5.89 (d, 1H), 7.90 (d, 1H), 8.17 (s, 1H); UV (Ethanol) λ_{max} 244, 283 nm. Compound **11a** was then converted to **12a** by procedure D (75% yield). The sulfone methyl singlet appears at 3.34 ppm and the UV_{max} shifts to 269 nm. The IR shows absorbance due to the sulfone group at 1306 and 1130 cm^{-1}. The sulfone **12a** was converted to **13a** by Procedure E (60% yield) to provide **13a:** m.p. 94-98 °C dec.; ^{1}H NMR (Me_2SO-d_6) δ 1.65-2.2 (m, 7H), 3.61 (m, 2H), 3.93 (m, 1H), 4.17(m, 1H), 4.55 (m, 1H), 4.85-5.45 (m, 3H), 5.35 (s, 2H), 5.83 (d, 1H), 7.44 (m, 5H), 8.02 (d, 1H), 8.19 (s, 1H); UV (Ethanol) λ_{max} 274 nm. The 2-oxo compound **14** was prepared from **13a** by Procedure F (58% yield) and was crystallized from Isopropanol/diethyl ether to provide white crystals: m.p. 167-170 °C; ^{1}H NMR (Me_2SO-d_6) δ 1.72-2.17 (m, 7H), 3.59 (m, 2H), 3.93 (m, 1H), 4.08 (m, 1H), 4.48 (m, 1H), 4.74 (m, 1H), 5.08 (m, 1H), 5.32 (m, 1H), 5.70 (d, 1H), 7.98 (s, 1H), 8.15 (m, 1H); UV (Ethanol) λ_{max} 249 (ε 9,110), 284.5 (8,260), 302.5 nm (6,890); IR (carbonyl) 1652 cm^{-1}.

Anal. Calcd. for $C_{14}H_{19}N_5O_5$: C, 49.85; H, 5.68; N, 20.76. Found: C, 49.22; H, 5.70; N, 20.06.

N^6-Cyclopentyl-2-oxo-9-(β-D-ribofuranosyl)purine (15). Compound **10b** was prepared from **3b** using procedure C (62% yield) and was deprotected using procedure B-1 (82% yield) to provide **11b:** m.p. 108-110 °C; ^{1}H NMR (Me_2SO-d_6) δ 1.61 (m, 9H), 2.56 (s, 3H), 3.58 (m, 1H), 3.92 (m, 1H), 4.11 (m, 1H), 4.56 (m, 1H), 5.00-5.41 (m, 3H), 5.83 (d, 1H), 7.77 (d, 1H), 8.20 (s, 1H); UV (Ethanol) λ_{max} 244, 280 nm. Compound **11b** was converted to **13b**

using In sequence Procedure D (70% yield) and Procedure E (50% yield). **13b:** m.p. 96-100 °C; ^{1}H NMR (Me_2SO-d_6) δ 1.60 (m, 9H), 3.62 (m, 2H), 3.91 (m, 1H), 4.42 (m, 1H), 4.62 (m, 1H), 5.13 (m, 3H), 5.33 (s, H), 5.80 (d, 1H), 7.38 (m, 5H), 7.67 (d, 1H), 8.14 (s, 1H); UV (Ethanol)λ_{max} 274.5 nm. Compound **13b** was then hydrogenated using Procedure F (76% yield) to obtain the 2-oxo compound **15**, which was crystallized from Isopropanol/diethyl ether to give white crystals: m.p. 186-188 °C; ^{1}H NMR (Me_2SO-d_6) δ 1.59-1.81 (m, 9H), 3.58 (m, 2H), 3.94 (m, 1H), 4.09 (m, 1H), 4.49 (m, 1H), 5.09-5.53 (m, 3H), 5.67 (d, 1H), 7.71 (m, 1H), 7.97 (s, 1H); UV (Ethanol) λ_{max} 248.5 (ε 9,410), 284 (8,210), 302.5 nm (7,860); IR (carbonyl) 1639 cm^{-1}.

Anal. Calcd. for $C_{15}H_{21}N_5O_5.H_2O$: C, 48.78; H, 5.73; N, 18.96. Found: C, 48.44; H, 6.17; N, 18.56.

N^6-Cyclohexyl-2-oxo-9-(β-D-ribofuranosyl)purine (16). Compound **11c** was prepared from **3c** by using In sequence Procedure C (70% yield) and Procedure B-1 (76% yield). **11c:** m.p. 107-109 °C; ^{1}H NMR (Me_2SO-d_6) δ 1.32-1.74 (m, 11H), 2.52 (s, 3H), 3.59 (m, 2H), 3.94 (m, 1H), 4.21 (m, 1H), 4.29 (m, 1H), 5.07-5.37 (m, 3H), 5.86 (d, 1H), 7.60 (d, 1H), 8.20 (s, 1H); UV (Ethanol) λ_{max} 244, 280 nm. Compound **11c** was then converted to **13c** using In sequence Procedure D (65% yield) and Procedure E (62% yield): m.p. 98-102 °C dec.; ^{1}H NMR (Me_2SO-d_6) δ 1.23-1.76 (m, 9H), 3.60 (m, 2H), 3.97 (m, 1H), 4.18 (m, 1H), 4.62 (m, 1H), 4.70-5.60 (m, 3H), 5.34 (s, 2H), 5.82 (d, 1H), 7.41 (m, 5H), 7.72 (d, 1H), 8.17 (s, 1H); UV (Ethanol) λ_{max} 275 nm. Compound **13c** was then hydrogenated to provide the 2-oxo compound **16**, using Procedure F (70% yield). Crystallization from Isopropanol/diethyl ether gave **16** as white crystals: m.p. 170-172 °C; ^{1}H NMR (Me_2SO-d_6) δ 1.32-1.88

(m, 11H), 3.60 (m, 2H), 3.95 (m, 1H), 4.10 (m, 1H), 4.49 (m, 1H), 5.12-5.38 (m, 3H), 5.69 (d, 1H), 7.64 (m, 1H), 7.97 (m, 1H); UV (Ethanol) λ_{max} 248.5 (ε 9,290), 284.5 (7,770), 302.5 nm (7,990); IR (carbonyl) 1643 cm^{-1}.

Anal. Calcd. for $C_{16}H_{23}N_5O_5.H_2O$: C, 50.12; H, 6.05; N, 18.27. Found: C, 49.72; H, 6.89; N, 17.37.

N^6-Cycloheptyl-2-oxo-9-(β-D-ribofuranosyl)purine (17). Compound **11d** was prepared from **9** using in sequence Procedure A, except in DMF at 90 °C for 2 h (71% yield) and Procedure B-1 (64% yield): m.p. 95-98 °C; ^1H NMR $\delta(Me_2SO-d_6)$ 1.57-1.80 (m, 13H), 2.54 (s, 3H), 3.62 (m, 2H), 3.89 (m, 1H), 4.16 (m, 1H), 4.60 (m, 1H), 5.05-5.38 (m, 3H), 5.85 (d, 1H), 7.6 (d, 1H), 8.23 (s, 1H); UV (Ethanol) λ_{max} 244, 283 nm. Compound **11d** was then converted to **12d** by Procedure D (75% yield) and subsequently the methylsulfonyl group was displaced by the benzyloxy anion using Procedure E (68% yield) to provide **13d:** ^1H NMR (Me_2SO-d_6) δ 1.55-1.76 (m, 13H), 3.62 (m, 2H), 3.94 (m, 1H), 4.21 (m, 1H), 4.55 (m, 1H), 5.01-5.49 (m, 3H), 5.35 (s, 2H), 5.82 (m, 1H), 7.41 (m, 5H), 7.67 (d, 1H), 8.17 (s, 1H); UV (Ethanol) λ_{max} 274 nm. Compound **13d** was then hydrogenated to the 2-oxo compound **17** using Procedure F (72% yield). The product crystallized from isopropanol/diethyl ether as light tan crystals: m.p. 155-157 °C; ^1H NMR (Me_2SO-d_6) δ 1.56 (m, 13H), 3.61 (m, 2H), 3.96 (m, 1H), 4.11 (m, 1H), 4.49 (m, 1H), 5.05-5.39 (m, 3H), 5.70 (d, 1H), 7.71 (m, 1H), 7.99 (s, 1H); UV (Ethanol) λ_{max} 248.5 (ε 10,000), 284 (8,830), 302 nm (8,170); IR (carbonyl) 1636 cm^{-1}.

Anal. Calcd. for $C_{17}H_{25}N_5O_5.H_2O$: C, 51.38; H, 6.34; N, 17.62. Found: C, 51.60; H, 6.92; N, 17.34.

N^6-(2-Decahydronaphthyl)-2-oxo-9-(β-D-ribofuranosyl)purine (18). Compound **11e** was prepared from **9** using in sequence Procedure A, except in DMF at 90 °C for 18 h (58% yield) and Procedure B-2 (60% yield): m.p. 110-114 °C dec.; ^{1}H NMR (Me_2SO-d_6) δ 1.56-1.76 (m, 17H), 2.53 (s, 3H), 3.64 (m, 2H), 3.95 (m, 1H), 4.15 (m, 1H), 4.60 (m, 1H), 5.04 (m, 1H), 5.19 (m, 1H), 5.44 (m, 1H), 5.85 (d, 1H), 7.64 (d, 1H), 8.24 (s, 1H); UV (Ethanol) λ_{max} 244, 283 nm. Compound **11e** was converted to **13e** using in sequence Procedure D (64% yield) and Procedure E (48% yield): m.p. 111-114 °C; ^{1}H NMR (Me_2SO-d_6) δ 1.54 (m, 17H), 3.61 (m, 2H), 3.93 (m, 1H), 4.18 (m, 1H), 4.58 (m, 1H), 5.34 (s, 2H), 4.90-5.58 (m, 3H), 5.83 (d, 1H), 7.42 (m, 5H), 7.61 (m, 1H), 8.17 (s, 1H); UV (Ethanol) λ_{max} 274.5 nm. Compound **13e** was then converted to the 2-oxo compound **18** using Procedure F (71% yield). The product crystallized from isopropanol/diethyl ether as white crystals: m.p. 175-180 °C dec.; ^{1}H NMR (Me_2SO-d_6) δ 1.54 (m, 17H), 3.59 (m, 2H), 3.96 (m, 1H), 4.11 (m, 1H), 4.50 (m, 1H), 5.07-5.35 (m, 3H), 5.69 (d, 1H), 7.64 (m, 1H), 7.97 (s, 1H); UV (Ethanol) λ_{max} 249 (ε 9,490), 283.5 (8,920), 302 nm (7,250); IR (carbonyl) 1636 cm^{-1}

Anal. Calcd. for $C_{20}H_{29}N_5O_5$: C, 57.27; H, 6.97; N, 16.69. Found: C, 57.79; H, 7.12; N, 16.39.

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SYNTHESIS OF *CIS* AND *TRANS* ISOMERS OF 4-CHLORO-L-PROLINE, 4-BROMO-L-PROLINE, AND 4-AMINO-L-PROLINE

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Summary

cis- and *trans*-4-Chloro- and 4-bromo-L-prolines have been synthesized stereospecifically, the key step being S_N2 displacement of a free or substituted 4-hydroxyl group in suitably protected 4-hydroxy-L-prolines. Similar displacements with azide ion followed by reduction provide convenient routes to *cis*- and *trans*-4-amino-L-proline. A less satisfactory pathway to *cis*-4-aminoproline is reduction of a 4-oximinoproline derivative. In the course of the syntheses, which involve a variety of protecting groups, 45 new L-proline derivatives have been prepared. Unexpected side reactions were the formation of *cis*-4-hydroxyprolinamide by the action of ammonia on *trans*-4-bromoproline, and the reduction by sodium borohydride of *N*-benzyloxycarbonyl-4-oximinoproline methyl ester to *N*-benzyloxycarbonyl-4-oximinoprolinol.

Introduction

Mauger and Witkop have recently reviewed proline and hydroxyproline chemistry with particular emphasis on analogues and homologues and their various biological ramifications.[1] We present here syntheses of the *cis* and *trans* isomers of 4-chloro-L-proline, 4-bromo-L-proline, and 4-amino-L-proline. A main aim has been to devise routes of preparative significance and this has required exploration of a variety of pathways. Starting material for all syntheses was natural *trans*-4-hydroxy-L-proline (I). Careful selection of the most appropriate protecting group for the secondary amino function was vital because reactions which proceed satisfactorily on monofunctional compounds often failed or gave unstable products in this series. Frequently, it was also necessary to mask the carboxyl group to avoid unwanted side reactions as well as to permit chromatographic purification of these very polar compounds. Preliminary announcement of some of our results was included in Mauger and Witkop's review.[1]

Discussion

S_N2 displacement of the secondary hydroxyl group in a suitably protected 4-hydroxy-L-proline would provide direct access to stereochemically defined 4-haloprolines. In general, for alcohols in which the transition states are unhindered, phosphorus halides produce inversion by an S_N2 mechanism, and thionyl halides substitute with retention by an S_Ni mechanism.[2] The presence of base in reactions

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with thionyl chloride promotes the S_N2 mechanism at the expense of S_Ni, although elimination may be a major competing reaction.

N-Tosyl*-*trans*-4-hydroxy-L-proline methyl ester (III) was readily prepared from the known[3] *N*-tosyl acid (II). The action of phosphorus pentachloride in chloroform on (III) gave an 83% yield of a chloro product, formulated as (XVII) in the *cis* (= allo) series on the above mechanistic grounds. Thionyl chloride in pyridine generated the same compound in 54% yield. Both to confirm the stereochemical assignment and to determine the yield with another reagent, the two-step replacement of OH by Cl using the method of Cramer and co-workers[4] was examined. This involves conversion of the alcohol group into its trichloroacetimidate by interaction of the alkoxide anion with CCl_3–CN. Treatment of the acetimidate with hydrogen chloride in ether forms the chloro compound with elimination of trichloroacetamide. In all known cases under these reaction conditions, this displacement proceeds with virtually quantitative inversion.[4]

Preparation of the trichloroacetimidate (XXIV) of the alcohol (III) proved difficult, and of many bases tried only sodium hydride was satisfactory. The action of hydrogen chloride in ether on (XXIV) gave a 54% yield of the chloro compound (XVII), and a further 30% of starting material was accounted for as the trichloroacetate (XXV) which must have been formed by reaction with water in the work-up. Hydrolysis of (XXIV) with aqueous acid provided an authentic specimen of (XXV).

The unprotected amino acid *cis*-4-chloro-L-proline (XV) was obtained almost quantitatively from its *N*-tosyl methyl ester (XVII) by alkaline hydrolysis to the acid (XVI) followed by detosylation with HBr/HOAc.

The key intermediate in the synthesis leading to the *trans*-4-chloro series was the known compound *N*-tosyl-*cis*-4-hydroxy-L-proline methyl ester (VIII).[5] This compound was obtained here by a more direct route through chromic acid oxidation of *N*-tosyl-*trans*-4-hydroxyproline (II) to the keto acid (XXVIII), reduction of which with sodium borohydride gave *N*-tosyl-*cis*-4-hydroxyproline (VII) as the single product in 90% yield. Such stereospecificity in hydride reductions of *N*-protected 4-oxoprolines is well known.[6] The *cis*-hydroxy acid (VII) was further characterized by cyclization to its lactone (XXVII) with dicyclohexylcarbodiimide. Esterification with diazomethane gave the ester (VIII). Thionyl chloride in pyridine or phosphorus pentachloride in chloroform converted this *cis*-hydroxy ester (VIII) into the corresponding *trans*-chloro derivative (XIV) in 92% and 88% yields respectively. Noticeably higher yields in these S_N2 reactions compared with those leading to the *cis*-series reflect differences in steric hindrance to chloride attack. Generation

* Tosyl = *p*-toluenesulphonyl.

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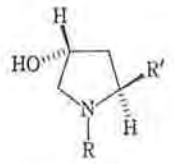
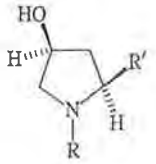
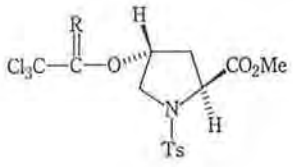


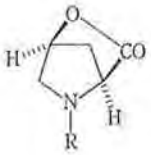
	R	R′
(I)	H	CO_2H
(II)	Ts	CO_2H
(III)	Ts	CO_2Me
(IV)	Ts	$CONH_2$
(V)	$PhSO_2$	CO_2H



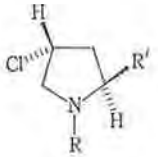
	R	R′
(VI)	H	$CONH_2$
(VII)	Ts	CO_2H
(VIII)	Ts	CO_2Me
(IX)	$PhSO_2$	CO_2H
(X)	$PhSO_2$	CO_2Me
(XI)	$PhSO_2$	$CONH_2$



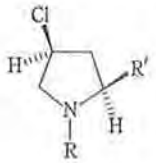
(XXIV) R = NH
(XXV) R = O



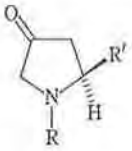
(XXVI) R = H
(XXVII) R = Ts



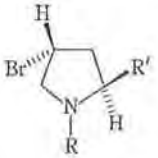
	R	R′
(XII)	H	CO_2H
(XIII)	Ts	CO_2H
(XIV)	Ts	CO_2Me



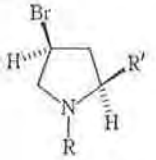
	R	R′
(XV)	H	CO_2H
(XVI)	Ts	CO_2H
(XVII)	Ts	CO_2Me



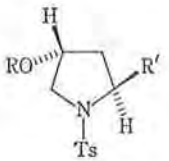
	R	R′
(XXVIII)	Ts	CO_2H
(XXIX)	Ts	CO_2Me
(XXX)	Ts	$CONH_2$
(XXXI)	$PhSO_2$	CO_2H
(XXXII)	Cbz	CO_2H
(XXXIII)	Cbz	CO_2Me
(XXXIV)	Cbz	CH_2OH



	R	R′
(XVIII)	H	CO_2H
(XIX)	Ts	CO_2H
(XX)	Ts	CO_2Me



	R	R′
(XXI)	H	CO_2H
(XXII)	Ts	CO_2H
(XXIII)	Ts	CO_2Me



	R	R′
(XXXV)	Ts	CO_2H
(XXXVI)	Ts	CO_2Me
(XXXVII)	Ts	$CONH_2$
(XXXVIII)	$MeSO_2$	CO_2Me

	R^1	R^2	R^3
(XXXIX)	Ts	CO_2Me	Ts
(XL)	$PhSO_2$	$CONH_2$	$PhSO_2$

of the *trans*-series requires attack from the unhindered face of the heterocyclic ring opposite to the CO_2Me group. Hydrolysis of (XIV) and detosylation gave *trans*-4-chloro-L-proline (XII), but in this case the intermediate *N*-tosyl-4-chloro acid (XIII) was very hygroscopic and difficult to manipulate.

Similar reactions led to both isomers of 4-bromo-L-proline. The *trans*-4-hydroxy derivative (III) was converted into the *N*-tosyl-*cis*-4-bromoproline methyl ester (XXIII) in 60% yield using phosphorus pentabromide in chloroform, and 14% yield with phosphorus tribromide in benzene. Removal of the protecting groups gave *cis*-4-bromo-L-proline (XXI) via the *N*-tosyl bromo acid (XXII).

Phosphorus pentabromide and the *cis*-4-hydroxy derivative (VIII) gave a 64% yield of the *trans*-4-bromo ester (XX), from which *trans*-4-bromo-L-proline (XVIII) was prepared in excellent yield via the *N*-tosyl bromo acid (XIX).

Other studies leading to the 4-bromo series involved the *trans*- and *cis*-*N*,*O*-ditosyl-4-hydroxy-L-proline methyl esters (XXXVI) and (XXXIX). These known compounds[5] were prepared here by treatment of the *N*-tosyl-4-hydroxy esters (III) and (VIII) with tosyl chloride in pyridine. The action of lithium bromide in acetone for 3 days on both (XXXVI) and (XXXIX) gave a 70% conversion into the 4-bromo series, but in each case the product was a mixture of the *cis* epimer (XXIII) and *trans* epimer (XX) in the ratio *c.* 2 : 5 respectively. Although the substitution of *O*-tosyl by bromide can be expected to proceed with inversion, further displacement of the 4-bromo atom by the excess of bromide leads to an equilibrium mixture of epimers. This is analogous to the displacement of *O*-tosyl groups by iodide ion in the *N*-Cbz*-4-hydroxyproline series.[5] Monitoring of the lithium bromide reactions by thin-layer chromatography revealed that the equilibration reaction proceeds at a similar rate to the *O*-tosyl displacement reaction so that this route cannot yield a single 4-bromo epimer. On the other hand the composition of the product mixture must be near the true thermodynamic equilibrium ratio.

So far the stereochemical assignment of these 4-haloprolines rests not on direct proof but on the expectation that the mechanism of displacement of secondary hydroxyl under the conditions used will follow its normal S_N2 course. Other observations support the assignments. Firstly, the higher relative yields in the displacement steps leading to *trans*-isomer (less hindered attack) rather than *cis*-isomer, and secondly, the greater relative proportion of the less hindered *trans*-4-bromo epimer in the thermodynamically equilibrated mixture of (XX) and (XXIII). Nuclear magnetic resonance spectra have been recorded for all the compounds in this paper in the expectation that correlations defining the *cis* and *trans* stereochemical families would emerge. This hope has been justified and the results fully confirm the stereochemistry already allotted. The detailed spectroscopic analyses and the ramifications concerning coupling constants and the Karplus equation in this series will be published separately.

The four new halogenated free amino acids (XII), (XV), (XVIII), and (XXI) are all well-crystalline, stable compounds, and are not hygroscopic.

Two obvious routes for the synthesis of 4-aminoprolines are S_N2 displacement of a suitable leaving group by a nitrogenous nucleophile, and reduction of the oxime

* Cbz = benzyloxycarbonyl.

of a protected 4-oxoproline. Success has been achieved by both routes but only after extensive variation of reaction conditions and protecting groups.

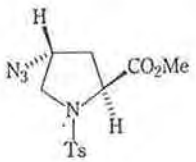
The use of ammonia as a nucleophile was unsatisfactory. When *trans*-4-bromoproline (XVIII) was treated with concentrated ammonium hydroxide, bromide ion was released quantitatively, but the product obtained was shown to be the hydrobromide of the hitherto unknown *cis*-4-hydroxyprolinamide (VI). This crystalline salt was too hygroscopic to be recrystallized, and the free base (VI) liberated by ion-exchange techniques was crystalline but unstable. It was characterized as its *N*,*O*-dibenzenesulphonyl derivative (XL). Proof of this unforeseen conversion of (XVIII) into (VI) was provided by an unequivocal synthesis of (XL). The known *N*-benzenesulphonyl-*trans*-4-hydroxy-L-proline (V)[7] was converted into its *cis* epimer (IX) by chromic acid oxidation to the keto acid (XXXI) followed by stereospecific sodium borohydride reduction. Diazomethane and methanol/ammonia gave in turn the *N*-benzenesulphonyl-*cis*-4-hydroxy ester (X) and amide (XI). The latter compound was not isolated but was treated directly with benzenesulphonyl chloride in aqueous base to yield authentic (XL).

Formation of *cis*-4-hydroxyprolinamide from *trans*-4-bromoproline and ammonium hydroxide is considered to take place by the following mechanism. Rather than S_N2 replacement of Br by NH_2, neighbouring group displacement by the *trans*-carboxylate anion occurs to yield the lactone (XXVI) which then undergoes ammonolysis to the hydroxy amide (VI). Provided the bromide displacement by carboxylate is a synchronous S_N2 process, this reaction supplies a chemical proof of stereochemistry for the 4-bromoprolines. Analogies exist for γ-lactone formation by intramolecular displacement of a *trans*-4-*O*-tosyl group by carboxylate anion.[8] There is negative evidence in our case to support the S_N2 mechanism over the alternative of S_N1 release of bromide ion followed by carboxylate attack on the C4 carbonium ion. When *cis*-4-bromoproline (XXI) was treated with ammonium hydroxide as for (XVIII), bromide ion was again released quantitatively, but the reaction product was a dark oil yielding no crystalline free base or solid derivative after benzenesulphonylation. Had an S_N1 mechanism operated with this epimer, some (VI) should have been formed and been isolable as (XL). There are always several competing reactions in the ammonolysis of halo acids to amino acids. Such side reactions do not prevent the process being a general method for the preparation of α-amino acids from α-halo acids, but they dominate our experiments.

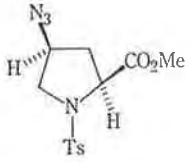
Displacement reactions with ammonia on the *N*,*O*-ditosyl methyl ester (XXXVI) were also unsuccessful, despite the fact that *O*-tosyl is typically a better leaving group than bromide[9] and that no complications due to carboxylate anion are possible. When (XXXVI) was treated with a solution of ammonia in methanol under mild pressure, only *N*,*O*-ditosyl-4-hydroxyprolinamide (XXXVII) was isolated. Repetitions of this reaction at pressures up to 100 atm and temperatures up to 100° failed to cause any displacement at C4. Neither did the reaction between (XXXVI) and sodium amide in liquid ammonia.

[7] Milne, H. B., and Peng, C.-H., *J. Am. chem. Soc.*, 1957, **79**, 639.
[8] Patchett, A. A., and Witkop, B., *J. Am. chem. Soc.*, 1957, **79**, 185.
[9] Gould, E. S., "Mechanism and Structure in Organic Chemistry." p. 261. (Holt, Rinehart & Winston: New York 1959.)

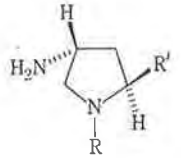
Azide ion, a more potent nucleophile, was satisfactory. Displacement with azide ion proceeds with inversion although the detailed mechanism probably involves an ion-pair intermediate rather than the classical S_N2 transition state.[10] The *trans*-*N*,*O*-ditosyl methyl ester (XXXVI) reacted with sodium azide in moist dimethylformamide to give the stable, crystalline, *N*-tosyl-*cis*-4-azido methyl ester (XLII) in 88% yield. The analogous reaction using exactly the same conditions on the corresponding 4-*O*-methanesulphonyl compound (XXXVIII), an oil prepared from (III) in the usual way, gave only a 54% yield of (XLII). Catalytic hydrogenation of the azide



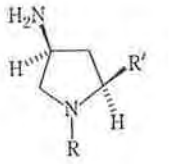
(XLI)



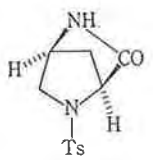
(XLII)



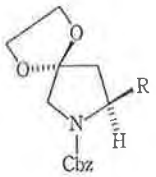
	R	R′
(XLIII)	H	CO_2H
(XLIV)	Ts	CO_2H
(XLV)	Ts	CO_2Me



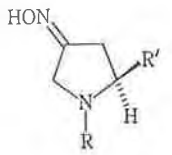
	R	R′
(XLVI)	H	CO_2H
(XLVII)	Ts	CO_2H
(XLVIII)	Ts	CO_2Me



(XLIX)



(L) R = CO_2Me
(LI) R = CH_2OH



	R	R′
(LII)	Ts	CO_2H
(LIII)	Ts	CO_2Me
(LIV)	Cbz	CO_2Me
(LV)	Cbz	CH_2OH

group in (XLII) yielded the expected *N*-tosyl-*cis*-4-amino ester (XLVIII) as an unstable viscous oil which was hydrolysed by alkali at room temperature to *N*-tosyl-*cis*-4-amino-L-proline (XLVII), a solid of high melting point. Reductive cleavage of the *N*-tosyl residue with hydrobromic acid/acetic acid gave *cis*-4-amino-L-proline (XLVI). This new dimino acid is crystalline, but is hygroscopic and discolours in air in a few days. Its spectroscopic properties are in full accord with the assigned structure. The hydrobromide salt is hygroscopic, and although the hydrochloride can be manipulated more readily it is not stable. Satisfactory analyses for the expected dihydrochloride have not been obtained. Repeated recrystallization causes it to decompose with formation of ammonium chloride. The free diamino acid is best preserved under nitrogen in sealed vessels after purification by ion-exchange methods.

[10] Weinor, H., and Sneen, R. A., *J. Am. chem. Soc.*, 1962, 84, 3599; 1965, 87, 292; Weiner, H., and Sneen, R. A., *Tetrahedron Lett.*, 1963, 1309; Larsen, J. W., and Sneen, R. A., *J. Am. chem. Soc.*, 1966, 88, 2593.

Direct confirmation of the *cis* stereochemistry was sought by cyclization reactions which would yield the *N*-tosyl lactam (XLIX). A variety of intramolecular cyclization attempts on the 4-amino ester (XLVIII) failed; the natural instability of this oil is apparently due to intermolecular polymerization. Attempts to induce cyclization of *N*-tosyl-*cis*-4-aminoproline (XLVII) with dicyclohexylcarbodiimide in organic solvents were also unsuccessful and the lactam (XLIX) was finally obtained by dehydration of (XLVII) in an aqueous medium using the water-soluble *N*-cyclohexyl-*N'*-(β-morpholinyl-4-ethyl)carbodiimide methyl *p*-toluenesulphonate. The product crystallized directly out of the aqueous reaction mixture.

The synthesis of *trans*-4-amino-L-proline paralleled that of the *cis* isomer. Displacement of the 4-*O*-tosyl group in the *cis*-*N*,*O*-ditosyl ester (XXXIX) by azide ion also proceeded in 88% yield to the crystalline *trans*-azido ester (XLI). Hydrogenation of (XLI) generated the oily, unstable *trans*-4-amino ester (XLV). Removal of protecting groups as before gave *N*-tosyl-*trans*-4-amino-L-proline (XLIV) and finally *trans*-4-amino-L-proline (XLIII). The stabilities of this compound and its hydrochloride and hydrobromide salts are similar to those of the *cis* compounds.

In other experiments it was found that the halogen atom in both *cis*- and *trans*-*N*-tosyl-4-chloroproline methyl esters (XVII) and (XIV) was not displaceable by azide ion, and that, although a reaction occurred when the *N*,*O*-ditosyl acid (XXXV) was treated with sodium azide in wet dimethylformamide, no azide absorption was present in the infrared spectrum of the reaction product.

cis-4-Aminoproline is also accessible, but in lower yield, by a route involving reduction of a 4-oximino group. *N*-Tosyl-4-oxoproline (XXVIII) readily formed the crystalline oxime (LII). Catalytic hydrogenation with platinum in ethanol/hydrochloric acid proceeded stereospecifically from the face of the proline ring opposite to the hindering carboxyl group and yielded (55%) the hygroscopic solid hydrochloride of *N*-tosyl-*cis*-4-aminoproline. Removal of HCl on an ion-exchange column gave the free base (XLVII), identical with that obtained above from the azide approach. The compound was further characterized as the hydrochloride of its methyl ester (XLVIII).

Problems associated with the choice of protecting groups are illustrated by the following results. The solid oxime (LIII) of *N*-tosyl-4-oxoproline methyl ester (XXIX) did not afford any satisfactory product despite an extensive series of reductive reactions including catalytic hydrogenation. This 4-keto ester (XXIX) was prepared from *N*-tosyl-*trans*-4-hydroxy-L-proline (II) both by oxidation to the keto acid (XXVIII) followed by esterification, and by the reverse order of reactions via the hydroxy ester (III); the former sequence gave a much higher overall yield. *N*-Tosyl-4-oxoprolinamide (XXX), prepared from the 4-hydroxy ester (III) by ammonolysis to the hydroxy amide (IV) followed by oxidation with chromic acid, not only failed to form an oxime, but decomposed under the reaction conditions.

N-Benzyloxycarbonyl was not useful as a protecting group for the oxime route. *N*-Benzyloxycarbonyl-4-oxo-L-proline (XXXII)[8] failed to yield an oxime, in contrast to the easy formation of the *N*-tosyl analogue (LII). The methyl ester (XXXIII) of the acid (XXXII) was prepared but it was an unstable oil. However,

it did yield the *N*-Cbz oxime (LIV), also as a relatively unstable oil. Like its crystalline *N*-tosyl relative (LIII) it failed to give any satisfactory product upon catalytic hydrogenation under a variety of conditions. On the other hand, sodium borohydride in buffered propan-2-ol reduced (LIV) to a white crystalline product having the correct molecular formula for *N*-benzyloxycarbonyl-4-amino-proline. Its spectroscopic properties did not, however, correspond to those expected for this amino compound, nor was it soluble in aqueous acid. The i.r. spectrum showed bands more in keeping with O–H rather than N–H stretching frequencies and no carbonyl absorption for an ester or a carboxyl group. Only two exchangeable protons were observable in the n.m.r. spectrum, and the other bands indicated the compound to be *N*-benzyloxycarbonyl-4-oxoprolinol oxime (LV), an isomer of *N*-benzyloxycarbonyl-4-aminoproline. An independent synthesis confirmed this structure. *N*-Benzyloxycarbonyl-4-oxoproline methyl ester (XXXIII) was converted into its ketal (L). Lithium borohydride reduction of the ester group in (L) yielded the ketalized prolinol (LI). Acid hydrolysis gave the free ketone (XXXIV) which on treatment with hydroxylamine hydrochloride in pyridine produced authentic (LV) identical to the sample above. Reduction of esters to alcohols by sodium borohydride is uncommon, but has been observed for molecules having electron-withdrawing α-substituents.[11]

Experimental

Melting points were determined on a Büchi melting point apparatus, and are uncorrected. Infrared spectra were recorded on a Perkin–Elmer 221 instrument as Nujol mulls unless otherwise indicated. Rotations were determined on a Hilger Mark III polarimeter with a 0·5-dm cell. Spence type H and neutral Woelm aluminas were used for chromatography. Microanalyses were carried out by Miss B. J. Stevenson of this Department and by the Australian Microanalytical Service, Melbourne. Nuclear magnetic resonance spectra have been recorded on a Varian Associates A60 instrument for all compounds described. In every case only absorption of expected chemical shift and area was observed. As implied above, the analysis of fine structure is complicated and the n.m.r. results form the subject matter of another paper. Microanalyses were not done on compounds (XI), (XIII), (XXXVIII), (XLIII), (XLV), (XLVI), and (XLVIII) because of difficulties in manipulation, and their n.m.r. spectra provided both the main criterion of purity, and the most direct structure confirmation. Identity of samples prepared by alternative routes was established by m.p., mixed m.p., and superposability of their i.r. and n.m.r. spectra.

(*a*) N-*Tosyl*-trans-*4-hydroxy*-L-*proline Methyl Ester* (*III*)

A solution of *N*-tosyl-*trans*-4-hydroxyl-L-proline[3] (II) (11·4 g) was treated with an excess of an ethereal solution of diazomethane. The reaction mixture was worked up in the usual way. N-*Tosyl*-trans-*4-hydroxy*-L-*proline methyl ester* crystallized from benzene as white needles (12·0 g, 90%), m.p. 103–104°, $[\alpha]_D^{20}$ −98·5° (1% in $CHCl_3$) (Found: C, 52·2; H, 5·8. $C_{13}H_{17}NO_5S$ requires C, 52·2; H, 5·7%). ν_{max} 3500, 1750 cm^{-1}.

(*b*) N-*Tosyl*-cis-*4-chloro*-L-*proline Methyl Ester* (*XVII*)

(i) Phosphorus pentachloride (4·5 g, 0·025 mole) was stirred into a solution of the ester (III) (3 g, 0·01 mole) in A.R. chloroform (30 ml). The reaction mixture was warmed gently, and, when all the phosphorus pentachloride had dissolved, it was heated under reflux for 0·5 hr. It was then cooled, diluted with chloroform (150 ml), washed with 5% sodium carbonate solution,

[11] House, H. O., "Modern Synthetic Reactions." p. 32. (Benjamin: New York 1965.); Gaylord, N. G., "Reduction with Complex Metal Hydrides." p. 500. (Interscience: New York 1956.)

brine, and dried ($MgSO_4$). The solvent was removed and the residue was purified by chromatography over deactivated alumina. N-*Tosyl*-cis-*4-chloro*-L-*proline methyl ester* crystallized from benzene/cyclohexane as white needles (2·65 g, 83%), m.p. 96·5–97·5°, $[\alpha]_D^{20}$ −46·6° (1·5% in $CHCl_3$) (Found: C, 49·2; H, 5·2; Cl, 10·9. $C_{13}H_{16}ClNO_4S$ requires C, 49·1; H, 5·1; Cl, 11·2%).

(ii) The ester (III) (3·0 g, 0·01 mole) was treated with dry pyridine (2 ml) followed by thionyl chloride (30 ml). The solution was heated under reflux for 2 hr and the excess of thionyl chloride was removed under reduced pressure. The residue was extracted with ether and the combined ethereal extracts were washed with brine and dried ($MgSO_4$). Removal of the solvent and crystallization of the yellow residue from benzene/cyclohexane gave the *cis*-chloro ester (XVII) as white needles (1·7 g, 54%), m..p 98–99°.

(iii) The finely powdered ester (III) (6·0 g) was suspended in dry ether (400 ml). The reaction mixture was refluxed under dry nitrogen until the supernatant was saturated. A suspension of 50% sodium hydride in paraffin oil (1·0 g) was added and the reaction mixture was heated under reflux for 1 hr. About half the volume of ether was removed by distillation. The white slurry produced was cooled to room temperature and trichloroacetonitrile (6 ml) was added to it with vigorous stirring. Chloroform (10 ml) was then introduced and the reaction mixture was stirred at room temperature for 3 hr before being filtered. Removal of the solvents from the filtrate gave a yellow oil which was dissolved in benzene/cyclohexane (1 : 1) and chromatographed over deactivated alumina. Elution with benzene/cyclohexane (5 : 1) gave N-*tosyl*-trans-*4-trichloroacetimidoxy*-L-*proline methyl ester* (XXIV) which crystallized from benzene/cyclohexane as colourless needles (6·95 g, 78·5%), m.p. 104–105°, $[\alpha]_D^{20}$ −78·5° (1·7% in $CHCl_3$) (Found: C, 40·8; H, 3·9; Cl, 23·9; N, 6·4. $C_{15}H_{17}Cl_3N_2O_5$ requires C, 40·6; H, 3·9; Cl, 24·0; N, 6·3%). ν_{max} ($CHCl_3$) 3340, 1745, 1665 cm^{-1}.

The foregoing compound (XXIV) (2·2 g) was dissolved in dry ether (100 ml) and a stream of pure dry nitrogen was bubbled through the solution. After 15 min the nitrogen stream was replaced by a slow stream of dry HCl gas. A white material which separated out during the first few minutes almost completely disappeared after 5 hr. The reaction mixture was heated under reflux for 3 hr and a stream of nitrogen was then passed through the turbid solution to remove excess of HCl. The ethereal solution was washed repeatedly with brine until neutral, and then dried ($MgSO_4$). The solvent was removed and the crystalline residue obtained was treated with cold ether (15 ml) and filtered from undissolved trichloroacetamide (0·44 g, 61%). Concentration of the filtrate and chromatography of the residue over deactivated alumina gave two fractions. Fraction 1 (eluted with benzene/cyclohexane, 4 : 1) gave *N*-tosyl-*cis*-4-chloro-L-proline methyl ester which crystallized from benzene/cyclohexane as colourless needles (0·86 g, 54%), m.p. 98–99°. Fraction 2 (eluted with benzene/ether, 1 : 1) gave N-*tosyl*-trans-*4-trichloroacetoxy*-L-*proline methyl ester* (XXV) which crystallized from benzene as colourless needles (0·67 g, 30%), m.p. 127–128°, $[\alpha]_D^{20}$ −82·8° (1·7% in $CHCl_3$) (Found: C, 40·8; H, 3·7; Cl, 23·9. $C_{15}H_{16}Cl_3NO_6S$ requires C, 40·5; H, 3·6; Cl, 23·5%). ν_{max} ($CHCl_3$) 1760, 1750 cm^{-1}.

(*c*) N-*Tosyl*-trans-*4-trichloroacetoxy*-L-*proline Methyl Ester* (*XXV*)

N-Tosyl-*trans*-4-trichloroacetimidoxy-L-proline methyl ester (XXIV) (0·44 g) was dissolved in ether (50 ml) and to this solution was added with stirring over 4 hr a 10% solution of acetic acid (50 ml). The ethereal phase was separated, washed with brine, and dried ($MgSO_4$). Removal of solvent and crystallization of the residue from benzene gave the trichloroacetate (XXV) as colourless needles (0·32 g, 72%), m.p. 127–128°.

(*d*) N-*Tosyl*-cis-*4-chloro*-L-*proline* (*XVI*)

N-Tosyl-*cis*-4-chloro-L-proline methyl ester (XVII) (3·2 g) in methanol (25 ml) was stirred at 0° while 1M NaOH (11 ml) was slowly added (10 min). After 1 hr the ice-bath was removed, and the reaction mixture was stirred at room temperature until all the solid had dissolved (3 hr). Methanol was removed under vacuum and the solution was cooled to 0° and acidified with 1M HCl. The resulting crystalline precipitate was collected and washed with a little ice-water. Crystallization from ethanol/water gave N-*tosyl*-cis-*4-chloro*-L-*proline* as colourless needles (2·9 g,

95%), m.p. 174–175°, $[\alpha]_D^{20}$ −49·6 (2% in EtOH) (Found: C, 47·4; H, 4·7; Cl, 11·5; N, 4·3. $C_{12}H_{14}ClNO_4S$ requires C, 47·4; H, 4·7; Cl, 11·5; N, 4·3%).

(*e*) cis-*4-Chloro*-L-*proline* (*XV*)

N-Tosyl-*cis*-4-chloro-L-proline (XVI) (2·8 g, 0·01 mole) and phenol (2·0 g, 0·02 mole) were dissolved in acetic acid containing hydrogen bromide (45%, 20 ml). The reaction flask was sealed and allowed to stand at room temperature for 26 hr.[12] The reaction mixture was poured into a stirred flask of dry ether (200 ml). The white, crystalline precipitate formed was filtered off, dissolved in water (50 ml), and adsorbed on a Dowex 50 cation-exchange column in the H^+ form. The column was washed thoroughly with distilled water and the amino acid was eluted with 0·4M NH_4OH. Removal of water under vacuum and crystallization of the residue from water/acetone gave cis-*4-chloro*-L-*proline* as white prisms (1·35 g, 98%), m.p. 224–225° (dec.), $[\alpha]_D^{20}$ −29·5° (1·5% in H_2O) (Found: C, 40·4; H, 5·4; Cl, 23·1; N, 9·5. $C_5H_8ClNO_2$ requires C, 40·2; H, 5·4; Cl, 23·7; N, 9·5%).

(*f*) N-*Tosyl-4-oxo*-L-*proline* (*XXVIII*)

Chromium trioxide in sulphuric acid solution[13] (32 ml) was added dropwise to a stirred solution of *N*-tosyl-*trans*-4-hydroxy-L-proline (II) (8·64 g) in A.R. acetone (150 ml), care being taken to ensure that the reaction temperature did not rise above 30°. Stirring was continued for a further 1 hr and excess of oxidant was destroyed by careful addition of methanol. Precipitated chromium salts were removed by filtration. The filtrate was concentrated (50 ml), diluted with chloroform (150 ml), washed with water (2 × 50 ml), and dried ($NaSO_4$). Removal of solvent gave N-*tosyl-4-oxo*-L-*proline* as a white solid which crystallized from ethyl acetate as white needles (7·1 g, 84%), m.p. 176–178°, $[\alpha]_D^{20}$ −1·2° (1% in EtOH) (Found: C, 50·7; H, 4·5; N, 4·8. $C_{12}H_{13}NO_5S$ requires C, 50·8; H, 4·6; N, 5·0%). ν_{max} 1750, 1710 cm^{-1}.

(*g*) N-*Tosyl*-cis-*4-hydroxy*-L-*proline* (*VII*)

The keto acid (XXVIII) (9·5 g, 0·034 mole) was dissolved in methanol (200 ml) and the solution was cooled to 0°. A cold solution of sodium borohydride (4 g) in water (15 ml) was added dropwise with stirring. The reaction mixture was left at 0° for 1 hr and the methanol was then removed. The residue was treated with 1M NaOH (70 ml) at room temperature for 0·5 hr and a clear solution resulted. This was cooled to 0°, acidified with 10M HCl, and extracted with ethyl acetate (3 × 100 ml). The combined ethyl acetate extracts were washed with brine and dried ($MgSO_4$). On reduction of volume, *N*-tosyl-*cis*-4-hydroxy-L-proline crystallized as shiny white prisms (8·6 g, 90%), m.p. 145–146°, $[\alpha]_D^{20}$ −74·8° (2·5% in EtOH) (lit.[5] m.p. 146–147°, $[\alpha]_D^{20}$ −43·9±1·0° (1% in EtOH)).

(*h*) N-*Tosyl*-cis-*4-hydroxy*-L-*proline Lactone* (*XXVII*)

The foregoing *cis*-hydroxy acid (VII) (0·86 g, 0·003 mole) was dissolved in methylene chloride (100 ml) and stirred with anhydrous magnesium sulphate (0·5 g) for 30 min. *N,N'*-Dicyclohexylcarbodiimide (0·62 g, 0·003 mole) was added and after 24 hr the reaction mixture was filtered. The residue obtained by evaporation of the filtrate was dissolved in benzene (15 ml) and chromatographed on deactivated alumina. The *lactone* (XXVII) crystallized from benzene/cyclohexane as colourless prisms (0·46 g, 57%), m.p. 106–107°, $[\alpha]_D^{20}$ +27·7° (3% in $CHCl_3$) (Found: C, 54·0; H, 5·0; N, 5·1. $C_{12}H_{13}NO_4S$ requires C, 54·0; H, 4·7; N, 5·2%). ν_{max} ($CHCl_3$) 1800 cm^{-1}.

(*i*) N-*Tosyl*-cis-*4-hydroxy*-L-*proline Methyl Ester* (*VIII*)

The *cis*-hydroxy acid (VII) (5·8 g) in methanol (50 ml) was esterified at 0° with excess of ethereal diazomethane. The reaction mixture was worked up in the usual way, and the *cis*-hydroxy

[12] Weisblat, D. I., Magerlein, B. J., and Myers, D. R., *J. Am. chem. Soc.*, 1953, 75, 3630.
[13] Bladon, P., Fabian, J. M., Henbest, H. B., Koch, H. P., and Wood, G. W., *J. chem. Soc.*, 1951, 2407.

ester (VIII) crystallized from benzene as white prisms (5·5 g, 90%), m.p. 100–101° raised to m.p. 104° by recrystallization, $[\alpha]_D^{20}$ −69·0° (3% in $CHCl_3$) (lit.[5] m.p. 103–104°, $[\alpha]_D^{20}$ −66·5±1·0° (1% in $CHCl_3$)).

(*j*) N-*Tosyl*-trans-*4-chloro*-L-*proline Methyl Ester* (*XIV*)

(i) Treatment of the *cis*-hydroxy ester (VIII) (3 g) with thionyl chloride and pyridine as in (*b*)(ii) gave N-*tosyl*-trans-*4-chloro*-L-*proline methyl ester* which crystallized from benzene/cyclohexane as white needles (2·9 g, 92%), m.p. 113–114°, $[\alpha]_D^{20}$ −110·3° (1·8% in $CHCl_3$) (Found: C, 49·0; H, 5·0; N, 4·5. $C_{13}H_{16}ClNO_4S$ requires C, 49·1; H, 5·1; N, 4·4%).

(ii) Treatment of the *cis*-hydroxy ester (VIII) (3 g) with phosphorus pentachloride in chloroform as in (*b*)(i) gave the *trans*-chloro compound which crystallized from benzene/cyclohexane as white needles (2·8 g, 88%), m.p. 113–114°.

(*k*) trans-*4-Chloro*-L-*proline* (*XII*)

The *trans*-chloro ester (XIV) (4·8 g) was hydrolysed as described above in (*d*). N-*Tosyl*-trans-*4-chloro*-L-*proline* (XIII) was obtained as a hygroscopic solid (4·5 g, 98%) which was very difficult to manipulate.

Reductive detosylation of this compound (XIII) was carried out as described above in (*e*). trans-*4-Chloro*-L-*proline* crystallized from water/acetone as white prisms [1·65 g, 73% from (XII)], m.p. 194–195° (dec.), $[\alpha]_D^{20}$ −55·6° (2% in H_2O) (Found: C, 40·4; H, 5·5; Cl, 22·9; N, 9·5. $C_5H_8ClNO_2$ requires C, 40·2; H, 5·4; Cl, 22·9; N, 9·5%).

(*l*) N-*Tosyl*-cis-*4-bromo*-L-*proline Methyl Ester* (*XXIII*)

(i) *N*-Tosyl-*trans*-4-hydroxy-L-proline methyl ester (III) (3·0 g, 0·01 mole) was dissolved in dry benzene (30 ml) and phosphorus tribromide (2·0 g, 0·007 mole) was added to this solution dropwise over a period of 10 min. The reaction mixture was allowed to stand at room temperature for 2 hr and then heated under reflux for 1 hr. It was cooled, washed with cold water, 5% Na_2CO_3, brine, and dried ($MgSO_4$). Solvent was removed and the residue was chromatographed over deactivated alumina. Two fractions were obtained. Fraction 1 (eluted with benzene/chloroform, 10 : 1) gave N-*tosyl*-cis-*4-bromo*-L-*proline methyl ester* which crystallized from benzene/cyclohexane as white needles (0·5 g, 14%), m.p. 78–79°, $[\alpha]_D^{20}$ −36·9° (2% in $CHCl_3$) (Found: C, 43·3; H, 4·6; Br, 22·3; N, 3·9. $C_{13}H_{16}BrNO_4S$ requires C, 43·1; H, 4·5; Br, 22·1; N, 3·9%). Fraction 2 (eluted with chloroform) gave the starting material (III) (2·1 g), m.p. 103–104°.

(ii) Phosphorus pentabromide (5·0 g, 0·01 mole) was slowly added with stirring and warming to a solution of the *trans*-hydroxy compound (III) (3·0 g, 0·01 mole) in A.R. chloroform (30 ml). When all the reagent had dissolved the flask was sealed, left at room temperature for 3 hr, and then heated to 60° for 0·5 hr. The reaction mixture was cooled, washed with 5% Na_2CO_3, brine, and then dried ($MgSO_4$). The solvent was removed and the residue was purified by chromatography over deactivated alumina. The *cis*-bromo compound (XXIII) crystallized from benzene/cyclohexane as white needles (2·2 g, 60%), m.p. 78–79°.

(*m*) cis-*4-Bromo*-L-*proline* (*XXI*)

The hydrolysis of the *cis*-bromo compound (XXIII) (4·35 g) was performed as described in (*d*). N-*Tosyl*-cis-*4-bromo*-L-*proline* (XXII) crystallized from acetone/ethanol/water as white prisms (3·85 g, 92%), m.p. 172–173°, $[\alpha]_D^{20}$ −34·3° (2% in EtOH) (Found: C, 41·7; H, 4·3; Br, 23·1. $C_{12}H_{14}BrNO_4S$ requires C, 41·4; H, 4·1; Br, 23·0%).

Reductive detosylation of compound (XXII) was carried out as described in (*e*). cis-*4-Bromo*-L-*proline* crystallized from water/ethanol/acetone as white needles (1·9 g, 96%), m.p. 167–168° (dec.), $[\alpha]_D^{20}$ −17·5° (2·5% in H_2O) (Found: C, 30·9; H, 4·2; Br, 41·4; N, 7·0. $C_5H_8BrNO_2$ requires C, 31·0; H, 4·2; Br, 41·2; N, 7·2%).

(*n*) N-*Tosyl*-trans-*4-bromo*-L-*proline Methyl Ester* (*XX*)

Synthesis of this compound from *N*-tosyl-*cis*-4-hydroxy-L-proline methyl ester (VIII) (6 g) and phosphorus pentabromide was carried out as described in (*l*)(ii) to give the trans-

bromo ester (XX) as white needles (4·65 g, 64%), m.p. 93–94°, $[\alpha]_D^{20}$ −90·3% (1·5% in $CHCl_3$) (Found: C, 43·5; H, 4·7; Br, 22·2; N, 3·8. $C_{13}H_{16}BrNO_4S$ requires C, 43·1; H, 4·5; Br, 22·1; N, 3·9%).

(*o*) trans-*4-Bromo*-L-*proline* (*XVIII*)

Hydrolysis of the *trans*-bromo ester (XX) (4·35 g) was accomplished as described in (*d*). N-*Tosyl*-trans-*4-bromo*-L-*proline* (XIX) crystallized from chloroform/light petroleum as colourless needles (4·0 g, 96%), m.p. 119–120°, $[\alpha]_D^{20}$ −92·5 (2% in EtOH) (Found: C, 41·2; H, 4·2; Br, 23·1; N, 4·1. $C_{12}H_{14}BrNO_4S$ requires C, 41·3; H, 4·1; Br, 23·0; N, 4·0%).

The reductive detosylation of compound (XIX) was performed as described in (*e*). trans-*4-Bromo*-L-*proline* crystallized from water/ethanol/acetone as white needles (1·9 g, 96%), m.p. 166–167° (dec.), $[\alpha]_D^{20}$ −38·2 (2% in H_2O) (Found: C, 31·0; H, 4·3; Br, 41·1; N, 7·0. $C_5H_8BrNO_2$ requires C, 31·0; H, 4·2; Br, 41·1; N, 7·2%).

(*p*) N,O-*Ditosyl*-trans-*4-hydroxy*-L-*proline Methyl Ester* (*XXXVI*)

N-Tosyl-*trans*-4-hydroxy-L-proline methyl ester (III) (8·5 g, 0·028 mole) was dissolved in dry A.R. pyridine (25 ml) and the solution was chilled in an ice-bath. A cold solution of *p*-toluenesulphonyl chloride (5·7 g, 0·03 mole) in dry A.R. pyridine (20 ml) was added and the reaction mixture was left at 0° for 3 days. Ice-cold 2M HCl (170 ml) was added and the resulting crystalline precipitate was collected and washed with 2M HCl (50 ml). Crystallization from ethyl acetate/ether (charcoal) gave *N,O*-ditosyl-*trans*-4-hydroxy-L-proline methyl ester as white needles (8·5 g, 66%), m.p. 95°, $[\alpha]_D^{20}$ −54·8° (2% in $CHCl_3$) (lit.[5] m.p. 94–95·5°, $[\alpha]_D^{20}$ −54·1±1·0° (1% in $CHCl_3$)).

(*q*) N,O-*Ditosyl*-cis-*4-hydroxy*-L-*proline Methyl Ester* (*XXXIX*)

Tosylation of the *cis*-4-hydroxy derivative (VIII) (4·5 g) was carried out as described in (*p*) to give the *cis*-ditosyl compound (XXXIX) as white needles (5·1 g, 75%), m.p. 94–95° raised to m.p. 98–99° by recrystallization, $[\alpha]_D^{20}$ −24·8° (2% in $CHCl_3$) (lit.[5] m.p. 123–124°, $[\alpha]_D^{20}$ −25·0±1·0° (1% in $CHCl_3$)) (Found: C, 53·0; H, 5·3. Calc. for $C_{20}H_{23}NO_7S_2$: C, 53·0; H, 5·1%).

(*r*) *Reaction of* trans- *and* cis-N,O-*Ditosyl-4-hydroxy*-L-*proline Methyl Esters with Lithium Bromide*

(i) The *trans*-ditosyl compound (XXXVI) (4·5 g, 0·01 mole) in acetone (30 ml) was treated with lithium bromide (2·5 g, 0·03 mole). The reaction mixture was refluxed for 3 days and the precipitated lithium *p*-toluenesulphonate (1·4 g) was filtered off. The filtrate was evaporated to dryness and the residue was dissolved in chloroform and chromatographed on alumina. Crystallization of the chromatographed material from benzene/cyclohexane gave as the first crop almost pure *N*-tosyl-*trans*-4-bromo-L-proline methyl ester (XX) (2·1 g) which was obtained pure after one further crystallization (1·9 g, 53%), m.p. 92–93°. The combined mother liquors gave, after addition of a little cyclohexane, *N*-tosyl-*cis*-4-bromo-L-proline methyl ester (XXIII) (0·9 g) which was obtained pure after one further crystallization (0·8 g, 21%), m.p. 77–78°.

(ii) The reaction of the *cis*-ditosyl compound (XXXIX) (4·5 g) with lithium bromide was carried out as described in (i) to give the *cis*-bromo ester (XXIII) (0·9 g, 25%), m.p. 77–78°, and the *trans*-bromo ester (XX) (1·8 g, 50%), m.p. 92°.

(*s*) *Reaction of* trans-*4-Bromo*-L-*proline with Aqueous Ammonia: Formation and Characterization of* (*VI*)

trans-4-Bromo-L-proline (XVIII) (0·5 g) was dissolved in conc. ammonium hydroxide (50 ml) and the reaction flask was sealed and allowed to stand at room temperature for 1 week. The solvent was removed under vacuum below 40° and the residual oil was dried over P_2O_5 under vacuum. After a few days the oil (0·56 g) began to crystallize in long needles. Attempts to recrystallize the extremely hygroscopic material failed. It was dissolved in water (10 ml) and adsorbed on a Dowex 50 cation-exchange column in the H^+ form. The column was washed with water until the eluate was neutral and then with 0·5M NH_4OH. The acidic water washings containing HBr were titrated with 0·1M NaOH. Consumption was 98% of the theoretically calculated

amount. The ammonium hydroxide eluate was concentrated under vacuum and the oily residue was dried under vacuum over P_2O_5. After 3 days the material started to crystallize in long plates (0·33 g). The compound was found to be unstable and therefore difficult to manipulate.

The foregoing compound (VI) (0·33 g) was dissolved in 1M NaOH (10 ml) and a solution of benzenesulphonyl chloride (1·0 g) in ether (15 ml) was added. The reaction mixture was shaken mechanically for 4 hr and the precipitated material was filtered, washed with ether, water, and then dried under vacuum. Crystallization from chloroform/light petroleum gave N,O-*dibenzenesulphonyl*-cis-*4-hydroxy*-L-*prolinamide* (XL) as white needles (0·84 g, 79% from (XVIII)), m.p. 184–185·5°, $[\alpha]_D^{25}$ −70·2° (1·8% in $CHCl_3$) (Found: C, 49·6; H, 4·6; N, 6·8. $C_{17}H_{18}N_2O_6S_2$ requires C, 49·7; H, 4·4; N, 6·8%). ν_{max} ($CHCl_3$) 3510, 3400, 1690 cm^{-1}.

(*t*) N-*Benzenesulphonyl*-cis-*4-hydroxy*-L-*proline Methyl Ester* (*X*)

N-Benzenesulphonyl-*trans*-4-hydroxy-L-proline (V) was prepared by a known method[7] in 85% yield, m.p. 150–151°, $[\alpha]_D^{20}$ −96·2° (2·5% in EtOH) (lit.[7] m.p. 143–144°) (Found: C, 48·9; H, 5·0; N, 5·1. Calc. for $C_{11}H_{13}NO_5S$: C, 48·7; H, 4·8; N, 5·2%).

Chromic acid oxidation of (V) (5·45 g) was carried out as described in (*f*). The *4-keto acid* (XXXI) crystallized from ethyl acetate/light petroleum as white needles (3·9 g, 73%), m.p. 175–176°, $[\alpha]_D^{20}$ −4·8° (2·5% in EtOH) (Found: C, 49·0; H, 4·3; N, 5·2. $C_{11}H_{11}NO_5S$ requires C, 49·0; H, 4·1; N, 5·2%).

Reduction of (XXXI) (4·05 g) with sodium borohydride was carried out as described in (*g*). The cis-*4-hydroxy derivative* (IX) crystallized from ethyl acetate/light petroleum as white needles (3·0 g, 74%), m.p. 113·5–114·5°, $[\alpha]_D^{20}$ −72·4 (2·5% in EtOH) (Found: C, 48·9; H, 5·1; N, 4·9. $C_{11}H_{13}NO_5S$ requires C, 48·7; H, 4·8; N, 5·2%).

Esterification of (IX) (2·7 g) was accomplished with diazomethane. N-*Benzenesulphonyl*-cis-*4-hydroxy*-L-*proline methyl ester* (X) crystallized from benzene/cyclohexane as colourless prisms (2·4 g, 85%), m.p. 104–105°, $[\alpha]_D^{25}$ −70·3° (2·6% in $CHCl_3$) (Found: C, 50·6; H, 5·5; N, 4·4. $C_{12}H_{15}NO_5S$ requires C, 50·5; H, 5·3; N, 4·9%).

(*u*) N,O-*Dibenzenesulphonyl*-cis-*4-hydroxy*-L-*prolinamide* (*XL*)

The ester (X) (1·43 g) was dissolved in methanol (20 ml) and the solution was added dropwise to liquid ammonia (40 ml) over a period of 20 min. The reaction mixture was stoppered and allowed to stand at room temperature for 2 days and then evaporated to dryness under vacuum to give a colourless material (1·3 g). Attempts to crystallize this *4-hydroxy amide* (XI) failed.

The compound (XI) (1·3 g) was dissolved in 1M NaOH (15 ml) and water (10 ml). To this solution benzenesulphonyl chloride (1·76 g) in ether (30 ml) was added, and the reaction mixture was shaken mechanically for 4 hr. The precipitated material was collected, dissolved in chloroform (100 ml), and the solution was dried ($MgSO_4$). Removal of solvent and crystallization of the residue from chloroform/light petroleum gave the prolinamide (XL) as white needles (1·72 g, 84% overall yield), m.p. 184·5–185·5°.

(*v*) *Reaction of* N,O-*Ditosyl*-trans-*4-hydroxy*-L-*proline Methyl Ester with Ammonia: Formation of* N,O-*Ditosyl*-trans-*4-hydroxy*-L-*prolinamide* (*XXXVII*)

Interaction of the ditosyl methyl ester (XXXVI) (1·2 g) with liquid ammonia was carried out as described in (*u*). Crystallization of the reaction product from methylene chloride gave the *prolinamide* (XXXVII) as white needles (0·94 g, 85%), m.p. 217·5° (Found: C, 52·2; H, 5·3; N, 6·2. $C_{19}H_{22}N_2O_6S_2$ requires C, 52·1; H, 5·1; N, 6·4%). ν_{max} 3445, 3320, 3270, 1670 cm^{-1}.

(*w*) N-*Tosyl*-cis-*4-azido*-L-*proline Methyl Ester* (*XLII*)

(i) The *trans*-N,O-ditosyl methyl ester (XXXVI) (3·0 g, 0·007 mole) was dissolved in dimethylformamide (30 ml), and sodium azide (0·6 g, 0·009 mole) in water (3 ml) was added. The reaction mixture was heated at 70° for 5 hr. It was cooled, and poured into a mixture of

saturated brine (500 ml) and water (100 ml), and extracted with ether (3 × 150 ml). The combined ether extracts were washed with saturated brine and dried ($MgSO_4$). Removal of the ether and crystallization of the residue from ether/light petroleum gave the cis-*azido ester* (XLII) as colourless needles (1·89 g, 88%), m.p. 69–70°, $[\alpha]_D^{21}$ −47·8° (2% in $CHCl_3$) (Found: C, 47·8; H, 5·1; N, 17·2. $C_{13}H_{16}N_4O_4S$ requires C, 48·1; H, 4·9; N, 17·3%). ν_{max} ($CHCl_3$) 2108, 1750 cm^{-1}.

(ii) The preparation of N-*tosyl*-O-*methanesulphonyl*-trans-*4-hydroxy*-L-*proline methyl ester* (XXXVIII) (5·1 g, 81%) from the *N*-tosyl methyl ester (III) (5·0 g) and methanesulphonyl chloride (2·0 g) was carried out as described in (*p*) for the *N*,*O*-ditosyl compound (XXXVI). The azide displacement reaction of this oily ester (XXXVIII) (2·0 g) was performed as in (i) above. The *cis*-azido ester (XLII) crystallized from ether/light petroleum as needles (0·93 g, 54%), m.p. 67–68°.

(*x*) N-*Tosyl*-cis-*4-amino*-L-*proline* (*XLVII*) *via the Azide Route*

The *cis*-azido ester (XLII) (1·0 g) in methanol (50 ml) was hydrogenated in the presence of 20% palladium on charcoal (0·12 g) at room temperature and 1 atm pressure. After 2 hr the catalyst was filtered off and the solvent was removed under vacuum to give N-*tosyl*-cis-*4-amino*-L-*proline methyl ester* (XLVIII) as a viscous oil (0·75 g, 82%), n_D^{18} 1·6450. The compound was found to be very unstable and was hydrolysed immediately after preparation.

The 4-amino ester (XLVIII) (3·0 g) in methanol (100 ml) was hydrolysed with 2·5M NaOH (15 ml) at room temperature for 6 hr. The reaction mixture was then chilled and acidified with 2M HCl to pH 6. It was concentrated and partitioned between water (pH 2) and chloroform. The aqueous phase was percolated through a Dowex 50 cation-exchange column in the H^+ form. The column was washed with distilled water (250 ml) and eluted with 2M NH_4OH containing 20% ethanol. Removal of solvent under vacuum from the ammoniacal eluate gave N-*tosyl*-cis-*4-amino*-L-*proline* as prisms (2·7 g, 94%), m.p. 278–280° (dec.). Crystallization from aqueous ethanol gave white needles, m.p. 284–285° (dec.), $[\alpha]_D^{20}$ −72° (0·4% in EtOH/H_2O) (Found: C, 50·2; H, 5·7; N, 9·9. $C_{12}H_{16}N_2O_4S$ requires C, 50·7; H, 5·6; N, 9·9%). ν_{max} 3580, 3360, 2750, 1610, 1580 cm^{-1}.

(*y*) cis-*4-Amino*-L-*proline* (*XLVI*)

The reductive detosylation of the amino compound (XLVII) (9·0 g) was carried out as described in (*s*) to give cis-*4-amino*-L-*proline* as a yellow solid (3·5 g, 85%). The diamino acid crystallized with difficulty from aqueous ethanol as white prisms, m.p. 191–193° (dec.), $[\alpha]_D^{20}$ −57·4° (3% in H_2O), ν_{max} 3400, 3310, 3220, 1600 cm^{-1}. It was found to be hygroscopic and unstable and could not be analysed satisfactorily. A mass spectrum confirmed the molecular weight of 130 (MS9 instrument). The *dihydrochloride* of (XLVI), m.p. 247°, prepared by evaporation of a solution in aqueous HCl, was also found to be unstable.

(*z*) N-*Tosyl*-cis-*4-amino*-L-*proline Lactam* (*XLIX*)

N-Cyclohexyl-*N'*-(β-morpholinyl-4-ethyl)carbodiimide methyl *p*-toluenesulphonate (0·465 g) was added to a stirred solution of N-tosyl-*cis*-4-amino-L-proline (XLVII) (0·285 g) in 30% aqueous ethanol (10 ml) at 70°, and the reaction mixture was maintained at this temperature for 7·5 hr. The crystalline product was filtered off, and the filtrate was extracted with chloroform (2 × 5 ml). Removal of solvent from the dried ($MgSO_4$) extract gave a white solid which was combined with the filtered crystals. Crystallization from ethanol/benzene gave the *lactam* (XLIX) as white prisms (0·15 g, 56%), m.p. 250–251°, $[\alpha]_D^{25}$ +26·7° (0·3% in EtOH) (Found: C, 53·9; H, 5·3; N, 10·2. $C_{12}H_{14}N_2O_3S$ requires C, 54·1; H, 5·3; N, 10·5%). ν_{max} 3220, 1710 cm^{-1}.

(*aa*) N-*Tosyl*-trans-*4-azido*-L-*proline Methyl Ester* (*XLI*)

Reaction of the *cis-N,O*-ditosyl ester (XXXIX) (3·0 g) with sodium azide in moist dimethylformamide was accomplished as described in (*w*)(i) to give the trans-*azido ester* (XLI) as white slender needles (1·9 g, 88·4%), m.p. 123°, $[\alpha]_D^{20}$ −45·5° (2% in $CHCl_3$) (Found: C, 48·2; H, 5·1; N, 17·4. $C_{13}H_{16}N_4O_4S$ requires C, 48·1; H, 4·9; N, 17·3%). ν_{max} ($CHCl_3$) 2119, 1750 cm^{-1}.

(*bb*) N-*Tosyl*-trans-*4-amino*-L-*proline* (*XLIV*)

The *trans*-azido ester (XLI) (4·0 g) in methanol (100 ml) was hydrogenated for 1 hr with 20% palladium on charcoal catalyst at room temperature and 5 atm hydrogen pressure to give N-*tosyl*-trans-*4-amino*-L-*proline methyl ester* (XLV) as a viscous oil (3·04 g, 82·5%), n_D^{22} 1·6490. This unstable amino ester (3·0 g) was hydrolysed as described in (*x*) to give N-*tosyl*-trans-*4-amino*-L-*proline* as a white solid (2·8 g, 96·5%). Crystallization from aqueous ethanol gave white prisms, m.p. 275–276° (dec.), $[\alpha]_D^{20}$ −62·7° (0·3% in EtOH/H_2O) (Found: C, 50·4; H, 5·6; N, 9·5. $C_{12}H_{16}O_4N_2S$ requires C, 50·7; H, 5·6; N, 9·9%). ν_{max} 3580, 3370, 2800, 1610, 1580 cm^{-1}.

(*cc*) trans-*4-Amino*-L-*proline* (*XLIII*)

Reductive detosylation of the amino compound (XLIV) (2·2 g) was performed as described in (*e*). trans-*4-Amino*-L-*proline* was obtained as an oil which crystallized slowly to white prisms (0·75 g, 80%), m.p. 229–230° (dec.), $[\alpha]_D^{21}$ −57·8° (1·2% in H_2O), ν_{max} 3400, 3305, 3220, 1610 cm^{-1}. It was found to be hygroscopic and unstable. A mass spectrum confirmed the molecular weight of 130. The *dihydrochloride* of (XLIII), m.p. 257–258°, was found to be unstable.

(*dd*) N,O-*Ditosyl*-trans-*4-hydroxy*-L-*proline* (*XXXV*)

The *trans-N,O*-ditosyl ester (XXXVI) (5·0 g) in methanol (100 ml) was hydrolysed in 1·2M NaOH (15 ml) at 0° for 48 hr. The reaction mixture was acidified to pH 4 at 0°. The crude product which precipitated was collected, dissolved in ethyl acetate, and the solution was washed with brine. Removal of solvent from the dried ($MgSO_4$) solution and crystallization of the residue from ethyl acetate/light petroleum gave N,O-*ditosyl*-trans-*4-hydroxy*-L-*proline* as white needles (3·1 g, 64%), m.p. 147–148°, $[\alpha]_D^{20}$ −60·2° (1% in EtOH) (Found: C, 51·9; H, 5·0. $C_{19}H_{21}NO_7S_2$ requires C, 51·9; H, 4·8%). ν_{max} ($CHCl_3$) 1725 cm^{-1}.

(*ee*) N-*Tosyl-4-oximino*-L-*proline* (*LII*)

(i) *N*-Tosyl-4-oxo-L-proline (XXVIII) (2 g, 0·007 mole) and hydroxylamine hydrochloride (2·5 g, 0·03 mole) were dissolved in a mixture of pyridine (10 ml) and absolute ethanol (10 ml) and the reaction mixture was heated under reflux for 2·5 hr. After removal of solvents the residue was partitioned between ethyl acetate (3 × 100 ml) and 1·3M HCl (120 ml) at 0°. The combined organic layers were washed with brine (2 × 50 ml) and dried ($MgSO_4$). Removal of solvent and crystallization of the residual oil from ethyl acetate/cyclohexane gave the oxime (LII) as white needles (1·7 g, 79%), m.p. 152–153°, $[\alpha]_D^{25}$ +4° (1% in EtOH) (Found: C, 48·4; H, 5·0. $C_{12}H_{14}N_2O_5S$ requires C, 48·3; H, 4·7%). ν_{max} ($CHCl_3$) 3685, 3600, 3050, 1760 cm^{-1}.

(ii) The keto acid (XXVIII) (1·5 g, 0·005 mole), methanol (120 ml), and water (4 ml) were placed in a flask which was flushed continuously with nitrogen. To the stirred solution was added hydroxylamine hydrochloride (1 g, 0·012 mole) and sodium bicarbonate (1·3 g). The reaction mixture was heated under reflux for 3 hr, and then stirred at room temperature for 24 hr. The methanol was removed and the residue was diluted with water and extracted with ethyl acetate (3 × 100 ml). The combined ethyl acetate extracts were washed with brine and dried ($MgSO_4$). Removal of the solvent and crystallization of the colourless residue from ethyl acetate/cyclohexane gave the oxime (LII) as white needles (1·2 g, 75%), m.p. 152–153°.

(*ff*) N-*Tosyl*-cis-*4-amino*-L-*proline* (*XLVII*) *and* cis-*4-Amino*-L-*proline* (*XLVI*) *via the Oxime Route*

The oxime (LII) (0·6 g) was dissolved in ethanol (20 ml) and 1M HCl (2 ml) and shaken with platinum oxide (0·2 g) under 5 atm hydrogen pressure for 5·5 hr. The reaction mixture was filtered and the solvent was removed. Crystallization of the residue from ethanol/ether gave N-*tosyl*-cis-*4-amino*-L-*proline hydrochloride* as white hygroscopic needles (0·35 g, 55%), m.p. 184–185° (Found: C, 44·7; H, 5·8; ionic Cl, 11·2; N, 8·4. $C_{12}H_{17}ClN_2O_4S$ requires C, 44·9; H, 5·3; ionic Cl, 11·1; N, 8·7%).

The above reaction was repeated on a 3·24-g scale and the crude hydrochloride was passed through an ion-exchange column as described in (*x*) to give the monosubstituted diamino acid (XLVII) (1·65 g, 54%), m.p. 281° (dec.). Electrophoresis at pH 1·9, −5°, and 1900 V in a buffer of acetic acid/formic acid/water (15 : 5 : 80) showed only a single spot 23 cm from the starting line after 2 hr. Colour was developed by the usual ninhydrin method.

Compound (XLVII) (0·86 g) was dissolved in 0·8M HCl in methanol (50 ml) and the solution was heated under reflux for 2 hr. Solvent was removed and crystallization of the residue from methanol/ether gave *N-tosyl-cis-4-amino-L-proline methyl ester hydrochloride* as needles 0·76 g, 75%), m.p. 221–222° (Found: C, 46·7; H, 5·8; N, 8·2. $C_{13}H_{19}ClN_2O_4S$ requires C, 46·6; H, 5·7; N, 8·4%).

Reductive detosylation of compound (XLVII) was then carried out as described in (*e*) and (*y*) to give the free diamino acid (XLVI).

(*gg*) N-*Tosyl-4-oxo-L-proline Methyl Ester* (*XXIX*)

(i) The 4-hydroxy ester (III) (3·5 g) was oxidized as described in (*f*) to give the *keto ester* (XXIX) which crystallized from benzene/cyclohexane as white needles (1·6 g, 48%), m.p. 103–104°, $[\alpha]_D^{20}$ +7·7° (2% in $CHCl_3$) (Found: C, 52·5; H, 5·1. $C_{13}H_{15}NO_5S$ requires C, 52·5; H, 5·1%). ν_{max} 1750, 1710 cm^{-1}.

(ii) The keto acid (XXVIII) (3·6 g) was esterified with ethereal diazomethane to give the keto ester (XXIX) (3·6 g, 96%), m.p. 103–104°.

(*hh*) N-*Tosyl-4-oximino-L-proline Methyl Ester* (*LIII*)

This preparation, from the keto ester (XXIX) (1 g) and hydroxylamine hydrochloride, was carried out as described in (*ee*)(i) to give the *oxime* (LIII) which crystallized from benzene/cyclohexane as white needles (0·81 g, 78%), m.p. 110–111°, $[\alpha]_D^{25}$ +9° (0·4% in EtOH) (Found: C, 50·2; H, 5·4; N, 8·5. $C_{13}H_{16}N_2O_5S$ requires C, 50·0; H, 5·2; N, 9·0%).

(*ii*) N-*Tosyl-4-oxo-L-prolinamide* (*XXX*)

Ammonolysis of the 4-hydroxy ester (III) (2·64 g) was performed as described in (*u*). The *hydroxy prolinamide* (IV) crystallized from methanol as white prisms (2·2 g, 88%), m.p. 204°, $[\alpha]_D^{25}$ −80° (0·3% in EtOH) (Found: C, 50·3; H, 5·5; N, 9·6. $C_{12}H_{16}N_2O_4S$ requires C, 50·7; H, 5·7; N, 9·9%). ν_{max} 3480, 3290, 3230, 1680 cm^{-1}.

Chromic acid oxidation of (IV) (1 g) in acetone/acetic acid was done as in (*f*) to give *N-tosyl-4-oxo-L-prolinamide* which crystallized from methanol/water as prisms (0·44 g, 44%), m.p. 154·5–155·5° (Found: C, 51·1; H, 5·3. $C_{12}H_{14}N_2O_4S$ requires C, 51·2; H, 5·0%). ν_{max} 3395, 3295, 3240, 1760, 1700 cm^{-1}.

(*jj*) N-*Benzyloxycarbonyl-4-oximino-L-proline Methyl Ester* (*LIV*)

Esterification of the keto acid[3] (XXXII) (10 g) with ethereal diazomethane gave the *keto ester* (XXXIII) as a colourless oil (10·2 g, 97%) (Found: C, 60·7; H, 5·5; N, 5·3. $C_{14}H_{15}NO_5$ requires C, 60·6; H, 5·5; N, 5·1%).

Interaction of the keto ester (XXXIII) (11·5 g) with hydroxylamine hydrochloride was carried out as described in (*ee*)(i) to give the *oxime* (LIV) as a colourless oil (9·2 g, 75%) (Found: C, 57·5; H, 5·7; N, 9·4. $C_{14}H_{16}N_2O_5$ requires C, 57·5; H, 5·5; N, 9·6%).

(*kk*) N-*Benzyloxycarbonyl-4-oximino-L-prolinol* (*LV*)

(i) A solution of sodium borohydride (0·5 g) in propan-2-ol (30 ml) was added to a solution of the oxime (LIV) (1·5 g) in propan-2-ol (20 ml). Disodium hydrogen phosphate (1·3 g) was added, and the mixture was stirred at room temperature for 16 hr. The reaction mixture was concentrated and partitioned between chloroform (3 × 50 ml) and water (50 ml). Removal of the solvent from the dried organic phase gave the *oximino prolinol* (LV) which crystallized from ethyl acetate/cyclohexane as white plates (0·83 g, 60%), m.p. 138–140°, $[\alpha]_D^{25}$ +11·3° (1·6% in

EtOH) (Found: C, 59·3; H, 6·1; N, 10·2; O, 24·5. $C_{13}H_{16}N_2O_4$ requires C, 59·1; H, 6·1; N, 10·6; O, 24·2%). ν_{max} 3470, 3300 cm^{-1}.

(ii) The keto ester (XXXIII) (0·72 g) and *p*-toluenesulphonic acid (0·07 g) were dissolved in ethylene glycol (50 ml) and the solution was heated at 100° for 1·5 hr. After partial removal of solvent the concentrate (10 ml) was poured into 20% aqueous Na_2CO_3 (30 ml). The oil which formed was extracted into ether (2 × 50 ml), and the combined extracts were dried ($MgSO_4$). Removal of ether and chromatography of the residue on deactivated alumina gave *N-benzyloxycarbonyl-4,4-ethylenedioxy-*L*-proline methyl ester* (L) as a colourless oil (0·54 g, 64%) (Found: C, 59·8; H, 6·1; N, 4·6. $C_{16}H_{19}NO_6$ requires C, 59·8; H, 6·0; N, 4·4%). ν_{max} ($CHCl_3$) 1745 cm^{-1}.

A solution of lithium borohydride (0·14 g) in 1,2-dimethoxyethane (10 ml) was slowly added to a chilled solution of (L) (0·7 g) in 1,2-dimethoxyethane (10 ml). The reaction mixture was left at 0° for 2 hr before addition of 7% aqueous $NaHCO_3$ (25 ml). It was extracted with ether (4 × 50 ml) and the combined extracts were dried ($MgSO_4$). Removal of solvent and chromatography of the residue on deactivated alumina gave *N-benzyloxycarbonyl-4,4-ethylenedioxy-*L*-prolinol* (LI) as a colourless oil (0·54 g, 84%) (Found: C, 60·9; H, 6·8. $C_{15}H_{19}NO_5$ requires C, 61·4; H, 6·5%). ν_{max} ($CHCl_3$) 3400 cm^{-1}.

The foregoing compound (LI) (1·3 g) in methanol (10 ml) and 1M HCl (8 ml) was heated under reflux for 1 hr. After removal of methanol the aqueous phase was diluted with water (20 ml) and extracted with chloroform (2 × 50 ml). Removal of solvent from the dried extracts and chromatography of the residue over deactivated alumina gave *N-benzyloxycarbonyl-4-oxo-*L*-prolinol* (XXXIV) as an oil (1·1 g, 100%) (Found: C, 62·2; H, 6·3; N, 5·8. $C_{13}H_{15}NO_4$ requires C, 62·6; H, 6·1; N, 5·6%). ν_{max} ($CHCl_3$) 3400, 1760 cm^{-1}.

The oxime (LV) was prepared from (XXXIV) (0·47 g) as described in (*ee*)(i). It crystallized from ethyl acetate/cyclohexane as white plates (0·15 g, 31%), m.p. 138–140°.

Acknowledgment

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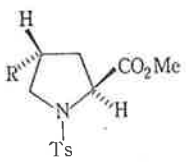
AUSTRALIAN JOURNAL OF CHEMISTRY

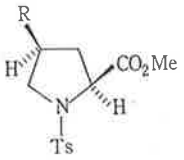
SOLVOLYTIC OXIDATION OF *O*-TOSYL-4-HYDROXYPROLINES IN DIMETHYL SULPHOXIDE*

By V. Nair† and A. V. Robertson†

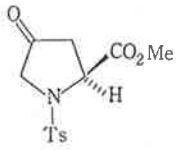
Dimethyl sulphoxide has been used widely as a solvent in nucleophilic substitution reactions,[1] because of its high polarity and low solvating power for anions.[2] Complications may arise due to side reactions such as elimination and oxidation. Elimination reactions of sulphonic esters to olefins in dimethyl sulphoxide have received considerable attention in recent years.[3–5] Very easy solvolytic oxidation of primary alkyl sulphonates, activated alkyl halides such as phenacyl halides, and α-halo esters has been reported.[6,7] Little elimination to olefin was observed with primary alkyl halides and sulphonic esters.[8] Sulphonic esters of secondary cyclic alcohols, however, have been reported to give mainly olefins, with ketones and alcohols as minor products.[3] The mechanism of oxidation involves S_N2 displacement of the sulphonate group by a solvent molecule, followed by collapse of the intermediate to ketone and dimethyl sulphide. Presence of base should then promote the formation of ketone at the expense of olefin by neutralization of liberated sulphonic acid, and this is observed.[3]



(I) R = TsO
(III) R = Cl



(II) R = TsO
(IV) R = Cl



(V)

We wish to report solvolytic oxidation of proline sulphonates and to emphasize the importance of steric factors in this reaction.

* Manuscript received February 22, 1967.
† Department of Organic Chemistry, University of Sydney.

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Treatment of the *trans*-4-hydroxyproline derivative (I) with hot dimethyl sulphoxide alone gave the 4-keto derivative (V) in 43% yield, and in 49% yield when collidine was present. The *cis* epimer (II) yielded the same product in 61% yield in dimethyl sulphoxide alone. The lower relative yield for the *trans* compound as compared with the *cis* compound is a result of greater steric hindrance to the development of an S_N2 transition state by attack of nucleophile from the same face of the heterocyclic ring as the carbomethoxyl group. This effect has been noted in S_N2 displacements of other 4-substituted prolines.[9]

N.m.r. examination of the crude reaction products from which (V) crystallized revealed no evidence of an olefinic product. In contrast, therefore, to previous reports that the predominant reaction of sulphonate esters of secondary cyclic alcohols in dimethyl sulphoxide is elimination,[3,10] solvolytic oxidation can be the major pathway. On the other hand, for reasons not yet clear, the 4-hydroxyproline series may be a special case. Drs J. E. Francis and B. Witkop (unpublished results cited by Robertson and Witkop[11]) failed to obtain dehydroproline compounds by any usual elimination procedure on 4-hydroxyproline derivatives. Yet Kenner and co-workers have recently dehydrated a 3-hydroxyproline derivative without difficulty.[12]

The 4-chloroproline epimers (III) and (IV)[9] were inert to hot dimethyl sulphoxide.

Experimental

Dimethyl sulphoxide was dried and distilled from calcium hydride. Product analyses were carried out by nuclear magnetic resonance spectroscopy in $CDCl_3$ with a Varian Associates A60 instrument.

Dimethyl Sulphoxide Oxidation of N,O-*Ditosyl*-trans-*4-hydroxy*-L-*proline Methyl Ester*

(i) The *trans*-ditosyl ester (I)[9,13] (0·5 g) in dimethyl sulphoxide (25 ml) was heated at 105° for 5 hr. The reaction mixture became light brown and the odour of dimethyl sulphide was noted. After being cooled, the mixture was poured into brine and extracted with ethyl acetate (3 × 100 ml). The combined extracts were washed with brine and dried ($MgSO_4$). Removal of solvent and trituration of the brown residual oil with ethyl acetate/ether gave *N*-tosyl-4-oxo-L-proline methyl ester[9] (V) as colourless prisms (0·075 g), m.p. 101–102°, undepressed on admixture with an authentic sample. N.m.r. spectra of both specimens were superposable, and details will be included in an n.m.r. survey of proline derivatives.[14] More keto ester crystallized upon concentration of the mother liquor (total yield 0·14 g).

(ii) Repetition exactly as in (i) except for the initial addition of collidine (0·3 ml) gave a total yield of colourless crystalline (V) of 0·16 g.

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Dimethyl Sulphoxide Oxidation of N,O-*Ditosyl*-cis-*4-hydroxy*-L-*proline Methyl Ester*

Solvolysis of the *cis*-ditosyl ester (II)[9,13] (0·5 g) as in (i) above gave a total yield of 0·20 g of keto ester (V).

Acknowledgment

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PROTON MAGNETIC RESONANCE SPECTRA, CONFIGURATION, AND CONFORMATION OF 4-SUBSTITUTED PROLINES

By R. H. Andreatta,*† V. Nair,*‡ and A. V. Robertson*

[*Manuscript received May* 10, 1967]

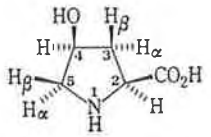
Summary

The proton magnetic resonance spectra of a series of 4-oxoprolines, and *cis*- and *trans*-4-substituted prolines, have been analysed. Correlations permitting assignment of configuration from the spectra are presented. The difficulty of deducing precise conformations from the spectral parameters is emphasized.

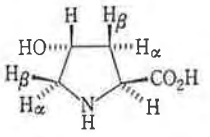
Introduction

We recently reported the stereospecific synthesis of a series of L-proline derivatives with a variety of substituents at C4 in both *cis* and *trans* configurations.[1] Assignment of configuration to these epimers rests so far mainly on the expectation that the substitution reactions employed in introducing the groups at C4 would follow an S_N2 path from 4-hydroxyproline derivatives of known stereochemistry. P.m.r. spectra were recorded for all compounds to confirm purity and general constitution.[1] About half of the spectra are susceptible to extensive analysis, the remainder being unsuitable for various reasons such as the obscuring of fine structure by methyl signals from some protecting groups, complexity of overlapping multiplets, and poor solubility of the compound. Correlations emerge that secure the stereochemistry already proposed.

These studies are relevant to the complete p.m.r. analyses of *cis*- ("allo" series) and *trans*- ("normal" series) 4-hydroxyproline, (I) and (II) respectively, and other



(I)



(II)

proline derivatives by Abraham and co-workers.[2–5] These analyses were complicated because at the time there was no obvious way of assigning the relative chemical

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† U.S.P.H.S. Postdoctoral Fellow, 1964–65.
‡ U.S.P.H.S. Postdoctoral Fellow, 1966.

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shifts of H 3α and H 3β, and of H 5α and H 5β. Analyses for all coupling constants were made,[2] and by assuming modified forms of the Karplus equation for the various H–C–C–H fragments, the *cis* or *trans* relationship of vicinal pairs of protons was deduced. The relative chemical shifts of protons within the two methylene groups then followed after inclusion of other considerations such as long-range coupling and possible conformations. Despite their use of empirically modified versions of the Karplus equation, and the existence of an internal crosscheck for self-consistency, the problems of using the Karplus equation in detail on such systems are formidable. Uncertainties arise due to ring strain, hybridization, presence of a hetero atom in the ring, and the presence and orientation of electronegative and charged substituents.[6] The conformations proposed[3,4] are difficult to accept at face value from the perspective of general organic chemistry. It was claimed that the heterocyclic rings in these amino acids exist in neutral solution in the envelope conformation,[7] with angles of buckle of 70° and 53° for *cis*- and *trans*-4-hydroxyproline respectively. Abraham and McLauchlan noted that the X-ray structure of crystalline *trans*-4-hydroxyproline showed only a 17° buckle.[8] Instead of concluding that their deductions about conformations should be viewed with some hesitation, they merely remarked that the disparity demonstrated the caution needed in extrapolating conformational results from crystal measurements to solution.[3]

Difficulty in assigning configuration by Karplus-type considerations is apparent in the epimers of 3-methylproline[9,10] and 3-hydroxyproline[11] where J_{cis} and J_{trans} for $J_{2,3}$ are both small and sometimes of almost equal magnitude. Similar problems exist in our work on the 3,4-epoxyprolines and 3,4-dihydroxyprolines.[12] The number and variety of 4-substituted prolines discussed here ensures that the correlations between configuration and p.m.r. data are well founded.

Experimental

Spectra for all compounds were recorded on a Varian Associates A60 spectrometer at ordinary probe temperature unless noted otherwise. In several cases the low intensity components of multiplets were unobservable with ordinary A60 sensitivity, and the SUPER SNAIL procedure[13] was used to enhance sensitivity when necessary. Experimental errors are estimated as $\pm 0{\cdot}1$ c/s for coupling constants and $\pm 0{\cdot}01$ p.p.m. for chemical shifts. Chemical shifts in deuterium oxide solutions are referred to the methyl peak of internal sodium 3-trimethylsilylpropyl-1-sulphonate, and in other solutions to internal tetramethylsilane.

A few of the compounds examined were not described in our synthetic paper[1] and their preparation is given here. Microanalyses were done by Miss B. J. Stevenson of this Department.

N-*Benzenesulphonyl-4-oxo-*L*-proline Methyl Ester* (*V*)

Esterification of the corresponding keto acid (III)[1] in methanol with ethereal diazomethane gave the *ester* (V) which crystallized from benzene–cyclohexane as white needles (yield 82%),

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m.p. 83·5–84·5° (Found: C, 50·8; H, 4·6; N, 4·9. $C_{12}H_{13}NO_5S$ requires C, 50·9; H, 4·6; N, 4·9%).

N-*Benzenesulphonyl*-trans-*4-hydroxy*-L-*proline Methyl Ester* (*XXII*)

Esterification of the corresponding acid (XX) with ethereal diazomethane gave the *ester* (XXII) which crystallized from benzene as white prisms (yield 92%), m.p. 121–122° (Found: C, 50·6; H, 5·4; N, 4·8. $C_{12}H_{15}NO_5S$ requires C, 50·5; H, 5·3; N, 4·9%).

N-*Benzenesulphonyl*-cis-*4-chloro*-L-*proline Methyl Ester* (*XIII*)

This compound was prepared by S_N2 displacement of the 4-hydroxyl group in the *trans* derivative (XXII) with phosphorus pentachloride as previously described for the *N*-tosyl analogue.[1] The cis-*4-chloro ester* (XIII) crystallized from benzene–cyclohexane as white needles (yield, 81%), m.p. 83–84° (Found: C, 47·8; H, 4·9; N, 4·3. $C_{12}H_{14}ClNO_4S$ requires C, 47·5; H, 4·6; N, 4·6%).

N-*Benzenesulphonyl*-cis-*4-chloro*-L-*proline* (*XI*)

Hydrolysis[1] of the ester (XIII) gave the cis-*4-chloro acid* (XI) which crystallized as a monohydrate from chloroform–carbon tetrachloride in white needles (yield, 89%), m.p. 83–84° (Found: C, 43·3; H, 4·6; Cl, 11·7; N, 4·8. $C_{11}H_{12}ClNO_4S,H_2O$ requires C, 43·0; H, 4·5; Cl, 11·6; N, 4·6%).

N,O-*Ditosyl*-cis-*4-hydroxy*-L-*proline* (*X*)

Hydrolysis of the corresponding methyl ester[1] gave the *acid* (X) which crystallized from ethyl acetate–cyclohexane in white prisms (yield, 83%), m.p. 181–182° (Found: C, 52·1; H, 5·2. $C_{19}H_{21}NO_7S_2$ requires C, 51·9; H, 4·8%).

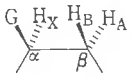
Method of Analysis

Chemical shifts and coupling constants of the heterocyclic ring protons for the 4-oxoprolines (III)–(VI), the *cis*-4-substituted prolines (VII)–(XIX), and the *trans*-4-substituted prolines (XX)–(XXXI) are tabulated below. Chemical shifts of methyl signals from ester and tosyl groups are included because they absorb in the same region. Aromatic and exchangeable proton signals are not listed. The general assignment of the multiplets to protons on C2, C3, C4, and C5 was obvious from the relative chemical shifts, integrated areas, and amount of fine structure. For the ketones, the C2 and C3 protons produce a straightforward ABX pattern. All other compounds give an ABMX system[14] for H3α, H3β (AB part), H2(M), and H4(X), and the X multiplet is further split by the ABX pattern involving H4, H5α, H5β. For clarity the symbols A and B are reserved for the C3 protons and A′ and B′ for the C5 protons. As usual A is by definition downfield from B, and A′ from B′. The ABMX patterns were analysed following the procedure of Abraham and McLauchlan.[2] The AB portion containing 16 lines can be treated as the 8-line ABX pattern with each line doubled by M, or the 8-line ABM pattern with each line doubled by X. The doublings "d_{AM}" and "d_{BM}" (due to, but not equal to J_{AM} and J_{BM}) are line separations in the M doublet of doublets. Subtraction of these doublings from the full AB part left the ABX pattern, analysis of which gave the H3–H4 couplings J_{AX} and J_{BX} and the doublings "d_{AX}" and "d_{BX}". Subtraction of d_{AX} and d_{BX} from the full AB part then yielded the ABM pattern, analysis of which gave the

[14] Pople, J. A., and Schaefer, T., *Molec. Phys.*, 1960, **3**, 547.

H3–H2 couplings J_{AM} and J_{BM}. The major problem in the analyses was as usual the correct assignment of observed lines; the combined ABMX and A′B′X systems give a total of 44 transitions. Difficulties surmounted include many accidental and unresolvable coalescences in the AB part, compounds with such a small chemical shift difference between A and B (δ_{AB}) that observation of the low intensity components demanded very high sensitivity,[13] obscuring of fine structure by methyl signals, the necessity of identifying methyl spinning sidebands amongst multiplet fine structure, and sometimes long-range coupling between H3 and H5 which broadened or doubled lines in the AB and A′B′ parts. The X multiplet for H4 of 16 lines was of little value during the analyses because of its complexity. Analysis of the 8 line A′B′ system for the A′B′X parameters was straightforward because $\delta_{A'B'}$ was generally large and all components were readily observable; occasional overlapping by ester methyl signals was only a minor complication. In some cases, discussed in detail below, spectra were deceptively simple because δ_{AB} or $\delta_{A'B'}$ was near zero and not all parameters were obtainable. In one case, (XV), the chemical shift difference $\delta_{A'X}$ was so small that the H4–H5 system was really ABC, and this was not analysed.

One problem remains after obtaining all values in terms of A, B, M, X, A′, and B′. It is not obvious whether A and B are H3α and H3β respectively, or vice versa; likewise for A′, B′ and H5α, H5β. Present chemical shift theory is not adequate to predict this with confidence.[15a] However, there are several studies on *rigid* cyclic molecules containing the monosubstituted ethane fragment shown where the proton H_B, *cis* to and eclipsed by the substituent group G, is *upfield* from H_A, *trans* and gauche to G. The assignments follow from the fact that the upfield proton has a vicinal coupling constant noticeably smaller than the downfield proton, and both couplings are of the right order for secure application of the principle of the Karplus equation for the dihedral angles involved, that is, $J_{AX} = J_{cis} > J_{BX} = J_{trans}$. In some cases the validity of using the Karplus principle has been checked by measuring J_{cis} and J_{trans} for corresponding 1,2-disubstituted pairs of isomers of known stereochemistry. The systems include polychloronorbornenes,[16] norbornenes,[17] succinic anhydrides,[18] acenaphthenes,[19] and β-lactams.[20] The range of substituents includes Me, Cl, Br, OH, OAc, NH_2, NHAc, CN, CO_2H, CO_2Et, CHO, COMe, Ph. In these surveys of four- and five-membered rings, CO_2H was the only group showing deviation from the general rule. In two cases[16,17] its effect followed the rule, but in acenaphthene-1-carboxylic acid the chemical shifts of H_A and H_B interchange.[19] Of the many factors contributing to the total screening of nuclei H_A and H_B, those mainly responsible for selectively shielding H_B with respect to H_A are direct



[15] Jackman, L. M., and Sternhell, S., "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry." (*a*) Ch. 2-2; (*b*) Ch. 4-4. (Pergamon Press: London, in press.)

[16] Williamson, K. L., *J. Am. chem. Soc.*, 1963, 85, 516.

[17] Laszlo, P., and Schleyer, P. v. R., *J. Am. chem. Soc.*, 1963, 85, 2709.

[18] Erickson, L. E., *J. Am. chem. Soc.*, 1965, 87, 1867.

[19] Fay, C. K., Sternhell, S., and Westerman, P. W., unpublished data.

[20] Barrow, K., and Spotswood, T. M., *Tetrahedron Lett.*, 1965, 3325.

electrostatic shielding by the G–C_α dipole[21–23] (contributing to the local diamagnetic shielding term[15a]) and long-range diamagnetic anisotropy of the G–C_α bond (contributing to the neighbouring diamagnetic shielding term[15a]). Both effects involve the relative geometry of H_A and H_B to G, but precise calculations are impossible at the moment for differences of such small relative magnitude in the overall chemical shift (e.g. see δ_{AB} and $\delta_{A'B'}$ in Tables 1, 3, and 5). Solvent effects such as preferential solvation on one face of the ring could be of similar magnitude. Even if valid predictions could be made for spherically or cylindrically symmetrical substituents (Cl, CN), the complexity of the problem is markedly increased for others such as CO_2H or Ph, where questions of rotamers and their cones of shielding or deshielding arise.

In applying the semi-empirical rule that the upfield proton is the one *cis* to G, it is essential to consider the planarity and rigidity of the system at hand. The *cis* and *trans* coupling constants change rapidly as H_B and G become less eclipsed, and J_{cis} may be only just larger or even smaller than J_{trans} (e.g. dioxolanes and cyclic carbonates,[24] cyclobutanones[25]).

Chemical shifts for the α and β protons on C3 and C5 in the present series have mostly been assigned by selecting the upfield components B and B′ as the protons *cis* to vicinal substituents, after consideration of possible conformations.

4-Oxoprolines (Tables 1 and 2)

These four compounds gave spectra with similar fine structure. The ring-proton signal at lowest field is a one-proton doublet of doublets for H2. This is the X part of the ABX pattern with the C3 protons as the AB part at highest field. The C5 protons are chemically equivalent and absorb as a two-proton singlet. Carbon atoms C3, C4, C5 must be coplanar because of the carbonyl group.[7,26] The nitrogen bonds may not be exactly planar since a sulphonamido group is not as flat as a carboxamido group. For example, the X-ray structure of an *N*-tosylprolyl peptide gave the sum of the bond angles of the prolyl nitrogen as 347·5°.[27] Nevertheless this is closer to sp^2 geometry (360°) than sp^3 (328·5°). N.m.r. studies also show that free rotation within the sulphonamido group does not occur at ordinary temperature.[28] Combination of a nitrogen atom of high sp^2 character (inhibiting sp^3 lone pair inversion) with the sp^2 C4 must produce a hetero ring which is very rigid and almost planar. Substituents on C2 and C3 are therefore in a well-eclipsed conformation and from the rule in the previous section the upfield proton B is assigned as H3β. Magnitudes of vicinal couplings actually happen to fit well for the normal version of the Karplus equation.

[21] Buckingham, A. D., *Can. J. Chem.*, 1960, **38**, 300.
[22] Schweizer, M. P., Chan, S. I., Helmkamp, G. K., and Ts'o, P. O. P., *J. Am. chem. Soc.*, 1964, **86**, 696.
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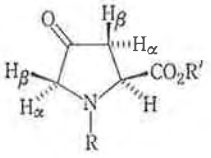
[24] Anet, F. A. L., *J. Am. chem. Soc.*, 1962, **84**, 747.
[25] Braillon, B., Salaun, J., Gore, J., and Conia, J.-M., *Bull. Soc. chim. Fr.*, 1964, 1981.
[26] Brutcher, F. V., Roberts, T., Barr, S. J., and Pearson, N., *J. Am. chem. Soc.*, 1959, **81**, 4915.
[27] Fridrichsons, J., and Mathieson, A. M., *Acta crystallogr.*, 1962, **15**, 569.
[28] Moriarty, R. M., *J. org. Chem.*, 1965, **30**, 600.



(III)–(VI)

	R	R′
(III)	$PhSO_2$	H
(IV)	Ts	H
(V)	$PhSO_2$	Me
(VI)	Ts	Me

TABLE 1

CHEMICAL SHIFT DATA FOR 4-OXOPROLINES

Compound	Solvent	Chemical Shifts, δ Scale (p.p.m.)						δ_{AB} (c/s)	$\delta_{A'B'}$ (c/s)
		Me	A H3α	B H3β	X H2	A′ H5α	B′ H5β		
(III)	C_5H_5N	—	2·99	2·87	5·23	4·18	4·18	7·0	0
(IV)	C_5H_5N	2·16	2·98	2·87	5·25	4·20	4·20	6·6	0
(V)	$CDCl_3$	3·60	2·80	2·56	4·84	3·83	3·83	14·6	0
(VI)	$CDCl_3$	2·41, 3·60	2·78	2·56	4·83	3·82	3·82	13·1	0

TABLE 2

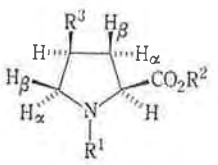
COUPLING CONSTANT DATA FOR 4-OXOPROLINES

Compound	Coupling Constants (c/s)			$\frac{J_{AX}}{J_{BX}}$
	J_{AB}	J_{AX}	J_{BX}	
(III)	17·8	9·2	2·7	3·4
(IV)	17·8	9·0	2·9	3·1
(V)	18·6	9·5	3·0	3·2
(VI)	18·4	9·3	3·1	3·0

cis-4-SUBSTITUTED PROLINES (TABLES 3 AND 4)

The *cis* compounds examined include 10 *N*-benzenesulphonyl or *N*-tosyl acids or esters (VII)–(XVI), and 4-chloro-, 4-bromo-, and 4-amino-proline (XVII)–(XIX) which would exist as the zwitterions. Fine structure in the spectrum of (XIX) was little different from that of its dihydrochloride, which must have both nitrogen atoms as cations. The last entries in Tables 3 and 4 are the data of Abraham *et al.* for *cis*-4-hydroxyproline. Discussion is most conveniently presented in terms of general trends and their exceptions.

The H2 resonance was a clear and obvious doublet of doublets in all cases but (IX) and (XIX) which gave a 1 : 2 : 1 triplet. The chemical shifts of H2 and H4 were well separated except for (IX) and the dihydrochloride of (XIX) so that fine structure in the multiplets rarely overlapped. The H2 signal was downfield from the H4 signal except for the 4-OTs compound (X) and the free amino acids (XVII), (XVIII), (I). The four couplings to H4 were of such size that its *prima facie* absorption was a 1 : 4 : 6 : 4 : 1 quintet [exceptions: (IX), (XV), (XVII), (XVIII),



(VII)–(XIX)

	R^1	R^2	R^3
(VII)	$PhSO_2$	H	OH
(VIII)	Ts	H	OH
(IX)	$PhSO_2$	Me	OH
(X)	Ts	H	OTs
(XI)	$PhSO_2$	H	Cl
(XII)	Ts	H	Cl
(XIII)	$PhSO_2$	Me	Cl
(XIV)	Ts	Me	Cl
(XV)	Ts	Me	Br
(XVI)	Ts	Me	N_3
(XVII)	H	H	Cl
(XVIII)	H	H	Br
(XIX)	H	H	NH_2

TABLE 3

CHEMICAL SHIFT DATA FOR CIS-4-SUBSTITUTED PROLINES

Compound	Solvent	Chemical Shifts, δ Scale (p.p.m.)							δ_{AB} (c/s)	$\delta_{A'B'}$ (c/s)
		Me	A H 3α,	B H 3β[a]	M H 2	X H 4	A′ H 5α,	B′ H 5β[b]		
(VII)	C_5H_5N	—	2·44	2·29	4·82	4·42	3·77	3·77	8·8	0
(VIII)	C_5H_5N	2·23	2·52	2·37	4·87	4·44	3·76	3·76	8·7	0
(IX)	$CDCl_3$	3·72	2·15	2·06	4·35	4·32	3·46	3·40	5·5	3·9
(X)	C_5H_5N	2·20, 2·23	2·70	2·42	4·93	5·30	3·98	3·98	16·9	0
(XI)[c]	$CDCl_3$	—	2·60	2·42	4·53	4·21	3·87	3·54	10·8	19·6
(XII)	C_5H_5N	2·24	2·77	2·62	4·90	4·40	4·11	3·78	8·7	20·0
(XIII)	$CDCl_3$	3·70	2·61	2·37	4·54	4·27	3·90	3·52	14·4	22·7
(XIV)	$CDCl_3$	2·42, 3·73	2·59	2·34	4·52	4·22	3·88	3·50	14·9	22·8
(XV)	$CDCl_3$	2·42, 3·73	2·73	2·42	4·48	*c.* 4·1	*c.* 3·95	*c.* 3·6	18·8	*c.* 20
(XVI)	$CDCl_3$	2·42, 3·73	2·32	2·19	4·48	4·07	3·60	3·32	7·9	16·5
(XVII)[d]	D_2O	—	2·88	2·68	4·57	4·86	3·78	3·78	11·8	0
(XVIII)	D_2O	—	2·98	2·72	4·36	4·82	3·87	3·87	15·9	0
(XIX)	D_2O	—	2·64	1·91	4·08	3·84	3·46	3·20	44·8	15·4
(XIX)[e]	D_2O	—	2·58	1·87	3·99	3·92	3·51	3·28	42·8	13·8
(I)[f]	D_2O	—	2·48	2·22	4·21	4·57	3·42	3·36	15·6	3·6

[a] Assignment of A and B between H 3α, H 3β discussed in text. [b] Assignment of A′ and B′ between H 5α, H 5β discussed in text. [c] Spectrum taken at 70° for solubility reasons. [d] Spectrum of hydrobromide salt, taken at 5° to remove overlapping by HDO peak. [e] Spectrum of dihydrochloride salt. [f] Data of Abraham *et al.* for *cis*-hydroxyproline.[4]

TABLE 4

COUPLING CONSTANT DATA FOR CIS-4-SUBSTITUTED PROLINES

Compound	Splitting Patterns[a]	Coupling Constants (c/s)								$\frac{J_{AM}}{J_{BM}}$	$\frac{J_{AX}}{J_{BX}}$	$\frac{J_{A'X}}{J_{B'X}}$
		J_{AB}	J_{AM}	J_{BM}	J_{AX}	J_{BX}	$J_{A'X}$	$J_{B'X}$	$J_{A'B'}$			
(VII)	ABMX, AA′X	12·7	3·5	9·5	3·2	5·6	4·2[b]		c	0·37	0·57	c
(VIII)	ABMX, AA′X	13·0	3·5	9·5	3·4	6·0	4·4[b]		c	0·37	0·58	c
(IX)	ABMX, ABX	13·6	6·8	5·2	4·1	2·5	1·4	5·2	10·2	1·31	1·64	0·27
(X)	ABMX, AA′X	14·1	2·3	9·9	3·0	6·0	4·0[b]		c	0·23	0·50	c
(XI)	ABMX, ABX	13·6	9·0	4·9	5·8	5·4	5·8	5·1	11·2	1·84	1·07	1·14
(XII)	ABMX, ABX	13·8	8·3	5·7	6·1	5·4	5·8	5·3	10·9	1·45	1·13	1·10
(XIII)	ABMX, ABX	13·5	8·6	4·8	6·0	4·8	5·8	4·6	11·0	1·79	1·25	1·26
(XIV)	ABMX, ABX	14·2	8·9	5·3	5·5	4·9	5·9	5·1	11·2	1·68	1·12	1·16
(XV)	ABMX, ABC	13·5	8·4	6·0	6·8	6·0	d	d	d	1·40	1·13	c
(XVI)	ABMX, ABX	13·4	8·6	4·6	5·8	4·4	5·8	4·2	10·9	1·87	1·32	1·38
(XVII)[e]	ABMX, AA′X	15·0	10·5	3·3	5·6	1·4	3·0[b]		c	3·18	4·0	c
(XVIII)	ABMX, AA′X	14·6	9·9	4·1	5·9	2·1	3·8[b]		c	2·41	2·81	c
(XIX)	ABMX, ABX	13·4	8·9	7·5	6·3	6·1	5·8	6·0	11·5	1·19	1·03	0·97
(XIX)[f]	ABMX, ABX	14·4	8·5	9·3	7·3	7·1	6·7	6·8	12·5	0·91	1·03	0·98
(I)[g]	ABMX, ABX	14·2	10·5	3·5	4·7	2·1	0·9	4·6	12·5	3·00	2·24	0·20

[a] General spin classification for H 2, H 3α, H 3β, H 4 system and H 4, H 5α, H 5β system respectively. [b] $\frac{1}{2}(J_{A'X}+J_{B'X})$; separate values unobtainable. [c] Parameter is unobtainable. [d] ABC system not analysed. [e] Hydrobromide salt. [f] Dihydrochloride salt. [g] Data of Abraham *et al.* for *cis*-hydroxyproline.[4]

(I)]. In several cases, e.g. (XI), the appearance of the 16 transitions in the quintet was really first order, although in others, e.g. (X), it was more ragged. The pattern for H 5α and H 5β was usually the expected 8-line system, but was only a clean doublet in the acids (VII), (VIII), (X), (XVII), (XVIII). In these five, the 3-spin system for the H 4 and H 5 protons has been classified (Table 4) under the AA'X[29] general type. That is, H 5α and H 5β are chemically equivalent but need not be magnetically equivalent provided ($J_{A'X}-J_{B'X}$) remains small with respect to $J_{A'B'}$. In the present context, this classification cannot be distinguished from one with the protons magnetically equivalent but not exactly chemically equivalent, that is, $J_{A'X} = J_{B'X}$ with $\delta_{A'B'}$ non-zero but small (a general ABX type with deceptive simplicity[30]). In neither classification can separate values for $J_{A'X}$, $J_{B'X}$, and $J_{A'B'}$ be obtained. None of the $CDCl_3$ spectra have this doublet pattern for the H 5 protons. The 16-line system for H 3α, H 3β at highest field was always complex, the smallest δ_{AB} being 5·5 c/s. No long-range coupling was evident for any of the new *cis* compounds, although for (I) a long-range coupling $J_{A'B}$ of 2 c/s was observed and assigned as $J_{5\alpha,3\beta}$.

Assignment of individual chemical shifts to the C 3 and C 5 protons remains. Taking the H 3α, H 3β problem first, it will be noted that if (IX) and (XIX) are neglected, there is always a marked difference in the size of J_{AM} and J_{BM}. The larger values range from 8·4 to 10·5 and the smaller values from 2·3 to 6·0 c/s. These are sufficiently different and of the right order to use the rule above with confidence and assign the upfield proton B as H 3β, *cis* to the C 2 acid or ester substituent, and A as H 3α. Three exceptions stand out (see J_{AM}/J_{BM} column): for the acids (VII), (VIII), and (X) the assignment should clearly be reversed so that A is H 3β and B is H 3α. The situation is more tenuous for the C 5 methylene group since for the compounds where $J_{A'X}$ and $J_{B'X}$ could be separated, the values are all of similar and moderate size apart from (IX) and (I). The dihedral angles along the C 4–C 5 bond are clearly different from those along the C 2–C 3 bond. However, we suggest that it is reasonable to assume that the upfield proton will still be the one *cis* to the 4-substituent, even though it may not be fully eclipsed, and on this basis A' is H 5α and B' is H 5β. These assignments agree with those of Abraham and co-workers for (I).

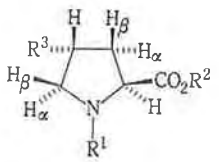
trans-4-Substituted Prolines (Tables 5 and 6)

The *trans* compounds examined include nine *N*-benzenesulphonyl or *N*-tosyl acids or esters, (XX)–(XXVIII), and the zwitterions (XXIX)–(XXXI). The last entries in Tables 5 and 6 are the data of Abraham *et al.* for *trans*-4-hydroxyproline. The *N*-protected compounds have quite different spectra from the free amino acids and discussion of each is presented separately.

For the *N*-protected compounds the H 2 resonance was a clean 1 : 2 : 1 triplet in all cases. The H 2 and H 4 multiplets overlap in all cases except (XXIV), so that although the H 2 triplet components were obvious, no definite shape could be ascribed

[29] Schaefer, T., *Can. J. Chem.*, 1962, **40**, 1678.

[30] Abraham, R. J., and Bernstein, H. J., *Can. J. Chem.*, 1961, **39**, 216.



(XX)–(XXXI)

	R^1	R^2	R^3
(XX)	$PhSO_2$	H	OH
(XXI)	Ts	H	OH
(XXII)	$PhSO_2$	Me	OH
(XXIII)	Ts	Me	OH
(XXIV)	Ts	H	OTs
(XXV)	Ts	Me	Cl
(XXVI)	Ts	H	Br
(XXVII)	Ts	Me	Br
(XXVIII)	Ts	Me	N_3
(XXIX)	H	H	Cl
(XXX)	H	H	Br
(XXXI)	H	H	NH_2

TABLE 5

CHEMICAL SHIFT DATA FOR TRANS-4-SUBSTITUTED PROLINES

Compound	Solvent	Chemical Shifts, δ Scale (p.p.m.)							δ_{AB} (c/s)	$\delta_{A'B'}$ (c/s)
		Me	A H3α, H3β[a]	B	M H2	X H4	A′ H5α, H5β[b]	B′		
(XX)	C_5H_5N	—	2·51	2·51	4·94	4·73	3·96	3·75	0	12·8
(XXI)	C_5H_5N	2·20	2·46	2·46	4·91	4·77	3·92	3·68	0	14·5
(XXII)	$CDCl_3$	3·73	2·13	2·13	4·38	4·44	3·59	3·40	0	11·5
(XXIII)	$CDCl_3$	2·41, 3·73	2·09	2·09	4·38	4·44	3·58	3·36	0	13·1
(XXIV)	C_5H_5N	2·28, 2·28	2·63	2·63	4·82	5·46	4·02	4·02	0	0
(XXV)	$CDCl_3$	2·43, 3·76	2·40	2·40	4·44	4·47	3·92	3·61	0	18·9
(XXVI)	$CDCl_3$	2·43	2·57	2·57	4·48	4·46	4·04	3·62	0	25·0
(XXVII)	$CDCl_3$	2·43, 3·77	2·48	2·48	4·46	4·44	4·01	3·66	0	21·1
(XXVIII)	$CDCl_3$	2·40, 3·70	2·21	2·21	4·30	4·25	3·70	3·42	0	16·7
(XXIX)	D_2O	—	2·78	2·55	4·55	4·93	3·85	3·75	13·7	6·3
(XXX)[c]	D_2O	—	2·73	2·64	4·51	4·85	3·93	3·78	11·2	9·2
(XXXI)[d]	D_2O	—	2·83	2·71	4·86	4·38	4·05	3·67	7·3	22·8
(II)[e]	D_2O	—	2·40	2·12	4·34	4·65	3·48	3·34	16·8	8·4

[a] Assignment of A and B between H3α, H3β discussed in text. [b] Assignment of A′ and B′ between H5α, H5β discussed in text. [c] Spectrum taken at 70° to remove overlapping by HDO peak. [d] Spectrum of dihydrobromide salt. [e] Data of Abraham *et al.* for *trans*-hydroxyproline.[4]

TABLE 6

COUPLING CONSTANT DATA FOR TRANS-4-SUBSTITUTED PROLINES

Compound	Splitting Patterns[a]	Coupling Constants (c/s)								$\frac{J_{AM}}{J_{BM}}$	$\frac{J_{AX}}{J_{BX}}$	$\frac{J_{A'X}}{J_{B'X}}$
		J_{AB}	J_{AM}	J_{BM}	J_{AX}	J_{BX}	$J_{A'X}$	$J_{B'X}$	$J_{A'B'}$	$J_{3,B'}$[b]			
(XX)	AA′MX, ABX	c	7·3[d]		4·2[e]		4·2	3·0	10·3	—	c	c	1·40
(XXI)	AA′MX, ABX	c	7·9[d]		4·6[e]		4·6	3·2	10·8	0·6	c	c	1·44
(XXII)	AA′MX, ABX	c	7·6[d]		4·2[e]		4·2	2·1	11·2	—	c	c	2·00
(XXIII)	AA′MX, ABX	c	8·0[d]		4·3[e]		4·3	2·2	11·3	0·6	c	c	1·96
(XXIV)	AA′MX, AA′X	c	7·8[d]		3·8[e]		2·8[f]		c	—	c	c	c
(XXV)	AA′MX, ABX	c	7·7[d]		4·6[e]		4·7	2·5	11·9	0·6	c	c	1·88
(XXVI)	AA′MX, ABX	c	7·4[d]		4·8[e]		4·9	3·3	11·7	—	c	c	1·49
(XXVII)	AA′MX, ABX	c	7·3[d]		4·9[e]		4·8	3·8	11·6	—	c	c	1·27
(XXVIII)	AA′MX, ABX	c	7·4[d]		4·8[e]		4·8	3·1	11·3	0·5	c	c	1·55
(XXIX)	ABMX, ABX	15·2	7·5	10·7	1·9	5·1	4·4	2·2	13·2	1·1	0·69	0·37	2·00
(XXX)	ABMX, ABX	14·5	7·0	10·6	1·7	5·3	4·6	2·3	13·4	1·1	0·66	0·32	2·00
(XXXI)[g]	ABMX, ABX	15·6	6·5	9·9	8·9	5·5	7·2	5·6	12·4	1·0	0·66	1·62	1·29
(II)[h]	ABMX, ABX	14·1	7·7	10·4	1·4	4·3	4·1	1·2	12·7	1·6	0·74	0·33	3·42

[a] General spin classification for H2, H3α, H3β, H4 system and H4, H5α, H5β system respectively. [b] Long-range coupling, see text. [c] Parameter is unobtainable. [d] $\frac{1}{2}(J_{AM}+J_{BM})$; separate values unobtainable. [e] $\frac{1}{2}(J_{AX}+J_{BX})$; separate values unobtainable. [f] $\frac{1}{2}(J_{A'X}+J_{B'X})$; separate values unobtainable. [g] Dihydrobromide salt. [h] Data of Abraham *et al.* for *trans*-hydroxyproline.[4]

to the band envelope for H4 absorption. The pattern for H5α and H5β was an 8-line system always except for (XXIV) where it was a two-proton doublet. In all cases absorption for H3α and H3β at highest field was a simple doublet of doublets. An intensive search at high sensitivity revealed no low intensity satellites on either side of this quartet. The interpretation at first glance would be that protons A and B have become chemically equivalent and both are magnetically equivalent with regard to M and X. The four-spin system involving H2, H3, and H4 appears to have changed from ABMX to the general A_2MX type. This classification is not unique since for practical purposes the same spectrum would be observed if any or all of δ_{AB}, (J_{AM}–J_{BM}), or (J_{AX}–J_{BX}) were non-zero but small with respect to J_{AB}. That is, a small element of deceptive simplicity could not be experimentally detected. The slightly more general spin classification of AA'MX has therefore been used in Table 6. Individual values of the vicinal couplings cannot be obtained, nor can J_{AB}. However, J_{AM} and J_{BM} must both be of similar size near their mean, otherwise the H2 band would have to be a quartet instead of a triplet. A similar comment cannot be made in regard to J_{AX}, J_{BX}, and their mean; this would require definition of the structure in the complex and obscured H4 band. Long-range coupling was clearly observable in four favourable cases as a broadening of the B' transitions compared to A'. It is argued below that the coupling is $J_{AB'}$, not $J_{BB'}$. Experimental confirmation of this point was impossible since each line of the quartet for A and B contains an intense A and B transition and no selective broadening of one line with respect to another can occur. Compound (XXIV) exhibits almost every effect that can simplify spectra in this series. Signals for H2, H3, and H5 are all first order in appearance, being a one-proton doublet, a two-proton doublet of doublets, and a two-proton doublet respectively. Transitions overlap for H4 to give an unresolvable fine structure, but as noted above, (XXIV) is the only *N*-protected compound having the H2 and H4 signals well separated. As in its *cis*-epimer (X), the 4-*O*-tosyl substituent promotes abnormal n.m.r. behaviour because of its bulk and anisotropy.

For the free amino acids, the chloro- and bromo-prolines (XXIX) and (XXX) have very similar spectra to (II). The H2 signal is a quartet at higher field than the complex H4 band. Significant magnitudes for δ_{AB} and $\delta_{A'B'}$ ensure the complexity needed in the AB and A'B' regions for complete analysis. The long-range coupling was observed as a doubling (not just a broadening) of the B' and A lines, as for (II). The spectrum of the 4-amino compound (XXXI),2HBr was different in several ways. The H2 signal was a triplet downfield from the H4 signal, and the latter had a definite "quintet" appearance. The δ_{AB} of 7·3 c/s was the smallest for the free *trans* amino acids and contrasts with its *cis*-epimer (XIX) where δ_{AB} was the largest. Long-range coupling from B' was evident, but whether to A or B was impossible to establish with certainty on account of overlapping in the AB region. All quoted long-range couplings are only measurements of line broadenings or line separations. Abraham and McLauchlan have shown that the true coupling constants would be larger.[2]

In assigning the individual chemical shifts to the C3 and C5 protons, all compounds will be discussed together and the latter methylene group will be taken first. Selection of the upfield H5 proton as the one *cis* to the 4-substituent predicts A' to be H5β and B' to be H5α. In all cases the upfield proton has the smaller vicinal

coupling (see $J_{A'X}/J_{B'X}$ column) although $J_{A'X}$ would be classed as medium in size rather than large. The C4–C5 fragment is apparently not in a fully eclipsed conformation, but apart from (XXXI),2HBr where the 4-substituent is a nitrogen cation, the constants and presumably the conformations for this part of the hetero ring are very similar. For the C3 methylene group, no assignment is needed in the *N*-substituted compounds where H3α and H3β are chemically equivalent. In the zwitterions, no safe use can be made of the rule that nominates the upfield proton to be *cis* to the vicinal substituent, since H3α is *cis* to the 4-substituent whereas H3β is *cis* to the 2-substituent. To proceed, advantage is taken of the long-range coupling. The only reasonable explanation for this interaction through four single bonds is according to the W-rule (extended planar zigzag path), which is very sensitive to stereochemistry.[15b,31,32] In (XXIX), (XXX), and (I) the coupling is definitely $J_{AB'}$. For the *N*-substituted compounds where no distinction could be observed between $J_{AB'}$ and $J_{BB'}$, it should be safe to extrapolate from the zwitterions, since as reasoned above the conformations around C4 are similar. That is, all long-range couplings should be $J_{AB'}$. The W-rule then requires that atoms A–C3–C4–C5–B′ have a high degree of coplanarity. Inspection of Dreiding models shows only two possibilities, both with C4 projecting out of the plane formed by C5–N–C2–C3. The first, in which C4 projects up, has H3β and H5β approaching coplanarity and the 4-substituent is pseudo-equatorial. However, the geometry between H3α and H4, and between H4 and H5α, is then pseudo-*trans*-diaxial, and two large couplings involving X would have to be present. But this is not observed. The two large couplings observed for M cannot be accommodated on the first model. In addition, it requires B′ to be H5β, which is opposite to the previous prediction. The second model, in which C4 projects down, has H3α and H5α approaching coplanarity and the 4-substituent is pseudo-axial. Proton H4 is then pseudo-equatorial and its four couplings J_{AX}, J_{BX}, $J_{A'X}$, $J_{B'X}$ should all be of small to medium size, as observed. Furthermore, the C2–C3 dihedral angles are such that H2 and H3α are not far from being eclipsed, whilst H2 and H3β are pseudo-*trans*-diaxial, and the two large couplings found for J_{AM} and J_{BM} would be expected. Therefore, A is H3α, B is H3β, A′ is H5β, and B′ is H5α. The arguments based on long-range coupling and on the upfield/*cis* proton rule are independent and in harmony: both assign B′ as H5α. These assignments confirm those previously allotted to (II).[4]

Discussion

The geminal coupling constants all fit well for their environment both in terms of theoretical prediction[33] and empirical analogy.[34] No sign determinations have been made but all J_{gem} are presumably negative. The value of *c.* −18 c/s for $J_{3\alpha,3\beta}$ in the 4-ketones (Table 2) is typical for a methylene group adjacent to a carbonyl in a five-membered ring.[34,35] Values of $J_{3\alpha,3\beta}$ in the *cis* and *trans* prolines (Tables 4

[31] Barfield, M, *J. chem. Phys.*, 1964, **41**, 3825.
[32] Sternhell, S., *Rev. pure appl. Chem.*, 1964, **14**, 15.
[33] Bothner-By, A. A., *Adv. magn. Resonance*, 1965, **1**, 195.
[34] Cookson, R. C., Crabb, T. A., Frankel, J. J., and Hudec, J., *Tetrahedron*, 1966, Suppl. No. 7, 355.
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and 6) are near the more negative end of the range (mostly −13·5 to −15 c/s) for saturated five-membered rings,[34] which can be attributed to the presence of suitably oriented electron-withdrawing β-substituents.[33] In the *N*-substituted compounds, $J_{5\alpha,5\beta}$ is mostly in the range −10·5 to −11·5 c/s, which is slightly more positive than J_{gem} in cyclopentanes. This can be accounted for by a small back-donation from the suitably oriented α-hetero-atom.[33] The zwitterions have a $J_{5\alpha,5\beta}$ which is about 1 c/s more negative, reflecting the absence of a lone pair on the quaternized nitrogen atom for back-donation.

Vicinal coupling constants have been given much attention in previous sections and little needs to be added. The many effects[33] which can influence the magnitude of J_{vic} cannot be dissected from each other in this series. Broadly though, the trends which can be perceived are explicable. The change in J_{AM}/J_{BM} in passing from the 4-ketones to the 4-monosubstituted compounds shows the increased flexibility of the rings. By and large the general principle of the Karplus equation can be applied in the sense that $J_{cis} > J_{trans}$ since *cis* protons have to be eclipsed or nearly so in five-membered rings. Reasons for apparent exceptions are readily found in particular cases, e.g., $J_{AM} = J_{cis} < J_{BM} = J_{trans}$ for the *trans* zwitterions, but the dihedral angles are deduced to be, say, 20–30° and 140–150° respectively.

Atypical parameters of the 4-*O*-tosyl epimers (X) and (XXIV) and of the 4-aminoprolines (XIX) and (XXXI) are not surprising: they simply indicate numerically that major structural alterations produce gross effects. Such compounds give a valuable demonstration of the need to confine extrapolation in n.m.r. studies to really close relatives. The spectra of these "misfits" are not needed to define their stereochemistry. The 4-OTs compounds were prepared from 4-hydroxy compounds of known stereochemistry, and there is a chemical proof securing configurations in the 4-amino series.[1]

Solvent effects were small. Most of the $CDCl_3$ spectra are of esters, and all of the pyridine spectra are of acids which would be ionized. Presence of the carboxylate anion apparently exerts little influence on conformation. For the free hydroxyprolines, an absence of marked effect has been noted in passing from neutral to acid solution (carboxylate anion to carboxyl group);[4] similarity of spectra for (XVII),HBr and (XVIII) reinforce this point Many trends remain in passing from the *N*-substituted compounds to the zwitterions in D_2O.

It is a tautology to remark that the specific variation in chemical shifts and coupling constants from compound to compound is simply a function of the particular substituents, but in a general sense it is hard to conclude anything more useful. These small molecules are in fact extremely complicated in terms of the variety of structural effects influencing their n.m.r. spectra. The features that determine the tabulated parameters include substituent electronegativities, presence of dipoles and formal charges, possibilities for hydrogen bonding, bond angles, bond lengths, bond anisotropies, hybridization, solvation, etc., and are too subtle to be separated yet in any quantitative fashion. Some of these effects are interdependent, and all contribute to defining the preferred conformation of such molecules. At the moment the translation of an n.m.r. spectrum of such complexity into an explicit expression of molecular conformation is an exercise that demonstrates courage but poor judgment.

We conclude by listing the most obvious correlations between configuration of the 4-substituent and the general appearance and detailed analyses of the spectra.

For N-*Substituted Compounds*

(i) In the *cis* series, $J_{AM} \neq J_{BM}$ and H2 absorbs typically as a doublet of doublets. In the *trans* series these couplings are equal or almost equal so that H2 absorption is always a 1 : 2 : 1 triplet.

(ii) In the *cis* series, H2 and H4 signals are well separated from each other, but in the *trans* series they overlap. This is caused mainly by a downfield shift of H4 in passing from the *cis* to the *trans* epimer while the H2 position remains approximately constant: compare (VII) with (XX), (XIV) with (XXV), etc.

(iii) The appearance of the H4 signal in the *cis* series is a 1 : 4 : 6 : 4 : 1 quintet (clean to ragged in detail). A description of the H4 band shape in the *trans* series is unavailable due to overlapping.

(iv) Apart from (XXIV) (which is exceptional in several ways), the C5 protons are not chemically equivalent in the *trans* series but they may be in the *cis* series.

(v) The C3 protons are always chemically equivalent in the *trans* series and never in the *cis* series.

(vi) For pairs of epimers, the ratio $J_{A'X}/J_{B'X}$ is always smaller for the *cis* compound, e.g. (IX) and (XXII), (XIV) and (XXV), (XVI) and (XXVIII).

(vii) Long-range coupling was sometimes observed for *trans* but never in *cis* compounds.

For the Free Amino Acids

Correlations (iv) and (vi) hold together with:

(viii) In the *trans* series, $J_{AM} < J_{BM}$, and the reverse trend operates for the *cis* series. See J_{AM}/J_{BM} column; (XIX),2HBr is anomalous.

(ix) Except for the 4-amino epimers, J_{AX}/J_{BX} is more than unity for the *cis* series, and less than unity for the *trans* series.

(x) A long-range coupling $J_{AB'}$ is typical of the *trans* series. The only *cis* compound with observed long-range coupling is (I).[4]

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CYCLOADDITION OF 1-AZIRINES WITH NITRILE OXIDES. FORMATION OF CARBODIIMIDES.

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In the course of our work on the symmetry-allowed thermal [$\pi^4 + \pi^2$] cycloadditions of the rigid C=N bond of 1-azirines,[1,2] we examined a 1,3-dipolar cycloaddition to the three-orbital 4π electron system of nitrile oxides.[3,4] We discovered that aromatic nitrile oxides react exothermically with 1-azirines to furnish carbodiimides in > 80% isolated yields.

Thus when 3-methyl-2-phenyl-1-azirine (I)[1] was treated with 2,4,6-trimethylbenzonitrile oxide[5] in anhydrous ether at 0° for 15 min, and the product carefully purified by preparative layer chromatography (controlled atmosphere development), the carbodiimide (II) was obtained as white plates, mp 39-41 (decomp.). Its infrared spectrum (Nujol) showed N=C=N absorption at 2120 cm^{-1} and C=O absorption at 1675 cm^{-1}. Its nmr spectrum (in dry $CDCl_3$)[8] showed absorptions at (δ values) 1.54 (d, J = 7.0 Hz, 3H), 2.20 (s, 6H), 2.29 (s, 3H), 4.93 (q, J = 7.0 Hz, 1H), 6.78 (s, br, 2H), and 7.22 to 8.05 (m, 5H).

The carbodiimide was highly hygroscopic, and hydrolysis to the urea (III) mp 196°, proceeded rapidly and quantitatively.

A possible mechanism for the formation of the carbodiimide (Fig. 1) assumes the initial formation of a cycloadduct which undergoes ring cleavage and a 1,2-shift of the R group. The initially formed cycloadduct is capable of valence tautomerism.

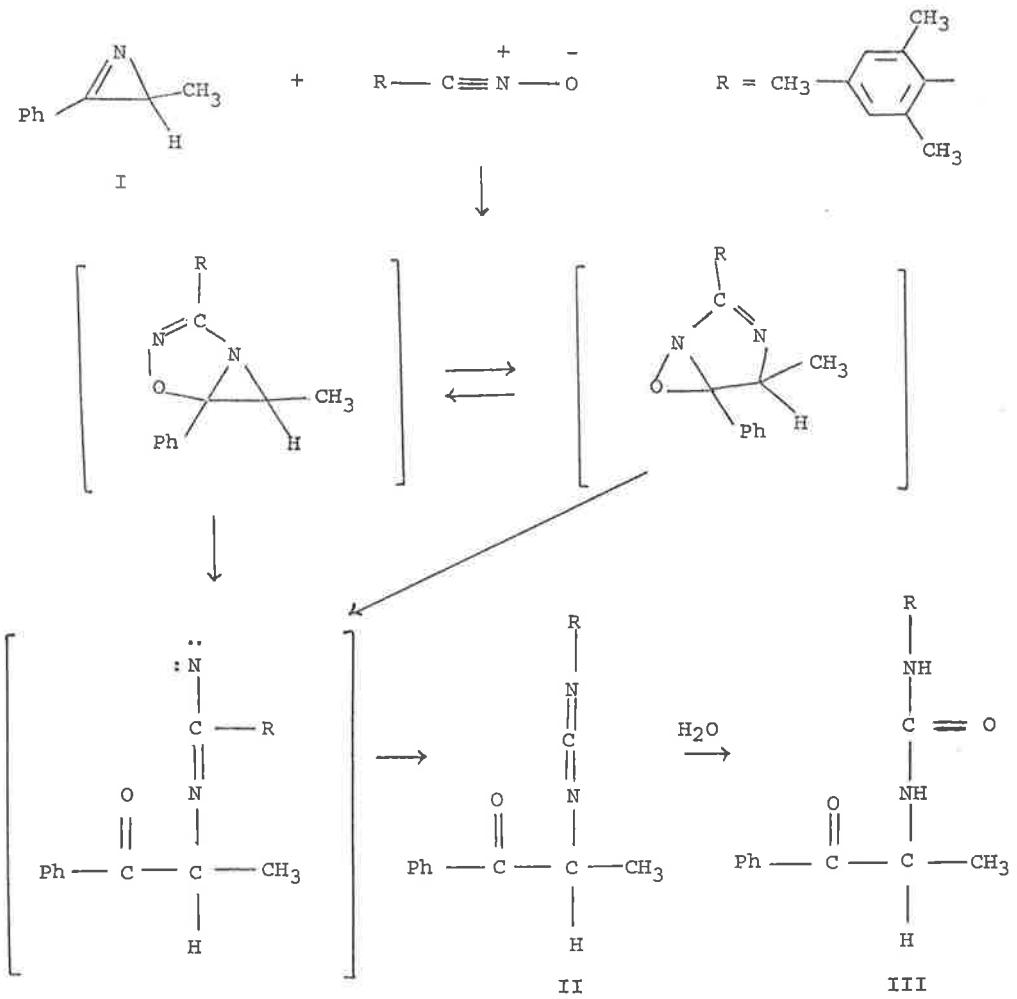

Figure 1

The generality of this transformation was established by preparation of carbodiimides from 2,3-diphenyl-1-azirine and 2-phenyl-1-azirine.[10] Further investigation on the direct spectral observation of the initial cycloadducts is in progress.

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(8) The abbreviations, s = singlet, d = doublet, q = quartet, and br = broad.

(9) Elemental analyses for urea derivatives were satisfactory and the infrared and nmr spectra were consistent with the assigned structures. Satisfactory elemental analyses could not be obtained for the carbodiimides because of their hygroscopic tendency and instability.

(10) A. Hassner and F. W. Fowler, J. Amer. Chem. Soc., 90, 2869 (1968).

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Ring Expansion of 1-Azirines to Azepines *via* Cycloaddition

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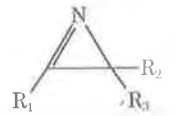
The chemistry of heterotropilidenes has received considerable impetus in recent years due in large part to the elegant synthetic contributions of Paquette and coworkers.[1] In the course of our work on the chemistry of 1-azirines,[2-5] we examined some symmetry-allowed thermal [$\pi^4 + \pi^2$] cycloadditions of the rigid C=N double bond with dienes. We discovered, as reported briefly earlier,[6] that cyclopentadienones reacted readily with 1-azirines (**1**) to furnish in good yields azatropilidenes.

When 2-phenyl-1-azirine (**1a**) was treated with 2,5-dimethyl-3,4-diphenylcyclopentadienone in benzene at reflux temperatures for 4 days, a relatively stable, pale yellow, crystalline compound was isolated in 65% yield. Mass spectral data and elemental analysis were consistent with the molecular formula $C_{26}H_{23}N$. The in-

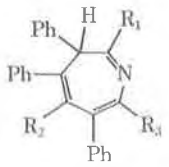
(1) L. A. Paquette in "Nonbenzenoid Aromatics," Vol. I, J. P. Snyder, Ed., Academic Press, New York, N. Y., 1969.
(2) V. Nair, *J. Org. Chem.*, **33**, 2121 (1968).
(3) G. Smolinsky and C. A. Pryde, *ibid.*, **33**, 2411 (1968).
(4) N. J. Leonard and B. Zwanenburg, *J. Amer. Chem. Soc.*, **89**, 4456 (1967).
(5) A. Hassner and F. W. Folwler, *ibid.*, **90**, 2869 (1968).
(6) A preliminary report of our results was announced in the 15th Annual Report of the Petroleum Research Fund, 1970. After this manuscript was submitted for publication, a communication on the cycloaddition of azirines to cyclopentadineones by D. J. Anderson and A. Hassner appeared in *J. Amer. Chem. Soc.*, **93**, 4339 (1971).



1a, $R_1 = Ph$; $R_2 = H$; $R_3 = H$
b, $R_1 = Ph$; $R_2 = CH_3$; $R_3 = H$
c, $R_1 = Ph$; $R_2 = Ph$; $R_3 = H$

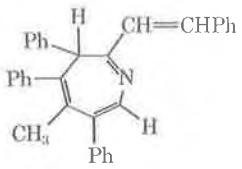
frared spectrum showed no carbonyl or NH absorption. The ultraviolet spectrum in CH_2Cl_2 exhibited absorption maxima at 302 nm (log ϵ 4.03), 270 (4.21), and 235 (4.58). The nmr spectrum ($CDCl_3$) at room temperature showed singlets at δ 1.77 (3 H) and 2.27 (3 H), 5.28 (1 H) and 6.94 (1 H), and a complex multiplet between 7.05 and 7.36 (15 H). The singlet at δ 2.27 disappeared within 20 min at 80° on D_2O exchange.[7] It could not be hydrogenated easily.[8] Attempted cycloadditions with tetracyanoethylene and 1,3-diphenylisobenzofuran were unsuccessful.

The data presented above together with the mechanistic rationalization suggested below led to the 3*H*-azepine (**2a**) as a plausible structure.



2a, $R_1 = R_2 = CH_3$; $R_3 = H$
b, $R_1 = R_2 = Ph$; $R_3 = H$
c, $R_1 = R_2 = R_3 = CH_3$
d, $R_1 = R_2 = Ph$; $R_3 = CH_3$
e, $R_1 = R_2 = CH_3$; $R_3 = Ph$
f, $R_1 = R_2 = R_3 = Ph$

The protons responsible for the rapid deuterium exchange are those of the 2-methyl group. Thus, when the compound was heated with benzaldehyde in the presence of pyrrolidine, a smooth condensation to the 2-styryl derivative (**3**) occurred.

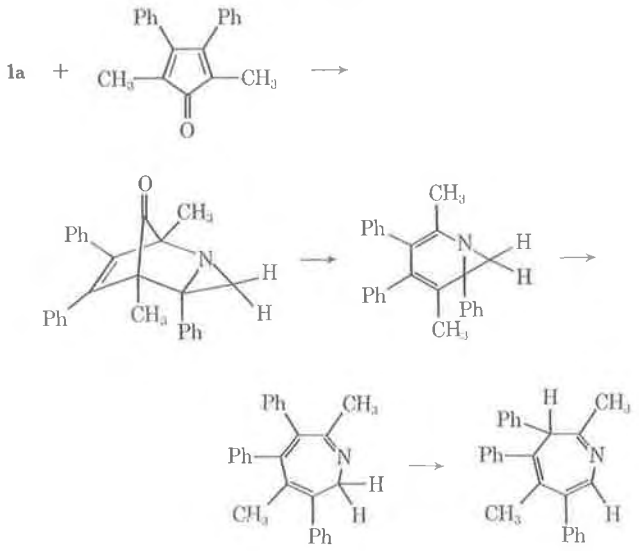


3

The generality of this transformation was established by preparation of compounds **2c** and **2e** from azirines **1b** and **1c** and 2,5-dimethyl-3,4-diphenylcyclopentadienone, and **2b**, **2d**, and **2f** from 2,3,4,5-tetraphenylcyclopentadienone and azirines **1a**, **1b**, and **1c**.

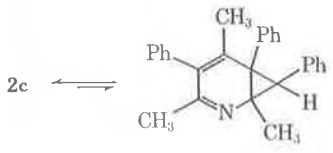
A possible mechanism for the formation of the azepine (Scheme I) assumes a normal Diels–Alder cycloaddition to furnish a strained adduct which undergoes a cheletropic fragmentation[9,10] to give an azanorcaradiene. The symmetry-allowed electrocyclic rearrangement of the azanorcaradiene to its valence tautomer, the azacycloheptatriene (or 2*H*-azepine)[11] is followed by a 1,5-suprafacial sigmatropic shift of the 2 hydrogen to give apparently the thermodynamically more stable 3*H*-azepine.

SCHEME I



Several interesting aspects of the chemistry of these azepines need explanation. Their inability to react with dienophiles or as dienophiles in the Diels–Alder fashion is the result of considerable steric crowding from the spatially large phenyl and methyl substituents. The ultraviolet and nmr spectra reflect not only differences arising from substituents but also any changes in preferred geometry resulting from the crowding.

Of particular interest in our informative D_2O exchange experiments was the observation that the azepine **2c** underwent deuterium exchange not only at the 2-methyl group (20 min at 80°) but also at the 7-methyl group, although the latter exchange was very slow (24 hr at 80°). In contrast, azepine **2d** did not show any tendency to exhibit this behavior at the 7-methyl group. One possible explanation for this is that **2c** undergoes this exchange *via* its valence tautomer **4**, which may be present in very small amounts in equilibrium with the azacycloheptatriene **2c**. This valence tautomerism may not be possible in **2d** because of steric crowding.

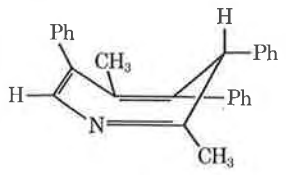


4

Initial variable-temperature nmr studies (−100 to 130°) suggest that these azepines (**2a–f**) exist predominantly in one conformation at room temperature and that the energy of activation for the flipping process is high.[12] Of the two conformations **5** and **6** (for **2a**), it would be reasonable to suggest that the preferred

(7) (a) L. A. Paquette, *J. Org. Chem.*, **28**, 3590 (1963); (b) T. J. van Bergen and R. M. Kellog, *ibid.*, **36**, 978 (1971).
(8) Prolonged hydrogenation in the presence of Pt gave a very complex mixture of products which could not be easily handled.
(9) R. B. Woodward and R. Hoffmann, *Angew. Chem., Int. Ed. Engl.*, **8**, 781 (1969).
(10) M. A. Battiste, *Chem. Ind. (London)*, 550 (1961).
(11) G. Maier, *Angew. Chem., Int. Ed. Engl.*, **6**, 402 (1967).
(12) A. Mannschreck, G. Rissmann, F. Vögtle, and D. Wild, *Chem. Ber.*, **100**, 335 (1967).

conformation would be **5**, where the bulky phenyl group at C-3 occupies the equatorial position.



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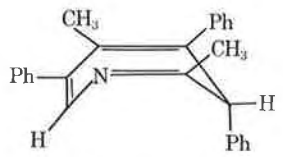

6

Experimental Section

2,5-Dimethyl-3,4,6-triphenyl-3*H*-azepine (2a).—A solution of 468 mg (4 mmol) of 2-phenyl-1-azirine (**1a**)[5] in 10 ml of benzene was treated with a solution of 520 mg (2 mmol) of 2,5-dimethyl-3,4-diphenylcyclopentadienone[13] in 10 ml of benzene.[14] The reaction mixture was heated under reflux for 4 days and then separated by preparative layer chromatography using silica gel PF_{254} with 50% benzene–pentane as the developing solvent. The azepine **2a** crystallized slowly from pentane to give 458 mg of pale yellow plates (65% yield based on the cyclopentadienone): mp 133–134°; uv $\lambda_{max}^{CH_2Cl_2}$ 235 nm (log ϵ 4.58), 270 (4.21), and 302 sh (4.03); nmr $\delta_{TMS}^{CDCl_3}$ 1.77 (s, 3 H), 2.27 (s, 3 H), 5.28 (s, 1 H), 6.94 (s, 1 H), 7.05–7.36 (m, 15 H).

Anal. Calcd for $C_{26}H_{23}N$: C, 88.59; H, 7.43; N, 3.97. Found: C, 88.21; H, 7.05; N, 3.93.

5-Methyl-3,4,6-triphenyl-2-styryl-3*H*-azepine (3) was formed when a solution of the azepine **2c** (100 mg) in benzene (10 ml) was heated under reflux for 4 days with an excess of a mixture of benzaldehyde and pyrrolidine. The solvent and excess reagents were removed under reduced pressure and the residue was chromatographed on preparative plates using silica gel PF_{254}. The styryl derivative **3** crystallized slowly from pentane to give 44 mg of bright yellow rods (36%): mp 161–163°; uv $\lambda_{max}^{CH_2Cl_2}$ end absorption, 283 nm (log ϵ 4.51), 315 (4.26), and 375 (4.16); nmr $\delta_{TMS}^{CDCl_3}$ 1.77 (s, 3 H), 5.81 (s, 1 H), 6.93 (d, 1 H), 7.17–7.38 (m, 22 H).

Anal. Calcd for $C_{33}H_{27}N$: C, 90.57; H, 6.22; N, 3.20. Found: C, 90.74; H, 5.92; N, 3.35.

2,3,4,5,6-Pentaphenyl-3*H*-azepine (2b) was prepared by reaction of 2-phenyl-1-azirine (**1a**) and tetraphenylcyclopentadienone in refluxing mesitylene. The azepine **2b** crystallized from benzene–hexane as yellow plates (90%): mp 217–218°; uv $\lambda_{max}^{CH_2Cl_2}$ 235 nm (log ϵ 4.46), 270 (4.52), and 325 (3.99); nmr $\delta_{TMS}^{CDCl_3}$ 6.45 (s, 1 H), 6.79–7.83 (m, 26 H).

Anal. Calcd for $C_{36}H_{27}N$: C, 91.30; H, 5.74; N, 2.96. Found: C, 90.23; H, 5.21; N, 3.00.

2,5,7-Trimethyl-3,4,6-triphenyl-3*H*-azepine (2c) was prepared from 3-methyl-2-phenyl-1-azirine (**1b**)[2] and 2,5-dimethyl-3,4-diphenylcyclopentadienone. The azepine **2c** crystallized from benzene–pentane as pale yellow plates (69%): mp 182–183°; uv $\lambda_{max}^{CH_2Cl_2}$ 233 nm (log ϵ 4.24), 270 (4.18), and 305 sh (4.03); nmr $\delta_{TMS}^{CDCl_3}$ 1.51 (s, 3 H), 1.57 (s, 3 H), 2.18 (s, 3 H), 5.16 (s, 1 H), 6.74–7.39 (m, 15 H).

Anal. Calcd for $C_{27}H_{25}N$: C, 89.21; H, 6.93; N. 3.85. Found: C, 88.90; H, 6.96; N, 3.79.

7-Methyl-2,3,4,5,6-pentaphenyl-3*H*-azepine (2d) was prepared from 3-methyl-2-phenyl-1-azirine (**1b**) and tetraphenylcyclopentadienone in 84% yield as pale yellow rods: mp 208°; uv $\lambda_{max}^{CH_2Cl_2}$ 235 nm (log ϵ 4.48), 270 (4.54), 350 (3.89); nmr $\delta_{TMS}^{CDCl_3}$ 1.80 (s, 3 H), 6.28 (s, 1 H), 6.83–7.83 (m, 25 H).

Anal. Calcd for $C_{37}H_{29}N$: C, 91.14; H, 6.00; N, 2.86. Found: C, 91.85; H, 6.47; N, 2.45.

A minor product of this reaction (<5% yield) was a very pale yellow crystalline compound, mp 198–201°, which had the molecular formula $C_{37}H_{29}N$ (microchemical analysis and mass spectrometry) and the following spectral characteristics: uv $\lambda_{max}^{CH_2Cl_2}$ 232 nm (log ϵ 4.49), 270 sh (4.33), 292 (4.38), and 325 (4.23); nmr $\delta_{TMS}^{CDCl_3}$ 2.20 (s, 3 H), 5.61 (s, 1 H), 6.68–7.33 (m, 25 H).[15]

Anal. Calcd for $C_{37}H_{29}N$: C, 91.14; H, 6.00; N, 2.86. Found: C, 91.14; H, 5.79; N, 2.94.

2,5-Dimethyl-3,4,6,7-tetraphenyl-3*H*-azepine (2e) was prepared from 2,3-diphenyl-1-azirine (**1c**)[5] and 2,5-dimethyl-3,4-diphenylcyclopentadienone in 58% yield as pale yellow plates: mp 186–188°; uv $\lambda_{max}^{CH_2Cl_2}$ 242 nm (log ϵ 4.16), 270 (4.22), and 312 (4.11); nmr $\delta_{TMS}^{CDCl_3}$ 1.63 (s, 3 H), 2.26 (s, 3 H), 5.27 (s, 1 H), 6.40–7.38 (m, 20 H).

Anal. Calcd for $C_{32}H_{27}N$: C, 90.31; H, 6.40; N, 3.29. Found: C, 90.16; H, 6.90; N, 3.15.

2,3,4,5,6,7-Hexaphenyl-3*H*-azepine (2f) was prepared from 2,3-diphenyl-1-azirine (**1c**) and tetraphenylcyclopentadienone in 91% yield as pale yellow plates: mp 227°; uv $\lambda_{max}^{CH_2Cl_2}$ 243 nm (log ϵ 4.42), 270 (4.53), and 350 (4.12); nmr $\delta_{TMS}^{CDCl_3}$ 6.40 (s, 1 H), 6.74–7.86 (m, 30 H).

Anal. Calcd for $C_{42}H_{31}N$: C, 91.76; H, 5.68; N, 2.56. Found: C, 91.78; H, 5.65; N, 2.82.

Registry No.—**2a**, 33070-60-9; **2b**, 33070-61-0; **2c**, 33070-62-1; **2d**, 33070-63-2; **2d**, 4*H*-azepine isomer, 33070-64-3; **2e**, 33070-65-4; **2f**, 33070-66-5; **3**, 33070-67-6.

Acknowledgment.—Acknowledgment is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society (Grant No. 1871-G1), for partial support of this research.

(13) (a) F. W. Graw, *J. Chem. Soc.*, **95**, 2131 (1909); (b) C. F. H. Allen and J. A. Van Allan, *J. Amer. Chem. Soc.*, **64**, 1260 (1942).

(14) A twofold excess of the azirine was used in all runs because of the instability of the azirines at elevated temperatures.

(15) This compound is tentatively assigned the 4*H*-azepine isomer of **2d** on analytical and spectral evidence. Further support for this structure came from D_2O exchange studies, which indicated rapid exchange of the methyl group.

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The Reaction of 1-Azirines with 1,3-Diphenylisobenzofuran. Ring Expansion to Isoquinoline, Dihydroisoquinoline, and Azanorcarane Derivatives

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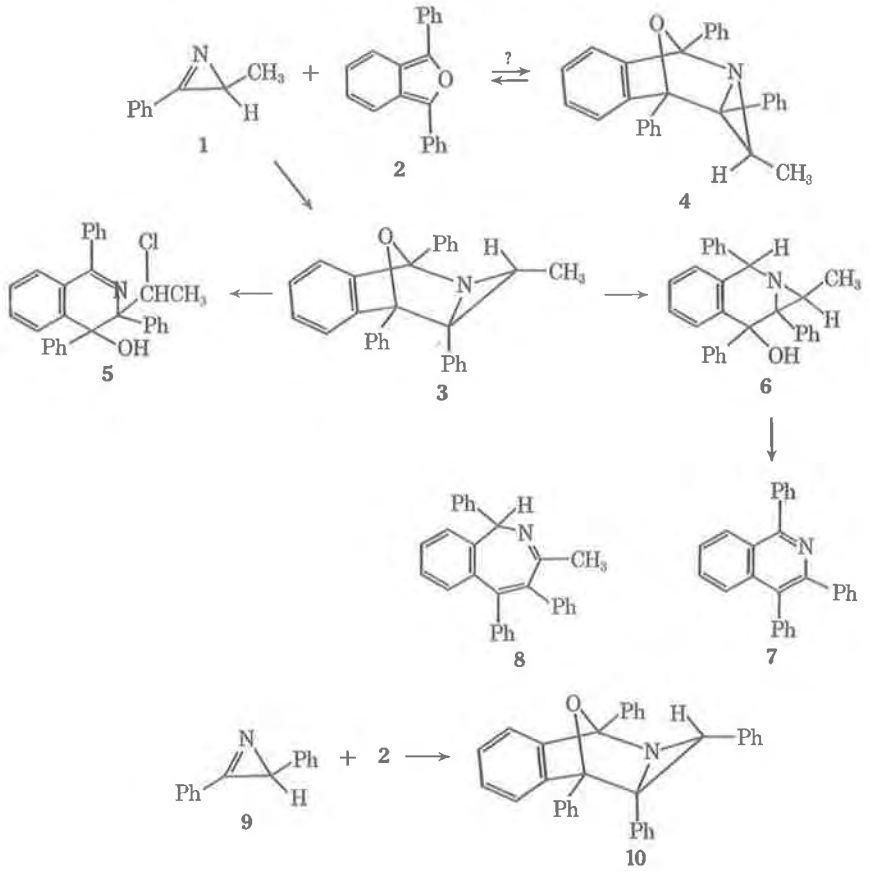
Received January 28, 1972

Under appropriate reaction conditions advantage can be taken of the inherent reactivity of the rigid C=N bond of 1-azirines to effect cycloadditions. The 2-π electrons of this system can participate in thermally allowed [$_\pi 4 + {}_\pi 2$] reactions as dienophiles[1,2] or as dipolarophiles.[3-5] Thus, reaction of 1-azirines with cyclopentadienones proceeds *via* the cycloadduct to furnish after decarbonylation, valence tautomerism, and 1,5-sigmatropic shift, 3*H*-azepine derivatives. 1,3-Dipolar cycloaddition to the three-orbital 4-π electron system of diazomethane and nitrile oxides transforms these 1-azirines into allylic azides and carbodiimides, respectively. The apparent photochemical [2 + 2] cycloaddition with electron-deficient olefins actually proceeds through thermal addition of a 1,3-dipolar species generated by cleavage of the electronically excited singlet state of the appropriate azirine.[6]

(1) V. Nair, *J. Org. Chem.*, **37**, 802 (1972).
(2) A. Hassner and D. J. Anderson, *J. Amer. Chem. Soc.*, **93**, 4339 (1971).
(3) V. Nair, *J. Org. Chem.*, **33**, 2121 (1968).
(4) V. Nair, *Tetrahedron Lett.*, 4831 (1971).
(5) R. B. Woodward and R. Hoffmann, *Angew. Chem., Int. Ed. Engl.*, **8**, 781 (1969).
(6) A. Padwa and J. Smolanoff, *J. Amer. Chem. Soc.*, **93**, 548 (1971).



As part of this program, we embarked on some further cycloadditions of 1-azirines with dienes. We were particularly interested in the isolation and examination of the initially formed cycloadduct, a feature that had been absent from our previous studies on cycloadditions due to the inherent instability of these adducts. We selected to examine the cycloaddition of the reactive diene[7] 1,3-diphenylisobenzofuran (2)[8] with a model azirine, 3-methyl-2-phenyl-1-azirine (1).[3] When azirine 1 was treated with 2 in toluene at reflux temperatures for 18 hr, column chromatography and crystallization furnished a white, crystalline compound in 73% isolated yield. Mass spectral data and elemental analysis were consistent with the molecular formula $C_{29}H_{23}NO$. The nmr spectrum (in $CDCl_3$) showed absorptions at δ 1.05 (d, J = 5.8 Hz, 3 H) and 3.52 (q, J = 5.8 Hz, 1 H) and multiplets in the aromatic region between δ 6.48 and 7.96 (19 H, aromatic).

On the basis of the spectral evidence and the chemical transformations discussed below, the compound was assigned the cycloadduct structure 3. The exo stereochemistry was inferred from its nmr spectrum, which showed considerable deshielding (>1 ppm) of the aziridine hydrogen (δ 3.52) by the oxido bridge, implying that this hydrogen is syn to the oxygen. Further support for this assignment comes from work on cyclopropene adducts with 1,3-diphenylisobenzofuran by Cava,[9] Breslow,[10] Battiste,[11] and coworkers.

The lone pair of electrons on the nitrogen of the cycloadduct 3 undergoes protonation readily in anhydrous HCl–benzene and the protonated species suffers subsequent cleavage to furnish the HCl salt of 5, from which the free base 5 can be easily obtained. No dihydroazepine derivatives were isolated or detected. The protonated species resulting from 3 undergoes C–N and not C–C bond cleavage. There is discrimination between the two C–N bonds and cleavage of presumably the weaker aziridine bond takes place, giving the dihydroisoquinoline derivative 5. That 5 is indeed the product of this ring cleavage was substantiated further by examination of the nmr spectrum of 5 HCl, which showed a downfield shift for both the H and CH_3 (of $ClCHCH_3$) consistent with a positive center situated β to the carbon carrying them.

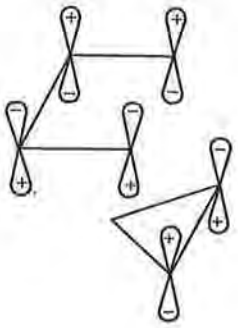
Reductive cleavage of the exo adduct 3 with $LiAlH_4$ gave a compound to which we have assigned the benzoazanorcarane structure 6 on the basis of its spectral and analytical data. Its nmr spectrum showed considerable deshielding of the benzylic hydrogen (δ 5.68), suggesting that the central six-membered ring retains its boat conformation, the deshielding being from the hydroxyl group. When 6 was treated with anhydrous HCl at room temperature, a white, crystalline compound precipitated out of the reaction mixture within a few minutes. Its nmr spectrum was consistent with its being the hydrochloride salt of 6. Treatment of 6 with anhydrous HCl in refluxing benzene converted it to the triphenylisoquinoline 7.

When 2,3-diphenyl-1-azirine (9) was treated with 1,3-diphenylisobenzofuran (2), conversion to the exo adduct 10 occurred in ~70% yield.

The isolation of the exo adducts exclusively from these Diels–Alder reactions may be explained in terms

(7) Attempted cycloaddition with furan and with butadiene derivatives, such as methyl *trans*-2,4-pentadienoate, were unsuccessful.
(8) M. S. Newman, *J. Org. Chem.*, **26**, 2630 (1961).
(9) M. P. Cava and K. Narasimhan, *ibid.*, **36**, 1419 (1971).
(10) R. Breslow, G. Ryan, and J. T. Groves, *J. Amer. Chem. Soc.*, **92**, 988 (1970).
(11) M. A. Battiste and C. T. Sprouse, *Tetrahedron Lett.*, 4661 (1970).

of an unfavorable increase in energy for the endo transition state as a result of secondary orbital interactions (**11**).[5] In **11**, a mixing of the highest occupied diene



11

orbital with the lowest unoccupied cyclopropene or azirine orbital occurs.

It is possible that the endo adduct **4** is formed to a small extent but is unstable and undergoes a retro Diels–Alder reaction.[9]

We are currently examining the possible dehydrative rearrangement of the azanorcarane **6** to the 2*H* azepine **8**.

Experimental Section

Reaction of 3-Methyl-2-phenyl-1-azirine with 1,3-Diphenylisobenzofuran. Formation of Exo Adduct 3.—A solution of 1.048 g (8 mmol) of 3-methyl-2-phenyl-1-azirine (**1**)[3,12] in 10 ml of toluene was treated with a solution of 1.620 g (6 mmol) of 1,3-diphenylisobenzofuran (**2**)[8] in 15 ml of toluene. The reaction mixture was heated under reflux for 18 hr and then chromatographed over silica gel. Unreacted 1,3-diphenylisobenzofuran was eluted with pentane and the adduct with 10% ether–pentane. Crystallization from ether–pentane gave the exo adduct **3** as white plates (1.75 g, 73%): mp 192–194°; nmr $\delta_{TMS}^{CDCl_3}$ 1.05 (d, J = 5.8 Hz, 3 H), 3.52 (q, J = 5.8 Hz, 1 H), 6.48–7.96 (m, 19 H).

Anal. Calcd for $C_{29}H_{23}NO$: C, 86.75; H, 5.77; N, 3.49. Found: C, 86.43; H, 5.51; N, 3.53.

Thermal Stability of Exo Adduct 3.—The adduct **3** in $CDCl_3$ was heated in a sealed nmr tube at 100° and the reaction was monitored by periodic nmr spectral determinations. Even after 1 week, about 85 ± 5% of **3** remained undestroyed.

3-Chloroethyl-4-hydroxy-1,3,4-triphenyl-3,4-dihydroisoquinoline (5).—A solution of 500 mg of the adduct **3** in 5 ml of anhydrous benzene was treated with 10 ml of a saturated solution of anhydrous HCl in benzene. The reaction mixture darkened immediately. After the mixture was stirred for 3 hr, the yellow crystalline compound that precipitated out (**5**·HCl) was collected (510 mg): mp 168°; nmr $\delta_{TMS}^{CD_3OD}$ 1.43 (d, J = 6.2 Hz, 3 H), 5.24 (s, broad, 2 H), 6.06 (q, J = 6.2 Hz, 1 H), 6.58–7.95 (19 H).

The product from the foregoing reaction was dissolved in 5 ml of methanol and treated with 20 ml of 2 *N* aqueous NaOH. The reaction mixture was diluted with 100 ml of water and extracted with benzene (3 × 50 ml). The combined organic extract was washed with water and dried (Na_2SO_4). The solution was concentrated and treated with pentane when pale yellow plates of the dihydroisoquinoline **5** crystallized out (335 mg, 76%): mp 178–180°; nmr $\delta_{TMS}^{CDCl_3}$ 1.28 (d, J = 6.2 Hz, 3 H), 4.10 (s, 1 H), 4.72 (q, J = 6.2 Hz, 1 H), 6.84–8.00 (m, 19 H).

Anal. Calcd for $C_{29}H_{24}NOCl$: C, 79.53; H, 5.52; N, 3.20. Found: C, 79.50; H, 5.32; N, 3.22.

Reductive Cleavage of Exo Adduct 3 with $LiAlH_4$. Isolation of Benzoazanorcarane (6).—A solution of 300 mg of the adduct **3** in 5 ml of anhydrous ether was reduced with $LiAlH_4$. Purification of the product by preparative layer chromatography on silica gel PF_{254} with 50% benzene–pentane as the developing solvent gave benzoazanorcarane (**6**) as a viscous, pale yellow oil which crystallized slowly from ether–pentane as pale yellow plates (280 mg, 93%): mp 85°; nmr $\delta_{TMS}^{CDCl_3}$ 0.95 (d, J = 5.5 Hz, 3 H), 2.32 (q, J = 5.5 Hz, 1 H), 2.52 (s, broad, 1 H), 5.69 (s, 1 H), 6.60–7.90 (m, 19 H).

Anal. Calcd for $C_{29}H_{25}NO$: C, 86.32; H, 6.24; N, 3.47. Found: C, 86.62; H, 6.41; N, 3.50.

Treatment of Benzoazanorcarane (6) with Anhydrous HCl in Benzene. Isolation of Isoquinoline (7).—A solution of 403 mg (1 mmol) of **6** in 20 ml of anhydrous benzene was treated with anhydrous HCl at reflux temperatures for 0.5 hr. The solution was concentrated and subjected to preparative layer chromatography using silica gel PF_{254} with 50% ether–pentane as the developing solvent. The isoquinoline (**7**) crystallized from ether–pentane as pale yellow plates (197 mg, 55%): mp 184–185; nmr $\delta_{TMS}^{CDCl_3}$ 7.08–8.18 (m, 19H).

Anal. Calcd for $C_{27}H_{19}N$: C, 90.72; H, 5.36; N, 3.92. Found: C, 90.79; H, 5.59; N, 3.83.

In a separate experiment the benzoazanorcarane (**6**) was treated with anhydrous HCl in benzene at 25°, and the precipitated white crystalline compound (**6** HCl) was collected: mp 178–181°; nmr $\delta_{TMS}^{CD_3OD}$ 1.45 (d, J = 5.8 Hz, 3 H), 5.12 (s, broad, 2 H), 5.94 (q, J = 5.8 Hz, 1 H), 6.60–8.13 (m, 20 H). Basification of this salt gave **6** quantitatively.

Reaction of 2,3-Diphenyl-1-azirine (9) with 1,3-Diphenylisobenzofuran. Formation of Exo Adduct 10.—A solution of 386 mg (2 mmol) of 2,3-diphenyl-1-azirine (**9**)[13] and 405 mg (1.5 mmol) of 1,3-diphenylisobenzofuran (**2**) was heated under reflux for 44 hr and then chromatographed using preparative plates (silica gel PF_{254}). Crystallization from ether–pentane gave the exo adduct **10** as white plates (490 mg, 70.5%): mp 198–200°; nmr $\delta_{TMS}^{CDCl_3}$ 4.52 (s, 1 H), 6.27–7.97 (m, 24 H).

Anal. Calcd for $C_{34}H_{25}NO$: C, 89.03; H, 5.00; N, 2.78. Found: C, 88.62; H, 5.22; N, 2.70.

Registry No.—**2**, 5471-63-6; **3**, 34806-16-1; **5**, 34806-17-2; **5** HCl, 34806-18-3; **6**, 34792-35-3; **6** HCl, 34792-36-4; **7**, 30081-56-2; **10**, 34806-20-7.

Acknowledgment.—Acknowledgment is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society (Grant No. 1871-G1), for partial support of this research.

(12) An excess of the azirine was used in all runs because of the instability of the azirines at elevated temperatures.

(13) A. Hassner and F. W. Fowler, *J. Amer. Chem. Soc.*, **90**, 2869 (1968).

Tetrahedron Letters No. 16, pp 1487 - 1490, 1974. Pergamon Press. Printed in Great Britain.

THIADIAZEPINONES: SYNTHESIS AND STABILITY

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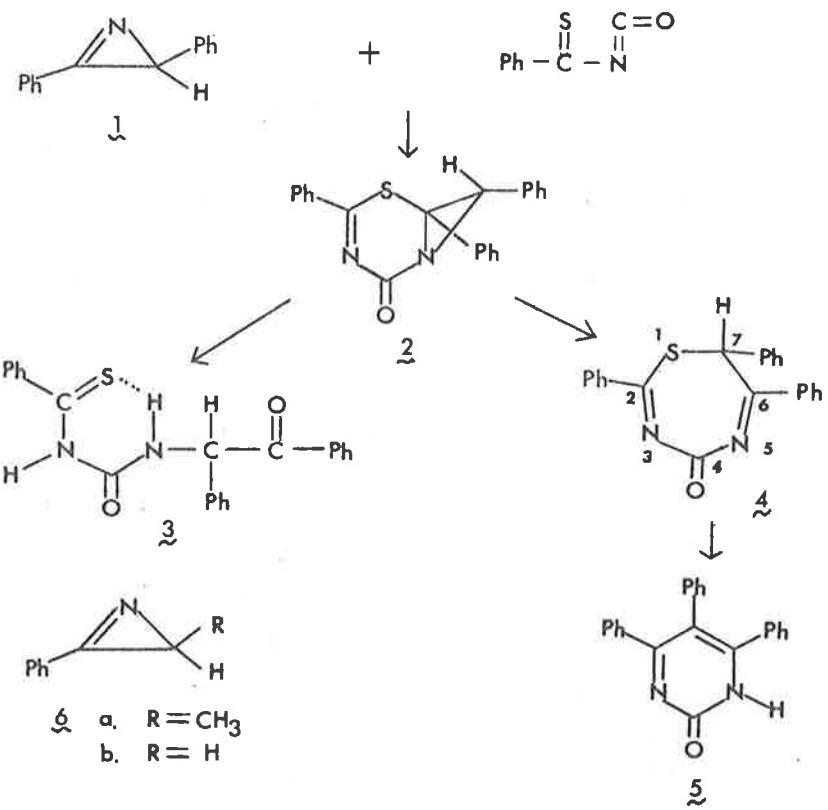
The hypnotic and sedative properties of diazepines and benzodiazepines have resulted in a considerable amount of synthetic, pharmacological, and clinical activity in this area of chemistry.[1-4] Our recent contributions in the area of thermal symmetry-allowed cycloadditions of 1-azirines[5,6] prompted the investigation of the interaction of 1-azirines with isocyanates, and in particular, thiobenzoyl isocyanate.[7-10] We discovered that 1-azirines participated in cycloadditions with thiobenzoyl isocyanate under room temperature conditions to give exclusively [4 + 2] cycloadduction.[11] Thermolytic rearrangement of these cycloadducts gave thiadiazepinones.

Thus, when 2,3-diphenyl-1-azirine (1) was treated with thiobenzoyl isocyanate in p-xylene at room temperature for 12 hr, and the product carefully purified by preparative layer chromatography, the cycloadduct (2) was obtained as white rectangular crystals in 85% yield, mp 154-155. Substantiation of this structure came from analytical and spectroscopic data and chemical transformations. The cycloadduct gave a mass spectral parent ion current at m/e 356 and fragments corresponding to the azirine and the thiobenzoyl isocyanate moieties. Its infrared spectrum (Nujol) showed amide carbonyl absorption at 1720 cm^{-1} and C=N absorption at 1550 cm^{-1}. Its ^{1}H nmr spectrum (in $CDCl_3$) showed considerable deshielding of the aziridine proton (singlet at δ 4.46)[12] and the aromatic protons appeared as a multiplet between δ 7.10 and 8.17. Its ^{13}C nmr spectrum was consistent with the assigned structure.

The regiospecificity of the addition as well as the structure was confirm-



ed by the formation of urea (3) (yellow plates, mp 199-201) on acid hydrolysis of (2). A remarkable observation in the ^{1}H nmr spectrum of (3) was the relatively very slow rate of deuterium exchange of one of the urea hydrogens[13] suggesting the presence of intramolecular hydrogen bonding as shown in structure (3). That this was indeed the case was shown by the diagnostic infrared shift of the hydrogen bonded N-H to 2400 cm^{-1} on deuteration.[14,15]

Controlled thermolysis of the cycloadduct (2) at 80°C gave (4) as yellow prisms, mp 165-167, in 67% yield. The thiadiazepinone structure proposed for (4) was consistent with its mass spectrum (m/e 356, 324, 253, 193, 163, 121, 103), its infrared spectrum in Nujol (1725, 1650 cm^{-1}) and its ^{1}H nmr spectrum in $CDCl_3$

[δ 7.22 to 8.40 (m, 15H), 8.62 (s, 1H)].[16] The ^{13}C nmr spectrum (in $CDCl_3$) provided final spectroscopic confirmation [δ 91.67 (C-7), singlets between 127.44 and 139.42 (phenyl carbons), 162.94 (C-6), 189.27 (C-2), 194.12 (C-4)].

Prolonged thermolysis of (4) resulted in extrusion of elemental sulfur to give a pyrimidine derivative which exists predominantly in the keto form (5).

These studies were extended to two other representative 1-azirines, 3-methyl-2-phenyl-1-azirine (6a) and 2-phenyl-1-azirine (6b).

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12. This type of deshielding has been observed on a number of occasions by us and others - see Reference 5.

13. The urea hydrogens exhibit broad resonances (in $CDCl_3$) at δ 9.87 (singlet) and at δ 10.47 (doublet, J = 6.9 Hz).

14. L. J. Bellamy, "The Infrared Spectra of Complex Molecules", Wiley, New York, N.Y., 1960, p. 207.

15. This marked difference in rate of deuterium exchange is also present in the urea from the benzoyl isocyanate adduct. Exchange of the hydrogen bonded N-H in D_2O is rapid as expected when a drop of triethylamine is added.

16. The marked downfield shift of the C-6 hydrogen is unusual but not entirely without precedence as we have observed this type of behavior with phenyl substituted azepines - see Reference 5.

17. All new compounds gave satisfactory elemental analyses.

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Regioselective [4 + 2] and [2 + 2] Cycloadditions of 1-Azirines to Heterocumulenes. Formation and Rearrangements of the Cycloadducts

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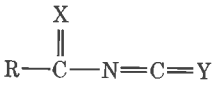
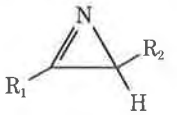
The cycloaddition of 1-azirines to some heterocumulenes is presented. The thermal reaction of representative 1-azirines (**4**) to thiobenzoyl isocyanate (**2**) results in exclusive [4 + 2] cycloaddition. The regiospecificity of the reaction was confirmed by hydrolysis of the cycloadducts **5** to the ureas **6**. Controlled thermolysis of **5a** results in the formation of a novel seven-membered-ring system, a thiadiazepinone (**7**). Compound **7** undergoes a sulfur extrusion reaction thermally to give a pyrimidine ring system (**8**). Benzoyl isocyanate (**1**) also gave [4 + 2] cycloaddition products (**9**). Benzoyl isothiocyanate (**3**), however, gave products (**12**) resulting apparently from a regiospecific [2 + 2] cycloaddition about the C=S bond. The nature of the transition state for the initial [2 + 2] addition is discussed. Structural identification came from mass spectral and nmr studies, particularly ^{13}C nmr.

Heterocumulenes containing a carbonyl or related unsaturation adjacent to the cumulative bonds such as **1**, **2** and **3**, offer the possibility of entry into complex heterocyclic



1, R = Ph; X = O; Y = O
2, R = Ph; X = S; Y = O
3, R = Ph; X = O; Y = S

systems through thermal symmetry-allowed [$_\pi4_s$ + $_\pi2_s$] or [$_\pi2_s$ + $_\pi2_a$] pericyclic reactions. The small ring nitrogen heterocycle, 1-azirine (**4**), may participate as a component in



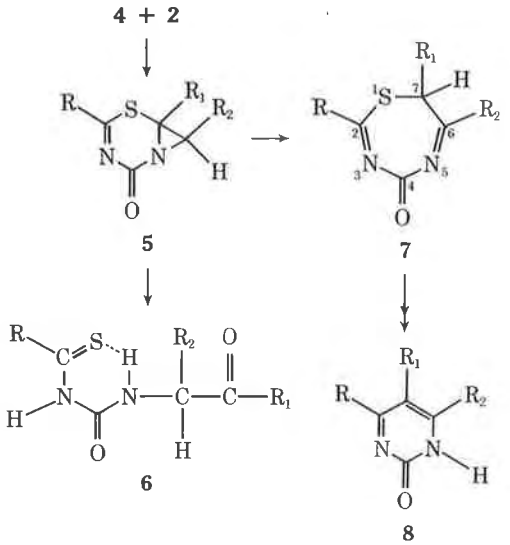
4a, R_1 = Ph; R_2 = Ph
b, R_1 = Ph; R_2 = CH_3
c, R_1 = Ph; R_2 = H

these cycloadditions by utilizing its reactive π bond.[1-4] The possibility of regioselectivity resulting from the inherent polarization in both components enhances the complexity of these reactions. We wish to report on such cycloadditions and to provide evidence that minor structural changes in the heterocumulenes can produce gross changes, not only in the preferred mechanistic pathway for the formation of the adducts, but also in the thermal stability of the final products. A brief announcement of some of our results was made earlier.[11]

Results and Discussion

Thiobenzoyl isocyanate (**2**) can be generated from 2-phenylthiazoline-4,5-dione by thermal extrusion of carbon monoxide.[5-8] When a solution of freshly generated **2** in *p*-xylene was treated with 2,3-diphenyl-1-azirine (**4a**)[9,10] at room temperature for 12 hr, and the reaction mixture after solvent removal was subjected to preparative layer chromatography, a white crystalline compound was obtained, mp 154–155°.[11] Its mass spectral parent ion (*m/e* 356) and fragmentation pattern established the presence of the azirine and thiobenzoyl isocyanate moieties within the structure and that the yield of adduct was high (85%). At least three possibilities exist for the mode of addition:[12] (i) $_\pi4_s$ + $_\pi2_s$ cycloaddition, (ii) $_\pi2_s$ + $_\pi2_a$ adduction, (iii) initial nucleophilic attack by the lone pair of the azirine nitrogen on the highly reactive electrophilic carbon of the carbonyl of the isocyanate and subsequent 1,3-bond scission and cyclization in one or more ways. That the product was actually the result of an exclusive [$_\pi4_s$ + $_\pi2_s$] cycloaddition (**5a**) came from its PFT carbon-13 nmr spectral evidence. The aziridine carbons appeared at δ 53.31 and 56.60, the carbonyl carbon at 173.46, and the imine carbon at 162.94.

The question of the direction or regiospecificity of the cycloaddition and further substantiation of structure was provided in an elegant way by the acid-catalyzed hydrolysis of **5a** to the urea **6a**, yellow plates, mp 199–201°. Dramatic



proof for this mode of ring opening was provided by the observation of three different carbonyl-type carbons (>C=O, N—C(=O)—N, C=S) as suggested by chemical shift correlations in the ^{13}C nmr spectrum. Further confirmation

was provided by the ^1H nmr spectrum of **6a** which showed the two urea N–H absorptions at δ 9.87 (singlet) and 10.47 (doublet, J = 6.9 Hz). A remarkable observation in the ^1H nmr study was the very slow rate of deuterium exchange of the N–H at δ 10.47 suggesting the presence of intramolecular hydrogen bonding. That this was indeed the case was shown by the diagnostic infrared shift of the hydrogen bonded N–H to 2400 cm^{-1} on deuteration.[13,14]

When the cycloadduct **5a** was subjected to thermolysis at 80°, a yellow crystalline compound, mp 165–167°, was isolated after chromatographic purification in 67% yield. Its 70-eV mass spectrum suggested that a rearrangement without fragmentation had occurred. The infrared spectrum showed no N–H absorption but peaks at 1725 and 1650 cm^{-1}. Its ^{13}C nmr spectrum (in $CDCl_3$) suggested the structure **7a** with δ 91.67 (C-7), singlets between 127.44 and 135.42 (phenyl carbons), 139.42 (C-6), 162.94 (C-2), 194.12 (C-4).

Prolonged thermolysis of **5a** at higher temperatures (110°) resulted in the removal of elemental sulfur and the eventual formation of a pyrimidone **8a.** That **7a** was indeed the intermediate in this sulfur extrusion reaction was confirmed not only by its isolation from the reaction mixture but also by its actual quantitative conversion to **8a** at 110°.

The differences in the stability of the cycloadducts derived from the three azirines bear consideration. The reactivity toward hydrolytic cleavage is in the direction **5a** < **5b** < **5c.** Compound **5c** undergoes hydrolysis even on silica gel columns whereas compound **5a** has to be heated at 55° for at least several hours. Whereas this acid-catalyzed hydrolysis proceeds quantitatively for **5b** and **5c** the lower yield (49%) in the case of **5a** is a reflection of the competitive ring opening reaction to **7a.** This rearrangement reaction is relatively unimportant for **5b** and **5c** even at elevated temperatures (138°, *p*-xylene reflux). Thermally **5b** and **5c** are much more stable than **5a.**

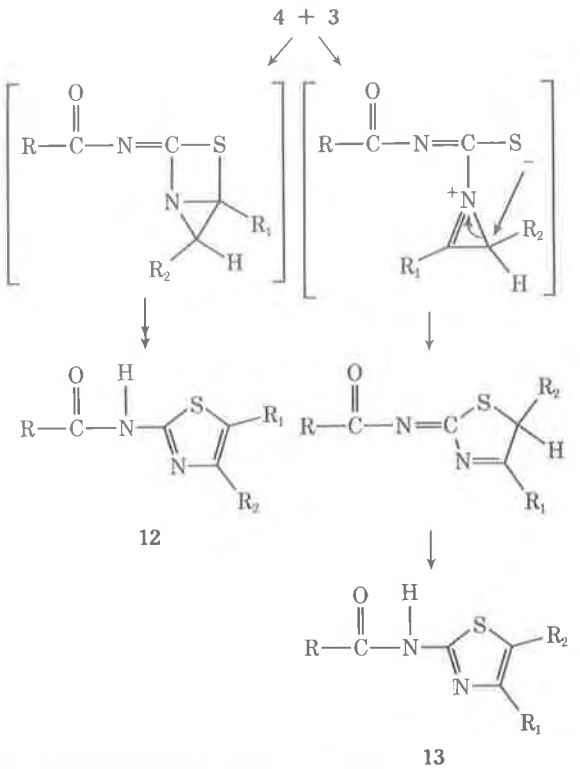
Our results with **2** prompted the investigation of the reaction of benzoyl isocyanate (**1**)[8] with 1-azirines (**4**). We discovered that the behavior of benzoyl isocyanate toward **4** paralleled those of thiobenzoyl isocyanate and [4 + 2] cycloaddition products **9** were isolated.[15] These compounds

Table I
Thermal Decomposition of 0.572 *M* Cycloadduct (9b) at 70°

Time, hr	*M* concn of **9b**	Dec, %
0	0.572	0
0.5	0.482	15.8
1.0	0.431	24.6
1.5	0.391	31.6
2.0	0.361	36.9
2.5	0.340	40.5
4.5	0.301	47.4
6.5	0.281	50.8
8.0	0.274	51.7
16.0	0.274	51.7

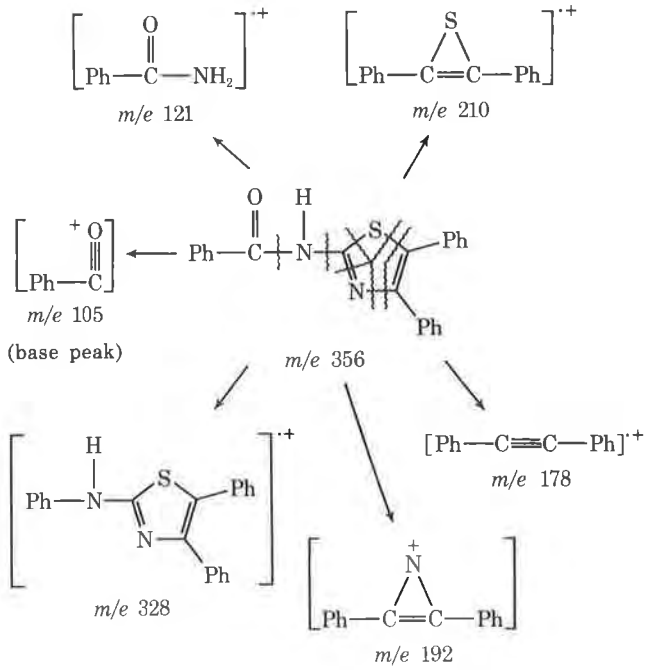
could be hydrolyzed to the ureas **10** under acid-catalyzed conditions. Thermolysis to **11** was not observed. At 70° a clean retro [4 + 2] pericyclic reaction took place and equilibrium was attained after 8 hr with $K = 3.24 \pm 0.20 \times 10^{-1}$.

Benzoyl isothiocyanate (**3**) can be prepared by the reaction of benzoyl chloride and lead thiocyanate.[16,17] A literature search revealed that only a limited amount of work had been done in the area of cycloadditions to **3.** We attempted the reaction of **3** with 1-azirines, not only to establish its preferred mode of addition, but also as a comparison with the behavior of **1** and **2** where exclusive [4 + 2] cycloaddition was observed. Thus, when 2,3-diphenyl-1-azirine (**4a**) was treated with benzoyl isothiocyanate (**3**) in refluxing benzene for 12 hr, preparative layer chromatography gave a white crystalline cycloadduct in 68% conversion, mp 143–144°. Mass spectral data and elemental analysis were consistent with the molecular formula $C_{22}H_{16}N_2OS$. The infrared spectrum showed diagnostic absorptions at 3270, 1675, and 1550 cm^{-1}. Its ^1H nmr spectrum (in $CDCl_3$) showed aromatic absorptions and a broad singlet (1 H) at δ 11.15 which underwent rapid exchange with D_2O. The PFT ^{13}C nmr spectrum (in $CDCl_3$)[27] showed singlets in the phenyl carbon region and resonances at δ 144.51, 157.46, and 165.33. This inconsistency in the nmr spectral data with a [4 + 2] cycloadduct was also apparent in the mass spectrum. Collectively, the data were consistent with benzamide bearing a thiazole ring system on the amide nitrogen. Two plausible structures are **12** and **13.** Compound **12** is

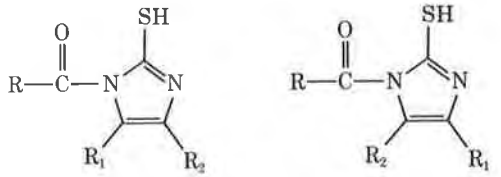


the eventual result of a $[\pi 2_s + \pi 2_a]$ cycloaddition and hydrogen shift(s). Compound **13** results from initial nucleophilic attack, 1,3-bond scission and cylization, and a 1,5-sigmatropic hydrogen shift. Both structures are consistent with the mass spectral data, *e.g.,* for **12a**[18] (Scheme I).

Scheme I



Mass spectral data, however, rules out structures **14** and **15**, the result of addition across the C=N bond of **3**.



14 **15**

Final structural confirmation came from the ^{1}H nmr spectrum of **12c** which showed the 4-H absorption as a singlet at δ 7.08. From comparison of a number of known thiazole derivatives it is clear that this absorption would be about 0.5 ppm upfield if the structure was **13c**.[19-21]

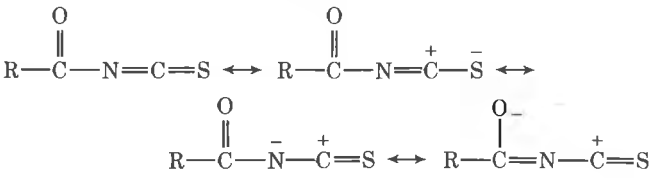
The marked difference in behavior between the exclusive [4 + 2] cycloaddition observed for benzoyl isocyanate (**1**) and thiobenzoyl isocyanate (**2**) and the apparent [2 + 2] cycloaddition in a regiospecific manner to the C=S bond of **3** requires explanation. Orbital symmetry analysis[12,22] reveals a possible concerted [$_\pi 2_s$ + $_\pi 2_a$] pathway but does not explain why the replacement of O by S produces such a marked change in mechanism. A striking clue to the nature of the transition state came from solvent polarity studies with **4c** at 75° (Table II) which showed a dramatic increase

Table II
Reaction of 2-Phenyl-1-azirine (4c) with Benzoyl Isothiocyanate at 75°

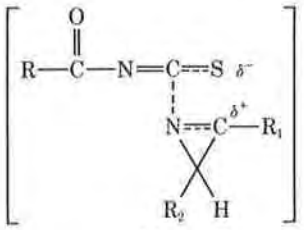
Solvent	Dielectric constant	Reaction time, hr	% yield of **12c**
Benzene	2.3	2	13.4 ± 1.5
Ethyl acetate	6.0	2	19.3 ± 1.5
Nitrobenzene	34.8	2	42.7 ± 1.5

in product yield with increase in the dielectric constant of the solvent. We interpret this solvent dependency as reflecting the presence of a polar transition state in the pathway to the formation of the initial cycloadduct. The polarization of **3** (Scheme II) is similar to **1** except for the greater

Scheme II



ability of sulfur to stabilize a negative charge.[17] A dipolar transition state such as **16** could conceivably account not



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only for the solvent dependency but also for the marked difference in behavior between **1**, **2**, and **3**. Whether such a transition state would transform into a relatively stable dipolar intermediate[23] so as to favor a two-step combination is not known.

Experimental Section

General. All melting points are uncorrected. The ir spectra were recorded on a Beckman IR-20A. The nmr spectra were determined at 60 MHz with a Varian A-60 nmr spectrometer with TMS as the internal reference and with a Bruker HX-90E PFT nmr spectrometer interfaced with a Nicolet 1080 computer and disk unit. The mass spectra were obtained on a Hitachi RMU-6E mass spectrometer using direct inlet and an ionization energy of 70 eV. Elemental analyses were performed by the University of Iowa Microanalytical Service.

2,3-Diphenyl-1-azirine (**4a**) and 2-phenyl-1-azirine (**4c**) were prepared by a modification of the literature method.[9,10] 3-Methyl-2-phenyl-1-azirine (**4b**) was prepared by the method of Nair.[24] 2-Phenylthiazoline-4,5-dione was prepared by the method of Goerdeler, *et al.*[5,7,25] Thiobenzoyl isocyanate was generated by thermolysis of 2-phenylthiazoline-4,5-dione in *p*-xylene at 120° for 5 min and used *in situ*. Benzoyl isocyanate was prepared from benzamide and oxalyl chloride by established methods.[8,26] Benzoyl isothiocyanate can be obtained from the reaction of benzoyl chloride and lead thiocyanate.[16]

Reaction of 2,3-Diphenyl-1-azirine (4a) with Thiobenzoyl Isocyanate (2). A solution of thiobenzoyl isocyanate (**2**) in *p*-xylene generated from 2.865 g (15 mmol) of 2-phenylthiazoline-4,5-dione was treated at 25° with 2.42 g (12.5 mmol) of 2,3-diphenyl-1-azirine (**4a**) and the reaction mixture was stirred for 4 hr at 25°. The precipitated material was filtered off and chromatographed using a silica gel column. The product was eluted with ether. Crystallization from ether gave 3.673 g (85% yield based on 1-azirine) of **5a** as white rectangular crystals: mp 154–155°; ir ν_{max} (Nujol) 1720 (C=O), 1550 (C=N) cm^{-1}; ^{1}H nmr δ_{TMS} ($CDCl_3$) 4.46 (s, 1 H), 7.10–8.17 (m, 15 H); ^{13}C nmr δ_{TMS} ($CDCl_3$) 53.31, 56.60, 127.55, 127.82, 128.46, 128.95, 129.22, 129.38, 129.54, 132.94, 134.18, 135.48, 136.93, 162.94, 173.46; mass spectrum *m/e* 356 (M^+), 324 (M^+ − S), 296 (M^+ − S–CO), 253 (M^+ − PhCN), 193 (azirine), 163 (isocyanate), 103 (PhCN).

Anal. Calcd for $C_{22}H_{16}N_2OS$: C, 74.13; H, 4.52; N, 7.86. Found: C, 73.89; H, 4.47; N, 8.05.

Reaction of 3-Methyl-2-phenyl-1-azirine (4b) with Thiobenzoyl Isocyanate (2). The azirine (**4b**) (0.524 g, 4 mmol) was treated with thiobenzoyl isocyanate (**2**) as described above and the reaction mixture was stirred at 25° for 24 hr. The product was separated by column chromatography on silica gel with 50% ether–pentane as the eluent. Crystallization from ether–pentane gave colorless rectangular crystals of **5b** (0.841 g, 72%): mp 96–98°; ir ν_{max} (Nujol) 1719 (C=O), 1560 (C=N) cm^{-1}; ^{1}H nmr δ_{TMS} ($CDCl_3$) 1.15 (d, J = 5.8 Hz, 3 H), 3.42 (q, J = 5.8 Hz, 1 H), 7.18–8.15 (m, 10 H); ^{13}C nmr δ_{TMS} ($CDCl_3$) 14.73, 47.85, 54.16, 127.14, 128.76, 129.35, 134.15, 135.07, 137.39, 171.70, 173.15; mass spec-

trum *m/e* 251 (M^+ − HNCO), 191 (M^+ − PhCN), 163 (isocyanate), 148 ($Ph(C_2S)CH_3$), 131 (azirine), 103 (PhCN) (product partly rearranged under operating conditions).

Anal. Calcd for $C_{17}H_{14}N_2OS$: C, 69.36; H, 4.79; N, 9.52. Found: C, 69.50; H, 4.90; N, 9.24.

Reaction of 2-Phenyl-1-azirine (4c) with Thiobenzoyl Isocyanate (2). The azirine (**4c**) (0.351 g, 3 mmol) was treated with **2** and chromatographed as described above to give 0.172 g (20%) of **5c** as colorless rectangular crystals: mp 128–129°; ir ν_{max} (Nujol) 1710 (C=O), 1560 (C=N) cm^{-1}; ^1H nmr δ_{TMS} ($CDCl_3$) 2.80 (s, 1 H), 3.13 (s, 1 H), 7.22–8.20 (m, 10 H); mass spectrum *m/e* 280 (M^+), 248 (M^+ − S), 177 (M − PhCN), 163 (isocyanate), 117 (azirine), 103 (PhCN).

Anal. Calcd for $C_{16}H_{12}N_2OS$: C, 68.55; H, 4.32; N, 9.99. Found: C, 68.21; H, 4.21; N, 9.99.

When the silica gel column was eluted with CH_2Cl_2, 0.344 g (38.5%) of **6c** was obtained as yellow needles: mp 166–167°; ir ν_{max} (Nujol) 3240, 3120 (N–H), 1690 (br) (C=O), 1535 cm^{-1}; ^1H nmr δ_{TMS} ($CDCl_3$) 4.87 (d, J = 5.0 Hz, 2 H), 7.25–8.17 (m, 10 H), 10.06 (s, br, 1 H, exchanges in D_2O), 10.69 (t, J = 5.0 Hz, 1 H, very slow D_2O exchange); ^{13}C nmr δ_{TMS} ($CDCl_3$) 47.63, 127.03, 128.06, 128.65, 128.97, 132.16, 134.04, 134.58, 154.00, 193.06, 200.93; mass spectrum *m/e* 298 (M^+), 264 ($M^+ - H_2S$), 193 (M^+ − PhCO), 177 (M^+ − PhCS), 161 ($PhCOCH_2NCO$), 137 ($PhCSNH_2$), 121 (PhCS), 105 (PhCO).

Anal. Calcd for $C_{16}H_{14}N_2O_2S$: C, 64.40; H, 4.71; N, 9.39. Found: C, 64.38; H, 4.81; N, 9.21.

Thermolysis of Cycloadduct (5a) at 80°. Formation and Isolation of Thiadiazepinone (7a). A solution of 0.250 g (0.7 mmol) of **5a** in 10 ml of benzene was heated under reflux for 6 hr. The solvent was removed and the residue was chromatographed on preparative layer silica gel PF_{254} plates with 50% ether–pentane as the developing solvent. The thiadiazepinone (**7a**) crystallized from ether–pentane as yellow prisms (0.168 g, 67%): mp 165–167°; ir ν_{max} (Nujol) 1725 (C=O), 1650 (C=N) cm^{-1}; ^1H nmr δ_{TMS} ($CDCl_3$) 7.22–8.40 (m, 15 H), 8.62 (s, 1 H); ^{13}C nmr δ_{TMS} ($CDCl_3$) 91.67, 127.44, 128.84, 129.22, 132.02, 135.42, 139.42, 162.94, 194.12; mass spectrum *m/e* 356 (M^+), 324 (M^+ − S), 296 (M^+ − S–CO), 253 (M^+ − PhCN), 193 (azirine), 163 (isocyanate), 121 (PhCS), 103 (PhCN).

Anal. Calcd for $C_{22}H_{16}N_2OS$: C, 74.13; H, 4.52; N, 7.86. Found: C, 73.93, H, 4.43; N, 7.71.

Thermolysis of Cycloadduct (5a) at 110°. Isolation of Pyrimidone (8a). A solution of 0.965 g (2.6 mmol) of **5a** in 20 ml of toluene was heated under reflux for 24 hr. The pyrimidone (**8a**) crystallized directly out of the reaction mixture as yellow needles (0.475 g, 54%): mp 274–278°; ir ν_{max} (Nujol) 3340 (br, N–H), 1645 (C=O), 1590 (C=N) cm^{-1}; ^1H nmr δ_{TMS} (CF_3CO_2H) 7.10–8.20 (m, 16 H); mass spectrum *m/e* 324 (M^+), 296 (M^+ − CO), 193 (azirine), 103 (PhCN).

Anal. Calcd for $C_{22}H_{16}N_2O$: C, 81.46; H, 4.94; N, 8.64. Found: C, 81.25; H, 5.22; N, 8.33.

Thermolysis of Thiadiazepinone (7a). Isolation of Pyrimidone (8a). A solution of 0.08 g (0.225 mmol) of **7a** in 15 ml of toluene was heated under reflux for 24 hr. The solvent was then removed and the residue was crystallized from dichloromethane–ether to give **8a** as yellow needles (0.063 g, 86.5%): mp 274–278°.

Hydrolysis of Cycloadduct (5a). A suspension of **5a** (0.200 g, 0.56 mmol) in 15 ml of 1 *M* HCl was stirred at 55° for 24 hr. The yellow solid formed was filtered, washed with water, and purified by preparative plates (silica gel PF_{254}) using ether as the developing solvent. The urea **6a** crystallized from ethanol as yellow plates (0.103 g, 49%): mp 199–201°; ir ν_{max} (Nujol) 3240, 3105 (NH), 1700 (C=O), 1690 (C=O) cm^{-1}; ^1H nmr δ_{TMS} ($CDCl_3$) 6.58 (d, J = 6.9 Hz, 1 H), 6.8–8.17 (m, 15 H), 9.87 (s, br, 1 H, exchanges with D_2O), 10.47 (d, br, J = 6.9 Hz, 1 H, very slow exchange with D_2O); ^{13}C nmr δ_{TMS} ($CDCl_3$) 59.44, 127.41, 128.16, 128.81, 131.40, 133.72, 134.04, 136.31, 136.46, 141.97, 152.16, 195.11, 202.17; mass spectrum *m/e* 374 (M^+), 340 ($M^+ - H_2S$), 269 (M^+ − PhCO), 253 (M^+ − PhCS), 163 (PhCSNCO).

Anal. Calcd for $C_{22}H_{18}N_2O_2S$: C, 70.57; H, 4.85; N, 7.48. Found: C, 70.69; H, 4.90; N, 7.36.

Hydrolysis of cycloadduct (5b) (0.200 g, 0.68 mmol) with 1 *M* HCl gave the urea **6b** (0.195 g, 92%): mp 136–138°; ir ν_{max} (Nujol) 3240, 3145 (NH), 1702 (br, C=O) cm^{-1}; ^1H nmr δ_{TMS} ($CDCl_3$) 1.51 (d, J = 7.0 Hz, 3 H), 5.53 (m, J = 6.9 Hz, 7.0 Hz, 1 H), 7.12–8.16 (m, 10 H), 10.39 (s, br, 1 H, exchanges with D_2O), 10.78 (d, br, J = 6.9 Hz, 1 H, very slow exchange with D_2O); ^{13}C nmr δ_{TMS} ($CDCl_3$) 19.31, 51.89, 127.14, 128.49, 128.76, 130.59, 132.05, 133.88, 142.24, 153.63, 197.86, 201.15; mass spectrum *m/e* 312 (M^+), 278 ($M^+ - H_2S$), 207 (M^+ − PhCO), 191 (M^+ − PhCS), 163 (PhCSNCO).

Anal. Calcd for $C_{17}H_{16}N_2O_2S$: C, 65.36; H, 5.16; N, 8.97. Found: C, 65.21; H, 5.21; N, 8.60.

Reaction of 3-Methyl-2-phenyl-1-azirine (4b) with Benzoyl Isocyanate (1). To a solution of 0.588 g (4 mmol) of benzoyl isocyanate in 5 ml of benzene was added 0.524 g (4 mmol) of the azirine (**4b**) in 5 ml of benzene. The reaction mixture was stirred at 25° for 20 hr and then chromatographed on a silica gel column using 50% ether–pentane as the eluting solvent for the product. The cycloadduct (**9b**) crystallized from ether-pentane as white needles (0.510 g, 45.5%): mp 111–113°; ir ν_{max} (Nujol) 1730 (C=O), 1610 (C=N) cm^{-1}; ^1H nmr δ_{TMS} ($CDCl_3$) 1.17 (d, J = 5.8 Hz, 3 H), 3.16 (q, J = 5.8 Hz, 1 H), 7.22–8.25 (m, 10 H); ^{13}C nmr δ_{TMS} ($CDCl_3$) 13.59, 44.45, 78.86, 127.14, 128.65, 129.19, 129.52, 129.73, 132.32, 134.48, 163.39, 167.17; mass spectrum *m/e* 147 (PhCONCO), 131 (azirine), 105 (PhCO), 103 (PhCN).

Anal. Calcd for $C_{17}H_{14}N_2O_2$: C, 73.36; H, 5.07; N, 10.06. Found: C, 73.07; H, 4.98; N, 10.21.

Reaction of 2-Phenyl-1-azirine (4c) with Benzoyl Isocyanate (1). The azirine (**4c**) (0.588 g, 4 mmol) was treated with **1** in benzene at 25° for 7 hr. Subsequent column chromatography resulted in hydrolysis of the cycloadduct **9c** to the urea **10c.** The urea **10c** was eluted from the column with CH_2Cl_2 and crystallized from ethanol as white needles (0.467 g, 40%): mp 146–147°; ν_{max} (Nujol) 3250 (NH), 1710 (C=O), 1690 (C=O), 1545 (amide II band); ^1H nmr δ_{TMS} ($CDCl_3$) 4.82 (d, J = 5.0 Hz, 2H), 7.16–8.18 (m, 10 H), 9.59 (d, br, J = 5.0 Hz, 1 H, very slow exchange with D_2O), 11.24 (s, br, 1 H, rapid D_2O exchange); mass spectrum *m/e* 282 (M^+), 177 (M^+ − PhCO), 147 (PhCONCO), 121 ($PhCONH_2$), 105 (PhCO).

Anal. Calcd for $C_{16}H_{14}N_2O_3$: C, 68.08; H, 5.00; N, 9.92. Found: C, 68.43; H, 5.13; N, 10.09.

Reaction of 2,3-diphenyl-1-azirine (4a) with benzoyl isocyanate (1) was carried out as described above using 0.588 g (4 mmol) of **1** and 0.772 g (4 mmol) of **4a.** The adduct **9a** crystallized from ether–pentane as white needles (0.082 g, 6%): mp 133–134°; ir ν_{max} (Nujol) 1730 (C=O), 1565 (C=N) cm^{-1}; ^1H nmr δ_{TMS} ($CDCl_3$) 4.23 (s, 1 H), 7.05–8.24 (m, 15 H); mass spectrum *m/e* 193 (azirine), 147 (PhCONCO), 105 (PhCO), 103 (PhCN).

Anal. Calcd for $C_{22}H_{16}N_2O_2$: C, 77.63; H, 4.71; N, 8.23. Found: C, 77.50; H, 4.64; N, 8.11.

Hydrolysis of the Cycloadduct 9b. A suspension of **9b** in 10 ml of 1 *M* HCl was stirred at 25° for 18 hr. The white precipitate that resulted was filtered off, washed with water, and recrystallized from ethanol to give white needles (0.183 g, 86%): mp 137–138°; ir ν_{max} (Nujol) 3270, 3140 (NH), 1710 (C=O), 1680 (C=O), 1550 (amide II); ^1H nmr δ_{TMS} ($CDCl_3$) 1.53 (d, J = 6.9 Hz, 3 H), 5.54 (m, J = 6.9 Hz, 7.0 Hz, 1 H), 7.16–8.20 (m, 10 H), 9.63 (d, br, J = 7.0 Hz, 1 H, very slow exchange with D_2O), 10.08 (s, br, 1 H, rapid exchange with D_2O); mass spectrum *m/e* 296 (M^+), 191 (M^+ − PhCO), 147 (PhCONCO), 121 ($PhCONH_2$), 105 (PhCO).

Anal. Calcd for $C_{17}H_{16}N_2O_3$: C, 68.90; H, 5.44; N, 9.45. Found: C, 68.57; H, 5.41; N, 9.48.

Thermolysis of Cycloadduct 9b. A solution of 0.278 g (1 mmol) of **9b** in 10 ml of toluene was heated under reflux for 2 hr and then separated on a silica gel column. Azirine (**4b**) (0.115 g) was eluted with 50% ether–pentane and benzamide (0.105 g) was eluted with 10% methanol–dichloromethane.

Kinetic measurements for the thermal decomposition of **9b** were done with a 0.572 *M* solution in *dry* $CDCl_3$ at 70° in a sealed (under N_2) nmr tube. The decomposition rate was followed by ^1H nmr. Careful and repeated integrations were done on the methyl groups of **9b** and **4b** (δ 1.17 and 1.36) and an internal cross-check with the aziridine proton of **9b** and the C-3 proton of **4b** (δ 3.1ξ and 2.28) was also done. These results are shown in Table I.

Reaction of 2,3-Diphenyl-1-azirine (4a) with Benzoyl Isothiocyanate (3). To a solution of 0.489 g (3 mmol) of benzoyl isothiocyanate (**3**) in 10 ml of benzene was added 0.386 g (2 mmol) of 2,3-diphenyl-1-azirine (**4a**) in 5 ml of benzene and the reaction mixture was heated under reflux for 12 hr. The solvent was removed and the residue was chromatographed on preparative plates carrying silica gel PF_{254} with 50% ether–pentane as the developing solvent. The cycloadduct (**12a**) crystallized from ether–pentane as white needles (0.483 g, 67.5%): mp 143–144°; ir ν_{max} (Nujol) 3270 (NH), 1675 (C=O), 1550 (amide II) cm^{-1}; ^1H nmr δ_{TMS} ($CDCl_3$) 6.85–7.95 (m, 15 H), 11.15 (s, br, 1 H, rapid exchange with D_2O); ^{13}C nmr δ_{TMS} ($CDCl_3$) 127.25, 127.57, 128.22, 128.76, 129.51, 131.83, 132.05, 132.54, 134.37, 144.51, 157.46, 165.33; mass spectrum *m/e* 356 (M^+), 328, 210, 192, 178, 165, 121, 105.

Anal. Calcd for $C_{22}H_{16}N_2OS$: C, 74.13; H, 4.52; N, 7.86. Found: C, 74.15; H, 4.64; N, 8.03.

Reaction of 3-Methyl-2-phenyl-1-azirine (4b) with Benzoyl Isothiocyanate (3). The cycloaddition was carried out as described above to give **12b** in 65% conversion as white needles: mp 138–139.5°; ir ν_{max} (Nujol) 3170 (NH), 1680 (C=O), 1545 (amide II) cm^{-1}; ^1H nmr δ_{TMS} ($CDCl_3$) 1.97 (s, 3 H), 7.24–8.10 (m, 10 H), 12.01 (s, br, 1 H, rapid exchange with D_2O); ^{13}C nmr δ_{TMS} ($CDCl_3$) 15.27, 126.28, 127.52, 128.17, 128.81, 129.03, 132.27, 132.80, 141.87, 157.62, 165.98; mass spectrum *m/e* 294 (M^+), 266, 191, 148, 121, 116.

Anal. Calcd for $C_{17}H_{14}N_2OS$: C, 69.36; H, 4.79; N, 9.52. Found: C, 69.35; H, 5.08; N, 9.34.

Reaction of 2-phenyl-1-azirine (4c) with benzoyl isothiocyanate (3) was carried out as described above but for 48 hr at 25°. The adduct **12c** was obtained in 15% yield as pale yellow needles: mp 212–213°; ir ν_{max} (Nujol) 3165 (NH), 1685 (C=O), 1570 (amide II) cm^{-1}; ^1H nmr δ_{TMS} ($CDCl_3$) 7.08 (s, 1 H), 7.21–8.15 (m, 10 H), 12.75 (s, br, 1 H, rapid exchange with D_2O); ^{13}C nmr δ_{TMS} ($CDCl_3$) 126.12, 127.90, 128.33, 128.87, 129.14, 136.67, 132.80, 133.02, 143.60, 159.51, 166.03; mass spectrum *m/e* 280 (M^+), 252, 134, 121, 105.

Anal. Calcd for $C_{16}H_{12}N_2OS$: C, 68.55; H, 4.32; N, 9.99. Found: C, 68.64; H, 4.63; N, 9.78.

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Registry No.—**1,** 4461-33-0; **2,** 3553-61-5; **3,** 532-55-8; **4a,** 16483-98-0; **4b,** 16205-14-4; **4c,** 7654-06-0; **5a,** 52920-29-3; **5b,** 52977-07-8; **5c,** 52920-30-6; **6a,** 52920-31-7; **6b,** 52920-32-8; **6c,** 52920-33-9; **7a,** 52920-34-0; **8a,** 52920-35-1; **9a,** 52920-36-2; **9b,** 52920-37-3; **10b,** 52920-38-4; **10c,** 52920-39-5; **12a,** 52920-40-8; **12b,** 52920-41-9; **12c,** 52920-42-0.

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Carbon Disulfide as a 2-π Component in Its Cycloaddition with 1-Azirines

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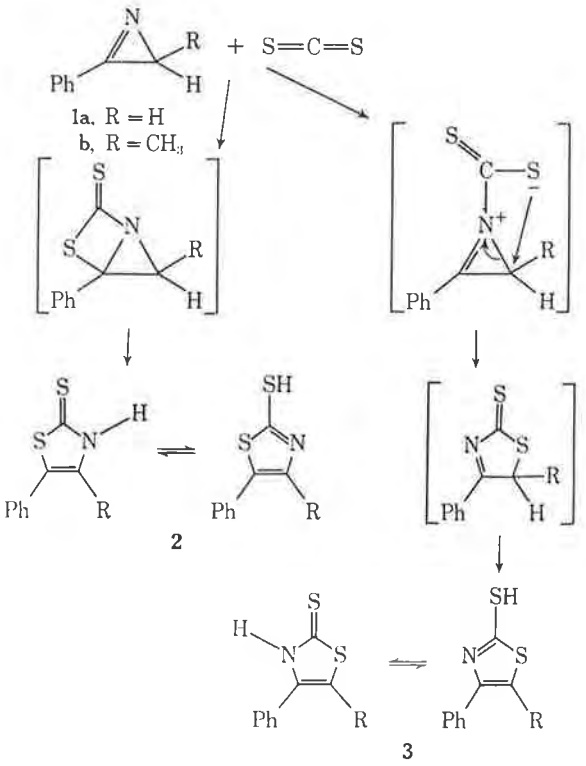
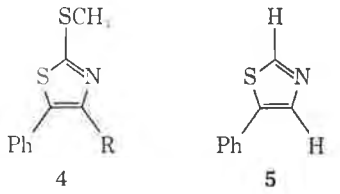
Thermally induced [2 + 2] cycloadditions are rarely encountered. Orbital symmetry analysis reveals that additions of this type that involve relatively low activation energies require special inherent geometric and/or electronic properties of the component(s).[1] The dienophilic and dipolarophilic character of 1-azirines in their thermal cycloadditions has already been established.[2-7] Most reactions of carbon disulfide proceed from an initial nucleophilic attack on carbon.[8-11] The few cycloadditions known are 1,3 dipolar in nature with carbon disulfide as the dipolarophile.[12,13]

We wish to report on the reaction of carbon disulfide with 1-azirines and discuss its mechanistic implications.

When 2-phenyl-1-azirine was dissolved in an excess of carbon disulfide and heated at 100° for 3 hr in a Carius tube, pale yellow needles were obtained, mp 208–209°. Mass spectral data (M^+ at *m/e* 193) and elemental analysis were consistent with the molecular formula $C_9H_7NS_2$, and therefore a 1:1 adduct. The infrared spectrum showed absorptions at 3110 (NH), 1610 (C=C), 1500 (C=S), 1060, 1040 cm^{-1} (C–S–C). Resonances in its ^1H NMR spectrum (in DMSO-d_6) were centered at δ 7.43 (5 H), 7.79 (1 H), and 13.27 (1 H). The broad absorption peak at δ 13.27 underwent rapid exchange with D_2O. Its pulse Fourier transform ^{13}C NMR spectrum (in DMSO-d_6) showed singlets in the phenyl carbon region and a singlet at δ 187.34 which we attribute to a C=S carbon.[14] Collectively, the data are consistent with a thiazole ring system. The adduct could be methylated with methyl iodide in the presence of 1 *M* NaOH. Two plausible structures are **2** and **3**. Compound **2** could conceivably be the eventual result of a [2 + 2] cycloaddition and hydrogen shift(s). Compound **3** (thioenol form) might result from initial nucleophilic attack by the lone pair of the azirine nitrogen on the reactive electrophilic carbon of carbon disulfide followed by 1,3-bond scission, cyclization, and 1,5 sigmatropic rearrangement.



Spectroscopic data did not provide an unambiguous assignment. Structural differentiation came from treatment of the adduct with nitric acid,[15] which gave the known 5-phenylthiazole (**5**), mp 45°.[16] Compound **5** must arise from



2 by a desulfurization reaction. Our spectroscopic data suggest that **2** exists predominantly in the thioketo form.

The formation of the adduct **2** appears therefore to proceed via a regioselective cycloaddition of carbon disulfide to the π bond of the 1-azirine. To our knowledge this is the first example of such an addition of carbon disulfide to a C=N bond. Whether this combination involves a concerted [$_\pi2_s + _\pi2_a$] pathway or a stepwise mechanism involving a dipolar transition state is not known.

These studies were extended to another representative azirine, 3-methyl-2-phenyl-1-azirine (**1b**). Similar results were observed.

Experimental Section

Reaction of 2-Phenyl-1-azirine (1a) with Carbon Disulfide. A mixture of 0.468 g (4 mmol) of 2-phenyl-1-azirine and 1.00 g (13.2 mmol) of carbon disulfide in a Carius tube was heated at 100° for 3 hr. Excess CS_2 was removed and the resultant solid material crystallized from dichloromethane–ether to give 0.182 g (24%) of **2a** as pale yellow needles: mp 208–209°; ir ν_{max} (Nujol) 3110, 1610, 1500, 1270, 1060, 1040, 750 cm^{-1}; ^1H NMR δ_{Me_4Si} (DMSO-d_6) 7.43 (s, br, 5 H), 7.79 (s, 1 H), 13.27 (s, br, 1 H, exchanges with D_2O); ^{13}C NMR δ_{Me_4Si} (DMSO-d_6) 125.09, 128.06, 129.08, 129.41, 129.89, 187.34; mass spectrum (70 eV) *m/e* 193 (M^+), 161 (M^+ − S), 134 [Ph-(c-C_2S)-H; c-C_2S-azirine ring], 121 (PhCS), 102 (PhC≡CH), 91, 77.

Anal. Calcd for $C_9H_7NS_2$: C, 55.93; H, 3.65; N, 7.25. Found: C, 55.90; H, 4.03; N, 7.34.

Methylation of Thiazole (2a). To a suspension of 0.120 g (0.62 mmol) of **2a** in 6 ml of 1 *M* NaOH was added 0.110 g (0.78 mmol) of methyl iodide in 4 ml of 1 *M* NaOH, and the reaction mixture was stirred at room temperature for 3 hr. The reaction mixture was then brought to pH 7 with dilute acetic acid and then extracted with dichloromethane. The combined extracts were dried (Na_2SO_4) and the solvent was then removed in vacuo. The residual material was purified by preparative layer chromatography using silica gel PF_{254} plates with 50% ether–pentane as the developing solvent. The thiazole thioether (**4a**) was obtained as a pale yellow oil (0.089 g, 70%); ^1H NMR δ_{Me_4Si} ($CDCl_3$) 2.68 (s, 3 H), 7.21–7.48 (m, 5 H), 7.77 (s, 1 H); mass spectrum (70 eV) *m/e* 207.

Anal. Calcd for $C_{10}H_9NS_2$: C, 57.94; H, 4.38; N, 6.76. Found: C, 57.60; H, 4.07; N, 6.67.

Desulfurization of Thiazole (2a). A suspension of 0.150 g (0.78 mmol) of **2a** in 10 ml of water and 4 ml of concentrated nitric acid was stirred at room temperature for 4 hr. The reaction mixture was neutralized with 10 *M* NaOH and extracted with chloroform. The combined extracts were washed with water and dried (Na_2SO_4). Removal of solvent and purification of the product by preparative layer chromatography on aluminum oxide PF_{254} plates with 50% ether–pentane as the developing solvent gave 0.064 (51%) of 5-phenylthiazole (**5**) as white prisms: mp 45° (lit.[16] mp 45–46°); ^1H NMR δ_{Me_4Si} ($CDCl_3$) 7.23–7.58 (m, 5 H), 8.06 (s, 1 H), 8.75 (s, 1 H); mass spectrum (70 eV) *m/e* 161 (M^+), 134 [Ph-(c-C_2S)-H], 102 (PhC≡CH).

Anal. Calcd for C_9H_7NS: C, 67.05; H, 4.38; N, 8.69. Found: C, 66.72; H, 4.15; N, 8.53.

Reaction of 3-Methyl-2-phenyl-1-azirine (1b) with Carbon Disulfide. The azirine **1b** (0.524 g, 4 mmol) was dissolved in car-

bon disulfide (1.00 g, 13.2 mmol) and heated at 100° as described above for 1a. The thiazole 2b crystallized from dichloromethane–ether as pale yellow needles (0.324 g, 39%): mp 223–224°; ir ν_{max} (Nujol) 3120, 1605, 1505, 1090, 1075, 710 cm^{-1}; ^1H NMR δ_{Me_4Si} (DMSO-d_6) 2.23 (s, 3 H), 7.36 (s, br, 5 H), 13.09 (s, br, 1 H, exchanges with D_2O); ^{13}C NMR δ_{Me_4Si} (DMSO-d_6) 12.46, 122.50, 127.95, 128.60, 128.97, 134.10, 186.21; mass spectrum (70 eV) *m/e* 207 (M^+), 175 ($M^+ - S$), 148 [Ph-(c-C_2S)-CH_3], 121 (PhCS), 116 (PhC≡CCH_3), 91, 77.

Anal. Calcd for $C_{10}H_9NS_2$: C, 57.94; H, 4.38; N, 6.75. Found: C, 58.00; H, 4.81; N, 6.64.

Methylation of Thiazole 2b. The thiazole 2b (0.238 g, 1.2 mmol) was methylated with methyl iodide (0.200 g, 1.4 mmol) in 15 ml of 1 *M* NaOH as described above for 2a. The thiazole thioether 4b was obtained as a pale yellow oil (0.220 g, 87%); ^1H NMR δ_{Me_4Si} ($CDCl_3$) 2.42 (s, 3 H), 2.64 (s, 3 H), 7.31 (s, br, 5 H); mass spectrum (70 eV) *m/e* 221 (M^+).

Anal. Calcd for $C_{11}H_{11}NS_2$: C, 59.69; H, 5.01; N, 6.33. Found: C, 59.33; H, 4.95; N, 6.16.

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Registry No.—1a, 7654-06-0; 1b, 16205-14-4; 2a, 25445-02-7; 2b, 7725-94-2; 4a, 25445-03-8; 4b, 54410-38-7; 5, 1826-13-7; carbon disulfide, 75-15-0.

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Transformation of 1-Azirines to 1*H*-Indoles with Benzyne. Evidence for the Intermediacy of the 3*H*-Indole System

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The synthetic capabilities of *o*-benzyne have been examined and utilized effectively in recent years.[1] It appears to possess a symmetric singlet ground state,[2] behaves as a highly reactive ethylenic component, and participates in cycloadditions with olefins and dienes in a [2 + 2], [2 + 4], or "ene" fashion.[1-10] Although the reaction of benzyne with enamines has been studied,[5,16] little is known about the reactivity of benzyne toward C=N bonds. We wish to report on the reaction of benzyne with the reactive C=N bond of the 1-azirine ring system.

Results and Discussion

2,3-Diphenyl-1-azirine (1) reacts with excess *o*-benzyne, generated by the thermal decomposition of benzenediazonium 2-carboxylate,[11] to give two products. The major product, a 1:1 adduct produced in 50% yield, was identified as

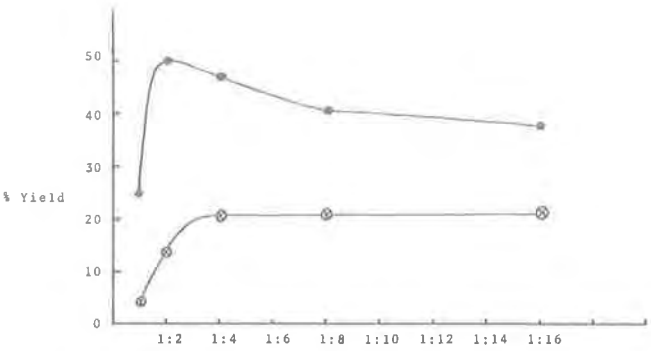
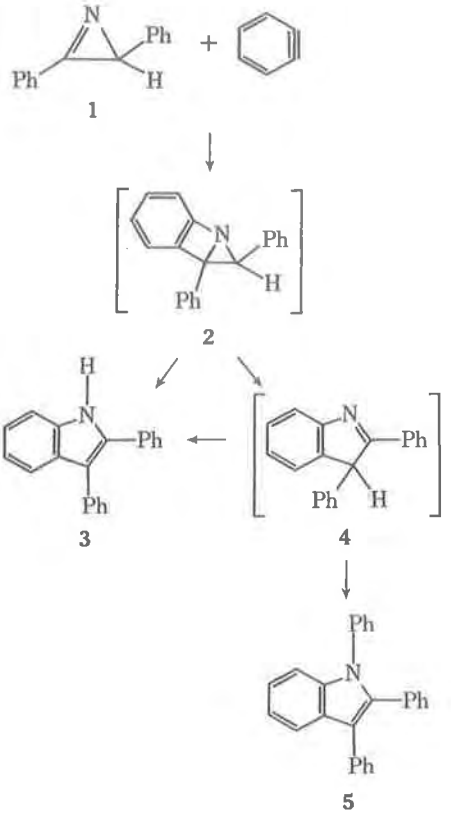
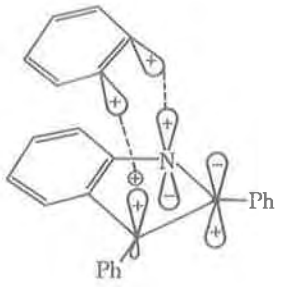


Figure 1. Millimolar ratio of azirine (1) to benzyne: ⊕, indole **5**; •, indole **3**.

2,3-diphenylindole[12] (**3**). A 1:2 adduct of azirine and benzyne, identified as 1,2,3-triphenylindole (**5**),[13] was isolated in 14% yield. Increase in the concentration of benzyne was accompanied by an increase in the yield of **5** as shown in Figure 1. Under the same conditions 2,3-diphenylindole was found to be relatively inert to benzyne, and no triphenylindole (**5**) could be isolated even after extended reaction times.

The mechanism of formation of 2,3-diphenylindole (**3**) may require initial formation of **2**, the result of 1,2 addition on the azirine ring system. Initial 1,3 addition may be ruled out by the isolation of 2-methyl-3-phenylindole (**8**) from the reaction of azirine **7a** and benzyne, although it is possible that more than one mechanism is operating depending on the substituent of carbon 3 of the azirine. It is known that the [2 + 2] cycloaddition between benzyne and simple olefins occurs in a nonconcerted fashion.[3,8-10] It can be presumed then that following the stepwise [2 + 2] cycloaddition of benzyne to the 1-azirine and the formation of intermediate **2**, two reaction pathways for partitioning of this intermediate are available. A 1,2-hydrogen shift to the nitrogen would give the stable aromatic indole **3**. A 1,2 shift to carbon would give the 3*H*-indole system **4**, which can be trapped by benzyne to give the 1,2,3-triphenylindole (**5**). The conversion of indolenine (**4**) to the indole **3** is a symmetry-forbidden process and it appears likely that in the presence of a large excess of benzyne, partitioning of **4** to **3**, a symmetry-forbidden process, is less favorable than the alternate symmetry-allowed "ene" reaction (see **6**) to give **5**.[6,10,15] That this may indeed be the case is borne out by





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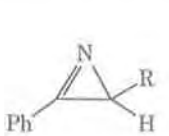
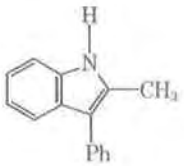
the observation illustrated in Figure 1. The yield of **5** reaches a steady maximum value when large excesses of benzyne are used implying efficient trapping of **4**. Some natural partitioning of **4** to **3** cannot still be ruled out. Interestingly, no "ene" product from **1** and benzyne was isolated.

When 3-methyl-2-phenyl-1-azirine (**7a**) was treated with excess benzyne, the only isolable product was 2-methyl-3-phenylindole (**8**). Under the same conditions, 2-phenyl-1-azirine (**7b**) gave only polymeric products.



7a, R = CH_3
b, R = H



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Experimental Section

Benzenediazonium 2-carboxylate was prepared by the method of Friedman.[11]

Reaction of 2,3-Diphenyl-1-azirine (1) with Benzyne. To a solution of 0.386 g (2 mmol) of 2,3-diphenyl-1-azirine (**1**) in 20 ml of dichloroethane was added 0.592 g (4 mmol) of benzene–wet benzenediazonium 2-carboxylate, and the reaction mixture was heated under reflux for 5 hr. The solvent was then carefully removed in vacuo and the residual material was chromatographed on silica gel PF_{254} plates with 30% ether–pentane as the developing solvent. The top band (R_f 0.85) was cut out and eluted with ether to give, after solvent removal and drying, white crystals (0.097 g, 14%): mp 185–186° (lit.[13] mp 186°); ^1H NMR δ_{Me_4Si} ($CDCl_3$) 7.07 (s, 5 H), 7.16–7.85 (m, 14 H); ^{13}C NMR δ_{Me_4Si} ($CDCl_3$) 110.6, 116.7, 119.6, 120.9, 122.8, 125.9, 127.1, 127.4, 127.6, 127.9, 128.3, 129.1, 130.2, 131.2, 131.6, 134.9, 137.1, 137.9, 138.1; mass spectrum (70 eV) *m/e* 345 (M^+).

The middle band (R_f 0.55) was cut out and eluted with ether to give 0.289 g of pale yellow oil after solvent removal. The oil crystallized from ether–pentane as colorless, rectangular crystals (0.267 g, 50%): mp 122–123° (lit.[12] mp 123–124°); ^1H NMR δ_{Me_4Si} ($CDCl_3$) 7.21–7.72 (m, 14 H), 7.95 (s, br, 1 H); ^{13}C NMR δ_{Me_4Si} ($CDCl_3$) 110.9, 115.0, 119.7, 120.4, 122.6, 126.2, 127.7, 128.2, 128.5, 128.7, 130.2, 132.6, 134.1, 135.1, 135.9; mass spectrum (70 eV) *m/e* 269 (M^+).

Attempted Reaction of 2,3-Diphenylindole (3) and Benzyne. To a solution of 0.538 g (2 mmol) of 2,3-diphenylindole (**3**) in 10 ml of dichloroethane was added approximately 0.60 g (4 mmol) of benzenediazonium 2-carboxylate and the reaction mixture was heated under reflux for 5 hr. Solvent removal and chromatographic separation gave 0.489 g (91% recovery) of **3**. No 1,2,3-triphenylindole was isolated. Some decomposition of **3** does occur in the presence of benzyne (see Figure 1).

Reaction of 3-Methyl-2-phenyl-1-azirine (7a) with Benzyne. The azirine **7a** (0.393 g, 3 mmol) in 20 ml of dichloroethane was treated with 0.90 g (6 mmol) of benzenediazonium 2-carboxyl-

ate, and the reaction mixture was heated under reflux for 4 hr. Solvent removal and chromatographic separation gave 0.198 g (32%) of 2-methyl-3-phenylindole (**8**): mp 58° (lit.[14] mp 59–60°); ^{1}H NMR δ_{Me_4Si} ($CDCl_3$) 2.28 (s, 3 H), 7.05–7.76 (m, 10 H); mass spectrum (70 eV) *m/e* 207 (M^+).

Registry No.—**1**, 16483-98-0; **3**, 3469-20-3; **5**, 54879-94-6; **7a**, 16205-14-4; **8**, 4757-69-1; benzyne, 462-80-6.

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The Reaction of 1-Azirines with 2-Pyridyl Isothiocyanate: Possible Approaches to Benzodiazepine and Benzotriazepine Derivatives

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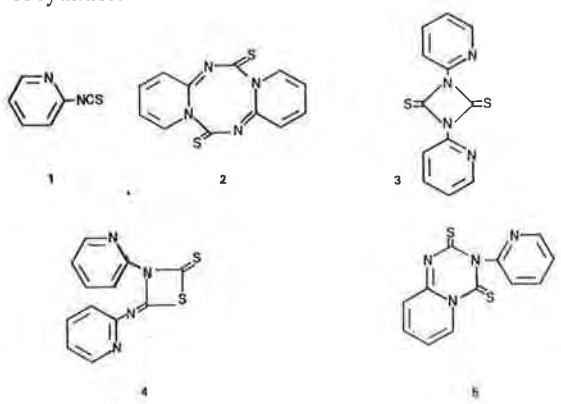
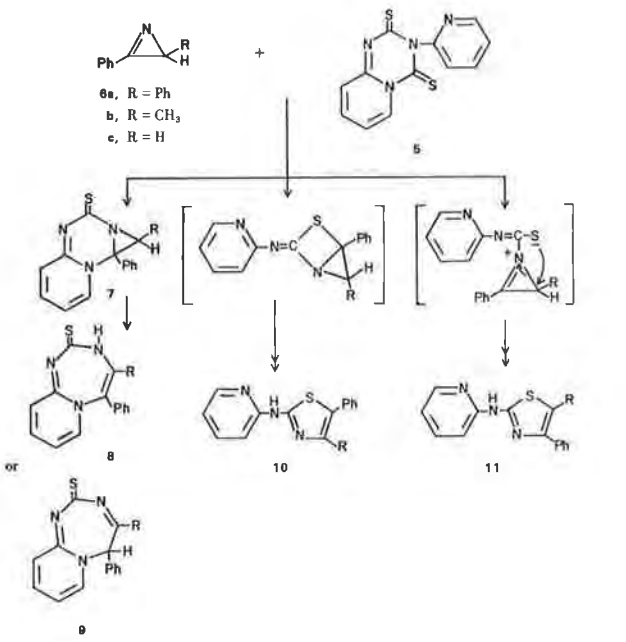
The structure of the dimer (**5**) from 2-pyridyl isothiocyanate (**1**) has been confirmed by ^{13}C nmr spectral studies. The cycloaddition of 2-pyridyl isothiocyanate with 1-azirines results in the formation of thiazoles (**10**). Thermal decomposition of the vinyl azide (**14**) gives the pyrrole (**15**) and the pyridazine (**16**) instead of 2-(2-pyridyl)-1-azirine (**12**).

J. Heterocyclic Chem., 13, 873 (1976).

2-Pyridyl isothiocyanate (**1**) is produced by the reaction of 2-aminopyridine and carbon disulfide in the presence of triethylamine (1,2). At room temperature this compound exists as a dimer. Several possibilities for the structure of the dimer are available in the form of structures **2**, **3**, **4** and **5**. That the correct structure of the dimer was **5**, the result of [4 + 2] cycloaddition of monomers, came from ^{1}H nmr studies (3) and by recognition of similar dimerization of imidoyl isothiocyanates (4). We have confirmed this by ^{13}C nmr spectral analysis at room temperature which clearly showed the presence of an unsymmetrical structure - two C=S carbons and ten aromatic ring carbons. Furthermore, when the probe temperature was increased to 70°, the spectrum changed dramatically and showed only six non-equivalent carbons consistent with dissociation of the dimer to monomeric pyridyl isothiocyanate.



We were particularly interested in the enophilic properties of **1** because of the potential for entry into the benzotriazepine system through cycloaddition with 1-azirines. However, when 2,3-diphenyl-1-azirine (**6a**) in toluene was treated with a half molar ratio of 2-(2-pyridyl)pyrido-[1,2-*a*][1,3,5]triazine-1,3-dithione (**5**) under reflux for 4 hours, an adduct m.p. 203° was obtained in 73% yield. Its mass spectral parent ion peak and elemental analysis were consistent with the molecular formula $C_{20}H_{15}N_3S$. Several possibilities exist for the structure of the adduct. A [4 + 2] cycloaddition would furnish **7a** or its rearranged products **8a** and **9a**. Product **10a** is the eventual result of



a [$\pi 2_s + \pi 2_a$] cycloaddition and hydrogen shift(s). Compound **11a** results from initial nucleophilic attack, 1,3-bond scission and cyclization, and a 1,5-sigmatropic shift. Spectroscopic and related data allowed differentiation among these and other possibilities. Infrared data which shows N-H absorption rule out structures **7a** and **9a**. Carbon-13 nmr spectral analysis (5) showed no C=S carbon, removing structure **8a** as a possibility. The mass spectrum exhibited a fragmentation pattern characteristic of a thiazole ring system (particularly the thiirene peak at m/e 210 (6). Mass spectral data, however, rule out imidazole derivatives, the eventual result of cycloaddition across the C=N bond of pyridyl isothiocyanate. Final confirmation of the structure of the adduct as **10a** came from careful comparison of the ^{1}H nmr spectra of known thiazole derivatives (7,8). Thiazoles **10b** and **10c** were obtained from azirines **6b** and **6c**, respectively. As expected, these cycloaddition products were thermally very stable even at relatively high temperatures (145°).

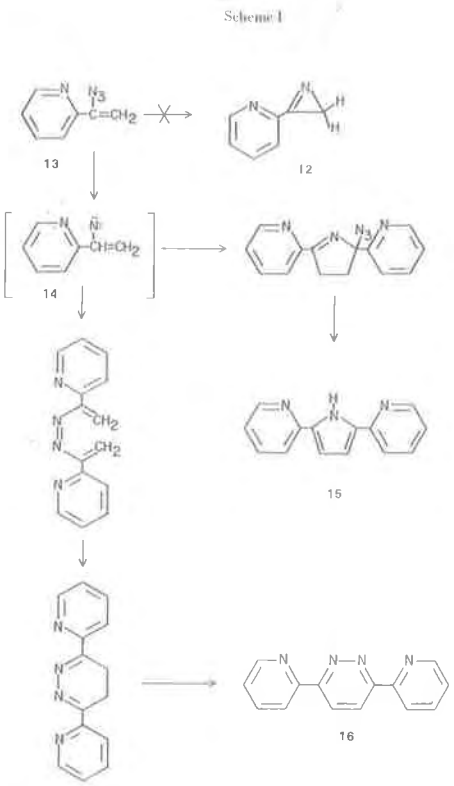
The formation of [2 + 2] type cycloadducts rather than [4 + 2] cycloadducts from the reaction of 2-pyridyl isothiocyanate and 1-azirines is puzzling but not entirely unexpected in the light of results with benzoyl isothiocyanate (8). It can be stated finally that in reactions of 1-azirines with heterocumulenes containing the C=S bond, preferential addition in a regiospecific manner to the C=S bond occurs probably through a dipolar transition state where the inherent ability of sulfur to stabilize the residual negative charge results in a lower energy electronic pathway to the cycloadducts.

Another possible one-step approach to benzotriazepine (and benzodiazepine) derivatives would be through a conjugated azirine system where part of this structure would behave as a diene. A molecular system that would behave not only as a very reactive ene but also as a possible diene is 2-(2-pyridyl)-1-azirine (**12**). However, when the synthesis of this azirine was attempted starting with 2-vinyl pyridine (see Scheme I), the corresponding vinyl azide **13** decomposed spontaneously and exothermically at room temperature to give a considerable amount of intractable polymeric material and two crystalline compounds in low yields.

The first compound (m.p. 94-95°) showed a mass spectral molecular ion peak at 221 and subsequent elemental analysis suggested the molecular formula $C_{14}H_{11}N_3$. Its infrared spectrum showed N-H absorption at 3455 cm^{-1}. Its ^{1}H nmr spectrum showed 10 hydrogens in the aromatic region in the ratio of 1:1:2:1 and an exchangeable hydrogen at δ 10.52. The ^{13}C nmr spectrum showed only 7 carbon absorptions (between 109 and 150 ppm) suggesting a symmetrical structure. Collectively, the data are consistent with the compound being 2,5-bis-(2-pyridyl)pyrrole (**15**).

The structure of the second compound was established

Scheme I



from mass spectral and spectroscopic data as 3,6-bis-(2-pyridyl)pyridazine (**16**). An authentic sample of this, prepared from 2-cyanopyridine, confirmed the structure (9).

On the basis of some previous work on the thermal decomposition of α-styryl azide (10), it is apparent that the formation of **15** and **16** does not occur *via* 2-(2-pyridyl)-1-azirine (**12**). The formation of both compounds, however, can be rationalized as occurring through the intermediacy of the pyridyl nitrene **14** (10). Cycloadditive capture of this fugitive species by the vinyl azide **13** and subsequent elimination of hydrogen azide would furnish **15**. Dimerization of **14**, followed by electrocyclization, and air oxidation would produce **16**.

EXPERIMENTAL

Dimer of 2-Pyridyl Isothiocyanate (**5**).

A mixture of 2-aminopyridine (12.54 g.), carbon disulfide (12.00 g.), triethylamine (22.00 g.), 10 ml. of absolute ethanol was stirred at room temperature for 2 days. The product which had separated was filtered and washed with a little ethanol and with acetone affording triethyl ammonium *N*-2-pyridyl dithiocarbamate as yellow prisms (29.60 g., 82%), m.p. 87-88° (lit. (1) m.p. 88-89°).

Triethylammonium *N*-2-pyridyl dithiocarbamate (25.00 g.) was suspended in 200 ml. of benzene and a solution of 12.5% phosgene in benzene (120 ml.) was added dropwise with stirring during 1.5 hours at 0°. After being stirred overnight at room temperature,

the solid was filtered, washed with benzene, air-dried, and triturated with 200 ml. of water. The residue was filtered and washed with water and finally with 50 ml. of acetone, affording the dimer of 2-pyridyl isothiocyanate as a brick-red solid (14.50 g., 70.6%), m.p. 106-107° (lit. (1) m.p. 110-111°); ^{1}H nmr δ TMS (DMSO-d_6): 7.13-7.55 (m, 4H), 7.90-8.21 (m, 2H), 8.53-8.64 (m, 1H), 9.19-9.27 (m, 1H); ^{13}C nmr δ TMS (DMSO-d_6): 116.78, 123.53, 124.07, 124.23, 133.02, 139.28, 143.75, 146.94, 149.58, 155.78, 173.21, 178.39.

The dimer (0.10 g.) in DMSO-d_6 (1 ml.) was heated at 80° for a few minutes until the solution became yellow, and was checked by ^{13}C nmr at 75°. The monomeric 2-pyridyl isothiocyanate showed the following absorptions: ^{13}C nmr δ TMS (DMSO-d_6): 114.71, 119.45, 135.11, 138.23, 146.88, 152.86.

Reaction of 2,3-Diphenyl-1-azirine (**6a**) with 2-Pyridyl Isothiocyanate.

To a solution of 0.772 g. (4 mmoles) of 2,3-diphenyl-1-azirine in 15 ml. of toluene was added 0.544 g. (2 mmoles) of the dimer of 2-pyridyl isothiocyanate **5**, and the reaction mixture was heated under reflux for 4 hours. The solvent was removed *in vacuo* and dichloromethane-ether was added for crystallization. The crude product was recrystallized from dichloromethane-ether, filtered, and washed with ether to give 0.957 g. (72.7%) of white powderish crystals, m.p. 202-203°; ir ν max (Nujol): 3250 (N-H), 3195 (N-H), 1615 (C=N), 1555, 1485, 1420, 1335 cm^{-1}; ^{1}H nmr δ TMS (DMSO-d_6): 6.82-7.86 (m, 13H), 8.30 (d, 1H), 11.44 (s, br, 1H, exchange with deuterium oxide); ^{13}C nmr δ TMS (DMSO-d_6): 110.85, 115.92, 123,36, 127.30, 128.11, 128.44, 128.71, 129.19, 132.70, 135.34, 137.88, 143.59, 146.40, 151.63, 157.46; mass spectrum: (70 eV, direct inlet) m/e 329 (M^+), 210 [Ph-(c-C_2S)-Ph], 178 (Ph-C≡C-Ph), 151 [M^+ - Ph-(c-C_2S)-Ph], 121 (PhCS), 103 (PhCN), 78, 77, 44.

Anal. Calcd. for $C_{20}H_{15}N_3S$: C, 72.9; H, 4.6; N, 12.7. Found: C, 72.8; H, 4.6; N, 12.7.

Reaction of 3-Methyl-2-phenyl-1-azirine (**6b**) with 2-Pyridyl Isothiocyanate.

To a solution of 0.524 g. (4 mmoles) of 3-methyl-2-phenyl-1-azirine in 15 ml. of toluene was added 0.544 g. (2 mmoles) of **5**, and the reaction mixture was heated under reflux for 6 hours. The solvent was removed and the residue was chromatographed on preparative plates carrying silica gel PF_{254} with 30% dichloromethane-ether as the developing solvent. The middle band (R_f = 0.54) was removed, washed with 5% methanol-dichloromethane, and crystallized from dichloromethane-ether to give white crystals (0.372 g., 34.8%), m.p. 199-200°, ir ν max (Nujol): 3230 (N-H), 3160 (N-H), 1620 (C=N), 1600, 1525, 1480, 1410, 1370, 900 cm^{-1}; ^{1}H nmr δ TMS (DMSO-d_6): 2.37 (s, 3H), 6.79-7.85 (m, 8H), 8.33 (d, 1H), 11.27 (s, br, 1H, exchange with deuterium oxide); ^{13}C nmr δ TMS (DMSO-d_6): 16.07, 110.80, 115.81, 121.85, 126.44, 128.22, 128.71, 132.91, 137.77, 141.76, 151.68, 156.86; mass spectrum: (70 eV, direct inlet) m/e 267 (M^+), 151 (M^+ - Ph-C≡C-CH_3), 148 [Ph-(c-C_2S)-CH_3], 121 (PhCS), 116 (Ph-C≡C-CH_3), 103 (PhCN), 78 (2-pyridyl+), 77, 51, 32.

Anal. Calcd. for $C_{15}H_{13}N_3S$: C, 67.4; H, 4.9; N, 15.7. Found: C, 67.0; H, 4.8; N, 15.4.

Reaction of 2-Phenyl-1-azirine (**6c**) with 2-Pyridyl Isothiocyanate.

To a solution of 0.468 g. (4 mmoles) of 2-phenyl-1-azirine in 15 ml. of toluene was added 0.544 g. (2 mmoles) of **5** and the reaction mixture was heated under reflux for 20 hours. The solvent was removed and the residue was separated by preparative layer chromatography (silica gel PF_{254}) using 20% dichloromethane-ether as the developing solvent. The middle band (R_f = 0.65) was removed, washed with 10% methanol-dichloromethane, and evaporated to give 0.302 g. of crude product. It was recrystallized with dichloromethane-ether to give 0.289 g. (28.5%) of pale yellow crystals, m.p. 223-224°; ir ν max (Nujol): 3240 (N-H), 3180 (N-H), 1610 (C=N), 1540, 1430 cm^{-1}; ^{1}H nmr δ TMS (DMSO-d_6): 6.80-7.88 (m, 9H), 8.45 (d, 1H), 11.32 (s, br, 1H, exchange with deuterium oxide); mass spectrum: (70 eV, direct inlet 200°) m/e 253 (M^+), 237, 136, 134 [Ph-(c-C_2S)-H], 121 (PhCS), 103 (PhCN), 94, 78, 77, 51.

Anal. Calcd. for $C_{14}H_{11}N_3S$: C, 66.4; H, 4.4; N, 16.6. Found: C, 66.3; H, 4.5; N, 16.3.

Attempted Synthesis of 2-(2-Pyridyl)-1-azirine. Isolation of 2,5-bis-(2-Pyridyl)pyrrole (**15**) and 3,6-bis-(2-Pyridyl)pyridazine (**16**).

Bromine (60.00 g., 0.375 mole) in 60 ml. of carbon tetrachloride was added slowly to a stirred, cooled (5-10°) solution of 2-vinyl pyridine (39.20 g., 0.375 mole) in 300 ml. of carbon tetrachloride. After the addition was complete, the reaction mixture was stirred at room temperature for 1 hour. A slight amount of dark yellow precipitate which formed during the reactions was removed, and the resulting solution was concentrated *in vacuo* to give a viscous oil, ^{1}H nmr δ TMS (deuteriochloroform): 3.92-4.63 (m, 2H), 5.17-5.42 (m, 1H), 7.01-7.75 (m, 4H).

The foregoing oil in 400 ml. of DMSO was placed in a three necked 1000 ml. flask fitted with a heavy-duty mechanical stirrer and a nitrogen gas inlet tube. With the aid of an ice bath, the solution was maintained at 10-15° during the addition of 40 g. of sodium azide, and the reaction mixture was stirred at room temperature for 15 hours. The reaction mixture was cooled to 10°. Stirring was continued at ambient temperature for an additional 18 hours, and the sluggish mixture was poured into 500 ml. of cold water. Extraction with ether, drying (sodium sulfate) of the ethereal solution, and evaporation of the solvent gave a thick oil, which immediately started decomposing at room temperature with effervescence. Ether (20 ml.) was added to this product and it was placed in the hood for 2 hours at room temperature. Then it was concentrated *in vacuo*, and separated by column chromatography (alumina activity I) using ether-pentane mixture as the eluent. The first eluate gave a colorless needle type crystalline compound (4.58 g., 12.3%), m.p. 94-95°; ir ν max (Nujol): 3455 (N-H), 1580, 1550, 1420, 765 cm^{-1}; ^{1}H nmr δ TMS (deuteriochloroform): 6.71 (d, 2H), 6.83-7.06 (m, 2H), 7.36-7.57 (m, 4H), 8.41-8.54 (m, 2H), 10.52 (s, br, 1H, exchanges with deuterium oxide); ^{13}C nmr δ TMS (deuteriochloroform): 108.98, 118.24, 120.67, 133.07, 136.15, 149.04, 150.10; mass spectrum: (70 eV, direct inlet) m/e 221 (M^+), 117, 89, 78, 63, 51, 39.

Anal. Calcd. for $C_{14}H_{11}N_3$: C, 76.1; H, 5.0; N, 19.0. Found: C, 76.4; H, 5.0; N, 19.2.

The second eluate, after solvent removal, gave light orange rectangular crystals, and the melting point of the compound was quite broad. Purification by silica gel plates and subsequent recrystallization of the compound gave colorless needles (1.25 g., 2.9%), m.p. 179-180° (lit. (9) 180°). An authentic sample of 3,6-bis-(2-pyridyl)pyridazine (**16**), prepared from 2-cyanopyridine according to literature methods (9), was found to be identical to that isolated above.

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Ozonolysis of 3,4-Dehydroproline

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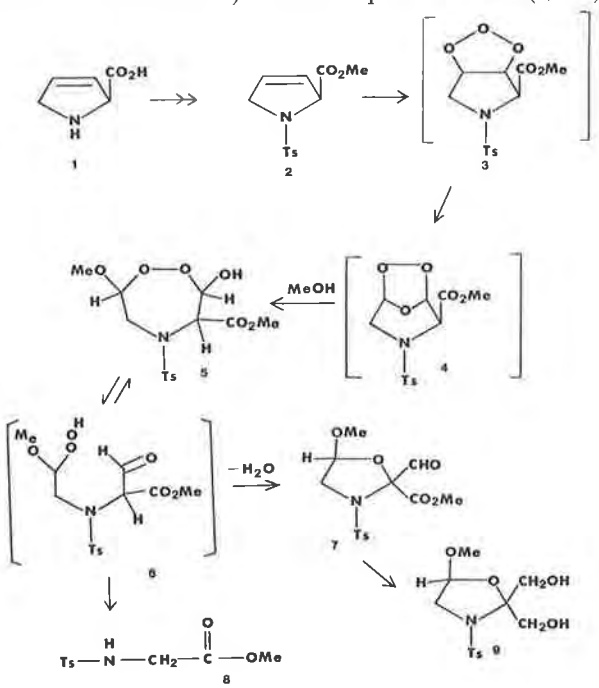
Ozonolysis of protected 3,4-dehydro-DL-proline (**2**) in methanol gives as the initially isolable product, a seven-membered ring cyclic peroxide (**5**). Compound **5** undergoes rearrangement thermally to give methyl *N*-tosylglycinate (**8**) and a stereochemical mixture of oxazolidine aldehyde esters (**7**). Structural evidence for **7** came from detailed ^{13}C nmr studies of **7** and its reduction product (**9**).

J. Heterocyclic Chem., **14**, 313 (1977).

3,4-Dehydroproline is a synthetic imino acid (1) with interesting biological properties. It is a proline antagonist and inhibits the growth of a number of microorganisms (2-5). It is readily incorporated in both plant and bacterial protein replacing an equimolar amount of proline (6). 3,4-Dehydroproline is a substrate for snake venom amino acid oxidases (7,8). Our interest in the oxidation products (9,10,11) of 3,4-dehydroproline prompted examination of the reaction of ozone with this compound (12,13).

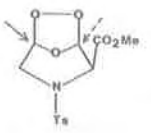
We discovered that at -60° appropriately protected 3,4-dehydro-DL-proline (**2**) in methanol reacts slowly with ozone to give as the initially isolable but unstable product, a seven-membered ring cyclic peroxide (**5**) (14). The structure proposed for this compound is consistent with its ir spectrum, its ^1H nmr, and its decomposition products. At room temperature (and much more rapidly at higher temperatures), the cyclic peroxide undergoes a rearrangement and dehydration to give a colorless oil with molecular formula $C_{14}H_{17}NSO_7$. Its ^1H nmr showed resonances for the tosyl group protons, two sets of methyl groups, an ABX multiplet, and a single absorption peak at δ 9.72 indicative of an aldehyde. Its ^{13}C nmr spectrum correlated well with the proton spectrum but for one difference. Whereas the ^1H spectrum appeared to be that of a single compound the ^{13}C spectrum showed all the features of being that of a mixture, very likely a stereochemical one. When this compound was reduced with sodium borohydride the ^{13}C nmr spectrum of the product was considerably simpler than that of its precursor and consistent with introduction of symmetry into the structure. A striking clue to the structure of the latter product came from the off-resonance decoupled ^{13}C nmr spectrum (see Experimental Section). On the basis of this, the reduction product was assigned the oxazolidine structure (**9**). Further substantiation of this came from elemental analysis, and mass spectral and ^1H nmr data. The product from rearrangement and dehydration of the cyclic peroxide (**5**) must therefore be the oxazolidine (**7**).

A second product from the thermal breakdown of the cyclic peroxide (**5**) was a white crystalline compound, m.p. 81-83°. Its 70 eV mass spectrum and elemental analysis established its molecular formula as $C_{10}H_{13}NSO_4$. Its infrared spectrum (Nujol) showed diagnostic absorption peaks at 3275 and 1725 cm^{-1} and its ^1H nmr spectrum (in deuteriochloroform) exhibited peaks at 2.41 (s, 3H),



3.60 (s, 3H), 3.77 (d, 2H), 5.45 (t, br, 1H, exchanges with deuterium oxide), and 7.15-7.89 (m, 4H). Collectively, the data are consistent with the compound being methyl *N*-tosylglycinate (**8**). An independent synthesis of **8** confirmed its structure (17). Acid hydrolysis of **5** also gave the glycine derivative (**8**).

A plausible reaction pathway for the ozonolysis of 3,4-dehydroproline in the presence of a participating solvent, methanol, is *via* capture of the secondary ozonide (**4**) by methanol. Nucleophilic attack of methanol on the ozonide (**4**) can occur on either of the two ozonide carbons. However, attack at one of the carbons (shown by dashed arrow) is subject to considerable steric hindrance so that the ring expansion of the ozonide (**4**) brought about by methanol must give the cyclic peroxide (**5**) as the predominant product. The two products from the thermal breakdown of **5** can be rationalized as occurring through the intermediacy of **6**, a reactive hydroperoxide. Dehydrative cyclization of the hydroperoxide then would produce the stereochemical mixture of oxazolidine aldehyde esters (**7**). Although the detailed pathway for the formation of **8** from **5** is not known, it is suggested that **8** might arise from **6** by C-N bond fission.



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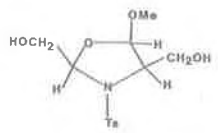

11

Carbon magnetic resonance data provided excellent indirect support for the structure of **5**. Its rearrangement, dehydration, and subsequent reduction would produce **9**. The isomer of **5** with the methoxyl and hydroxyl groups interchanged would give **11**. Both the broad-band ^1H noise decoupled ^{13}C nmr spectrum and the splitting pattern observed for the off-resonance decoupled spectrum are inconsistent with **11** but totally consistent with **9**. The mass spectrum also supports structure **9**.

EXPERIMENTAL

3,4-Dehydro-DL-proline (**1**) and *N*-Tosyl-3,4-dehydro-DL-proline Methyl Ester (**2**).

These two compounds were prepared as described previously (15,16). The protected dehydroproline (**2**) showed the following carbon-13 resonances; ^{13}C nmr δ TMS (deuteriochloroform): 21.58, 52.64, 55.18, 68.11, 124.72, 127.58, 128.68, 129.85, 135.18, 143.89, 170.27.

Ozonolysis of *N*-Tosyl-3,4-dehydro-DL-proline Methyl Ester (**2**).

Into a solution of 1.124 g. (4 mmoles) of *N*-tosyl-3,4-dehydroproline methyl ester (**2**) in 100 ml. of methanol was bubbled ozone from a Walsbach ozone generator for 30 minutes at -60°. The dry ice-acetone bath was then removed and the light blue solution was purged with nitrogen and allowed to attain room temperature. The solvent was then removed *in vacuo* at 25° and the residual material was chromatographed on preparative layer silica gel PF_{254} plates using 50% dichloromethane-ether as the developing solvent. The cyclic peroxide (**5**) was obtained as a colorless oil (0.795 g., 55%); ir ν max (neat): 3400, 1730 cm^{-1}; ^1H nmr δ TMS (deuteriochloroform): 2.39 (s, 3H), 3.36-4.05 (m, 9H), 5.00 (m, 2H), 6.31 (br, s, 1H, exchanged with deuterium oxide), 7.18-8.10 (m, 4H).

Anal. Calcd. for $C_{14}H_{19}NSO_8$: C, 46.53; H, 5.30; N, 3.88. Found: C, 46.92; H, 5.51; N, 3.50.

Thermolytic Rearrangement of **5**.

The cyclic peroxide (**5**) (200 mg.) in 10 ml. of toluene was heated under reflux for 6 hours. After evaporation of solvent the residue was chromatographed on preparative layer plates (silica gel PF 254) using 35% ether-pentane as the developing solvent. The top band (Rf 0.7) gave 30 mg. (22%) of methyl *N*-tosylglycinate (**8**) as white plates, m.p. 81-83° (Lit. (17) m.p. 89-91°); ir ν max (Nujol): 3275, 1725, 1590 cm^{-1}; ^1H nmr δ TMS (deuteriochloroform): 2.41 (s, 3H), 3.60 (s, 3H), 3.77 (d, 2H), 5.45 (t, br, 1H), 7.15-7.89 (m, 4H); mass spectrum: (direct inlet) m/e 243 (M^+), 184 (M^+-CO_2CH_3), 155 (Ts).

Anal. Calcd. for $C_{10}H_{13}NSO_4$: C, 49.50; H, 5.35; N, 5.76. Found: C, 49.50; H, 5.14; N, 5.43.

The middle band (Rf 0.4) gave 106 mg. (56%) of the oxazolidine (**7**) as a colorless oil; ir ν max (Nujol): 1735, 1580, 1435, 1320, 1145 cm^{-1}; ^1H nmr δ TMS (deuteriochloroform): 2.42 (s, 3H), 3.39 (s, 3H), 3.42-3.75 (m, 2H), 3.80 (s, 3H), 5.32 (q, 1H), 7.23-7.86 (m, 4H), 9.72 (s, 1H); ^{13}C nmr δ TMS (deuteriochloroform): 21.58, 52.45, 52.77, 52.90, 56.28, 56.61, 95.02, 96.06, 102.81, 104.50, 127.25, 127.71, 129.66, 129.79, 135.69, 144.60, 166.89, 190.88; mass spectrum: m/e 314 (M^+-HCO), 283, 243, 184, 155, 139, 91.

Anal. Calcd. for $C_{14}H_{17}NSO_7$: C, 48.97; H, 4.99; N, 4.08. Found: C, 49.50; H, 5.51; N, 3.01.

Reduction of Oxazolidine (**7**) with Sodium Borohydride.

A solution of 343 mg. (1 mmole) of **7** in 20 ml. of methanol was treated with 380 mg. (10 mmoles) of sodium borohydride in 10 ml. of methanol at 0°. The reaction mixture was stirred for 30 minutes at 0° and then allowed to stir at room temperature for 4 hours. It was subsequently acidified with 1*M* hydrochloric acid and concentrated. The residual aqueous solution was extracted with dichloromethane and the combined extracts were dried (sodium sulfate). Removal of solvent gave the reduced oxazolidine (**9**) as white crystals (210 mg., 66%), m.p. 115-117°; ir ν max (Nujol): 3360, 1590, 1180, 1075, 1035 cm^{-1}; ^1H nmr δ TMS (deuteriochloroform): 2.40 (s, 3H), 2.67 (s, br, 2H), 3.28-3.95 (m, 6H), 3.39 (s, 3H), 5.16 (q, 1H), 7.20-7.85 (m, 4H); ^{13}C nmr δ TMS (deuteriochloroform): 21.58 (q, CH_3), 54.14 (q, OCH_3), 55.76 (t, 5-CH_2), 64.41 (t, CH_2OH), 101.45 (s, 2-C), 101.90 (d, 4-CH), 127.64 (d), 129.79 (d), 136.22 (s), 144.28 (s) (aromatic carbons); mass spectrum: (direct inlet) m/e 227 (M^+-$HOCH_2$-$COCH_2OH$), 171 (NH_2-Ts), 155 (Ts), 91.

Anal. Calcd. for $C_{13}H_{19}NSO_6$: C, 49.20; H, 6.03; N, 4.41. Found: C, 49.27; H, 5.85; N, 4.20.

Acid-Catalyzed Hydrolysis of Cyclic Peroxide (**5**).

To a solution of 340 mg. of **5** in 10 ml. of methanol was added 10 ml. of 2*M* hydrochloric acid and the reaction mixture was stirred at room temperature for 1 hour. The reaction mixture was neutralized with aqueous sodium bicarbonate and extracted with dichloromethane. The combined extracts were dried (sodium

sulfate), concentrated, and then chromatographed on preparative plates (silica gel PF 254) using 10% dichloromethane-ether as the developing solvent. The top band (Rf 0.85) gave, after extraction, methyl *N*-tosylglycinate (**8**) as white plates (93 mg., 41%), m.p. 81-83°.

Acknowledgments.

We thank the National Science Foundation for a grant towards the purchase of a PFT nmr spectrometer. RHW thanks the 3M Compnay for a 3M Research Fellowship.

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OFFPRINTS FROM SMALL RING HETEROCYCLES V42, PT. 1
EDITED BY DR. ALFRED HASSNER

CHAPTER II

Azirines

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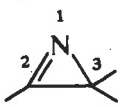
I. INTRODUCTION

The chemistry of the small ring heterocycle, azirine, has fluorished with considerable intensity in the past decade or so because of its theoretical, mechanistic, and synthetic applications. The theoretical and mechanistic interests are associated with the structure, stability, and inherent strain energy of the azirines, and the ability of the system to participate in and direct in several distinct ways the course of many mechanistically significant reactions. The synthetic potential for their transformations into other heterocyclic systems and for incorporation into compounds containing certain desirable functions is impressive.

There are two isomeric azirines **1** and **2**, and these are referred to as 1-azirine and 2-azirine, respectively, in this chapter. The 2-azirine ring system is of interest theoretically. It represents a cyclic conjugated system with 4π electrons and according to Hückel's rule would not be predicted to be stabilized by electron delocalization. Simple molecular orbital (MO) calculations on the parent 2-azirine system shows $DE_\pi \simeq 0.00\beta$ ($\alpha_N = \alpha_C + 1.5\beta$; $\beta_{C-N} = \beta_{C-C}$).[1,2] The corresponding uncyclized enamine has $DE_\pi \simeq 0.30\beta$, suggesting that cyclic conjugation results in destabilization. Therefore the 2-azirine system has been classified as antiaromatic.[3] Although the intermediacy of 2-azirines has been invoked in several attempts at their synthesis, this ring system, because of its inherent instability, has eluded isolation until 1981. For this reason this chapter on azirines will be devoted almost entirely to the chemistry of 1-azirines except for a brief mention of the attempted synthesis of the isomeric ring system. A number of reviews on azirines have appeared during the past few years.[4,42,50,53,172,212]



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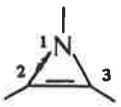


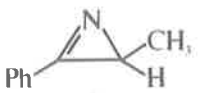
2

This chapter discusses nomenclature, physical properties, spectroscopic data, and particularly syntheses and reactions of 1-azirines. Tables of all known 1-azirines together with their melting points or boiling points and literative citation are also included. The references at the end of the chapter cover mainly the literature to 1981. In general, only literature references directly covering aspects of 1-azirine chemistry are cited.

II. NOMENCLATURE

The two isomeric azirine ring systems (**1** and **2**) have been designated as 2*H*- and 1*H*-azirine, respectively, by the Ring Index of the American Chemical Society[5] and *Chemical Abstracts*. For example, the azirine **3** is named 2-methyl-3-phenyl-2*H*-

azirine. In a system of nomenclature that has been used more frequently, the position of the double bond is designated: thus **1** and **2** are referred to as 1-azirine and 2-azirine, respectively. Compound **3** then is named 3-methyl-2-phenyl-1-azirine, and this nomenclature is employed throughout the chapter. In compounds **4–7**, however, both nomenclatures are shown for completeness. The stereochemistry at C-3 of the azirines is not implied necessarily by the structural representations.



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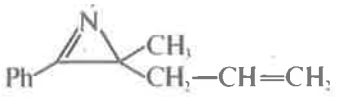
2-Methyl-1-azirine
[3-Methyl-2*H*-azirine]



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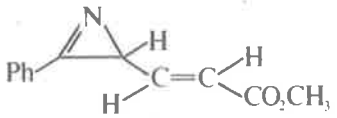
3-Allyl-3-methyl-2-phenyl-1-azirine
[2-Allyl-2-methyl-3-phenyl-2*H*-azirine]



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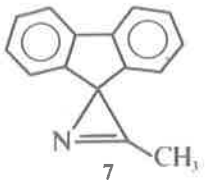
Methyl (E)-2-phenyl-1-azirine-3-acrylate
[Methyl (E)-3-phenyl-2*H*-azirine-2-acrylate]



7

3,3-(2,2′-Biphenylene)-2-methyl-1-azirine
[2,2-(2,2′-Biphenylene)-3-methyl-2*H*-azirine]

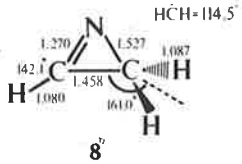
III. PHYSICAL PROPERTIES AND SPECTROSCOPIC DATA

1. Theoretical Calculations

Pople et al.[6] have carried out calculations on the parent 1-azirine (**8**) and 2-azirine (**9**) systems. For 1-azirine (**8**), an overall C_s symmetry was assumed. The structure showed a C–C bond shorter and a C–N bond longer than in acyclic molecules. For 2-azirine (**9**), when nonplanarity at nitrogen and a C_s symmetry are considered, the C–N bond again is found to be slightly lengthened, while the C=C bond is somewhat shorter than in cyclopropene.[6] The angle between the plane of the N–H bond and the ring plane is 72.1°. When compared to aziridines, this $\hat{HNp}1$ angle is found to be larger. Calculations carried out by Clark[7] showed a similar effect. Clark rationalized this result by suggesting that in its planar form the 2-azirine (**9**) is unstable because

of antiaromaticity arising from the delocalization of its 4π- electrons. Clark[7] calculated the inversion barrier in 2-azirine to be 35 kcal/mole, some 20 kcal/mole higher than the inversion barrier in aziridines, again supporting the idea of the instability of the planar antiaromatic form. Pople and his co-workers[6] calculated the ground state energy of 1-azirine and found it to be 40.5 kcal/mole less than that of 2-azirine, whereas Clark[7] obtained 27 kcal/mole for this difference. Apparently, 2-azirine is unstable, both because of ring strain and an energetically unfavorable π-electron structure.

Bond lengths and bond angles for an azirine and its palladium complex have been measured by Hassner and his coworkers[260] from X-ray data, and by Taniguchi and his coworkers[275] from X-ray data.



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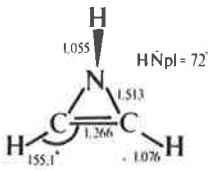

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2. Physical Characteristics

1-Azirines crystallize as colorless or pale yellow crystals. The lower molecular weight azirines are colorless or pale yellow liquids that can be purified relatively easily by fractional distillation under reduced pressures. 1-Azirines have sharp unpleasant odors and are skin irritants.

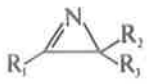
3. Infrared Spectral Data

2-Aryl-1-azirines show in the ir spectrum a strong C=N stretching absorption at about 1740 cm^{-1}. 2-Alkyl-substituted 1-azirines show this absorption at about 1775 cm^{-1}. Both absorptions are about 100 cm^{-1} higher than those observed for aromatic and aliphatic Schiff bases.[10] The spectra of 1-azirines with a hydrogen at the 2-position exhibit markedly different C=N absorptions compared to 2-substituted 1-azirines, with values around 1650 cm^{-1}. Typical C=N stretching frequencies of some representative 1-azirines are shown in Table 1.

4. Electronic Absorption Spectra of 1-Azirines

Table 2 summarizes the uv absorption spectra of some selected 1-azirines. 2-Alkylated 1-azirines show only a weak uv peak at about 230 nm. 2-Arylazirines exhibit an intense uv absorption peak at about 240 nm ($\epsilon > 13{,}000$). There is an inflection on the long wavelength side of the principal absorption band in these

TABLE 1. IR SPECTRAL DATA (NEAT) FOR SELECTED 1-AZIRINES[8, 9]



R_1	R_2	R_3	C=N Absorption (cm^{-1})
Ph	H	H	1740
Ph	CH_3	H	1740
$PhCH_2$	H	H	1780
n-Bu	H	H	1776
H	$CH_3CH_2CH_2$	H	1650
H	Ph	H	1655
H	C_2H_5	C_2H_5	1665

compounds (*ca.* 285 nm). This weak band shifts to shorter wavelengths (blue shift) with increasing polarity of the medium, suggesting that it is associated with an $n\pi^*$ transition.

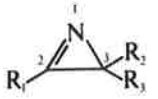
5. Nuclear Magnetic Resonance Spectral Data

Both ^1H and ^{13}C nmr data have been utilized extensively in 1-azirine chemistry. ^{13}C nmr spectroscopy can be particularly useful, not only for determining the structural characteristics of 1-azirines, but also for working out the structures of

TABLE 2. UV ABSORPTION SPECTRA OF SOME 1-AZIRINES[11-13]

R_1	R_2	R_3	Solvent	λ_{max} (nm)	ϵ
n-Bu	H	H	Ethanol	229	112
Ph	H	H	Ethanol	242	13,000
				287	1,000
Ph	Ph	H	Ethanol	245	23,600
				285	1,500
				305	1,050
Ph	CH_3	CH_3	Ethanol	245	15,200
				277	1,500
				286	1,040
Ph	Ph	Ph	Ethanol	250	24,500
				285	1,400
				310	1,100
Ph	PhCO	H	Ether	247	30,000
				324	165

TABLE 3. ^{13}C CHEMICAL SHIFTS OF 1-AZIRINES[15, 16]



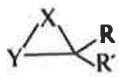
			Chemical shift, δ (ppm), TMS as internal standard			
R_1	R_2	R_3	C_2	C_3	CH_3	Phenyl carbons
Ph	H	H	165.7	19.6	–	126.0, 129.2, 129.5, 132.8
Ph	CH_3	H	172.4	27.5	18.9	126.2, 129.2, 132.7
Ph	Ph	H	163.8	34.6	–	124.6, 127.1, 128.4, 129.2, 129.3, 129.8, 133.1, 141.1
Ph	Ph	CH_3	169.2	39.1	21.0	124.3, 127.8, 128.2, 129.3, 129.4, 129.5, 132.9, 144.0
Ph	Ph	Ph	166.7	44.7	–	124.0, 127.2, 128.2, 128.3, 129.4, 129.7, 133.4, 141.8
CH_3	Ph	Ph	167.3	42.6	12.5	126.8, 127.9, 128.3, 142.1
CH_3	CH_3	Ph	169.9	35.6	12.1, 20.9	125.6, 126.2, 128.1, 144.3
CH_3	H	Ph	164.2	33.3	12.5	125.5, 126.6, 128.1, 141.2
H	Ph	Ph	163.2	39.3	–	127.3, 128.2, 128.4, 141.8
H	CH_3	Ph	165.9	31.9	21.7	126.0, 126.6, 128.1, 144.1
H	H	Ph	160.6	28.7	–	125.6, 127.0, 127.9, 140.4

its reaction products. The ^{13}C nmr chemical shifts of some representative azirines reported by Nair[15] and by Taniguchi et al.[16] are presented in Table 3.

The chemical shift of carbon-3 is in the range of ~ 19 to ~ 45 ppm; Table 4 compares this chemical shift with those of other three-membered cyclic compounds. Carbon-3 of 1-azirines resonates at a higher field than the ring carbons of oxiranes and aziridines but at a lower field than those of cyclopropanes.

A striking difference in chemical shift exists between the heterocyclic ring carbons 2 and 3 of 1-azirines. Carbon-2 appears in the imine region of the ^{13}C spectrum (i.e., 160–170 ppm). For example, the imino carbons of acetophenone methylimine and benzalaniline occur at 166.7 and 159.5 ppm, respectively.[16]

TABLE 4. ^{13}C CHEMICAL SHIFTS OF AZIRINE RING CARBONS COMPARED TO OTHER THREE-MEMBERED CYCLIC RING CARBONS[16–19]



		Chemical shift, δ (ppm) –X–Y–			
R	R′	–CH=N–	$-CH_2-CH_2-$	$-CH_2-O-$	$-CH_2-NH-$
H	H	–	– 2.6	40.8	–
Ph	H	28.7	15.9	52.2	–
Ph	CH_3	31.9	–	56.7	36.3
Ph	Ph	39.3	30.3	61.7	44.0

Examination of ^{13}C–^{1}H coupling constants allows an approximate determination of the percentage of *s* character in the exocyclic orbitals of small ring systems.[2, 20–22] For 1-azirines J (C_3–H) of 186–187 Hz have been observed, which indicate about 37% *s* character in the exocylic σ bonds. This is consistent with the expected greater amount of *p* character of the endocyclic orbitals.[23] In 3-phenyl-1-azirine, the C_2–H coupling constant is 242.5 Hz, which corresponds to about 49% *s* character of the C–H bond.[16] Even if the effect of an electronegative nitrogen atom is taken into account, the *sp*-like hybridization of the exocyclic orbital of carbon-2 is still appreciable. Collectively, these data also suggest that the nitrogen hybridization approaches *sp* in character.[2]

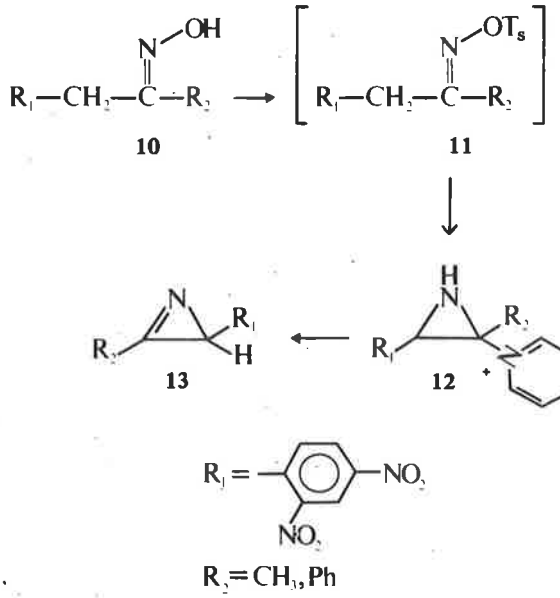
IV. SYNTHESIS OF 1-AZIRINES

A number of general methods are available for the synthesis of 1-azirines. These include the modified Neber reaction, thermolysis and photolysis of vinyl azides and isoxazoles, and thermolysis of oxazaphospholines. All these methods are discussed in this section, and representative examples to illustrate each procedure are mentioned. Tables 11–15 at the end of the chapter present specific examples of 1-azirines that have been synthesized, together with the literature citations of the procedures used. Perusal of these tables will show that 1-azirines with many diverse substitution patterns are known. Substitutions at the 2-position include examples with aryl and alkyl groups, vinyl groups, amino groups, and fluoro groups. Numerous examples of 3-substituted azirines have been synthesized, including such groups as aryl, alkyl, aralkyl, vinyl, allyl, akenyl, hydroxymethyl, halogeno, carboxylic ester, aldehydo, keto, imino, and phthalimido. Spiro- and ring-fused azirines also are known. A number of 2-unsubstituted azirines have been synthesized. It is clear that choice of the method to be used for synthesis of a particular azirine is dependent on the structural characteristics of the azirine and the availability of precursor compounds.

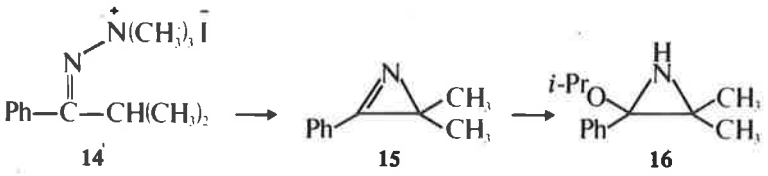
1. Neber and Related Reactions

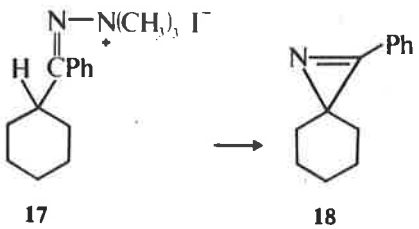
In 1932 Neber and his co-workers suggested for the first time the intermediate formation of a 1-azirine in the conversion of oxime *p*-toluene-sulfonates **11** to aminoketones with base.[25, 26] The structure of the 1-azirine intermediate was confirmed by Cram and Hatch in 1953.[27] They found that in the presence of tosyl chloride and pyridine, the oxime **10** is converted via **11** into aziridines **12**, and 1-azirines **13** could then be prepared by treating **12** with sodium carbonate.

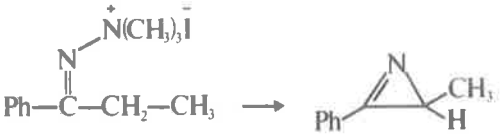
However, the Neber reaction lacked generality, and several modified Neber reactions have been developed during more recent years. For example, the synthesis of 3,3-dimethyl-2-phenyl-1-azirine (**15**) was carried out by the reaction of the dimethylhydrazone methiodide (**14**) with sodium isopropoxide in isopropanol.[28]



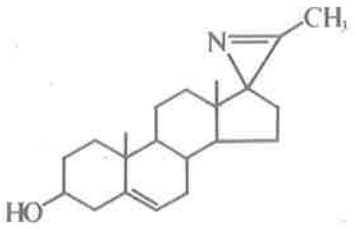
This method was applied successfully to the preparation of certain spiroazirines. For example, Sato[24] found that treatment of **17** with sodium isopropoxide resulted in an 80% yield of the spiro-1-azirine **18**. However, because of the formation of alkoxyaziridine during the reaction with sodium isopropoxide, only poor yields of the azirine **20** were obtained by this method. A more practical synthesis was developed[30–32] using dimethylsulfinyl carbanion as the base and dimethylsulfoxide as solvent. Using this modified Neber reaction, Nair[30] prepared pure 3-methyl-2-phenyl-1-azirine (**3**) in 63% yield from propiophenone dimethylhydrazone methiodide (**19**). Synthesis of the steroidal spiroazirine (**20**) using this method also has been reported.[31, 32] Padwa and Carlsen recently reported the preparation of a series of 3-allyl-substituted 1-azirines (**21**) by a modified Neber reaction.[29, 33] Acetylenic 1-azirines (e.g., **22**) also have been reported.[33]





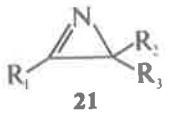


19 3



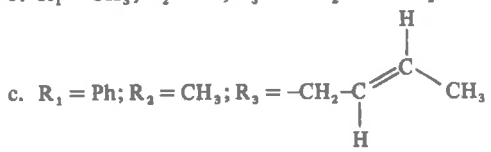
20

However, these modified Neber reactions do not always ensure the preparation of 1-azirines.[24, 34] For example, attempts by Sato[24] to prepare 2-phenyl-1-azirine from acetophenone dimethylhydrazone methiodide resulted in the formation of 2,4-diphenylpyrrole. But the isolation of 2-phenyl-1-azirine in low yields by this method confirms the intermediate formation of the azirine in this preparation.[41]

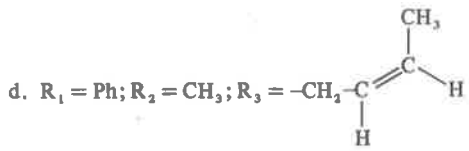


21

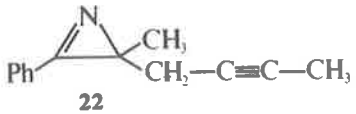
a. $R_1 = Ph; R_2 = CH_3; R_3 = -CH_2-CH=CH_2$ (5)
b. $R_1 = CH_3; R_2 = Ph; R_3 = -CH_2-CH=CH_2$



c. $R_1 = Ph; R_2 = CH_3; R_3 =$



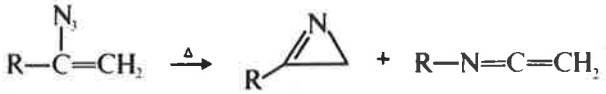
d. $R_1 = Ph; R_2 = CH_3; R_3 =$



22

2. Thermolysis and Photolysis of Vinyl Azides

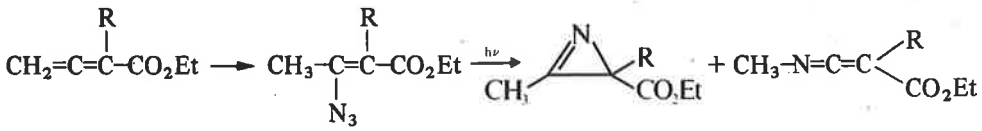
In 1961 Smolinsky reported the first general synthesis of 1-azirines by the vapor phase thermolysis of vinyl azides (**23**).[35, 36] Moderate yields (50–60%) of the 1-azirines **24** were obtained together with small amounts (5%) of the ketenimines **25**. The latter appear to be formed by migration of the group that is alpha to the azido function in a Curtius-type rearrangement.



23 **24** **25**

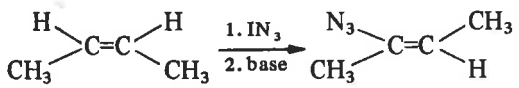
a. R = Ph
b. R = *o*-$CH_3C_6H_4$
c. R = *n*-C_4H_9

The photolysis of vinyl azides also produces azirines. Harvey and Ratts[37] reported the synthesis of 1-azirines (**28**) through photolysis of β-azidocrotonates (**27**). The vinyl azides **27** were prepared by the addition of sodium azide in THF-H_2O to the allenic esters **26**. Ketenimines (**29**) were produced also in the photolysis step.



26 **27** **28** **29**

Advances in the preparation of vinyl azides[38–40] have made the thermolysis or photolysis of vinyl azides the preferred general method for the synthesis of 1-azirines (see, e.g., references 8, 9, 35–37, 42–49). Hassner and his co-workers discovered[39, 40] that iodine azide, generated from iodine monochloride and sodium azide, adds regiospecifically[51] to many olefins to give high yields of β-iodoazides (Hassner reaction). Elimination of hydrogen iodide from the iodoazide with base occurs preferentially in the direction of the azide function to give good yields of vinyl azides. Thus, a terminal olefin such as 1-hexene gave 2-azidohexene rather than the isomeric 1-azidohexene. A vicinally disubstituted olefin such as *cis*-2-butene (**30**) resulted in stereospecific formation of *trans*-2-azido-2-butene (**31**). A conjugated olefin such as *trans*-methyl cinnamate (**32**) gave *cis*-azidocinnamate **33**. Steric effects in some cases may be dominant in determining the position of the azido group. For example, the *t*-butylethylene **34** gave the vinyl azide **35** rather than the vinyl



30 **31**

$$PhCH{=}CHCO_2CH_3 \xrightarrow[2.\ base]{1.\ IN_3} Ph{-}\underset{\displaystyle N_3}{\overset{|}{C}}{=}CHCO_2CH_3$$

trans-**32** *cis*-**33**

azide **36**, the expected, electronically favored product. Cyclic olefins such as indene, 1,2-dihydronaphthalene, and cyclooctene gave the corresponding vinyl azides. However, the iodine azide adducts from cyclopentene and cyclohexene produced allyl azides. Trisubstituted olefins reacted with iodine azide regiospecifically so that the azido function occupied the tertiary position. The absence of a hydrogen geminal to the azido group precluded the synthesis of vinyl azides from these adducts.

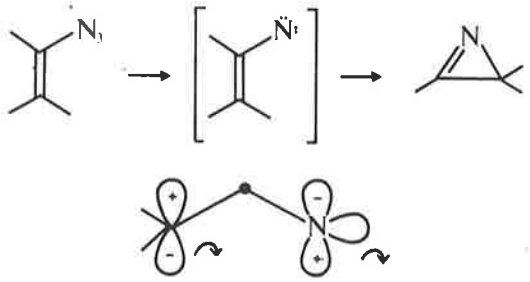
t-BuCH=CH$_2$	t-BuCH=CHN$_3$	t-BuC(N$_3$)=CH$_2$
34	**35**	**36**

Vinyl azides such as 1-azidostyrenes are conveniently prepared by bromine addition to the styrenes followed by azide ion displacement and elimination of hydrogen bromide.[43] This method is particularly useful for the synthesis for 2-phenyl-1-azirines.

Free-radical addition of bromine azide complements the iodine azide method for the synthesis of some vinyl azides. Thus, 2-azidostyrene can be conveniently prepared through free-radical addition of bromine azide to styrene followed by base treatment of the resulting bromoazide.[52]

Vinyl azides can be prepared by a number of other methods. For example, treatment of epoxides with azide ions and dehydration of the resulting azidoalcohols gives vinyl azides. The β-hydroxyazide precursors can also be prepared by the reduction of α-azidoketones with sodium borohydride.[53] The displacement of activated vinyl halides and sulfinates has been utilized for the synthesis of some vinyl azides.[54–56] As mentioned previously, treatment of allenic esters with sodium azides gives vinyl azides.[37] When hydrazoic acid is added to conjugated acetylenes, vinyl azides are formed.[57] The base-catalyzed reaction of α-azido esters and ketones with aromatic aldehydes has been developed as a good method for the synthesis of some vinyl azides.[58–60]

Several mechanisms can be postulated for the formation of 1-azirines from the thermolysis or photolysis of vinyl azides.[8,54] One attractive pathway involves formation of a transient vinyl nitrene species by loss of molecular nitrogen from the thermally or photolytically excited vinyl azide.[61] If the 1-azirine is formed from singlet vinyl nitrene, this conversion is a symmetry-allowed conrotatory electrocyclization (Scheme 1).[62] Although evidence for the intermediacy of a nitrene in the formation of 1-azirines is not available, the formation of certain side products provides some support for the transient existence of this fugitive species. For example, the formation of ketenimine, indole, and dihydropyrazine can be reason-



Scheme 1 A possible mechanism for the synthesis of 1-azirines from vinyl azides.

ably assumed to arise from intermediate nitrene species.[54] Two further studies relevant to this should be mentioned. Nair and Kim[67] reported that the vinyl azide **37** decomposes spontaneously and exothermically at room temperature to give intractable polymers and two crystalline compounds (**40** and **43**). The formation of both compounds can be rationalized as occurring through the intermediacy of the vinyl nitrene **38**. A thermally allowed [4 + 2] capture of this fugitive species by the vinyl azide **37** may lead to a pyrroline **39** which on subsequent elimination of hydrogen azide would furnish **40**. Dimerization of the vinyl nitrene and electrocyclization of the resulting triene **41** would give **42**, which would undergo rapid air oxidation to the aromatic compound **43**. α-Azidostyrene also decomposes on storage and produces 2-phenyl-1-azirine, 3,6-diphenylpyridazine, and 2,5-diphenylpyrrole.[68]

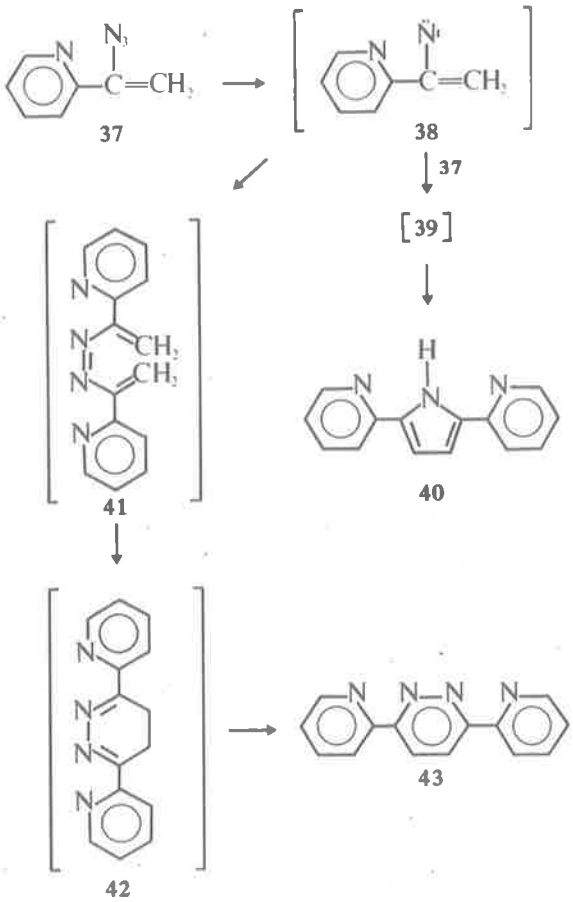
The thermolysis and photolysis of vinyl azides have been utilized extensively for the preparation of 1-azirines, and some representative examples are discussed below.

Preparation of the parent 1-azirine **8** by flash-vacuum pyrolysis of vinyl azide has been reported.[63] The azirine was characterized by its rotational spectrum. It can be trapped at liquid nitrogen temperatures but decomposes at higher temperature to acetonitrile.

Hassner and Fowler[8] have reported the preparation of a number of 1-azirines (**44**) in good to excellent yields by photolysis of the corresponding vinyl azides at 3500 Å. 2,3-Diphenyl-1-azirine is conveniently prepared by the thermolysis of 1-azido-1,2-diphenylethylene.[39] Hassner and Fowler[8] also prepared the first ring-fused 1-azirines. For example, photolysis of 1-azidocyclooctene gave 9-azabicyclo[6.1.0]non-1(9)-ene (**45**) in 93% yield.

Smolinsky and Pryde[54] prepared the spiroazirine **47a** by thermolysis of 9-(1-azidoethylidene)fluorene (**46a**). However, the related azirine **47b** unsubstituted at the 2-position could be obtained only by irradiation of the vinyl azide **46b** at $-15°$.[48] Other spiroazirines also have been synthesized and are mentioned in Table 15.

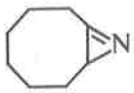
Perfluoro-2-azidopropene (**49**) prepared from perfluoropropene (**48**) undergoes thermolysis to give the perfluoroazirine **50**.[64–66] In the presence of catalytic amounts of HF, this azirine is converted to the thermodynamically more stable isomer **51**.



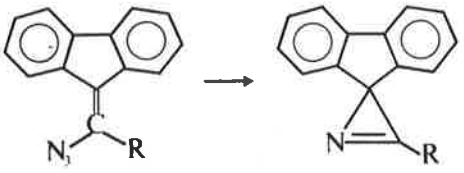
37 38 [39] 40 41 42 43

44

a. $R_1 = Ph; R_2 = H; R_3 = H$ **(24a)**
b. $R_1 = Ph; R_2 = CH_3; R_3 = H$ **(3)**
c. $R_1 = n\text{-Bu}; R_2 = H; R_3 = H$
d. $R_1 = C_2H_5; R_2 = C_2H_5; R_3 = H$
e. $R_1 = PhCH_2; R_2 = H; R_3 = H$
f. $R_1 = PhCHCH_2; R_2 = H; R_3 = H$
g. $R_1 = Ph; R_2 = CO_2Me; R_3 = H$
h. $R_1 = Ph; R_2 = Ph; R_3 = H$

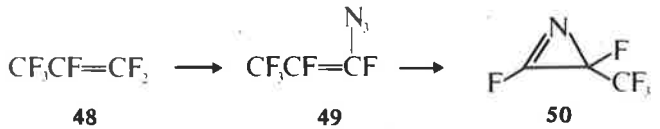


45

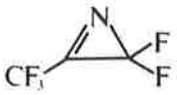


46
a. R = CH_3
b. R = H

47
a. R = CH_3
b. R = H



48 49 50

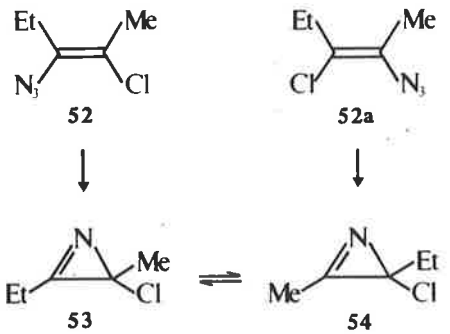


51

It was originally believed that thermolysis or photolysis of terminal vinyl azides did not give azirines.[54] However, the intermediacy of the 2-unsubstituted 1-azirines was implied in a number of studies of decomposition of terminal vinyl azides.[8, 54, 69] It was later reported that both photolysis and pyrolysis of terminal vinyl azides can result in the formation and isolation of 1-azirines.[9, 49] 2-Unsubstituted 1-azirines are thermally unstable, and their preparation and isolation generally requires photolysis at low temperatures.

The preparation of some fatty acid azirines has been reported.[70]

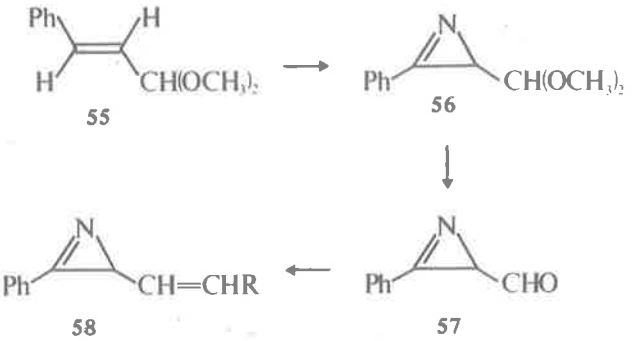
Ciabattoni and Cabell reported the synthesis and thermal isomerization of 3-chloro-1-azirines.[71] When a solution of **52** was photolyzed at 3500 Å at $-40°$, the 1-azirine **53** was formed exclusively as evidenced by nmr spectral data. When the solution of **53** was warmed in the nmr probe, the appearance and growth of new peaks corresponding to **54** was noted. Similarly, the vinyl azide **52a** gave the 1-azirine **54**, which underwent interconversion to **53**. The activation energy E_a for the isomerization of **53** → **54** was 15 kcal/mole with $\Delta S^{\ddagger}$ ($-15°$) = -15 eu.



52 52a

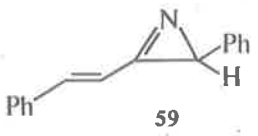
53 54

Padwa and his co-workers[72–74] synthesized the azirine **57** containing a carboxaldehyde at the 3-position. Cinnamaldehyde dimethylacetal (**55**), when treated with iodine azide followed by dehydrohalogenation, thermolysis, and aqueous hydrolysis, gave **57**. Azirine **57** served as a convenient starting material for the synthesis of a series of vinyl-substituted azirines **58**.[73] For example, when **57** was treated with the Wittig reagent, carbomethoxymethylenetriphenylphosphorane in benzene, methyl (E)-2-phenyl-1-azirine-3-acrylate (**58a**) was formed in quantitative yield. A similar set of Wittig reactions gave azirines the **58b–58e**.



a. R = CO_2CH_3
b. R = CN
c. R = C(=O)–Ph
d. R = CHO
e. R = Ph

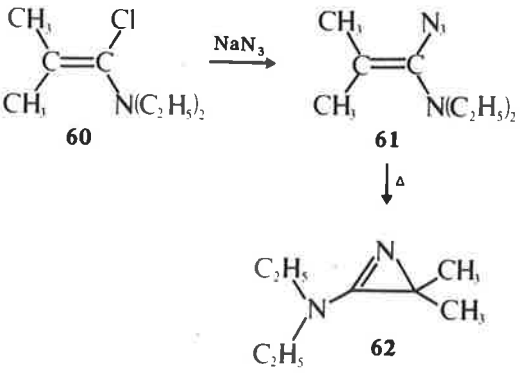
Hassner and Keogh[75] prepared the 2-vinyl-substituted azirine **59** by addition of IN_3 to diphenylbutadiene followed by HN_3 elimination and thermolysis.



Another interesting class of azirines, 2-amino-1-azirines, has been reported by Ghosez and his co-workers.[76–78] These compounds were prepared from α-chloroenamines by reaction with sodium azide as shown here for **62**.

Synthesis of a bisazirine by the vinyl azide route has been reported.[96]

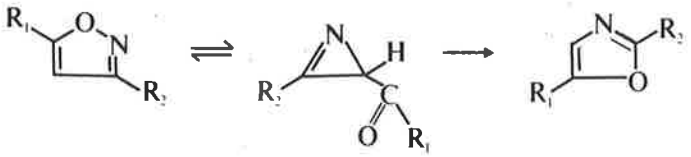
Although the thermolysis or photolysis of vinyl azides offers a convenient entry to many 1-azirines, the yields in these transformations are not always good. In some cases, catalysis by tertiary amines gives higher yields. For example, it has been reported that diazabicyclo[2.2.2]octane (DABCO) not only accelerates the conversion of vinyl azides to 1-azirines, but also inhibits the formation of some of the by-products of the reaction.[79]



60 61 62

3. Photolysis and Thermolysis of Isoxazoles

In some very elegant photochemical work, Ullman and Singh reported that 1-azirines could be generated from isoxazoles.[13, 80, 81] Irradiation of 3,5-diphenylisoxazole (**64a**) (λ_{max}^{ether} 245 nm, ϵ 22,000; 265 nm, ϵ 24,000) in ether solution with 2537 Å light led to the formation of 2,5-diphenyloxazole (**65a**) (λ_{max}^{ether} 302 nm, ϵ 30,000; 315 nm, ϵ 27,600). However, when the reaction was interrupted before completion, an intermediate whose structure proved to be the azirine **63a** (λ_{max}^{ether} 247 nm, ϵ 24,300; 350 nm, ϵ 150) was isolated. Investigation of the effect of wavelength revealed a striking dependence of photochemistry on wavelength. Irradiation of **64** at 2537 Å produced the 1-azirines **63**, which rearranged to the oxazoles **65**, whereas irradiation of the 1-azirines **63** with 3340 Å light resulted in their conversion to the isoxazoles **64**. It has also been observed that photolysis of 3,4,5-triphenylisoxazole gives 3-benzoyl-2,3-diphenyl-1-azirine, 2,4,5-triphenyloxazole, and *N*-phenylbenzoylphenylketenimine.[83] Further mechanistic aspects of isoxazole photochemistry have been reported.[86, 87]



64 63 65

a. $R_1 = R_2 = Ph$
b. $R_1 = Ph$; $R_2 = p\text{-}C_6H_4\text{–}OCH_3$
c. $R_1 = Ph$; $R_2 = CH_3$
d. $R_1 = CH_3$; $R_2 = Ph$

At relatively high temperatures (~ 200°), Singh and Ullman[13] found that 3-benzoyl-2-phenyl-1-azirine (**63a**) can be converted to 3,5-diphenylisoxazole (**64a**). It is likely that at these temperatures the azirine and isoxazole are in equilibrium,

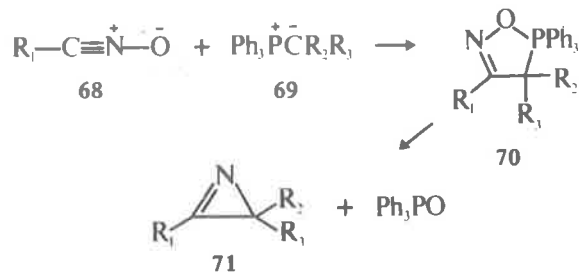
as evidenced by the preparation of several 1-azirine 3-carboxylates (**67**) from the corresponding isoxazoles by Nishiwaki and his co-workers.[84,85] 2-Amino-1-azirines can be prepared by the thermolysis or photolysis of amino-substituted isoxazoles.[92]

66 **67**

a. Ar = Ph; R = CH_3
b. Ar = Ph; R = C_2H_5
c. Ar = Ph; R = *n*-Bu
d. Ar = *p*-C_6H_4–CH_3; R = CH_3
e. Ar = *p*-C_6H_4–Cl; R = CH_3
f. Ar = *p*-C_6H_4–OCH_3; R = CH_3
g. Ar = *p*-C_6H_4–NO_2; R = CH_3

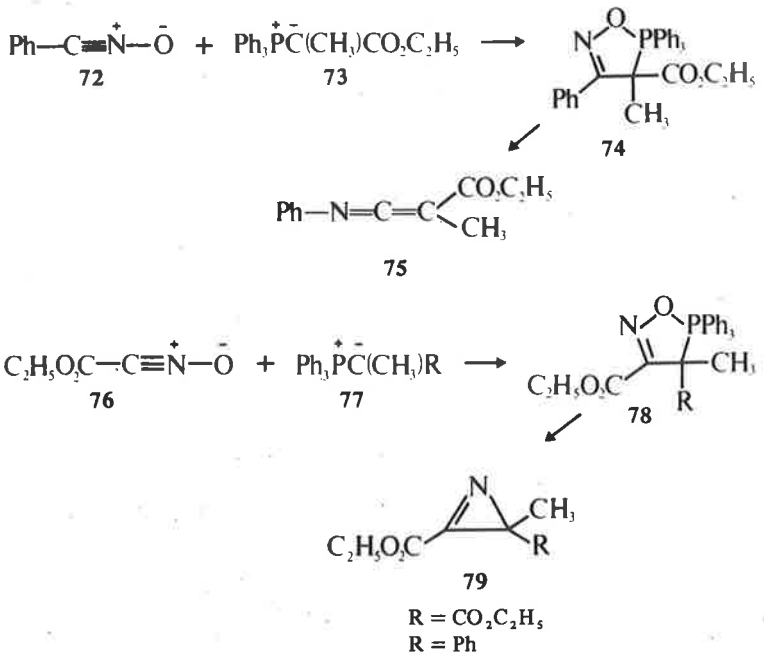
4. Thermolysis of Oxazaphospholines

Huisgen and Wulff,[88,89] and Bestmann and Kunstmann[90,91] discovered that nitrile oxides add smoothly to phosphorous ylides to gives oxazaphospholines **70**. Thermolysis of **70** results in elimination of triphenylphosphine oxide and formation of 1-azirines (**71**). The method is dependent on the availability and structure of both the nitrile oxide and the phosphorous ylide. Electron-withdrawing groups on the phosphorous ylide (e.g., carbomethoxy in **73**) give rise to unstable oxazaphospholines (**74**), which convert to the ketenimine **75** at the expense of 1-azirine formation. The presence of electron-withdrawing groups on the nitrile oxide (e.g., **76**) also results in unstable oxazaphospholines (**78**), but these do convert to 1-azirines (**79**), albeit in low yields.

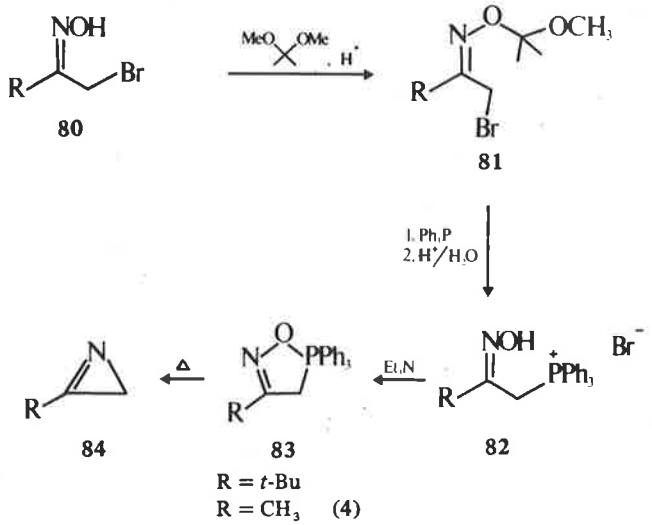


68 **69** **70** **71**

R_1 = Ph; R_2 = R_3 = H (**24a**)
R_1 = Ph; R_2 = CH_3; R_3 = CH_3 (**15**)
R_1 = Ph; R_2 = CH_3; R_3 = C_2H_5
R_1 = Ph; R_2 = CH_3; R_3 = H (**3**)
R_1 = *p*-C_6H_4–Cl; R_2 = CH_3; R_3 = H
R_1 = Ph; R_2 = CH_3; R_3 = Ph

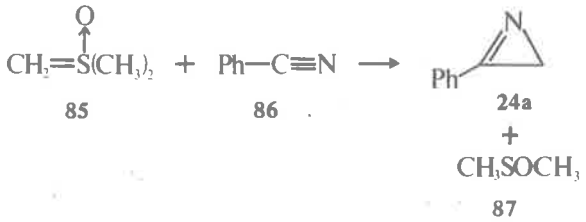


Hassner and Alexanian[82] used α-bromoketoximes (**80**) to prepare oxazaphospholines (**83**). They applied their procedure for the synthesis of azirines that are not easily accessible via the more general vinyl azide procedure (e.g., 2-*t*-butylazirine **84**). This method also avoids the necessity of handling potentially explosive low molecular weight vinyl azides in the synthesis of simpler 1-azirines. It also allows the preparation of 1-azirines with specific labeling (e.g., deuterium) at the 3-position.[82]



5. Addition of Methylene to Nitriles

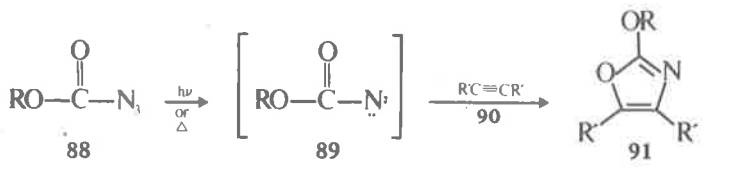
The reaction of "methylene transfer" reagents with nitriles offers a simple and direct approach to the synthesis of 1-azirines. However, very little work has been done in this area. There is one report of the reaction of dimethyloxosulfonium methylide (**85**) with benzonitrile (**86**) to give 2-phenyl-1-azirine (**87**) in low yield.[93]



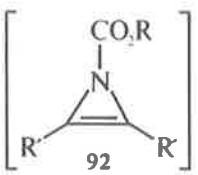
V. ATTEMPTED APPROACHES TO 2-AZIRINES

Although the 2-azirine ring system has been invoked as a transient intermediate in a number of studies directed towards its synthesis, it has been detected only recently and has not yet been isolated. Yamada, Mizoguchi, and Ayata[94] originally suggested that treatment of *1H*-1,2,3-triazole-4,5-dicarboxylic acid with acetic anhydride resulted in a 2-azirine system. However, further investigation of this reaction by Anderson, Gilchrist, and Rees[95] showed the product of this reaction to be an oxazole.

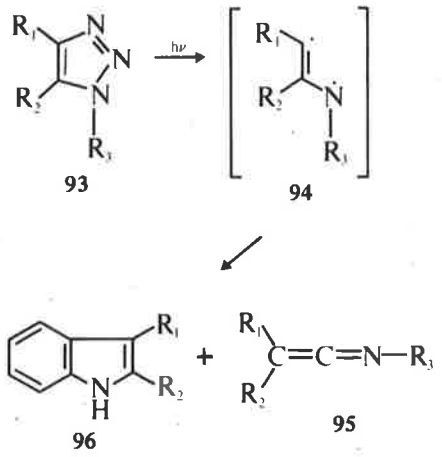
Huisgen and Blaschke[97] and Meinwald and Aue[98] studied the addition of nitrenes to acetylenes as an approach to obtaining 2-azirines. However, the addition of carbethoxy or carbomethoxy nitrene (**89**), generated thermally or photochemically from the corresponding azidoformate **88**, to acetylenes **90**, resulted in isolation of oxazoles **91**. The latter could arise by one or more of several pathways including one that involves addition of the nitrene **89** to **90** to produce the transient 2-azirine intermediate **92**.



R = CH_3, C_2H_5
R′ = CH_3, C_2H_5, Ph



Another direct route to 2-azirines by photolytic decomposition of *vic*-triazoles was examined by Burgess and his co-workers.[99] They envisioned that the 1,3-diradical **94** (or the related carbene) resulting from photochemical loss of nitrogen from **93** might undergo ring closure to a 2-azirine. However, irradiation of the triazoles **93** resulted in isolation of the ketenimine **95** and the indole **96**.



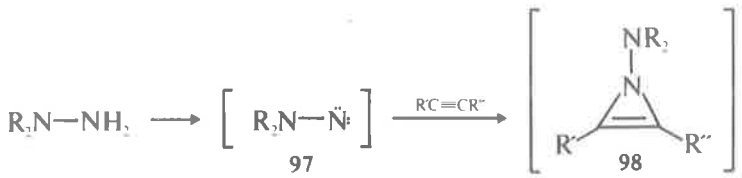
$R_1 = R_2 = R_3 = C_6H_5$
$R_1 = H; R_2 = R_3 = C_6H_5$
$R_1 = R_3 = C_6H_5; R_2 = H$

Fowler and Hassner[2] attempted the dehydrohalogenation of chloroaziridines as a method of generating 2-azirines but succeeded only in isolating oxazoles.

Phthalimidonitrene (**97**), generated by lead tetraacetate oxidation of *N*-aminophthalimide, reacts with acetylenes to give the 1-azirines **99**. This work provides good evidence of the probable intermediate formation of a 2-azirine system, **98**. Rees and his co-workers provided even more compelling evidence for the generation of the 2-azirine intermediate by examining the pyrolysis of 4-methyl-5-phenyl-1-phthalimido-1,2,3-triazole and 5-methyl-4-phenyl-1-phthalimido-1,2,3-triazole.[101] Both triazoles gave identical mixtures of 1-azirines and their pyrolysis products, indicating that the products are formed from a common intermediate (i.e., 2-methyl-3-phenyl-1-phthalimido-2-azirine).

The failure in all these studies to isolate the 2-azirine system is in complete agreement with theory, which predicts that the 2-azirine ring system is unstable because of ring strain and an electronically unfavorable structure. MO calculations show 2-azirine to be approx. 30 kcal less stable than 1-azirine.[102]

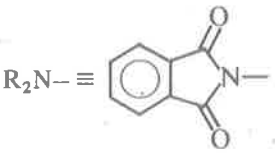
Taking advantage of donor-acceptor substituent stabilization, Regitz and co-workers[106] were able to detect the presence of 2-azirine **101** by the photoirradiation of α-diazoimine **100** in a CH_2Cl_2-glass at 77°K. The presence of **101** was surmised from its 1867 cm^{-1} infrared absorption.

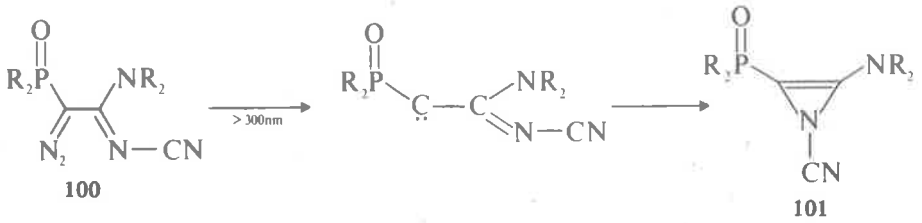


97 98

99

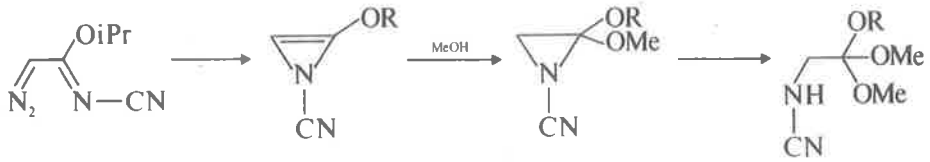
$R' = H; R'' = CH_3$
$R' = H; R'' = Pr$
$R' = H; R'' = n\text{-Bu}$
$R' = C_2H_5; R'' = C_2H_5$





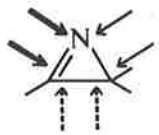
100 101

Similar C=C in frequencies (1880–1890 cm^{-1}) were observed on photolysis of an α-diazoiminoester in an argon matrix at 8°K. When the photolysis was carried out in methanol, one of the products was an ortho ester, a logical transformation product of a preliminarily formed 2-azirine.



VI. REACTIONS OF 1-AZIRINES

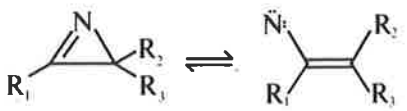
1-Azirines are reactive and versatile substrates because of certain inherent features within their structure. These include high ring strain, a reactive π bond, a lone pair of electrons on the nitrogen, and the ability to undergo ring cleavage on thermal or photochemical excitation to give such reactive fugitive species as vinyl nitrene, iminocarbene, and nitrile ylide. 1-Azirines are capable of acting in reactions as nucleophiles and electrophiles, as a 2π component in thermal cycloadditions, as precursors of vinyl nitrenes and iminocarbenes in thermal intramolecular reactions, as precursors of nitrile ylides, as a 4π component in photochemical cycloadditions, and as a substrate in metal-induced transformations.[42,50,53] These reactions can be regarded in general terms as involving the participation of the C=N, the C–C, or the C–N bond (see **1a**).



1a

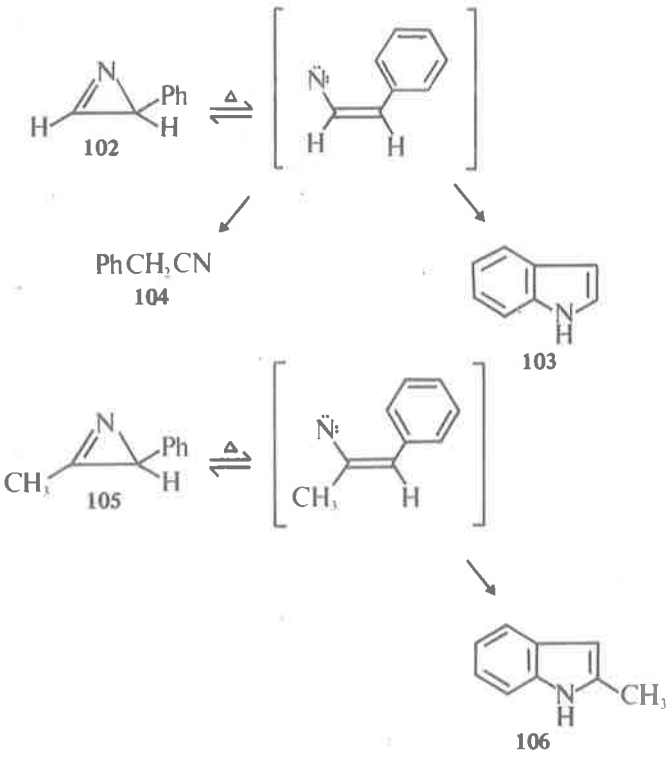
1. Thermal Decomposition and Rearrangement

Thermolysis of 1-azirines may involve C–N bond cleavage or C–C bond cleavage.[103] Ring opening involving the weaker C–N bond to give the vinyl nitrene is the reverse of the thermal electrocyclic closure (Scheme 2). The possibility that such an electrocyclic opening might be occurring during the pyrolysis of 1-azirines was implied in the work of Isomura, Kobayashi, and Taniguchi.[49] They reported that thermal decomposition of 3-phenyl-1-azirine (**102**) in refluxing hexadecane gave a 1:1 mixture of indole (**103**) and phenylacetonitrile (**104**) (Scheme 2a). When 2-methyl-3-phenyl-1-azirine (**105**) was similarly treated, only 2-methylindole (**106**) was isolated. A plausible mechanism for the formation of these products involves cleavage of the C–N bond to generate a vinyl nitrene. This intermediate can undergo insertion into the phenyl group to give indole, or it can rearrange to give phenylacetonitrile.



Scheme 2 Thermal equilibration between 1-azirine and vinyl nitrene.

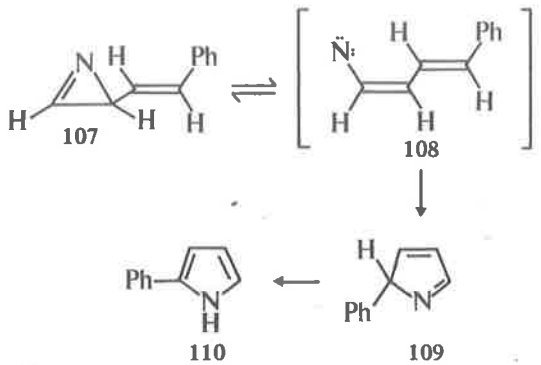
Thermal rearrangement of 2,3-diphenyl-1-azirine appears to be temperature dependent. When the azirine was heated at 250° for 3 hr in a sealed tube, 2-phenylindole, 2,3,4,5-tetraphenylpyrrole, 2,4,5-triphenylimidazole, and 1-benzyl-

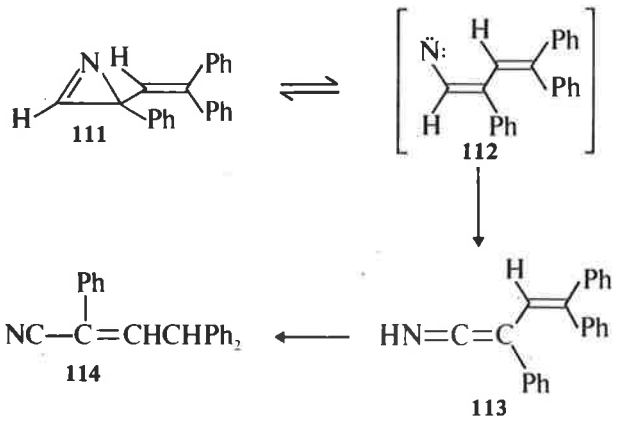


Scheme 2a Thermal rearrangement of 1-azirines to indoles.

2,4,5-triphenylimidazole were obtained as major products.[116] In contrast, thermolysis at 290° for 8 hr gave 2-phenylindole (54%) as the sole product.[117]

In a further study, Isomura, Okada, and Taniguchi examined the thermal rearrangement of 3-vinyl-azirines.[104] The results of this work also can be explained by C–N bond cleavage and formation of a transient vinyl nitrene. For example, the azirine **107** is converted thermally to 2-phenylpyrrole (**110**), presumably through the intermediacy of the vinyl nitrene **108**. The formation of nitrile **114** by thermolysis of the azirine **111** can be explained as proceeding through the nitrene **112**.





Padwa and his co-workers[73,105] examined the thermal rearrangement of ethyl-2-phenyl-1-azirine-3-(2-methylacrylate) (**115**). When this azirine was heated in xylene at 140° for 10 hr, the pyrrole **117** and the pyridine **118** were isolated. These transformations can best be rationalized in terms of an equilibration of the azirine with a transient vinyl nitrene **116**, which subsequently rearranges as shown in Scheme 3 to the products. The transient intermediacy of the vinyl nitrene was supported by trapping experiments. Thus, when the thermolysis of **115** was carried out in the presence of tris(dimethylamino)phosphine, the yields of **117** and **118** were significantly diminished and a 1:1 adduct of **116** and tris(dimethylamino)-phosphine (i.e., structure **119**) was isolated.

Ring expansion of a related system, 3-methyl-3-vinyl-2-dimethylamino-1-azirine has been reported by Ghosez et al.[77]

Thermolysis of iminoazirines results in the formation of pyrazoles (e.g., **120** → **121**).[72,105]

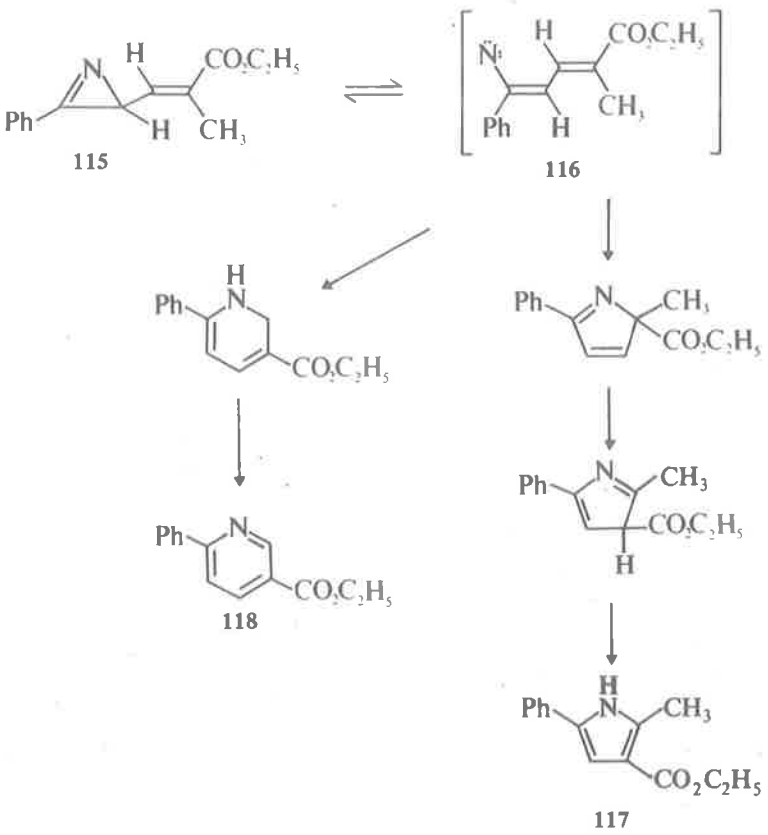
Rees and his co-workers[101,107] examined the thermal decomposition of 1-azirines **122** generated from the flash-vacuum pyrolysis of 1,2,3-triazoles at 400–500°. Their results (Scheme 4) also can be explained by initial carbon-nitrogen bond cleavage.

The pyrolysis of 2-phenyl-1-azirine-3-carboxamide (**123**) was studied by Nishiwaki and his co-workers.[108,109] The pyrazine-2,5-dicarboxamide **124** can be explained by invoking C–N or C–C bond cleavage (Scheme 5). Rupture of the C–N bond may lead to a diradical or a vinyl nitrene.

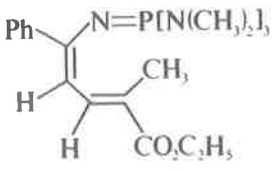
Although the azirinyl diene **125** was reported to produce an azepine (**127**) through intramolecular cyclization of the vinyl nitrene **126**,[105] the structure of this product has been subsequently shown to be the pyrrole **128**.[110]

However, when the azirine **129** was subjected to thermolysis, the azepine **130** was isolated.[111]

Padwa and Carlsen studied the interesting thermal rearrangements of 3-allyl-substituted azirines.[33,112,113] Thermolysis of 3-allyl-3-methyl-2-phenyl-1-azirine (**5**) in toluene at 195° for 180 hr gave 1-methyl-2-phenyl-3-azabicyclo[3.1.0]hex-2-ene (**132**) in 90% yield. On prolonged heating, compound **132** is converted to 3-methyl-2-phenylpyridine (**133**). When the azirine **21c** was subjected to similar thermolysis



Scheme 3 Thermal rearrangements of a 3-vinyl-1-azirine. (Adapted from reference 105 with permission from the American Chemical Society.)



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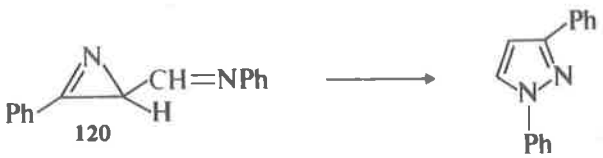
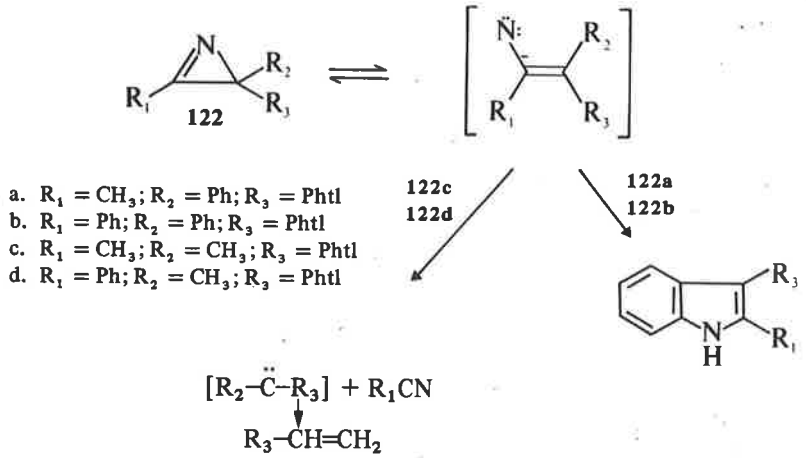


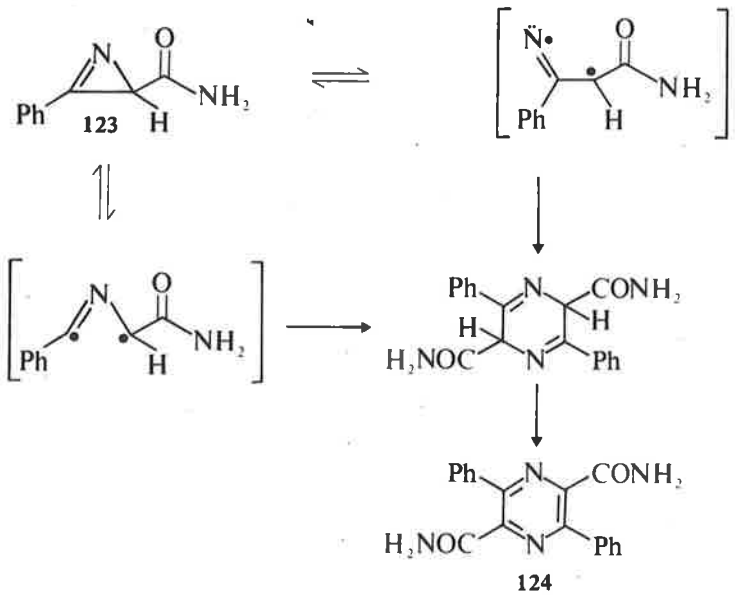
120 **121**



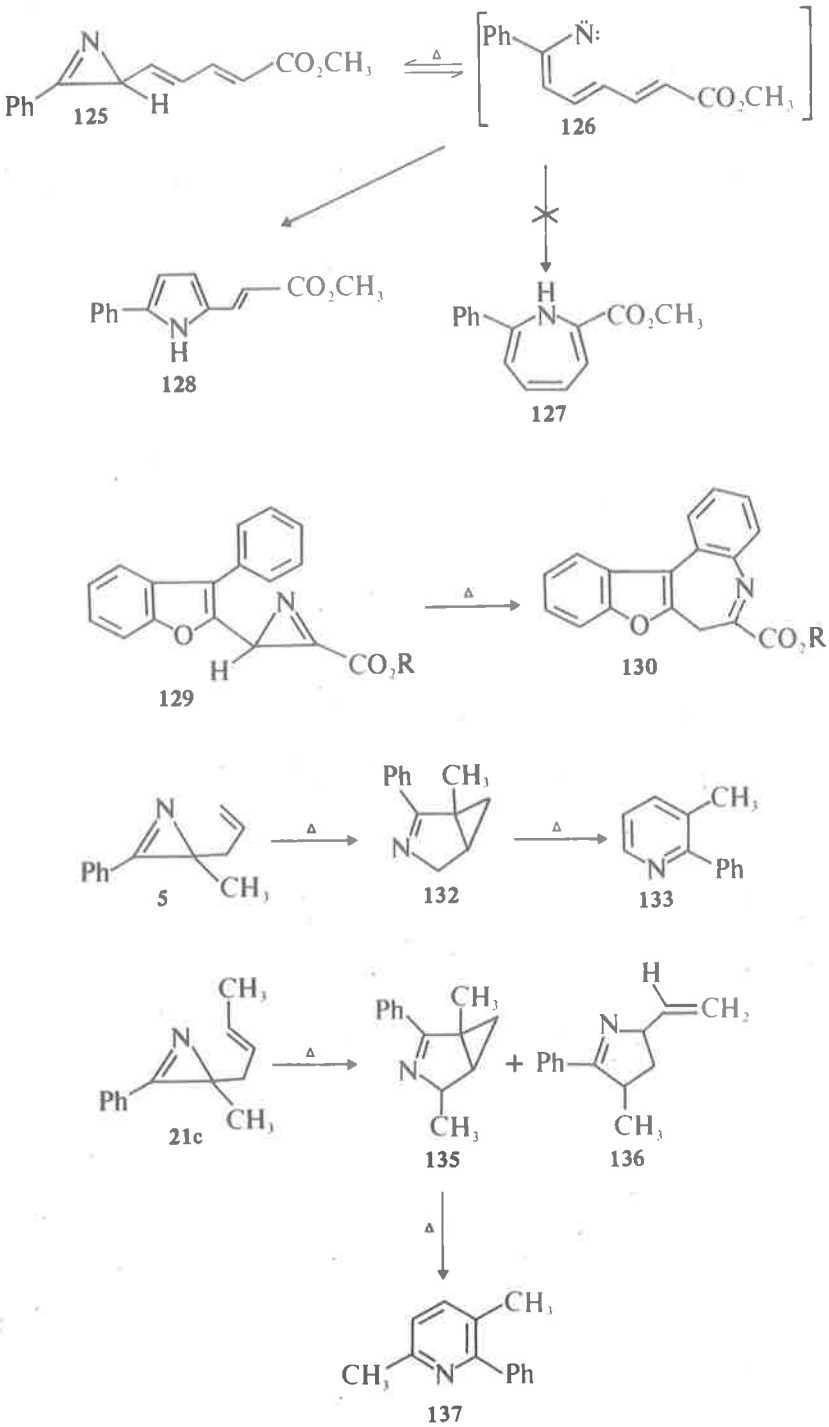
Scheme 4 Thermolysis of 1-azirines generated by flash-vacuum pyrolysis of triazoles (Phtl = phthalimido). (Adapted from reference 119 with permission from the American Chemical Society.)

conditions, the products **135** and **136** were isolated in 71 and 21% yields, respectively.

The formation of 3-azabicyclo[3.1.0]hex-2-enes probably involves initial C–N bond cleavage, and attack of the neighboring π system on the electrophilic singlet nitrene followed by bond reorganization (path 1, Scheme 6). An equally reasonable



Scheme 5 Pyrolysis of 2-phenyl-1-azirine-3-carboxamide.


Ph
N
H
CO2CH3
125
Δ
Ph
N:
CO2CH3
126
Ph
N
H
CO2CH3
128
H
Ph
N
CO2CH3
127
O
N
H
CO2R
129
Δ
O
N
CO2R
130
Ph
N
CH3
5
Δ
Ph
CH3
N
132
Δ
CH3
N
Ph
133
CH3
N
Ph
21c
Δ
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Δ
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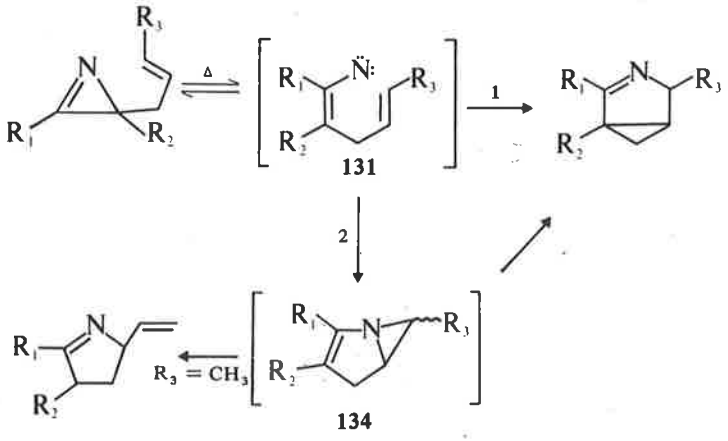
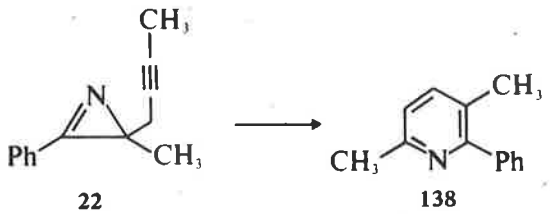
CH3
N
Ph
137



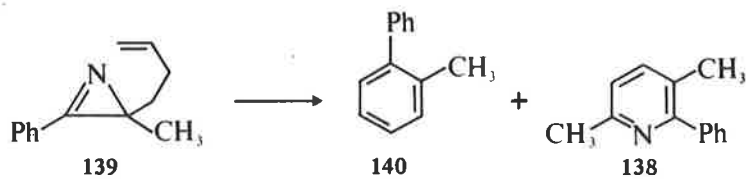
Scheme 6 Mechanism of formation of azabicyclohexenes from the thermolysis of 3-allyl-2-phenyl-1-azirines. (Adapted from reference 33 with permission from the American Chemical Society.)

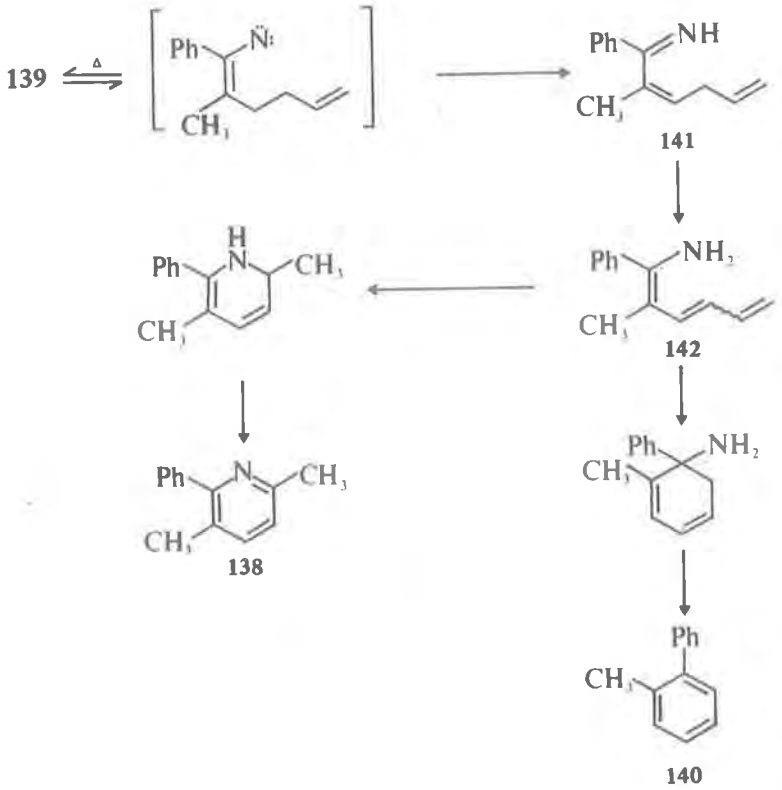
mechanism (path 2) involves intramolecular addition of the nitrene to the adjacent π bond followed by a 1,3-sigmatropic shift of the intermediate. Formation of the Δ^1-pyrroline ring system results from the latter intermediate probably by a homo[1,5]hydrogen migration.

Flash-vacuum pyrolysis (500° at 0.005 mm) of 3-(2-butynyl)-3-methyl-2-phenyl-1-azirine (**22**) gives 2,5-dimethyl-6-phenylpyridine (**138**), presumably through a vinyl nitrene intermediate.[33]



When but-3-enyl-substituted 1-azirines (e.g., **139**) are heated in toluene to 195°, 2-methylbiphenyl (**140**) and 2,5-dimethyl-6-phenylpyridine (**138**) are produced.[114,115] The mechanism of this thermolytic rearrangement (Scheme 7) can be explained by initial ring opening to the vinyl nitrene, followed by a 1,4-hydrogen transfer to produce an azatriene (**141**). This reactive system undergoes a 1,5-

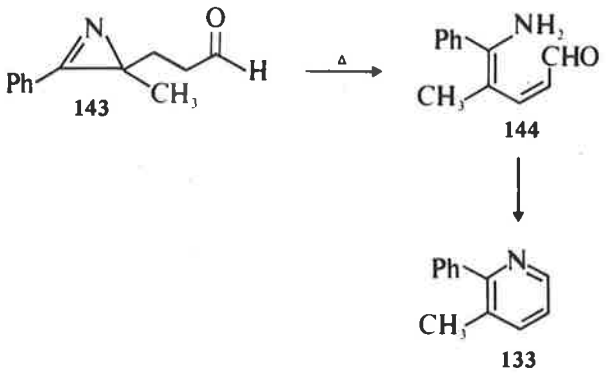




Scheme 7 Mechanism of thermal rearrangement of 3-(but-3-enyl)-2-phenyl-1-azirine. (Adapted from reference 115 with permission from the American Chemical Society.)

sigmatropic shift to give the thermodynamically more stable azatriene **142**, which can undergo cyclization and elimination to give the observed products. The proposed sequential 1,4- and 1,5-hydrogen transfers was supported by evidence from a related study (Scheme 8), where the azirine **143** is converted by ring cleavage and 1,4- and 1,5-hydrogen shifts to the dienaminal **144**. This intermediate cyclizes as expected to give the observed product, 2-phenyl-3-methylpyridine.

In almost all the aforementioned examples, thermolysis of the 1-azirine ring system examined led to products that could reasonably be explained as having arisen from initial C–N bond rupture. Excellent evidence for the occurrence of thermal C–C bond cleavage in the vapor phase pyrolysis of some 1-azirines was reported by Bergman and Wendling.[118,119] They studied the pyrolysis of 3-methyl-2-phenyl-1-azirine in the gas phase at 565° and 1 atm pressure of helium. The products were styrene (56%), benzonitrile (2%), and a reddish polymer. 3-Ethyl-2-phenyl-1-azirine gave similar results. The vapor phase pyrolysis of 3,3-dimethyl-2-phenyl-1-azirine at 472°, however, gave styrene (10%), benzonitrile (6%), ace-



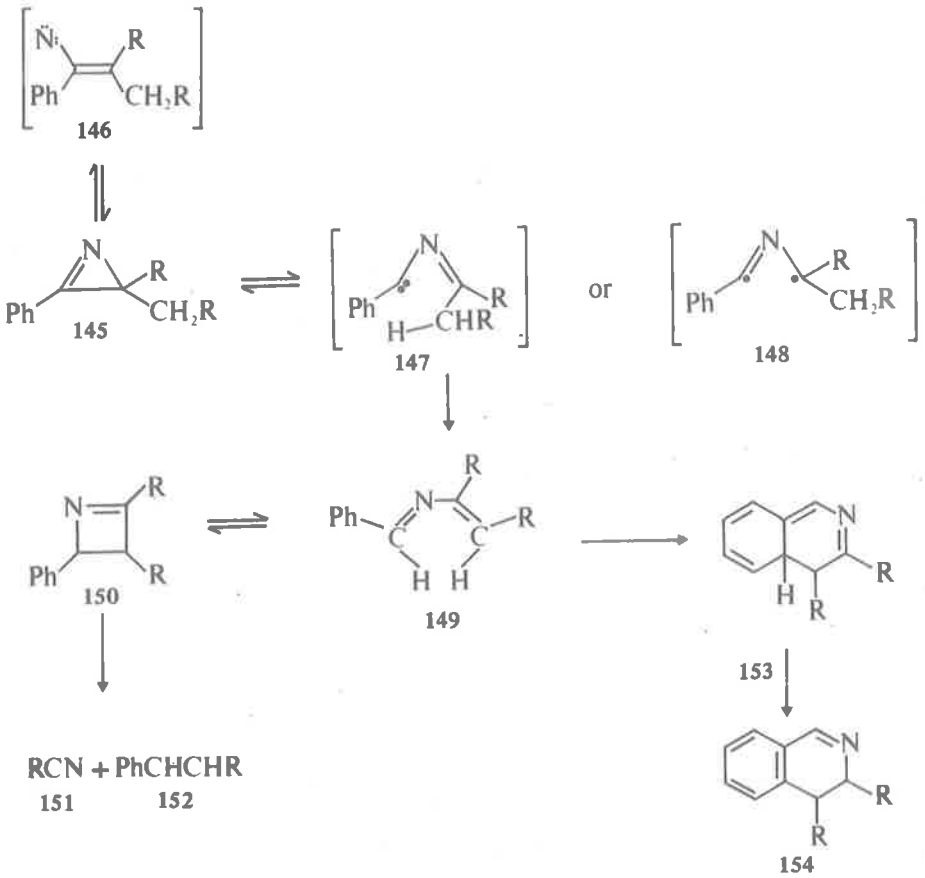
Scheme 8 Dienaminal formation in the thermolysis of the azirine **143**.

tonitrile (small and variable amounts), polymer (20%), and an azadiene (24%). When the pyrolysis temperature was raised to 545°, this azirine gave the following products: styrene (56%), benzonitrile (4%), 3-methyldihydroisoquinoline (5%), and polymer (32%).

From these and further supporting experiments, Bergman and Wendling proposed (Scheme 9) that the initial bond breaking involves the C–C bond and produces a vinyl carbene (**147**) or a 1,3-diradical species (**148**). Hydrogen abstraction by the carbene (or diradical) results in formation of the key intermediate in these reactions, that is, the azabutadiene **149**. An endothermic electrocyclization (4π electrons) may then generate a small steady state amount of azetine (**150**), which fragments to give the nitriles and styrenes observed. Electrocyclization involving 6π electrons produces **153**, which rearranges by a 1,5-sigmatropic hydrogen shift to give the dihydroisoquinoline **154**.

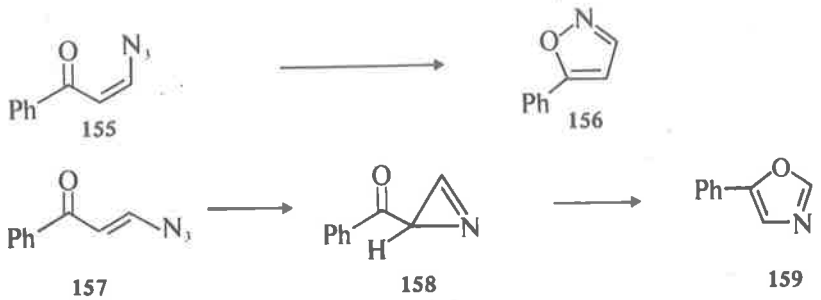
Further support for this mechanistic scheme was provided by Ghosez and his co-workers, who reported the isolation in high yield of an azabutadiene by the pyrolysis of 3,3-dimethyl-2-dimethylamino-1-azirine.[120] The absence of products arising from the vinyl nitrene **146** warrants discussion. It is reasonable to assume that C–N bond cleavage provides a lower energy pathway than C–C bond cleavage. However, the nitrenes formed in these cases apparently will not undergo 1,4-hydrogen abstractions (cf. Padwa and Kamigata[115]). Furthermore, 1,2-abstraction by the nitrene to form ketenimines occurs only when hydrogen is the group being transferred. Consequently, the only product path that seems to be available to the vinyl nitrene is regeneration of the azirine. Thus, reaction products with these azirines are observed only when pyrolysis temperatures are high enough to cause the rupture of the C–C bond.

Thermolytic products can be explained by invoking C–C bond cleavage of the 1-azirine ring system in other cases. For example, the *cis*-vinyl azide **155** is smoothly converted to the isoxazole **156** at room temperature, whereas the *trans* isomer **157** gives the oxazole **159**. In both cases, vinyl nitrenes are plausible intermediates. In the former case, the stereochemical arrangement of the intermediate vinyl nitrene

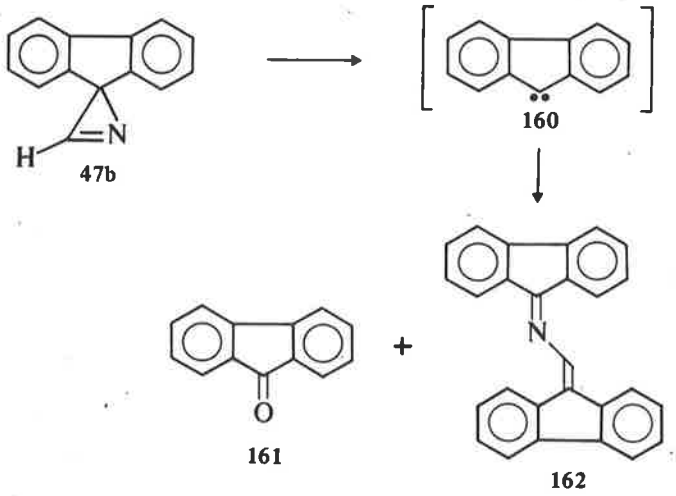


Scheme 9 Thermal C–C bond cleavage in the vapor phase pyrolysis of 1-azirines.

is favorable for a six-electron electrocyclization whereas in the latter case, a four-electron electrocyclization to the azirine **158** is probably the preferred pathway. Transformation of the azirine to the oxazole **159**, would require a C–C bond cleaveage.[121]



The azirine **47b** undergoes bond cleavage at both C–N and C–C to give products that can be explained as having arisen from the intermediate carbene **160**.[48]

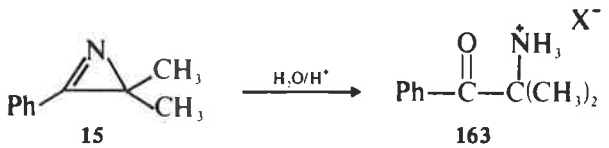


2. Reactions with Azirines as Nucleophiles

1-Azirines undergo a number of reactions in which the ring system plays the role of the nucleophile. The focal point of the initial nucleophilic step in these transformations is the heterocyclic nitrogen. The basicity of the nitrogen in azirines is much lower than in simple aliphatic amines. Calculations based on ^{13}C–H coupling constants[2,15,16] suggest a high degree of *s* character for the exocyclic bonds in this ring system. The basicity and nucleophilicity of 1-azirines appear to be comparable to that of simple aliphatic nitriles.[2]

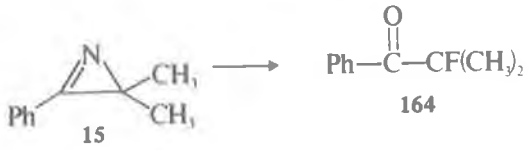
A. *Reactions Involving Acids and Derivatives*

The acid-catalyzed hydrolysis of 1-azirines to α-aminoketones is well established. In fact, in many reactions of 1-azirines where acid catalysis is used, formation of α-aminoketones is difficult to avoid. Hydrolysis of 2-substituted 1-azirines (e.g., **15**) gives α-aminoisobutyrophenone (**163**).[122] With 2-unsubstituted 1-azirines, the hydrolysis products would be aminoaldehydes. The acid-catalyzed methanolysis of azirine **15** gives the dimethyl ketal of **163**, quantitatively.



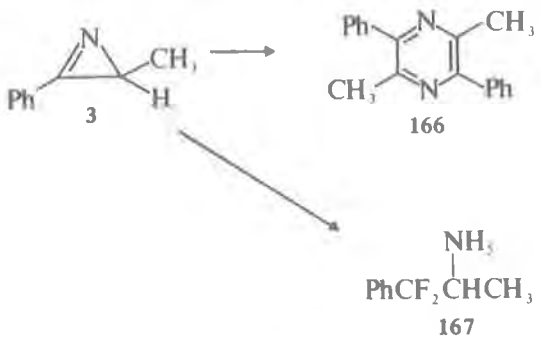
15 163

The reaction of HF in the presence of pyridine or triethylamine with 1-azirines gives products that depend on the structure of the 1-azirine as well as on the solvent used. For example, 3,3-dimethyl-2-phenyl-1-azirine (**15**) reacts with HF/pyridine in tetrahydrofuran to give the α-fluoroketone **164** almost quantitatively,[123] whereas 2-phenyl-1-azirine (**24a**) is converted by HF/pyridine in benzene to the difluoroamine **165** as the major product.[124] Variation of products results with the same azirine when different solvents are used. Thus, 3-methyl-2-phenyl-1-azirine **3** reacts with HF/pyridine in tetrahydrofuran to give the pyrazine **166** in 81% yield, whereas in benzene it is converted to the difluoroamine **167** in 75% yield.[125]



15 **164**

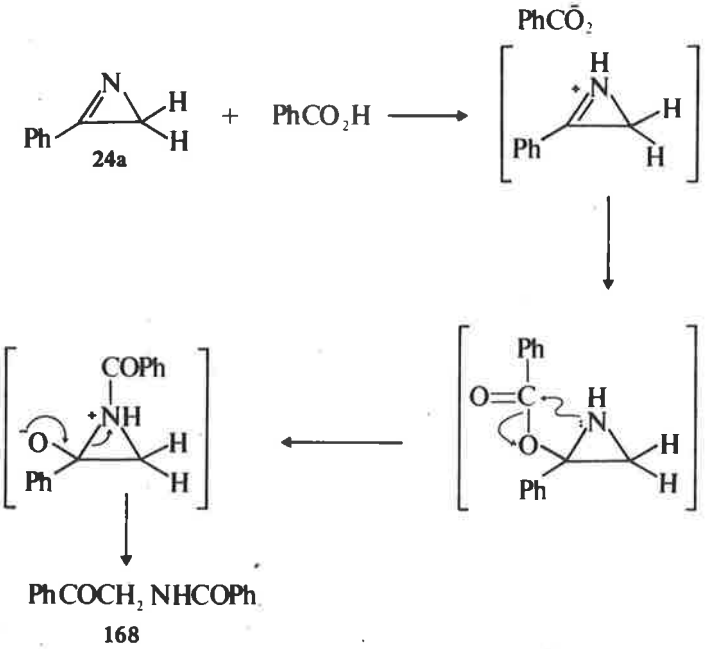
24a **165**



3 **166** **167**

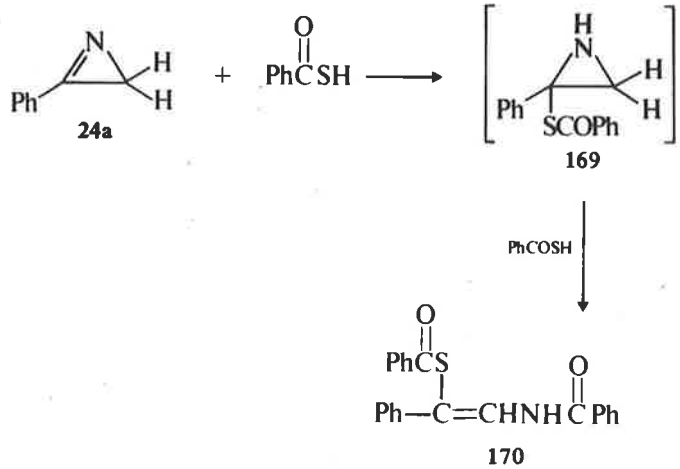
The reaction of 2-phenyl-1-azirine (**24a**) with benzoic acid gave *N*-benzoylphenacylamine (**168**).[126] The overall mechanism of the reaction in this case and in the two former examples involves initial protonation on nitrogen followed by addition of nucleophile to the azirinium ion, and finally ring opening. In the latter example (Scheme 10), a rearrangement following the nucleophilic attack must occur to account for the observed product **168**. α-Haloacids behave similarly.[131]

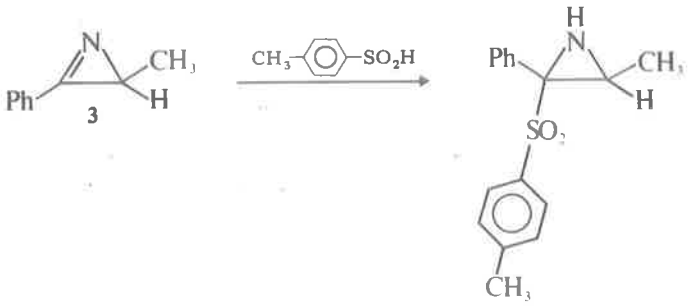
However, thiobenzoic acid reacted with azirine **24a** to give **170**, presumably through the intermediacy of the aziridine **169**.[126]



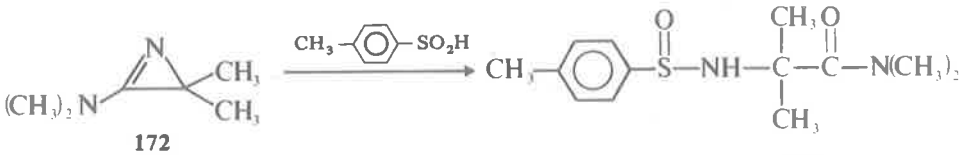
Scheme 10 Mechanism of reaction of 2-phenyl-1-azirine and benzoic acid.

Meek and Fowler[53] observed that the addition of *p*-toluenesulfinic acid to 3-methyl-2-phenyl-1-azirine (**3**) gave the sulfonylaziridine **171**. However, reaction of the 2-aminoazirine **172** with *p*-toluenesulfinic acid[127] gave the ring-opened product **173**. The reaction pathway followed in the latter case appears to be similar to that shown in Scheme 10 for the benzoic acid reaction with azirine (**24a**).





3 171



172 173

Sulfonic acids react with the aminoazirine **172** to give dimeric salts containing the piperazine ring.[127]

Activated phenols (e.g., **174**) react with the 2-aminoazirine **172** in boiling benzene to give the aniline derivatives **175**.[128] A plausible reaction mechanism is shown in Scheme 11: protonation of the azirine is followed by attack of the phenolate ion at the amidinium carbon atom. The resulting intermediate rearranges to a spiro-Meisenheimer complex, which undergoes ring opening to give the observed products **175**. Compound **175c** can also be produced by the reaction of **172** with 2,4-dinitrofluorobenzene.[128]

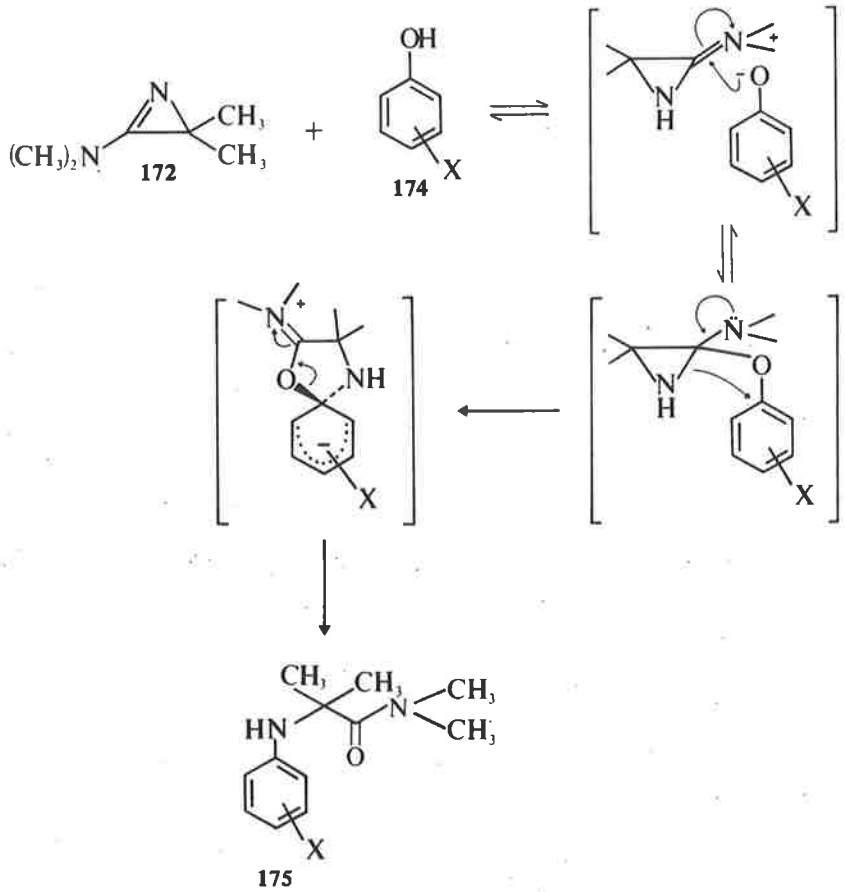
2-Formyl- and 2-acetylphenols (**176**) convert **172** to **177**.[128]

The 2-aminoazirine **172** reacts with formyl cycloalkanones **178** to give the 1 : 1 adducts **179** as shown in Scheme 12.[127]

The first example of the utilization of the protonated 1-azirine system for the synthesis of heterocyclic compounds was reported by Leonard and Zwanenburg.[122] They discovered that treatment of 3,3-dimethyl-2-phenyl-1-azirine (**15**) with anhydrous perchloric acid and acetone or acetonitrile gave the oxazolinium perchlorate **180** and the imidazolinium perchlorate **181**, respectively. Using elegant isotope labeling studies, they proposed that the mechanism of these conversions involved 1,3-bond cleavage of the protonated azirine and reaction with the carbonyl group (or nitrile) to produce a resonance-stabilized carbonium-oxonium ion (or carbonium-nitrilium ion), followed by attack of the nitrogen unshared pair of electrons to complete the cyclization (Scheme 13).

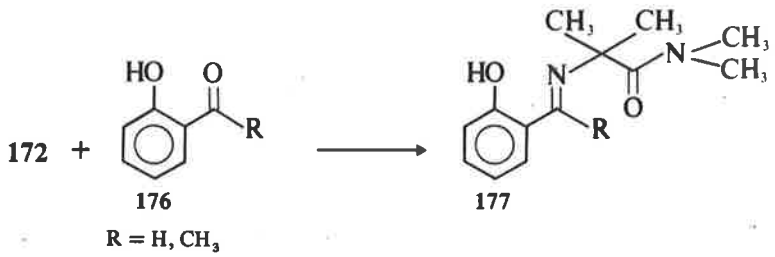
Similar results are also obtained when boron trifluoride etherate is substituted for perchloric acid or fluoroboric acid in these ring expansion reactions.[129]

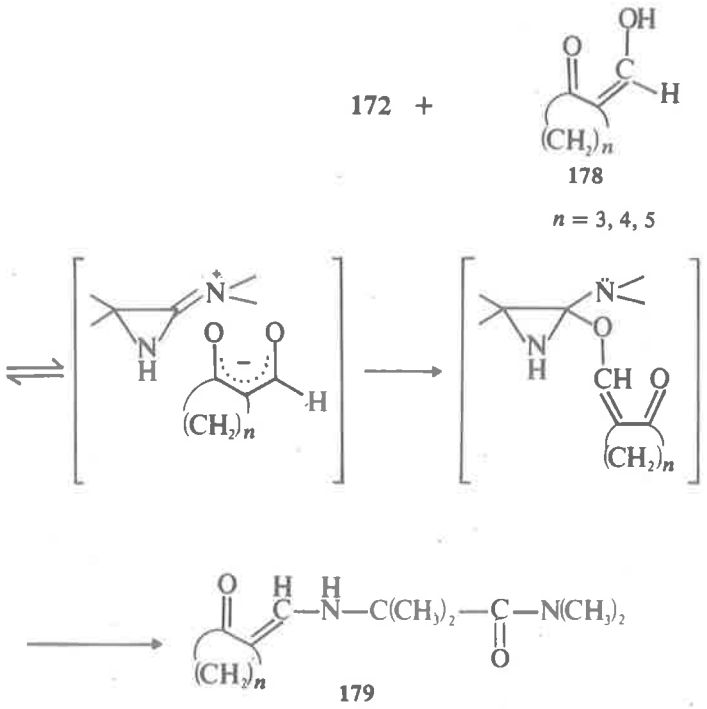
Leonard and Zwanenburg isolated the aziridine **182** from the reaction of azirine (**15**) and pyridinium perchlorate.[122] The structure of a similar product, prepared from Neber's azirine,[25] was proposed by Cram and Hatch.[27]



a. X = 2-nitro
b. X = 4-nitro
c. X = 2,4-dinitro
d. X = pentachloro

Scheme 11 Mechanism of reaction of 2-amino-1-azirines and activated phenols. (Adapted from reference 128 with permission from *Helvetica Chimica Acta*, Birkhauser Verlag.)



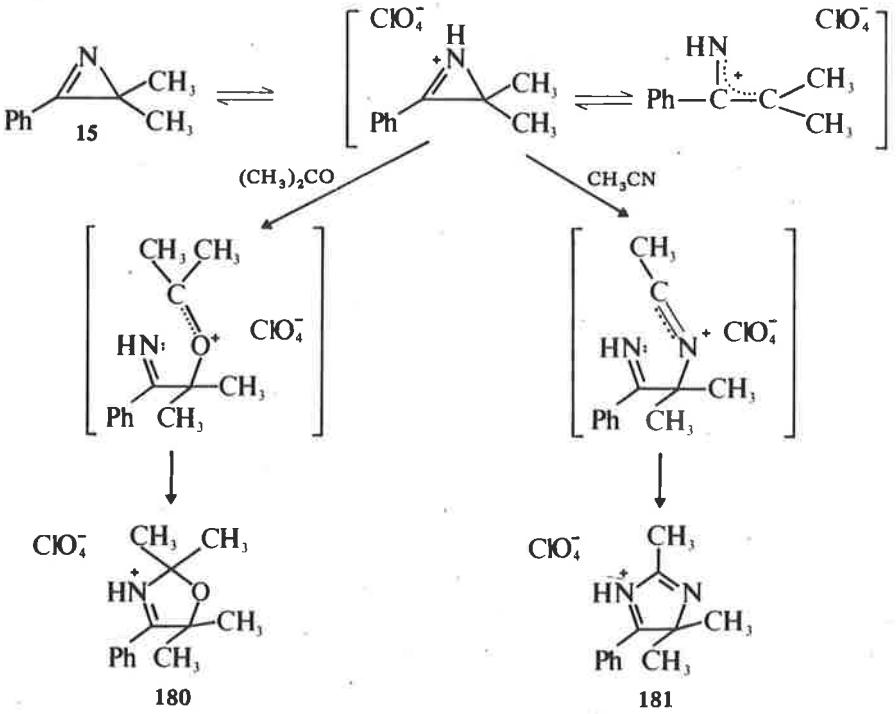


Scheme 12 Reaction of 2-amino-1-azirines with formyl cycloalkanones.

However, when Leonard, Muth, and Nair[130] treated the azirine **15** with anilinium perchlorate in acetonitrile at 0°, they found that it was quantitatively transformed into α-ammonium isobutyrophenone anil perchlorate (**186**). The probable first step in this conversion is the transfer of a proton to the azirine and attack by aniline on the iminium bond to give **184**. A second proton transfer from the anilinium to the more basic aziridine nitrogen would lead to intermediate **185**. Cleavage at the 1,2-bond of the strained ring to give a resonance-stabilized iminium ion would be followed by a final proton transfer to yield the product **186**. An intriguing feature of the mechanistic sequence is the effective transfer of all three protons from one nitrogen to the other (Scheme 14).

Reaction of azirine **63a** with hydrazine perchlorate gives the aminopyrazole **188**.[13] The mechanism is probably similar to that of the reaction of anilinium perchlorate and azirine (**15**). The intermediate **187** is therefore the precursor to the pyrazole **188**.

1-Azirines react with carboxylic acid chlorides in benzene to give aziridines, where RCOCl has been added to the C=N bond.[2,132,133] For example, Hassner and coworkers found that 3-methyl-2-phenyl-1-azirine (**3**) reacts with benzoyl chloride presumably through the azirinium ion **189** to give a stereoisomeric mixture of *N*-benzoyl-2-chloroaziridines (**190**). These unstable aziridines are converted in polar solvents or by heating into a mixture of oxazole (**191**) and dichloroamide (**192**)



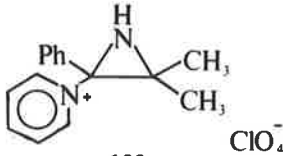
Scheme 13 Acid-catalyzed additions of acetone and acetonitrile to 1-azirines.

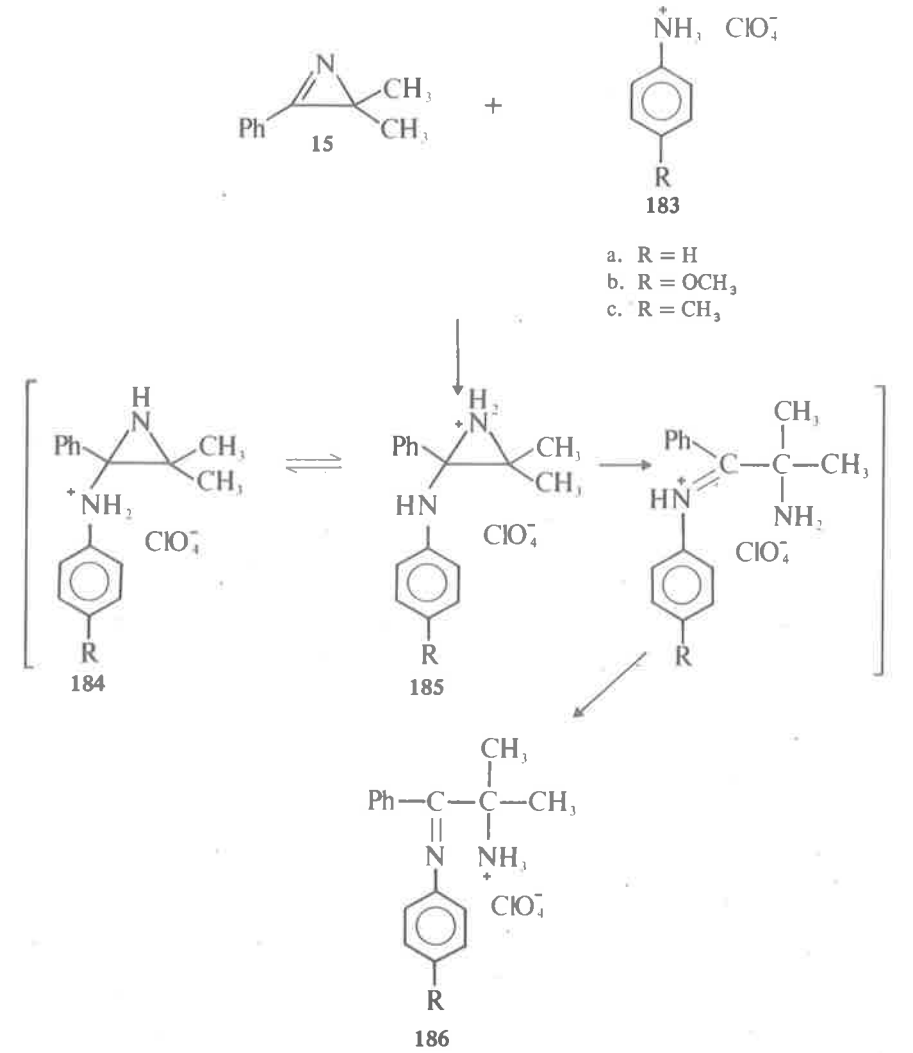
(Scheme 15). It should be noted that the rearrangement to the oxazole proceeds with opposite regiochemistry to that observed in the formally similar acid-catalyzed reaction of 1-azirines with ketones reported by Leonard and Zwanenburg.[122]

Sato and his co-workers[126] observed that the reaction of 2-phenyl-1-azirine (**24a**) with acid chlorides and anhydrides in the presence of triethylamine gave the oxazole directly. Thus **24a** was converted to the oxazole **193** when it was treated with acetic anhydride and triethylamine under reflux for 6 hr. Using a lower temperature and a shorter reaction time they were able to isolate the aziridine **194**.

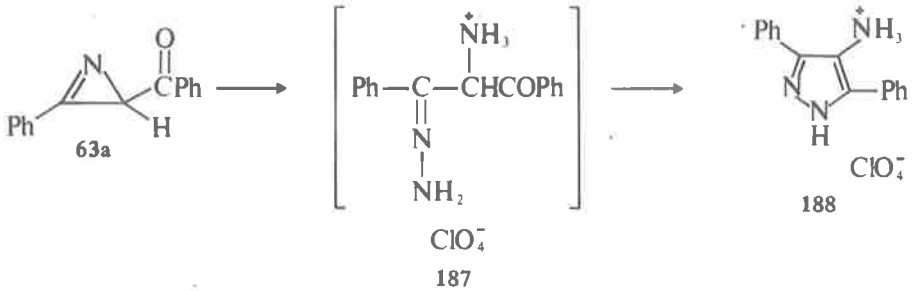
The reaction of phthalic anhydride with the azirine **24a** gives the ketoamide **196**. A likely intermediate in this conversion is **195**, which on hydrolysis gives the observed product.[126]

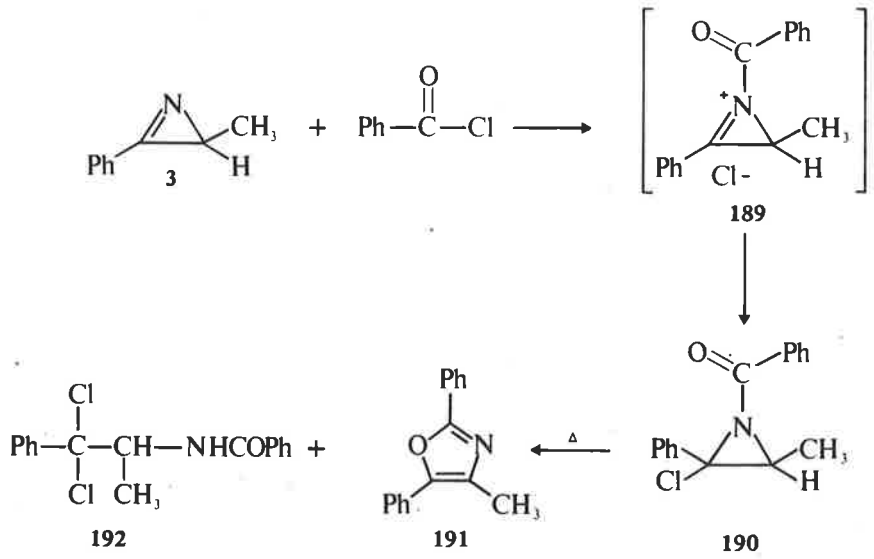
When azirine **3** was treated with an excess of benzenesulfonyl chloride in pyridine, a mixture of sulfonamides **198** and **199** was produced.[2] It is likely that





Scheme 14 Reaction of 1-azirines with anilinium perchlorates.



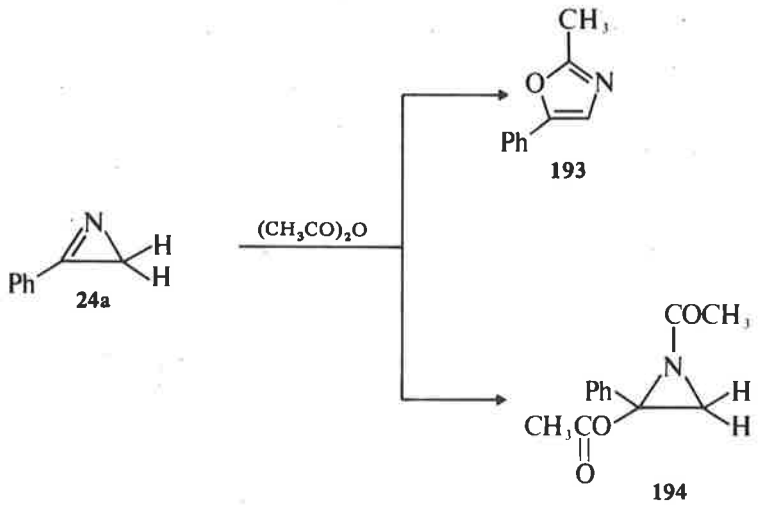


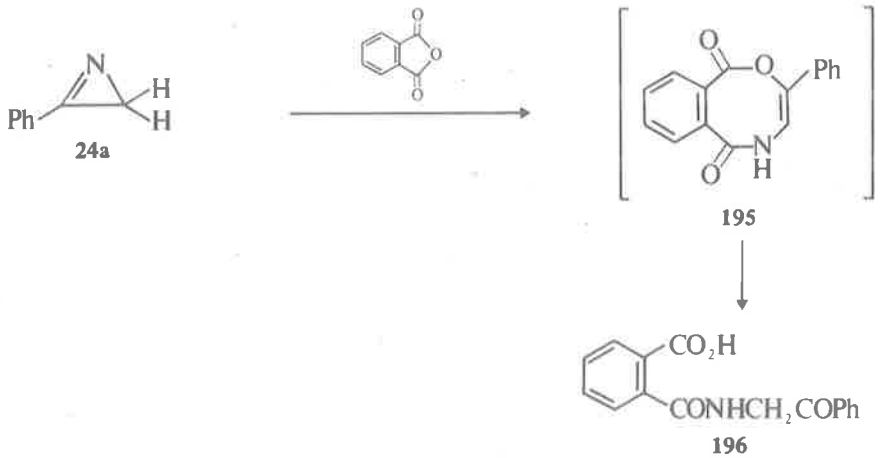
Scheme 15 Reaction of 1-azirines with carboxylic acid chlorides.

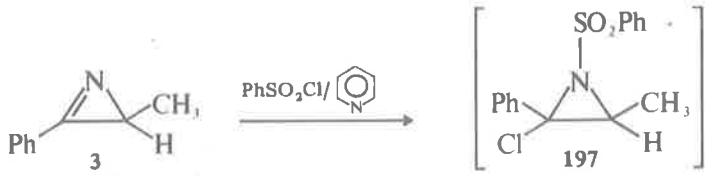
the chloroaziridine **197** is the precursor of **198**, since the rearrangement of *N*-sulfonylaziridines to vinyl sulfonamides is known.[134]

2-Dimethylamino-3,3-dimethyl-1-azirine **(172)** reacts with acid chlorides to give *N*-acylamidines (e.g., **200**) through 1,3-bond cleavage of the initially formed intermediate.[135] Carboxylic acid anhydrides, however, convert this azirine to diacylamino derivatives (e.g., **201**) in a reaction that involves 1,2-bond breaking.[135]

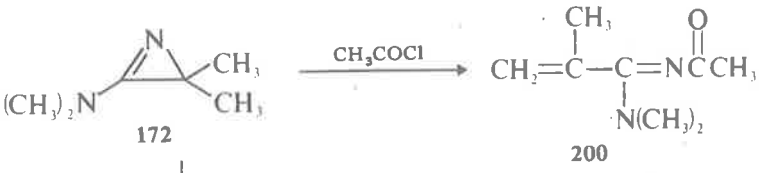
Deyrup and Szabo[136] have reported that alkylation of 1-azirines is possible with methyl triflate. Treatment of 2,3-diphenyl-1-azirine **(44h)** with methyl triflate

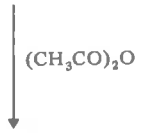


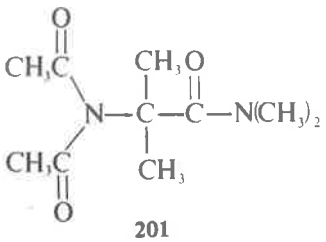

Ph
H
H
N
24a
O
O
O
O
O
Ph
N
H
195
CO2H
CONHCH2COPh
196


Ph
CH3
H
N
3
PhSO2Cl/
SO2Ph
Ph
CH3
Cl
H
197

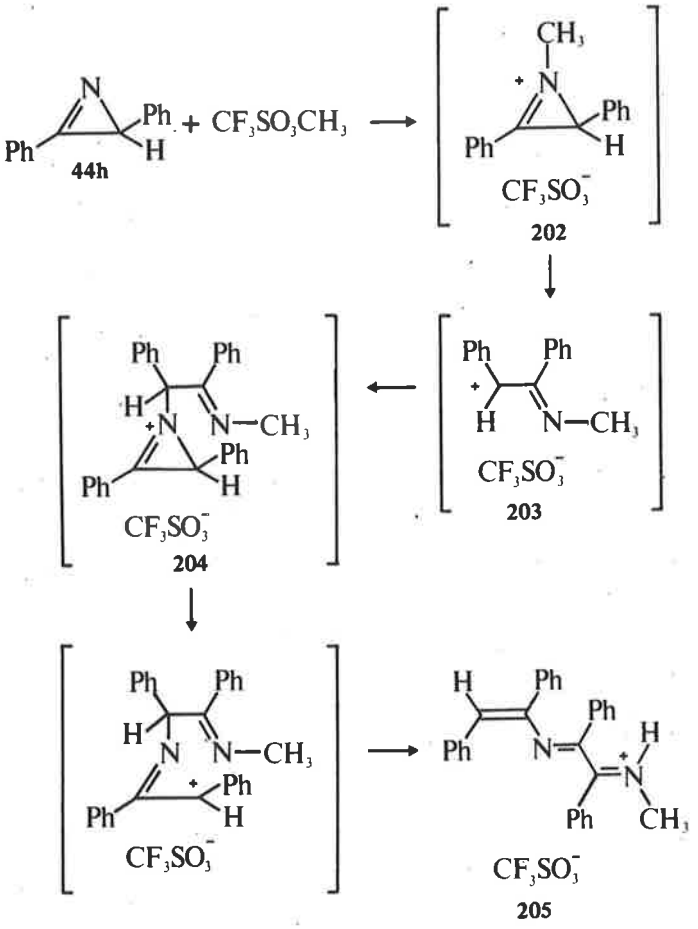

PhC=C(CH3)NHSO2Ph
Cl
198
+
PhC=C(CH3)N(SO2Ph)2
Cl
199


(CH3)2N
CH3
CH3
N
172
CH3COCl
CH2=C—C=NCCH3
CH3
O
N(CH3)2
200


(CH3CO)2O


CH3C
O
CH3
O
N—C—C—N(CH3)2
CH3C
CH3
O
201

in dichloromethane gave **205**. The mechanism of formation of **205** is shown in Scheme 16. The initial step involves alkylation of the azirine to generate intermediate **202**. Ring cleavage produces cation **203**, which alkylates a second molecule of azirine to give via **204** the observed product **205**.

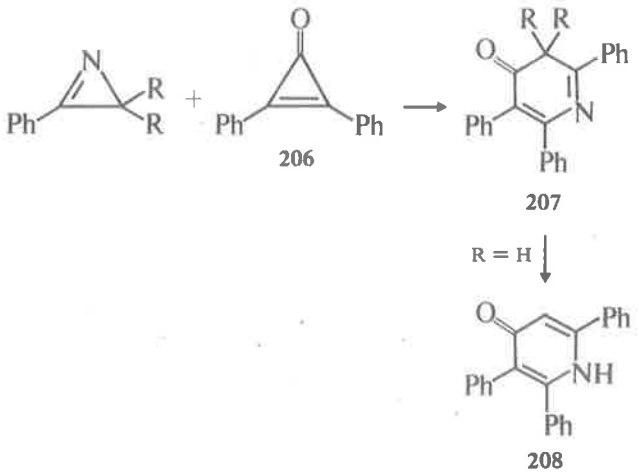


Scheme 16 Alkylation of 2,3-diphenyl-1-azirine with methyl triflate.

B. *Nucleophilic Reactions Involving Cyclopropenones and Cyclopropenyl Cations*

Hassner and Kascheres[137] found that diphenylcyclopropenone (**206**) reacts with 1-azirines to produce 4-pyridones (**208**). When R = CH_3, a prototropic shift is not possible and intermediate **207** can be isolated (Scheme 17).

Moerck and Battiste[138] reported that cyclopropenyl cations (**209**) convert 1-azirines (e.g., **24a**) to pyridines (**210**).



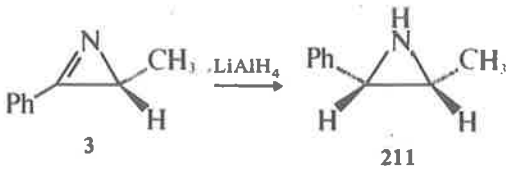
Scheme 17 Reaction of 1-azirines with diphenylcyclopropenone.

3. Reactions with Azirines as Electrophiles

1-Azirines also undergo reactions while participating as electrophiles. The electrophilicity of 1-azirines is associated with the polarized nature of the C=N bond.

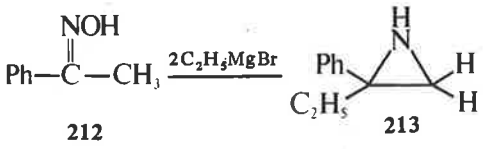
A. *Reactions Involving Organometallic Reagents*

Lithium aluminum hydride reduces 1-azirines in a highly stereospecific manner to give aziridines.[8,27] For example, 3-methyl-2-phenyl-1-azirine (**3**) is stereospecifically and quantitatively reduced to the *cis*-aziridine **211**. Approach of hydride occurs exclusively from the less hindered side of the azirine molecule. This reduction provides a useful preparation of *cis*-aziridines.



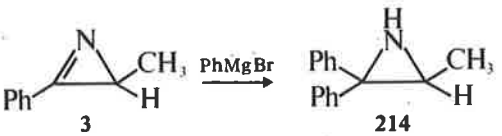
Sodium borohydride also has been reported to convert azirines to aziridines.[27]

Eguchi and Ishii[139] observed that the 1-azirine **24a** generated *in situ* from the oxime **212** reacts with a Grignard reagent to give aziridine **213**.



212 **213**

Hassner and Fowler[2] found that 3-methyl-2-phenyl-1-azirine (**3**) reacted readily with phenylmagnesium bromide to give 2,2-diphenyl-3-methylaziridine (**214**). The observed reactivity of 1-azirines toward Grignard reagents is an anomalous reaction of an imine. Generally, Grignard reagents react by α-hydrogen abstraction to give the enamine anion, which on work-up generates the starting imine. The failure of 1-azirines to follow this behavior can be explained in terms of the instability of the enamines that would be generated from α-hydrogen abstraction of 1-azirines. The reaction of 1-azirines with Grignard reagents exhibits similar stereospecificity as observed for hydride reductions.[146]



3 **214**

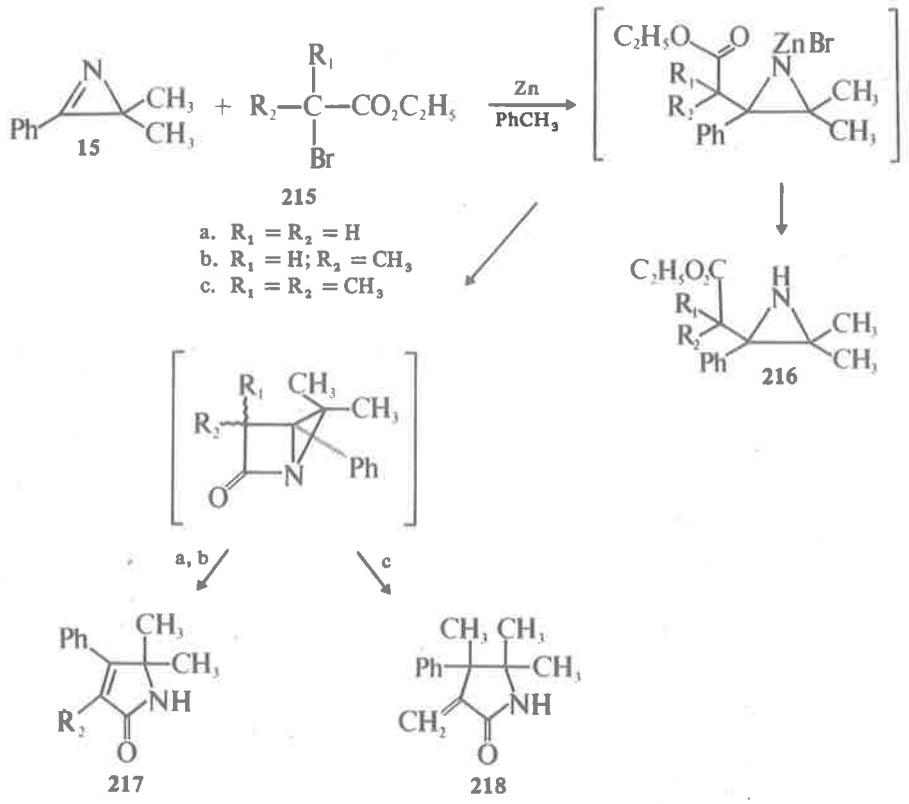
1-Azirines undergo the Reformatsky reaction.[140] For example, 3,3-dimethyl-2-phenyl-1-azirine (**15**) reacts with the α-bromoesters **215** to give **216** and **217** or **218** (Scheme 18).

B. *Reactions with Carbanions*

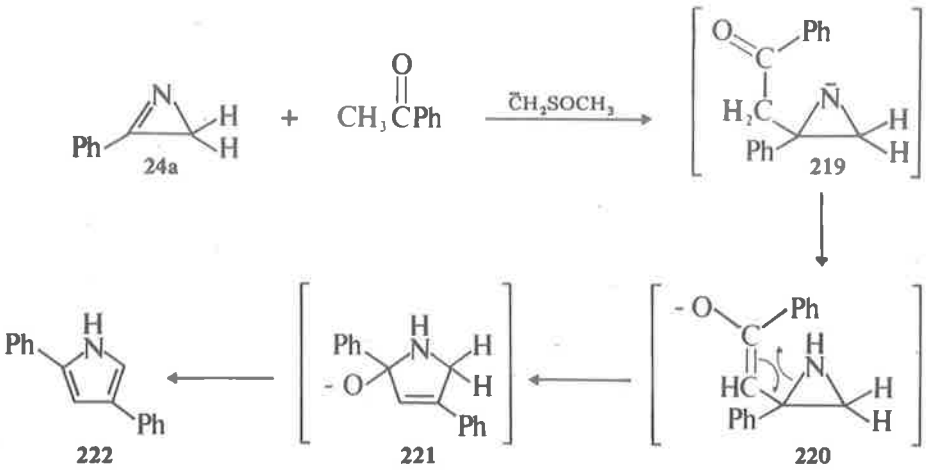
Sato, Kato, and Ohta[141,142] observed that 2-phenyl-1-azirine reacted with acetophenone in the presence of dimethylsulfinyl carbanion to give 2,4-diphenylpyrrole (**222**). A reasonable mechanism for this transformation involves initial nucleophilic attack by the enolate anion of acetophenone on the C=N bond to give **220** through **219**. Intermediate **220** undergoes 1,2-bond cleavage, cyclization, and hydroxyl group elimination to give **222** (Scheme 19).[53,141,142,158]

Benzyl cyanide reacts with azirine (**24a**) in the presence of dimethylsulfinyl carbanion to give 3,4-diphenyl-2-oxo-5-iminopyrroline (**224**), probably via intermediate **223**.

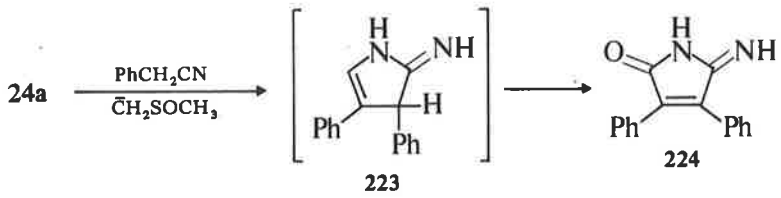
When no hydrogen is present at the 3-position of the azirine (i.e., with 3-disubstituted azirines), the reaction with carbanions produces different products.[143] For example, 3,3-dimethyl-2-phenyl-1-azirine (**15**) reacts with α-phenylethylacetate in dimethylsulfoxide and base to give **225**, **226**, and **227**.

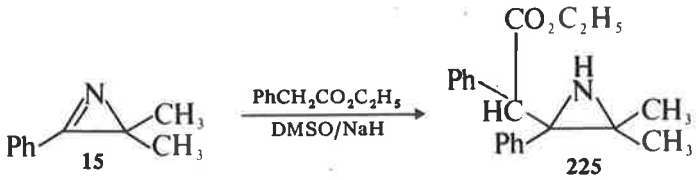


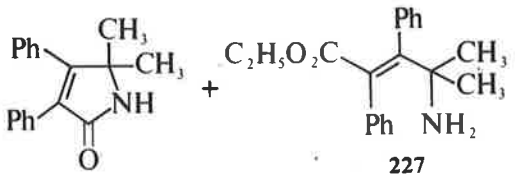
Scheme 18 Reformatsky reaction of 1-azirines. (Adapted from reference 140 with permission from Pergamon Press, Ltd.)



Scheme 19 Conversion of 1-azirines to pyrroles by reaction with carbanions. (Adapted from reference 53 with permission from Academic Press, Inc.)

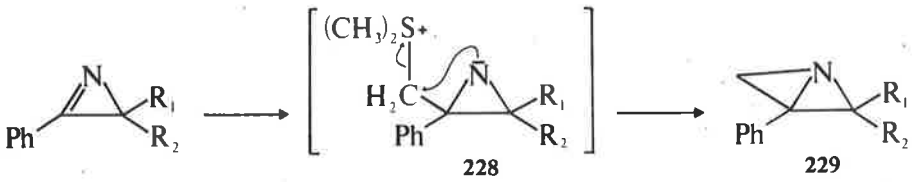






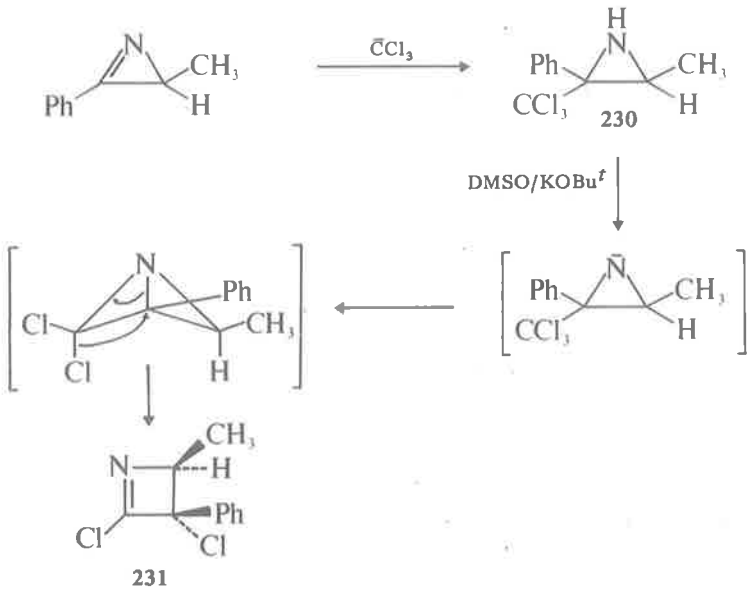
The reaction of activated methylene groups in β-dicarbonyl compounds with azirines can be conducted at room temperature and under nickel(II) catalysis to give high yields of pyrroles.[144]

Carbanions in the form of ylides also add to azirines. Hortmann and Robertson[145] reported the conversion of azirines (**3**, **15**, **24a**) with dimethylsulfonium methylide to 1-azabicyclobutanes (**229**) in good yields. The addition of the methylene group probably occurs by initial nucleophilic attack by the ylide to give the intermediate **228**, which cyclizes with expulsion of dimethyl sulfide.



3 $R_1 = H; R_2 = CH_3$
15 $R_1 = CH_3; R_2 = CH_3$
24a $R_1 = H; R_2 = H$

Addition of trichloromethide ion to azirine **3** was used by Hassner et al.[147] to generate, after work-up, the aziridine **230**. When this aziridine was treated with base, cyclization and rearrangement occurred and the azetine **231** was isolated (Scheme 20).[147]

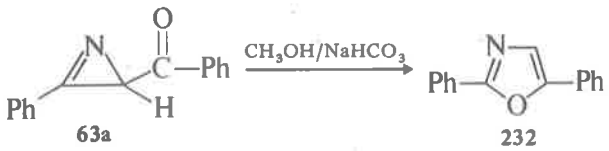


Scheme 20 Synthesis of 1-azetine from 1-azirine.

C. *Reactions with Alcohols*

1-Azirines react with alcohols in the presence of alkoxides to give alkoxyaziridines.[28,122] Further treatment with alcohol and alkoxide results in the formation of aminoketone acetals. Alkoxyaziridines are not isolated in general from the acid-catalyzed addition of methanol to azirines.[122]

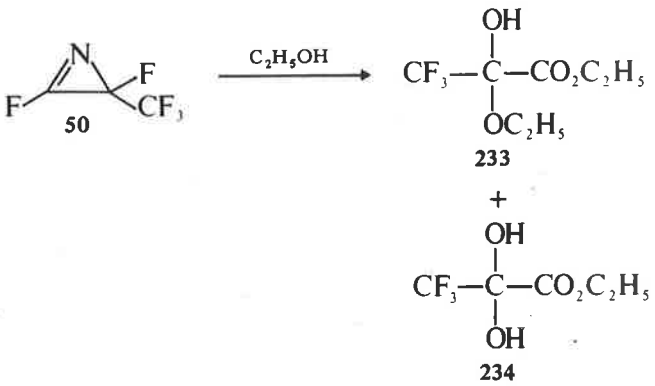
Oxazole **232** can be isolated in about 30% yield from the reaction of the azirine **63a** and weakly alkaline methanol.[13]



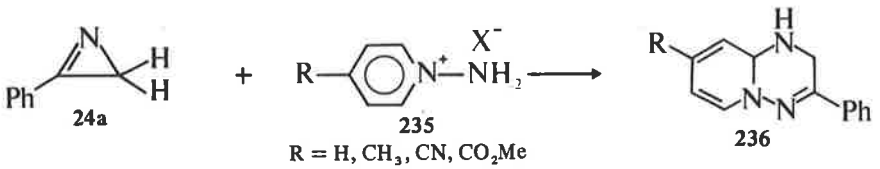
The perfluoroazirine **50** is converted by ethanolysis to ethyl-2-ethoxy-3,3,3-trifluoro-2-hydroxypropionate (**233**) and 3,3,3-trifluoro-2,2-dihydroxypropionate (**234**).[149]

D. *Reactions with Amines and Derivatives*

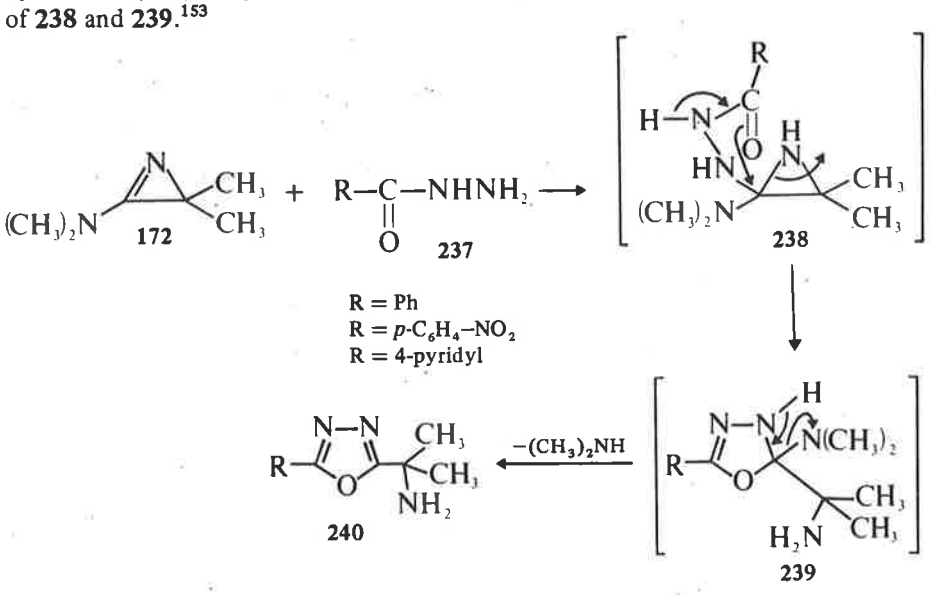
The reaction of aniline with 2-phenyl-1-azirine (**24a**) was examined by Smolinsky and Feuer.[148] They isolated, after mild acid hydrolysis, benzanilide and smaller amounts of 2,5-diphenylpyrazine and 3,4-dianilino-1,2,5-triphenylpyrrole.



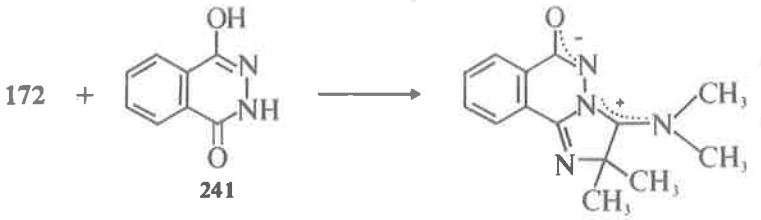
An interesting reaction of 1-azirines is with pyridine *N*-imines. It has been reported that the pyridine *N*-imine salts **235** react with 2-phenyl-1-azirine (**24a**) in the presence of base to give 1,9a-dihydro-2*H*-pyrido[1,2-b]as-triazines (**236**).[150–152] The mechanism of this transformation probably involves addition of the ylide from **235** to the C=N bond of the azirine followed by cyclization and rearrangement.



2-Dimethylamino-3,3-dimethyl-1-azirine (**172**) reacts with aromatic carbohydrazides (**237**) to give the oxadiazoles **240**, probably through the intermediacy of **238** and **239**.[153]

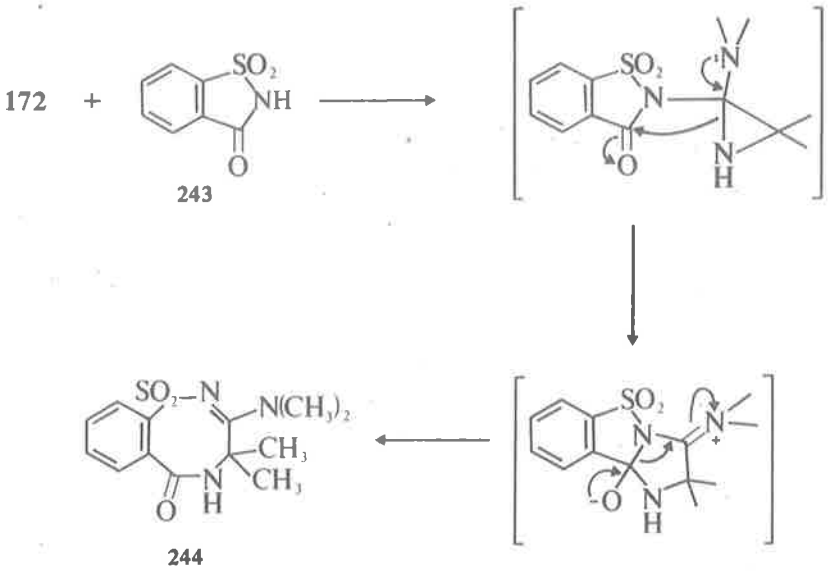


The amino azirine **172** also reacts with six-membered cyclic hydrazides (e.g., **241**) to give zwitterionic compounds (e.g., **242**).[154]



241 **242**

Another interesting reaction of the amino azirine **172** is with saccharin (**243**), where ring expansion to an eight-membered ring heterocycle **244** is observed.[155] The mechanism suggested for this transformation is shown in Scheme 21. Phthalimide undergoes a similar reaction with this amino azirine.[155]



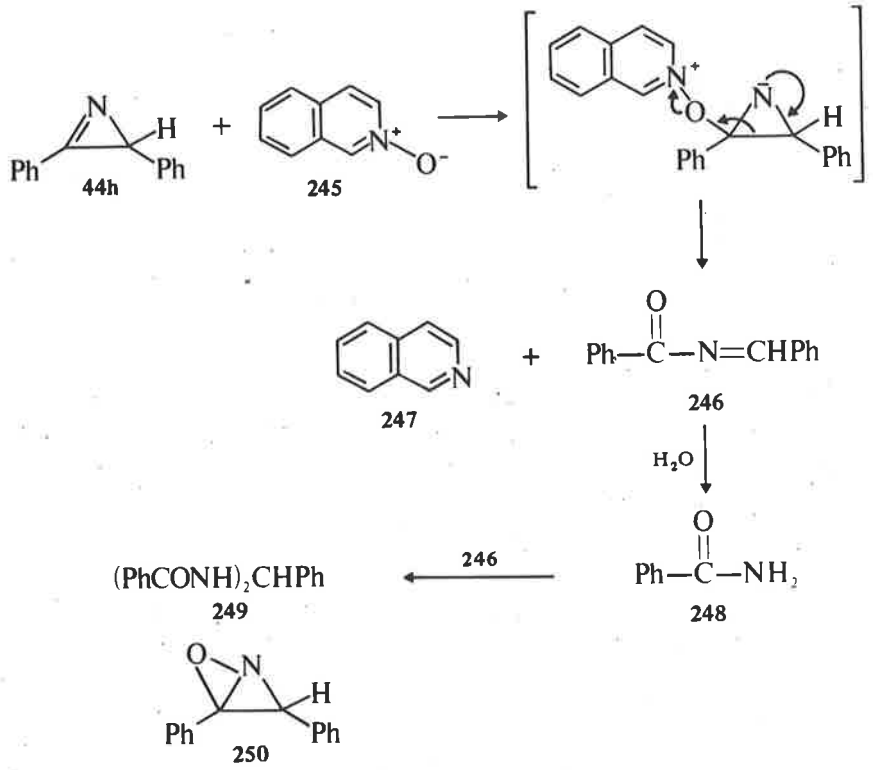
243 **244**

Scheme 21 Reaction of 2-amino-1-azirine with saccharin.

E. *Reactions with Nitrones*

Nitrones attack 1-azirines nucleophilically.[156] Thus, when 2,3-diphenyl-1-azirine (**44h**) was heated with isoquinoline *N*-oxide (**245**) in benzene at reflux temperatures, isoquinoline (**247**) and bis(benzamido)phenylmethane (**249**) were isolated in high yields. The reaction involves initial nucleophilic attack of the nitrone oxygen on the C=N bond of the azirine ring. This step bears some resemblance to the formation of alkoxyaziridines from the reaction of 1-azirines with alkoxide ion and

to the initial step of carbanion reaction with 1-azirines, both of which were mentioned previously. Bond reorganization results in formation of isoquinoline and a reactive imine intermediate (**246**). Partial hydrolysis of the imine **246** produces benzamide, which reacts further with **246** to produce **249** (Scheme 22). The bicyclic intermediate **250** may also be the precursor of **246** (cf. **228** → **229**).[4c] A similar reaction takes place when the 3-methyl analog of **44h** is treated with *m*-chloroperbenzoic acid to produce the methyl analog of **246**, presumably via an intermediate similar to **250**.[159]



Scheme 22 Reaction of 1-azirines with nitrone.

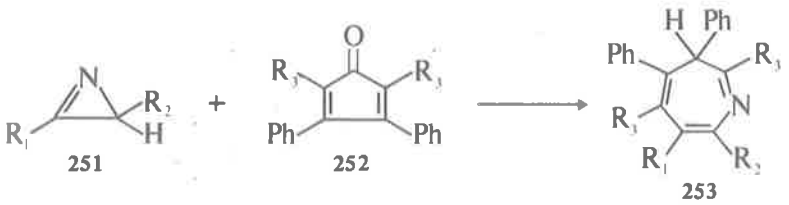
4. Thermal Cycloadditions

The 2π electrons of the carbon-nitrogen double bond of 1-azirines can participate in thermal symmetry-allowed [4 + 2] cycloadditions with cyclopentadienones, isobenzofurans, triazines, tetrazines, α-ketosulfenes, diazomethane, azomethine ylides, nitrile ylides, and nitrile oxides. Cycloadditions also occur with hetero-cumulenes such as ketenes, ketenimines, complex isocyanates, and carbon disulfide. 1-Azirines are reactive toward benzyne, and some 1-azirines form adducts with mesoionic compounds. It is possible also for the 2π electrons of 1-azirines to participate in "ene" reactions.

A. *Diels-Alder Reactions*

a. CYCLOPENTADIENONES

One of the first examples of cycloaddition of the 1-azirine ring system was reported independently by Nair[160] and by Hassner and Anderson.[161,162] They discovered that 3*H*-azepines (**253**) are formed directly when 1-azirines (**251**) and cyclopentadienones (**252**) are heated under reflux in benzene or toluene.



251 + **252** → **253**

a. $R_1 = Ph; R_2 = H$ (**24a**)
b. $R_1 = Ph; R_2 = CH_3$ (**3**)
c. $R_1 = Ph; R_2 = Ph$ (**44h**)
d. $R_1 = Ph; R_2 = CH_2OH$
e. $R_1 = PhCH_2; R_2 = H$ (**44e**)

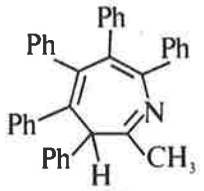
a. $R_3 = Ph$
b. $R_3 = CH_3$
c. $R_3 = C_2H_5$

a. $R_1 = Ph; R_2 = H; R_3 = Ph$
b. $R_1 = Ph; R_2 = CH_3; R_3 = Ph$
c. $R_1 = Ph; R_2 = Ph; R_3 = Ph$
d. $R_1 = Ph; R_2 = CH_2OH; R_3 = Ph$
e. $R_1 = PhCH_2; R_2 = H; R_3 = Ph$
f. $R_1 = Ph; R_2 = H; R_3 = CH_3$
g. $R_1 = Ph; R_2 = CH_3; R_3 = CH_3$
h. $R_1 = Ph; R_2 = Ph; R_3 = CH_3$
i. $R_1 = PhCH_2; R_2 = H; R_3 = CH_3$
j. $R_1 = Ph; R_2 = H; R_3 = C_2H_5$
k. $R_1 = Ph; R_2 = CH_3; R_3 = C_2H_5$

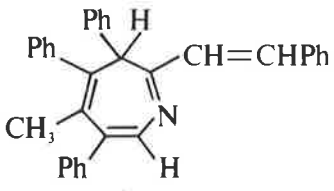
Hassner and Anderson showed that the cycloaddition occurs even with relatively unstable 1-azirines. These are generated *in situ* from the appropriate vinyl azide and reacted directly with the cyclopentadienones.[163]

Assignment of the 3*H*-azepine structure in the work of both Nair and Hassner and Anderson was facilitated by specific utilization of the cyclopentadienone, 2,5-dimethyl-3,4-diphenylcyclopentadienone (**252b**), and the azirine, 2-phenyl-1-azirine (**251a** = **24a**). The resulting azepine (**253f**) undergoes rapid deuterium exchange (D_2O) at the 2-methyl group, whereas deuterium exchange of methyl protons at other positions either were very slow or did not occur. Interestingly, a minor product identified as **254** and isolated in the reaction of azirine **251b** and cyclopentadienone **252a** showed as expected deuterium exchange at the methyl group. The azepine **253f** also underwent a smooth condensation with benzaldehyde in the presence of pyrrolidine to the styryl derivative **255**.[160] Further substantiation of structure came from nmr studies. In the azepine **253f**, the methyl resonance at δ 2.28 showed homoallylic coupling ($J = 0.8$ Hz) and in azepines **253e** and **253i** only singlets were observed for the benzylic protons.[164]

The mechanism of formation of the 3*H*-azepines merits discussion.[160,162,164] It is reasonable to assume that the first step of the cycloaddition is a symmetry-allowed $[\pi^4 s + \pi^2 s]$ process to furnish an *endo*-adduct (**256**).[165] At least two pathways are possible from this adduct to the observed 3*H*-azepine product (Scheme 23). Mechanism **a** involves cheletropic fragmentation of the adduct **256** to furnish

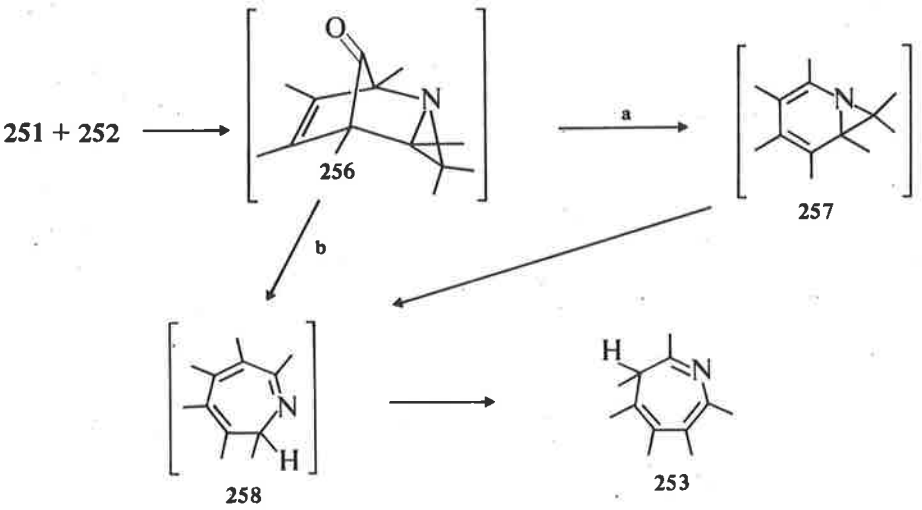


254



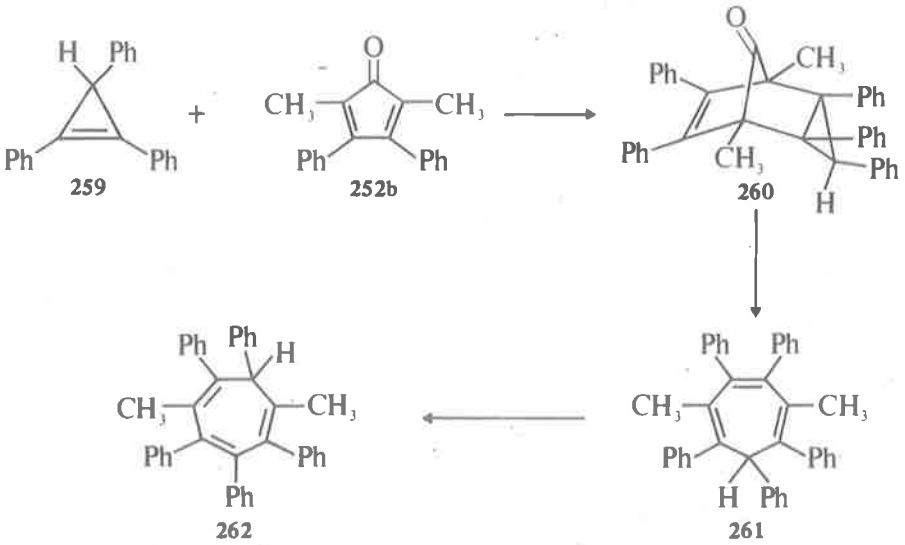
255

an azanorcaradiene (**257**). The symmetry-allowed disrotatory electrocyclic ring opening of the azanorcaradiene to its valence tautomer, the azacycloheptatriene (or 2*H*-azepine) **258**, is followed by a 1,5-suprafacial sigmatropic shift of the 2-hydrogen to give the thermodynamically more stable 3*H*-azepine **253**.[160] In mechanism **b**, loss of carbon monoxide from **256** occurs with participation of the aziridine carbon-nitrogen bond to afford **258**, which undergoes a 1,5-sigmatropic shift to give the 3*H*-azepine **253**.[162, 172]



Scheme 23 Mechanism of formation of 3*H*-azepines from 1-azirines and cyclopentadienones.

The main difference between these two mechanisms is that the elimination of carbon monoxide is concomitant with the disrotatory electrocyclic ring opening in mechanism **b**. Rate acceleration in the decarbonylation of *endo*-tricyclooctenones is known.[166, 167] Additional evidence for the concomitant participation of the three-membered ring in the decarbonylation of **256** came from two sets of experiments.[162] First, the cyclopropene adduct **260** from triphenylcyclopropene (**259** and **252b**) is converted on heating initially to the cycloheptatriene **261** and subsequently to the cycloheptatriene **262** (Scheme 24). Second, the cycloaddition of **259** and the 1,3-diphenylinden-2-one **263** gave the stable *exo*-adduct **264** and the cycloheptatriene **265** in a 1:4 ratio. It is likely that **265** was derived from the unstable *endo*-cycloadduct (Scheme 25).

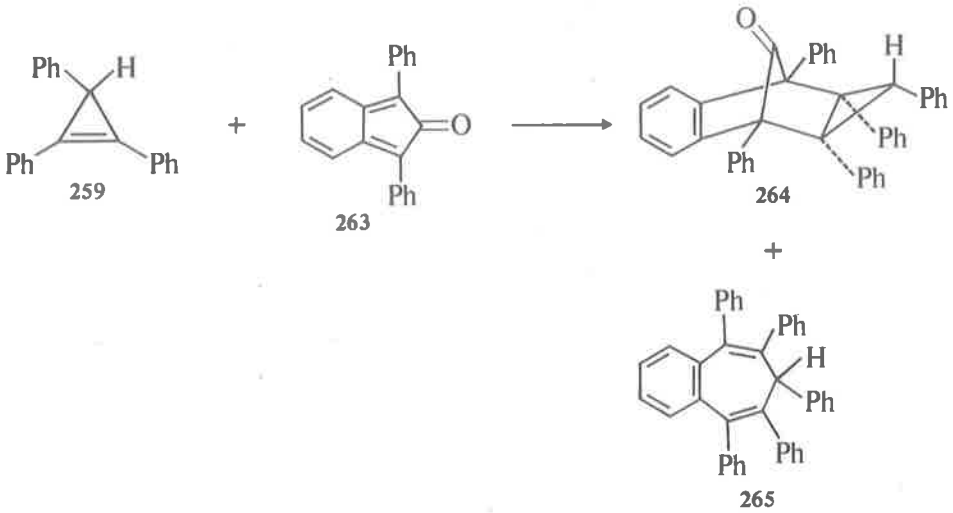


Scheme 24 Reaction of triphenylcyclopropene and 2,5-dimethyl-3,4-diphenylcyclopentadienone.

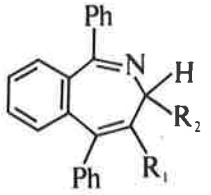
The 3*H*-azepine **254** isolated as a minor product in the reaction of azirine **251b** and cyclopentadienone **252a** must have arisen from the azepine **253b** by a further symmetry-allowed 1,5-sigmatropic shift. Hassner and Anderson[164] have provided evidence for this type of phenomenon.

The regiochemistry of these cycloadditions has been discussed.[164]

Hassner and Anderson[162] also reported apparently the first example of a stable 2*H*-azepine system **266** from the reaction of **263** and azirines **251a–251c**.



Scheme 25 Reaction of triphenylcyclopropene and 1,3-diphenylinden-2-one.

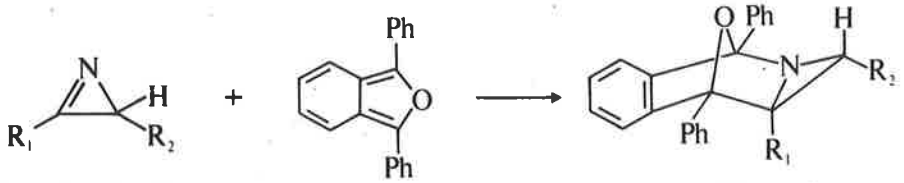


266

a. $R_1 = Ph; R_2 = H$
b. $R_1 = Ph; R_2 = CH_3$
c. $R_1 = Ph; R_2 = Ph$

b. ISOBENZOFURANS

1,3-Diphenylisobenzofuran (**268**) reacted readily with 3-methyl-2-phenyl-1-azirine (**3**) and with other azirines in refluxing toluene to give a cycloadduct (**269**), the primary product of a $[\pi^4 s + \pi^2 s]$ cycloaddition.[168,169] The adduct **269** was assigned the *exo* stereochemistry on the basis of its nmr data.



3 $R_1 = Ph; R_2 = CH_3$
267 $R_1 = H; R_2 = t\text{-Bu}$

268

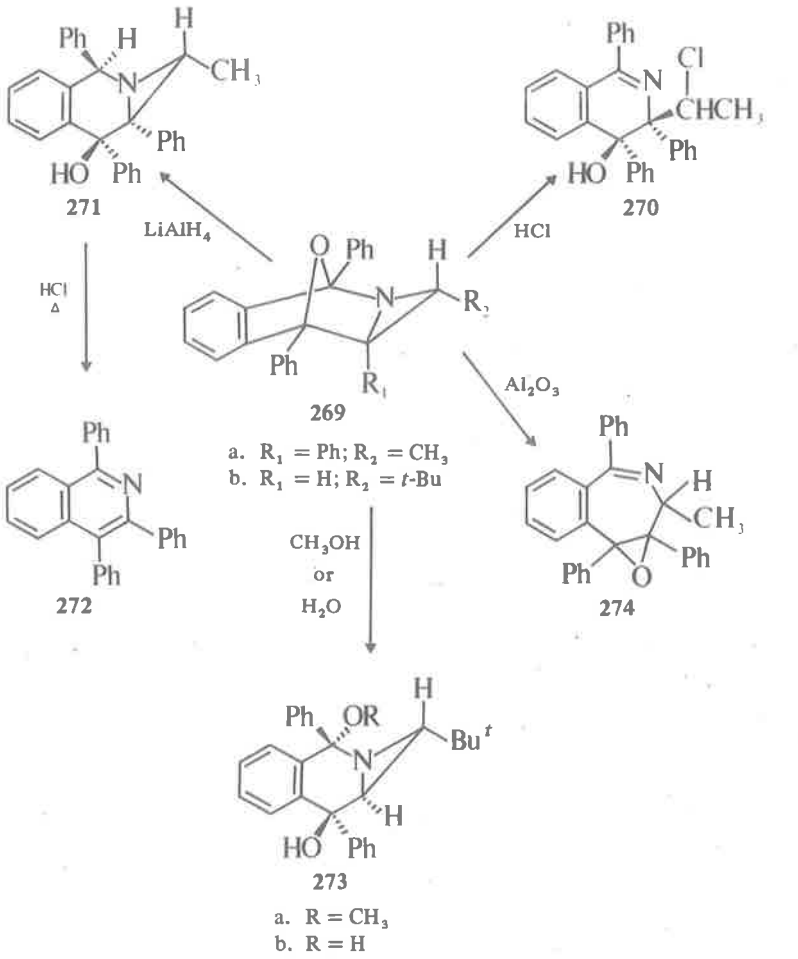
269

a. $R_1 = Ph; R_2 = CH_3$
b. $R_1 = H; R_2 = t\text{-Bu}$

The cycloadduct **269** undergoes a number of interesting reactions involving both the oxido bridge and the aziridine C–N bonds. Thus when **269a** was treated with anhydrous HCl in benzene, the hydrochloride salt of **270** was isolated. The reaction involves protonation of the aziridine nitrogen (easily monitored by ^{1}H nmr methods), followed by selective cleavage of one of the aziridine C–N bonds.[168] Reductive cleavage of adduct **269** with lithium aluminum hydride gave the benzoazanorcarane **271**. Attack of hydride is regiospecific and stereospecific.[169] Treatment of **271** with anhydrous HCl in refluxing benzene led to isolation of the triphenylisoquinoline **272**.[168] Other nucleophiles such as water and alcohols also cleave the oxido bridge at the benzylic position alpha to the aziridine nitrogen.[169] Thus, when **269b** was heated in methanol, compound **273a** was isolated. In the presence of silica gel and moist ether, **269b** was converted to **273b**.

An interesting isomerization reaction of the cycloadduct **269a** was reported by Hassner and Anderson.[169] When **269a** in benzene was stirred with Woelm neutral alumina, it was converted in good yields to the azepine **274**.

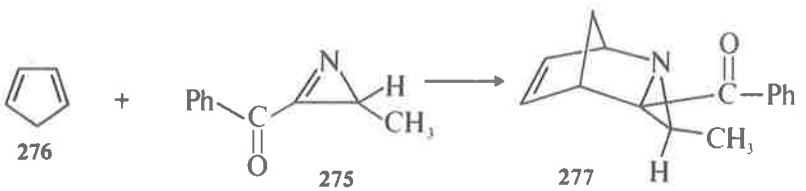
Although 1,3-diphenylisobenzofuran (**268**) reacts readily with 1-azirines monosubstituted at the 3-position, it is unreactive towards such 3-disubstituted azirines



as 3,3-dimethyl-2-phenyl-1-azirine (**15**).[169] Cyclopentadienones (**252**) are also unreactive toward this azirine.

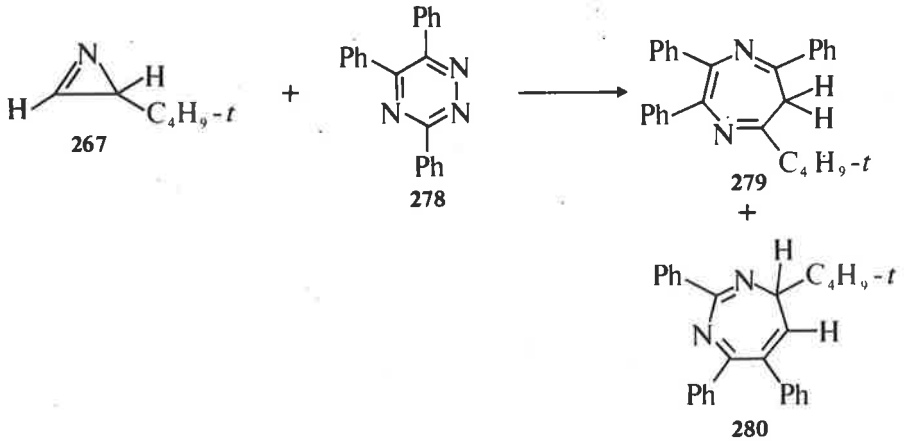
c. CYCLOPENTADIENE

3-Methyl-2-phenyl-1-azirine (**3**) and 2-phenyl-1-azirine (**24a**) are unreactive toward cyclopentadiene under a variety of conditions.[170] However, an electronically different azirine, 2-benzoyl-3-methyl-1-azirine (**275**), has been reported[171] to react with cyclopentadiene (**276**) to give the expected [4 + 2] cycloadduct **277**.



d. TRIAZINES

One example of the reaction of a triazine (**278**) with an azirine (**267**) has been reported.[172] The products, obtained only in low yields, are the diazepines **279** and **280**.

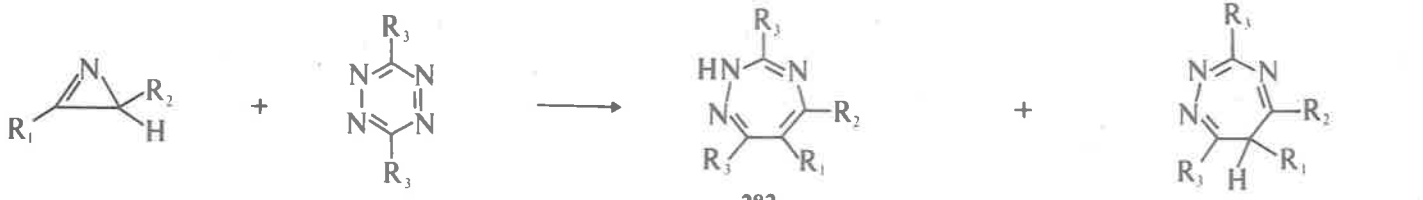


e. TETRAZINES

A more reactive system in which nitrogen is lost cheletropically after formation of the initial [4 + 2] cycloadduct is the tetrazine. Five research groups have reported on this cycloaddition.[173–177] A variety of heterocyclic products are produced depending on the structures of the azirine and tetrazine used and the reaction conditions. Azirines **24a**, **3**, and **267** react with the tetrazines **281a–281c** in toluene under reflux to give the triazepines **282** and **283**, the pyrimidines **284**, and the pyrazoles **285**. The tetrazine **281c** was the most reactive and gave the triazepine **282b** in 95% yield. Similarly, cycloaddition of **267** with **281c** gave the triazepine **282c** in 82% yield, whereas the reaction of **267** with **281a** afforded the pyrimidine **284c** in 92% yield.[174]

The triazepines **282** are the primary products of these reactions with tetrazines. Their formation occurs very likely from initial [4 + 2] cycloaddition followed by nitrogen elimination and electrocyclic ring opening, and then 1,5-sigmatropic shift of the intermediate 5*H*-1,2,4-triazepines. This pathway is analogous to that discussed for the cycloaddition with cyclopentadienones. A further symmetry-allowed 1,5-sigmatropic shift converts **282** to **283**. Both **282** and **283** may then undergo thermal fragmentations to give pyrimidines **284** (loss of :NH) or pyrazoles **285** (loss of R_2–C≡N).[172]

Anderson and Hassner had reported originally[174] the isolation of an unidentified product from these cycloadditions. This product appeared to be the major product from the azirine **3** and the tetrazines **281a** and **281b**. Elemental analysis showed 2 molecules of azirine and 1 molecule of tetrazine minus N_2 and CH_3CN. These products appear to result from the addition of the pyrazoles formed in the cyclo-



24a $R_1 = Ph; R_2 = H$
3 $R_1 = Ph; R_2 = CH_3$
267 $R_1 = H; R_2 = t\text{-}Bu$

281

a. $R_3 = Ph$
b. $R_3 = 4\text{-}CH_3\text{–}C_6H_4$
c. $R_3 =$ 

282

a. $R_1 = Ph; R_2 = H; R_3 = Ph$
b. $R_1 = Ph; R_2 = CH_3; R_3 =$ 
c. $R_1 = H; R_2 = t\text{-}C_4H_9; R_3 =$ 

283

a. $R_1 = Ph; R_2 = CH_3; R_3 = Ph$
b. $R_1 = Ph; R_2 = CH_3; R_3 = 4\text{-}H_3C\text{–}C_6H_4$
c. $R_1 = H; R_2 = t\text{-}C_4H_9; R_3 =$ 

284

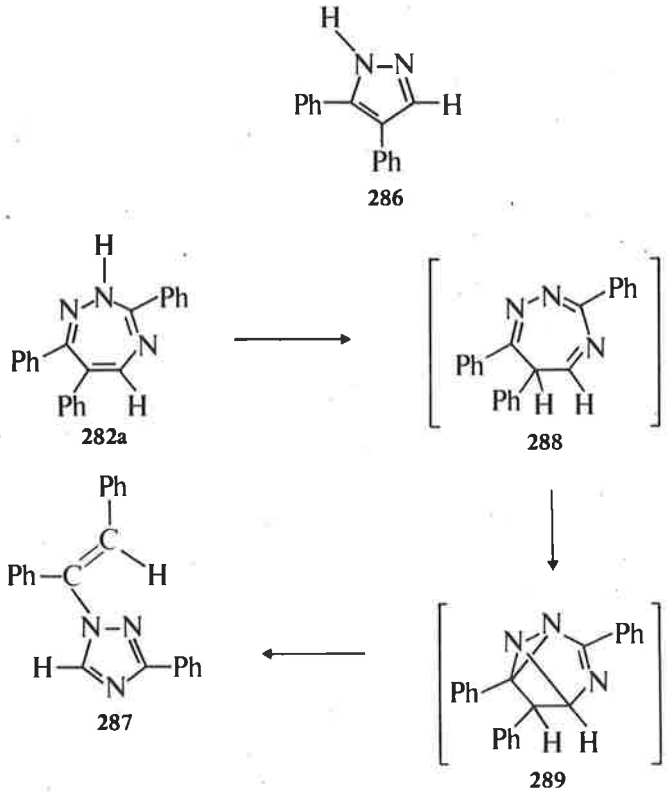
a. $R_1 = Ph; R_2 = H; R_3 = Ph$
b. $R_1 = Ph; R_2 = CH_3; R_3 = Ph$
c. $R_1 = H; R_2 = t\text{-}C_4H_9; R_3 = Ph$
d. $R_1 = Ph; R_2 = CH_3; R_3 = 4\text{-}H_3C\text{–}C_6H_4$

285

a. $R_1 = R_3 = Ph$
b. $R_1 = Ph; R_3 = 4\text{-}H_3C\text{–}C_6H_4$
c. $R_1 = Ph; R_3 =$ 

addition with the excess azirine present in the reaction mixture. This reaction is similar to the addition of amines to azirines, which has been mentioned already.

Nair reported on further details of the thermolysis of 2*H*-1,2,4-triazepines.[177] For example, when the triazepine **282a** was heated in refluxing mesitylene not only was **285a** formed (11%), but a second pyrazole **286** was also isolated in 29% yield. The pyrazoles are formed through elimination of HCN and PhCN from **282a** or an isomeric structure. In direct competition with nitrile extrusion in the thermolysis of **282a** is a remarkable skeletal rearrangement that gives a third product (**287**) in 28% yield. The formation of the triazolylstilbene **287** from **282a** requires an initial symmetry-allowed 1,5-sigmatropic shift of hydrogen to give **288**. Intermediate **288** can destroy itself by nitrile elimination to furnish **285a** and **286**, or it can undergo an intramolecular $[\pi^4a + \pi^2a]$ cycloaddition to give **289**, which subsequently rearranges in a reverse Diels-Alder fashion to **287**.

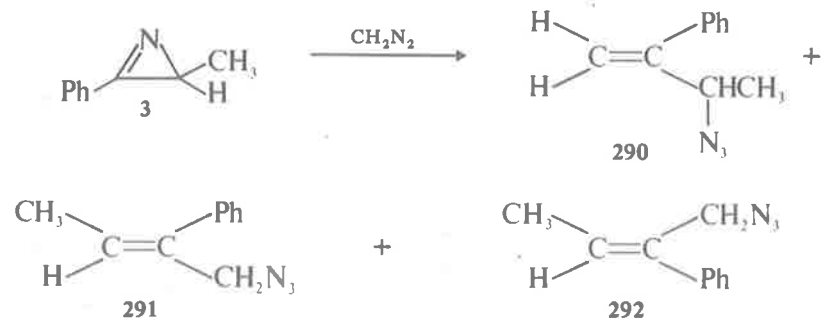


B. *1,3-Dipolar Cycloadditions*

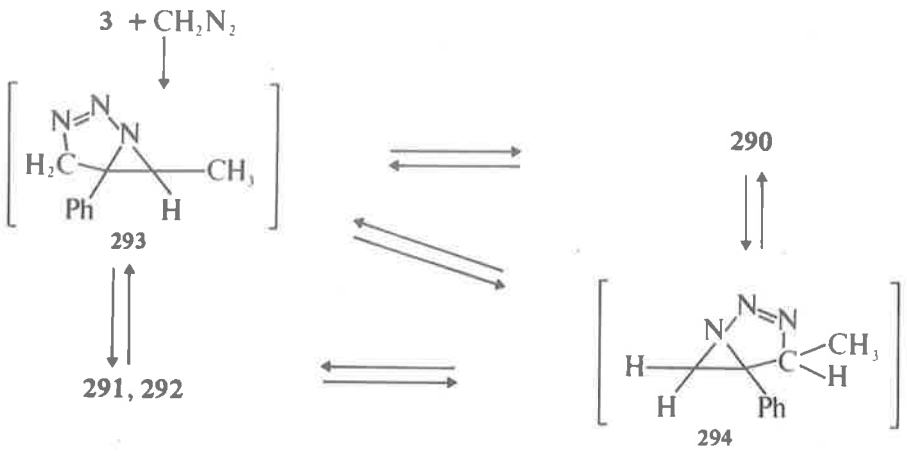
a. DIAZOMETHANE AND DERIVATIVES

The interaction of diazomethane with 1-azirines was the first example of 1,3-dipolar cycloaddition with this ring system. This reaction was reported by

Logothetis[178] and subsequently studied in more detail by Nair.[30,179] 3-Methyl-2-phenyl-1-azirine (**3**) reacts with diazomethane in ether at room temperature to give a 1.6:1:1 mixture of the allylic azides **290**, **291**, and **292**, respectively. Structural evidence for the allylic azides came from elemental analysis, ir spectra, and particularly the nmr data.[30]

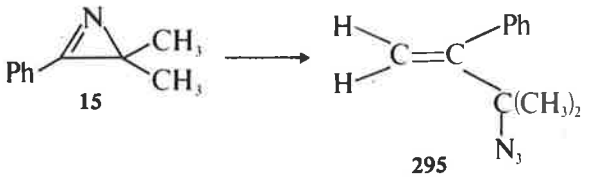


The mechanism of formation of the allylic azides (Scheme 26) is probably the result of at least a two-step process.[30,179] 1,3-Dipolar addition of diazomethane across the C=N bond of the azirine produces the triazoline adduct **293**. The adduct **293** can exist in equilibrium with its valence tautomer **294**, and the allylic azides can be produced from these triazolines by ring cleavage. Allylic azides are said to undergo isomerization very rapidly, and triazoline intermediates were proposed by Gagneux, Winstein, and Young[180] for the rapid equilibration of pentenyl and butenyl azides. Rapid equilibration of the allylic azides **290**, **291**, and **292** would explain both the appearance of the mixture as a single spot on thin layer chromatographic plates with several different solvent systems and our inability to separate these compounds.

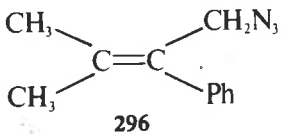


Scheme 26 Mechanism of formation of allylic azides from 1-azirines and diazomethane.

3,3-Dimethyl-2-phenyl-1-azirine (**15**) also reacts with diazomethane to give the allylic azides **295** and **296**, respectively, in a ratio of 1:3.[30] This is in contrast to the lack of reactivity of this azirine toward cyclopentadienones and isobenzofurans.

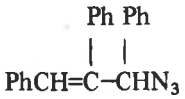


15 **295**



296

Bowie, Nussey, and Ward[181] reported that treatment of 2,3-diphenyl-1-azirine (**44h**) with phenyldiazomethane gave as the major product (70%) the vinyl azide **298**. The precursor to the vinyl azide **298** may very likely be the allyl azide **297**, formed as suggested in Scheme 26. The rearrangement of **297** to **298** represents a 1,3-sigmatropic proton shift. Although this is a symmetry-forbidden shift, the prolonged heating and/or the copper powder used in this reaction may have been responsible for conversion of the allyl azide **297** to the thermodynamically more stable vinyl azide **298** and analogous rearrangements have been reported.[181b]



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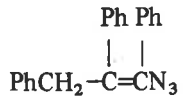
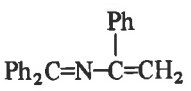


298

The behavior of diphenyldiazomethane toward 1-azirines follows a different pathway.[182] Diphenyldiazomethane acts as a source of diphenylcarbene in these reactions. Thus with 2-phenyl-1-azirine (**24a**), the primary product is 1,1,3-triphenyl-2-azabuta-1,3-diene (**299**). Electrophilic attack by diphenylcarbene on **299** followed by rearrangement produces three 1:2 adducts, **300**, **301**, and **302**.



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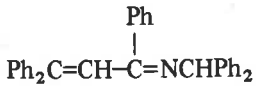
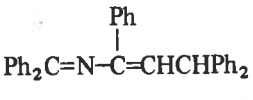
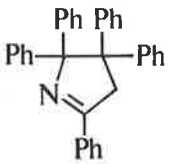


300



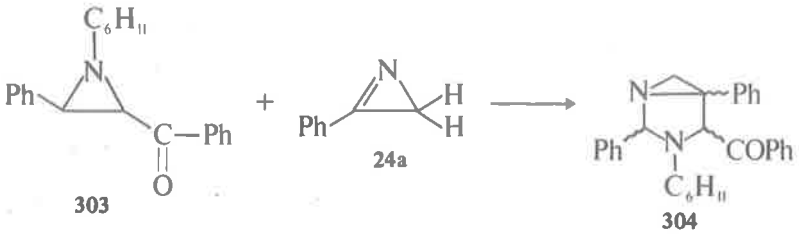
301

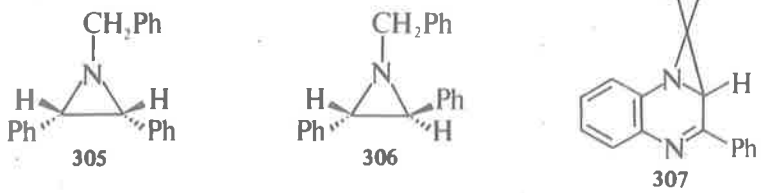


302

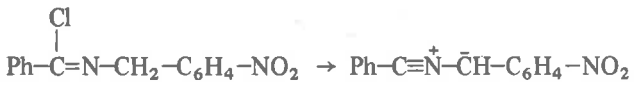
b. AZOMETHINE AND NITRILE YLIDES

Aziridines undergo thermal ring opening in a conrotatory manner to generate azomethine ylides.[183] These azomethine ylides are 4π components and can participate in [4 + 2] cycloadditions with 1-azirines as the 2π component. For example, the aziridine **303** reacts with 2-phenyl-1-azirine (**24a**) to give **304** as a stereochemical mixture. The *cis*-aziridine **305** also gives a mixture of two adducts. However, the *trans*-aziridine **306** and the fused aziridine **307** exhibit stereospecificity in their cycloadditions with **24a**.[184]



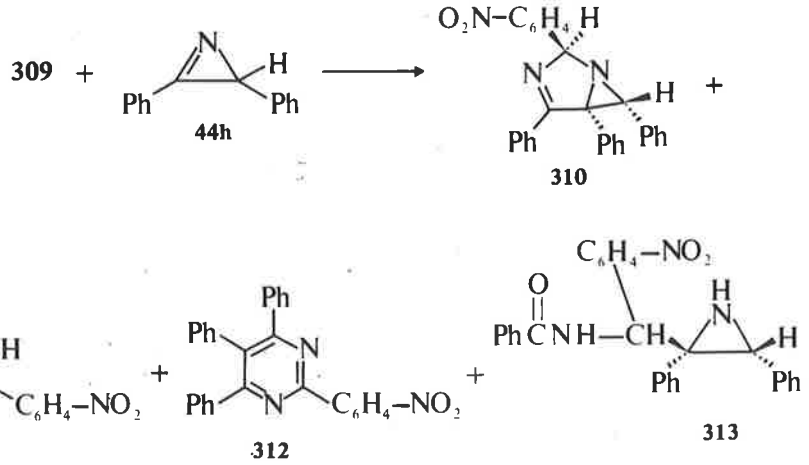


Benzonitrile ylide **309** is generated when **308** in benzene is treated with triethylamine. The nitrile ylide **309** reacts with 2,3-diphenyl-1-azirine (**44h**) to give 2-(*p*-nitrophenyl)-4,5,6-triphenyl-1,3-diazabicyclo[3.1.0]hex-3-ene (**310**). Under the basic conditions of the reaction mixture **310** is converted to the dihydropyrimidine **311**, which is subsequently oxidized to the pyrimidine **312**. Another product **313** was also isolated. Yields were low.[185]

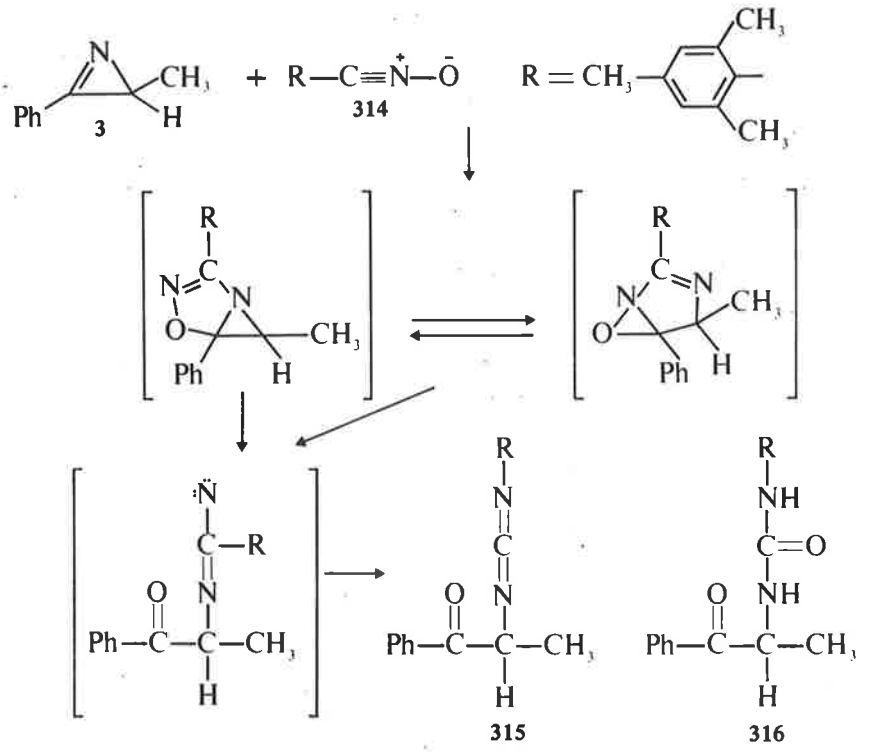


c. NITRILE OXIDES

The 4π-electron system of nitrile oxides can participate in 1,3-dipolar cycloaddition with 1-azirines. Nair[186] discovered that aromatic nitrile oxides react exothermically with 1-azirines to furnish carbodiimides in isolated yields exceeding 80%. Thus, when 3-methyl-2-phenyl-1-azirine (**3**) was treated with 2,4,6-trimethylbenzonitrile oxide (**314**) in anhydrous ether at 0° for 15 min, the carbodiimide **315** was isolated in almost quantitative yield. The carbodiimide was found to be highly hygroscopic, and hydrolysis to the urea **316** proceeded extremely rapidly and quantitatively.



A possible mechanism for the formation of the carbodiimide (Scheme 27) assumes the initial formation of a cycloadduct from a 1,3-dipolar addition between the nitrile oxide and the azirine. Ring cleavage of the bicyclic adduct or its valence tautomer is followed by a 1,2-migration of the R group of the nitrile oxide in a Beckmann-type rearrangement to give the carbodiimide.

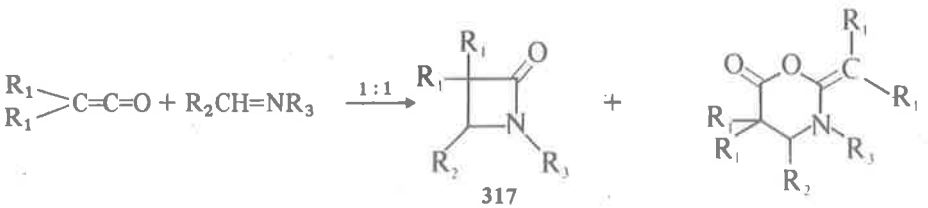


Scheme 27 Mechanism of formation of carbodiimides from 1-azirines and nitrile oxides.

C. *Cycloadditions with Heterocumulenes*

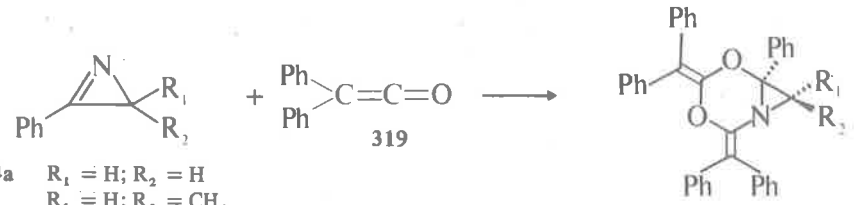
a. KETENES AND KETENIMINES

Ketenes generally react with a variety of imines to form as major products 1 : 1 adducts that are β-lactams (**317**), as well as 1 : 2 adducts possessing the dihydro-oxazinone structure **318**.[187]



317 **318**

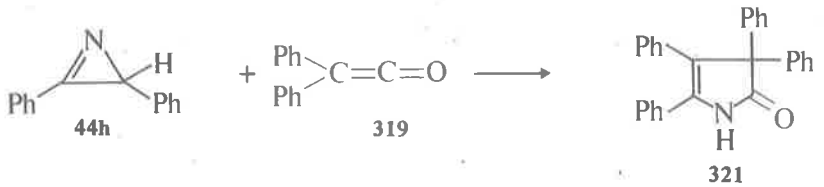
Hassner and his co-workers reported that the 1-azirines (**3**, **15**, **24a**) react with diphenylketene (**319**) to give the 1 : 2 adducts **320**.[188–190] Analogous cycloadditions were observed with *t*-butylcyanoketene. However, 2,3-diphenyl-1-azirine (**44h**) reacted with diphenylketene to give a 1 : 1 adduct (**321**).



319 **320**

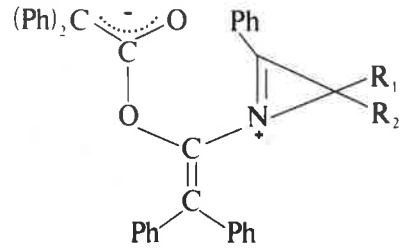
24a $R_1 = H; R_2 = H$
3 $R_1 = H; R_2 = CH_3$
15 $R_1 = CH_3; R_2 = CH_3$

a. $R_1 = H; R_2 = H$
b. $R_1 = H; R_2 = CH_3$ *cis* and *trans*
c. $R_1 = CH_3; R_2 = CH_3$

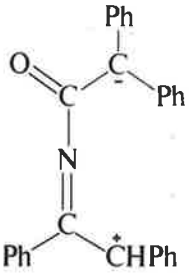


44h **319** **321**

The 1 : 2 adducts (bicyclic aziridines) **320** are different in structure from the 1 : 2 adducts **318** formed from simple imines and ketenes. It was suggested[188,190] that the formation of **320** proceeds via the intermediacy of a reactive azirinium ion **322**. The 1 : 1 adduct observed in the case of 2,3-diphenyl-1-azirine (**44h**) was interpreted as resulting from the intermediate **323**, where the presence of the 3-phenyl substituent in the azirine stabilizes the cationic site resulting from initial monoaddition to ketene.



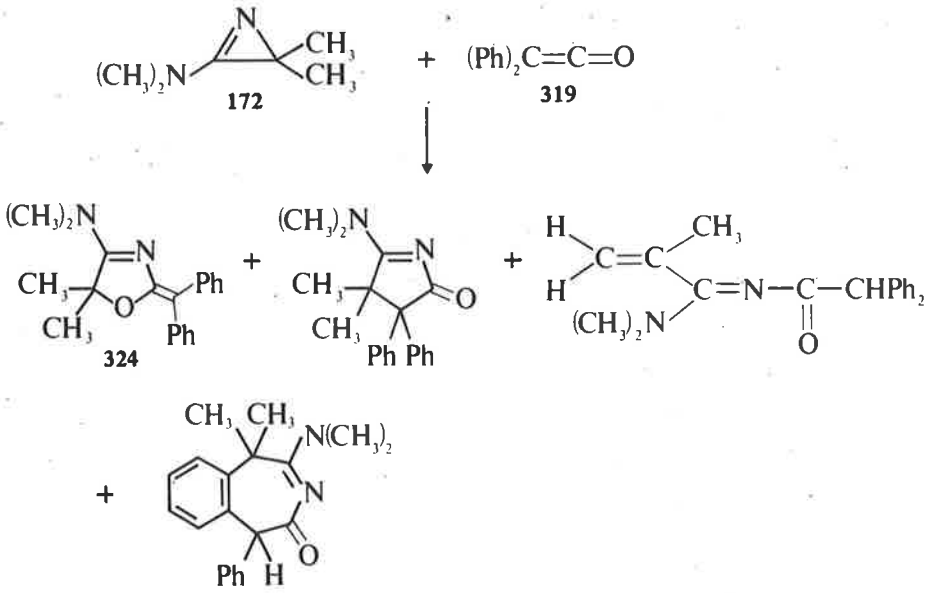
322



323

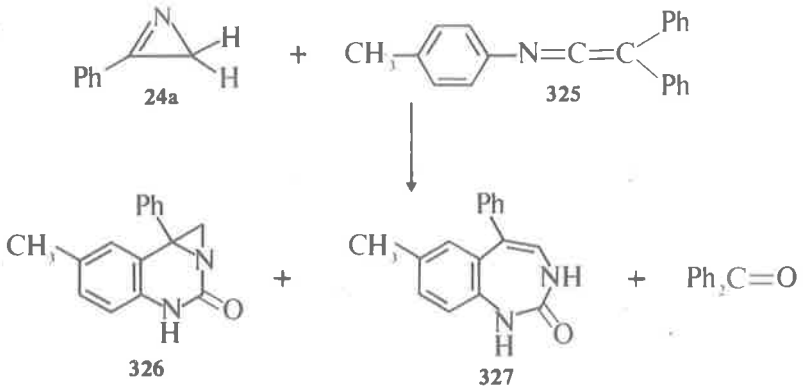
The reactions of these 1-azirines with ketenes represent nonconcerted additions and are formally different from the additions to 4π systems of dienes and 1,3-dipole compounds.

2-Amino-1-azirines behave somewhat differently from 2-aryl- and 2-alkyl-1-azirines, as mentioned previously. The aminoazirine **172** reacts with diphenylketene **(319)** in a nonconcerted manner to give the 3-oxazoline **324** as the major product.[191,196] Other products (generally minor) also have been reported from this and related reactions recently (Scheme 28).[196b]



Scheme 28 Reaction of aminoazirine and diphenylketene.

Ketenimine **(325)** reacts with 2-phenyl-1-azirine **(24a)** in refluxing benzene to give a mixture of the bicyclic aziridine **326** (15%), the benzodiazepinone **327** (15%), and benzophenone.[192] The benzodiazepinone **327** is a secondary product of this reaction and is produced from the thermal rearrangement of **326** (Scheme 29).



Scheme 29 Reaction of 1-azirine and ketenimine.

b. ISOCYANATES

Heterocumulenes containing a carbonyl or related unsaturation adjacent to the cumulative bonds usually possess high reactivity, and Nair and Kim[193,194] first reported on the interesting reactions of 1-azirines with such isocyanates.

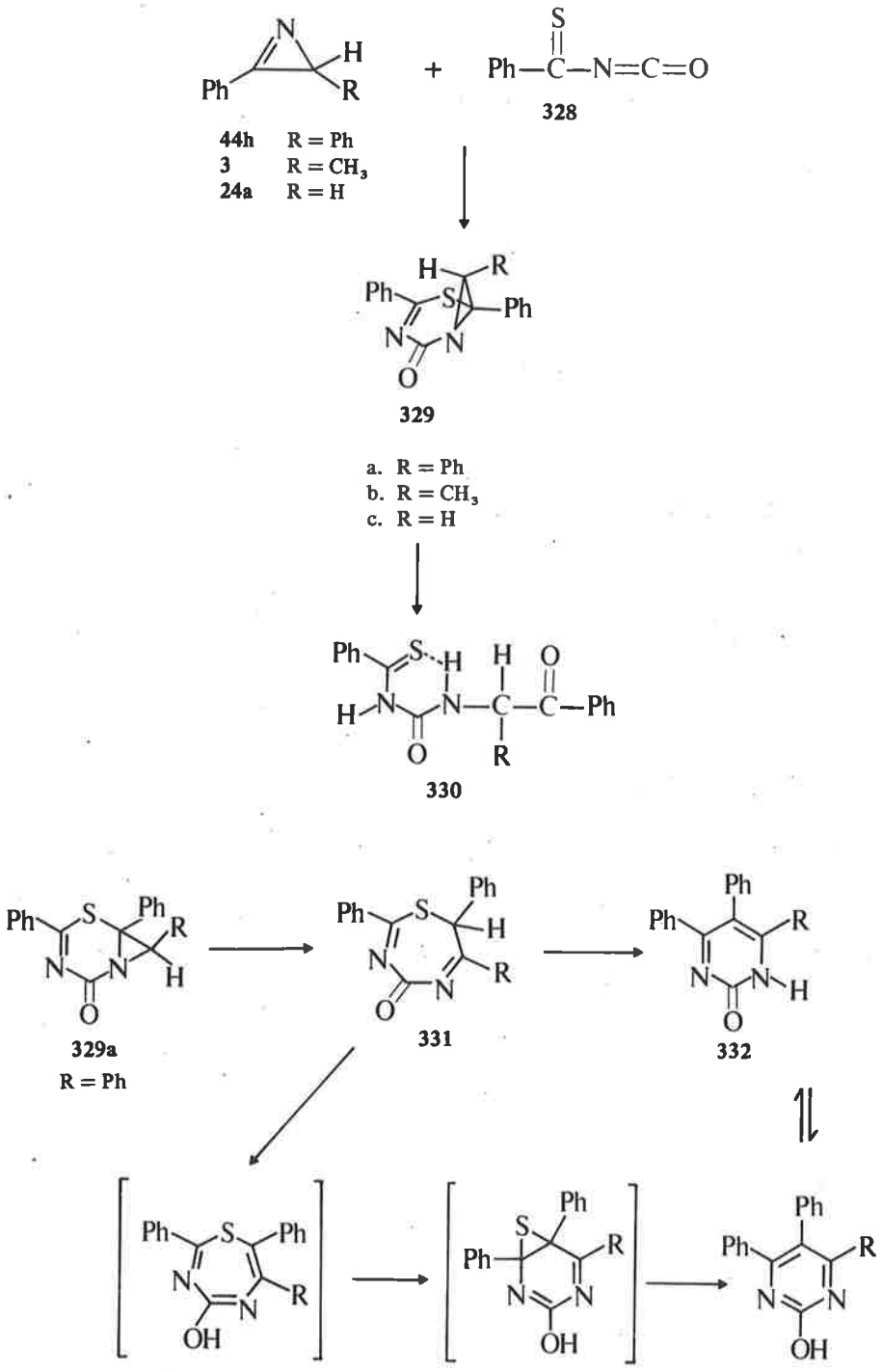
Thiobenzoyl isocyanate (**328**) can be generated from 2-phenylthiazoline-4,5-dione by thermal extrusion of carbon monoxide. Isocyanate **328**, prepared *in situ*, adds stereospecifically and regiospecifically at room temperature to give high yields of [4 + 2] cycloadducts, the bicyclic aziridines **329**.[172,193,194] The aziridines **329** undergo clean acid-catalyzed hydrolysis to the ureas **330**, providing excellent evidence for the regiospecificity of these cycloadditions.

The cycloadducts **329** exhibit other interesting behavior. For example, when **329a** was subjected to thermolysis in refluxing benzene, ring expansion to the novel thiadiazepinone **331** was observed. Prolonged thermolysis of **329a** at higher temperatures resulted in the removal of elemental sulfur and the formation of the pyrimidone **332**. The thiadiazepinone **331** was shown to be the intermediate in the thermal conversion of **329a** to **332**. A reasonable mechanism for the sulfur extrusion reaction is shown in Scheme 30. The initial formation of **331** is followed by a 1,5-sigmatropic shift and electrocyclization to a thiirane. Elimination of elemental sulfur is followed by tautomerization of the pyrimidine to the preferred pyrimidone structure **332**.[194]

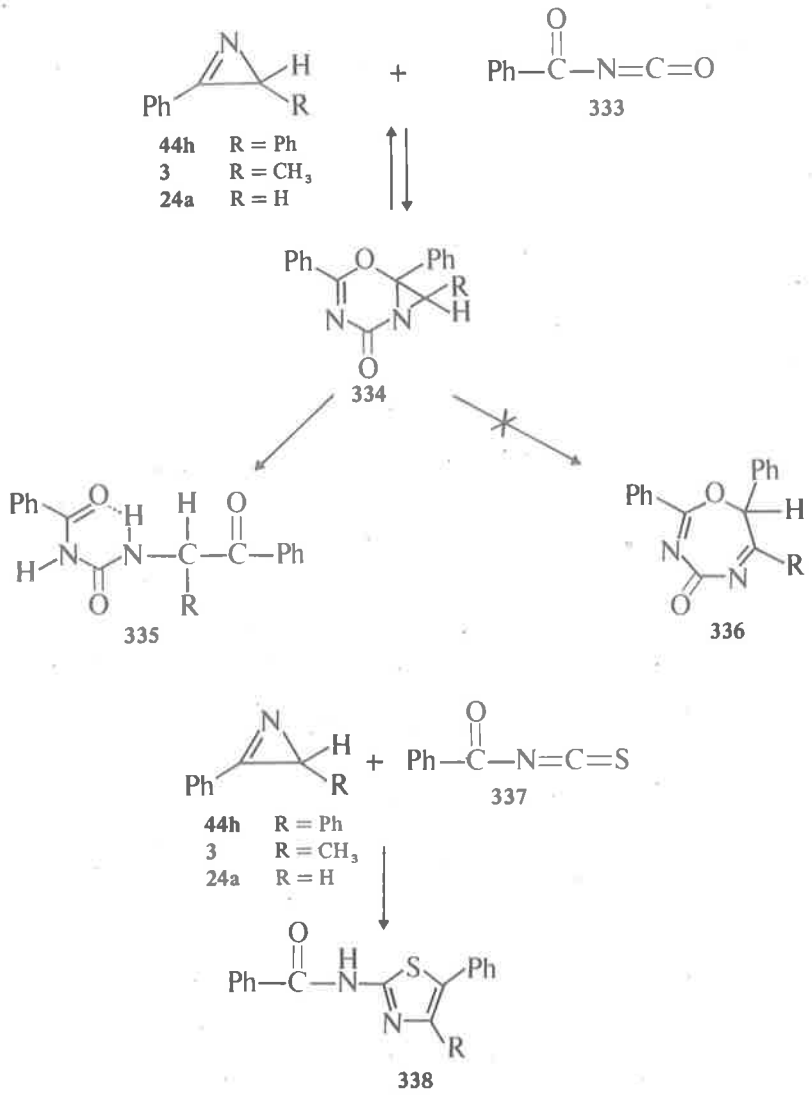
The behavior of benzoyl isocyanate (**333**) toward 1-azirines paralleled those observed with thiobenzoyl isocyanate, and [4 + 2] cycloadducts **334** were isolated.[194] Hydrolysis to the ureas **335** occurred under acid-catalyzed conditions. Thermolysis to **336** was not observed. However, at 70°, a clean retro[4 + 2] pericyclic reaction occurred.

Benzoyl isothiocyanate (**337**) also reacts with 1-azirines. The cycloaddition apparently occurs in a [2 + 2] fashion across the C=S bond to give thiazoles **338** as final products.[194]

The marked difference in behavior between the exclusive [4 + 2] cycloaddition observed for benzoyl isocyanate (**333**) and thiobenzoyl isocyanate (**328**) and the



Scheme 30 Thermal rearrangements of bicyclic aziridines.

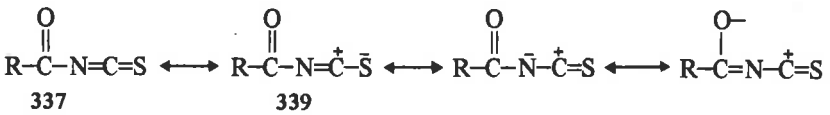


apparent [2 + 2] cycloaddition in a regiospecific manner to the C=S bond of **337** requires explanation. Orbital symmetry analysis[165] reveals a possible concerted $[\pi^2 s + \pi^2 a]$ pathway for addition involving the C=S bond. A striking clue to the nature of the transition state came from solvent polarity studies with **24a** at 75° (Table 5), which showed a dramatic increase in product yield with increase in the dielectric constant of the solvent.[194] This solvent dependency was interpreted as reflecting the presence of a polar transition state in the pathway to the formation of the initial cycloadduct. The polarization of **337** (Scheme 31) is similar to **333** except for the greater ability of sulfur to stabilize a negative charge[195] (see **339**). A dipolar transition state such as **340** could conceivably account not only for the

TABLE 5. REACTION OF 2-PHENYL-1-AZIRINE (24a) with BENZOYL ISOTHIOCYANATE (337) AT 75°

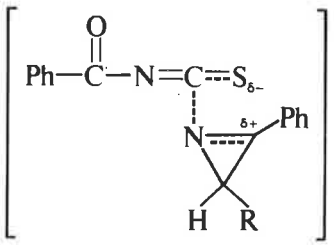
Solvent	Dielectric constant	Reaction time (hr)	Yield of 338 (R = H) (%)
Benzene	2.3	2	13.4 ± 1.5
Ethyl acetate	6.0	2	19.3 ± 1.5
Nitrobenzene	34.8	2	42.7 ± 1.5

solvent dependency but also for the marked difference in the behaviors of **328**, **333**, and **337**. Whether such a transition state would transform into a relatively stable dipolar intermediate to favor a two-step combination is not known.



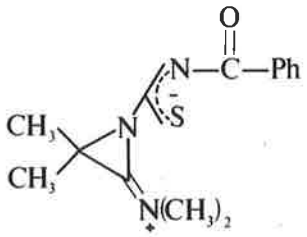
337 **339**

Scheme 31 Contributing resonance structures for benzoyl isothiocyanate. R = Ph.



340

Interestingly, 2-amino-1-azirine (**172**) reacts with benzoyl isothiocyanate to give the 1,4-dipolar compound **341**.[196]

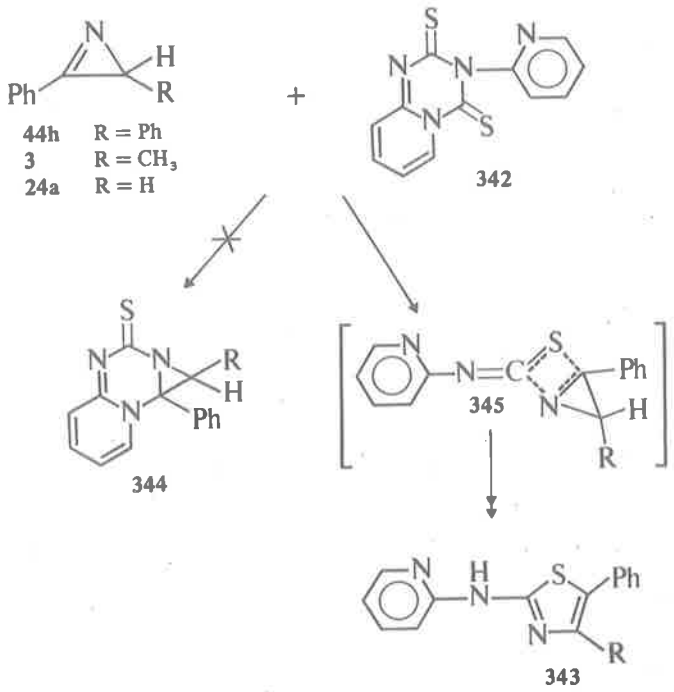


341

Although isothiocyanates such as methyl isothiocyanate, phenyl isothiocyanate, and *p*-nitrophenyl isothiocyanate react with 2-amino-1-azirines,[196,199] Kim and Nair[197] have found them to be normally unreactive toward 2-aryl-1-azirines.

Nair and Kim[198] also examined the reactivity of 2-pyridyl isothiocyanate toward 2-aryl-1-azirines. 2-Pyridyl isothiocyanate is produced by the reaction

of 2-aminopyridine and carbon disulfide in the presence of triethylamine. At room temperature, this compound exists as a dimer (**342**). When the azirines (**3**, **24a**, and **44h**) were heated in toluene under reflux with the **342**, thiazoles **343**, the result of regiospecific addition of the C=S bond of the monomer to the C=N bond of the azirines (see **345**), were isolated. Thiazoles arising from initial nucleophilic attack and 1,3-bond cleavage were not formed. Neither the product **344** nor its ring-expanded forms, the result of [4 + 2] cycloaddition, were isolated (Scheme 32).

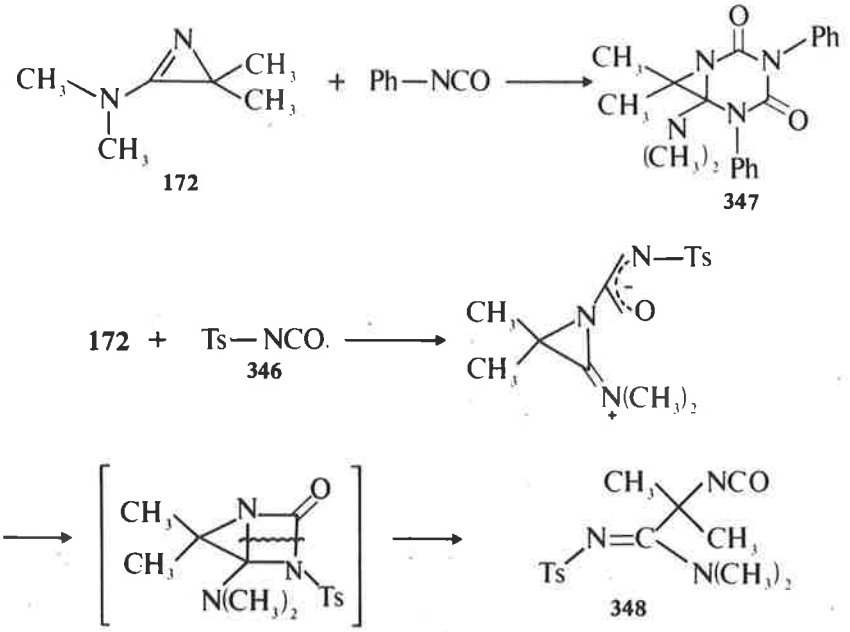


Scheme 32 Reaction of 2-pyridyl isothiocyanate with 1-azirines.

Simple aryl isocyanates such as phenyl isocyanate, *p*-methylphenyl isocyanate, *p*-chlorophenyl isocyanate, and *p*-nitrophenyl isocyanate are unreactive toward 2-aryl-1-azirines.[197] However, phenyl isocyanate has been reported to react at room temperature with the 2-amino-1-azirine **172** to give the 2:1 adduct **347**.[196] Adducts in a 3:1 ratio also have been reported.[191] Azirine **172** also reacts with *p*-toluenesulfonyl isocyanate (**346**) at room temperature to give the ring-opened 1:1 adduct **348** (Scheme 33).[196] The isocyanate **346** also has been found to react with 2-aryl-1-azirines.[172]

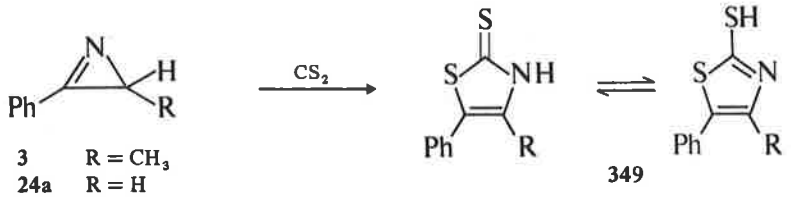
c. CARBON DISULFIDE

Carbon disulfide is a simple heterocumulene, and most of its reactions proceed from initial nucleophilic attack on the central carbon.[200] The few cycloadditions known are 1,3-dipolar in nature, with carbon disulfide as the dipolarophile.[201, 202]



Scheme 33 Reactions of 2-amino-1-azirines with isocyanates.

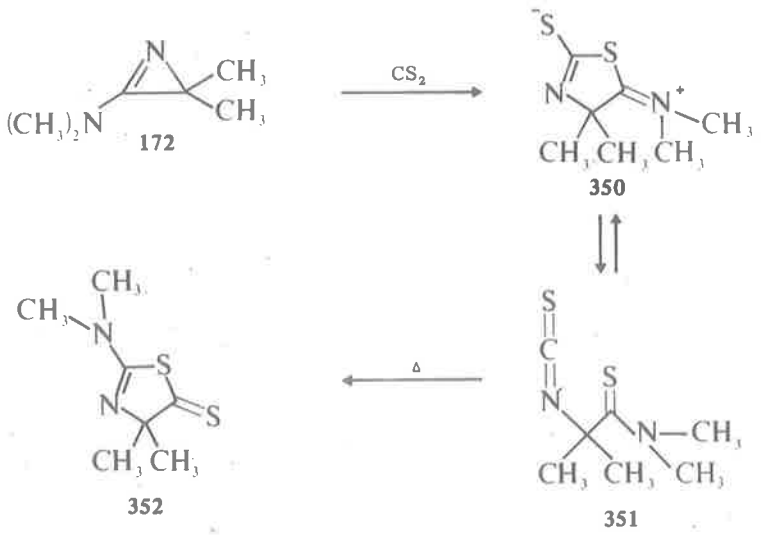
Nair and Kim[203] discovered that 2-phenyl-1-azirine (**24a**) and 3-methyl-2-phenyl-1-azirine (**3**) react with carbon disulfide in a sealed tube at 100° to give the thiazoles **349**. These products are the result of regiospecific cycloadditions of carbon disulfide to the π bond of the 1-azirines.



3 R = CH_3
24a R = H

In general, cycloaddition of 2-phenyl-1-azirine with heterocumulenes containing the C=S bond proceeds through a dipolar transition state where the ability of sulfur to stabilize the negative charge results in a lower energy electronic pathway to the cycloadducts.

2-Amino-1-azirines react differently with carbon disulfide. For example, 2-dimethylamino-3,3-dimethyl-1-azirine (**172**) reacts smoothly with carbon disulfide to give crystals that have the dipolar structure **350**.[204,205] In solution, the isomeric form **351** is the predominant structure. Thermolysis of the adduct leads to **352** in high yield.



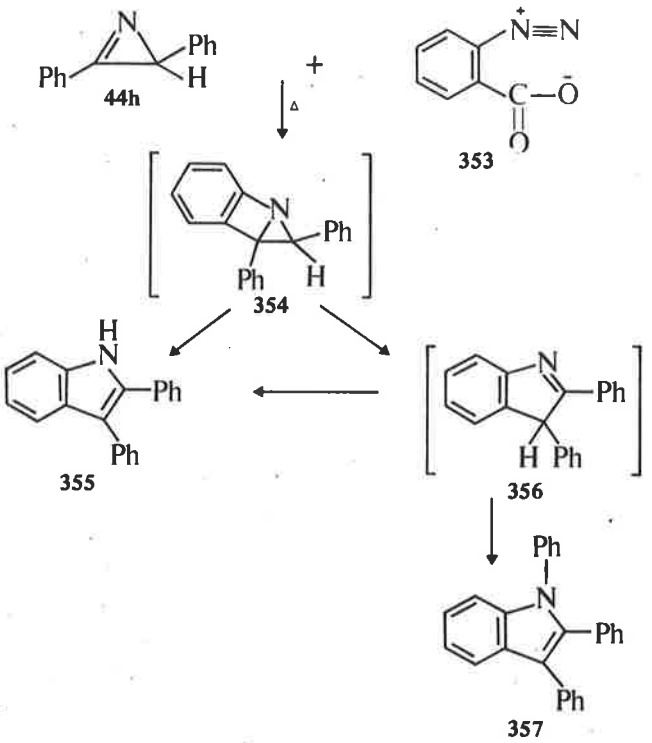
D. *Miscellaneous Cycloadditions*

a. BENZYNE

Nair and Kim[206] reported that 2,3-diphenyl-1-azirine (**44h**) reacts with *o*-benzyne, generated by the thermal decomposition of benzenediazonium 2-carboxylate (**353**), to give two products. The major product, a 1 : 1 adduct produced in 50% yield, was identified as 2,3-diphenylindole (**355**). A 1 : 2 adduct of azirine and benzyne, identified as 1,2,3-triphenylindole (**357**), was isolated in 14% yield. When the concentration of benzyne was increased, the yield of **357** also increased. 2,3-Diphenylindole (**355**) was found to be relatively inert to benzyne, and no triphenylindole **357** could be isolated from the reaction of **355** and benzyne even after extended reaction times. The mechanism of formation of 2,3-diphenylindole (**355**) (Scheme 34) may be interpreted as requiring the initial formation of **354**, the result of 1,2-addition on the azirine ring system. Moreover, as 2-methyl-3-phenylindole (**358**) is isolated from the reaction of 3-methyl-2-phenyl-1-azirine (**3**) and benzyne, initial 1,3-addition appears unlikely. Two reaction pathways are available for partitioning of intermediate **354**. Ring cleavage and a concomitant 1,2-hydrogen shift to the nitrogen would give the stable aromatic indole **355**. A similar 1,2-hydrogen shift to carbon would generate the 3*H*-indole system **356**, which can be trapped by benzyne to give the 1,2,3-triphenylindole **357**. The conversion of indolenine **356** to the indole **357** may proceed via a symmetry-allowed "ene" reaction (illustrated in **359**). An alternative explanation involves a competitive interaction of benzyne with the nitrogen of **354** leading via a zwitterion to **357**.[50]

b. ENE REACTION

When the azlactone **360** was heated under reflux in xylene with 2-phenyl-1-



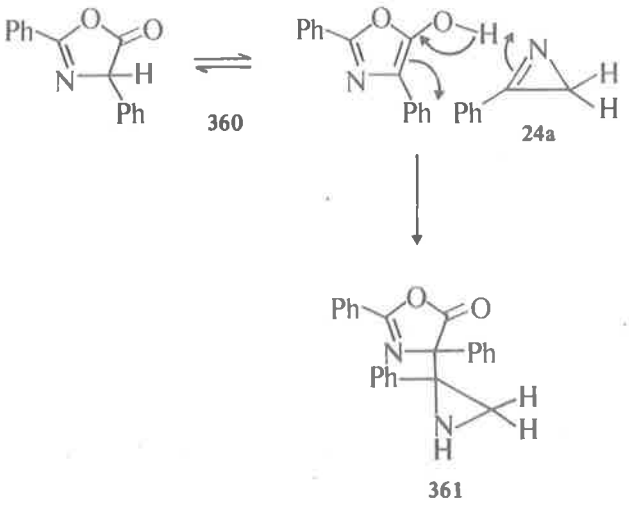
Scheme 34 Reaction of 2,3-diphenyl-1-azirine with benzyne. (Adapted from reference 206 with permission from the American Chemical Society.)

azirine (**24a**), the aziridine **361** was isolated in 92% yield.[185] Compound **361** is the product of an ene reaction as shown in Scheme 35.

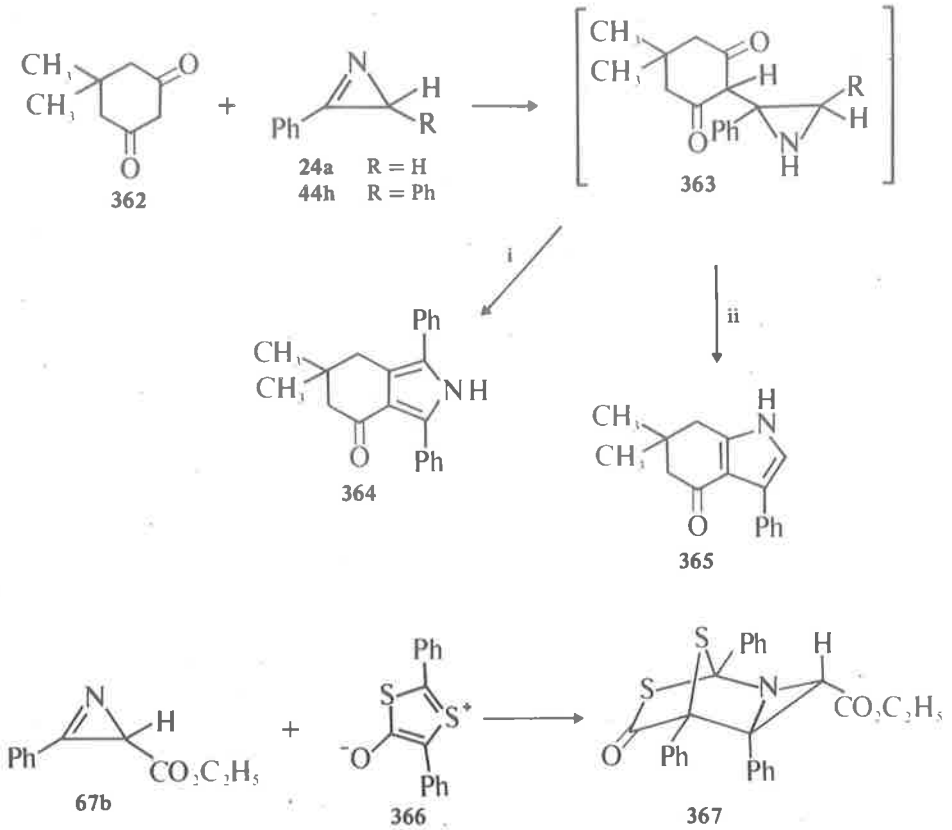
An ene reaction with dimedone also has been reported.[185] The ene product **363** is an intermediate in this case and undergoes C–C (i) or C–N (ii) bond cleavage to give, after H_2O elimination, the isolated products **364** or **365**, respectively.

c. MESOIONIC COMPOUNDS

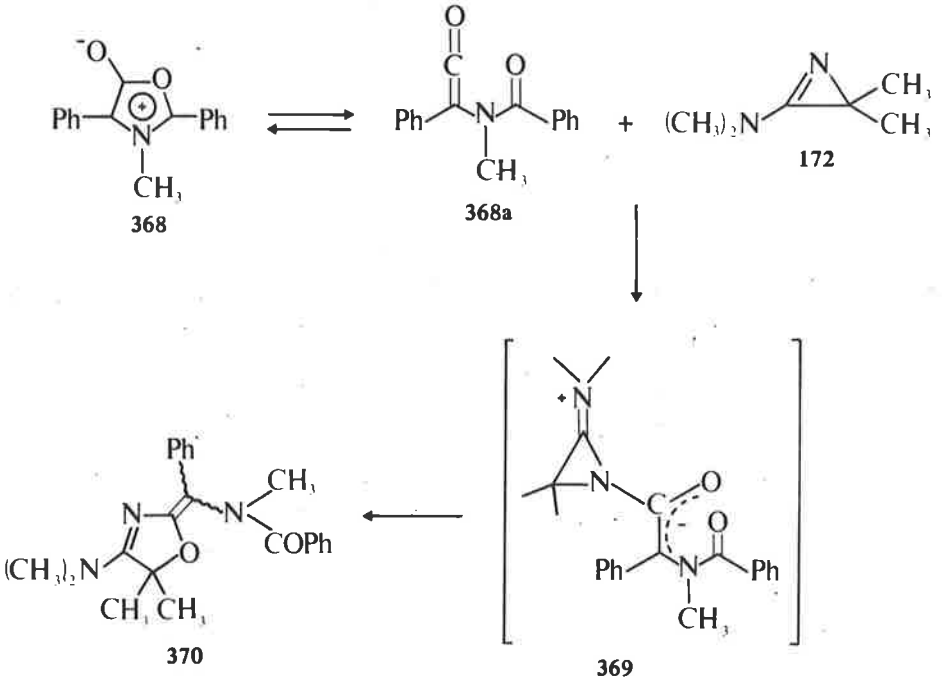
The mesoionic compound **366** adds to the azirine **67b** at 100° to give the adduct **367** in 92% yield.[207] The reaction is regiospecific and stereospecific.



Scheme 35 Ene reaction of 2-phenyl-1-azirine with an azlactone.



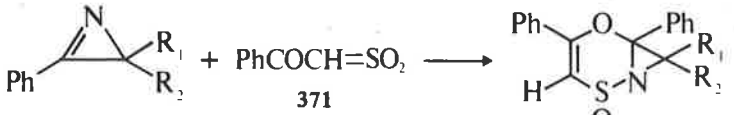
Lukac, Bieri, and Heimgartner[208, 209] reported that the 2-dimethylamino-1-azirine (**172**) reacts with the mesoionic oxazole **368** at room temperature to give the adduct **370**, presumably through the intermediacy of **369**. The corresponding mesoionic dithiole shows similar behavior toward 2-amino-1-azirines.[209]



368 **368a** **172** **370** **369**

d. α-KETOSULFENES

The reaction of benzoylsulfene **371** and related cyclic α-ketosulfenes was studied by Tsuge and Noguchi.[210] The α-ketosulfene **371** reacts with the azirines **3**, **15**, **24a**, and **44h** to give the [4 + 2] cycloadducts **372**.



371

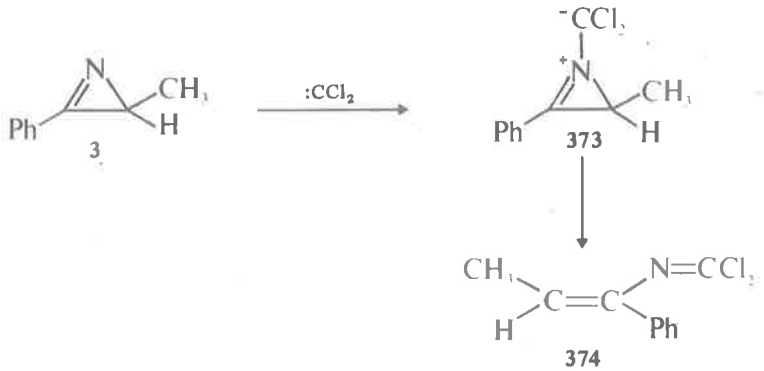
3	$R_1 = H; R_2 = CH_3$
15	$R_1 = CH_3; R_2 = CH_3$
24a	$R_1 = H; R_2 = H$
44h	$R_1 = H; R_2 = Ph$

372

e. CARBENES

Hassner et al.[147] showed that dichlorocarbene, generated from phenyl (trichloromethyl) mercury, reacts with 3-methyl-2-phenyl-azirine (**3**) to give the ring-opened product **374**. No azabicyclobutane was detected.[147] It was suggested that the

reaction involves initial nucleophilic attack by the azirine to generate the ylide **373**. This intermediate then undergoes ring opening with cleavage of the C–N bond to give **374**.



The conversion of 1-azirines with dimethylsulfonium methylide to give azabicyclobutanes[145] was mentioned previously.

The reaction of 1-azirines with diphenylcarbene[182] (generated by thermolysis of diphenyldiazomethane) was discussed in Section VI, 4, B on 1,3-dipolar cycloadditions.

5. Photochemical Reactions of 1-Azirines

Simple imines exhibit weak $n\pi^*$ absorption in the 235 nm region and generally are unreactive photochemically because of the deactivation of their excited state by (*E*)/(*Z*) isomerization. The C=N bond of 1-azirines, being part of a small heterocyclic system, cannot be deactivated in this manner after photochemical excitation. Considerable evidence has accrued to suggest that 1-azirines participate in photochemical reactions through initial ring cleavage. Many studies of the photoreactions of 1-azirines, both intermolecular and intramolecular, have been reported. Some photochemical reactions are described in two reviews.[211,212] This part of the chapter briefly discusses a wide variety of representative examples. The reader is referred to the original literature for more exhaustive coverage.

A. *Photochemical Excitation of 1-Azirines*

2-Aryl-1-azirines show a strong uv absorption at about 240 nm ($\epsilon > 10{,}000$) and a weak inflection on the long wavelength side of the principal band (*ca.* 285 nm, $\epsilon \sim 500$). The latter absorption is very likely associated with an $n\pi^*$ transition. It is likely that the first excited $n\pi^*$ singlet state of 2-aryl-1-azirines is responsible for its photochemistry. Padwa[211] and Schmid[212] and their co-workers have shown that 1-azirines undergo ring opening on $n\pi^*$ excitation to give nitrile ylides as reactive intermediates.

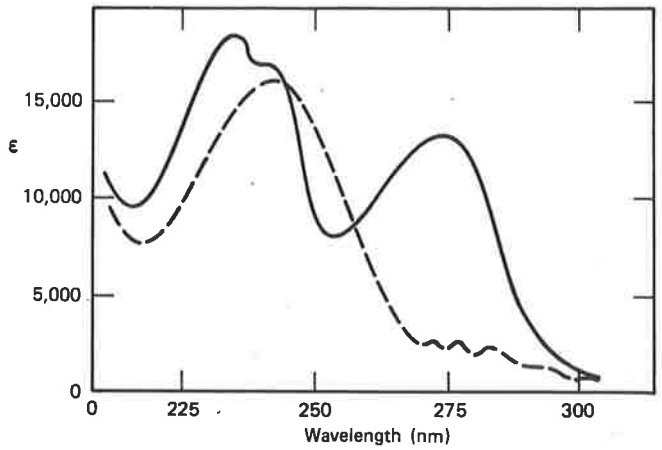
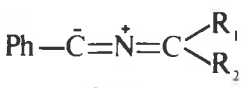


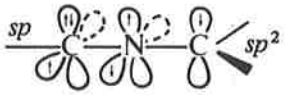
Figure 1. Irradiation (255 nm) of 3,3-dimethyl-2-phenyl-1-azirine in a rigid matrix at − 185°: solid curve, uv spectrum of azirine; dashed curve, uv spectrum of nitrile ylide. (Adapted from reference 213 with permission from *Helvetica Chimica Acta*, Birkhauser Verlag.)

Excellent direct experimental evidence for the generation of the nitrile ylides was provided by Schmid and his co-workers.[212–215] Irradiation of 3,3-dimethyl-2-phenyl-1-azirine (**15**) in a 2-methylpentane glass at − 185° with 255 nm light gave rise at 275 nm to a new absorption peak that was attributed to the nitrile ylide (Fig. 1). Similarly, irradiation of 2,3-diphenyl-1-azirine (**44h**) gave a nitrile ylide absorption at 350 nm. The dipolar species formed was shown to undergo photochemical but not thermal reversion to the starting azirine.[212,213] The absorption band due to the nitrile ylide disappeared slowly in the presence of a trapping agent such as a dipolarophile, suggesting that a thermally allowed 1,3-dipolar cycloaddition was occurring.

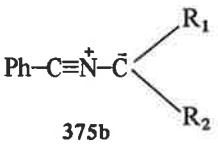
Structurally, nitrile ylides may be classified as nitrilium betaines, a class of 1,3-dipoles containing a central nitrogen atom and a π bond orthogonal to the 4π allyl system (**375a**).[211,216,217] However, a number of other forms (**375b–375e**) are possible for the ring-opened azirine. The structure may be partly diradical and partly zwitterionic.[218]



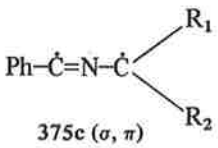
375a



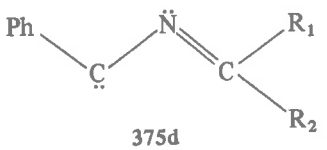
375a
Orbital representation.



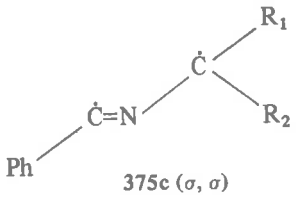
375b



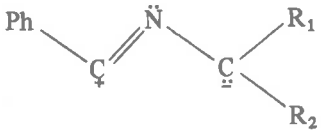
375c (σ, π)



375d



375c (σ, σ)

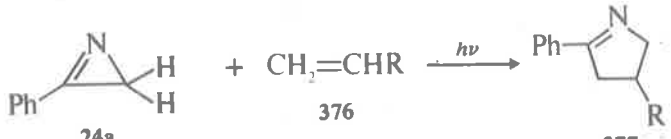


375e (bent 375b)

B. *Intermolecular Photochemical Reactions*

a. WITH ALKENES AND ALKYNES

Padwa and Smolanoff[219] reported that when a solution of 2-phenyl-1-azirine (**24a**) in excess methyl acrylate (**376a**) is photolyzed using a 450 W high pressure mercury lamp with a Vycor filter for 3 hr, 2-phenyl-4-carbomethoxy-Δ^1-pyrroline (**377a**) is produced in 80% yield. Similarly, when acrylonitrile (**376b**) was used as the olefin, Δ^1-pyrroline (**377b**) was isolated in 70% yield.



24a

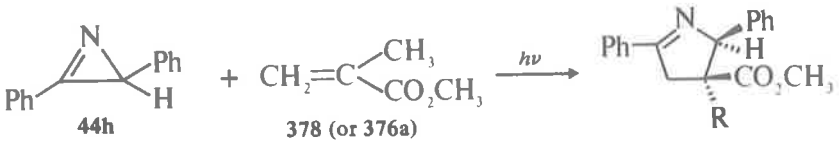
376

a. R = CO_2CH_3
b. R = CN

377

a. R = CO_2CH_3
b. R = CN

However, the photochemical addition of 2,3-diphenyl-1-azirine (**44h**) with methyl methacrylate (**378**) afforded a mixture of 2,5-diphenyl-4-methyl-4-carbomethoxy-Δ^1-pyrrolines **379** (40% yield) and **380** (60%).[220]

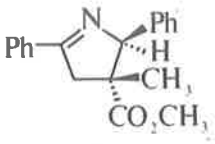


44h

378 (or 376a)

379, R = CH_3
(381, R = H)

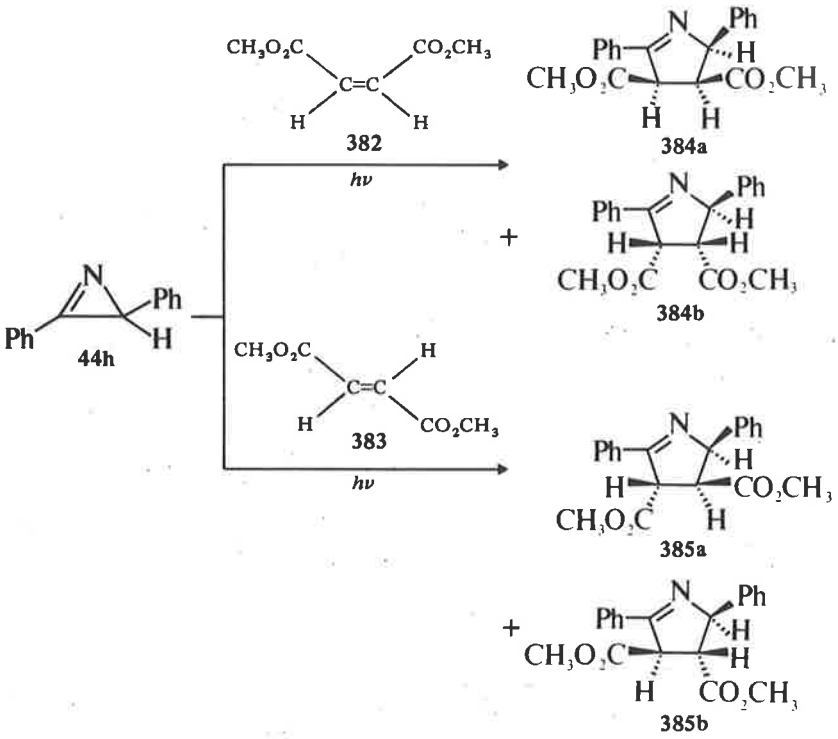
+



380

Irradiation of a mixture of **44h** and methyl acrylate (**376a**) led to a single photoproduct, 2,5-diphenyl-*cis*-4-carbomethoxy-Δ^1-pyrroline (**381**).

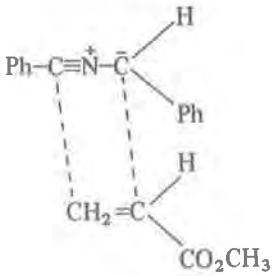
The photoadditions of 2,3-diphenyl-1-azirine (**44h**) also exhibit *syn* stereospecificity (Scheme 36). For example, irradiation of **44h** with maleic and fumaric acid esters **382** and **383** gave totally stereospecific addition, and the isomeric Δ^1-pyrrolines **384a**, **384b**, and **385a**, **385b** respectively, were isolated.



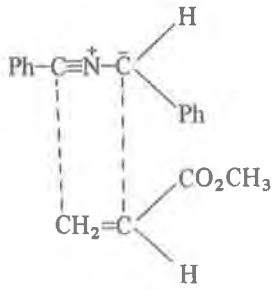
Scheme 36 Photochemical addition of 2,3-diphenyl-1-azirine with dimethyl maleate and dimethyl fumarate.

The photocycloaddition of 2-phenyl-1-azirines with electron-deficient olefins to produce Δ^1-pyrrolines generally shows characteristics of concerted reactions, including features of stereospecificity and regioselectivity. The reaction can be classified in simple terms as a thermal 1,3-dipolar cycloaddition of a nitrile ylide with a π bond.[221] If these dipolar additions proceed through a "two-plane" orientation complex, a number of possible arrangements can be drawn. For the reaction of diphenylazirine and methyl acrylate, two possible orientation complexes (**386** and **387**) exist. The interaction of the substituent groups in the *syn* complex **386** has both an attractive and a repulsive nature. These effects are relatively small in the *anti* complex **387**. However, the results of this photoaddition (*cis*-Δ^1-pyrroline, **381**, is the predominant product) suggest that the π overlap of the ester and phenyl groups in the *syn* complex more than compensates for the

adverse van der Waals repulsion of these substituents.[220,222] This, however, is not the case when the hydrogen in the position alpha to the carbomethoxyl is replaced by a methyl group. There is little discrimination between the *syn* and *anti* forms, and both products (**379** and **380**) are formed.

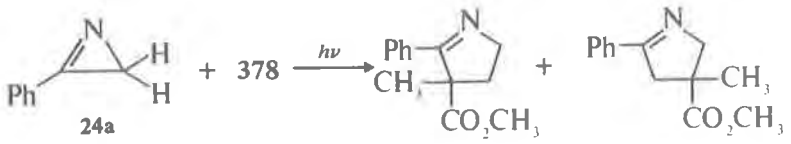


386



387

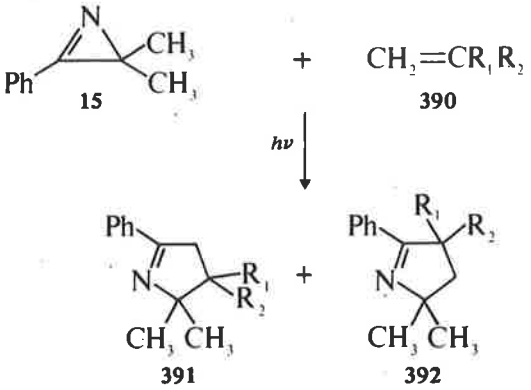
These photocycloadditions also exhibit regioselectivity. Frontier orbital theory has been used successfully to rationalize the observed regioselectivity of many 1,3-dipolar cycloadditions.[223] For example, with nitrile ylides the favored cycloadduct is that formed by the bonding of atoms with the largest coefficients in the dipole highest occupied molecular orbital (HOMO) and dipolarophile lowest unoccupied molecular orbital (LUMO). In the HOMO of the nitrile ylides under consideration, the electron density at the disubstituted carbon is somewhat greater than that at the trisubstituted carbon.[211] In electron-deficient olefins, the largest coefficient in the LUMO is on the unsubstituted carbon. This treatment adequately explains the photochemical reaction of diphenylazirine with methyl acrylate to produce only the 4-substituted regioisomer **381**. Padwa[211] explained the mixture of cycloadducts **388** and **389** in the reaction of 2-phenyl-1-azirine (**24a**) with methyl methacrylate by the lowering of the LUMO coefficient at the unsubstituted carbon atom of the dipolarophile by the presence of the methyl group. Apparently the terminal coefficients in the LUMO of methyl methacrylate are more nearly the same than they are for methyl acrylate, resulting in the observed loss of regioselectivity.



24a + **378** $\xrightarrow{h\nu}$ **388** + **389**

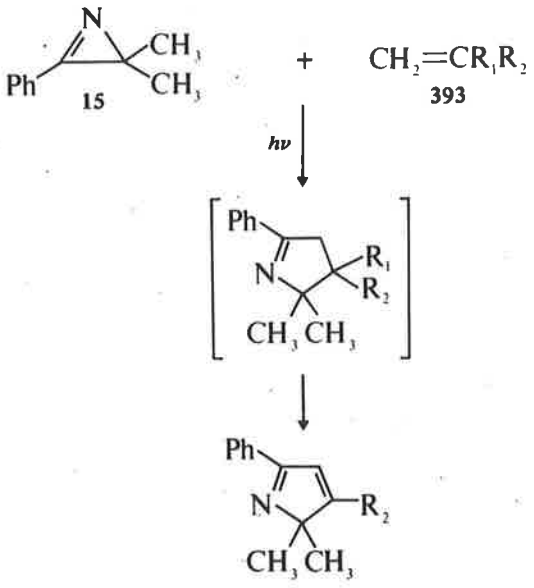
Regioselectivity is lost also in the photochemical cycloaddition of 3,3-dimethyl-2-phenyl-1-azirine (**15**) and diethylvinyl phosphonate or dimethylvinyl phosphine sulfide. The two Δ^1-pyrrolines **391** and **392** are formed in equal amounts.[212,224]

The photoaddition of azirine **15** to vinyl phosphonium salts (**393a** and **393b**) and to vinyl sulfones (**393c**) results in the isolation of the pyrroles **394a–394c** in the yields shown in Scheme 37. The initial photoadduct is presumably a Δ^1-



a. $R_1 = PO(OC_2H_5)_2$; $R_2 = H$
b. $R_1 = PS(CH_3)_2$; $R_2 = H$

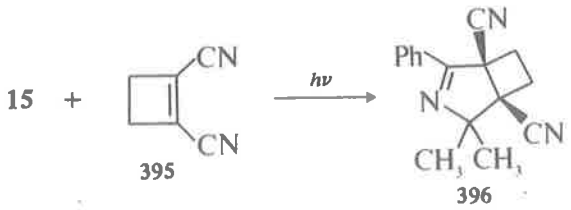
pyrroline from which the pyrroles are derived by elimination of $(Ph)_3P \cdot HBr$ or $PhSO_2H$.[225] α-Ethoxyacrylonitrile (**393d**) exhibits similar behavior,[226] eliminating acetic acid to produce the pyrrole **394d** (Scheme 37).

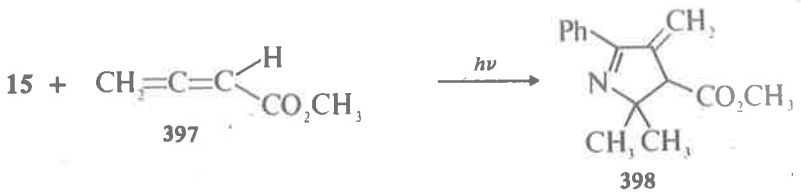


a. $R_1 = \overset{+}{P}(Ph)_3Br^-$; $R_2 = H$ (40%)
b. $R_1 = \overset{+}{P}(Ph)_3Br^-$; $R_2 = CH_3$ (26%)
c. $R_1 = SO_2Ph$; $R_2 = H$ (65%)
d. $R_1 = CN$; $R_2 = OCOCH_3$ (55%)

Scheme 37 2*H*-Pyrrole formation in the photocycloaddition of 3,3-dimethyl-2-phenyl-1-azirine and vinyl phosphonium salts, vinyl sulfones, and α-acetoxyacrylonitrile. (Adapted from reference 212 with permission from *Heterocycles*.)

1,2-Dicyanocyclobutene (**395**) reacts photochemically with azirine **15** to give in 68% conversion the bicyclic pyrroline **396**.[226] The allene **397** gives the pyrroline **398** in 90% yield.[212]





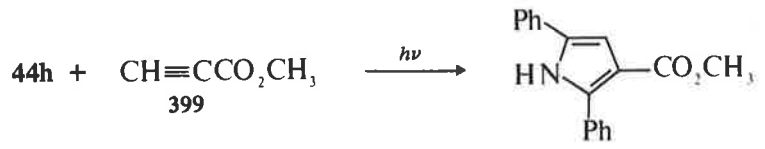
Other alkenes such as styrenes and vinylpyridines[212] are also reactive toward 1-azirines photochemically. Nonactivated alkenes such as cyclohexene are unreactive. The relative reactivities of a series of alkenes toward the nitrile ylide from 2,3-diphenyl-1-azirine (**44h**) are shown in Table 6.[233]

Alkynes also undergo cycloaddition to the nitrile ylides derived from 1-azirines. For example, the monosubstituted acetylene **399** adds to produce the pyrrole **400**, presumably via initial cycloaddition and subsequent 1,5-sigmatropic shift. When the 1,5-sigmatropic shift is prevented as in the reaction of the geminally disubstituted azirine **15** with dimethylacetylene dicarboxylate (**401**), the 2*H*-pyrrole **402** is the isolated product.[212,228,229]

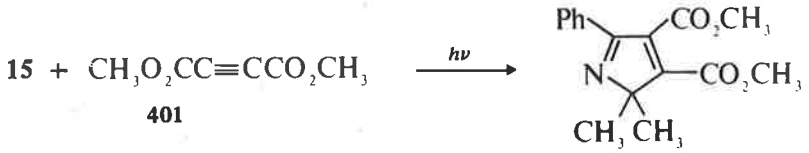
TABLE 6. RELATIVE REACTIVITY OF ALKENES TOWARD NITRILE YLIDE FROM AZIRINE **44h**.[a]

Dipolarophile	Relative rate
Methyl crotonate	1
Methylacrylonitrile	3.6
Methyl methacrylate	9
Diethyl maleate	135
Methyl acrylate	160
Dimethyl maleate	166
Acrylonitrile	180
Dimethyl acetylenedicarboxylate	540
Maleonitrile	2,300
Diethyl fumarate	56,000
Dimethyl fumarate	84,000
Fumaronitrile	189,000

[a] Adapted from reference 233 with permission from the American Chemical Society.

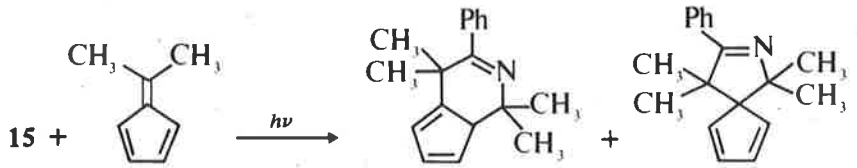


400



402

The reactions of the photochemically generated nitrile ylides with alkenes and alkynes represent thermally allowed [4 + 2] cycloadditions. Under appropriate conditions, these nitrile ylides can participate as 4π components in [6 + 4] cycloadditions. For example, irradiation of a 1:1 mixture of azirine **15** and 6,6-dimethylfulvene **403** in cyclohexane with Vycor filtered light gives two products (**404** and **405**) in a 3:1 ratio. Compound **405** is the result of a [4 + 2] cycloaddition similar to the reactions of alkenes already mentioned, and compound **404** represents a [6 + 4] adduct.[230]

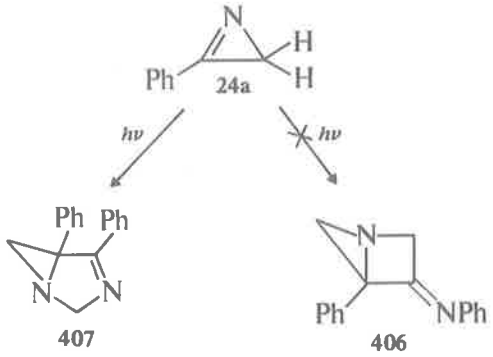


403 **404** **405**

b. WITH IMINES

Generally, imines such as benzylidene-methylamine do not react with 1-azirines under photochemical conditions. However, the nitrile ylides derived from 1-azirines are reactive toward the strained C=N bond of 1-azirines. The first report describing such a reaction was made by Woerner, Reimlinger, and Arnold.[231,232] However, the structure of the product from the photolysis of 2-phenyl-1-azirine (**24a**) was incorrectly assigned as an azabicyclopentane **406**. Padwa and his co-workers[233] subsequently showed that the photodimer isolated from this reaction was the diazabicyclohexane **407**.

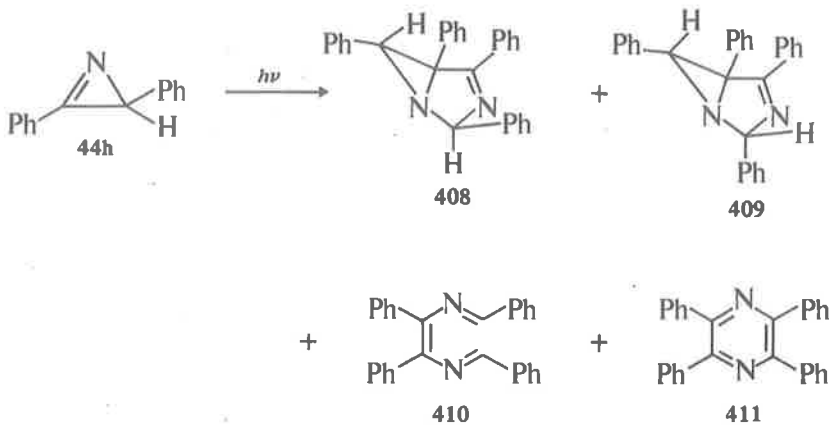
Further detailed analysis of this photodimerization was carried out with 2,3-diphenyl-1-azirine (**44h**).[233] Irradiation of azirine **44h** in cyclohexane for 17 hr with 300–340 nm light led to the complete disappearance of starting material and the formation of the photoadducts **408–411** (Scheme 38). The relative yields of these products varied as a function of time of irradiation. The formation of products **408** and **409** can be rationalized in terms of a 1,3-dipolar cycloaddition of the nitrile ylide $Ph\bar{C}{=}\overset{+}{N}{=}CHPh$ with the C=N bond of ground state azirine **44h**. This mechanism



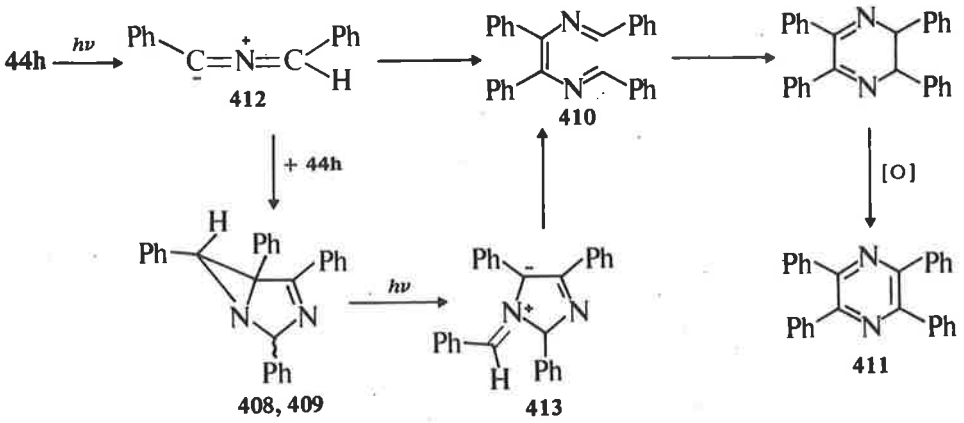
is consistent with Stern-Volmer plots obtained in these studies. On further irradiation, the stereoisomeric photodimers **408** and **409** are converted to the diazahexatriene **410**.[233,234] Schmid and his co-workers[215] have shown that the nitrile ylide **412** derived from **44h** can undergo quantitative dimerization to **410** at − 160°. Compound **410** can therefore be formed by both pathways. The intermediacy of the azomethine ylide **413** in the photochemical transformation of **408** and **409** to **410** was verified also by low temperature photolysis studies. It is very likely that formation of tetraphenylpyrazine (**411**) is due to the electrocyclization of **410** followed by oxidation (Scheme 39).

When a mixture of 2-phenyl-1-azirine (**24a**) and 2,3-diphenyl-1-azirine (**44h**) is photolyzed in such a way that only **44h** is excited (313 nm light), the sole products are 2-*exo*- and 2-*endo*-2,4,5-triphenyl-1,3-diazabicyclo[3.1.0]hex-3-ene (**414**).[227] These are cross-dimerization products.

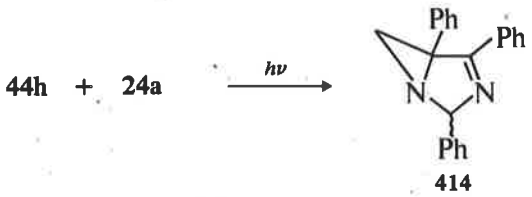
The photodimerization of these 1-azirines to 1,3-diazabicyclo[3.1.0]hex-3-enes appears to be a general reaction that exhibits some dependence on solvent, irradiation time, and substituents, mainly because of the inherent photochemical instability of the 1,3-diazabicyclohexenes.



Scheme 38 Products from the photodimerization of 2,3-diphenyl-1-azirine.



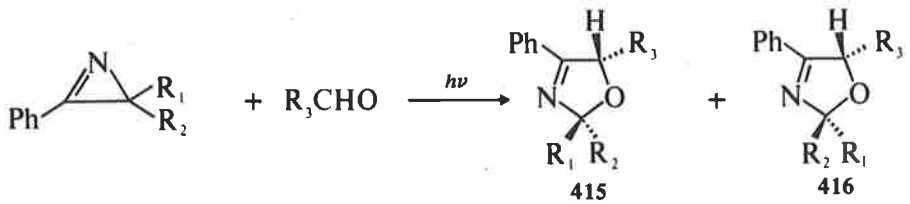
Scheme 39 Mechanism of photodimerization of 2,3-diphenyl-1-azirine. (Adapted from reference 211 with permission from the American Chemical Society.)



c. WITH ALDEHYDES, KETONES, AND α,β-UNSATURATED CARBONYL COMPOUNDS

Aldehydes, both aliphatic and aromatic, react regiospecifically with 2-phenylazirines under photolytic conditions to give 3-oxazoline derivatives exclusively and in isolated yields ranging approximately from 30 to 80%.[227,235,236] Where the possibility of stereochemistry exists, such as with 3-monosubstituted 1-azirines, both *cis*- and *trans*-isomeric 3-oxazolines are produced, with the *cis*- isomer being the major product (Scheme 40, Table 7).

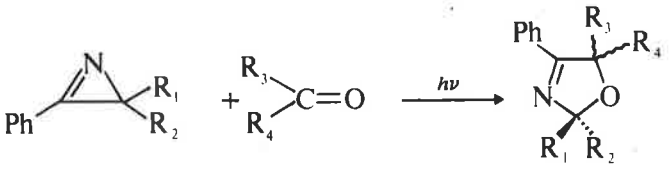
The reaction of ketones with these benzonitrile ylides is similar to the aldehyde reactions. Schmid and his co-workers reported[237,238] good yields of 3-oxazolines (**417**) generally from these reactions (Scheme 41, Table 8). Ketones with electron-withdrawing groups such as trifluoromethyl, ethoxycarbonyl, and nitrile in the



Scheme 40 General representation of the photoinduced reaction of 2-phenylazirines with aldehydes.

TABLE 7. ISOLATED YIELDS OF 3-OXAZOLINES IN THE PHOTOCYCLOADDITION OF 2-PHENYLAZIRINES WITH ALDEHYDES (SCHEME 40)[235,236]

Azirine	Aldehyde	3-Oxazoline	Yield (%)
$R_1 = R_2 = H$ **(24a)**	$R_3 = Ph$	$R_1 = R_2 = H; R_3 = Ph$	62
$R_1 = R_2 = H$ **(24a)**	$R_3 = p\text{-}CH_3\text{–}C_6H_4$	$R_1 = R_2 = H; R_3 = p\text{-}CH_3\text{–}C_6H_4$	54
$R_1 = R_2 = H$ **(24a)**	$R_3 = n\text{-}C_3H_7$	$R_1 = R_2 = H; R_3 = n\text{-}C_3H_7$	32
$R_1 = CH_3; R_2 = H$ **(3)**	$R_3 = Ph$	***cis*-416**	18
		***trans*-415**	9
		$R_1 = CH_3; R_2 = H; R_3 = Ph$	
$R_1 = Ph; R_2 = H$ **(44h)**	$R_3 = Ph$	***cis*-416**	27
		***trans*-415**	8
		$R_1 = Ph; R_2 = H; R_3 = Ph$	
$R_1 = Ph; R_2 = H$ **(44h)**	$R_3 = p\text{-}Cl\text{–}C_6H_4$	***cis*-416**	19
		***trans*-415**	7
		$R_1 = Ph; R_2 = H; R_3 = p\text{-}Cl\text{–}C_6H_4$	
$R_1 = Ph; R_2 = H$ **(44h)**	$R_3 = C_2H_5$	***cis*-416**	32
		***trans*-415**	13
		$R_1 = Ph; R_2 = H; R_3 = C_2H_5$	
$R_1 = Ph; R_2 = H$ **(44h)**	$R_3 = i\text{-}C_3H_7$	***cis*-416**	35
		***trans*-415**	9
		$R_1 = Ph; R_2 = H; R_3 = i\text{-}C_3H_7$	
$R_1 = R_2 = CH_3$ **(15)**	$R_3 = Ph$	$R_1 = R_2 = CH_3; R_3 = Ph$	60
$R_1 = R_2 = CH_3$ **(15)**	$R_3 = p\text{-}CH_3\text{–}C_6H_4$	$R_1 = R_2 = CH_3; R_3 = p\text{-}CH_3\text{–}C_6H_4$	70
$R_1 = R_2 = CH_3$ **(15)**	$R_3 = C_2H_5$	$R_1 = R_2 = CH_3; R_3 = C_2H_5$	74
$R_1 = R_2 = CH_3$ **(15)**	$R_3 = i\text{-}C_3H_7$	$R_1 = R_2 = CH_3; R_3 = i\text{-}C_3H_7$	80

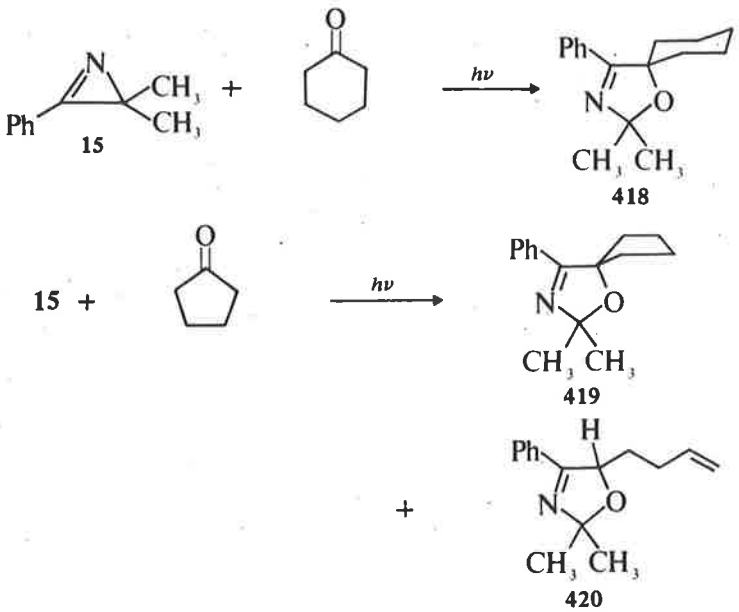


417

Scheme 41 General representation of the photoinduced reaction of 2-phenylazirines with ketones.

α-position react particularly smoothly. 3-Monosubstituted azirines such as **3** and **44h** react with unsymmetrical ketones to form mixtures of *cis*- and *trans*-3-oxazolines. It is of interest to compare these reactions with the acid-catalyzed addition of ketones to 1-azirines.

Cyclic ketones also react with 1-azirines under photochemical conditions (Scheme 42).[238] Irradiation of azirine **15** with cyclohexanone results in the formation of the spiro-3-oxazoline **418** in 86% yield. The photochemical behavior of **15** and cyclopentanone is dependent on the reaction conditions. When azirine **15** is irradiated and cyclopentanone slowly added, the spiro-3-oxazoline **419** is the major product. When cyclopentanone is irradiated first and the irradiation is continued with added azirine **15**, the only product isolated (80%) is the 3-oxazoline **420**. Product **420** must arise from the cycloaddition of the nitrile ylide with 4-pentenal. The latter is produced from cyclopentanone by Norrish type I cleavage and hydrogen transfer. Camphor and norcamphor also react after initial Norrish type I cleavage.



Scheme 42 Photochemical behavior 3,3-dimethyl-2-phenyl-1-azirine in the presence of cyclic ketones.

TABLE 8. ISOLATED YIELDS OF 3-OXAZOLINES (417) IN THE PHOTOCYCLOADDITION OF 2-PHENYLAZIRINES WITH KETONES.[237,238]

Azirine	Ketone	3-Oxazoline	Yield (%)
$R_1 = R_2 = CH_3$ (15)	$R_3 = R_4 = CH_3$	$R_1 = R_2 = R_3 = R_4 = CH_3$	98
$R_1 = CH_3; R_2 = H$ (3)	$R_3 = R_4 = CH_3$	$R_1 = R_3 = R_4 = CH_3; R_2 = H$	17
$R_1 = R_2 = CH_3$ (15)	$R_3 = CH_3; R_4 = Ph$	$R_1 = R_2 = R_3 = CH_3; R_4 = Ph$	84
$R_1 = R_2 = CH_3$ (15)	$R_3 = R_4 = Ph$	$R_1 = R_2 = CH_3; R_3 = R_4 = Ph$	88
$R_1 = R_2 = CH_3$ (15)	$R_3 = CF_3; R_4 = Ph$	$R_1 = R_2 = CH_3; R_3 = CF_3; R_4 = Ph$	80
$R_1 = Ph; R_2 = H$ (44h)	$R_3 = CF_3; R_4 = Ph$	$R_1 = R_4 = Ph; R_2 = H; R_3 = CF_3$	90
$R_1 = Ph; R_2 = H$ (44h)	$R_3 = CF_3; R_4 = CH_3$	$R_1 = Ph; R_2 = H; R_3 = CF_3; R_4 = CH_3$	65
$R_1 = R_2 = CH_3$ (15)	$R_3 = CH_3; R_4 = CO_2C_2H_5$	$R_1 = R_2 = R_3 = CH_3; R_4 = CO_2C_2H_5$	21
$R_1 = R_2 = CH_3$ (15)	$R_3 = R_4 = CO_2C_2H_5$	$R_1 = R_2 = CH_3; R_3 = R_4 = CO_2C_2H_5$	50
$R_1 = Ph; R_2 = H$ (44h)	$R_3 = R_4 = CO_2C_2H_5$	$R_1 = Ph; R_2 = H; R_3 = R_4 = CO_2C_2H_5$	59
$R_1 = R_2 = CH_3$ (15)	$R_3 = Ph; R_4 = CN$	$R_1 = R_2 = CH_3; R_3 = Ph; R_4 = CN$	65
$R_1 = Ph; R_2 = H$ (44h)	$R_3 = Ph; R_4 = CN$	$R_1 = R_3 = Ph; R_2 = H; R_4 = CN$	87
$R_1 = CH_3; R_2 = H$ (3)	$R_3 = Ph; R_4 = CN$	$R_1 = CH_3; R_2 = H; R_3 = Ph; R_4 = CN$	90

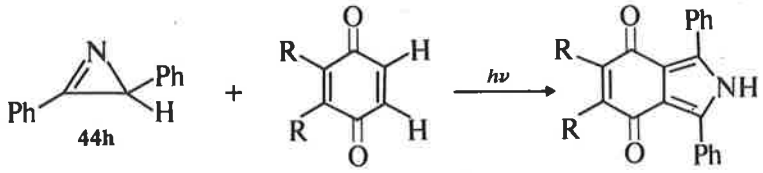
TABLE 9. PHOTOADDITION OF 3,3-DIMETHYL-2-PHENYL-1-AZIRINE WITH α,β-UNSATURATED CARBONYL COMPOUNDS.[a]

Compound	C=C Addition (%)	C=O Addition (%)
CH_2=CH–CHO	39	46
CH_3–CH=CH–CHO	–	84
C_2H_5O–CH=C(CH_3)–CHO	–	93
CH_2=CH–$COCH_3$	73	–
$(CH_3)_2$C=CH$COCH_3$	3	30
$(C_2H_5)_2$OP–CH=CH$COCH_3$	41	31
$Br^{\ominus}(Ph)_3\overset{\oplus}{P}$–CH=CH$COCH_3$	–	59
$(CH_3)_2$C=CH–COPO$(OCH_3)_2$	–	80

[a] Adapted from reference 212 with permission from *Heterocycles*.

α,β-Unsaturated carbonyl compounds may react with the benzonitrile ylides at the C=C component, at the C=O component, or at both, depending on the structural characteristics of the substrate. The reactions exhibit regiospecificity. In general, for relatively simple systems, the alkene and aldehyde groups appear to react at approximately equal rates, and both react faster than keto groups.[212] Steric hindrance and electronic factors may alter this order of reactivity. α,β-Unsaturated cyclic ketones such as cyclo-2-pentenone, cyclo-2-hexenone, and cyclo-2-heptenone react with azirine **15** exclusively at the C=C bond. Phosphorus-containing α,β-unsaturated compounds also react with 2-phenylazirines photolytically. Table 9 summarizes some data from representative α,β-unsaturated carbonyl systems.

1,4-Quinones (e.g., **421**) react at the C=C bond to give isoindolediones (**422**) in 30–40% yield.[239] Positions 5 and 6 in the quinone must be unsubstituted for the products **422** to form.

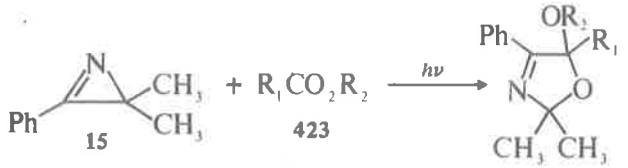


44h + **421** → **422**

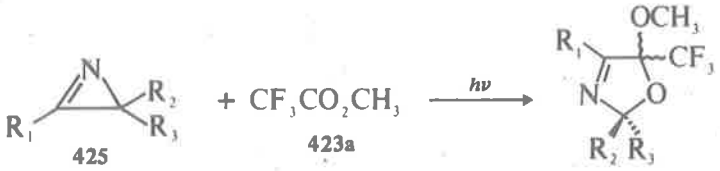
R = H, CH_3, OCH_3

d. WITH CARBOXYLIC ACID ESTERS, ANHYDRIDES, AND ACID CHLORIDES

Carboxylic acid esters (**423**), whose carbonyl groups are activated by electron-withdrawing groups in the alkyl or acyl moiety, react regiospecifically with benzonitrile ylides derived from 2-phenylazirines (e.g., **15**) to give 5-alkoxy-3-oxazolines (**424**).[240] Esters such as methyl acetate or methyl benzoate, which are not sufficiently activated, do not undergo these cycloadditions. Methyl trifluoroacetate was found to be the most reactive ester (Scheme 43). It underwent cycloaddition even to nitrile ylides derived from 2-alkylazirines.[241] Cycloadditions of 1-azirines that are monosubstituted at C-3 give rise to *trans-cis* mixtures of the 5-alkoxy-3-oxazolines.



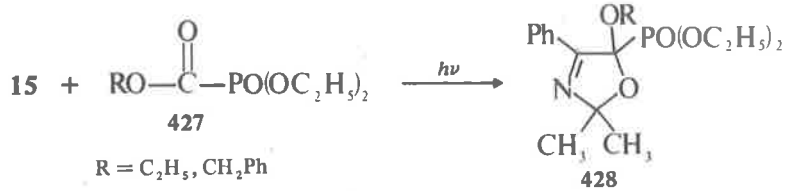
a. $R_1 = CF_3$; $R_2 = CH_3$
b. $R_1 = CH_2F$; $R_2 = CH_3$
c. $R_1 = CH_2Cl$; $R_2 = CH_3$
d. $R_1 = CH_2Br$; $R_2 = CH_3$
e. $R_1 = CO_2CH_3$; $R_2 = CH_3$
f. $R_1 = CN$; $R_2 = C_2H_5$
g. $R_1 = CH_3$; $R_2 = CH_2CF_3$
h. $R_1 = CH_3$; $R_2 = Ph$
i. $R_1 = CH_3$; $R_2 = CH{=}CH_2$
j. $R_1 = CH_3$; $R_2 = C{\equiv}CH$



a. $R_1 = Ph$; $R_2 = R_3 = H$ (24a)
b. $R_1 = Ph$; $R_2 = CH_3$; $R_3 = H$ (3)
c. $R_1 = Ph$; $R_2 = Ph$; $R_3 = H$ (44h)
d. $R_1 = R_2 = R_3 = Ph$
e. $R_1 = PhCH_2$; $R_2 = R_3 = H$ (44e)
f. $R_1 = R_2 = C_3H_7$; $R_3 = H$

Scheme 43 Methyl trifluoroacetate as a dipolarophile for nitrile ylides derived photochemically from 1-azirines.

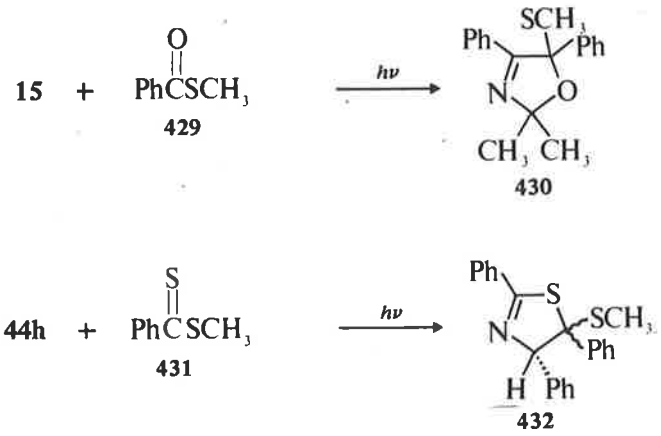
Ester carbonyl groups can be activated by the presence of other types of functionality. For example, ester carbonyls can be activated for photoaddition by the diethyl phosphonate residue. Thus, azirine **15** reacts photochemically with diethyl ethoxycarbonyl or benzyloxycarbonyl phosphonate (**427**) to give the corresponding 3-oxazolines (**428**) in almost quantitative yield.[212,224] The reaction is regiospecific, as expected.



$R = C_2H_5, CH_2Ph$

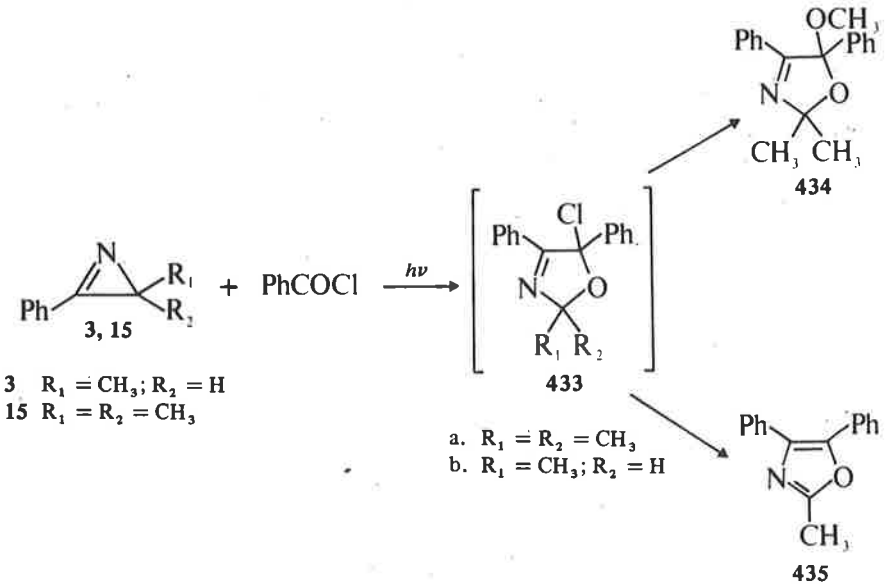
Thioesters are also reactive toward these nitrile ylides. *S*-Methyl thiobenzoate (**429**) reacts with azirine **15** to give the 3-oxazoline **430**.[240] However, in contrast

to the regiospecific addition with *S*-methyl thiobenzoate and other esters to produce Δ^3-oxazolines, the reaction of methyl dithiobenzoate **431** with 2,3-diphenyl-1-azirine (**44h**) proceeds with the inverse regiospecificity to give Δ^2-thiazolines (**432**). Since the azirine **44h** is monosubstituted at the 3-position, a mixture of *cis* and *trans* isomers is obtained (Scheme 44).[227,240]



Scheme 44 Photoinduced reaction of 2-phenylazirines with thioesters.

Cycloadditions have been observed also with acyl chlorides (Scheme 45).[242] Benzoyl chloride adds to the nitrile ylide from azirine **15** to give as the primary product the 5-chloro-4-phenyl-3-oxazoline **433a**. Because of its instability, this compound is best characterized as its 5-methoxy derivative **434**, obtained by



3 $R_1 = CH_3$; $R_2 = H$
15 $R_1 = R_2 = CH_3$

a. $R_1 = R_2 = CH_3$
b. $R_1 = CH_3$; $R_2 = H$

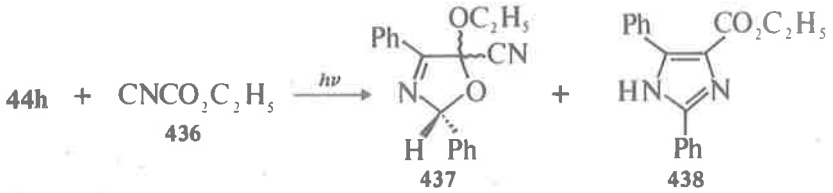
Scheme 45 Photochemical reaction of 2-phenylazirines with acid chlorides.

methanol treatment of **433a**. The primary adduct **433b**, which is monosubstituted at C-2, undergoes elimination of HCl on treatment with triethylamine to give the oxazole **435**. As discussed previously, the isomeric oxazole is produced in the thermal reaction of azirine **3** and benzoyl chloride.

A related photochemical reaction occurs with carboxylic acid anhydrides.[242]

e. WITH NITRILES

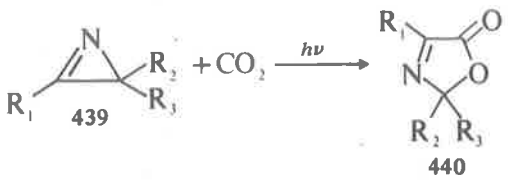
Under acid-catalyzed thermal conditions, 2-phenylazirines react readily with acetonitrile to furnish imidazolines. However, nitriles such as acetonitrile and benzonitrile are unreactive photochemically with 2-phenylazirines. "Activated" nitriles such as fluoroacetonitrile, trichloroacetonitrile, and 2- and 4-cyanopyridine react to give imidazoles.[212,226] Ethyl cyanoformate (**436**) interacts with azirine **44h** under photolytic conditions to give products of both carbonyl (**437**) and nitrile group (**438**) cycloadditions (Scheme 46).[237]



Scheme 46 Photocycloaddition of ethyl cyanoformate with 2,3-diphenyl-1-azirine.

f. WITH HETEROCUMULENES

The photoinduced combination of carbon dioxide with 2-phenyl- and 2-benzylazirines was first described by Schmid and his co-workers.[235] The reactions were carried out by passing carbon dioxide through benzene solutions of the 1-azirines under conditions of irradiation from a high pressure mercury lamp with a Vycor filter. Padwa and Wetmore[243] subsequently reported a similar photoaddition with carbon dioxide. The regiospecifically produced adducts in these reactions are 3-oxazolin-5-ones (Δ^3-oxazolin-5-ones), **440** (Scheme 47).



a. $R_1 = R_2 = Ph; R_3 = H$ (44h)	**440a** (65%)
b. $R_1 = Ph; R_2 = R_3 = CH_3$ (15)	**440b** (84%)
c. $R_1 = PhCH_2; R_2 = R_3 = H$ (44e)	**440c** (40%)
d. $R_1 = Ph; R_2 = CH_3; R_3 = H$ (3)	**440d** (88%)

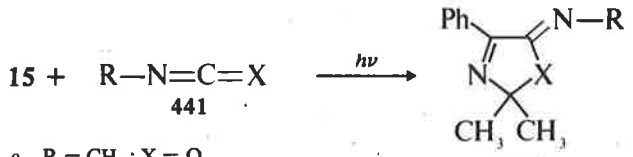
Scheme 47 Photoinduced combination of carbon dioxide with 2-arylazirines.

The photoaddition of carbon dioxide to these nitrile ylides is reversible, and irradiation of **440** results in generation of the starting nitrile ylides with quantum yields in the order of 0.3.[243]

When 3,3-dimethyl-2-phenyl-1-azirine (**15**) was photolyzed in the presence of carbon disulfide, only a 2:1 adduct, 5,5-spirobis(4,4-dimethyl-2-phenyl-2-thiazoline) could be isolated.[243]

Heterocumulenes such as isocyanates **441a** and **441b** and isothiocyanates **441c** and **441d** undergo photoreactions with 2-phenylazirines.[244,245] For isocyanates, reaction occurs at the C=O and isothiocyanates add at the C=S bond (Scheme 48). No photoreaction involving the C=N bond in these compounds was observed. A comparison of the thermal reaction with these heterocumulenes is of interest. Simple isocyanates and isothiocyanates such as **441a–441d** do not normally react under thermal conditions with 2-phenylazirines. However, the more reactive benzoyl isocyanate and benzoyl isothiocyanate are reactive under these conditions.

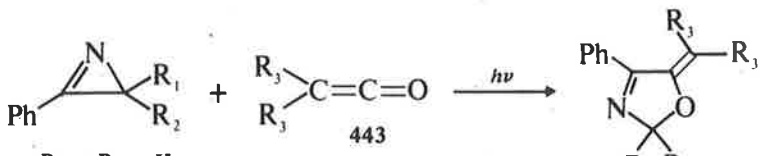
Carbodiimides (e.g., **441e**) undergo photoaddition to 2-phenylazirines (Scheme 48).[244,245]



a. $R = CH_3$; $X = O$
b. $R = Ph$; $X = O$
c. $R = CH_3$; $X = S$
d. $R = Ph$; $X = S$
e. $R = C_6H_4$–2-CH_3; $X = N$–C_6H_4–2-CH_3

Scheme 48 Reaction of isocyanates, isothiocyanates, and carbodiimides with 3,3-dimethyl-2-phenyl-1-azirine under photolytic conditions.

Ketenes react with 2-phenylazirines under thermal conditions (as previously discussed) to give, depending on the structure of the azirine, 1:2 adducts (bicyclic aziridine) or 1:1 adducts (pyrrolinones).[188–190] Photochemically, ketenes add to the nitrile ylides derived from 2-phenylazirines with participation of the C=O bond to give the 3-oxazolines **444**.[246]



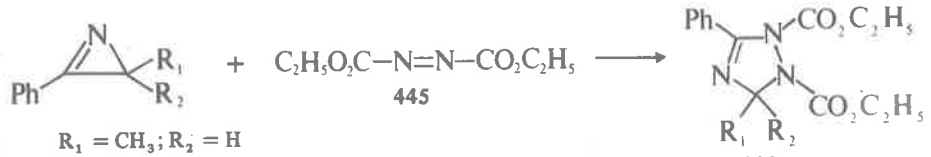
24a	$R_1 = R_2 = H$
3	$R_1 = CH_3$; $R_2 = H$
44h	$R_1 = Ph$; $R_2 = H$
15	$R_1 = R_2 = CH_3$

a. $R_3 = Ph$
b. $R_3 = H$

g. WITH AZO COMPOUNDS

2-Phenylazirines can interact photochemically with the N=N bond of azo compounds. Diethylazodicarboxylate (**445**) has been shown to react with 2-

phenylazirines (**3**, **15**, **24a**, **44h**) under irradiation to give Δ^3-1,2,4-triazolines (**446**) in good yields (*ca.* 50–70%).[247]



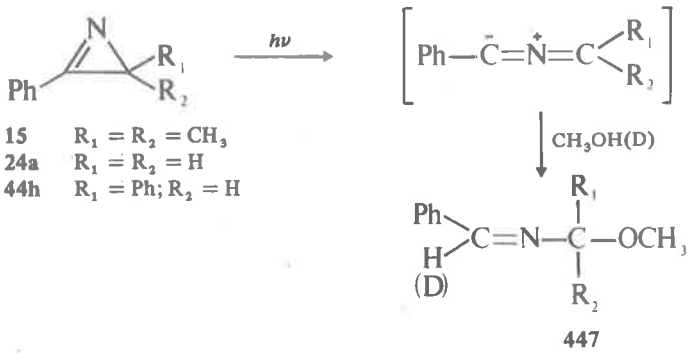
3 $R_1 = CH_3; R_2 = H$
15 $R_1 = R_2 = CH_3$
24a $R_1 = R_2 = H$
44h $R_1 = Ph; R_2 = H$

445

446

h. WITH ALCOHOLS

When 2-phenylazirines dissolved in methanol are photolyzed, almost quantitative yields of **447** are produced.[248,249] When deuterated methanol (CH_3OD) was used, the corresponding deuterated methoxyimines were produced (Scheme 49). Padwa and Smolanoff[248] suggested that these results provided good experimental evidence that in the HOMO of the nitrile ylide, the electron density at the disubstituted carbon is greater than at the trisubstituted carbon. The usefulness of these results in the explanation of the regiochemical data found in the photoaddition of 2-phenylazirines with dipolarophiles was discussed previously.



15 $R_1 = R_2 = CH_3$
24a $R_1 = R_2 = H$
44h $R_1 = Ph; R_2 = H$

447

Scheme 49 Photochemical addition of methanol to 2-phenylazirines.

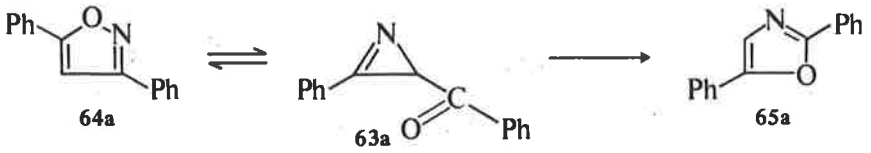
Products arising from initial protonation of the disubstituted carbon atom of these photochemically generated benzonitrile ylides have been observed in cycloadditions with ethyl cyanoacetate[240] and with ethyl acetoacetate.[238]

C. *Intramolecular Photorearrangements*

a. 3-AROYL-2-ARYL-1-AZIRINES

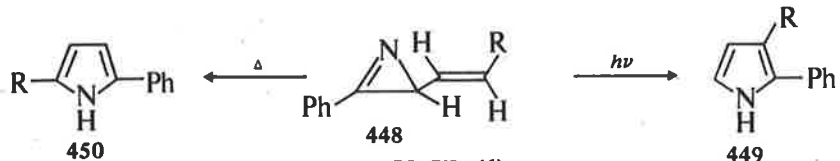
The first example of the intramolecular photochemical rearrangement of an azirine was reported by Ullman and Singh.[13,80,81] They discovered that the photo-

chemical behavior of 3-benzoyl-2-phenyl-1-azirine was markedly dependent on the wavelength of light used in the irradiation. With 3130 Å or shorter wavelength light (e.g., 2537 Å), **63a** rearranged almost quantitatively to the oxazole **65a**, whereas with 3340 Å light, quantitative conversion to the isoxazole **64a** was observed. Each reaction apparently proceeds with virtual exclusion of the other, since spectral monitoring produced nearly perfect isosbestic points. Using emission spectroscopy, sensitization experiments, quenching studies, and MO calculations, Singh and his co-workers[81] showed that a higher energy state associated with the nitrogen $n\pi^*$ transition results in azirine C–C bond cleavage and rearrangement to oxazole, whereas a lower energy excited state associated with the carbonyl $n\pi^*$ transition causes reorganization to the isoxazole.



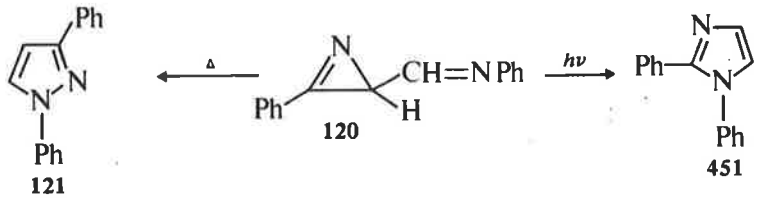
b. 3-VINYL-1-AZIRINES

Intramolecular rearrangement related to the conversion of **63a** to **65a** was reported by Padwa et al.[72, 73, 250] They found that irradiation of 3-vinyl-substituted azirines (**448**: *E* isomers) gave 2,3-disubstituted pyrroles (**449**). Thermolysis of **448**, however, results in the formation of 2,5-disubstituted pyrroles (**450**), as previously discussed. Photolysis of the 3-iminoazirine **120** gives the 1,2-diphenylimidazole exclusively, whereas its thermolysis affords 1,3-diphenylpyrazole (**121**).

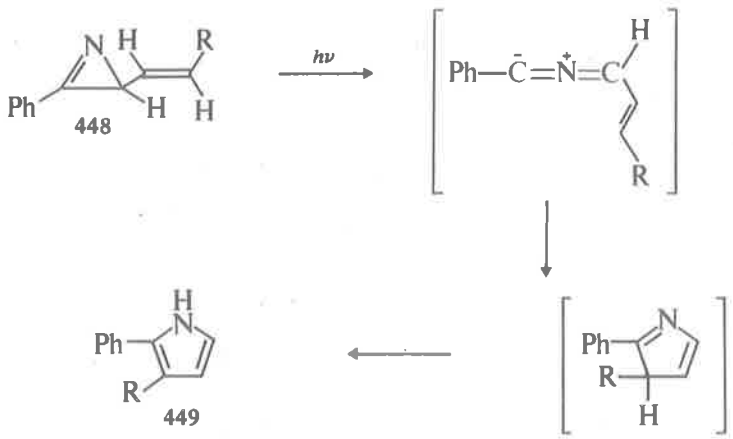


a. R = CO_2CH_3 (6)
b. R = CN
c. R = COPh
d. R = CHO

(**58a–58d**, *E* isomers)

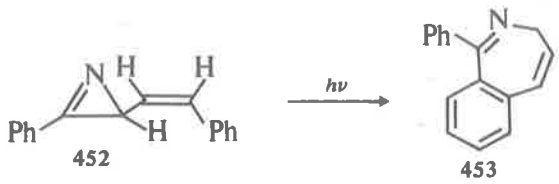


Evidence was provided[73] to support the suggestion that these photorearrangements proceed through the intermediacy of nitrile ylides (Scheme 50). Electrocyclization of the latter followed by a sigmatropic shift or shifts of the initially formed ring would give the observed products.



Scheme 50 Mechanism for the photorearrangement of 3-vinylazirines.

Photolysis of (*Z*)-2-phenyl-3-styryl-1-azirine **452** in contrast gave the benzazepine **453**. The isomeric *trans*-styrylazirine, however, produces 2,3-diphenylpyrrole as the major product. These results suggest that azirine ring cleavage and intramolecular cyclization proceed faster than isomerization of the styryl group. The formation of **453** from **452** also indicates that cyclization of the nitrile ylide from **452** to the seven-membered compound may be a faster process than the alternative cyclization to the five-membered pyrrole ring. Cyclization of the nitrile ylide from the *trans*-styrylazirine to a benzazepine is precluded on structural grounds. With naphthyl vinylazirines however, both (*Z*) and (*E*) isomers gave seven-membered rings, suggesting rapid C=C bond isomerization of the (*E*) isomers before azirine ring opening.[73]



c. 3-ALLYL-1-AZIRINES AND RELATED SYSTEMS

Padwa and Carlsen[29,251,252] examined the photochemistry of 3-allyl-1-azirines, particularly with respect to intramolecular cycloaddition. They reported that photolysis of 3-allyl-3-methyl-2-phenyl-1-azirine (**454a**) gave a 1:1 mixture of

azabicyclohexenes (**456a** and **457a**). On further irradiation, **457a** was quantitatively isomerized to **456a**. When the allylazirine **454b** (**21c**) was irradiated, the azabicyclohexene **456b** was produced as the primary photoproduct. Photolysis of the isomeric 3-allyl-2-methyl-3-phenyl-1-azirine **455a** gave **456a** and **457a**. The azirine **455b** gave the *endo* isomer **456b**, the thermodynamically less favored one, as the exclusive product on irradiation. From control experiments it was determined that azirines **454** and **455** were not being interconverted by a Cope rearrangement during the photolysis. The mechanism for the photoreactions has been proposed to proceed via C–C bond cleavage and generation of a bent nitrile ylide (carbene like) intermediate. Attack of the carbene carbon intramolecularly on the terminal position of the neighboring π bond generates a six-membered ring that may be regarded structurally as either a 1,3-dipole or a 1,3-diradical intermediate. Collapse of this intermediate results in the formation of the observed azabicyclohexenes. The photoconversion of **457** to **456** was explained in terms of the six-membered ring (Scheme 51). Formation of the thermodynamically less favored *endo* isomer **456b** from the irradiation of **454b** or **455b** was attributed to the greater torsional barrier in the transition state for cyclization to the *exo* isomer. Padwa and Carlsen classified these reactions in general terms as nonconcerted 1,1-cycloadditions.

A number of additional examples of these 1,1-cycloadditions, as well as spatial requirements and the role of substituents in controlling intramolecular cycloadditions, have been reported.[115,253–257] Several representative examples are discussed here.

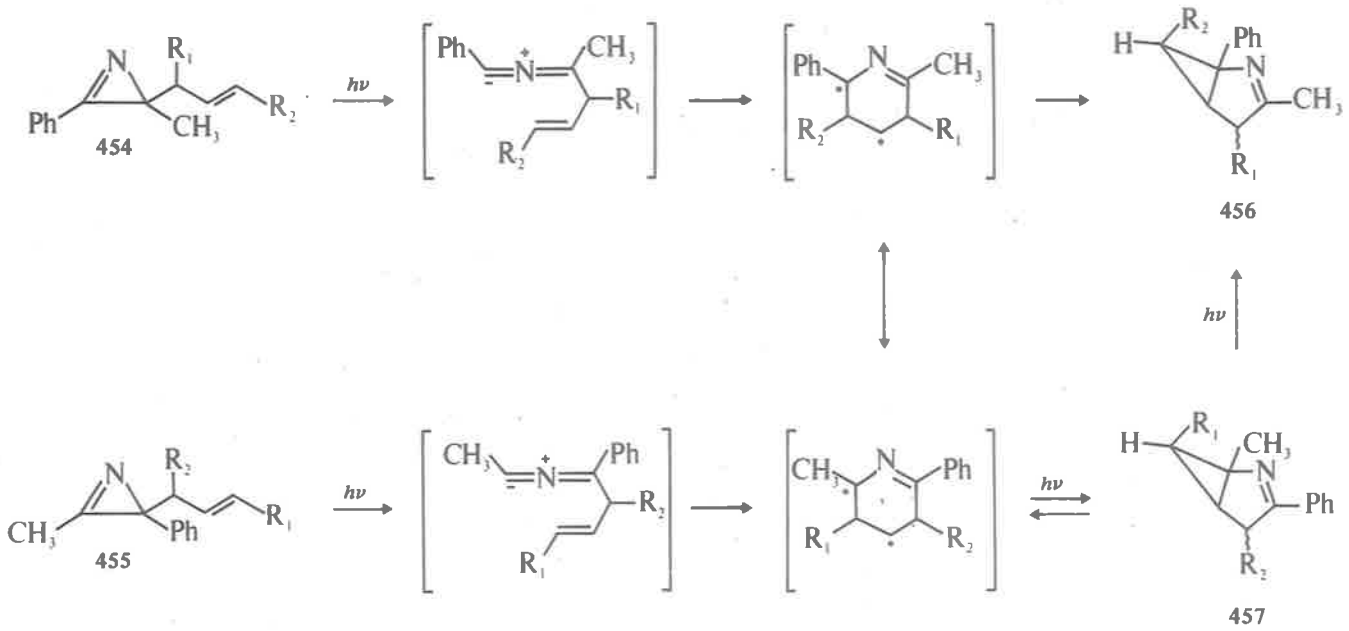
When the number of carbons between the azirine ring and the alkene moiety is increased from one (as in **454** and **455**) to three, 1,3-dipole cycloaddition is favored over the 1,1-cycloaddition. For example, the azirine **458** affords upon irradiation, a single photoproduct, the bicyclic pyrroline **459**.[115] Two interesting aspects of this conversion should be mentioned. First, the regiospecificity is opposite to that which would be expected on the basis of frontier orbital arguments. Padwa and Kamigata[115] attributed this change in regiospecificity to steric factors. Second, this intramolecular cyclization involves the cycloaddition of a nitrile ylide with an unactivated alkene, a substrate that is generally unreactive toward nitrile ylides in intermolecular cycloadditions.

The *o*-2-butenylphenyl-substituted 1-azirine **460**, however, gave a mixture of the *endo*- and *exo*-benzobicyclohexenes **462**, the product of 1,1-cycloaddition of the nitrile ylide **461**, in quantitative yield.[254,255]

The mode of cyclization of the related *o*-allyloxyphenyl-substituted azirine **463** appears to be markedly controlled by the nature of the substituent groups attached to carbon-3 of the azirine.[256]

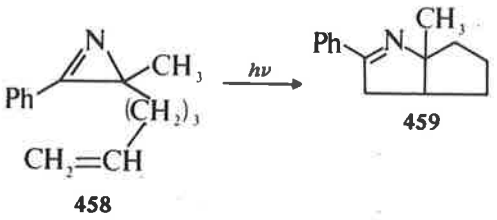
D. *Miscellaneous Photoreactions*

A number of other reactions of 2-phenylazirines induced by light have been investigated. For example, photolysis of a series of 3-hydroxymethyl-2-phenyl-1-azirine derivatives (**464**) was found to give 1-substituted 1-phenyl-2-azabutadienes

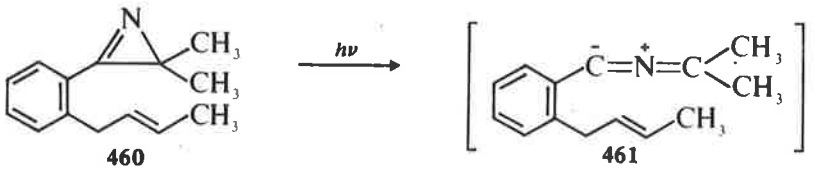


a. $R_1 = R_2 = H$
b. $R_1 = H; R_2 = CH_3$

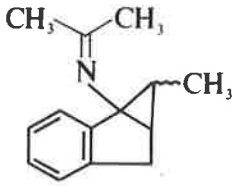
Scheme 51 Mechanism of formation of azabicyclohexenes from the photolysis of 3-allylazirines: asteriks indicates + or —. (Adapted from reference 29 with permission from the American Chemical Society.)



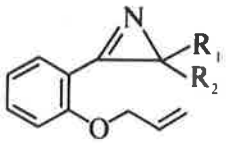
458 459



460 461

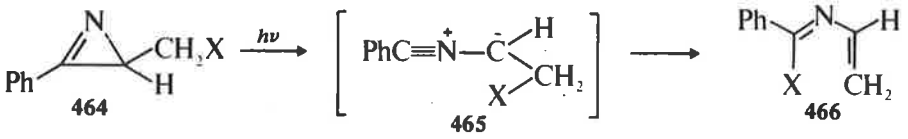


462



463

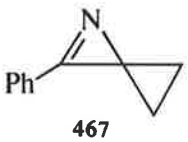
(466) in excellent yields. The conversion involves a 1,4-shift of the substituent X. The rate of the rearrangement was found to be directly related to the leaving group ability of X.[258]



464 465 466

X = Cl, Br, $OCOCH_3$, $OCOCF_3$, OCOAr

The photochemistry of the spiroazirine **467** has been reported.[249,259]



467

E. *Concluding General Remarks on the Photochemistry of 1-Azirines*

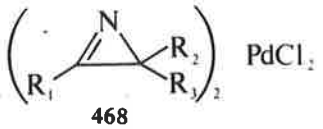
Spectroscopic data, sensitization and quenching experiments, MO calculations, and photoproduct analysis provide remarkably convincing evidence that 1-azirines, and particularly 2-phenyl-1-azirines, undergo photoreactions through initial 2–3 bond cleavage of the first excited $n\pi^*$ singlet state. This ring cleavage, which is photochemically reversible, produces a nitrile ylide that is the reactive participant in almost all the photochemical reactions, both intermolecular and intramolecular, discussed in this chapter. We have seen that the benzonitrile ylides derived from 2-phenylazirines can participate regiospecifically as 4π components in thermal 1,3-dipolar cycloadditions with species such as electron-deficient alkenes, as well as with carbonyl compounds, nitriles, imines, activated esters, azo compounds, and heterocumulenes. The benzonitrile ylides can also participate in thermal intramolecular reorganization processes such as 1,1-cycloadditions and 1,3-dipolar cyclizations. Ample precedent for some of these reactions can be found in the superb contributions of Huisgen and his co-workers on the chemistry of thermally generated nitrile ylides. The photochemical reactions of 1-azirines provide excellent routes to the synthesis of a wide variety of heterocyclic systems, particularly five-membered ring heterocycles.

6. Metal Complexes and Metal-Induced Reactions of 1-Azirines

The synthesis of metal-coordinated 1-azirines and the reactions of 1-azirines induced by metals have opened a new area in the chemistry of this small ring heterocycle. The mechanistic aspects of most of these reactions are not well understood. However, as this section of the chapter unfolds it will become apparent that many of the reactions mentioned here resemble previously discussed thermal and photochemical reactions of 1-azirines. Reactions of 1-azirines with some organometallic reagents (e.g., Grignard reagents, lithium aluminum hydride) and in the Reformatsky reaction were reviewed in an earlier part of this chapter.

A. *Synthesis of Stable Metal Complexes of 1-Azirines*

The synthesis of stable 2:1 complexes of 1-azirines with $AgSbF_6$, H_2PtCl_6, and $PdCl_2$ was achieved as early as 1971.[160b] The structure of the palladium and platinum complexes of azirines was elucidated by Hassner, Bunnell, and Haltiwanger[260] and confirmed by x-ray crystallography. These studies revealed coordination of the nitrogen of the azirine with palladium in a 2:1 azirine/$PdCl_2$ complex with a *trans* configuration about the planar palladium. It is of interest to compare these x-ray data with those reported recently for an uncomplexed 1-azirine.[275] Furthermore, the ir spectra of the series of 1-azirine–palladium chloride complexes **468** reported by Hassner et al.[260] showed strong C=N absorption bands in the 1760–1810 cm^{-1} region. This represents shifts of 30–40 cm^{-1} toward higher energy on complexation.



468

a. $R_1 = p\text{-}CH_3\text{–}C_6H_4$; $R_2 = R_3 = H$
b. $R_1 = p\text{-}OCH_3\text{–}C_6H_4$; $R_2 = R_3 = H$
c. $R_1 = Ph$; $R_2 = R_3 = CH_3$
d. $R_1 = Ph$; $R_2 = CH_3$; $R_3 = H$
e. $R_1 = Ph$; $R_2 = CO_2CH_3$; $R_3 = H$
f. $R_1 = Ph$; $R_2 = CH(OCH_3)_2$; $R_3 = H$
g. $R_1 = Ph$; $R_2 = CH_2OH$; $R_3 = H$
h. $R_1 = CH_3$; $R_2 = CH_3$; $R_3 = H$

These palladium complexes were found to exhibit relatively high stability toward air, moisture, and uv light. Thermolysis gave a complex mixture of products.

Stable zinc complexes of 2-amino-1-azirines have been reported.[261]

B. *Metal-Induced Reactions*

a. INSERTION REACTIONS

Alper and Prickett[262,263] studied the reaction of a series of azirines with diiron enneacarbonyl in benzene. They found that azirines undergo coupling and insertion reactions under these conditions to give diimine complexes (**469**) and ureadiiron complexes (**470**, **471**), as well as pyrroles and ketones. The yields of the diiron complexes **469–471** from three commonly encountered azirines are shown in Table 10. A mechanism for the formation of these products, which involves initial 1,3-bond cleavage and generation of a nitrene–iron carbonyl complex as intermediate, was proposed.

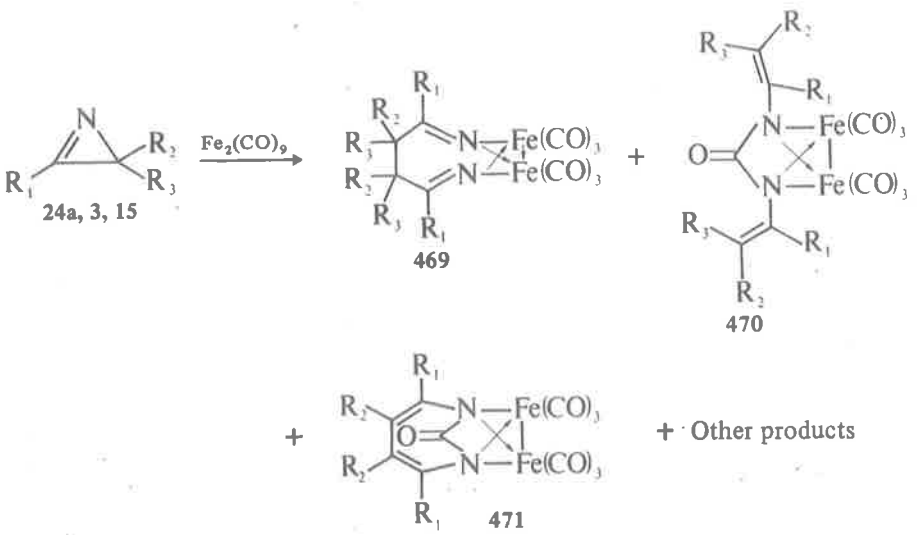
A related study was reported more recently by Schmid, Heimgartner, and their co-workers.[264]

b. DIMERIZATIONS

Reaction of 2-aryl-1-azirines with an equimolar amount of a group 6 metal carbonyl [$M(CO)_6$, M = Cr, Mo, W] gives 2,5-diarylpyrazines and isomeric dihydropyrazines in good yields (Scheme 52).[265,266] Conversion of 2-arylazirines

TABLE 10. YIELDS OF INSERTION AND COUPLING PRODUCTS **469–471** IN THE REACTION OF 1-AZIRINES WITH $Fe_2(CO)_9$

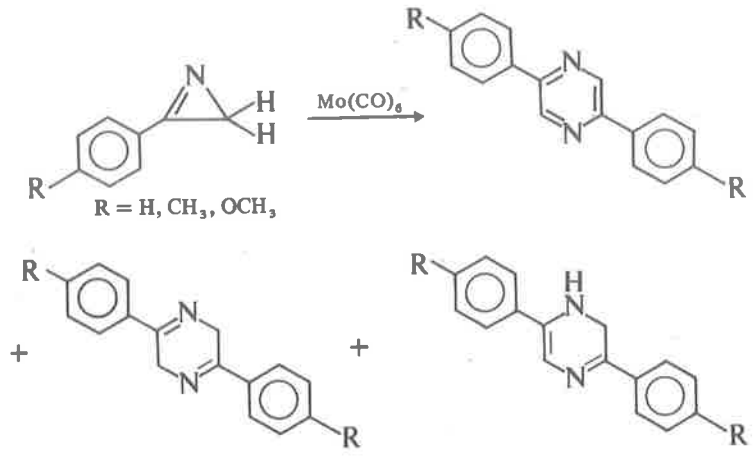
Azirine		Product	Yield (%)
24a	$R_1 = Ph$; $R_2 = H$; $R_3 = H$	**469**	7.8
		470	1.0
		471	3.0
3	$R_1 = Ph$; $R_2 = CH_3$; $R_3 = H$	**470**	9.1
15	$R_1 = Ph$; $R_2 = CH_3$; $R_3 = CH_3$	**470**	18.8



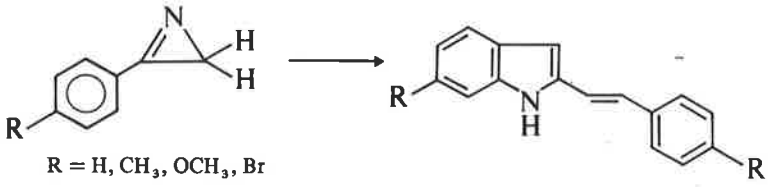
to 2,5-diarylpyrazines also has been reported.[267] The mechanisms of these reactions are poorly understood. It is interesting to compare these metal-induced dimerizations with the mechanistically well-established photochemical dimerizations of 1-azirines.

Dimerization reactions of 1-azirines with other transition metal compounds have been studied.[268]

Alper and Prickett[269,270] reported that 2-arylazirines can be converted to 2-styrylindoles with rhodium carbonyl compounds {e.g., $[Rh(CO)_2Cl]_2$ or $[(Ph_3P)_2Rh(CO)Cl]$} or with dicobalt octacarbonyl in benzene at room temperature (Scheme 53). The mechanism of this transformation is not understood. Although not formally a dimerization, the reaction is mentioned here mainly for convenience in presentation.



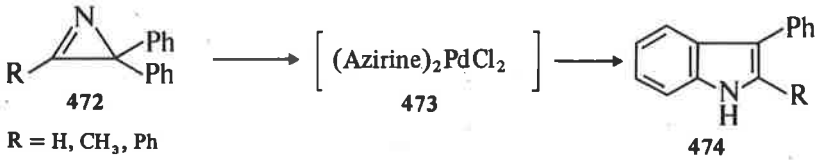
Scheme 52 Dimerization of 2-arylazirines induced by molybdenum hexacarbonyl.



Scheme 53 Intramolecular cyclization of 2-arylazirines by $[Rh(CO)_2Cl]_2$ and $Co_2(CO)_8$.

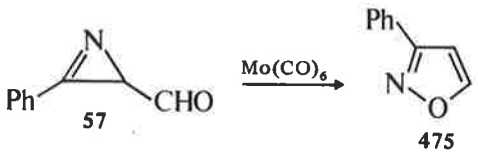
c. INTRAMOLECULAR CYCLIZATIONS

Taniguchi and his co-workers[271] showed that treatment of 1-azirines (**472**) with catalytic amounts of dichlorobis(benzonitrile)-palladium(II) at room temperature gave quantitative yields of the indoles **474**. These transformations presumably proceed through the intermediacy of the 2:1 azirine–palladium chloride complex **473**. Conversion of these azirines to indoles under uncatalyzed thermolytic conditions provides a mechanistically interesting comparison with the Pd(II)-catalyzed conversions. The C–N bond cleavage in the latter is apparently accelerated as a result of the coordination of the azirine to palladium. On the other hand, some platinum and palladium complexes of azirines were found to be extremely stable.[260]



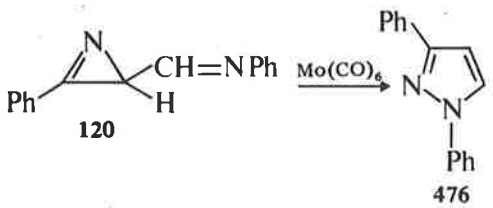
472 **473** **474**

When 3-formyl-2-phenyl-1-azirine (**57**) was treated with $Mo(CO)_6$ in tetrahydrofuran at room temperature for 5 hr, 3-phenylisoxazole (**475**) was isolated in 81% yield.[266] Photolysis converts **57** to **475** in 70% yield in 75 min. However, conversion of **57** to **475** by thermolysis requires 200° temperatures and reaction times of 3 days.[73]



57 **475**

Similarly, 1-azirines with *N*-arylimines at the 3-position (e.g., **120**), were cleanly converted to 1-aryl-3-phenylpyrazoles (e.g., **476**) in high yield by treatment with $Mo(CO)_6$.[266]

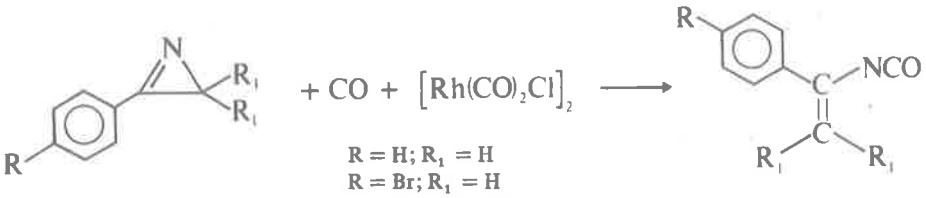


120 **476**

d. INTERMOLECULAR ADDITION REACTIONS

The reaction of 1-azirines with activated ketones to give pyrrole derivatives can be catalyzed by nickel(II) compounds. Excellent yields of pyrroles are generally obtained.[144] This and related conversions were discussed previously.

2-Aryl-1-azirines react with carbon monoxide at room temperature in the presence of chlorodicarbonyl rhodium(I) dimer to give isocyanates in yields of the order of 70–80% (Scheme 54). It was suggested that the isocyanates could arise either through carbonylation of a vinyl nitrene–rhodium complex or through carbonylation of a metallocyclic intermediate.[272]



Scheme 54 Carbonylation of 1-azirines catalyzed by rhodium(I).

An azirine-mediated formation of cyclopentadienone dimer from cyclopentadienyliron dicarbonyl dimer has been reported.[273]

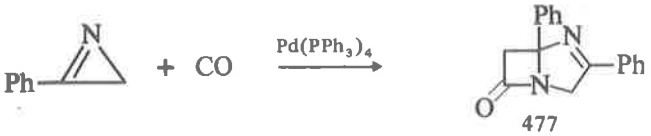
Heimgartner and his co-workers[274] studied the intermolecular cycloaddition of acetylenes with 2-phenylazirines induced by molybdenum hexacarbonyl. They isolated pyrrole derivatives that appear to arise from initial [2 + 2] cycloaddition followed by ring opening (Scheme 55).

15

Scheme 55 Addition of acetylene carboxylic esters to 2-phenylazirines induced by molybdenum hexacarbonyl.

7. Recent References

Recent work on 1-azirines is included in references 279–287. A noteworthy new development is the Pd catalyzed transformation of azirines to bicyclic β-lactams **477**,[279] as shown below:



477

VII. TABLES OF SYNTHETIC 1-AZIRINES

Tables 11–15 contain the structures, molecular formulas, melting points, boiling points, and literature references pertaining to preparation of most known 1-azirines. All boiling points given have the relevant pressures accompanying them in parentheses. For convenience in presentation, the compounds are classified according to the substitution at the 2-position of the azirine. These tables contain azirines with a wide range of interesting substituents at the 3-position. Ring-fused and spiroazirines also have been included.

TABLE 11. 2-ARYL-1-AZIRINES

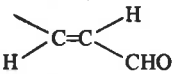
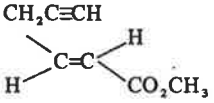
R_1	R_2	R_3	Molecular formula	m.p. or b.p. [°C (mm)]	Ref.
Ph	D	D	$C_8H_5D_2N$	–	82
4-F–C_6H_4	H	H	C_8H_6FN	63–66 (5.5)	43
4-Cl–C_6H_4	H	H	C_8H_6ClN	42.5–44.5	43
4-Br–C_6H_4	H	H	C_8H_6BrN	73–74.5	43
Ph	H	H	C_8H_7N	80 (10)	36
4-CF_3–C_6H_4	H	H	$C_9H_6F_3N$	42–44 (1.2)	43
Ph	CHO	H	C_9H_7NO	45–47	72–74
4-Cl–C_6H_4	CH_3	H	C_9H_8ClN	37–39 (0.05)	91
Ph	CH_3	H	C_9H_9N	96 (15)	8, 30
$PhCH_2$	H	H	C_9H_9N	74 (1.5)	8
4-CH_3–C_6H_4	H	H	C_9H_9N	75–76 (5)	43
4-OCH_3–C_6H_4	H	H	C_9H_9NO	101–102.5 (2.8) 29–31	43
Ph	CH_2OH	H	C_9H_9NO	58–59	249
4-Cl–C_6H_4	CO_2CH_3	H	$C_{10}H_8ClNO_2$	72–73	84
4-NO_2–C_6H_4	CO_2CH_3	H	$C_{10}H_8NO_4$	100	84
Ph	$COCH_3$	H	$C_{10}H_9NO$	–	81
Ph	CO_2CH_3	H	$C_{10}H_9NO_2$	98–102 (1) 45	8, 85
Ph	CH_3	CH_3	$C_{10}H_{11}N$	93.5–95 (15)	28, 122
Ph	CH=CHCN (*E* and *Z*)	H	$C_{11}H_8N_2$	–	73
Ph		H	$C_{11}H_9NO$	–	73
Ph	CH_2CHO	CH_3	$C_{11}H_{11}NO$	25 (0.05)	253
Ph	$CO_2C_2H_5$	H	$C_{11}H_{11}NO_2$	92–95 (0.5)	85
4-CH_3–C_6H_4	CO_2CH_3	H	$C_{11}H_{11}NO_2$	100–105 (0.6)	84
4-OCH_3–C_6H_4	CO_2CH_3	H	$C_{11}H_{11}NO_3$	108–112 (0.5)	84
Ph	CH_3	C_2H_5	$C_{11}H_{13}N$	37–40 (0.1)	91
Ph	$(CH_2)_3OH$	H	$C_{11}H_{13}NO$	–	249
Ph	$CH_2C{\equiv}CH$	CH_3	$C_{12}H_{11}N$	–	253
Ph		H	$C_{12}H_{11}NO_2$	–	73

TABLE 11. CONTINUED

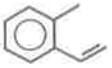
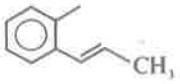
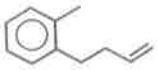
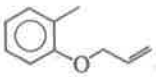
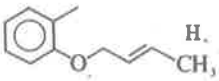
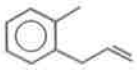
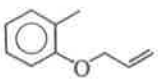
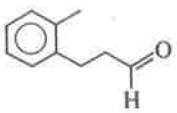
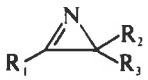
R_1	R_2	R_3	Molecular formula	m.p. or b.p. [°C (mm)]	Ref.
Ph	$-CH=NCH_2CH=CH_2$	H	$C_{12}H_{12}N_2$	–	105
Ph	$CH_2CH=CH_2$	CH_3	$C_{12}H_{13}N$	48–50 (0.04)	29
	CH_3	CH_3	$C_{12}H_{13}N$	43–45 (0.1)	255
	CH_3	H	$C_{12}H_{13}N$	57–60 (0.2)	255
	H	H	$C_{12}H_{13}N$	–	255
Ph	$-(CH_2)_2CHO$	CH_3	$C_{12}H_{13}NO$	–	115
	CH_3	H	$C_{12}H_{13}NO$	70–72 (0.01)	256
	H	H	$C_{12}H_{13}NO$	–	256
Ph	$-CH_2C\equiv CCH_3$	CH_3	$C_{13}H_{13}N$	80–81 (0.03)	33
Ph	$-(CH_2)_2CH=CH_2$	CH_3	$C_{13}H_{15}N$	69–71 (0.04)	115
	CH_3	CH_3	$C_{13}H_{15}N$	43–45 (0.3)	255
Ph	$-CH_2-\underset{\displaystyle CH_3}{\overset{\displaystyle CH_3}{C}}=CH_2$	CH_3	$C_{13}H_{15}N$	49 (0.01)	29
Ph	$-\underset{\displaystyle CH_3}{\underset{|}{CH}}-CH=CH_2$	CH_3	$C_{13}H_{15}N$	96–97 (4.0)	29
Ph	$-CH_2$, H, $C=C$, H, CH_3	CH_3	$C_{13}H_{15}N$	63–64 (0.04)	29
Ph	$-CH_2$, CH_3, $C=C$, H, H	CH_3	$C_{13}H_{15}N$	61–62 (0.1)	29
Ph	$-(CH_2)_3CHO$	CH_3	$C_{13}H_{15}NO$	–	115
	CH_3	CH_3	$C_{13}H_{15}NO$	75–77 (0.2)	256
	CH_3	CH_3	$C_{13}H_{15}NO$	–	255
Ph	$-CO_2(CH_2)_3CH_3$	H	$C_{13}H_{15}NO_2$	100–103 (0.5)	85
Ph	Ph	H	$C_{14}H_{11}N$	59–61	39
Ph	$-CH_2$, H, $C=C$, H, CO_2CH_3	CH_3	$C_{14}N_{15}NO_2$	–	253

TABLE 12. 2-ALKYL- AND 2-ARALKYL-1-AZIRINES



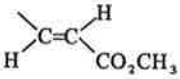
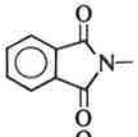
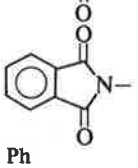
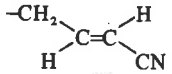
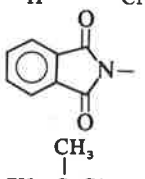
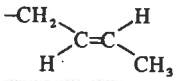
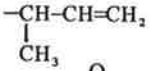
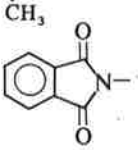
R_1	R_2	R_3	Molecular formula	m.p. or b.p. [°C (mm)]	Ref.
CH_3	H	H	C_3H_5N	42–43 (760)	82
F	CF_3	F	C_3F_5N	–	65, 66
CF_3	F	F	C_3F_5N	–	65, 66
C_2H_5	CH_3	Cl	C_5H_8ClN	–	71
CH_3	C_2H_5	Cl	C_5H_8ClN	–	71
CH_3	$CO_2C_2H_5$	H	$C_6H_9NO_2$	80–90 (70)	37
n-Bu	H	H	$C_6H_{11}N$	57 (54)	36
t-Bu	H	H	$C_6H_{11}N$	80 (760)	82
C_2H_5	C_2H_5	H	$C_6H_{11}N$	68 (130)	8
	CH_3	H	$C_7H_9NO_2$	–	75
CH_3	CH_3	$CO_2C_2H_5$	$C_7H_{11}NO_2$	95 (32)	37
CH_3		H	$C_{10}H_9NO$	40–41	81
$PhCH_2CH_2$	H	H	$C_{10}H_{11}N$	108–110 (10)	8
CH_3		H	$C_{11}H_8N_2O_2$	131	100
CH_3		CH_3	$C_{12}H_{10}N_2O_2$	78	100
CH_3	Ph	CH_2–CH=CH_2	$C_{12}H_{13}N$	52–53 (0.04)	29
CH_3		Ph	$C_{13}H_{12}N_2$	85 (0.02)	253
$CH_3CH_2CH_2$–		H	$C_{13}H_{12}N_2O_2$	46–47	100
CH_3		Ph	$C_{13}H_{15}N$	61–62 (0.05)	29
CH_3		Ph	$C_{13}H_{15}N$	66–67 (0.05)	29
CH_3		Ph	$C_{13}H_{15}N$	54–55 (0.05)	29
C_2H_5		C_2H_5	$C_{14}H_{14}N_2O_2$	–	100

TABLE 12. CONTINUED

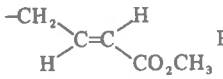
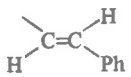
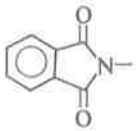
R_1	R_2	R_3	Molecular formula	m.p. or b.p. [°C (mm)]	Ref.
CH_3		Ph	$C_{14}H_{15}NO_2$	–	253
	Ph	H	$C_{16}H_{13}N$	67–68	75
CH_3		Ph	$C_{17}H_{12}N_2O_2$	119–120	102
C_8H_{17}	C_8H_{17}	H	$C_{18}H_{35}N$	121 (0.2)	70
$C_7H_{14}CO_2CH_3$	C_8H_{17}	H	$C_{19}H_{35}NO_2$	–	70
C_8H_{17}	$C_7H_{14}CO_2CH_3$	H	$C_{19}H_{35}NO_2$	–	70

TABLE 13. 2-AMINO-1-AZIRINES

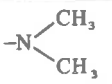
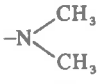
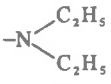
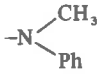
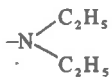
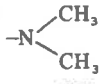
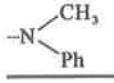
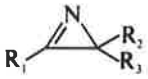
R_1	R_2	R_3	Molecular formula	m.p. or b.p. [°C (mm)]	Ref.
	CH_3	CH_3	$C_6H_{12}N$	–	76, 196b
	$-CH=CH_2$	CH_3	$C_7H_{12}N_2$	–	196b
	CH_3	CH_3	$C_8H_{16}N_2$	42 (1)	76
	CH_3	CH_3	$C_9H_{16}N_2$	48–49 (0.3)	76
	CH_3	H	$C_{10}H_{12}N_2$	71–74 (0.2)	78
	$-(CH_2)_5-$		$C_{11}H_{20}N_2$	62–63 (0.2)	76
	C_2H_5	Ph	$C_{12}H_{16}N_2$	–	196b
	Ph	H	$C_{15}H_{14}N_2$	94–96	78

TABLE 14. 2-UNSUBSTITUTED 1-AZIRINES



R_1	R_2	R_3	Molecular formula	m.p. or b.p. [°C (mm)]	Ref.
H	H	H	C_2H_3N	–	63
H	C_3H_7	H	C_5H_9N	–	9
H	C_2H_5	C_2H_5	$C_6H_{11}N$	61–62 (110)	9
H	Ph	H	C_8H_7N	–	49
H	C_3H_7	C_3H_7	$C_8H_{15}N$	63–64 (24)	276
H	Ph	CH_3	C_9H_9N	73–74 (3)	16
H	PhCH=CH– (*E* isomer)	H	$C_{10}H_9N$	–	104
H	PhCO	CH_3	$C_{10}H_9NO$	–	278
H	$PhCH_2CH_2$	H	$C_{10}H_{11}N$	–	9
H	Ph	C_2H_5	$C_{10}H_{11}N$	52–53 (1)	9
H	Ph	C_3H_7	$C_{11}H_{13}N$	80–82 (0.1)	277
H	Ph	Ph	$C_{14}H_{11}N$	–	9

TABLE 15. RING-FUSED AND SPIRO-1-AZIRINES; BIS-1-AZIRINES

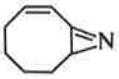
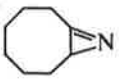
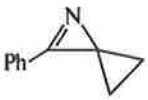
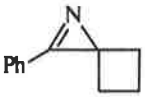
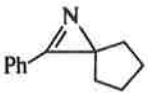
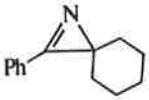
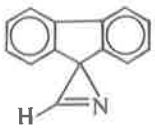
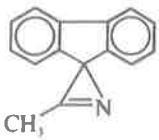
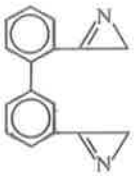
	Molecular formula	m.p. or b.p. [°C (mm)]	Ref.
	$C_8H_{11}N$	38 (0.2)	8
	$C_8H_{13}N$	76 (20)	8
	$C_{10}H_9N$	42–45 (0.1)	91
	$C_{11}H_{11}N$	–	249
	$C_{12}H_{13}N$	59–60 (0.1 mm)	249
	$C_{13}H_{15}N$	–	24

TABLE 15. CONTINUED

	Molecular formula	m.p. or b.p. [°C (mm)]	Ref.
	$C_{14}H_9N$	83–85 (decomp.)	48
	$C_{15}H_{11}N$	97–99	54
	$C_{16}H_{12}N_2$	84–85	96

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