The Synthesis and Palladium-Catalysed Coupling of 5-Trifluoromethanesulfonyluridine Derivatives

A Thesis Submitted Towards The Degree of

Doctor of Philosophy

by

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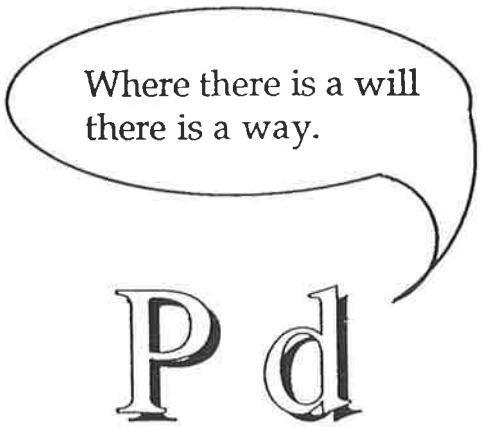
Where there is a will
there is a way.
P d

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Statement

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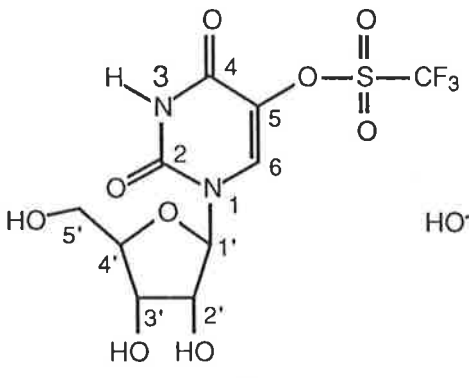
Abstract

A simple, high yielding procedure has been developed for the preparation of the novel 5-trifluoromethanesulfonyluridine **7** and 5-trifluoromethanesulfonyl-2'-deoxyuridine **8**. The application of these nucleoside triflates to palladium assisted coupling methodologies has been explored. In particular, the coupling of organostannane and copper derivatives has allowed for the introduction of structurally and functionally diverse hydrocarbons to the C5 centre.

A number of potentially biologically interesting 5-phenylalkynyl-2'-deoxyuridines have been prepared and considerable attention has been given to the effects of the aryl substituents on the preparation and potential activity of these nucleoside analogues. Approaches to a number of sugar modified C5-trifloxyl uridine derivatives have been explored, with particular emphasis given to those sugar modification that endow antiviral or anticancer activity upon the nucleoside.

Postulates for the hitherto undiscerned mechanism(s) of transmetallation and of some unexpected coupling products have been developed.



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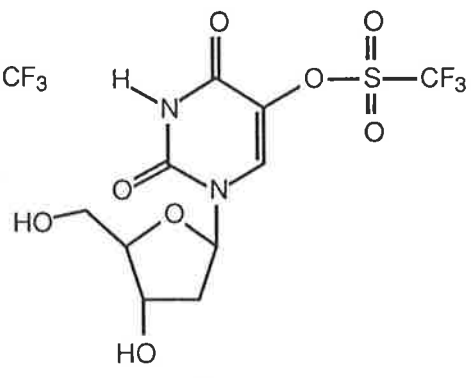

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Chapter 1

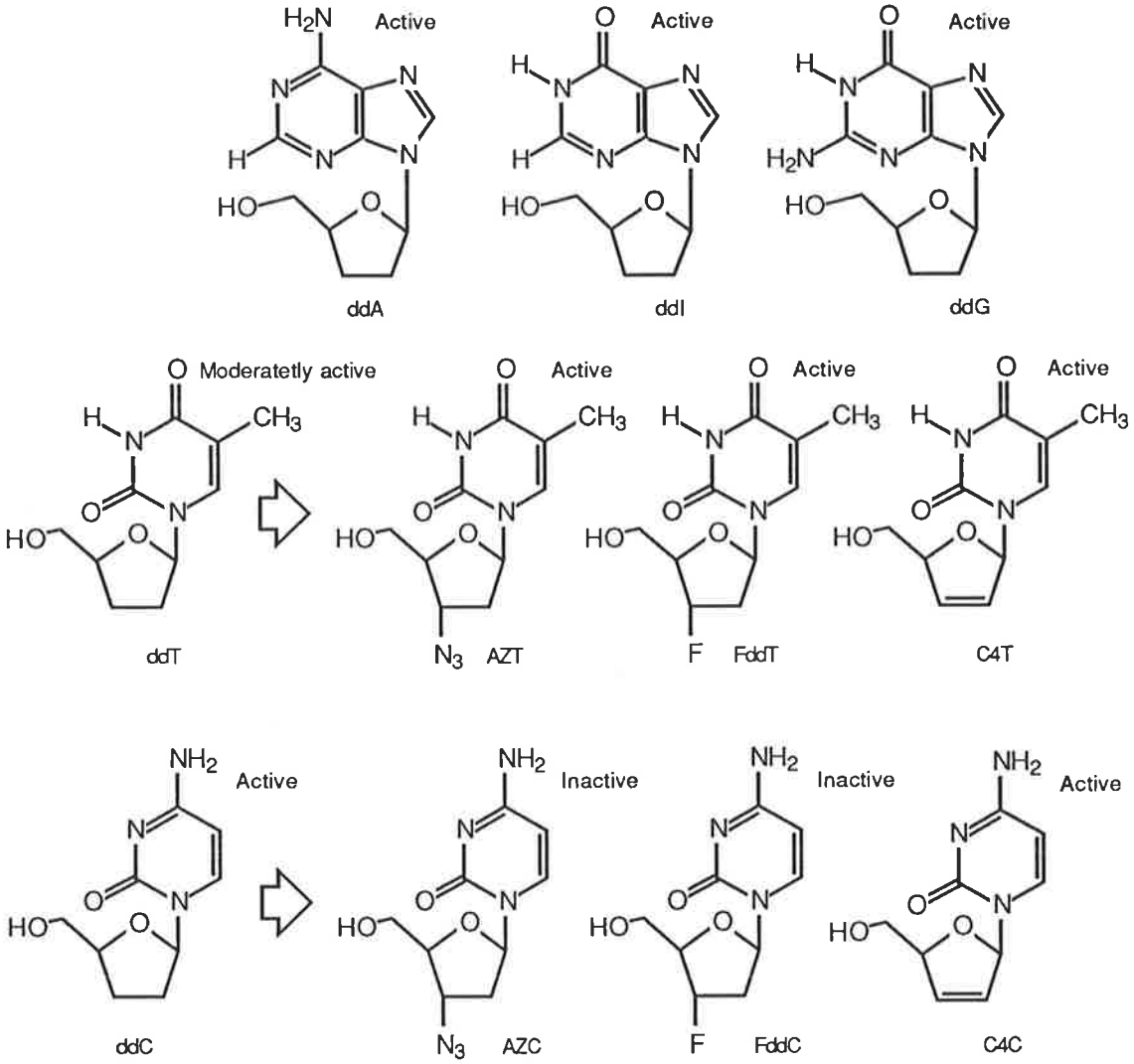
Introduction

The uncontrolled and unmitigated replication of viri and cancerous cells is the cause of many debilitating and often fatal diseases in humans. As the monomeric units of RNA and DNA, nucleotides play a key role in the replicative processes of biological systems. Appropriate modification of nucleotides and their nucleoside precursors has proved fruitful of recent in the development of effective antiviral and anticancer chemotherapies. These therapies are usually directed at the selective inhibition of viral and cancer cell replication.[1-3] Interest in this field has been intensified in view of the successful application of nucleoside analogues in the treatment of the Acquired Immune Deficiency Syndrome (AIDS).[1]

Despite this most recent interest in nucleoside analogues, from a more historical perspective, this area of chemistry has received considerably less attention in comparison to other classes of biological compounds such as alkaloids, carbohydrates, amino acids, steroids, terpenoids etc..

A large number of unnatural nucleoside derivatives have been shown to be active as inhibitors of the human immune deficiency viri (HIV) 1 & 2, the putative causative agents of AIDS.[1] The removal or substitution of the 2' and 3' hydroxyl groups appears to be of primary importance in deriving nucleosides that will be active against HIV. A broad spectrum of modified purine and pyrimidine based nucleosides have been prepared and tested, many show significant activity against HIV 1 & 2,[1,4,5] (Fig. 1). Whilst a large number of α and β-2' and/or 3' substituents have been tested, few have been shown to significantly increase the anti-HIV nature of the 2',3'-dideoxynucleosides. Two that have emerged as particularly effective are the 3'α-azido,[1,2,4,5] and the 3'α-fluoro groups.[3]

Figure 1
anti-HIV agents



ddA, 2',3'-dideoxyadenosine; ddI, 2',3'-dideoxyinosine; ddG, 2',3'-dideoxyguanosine; ddT,2',3'-dideoxythymidine; AZT, 3'-α-azido-2',3'-dideoxythymidine; FddT, 3'-α-flouro-2',3'-dideoxythymidine; C4T, 2',3'-didehydro-2',3'-dideoxythymidine; ddC, 2',3'-dideoxycytidine; AZC, 3'-α-azido-2',3'-dideoxycytidine; FddC, 3'-α-fluoro-2',3'-dideoxycytidine; C4C, 2',3'-didehydro-2',3'-dideoxycytdine.

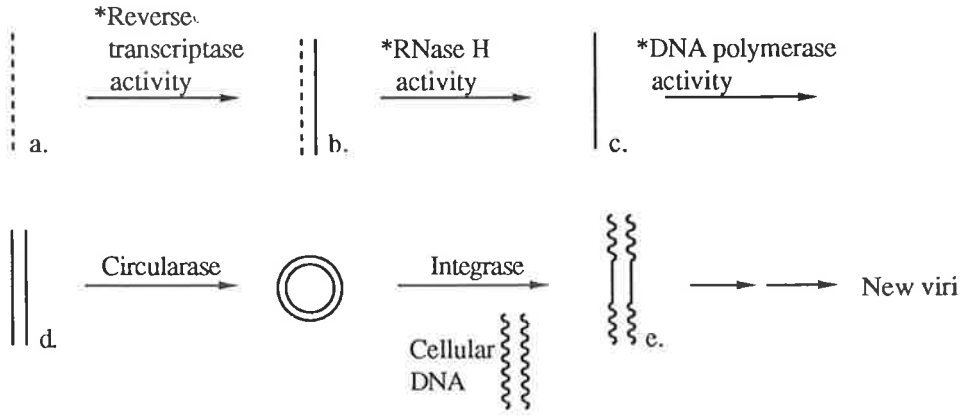
The replacement of the 2' and 3' hydroxyl groups with an endo cyclic double bond also endows some nucleosides with significant activity.[4,5] It is important to note, however, that the relationship between 2' or 3' substitution or dehydrogenation and activity is not always commutable from one nucleoside type to another, that is, the presence of a 3'α-azido or fluoro group, or the introduction of a 2',3' double bond only increases activity in specific compounds.

For instance, the introduction of any of the aforementioned functional groups onto the moderately active anti-HIV agent, ddT, to give AZT, FddT or D4T leads to a marked increase in activity in each case (Fig. 1). However, analogous substitutions in the case of the already highly potent 2',3'-dideoxycytidine (ddC) fails to give rise to any increase in activity. In fact a dramatic decrease in activity is observed for AZC and FddC, though C4C expresses comparable activity to ddC.[4,5] (Fig. 1).

The proposed mode of action of these dideoxynucleosides is as inhibitors of viral reverse transcriptase.[1,5-7] The function of viral reverse transcriptase is summarized in Scheme 1. After sequential phosphorylation by cellular kinases to give, ultimately, the dideoxynucleoside 5'-triphosphates, that is the dideoxynucleotides, these nucleotide analogues are able to compete with the natural nucleotides as substrates for viral reverse transcriptase. In fact some 2',3'-dideoxynucleotides have a greater affinity for this enzyme than do the natural nucleotides.[5] Also, the incorporation of these 2',3'-dideoxynucleotides into the viral DNA chain by the reverse transcriptase leads to termination of the DNA chain, since the absence of a 3'-hydroxyl means that a subsequent 3',5'-phosphodiester linkage cannot be formed.

Scheme 1

Funtions of viral reverse transcriptase*

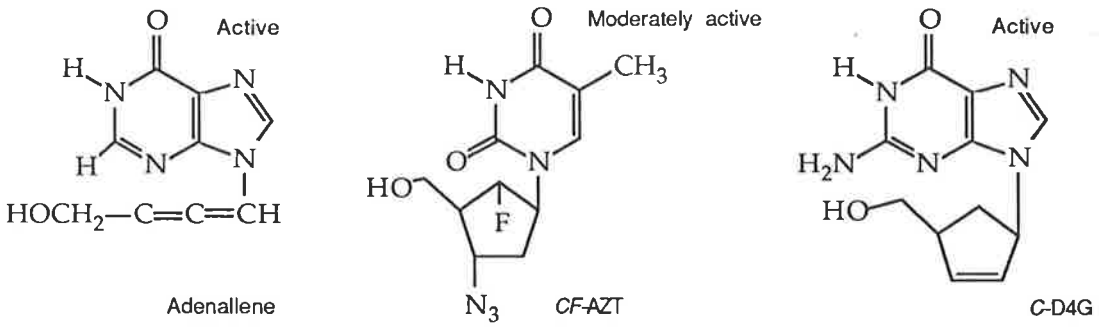


a. viral RNA; b. viral RNA/DNA complex; c. single stranded viral DNA; d. double stranded viral DNA; e. viral/cellular DNA product.

The selectivity of the 2',3'-dideoxynucleotides in inhibiting viral DNA synthesis and not cellular DNA synthesis is assumed to arise from two factors. Cellular DNA polymerase is much more substrate specific than is viral reverse transcriptase and is less likely to incorporate a modified nucleotide. Also, the host cell may have a greater capacity to repair the inclusion of a false nucleotide than does the virus.[8]

Other sugar modifications have been shown to be beneficial in the development of anti-HIV nucleoside analogues. The use of acyclic sugar mimics such as a 4-hydroxy-1,2-butadienyl group confers significant anti-HIV activity upon certain nucleoside analogues, for example, adenallene (Fig. 2).

Figure 2



Adenallene, 9-(4'-hydroxy-1',2'-butadienyl) adenine; *CF*-AZT, *carbofluorocyclic*-3'α-azido-2'-deoxythymidine; *C*-D4G, *carbocyclic*-2',3'-dideoxydidehydroguanosine (Carbovir).

An alternative stategy for modifying the sugar ring is to prepare nucleosides containing a carbocyclic sugar mimic.[1,9-12] Replacement of the ring oxygen with a methylene (CH_2) or fluoromethylene (CFH) group has given rise to nucleoside analogues which are metabolically more stable than the ribose equivalents which undergo cleavage of the glycosidic linkage by the phosphorylase enzymes.[13] These analogues have somewhat different potency compared to their ribose equivalents, either increased or decreased depending on the system. For example the carbocyclic analogue of AZT, *CF*-AZT,[14] is only a moderate inhibitor of HIV (Fig. 2).* By contrast the carbocylic-2',3'-didehydro-

* The abbreviation for the carbocyclic analogue is the same as that for the original nucleoside preceeded by a *C*-. The *C*- term is italicized so as to avoid confusion with the use of the C-

2',3'-dideoxyguanosine, C-D4G[1,10] is quite potent against HIV whereas the ribose equivalent, 2',3'-didehydro-2',3'-dideoxyguanosine (D4G, not shown), is almost completely inactive.[4,10] This is another example of how two relatively similar structural variations can have decidedly different effects on two different systems with regards to HIV inhibition.

An area of investigation that has, up until recently, received considerably less attention than has perhaps been justified has been the anti-HIV activity of nucleosides modified at both the sugar and base moieties. The nucleosides of this type that have been prepared and tested have included only rather simple base ring modifications such as the 5-chloro-3'-fluoro-2', 3'-dideoxyuridine, FClddU[4,15-7] (Fig. 3). This compound has been one of the few that has emerged as significant, in that whilst its potency against HIV was slightly decreased (slightly higher ED_{50}), its selectivity was dramatically enhanced (much higher CD_{50}) as compared to the parent 3'-fluoro-2',3'-dideoxyuridine, FddU,[4,18] giving a much higher selectivity index (SI) (Fig. 3). The selectivity index is the most common measure of the effectiveness of antiviral agents *in vitro*.

Undoubtedly the most interesting development as regards combined sugar and base ring modification has been the emergence of HEPT and related compounds HEPTa-c (Fig. 4). Most recently, Tanaka *et al*[19-22] have described the preparation and anti-HIV activity of over one hundred HEPT related compounds. Those compounds, illustrated as HEPTa-c, represent some of the most potent and selective HEPT analogues tested (*in vitro*), some expressing more potency and selectivity than does AZT.

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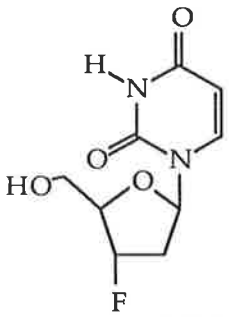
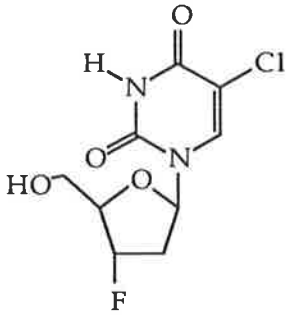
nucleoside which is applied to analogues in which the sugar moiety is joined to a carbon atom in the heterocyclic ring.[11]

Figure 3



FddU

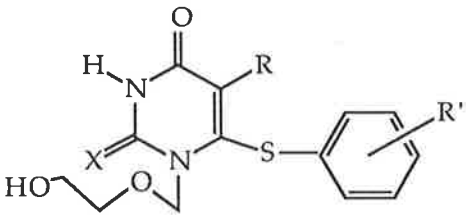


FCldd U

	ED_{50} (μM)	CD_{50} (μM)	SI
FddU	0.04	16.0	400
FddClU	0.38	535.0	1408

FddU, 3'-fluoro-2',3'-dideoxyuridine.
FCldd U, 5-chloro-3'-fluoro-2',3'-dideoxyuridine.
ED_{50}: 50% effective dose (the dose required to protect 50% of HIV infected cells against destruction).
CD_{50}: 50% cytotoxic dose (the dose required to reduce the viability of uninfected cells by 50%).
SI: selectivity index (the ratio of CD_{50} to ED_{50}).

Figure 4



	R	R'	X	SI
HEPT	M	H	O	106
HEPTa	*i*-Pr	3,5-Me_2	O	47400
HEPTb	E	3,5-Cl_2	O	1400
HEPTc	Et	3,5-Me_2	S	35500
AZT				1300

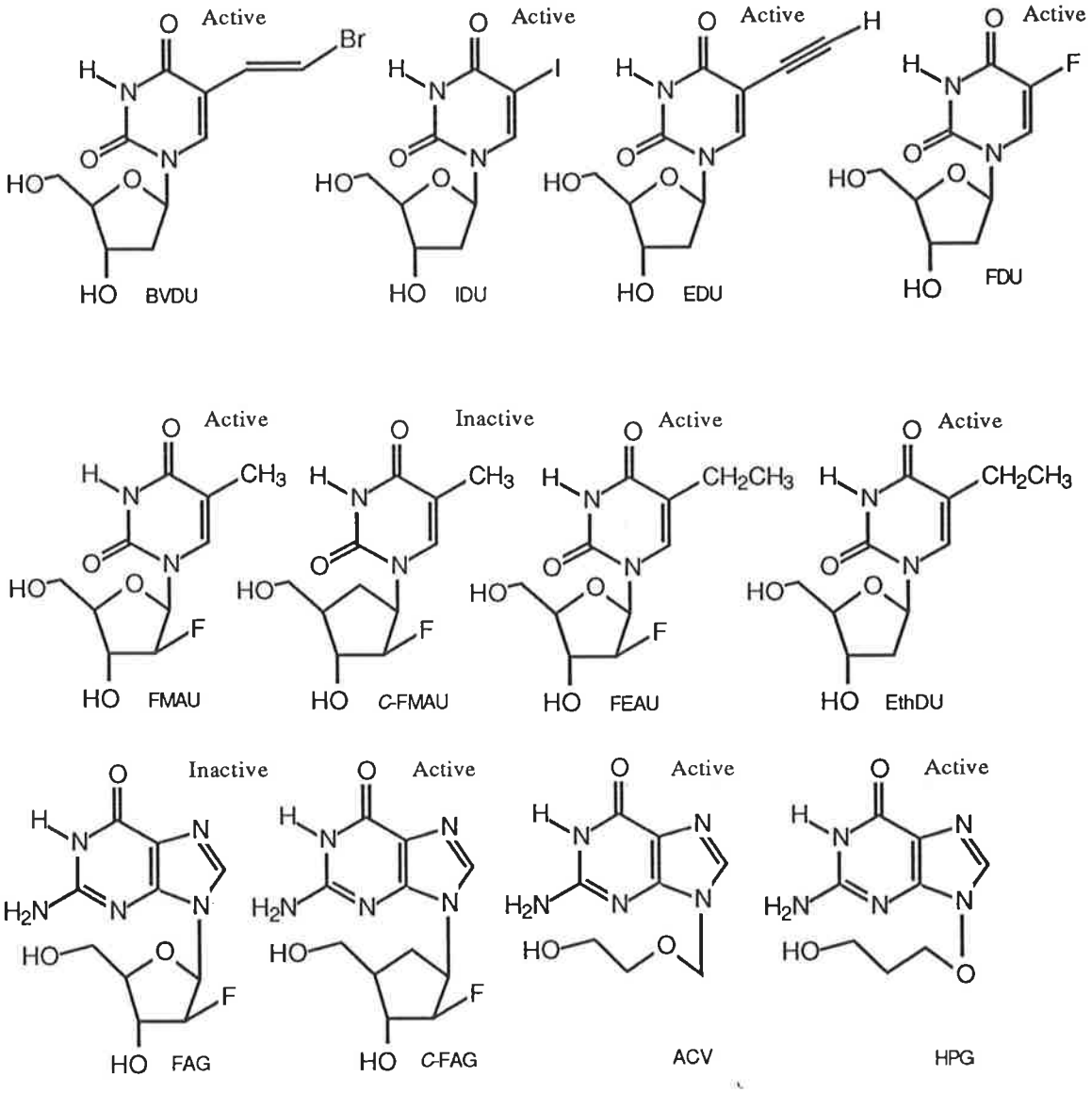
HEPT, 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine;
HEPTa, 6-[(3,5-dimethylphenyl)thio]-5-isopropyl-1-[(2-hydroxyethoxy)methyl]uracil;
HEPTb, 6-[(3,5-dichlorophenyl)thio]-5-ethyl-1-[(2-hydroxyethoxy)methyl]uracil;
HEPTc, 6-[(3,5-dimethylphenyl)thio]-5-ethyl-1-[(2-hydroxyethoxy)methyl]-2-thiouracil.

It should be noted that the discovery of AZT as a highly effective inhibitor of HIV infectivity and replication was rather serendipitous and predates the preparation and application of many other nucleoside analogues mentioned above to this field. Virtually all of the other nucleoside analogues mentioned above and many more that show activity against HIV have resulted from the preparation of a much larger body of compounds and a concerted analysis of their structure activity relationships.

For sometime now nucleoside analogues have been successfully applied as antiherpetics and as anticancer agents. Examples of some nucleoside analogues active against herpes are given in Fig. 5. Important modifications of the nucleosides appear to be substitution at the C5 position of the pyrimidine base rings with electron-withdrawing groups, the introduction of a 2'-β fluoride and the use of acyclic and carbocyclic sugar mimics, amongst others.

The C5 substitution of 2'-deoxyuridine has been exploited for some time now in the development of antiviral and anticancer agents. *E*-5-(2-Bromovinyl)-2'-deoxyuridine (BVDU)[23,24] and 5-iodo-2'-deoxyuridine (IDU)[25] are two potent antiherpetics that have found clinical applications. However many more C5 substituted 2'-deoxyuridines express significant antiviral/anticancer activity.[26-9] Structure activity relationship studies seem to indicate that the types of C5 substituents likely to confer activity are those which are electron-withdrawing, in conjugation with the base ring, lipophilic and not too sterically demanding (≤ 4 bonds).[27]

Figure 5
Antiherpetic and anticancer nucleoside derivatives:



BVDU, *E*-5-(2-bromovinyl)-2'-deoxyuridine; IDU, 5-iodo-2'-deoxyuridine; EDU, 5-ethynyl-2'-deoxyuridine; FDU, 5-fluoro-2'-deoxyuridine; FMAU, 2'-fluoro-5-methyl-arabinofuranosyl uracil; *C*-FMAU, *carbocyclic*-FMAU; FEAU, 2'-fluoro-5-ethyl-arabinofuranosyl uracil; EthDU, 5-ethyl-2'-deoxyuridine; FAG, 9-(2'-fluoroarabinofuranosyl) guanine; *C*-FAG, *carbocyclic*-FAG; ACV, 9-[(2-hydroxethoxy)methyl] guanine; HPG, 9-(3-hydroxypropoxy) guanine.

The introduction of a 2'β-fluoride has proved fruitful in conferring or enhancing antiherpetic activity in the case of many nucleoside analogues.[30-1] FMAU and FEAU are good examples of this (Fig. 5). The 2'β-fluoro analogue of thymidine, FMAU, is quite a potent antiherpetic, whilst obviously thymidine itself is not active. 5-Ethyl-2'-deoxyuridine (EthDU) is a potent inhibitor of the herpes symplex virus (HSV-1 & 2), this potency is retained in its 2'β-fluoro analogue and its selectivity is increased.

Nucleoside analogues bearing acyclic sugar mimics such as ACV,[33,34] and its more recently developed isomer 9-(3-hydroxypropoxy)guanine, HPG,[35] (Fig. 5) show selective antiherpetic activity.

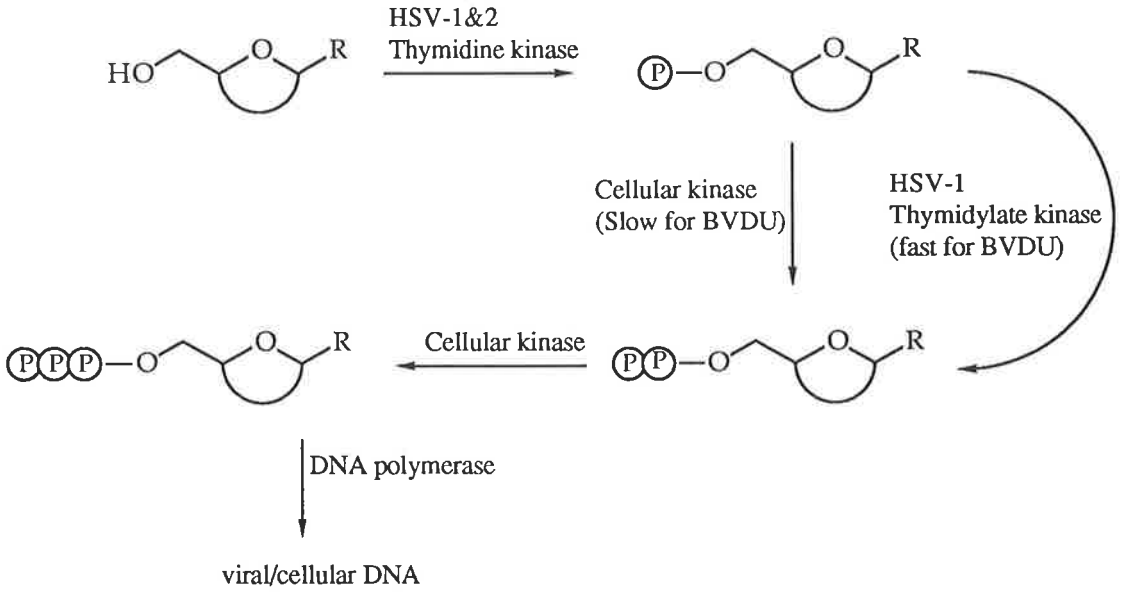
An interesting parallel between the structure activity relationship of carbocyclic nucleoside analogues expressing antiherpetic activity and those expressing anti-HIV activity is apparent.[9,10] FMAU, as mentioned above, is a potent, selective inhibitor of HSV 1 & 2, however carbocyclic-FMAU (C-FMAU) is a thousand times less active. On the other hand 9-(2'β-fluoroarabinofuranosyl)guanine (FAG) is almost inactive as an antiherpetic whereas its carbocyclic analogue, C-FAG, is a thousand times more active (Fig 5). This increased antiherpetic activity C-FMAU→FMAU and FAG→C-FAG parallels that observed in the anti-HIV case for similar purine and pyrimidine based nucleosides, where increases in activity were observed in going from C-AZT→AZT and D4G→C-D4G. In both cases the carbocyclic pyrimidine is much more active than the ribose equivalent but the carbocyclic purine is much less active than the ribose equivalent. No explanation is offered for this parallel, which may well be coincidental.

In general the mode of action of nucleoside analogues, such as those mentioned above, in their inhibition of the herpes simplex viri (HSV) 1 & 2 has been related to the fact that both these viri induce viral thymidine kinase (the

enzyme responsible for monophosphorylation of thymidine).[9,36] HSV thymidine kinase has a similar affinity for the aforementioned nucleoside analogues as it does for the natural nucleoside substrates, whereas the cellular kinases are significantly more selective for the natural substrates.[36] Thus selective phosphorylation of nucleoside analogues is achieved in infected cells over uninfected cells (Scheme 2). After monophosphorylation subsequent phosphorylation can be achieved by cellular and viral kinases to give, ultimately, the nucleoside triphosphates, that is, the nucleotide analogues.

Scheme 2

Proposed mode of action for anti-HSV 1 & 2 nucleoside analogues:



These nucleotide analogues show similar affinity towards viral DNA polymerase as do the natural nucleotides but, again, are selected against by the cellular DNA polymerase.[38-40] Hence the selectivity for the incorporation of nucleoside analogues into viral and not cellular DNA is two tiered. The modified nucleotide may act as either substrate[38,39] or inhibitor[38,40] of the viral DNA polymerase.

The inclusion of some of these modified nucleotides into the viral DNA chain may lead to "mistakes" in transcription and thus nonviable viral products.[41] In the case of nucleotide analogues such as ACV triphosphate where

no 3'-hydroxyl group exists the DNA chain is terminated upon its incorporation since, as seen previously, no new phosphodiester linkage can be formed. Furthermore, the viral DNA polymerase becomes irreversibly bound to the fraudulent nucleotide it has just incorporated.[9]

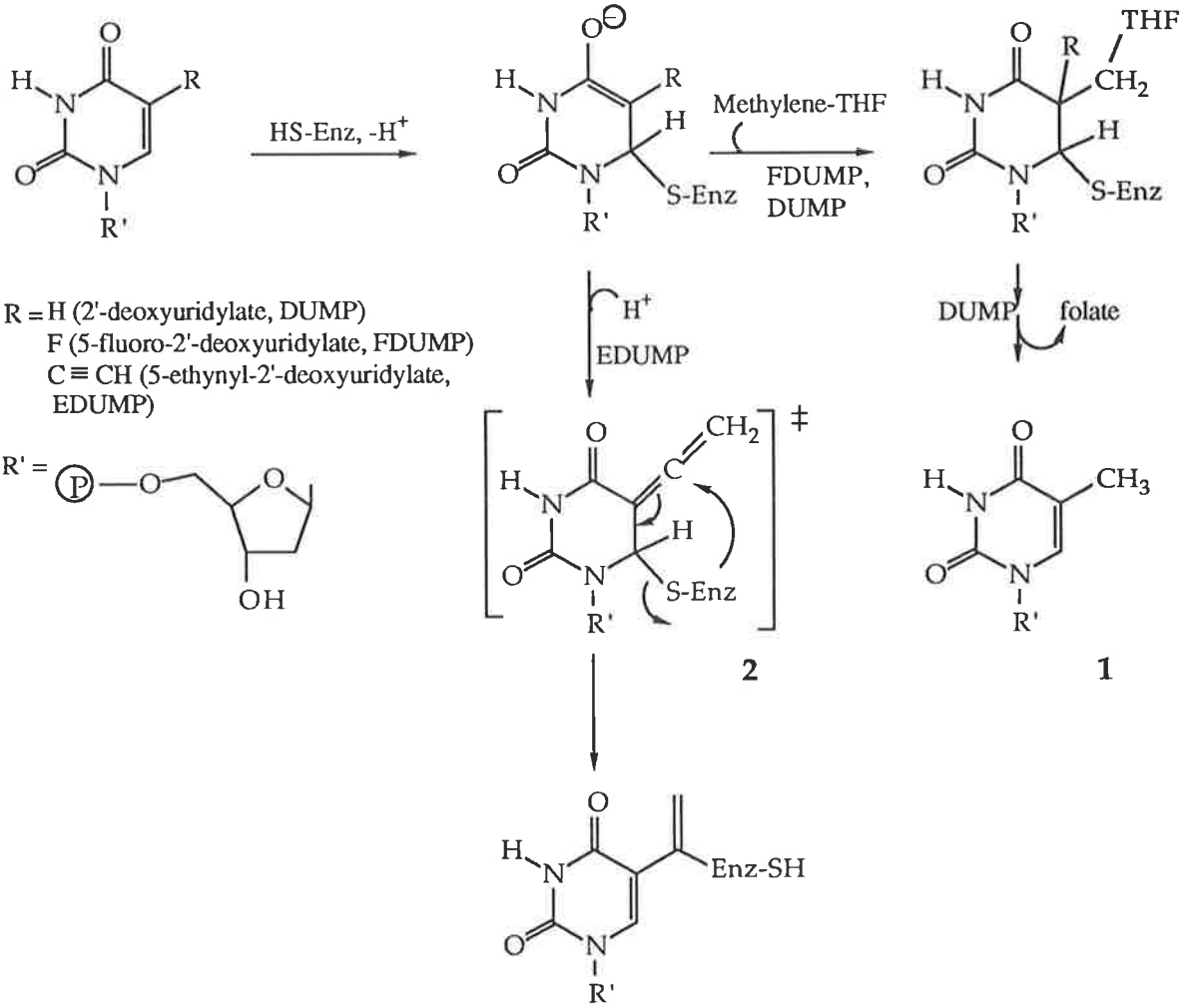
It should also be added that in the case of BVDU, the monophosphate is only effectively phosphorylated by viral thymidylate kinase to give the BVDU diphosphate (Scheme 2). HSV-2 does not induce viral thymidylate kinase. Thus BVDU is most effective against HSV-1 which does induce this enzyme.[42]

Another important enzyme that has been targeted in the development of antiviral and anticancer therapies has been thymidylate synthase.[43,44] This enzyme catalyses the conversion of 2'-deoxyuridine-monophosphate (2'-deoxyuridylate, dUMP) to thymidine-monophosphate (thymidylate). Cells that are rapidly dividing or cells in which a DNA virus is rapidly multiplying require an abundant supply of thymidylate. Thymidylate synthase has been identified as a primary site of action of 5-substituted-2'-deoxyuridine compounds in the inhibition of murine leukemia (L1210) cell growth.[45]

Two important examples of 5-substituted-2'-deoxyuridines of this class are 5-fluoro-2'-deoxyuridine, FDU,[43,44] and 5-ethynyl-2'-deoxyuridine, EDU, (Fig. 5).[23,45-6] 5-Fluoro-2'-deoxyuridine can be generated *in vivo* by glycosylation of 5-fluorouracil, this prodrug has been in clinical use for years. FDU undergoes monophosphorylation in the cell via cellular and viral thymidine kinases to give 5-fluoro-2'-deoxyuridylate (FDUMP). EDU only undergoes monophosphorylation with viral thymidine kinase (as with ACV and BVDU), so that EDUMP will only form in virally infected cells or virally induced cancer cells, that induce viral thymidine kinase (i.e. are TK^+).[47] Both these nucleoside monophosphate analogues have been shown to be mechanism-based inhibitors of thymidylate synthase (Scheme 3).[43,47-50]

Scheme 3

Mechanism based inhibition of thymidylate synthase:



As with the usual substrate of thymidylate synthase (DUMP) the analogues undergo the usual cysteine thiol Michael addition of the enzyme to the C6 position. The enolate thus generated is then thought to add to the electrophilic cofactor N^5,N^{10}-methylenetetrahydrofolate. Normally the C5 proton and the thiol enzyme would be eliminated and subsequent hydride transfer from the folate to the methylene group would give thymidylate **1** and folate. However in the case of FDUMP and EDUMP no C5 proton is present and the enzyme remains irreversibly bound to the substrate analogue to form a covalent complex.

In the case of EDUMP some evidence for an alternative mechanism also exists (Scheme 3).[47,49] In this mechanism the initially formed enolate leads to the formation of a transient allene **2**. This highly reactive species would readily

undergo nucleophilic attack from some other site within the enzyme to give a different complex with the enzyme and substrate analogue covalently bound. This alternative mechanism for EDUMP is consistent with other β,γ-acetylenic carbonyl suicide substrates.[48,49,51]

Other enzymes which require nucleosides or their 5'-mono, di or triphosphate equivalents as substrates and which are necessary for the *de novo* biosynthesis of DNA, such as ribonucleoside diphosphate reductase (RDPR), have been targeted in the development of anticancer chemotherapies[55] but they are not discussed here.

Clearly sugar and base derivatized nucleosides have, and will continue to have, an important role to play in the development of antiviral and anticancer agents. Many of the sugar ring modifications mentioned above have only somewhat limited general applicability, except for perhaps the removal of the 2' & 3' hydroxyl groups in the development of anti-HIV agents, making it virtually impossible to predict the effect of a certain structural change upon the activity. C5 substitution of pyrimidine base nucleosides has proved extremely fruitful in the development of biologically active compounds and certain generalization have been made about the nature of substituents likely to endow a nucleoside analogue with antiherpetic or anticancer activity.[27] However, the structural and functional group diversity of substituents tested to date have been somewhat limited by the chemistry employed in their introduction to the C5 centre. Also, only relatively few studies of antiviral/anticancer activity of nucleoside analogues modified in both the sugar and base moiety have been reported.

In view of these limitations it would appear advantageous to develop methods that would allow for the facile elaboration of a base ring, in particular C5 substitution of a pyrimidine base. It would also be of particular advantage if the precursor to C5 substitution was stable to the conditions of sugar ring

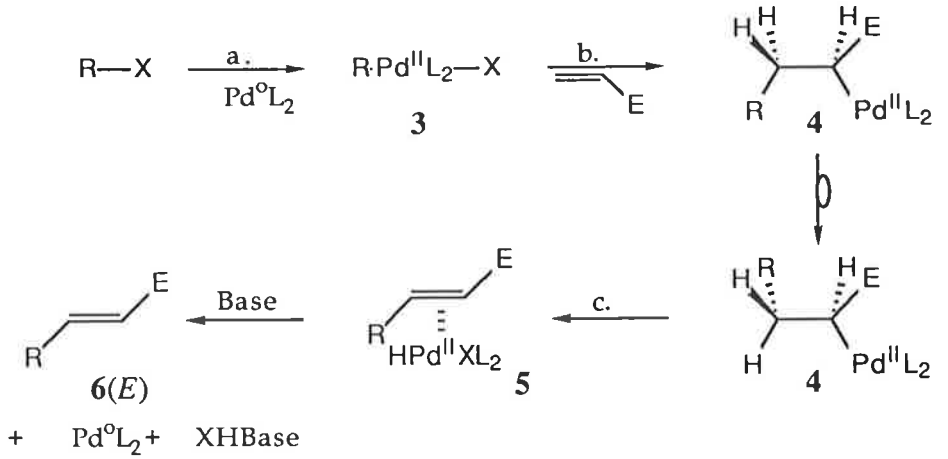
modification and that the method of C5 substitution be tolerant to a large range of functionality in order not to limit the type of modifications that can be applied to the sugar moiety.

The development of transition metal catalysts for organic synthesis has been a prominent feature of synthetic chemistry over the last fifteen years. Many of the techniques developed could potentially revolutionize more conventional strategies for the preparation of carbon frameworks. In particular, palladium-catalysed carbon-carbon bond formation has emerged as an extremely facile technique, tolerant of a large range of functional groups. A number of palladium-catalysed coupling techniques have been utilized in the modification of the purine and pyrimidine bases of nucleosides.[31-3,53-61]

Much of the early work in the development of palladium-catalysis for organic synthesis was initiated by Richard F. Heck.[62-4] The Heck reaction has become a popular synthetic tool in research laboratories as well as finding increasing application in the preparation of industrial and pharmaceutical compounds.[64]

The Heck reaction results from palladium's rather fortuitous combination of three important carbon-metal interactions, as shown in Scheme 4.[63] Firstly, oxidative addition, in this step zero valent palladium displaces the leaving group (iodide) with concomitant oxidation of the palladium to give a divalent σ-palladium complex **3**. This complex is capable of undergoing *syn*-addition to an activated double bond to give a different σ-palladium(II) complex **4**. *Syn*-elimination of the palladium and a β-hydride occurs readily to give the substituted olefin stereoselectively (*trans* isomer only) as a π-palladium(II) complex **5**. Displacement of the olefin by another ligand and reductive elimination of HX from the palladium by base gives the product olefin **6** and regenerates the zero valent catalyst.

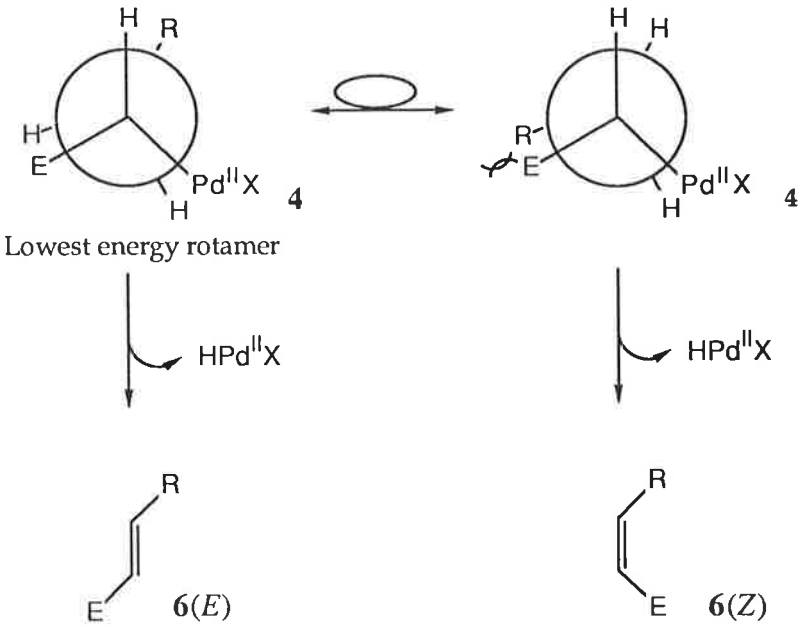
Scheme 4



a. Oxidative addition; b. *Syn*-addition; c. β–hydride elimination
R = aryl or alkenyl
X = halide (I or Br)
E = electron-withdrawing group

The *trans* only stereochemistry of **6** arises from the lowest energy conformation of **4** for the all eclipsed transition state of *syn*-elimination (Fig. 6).

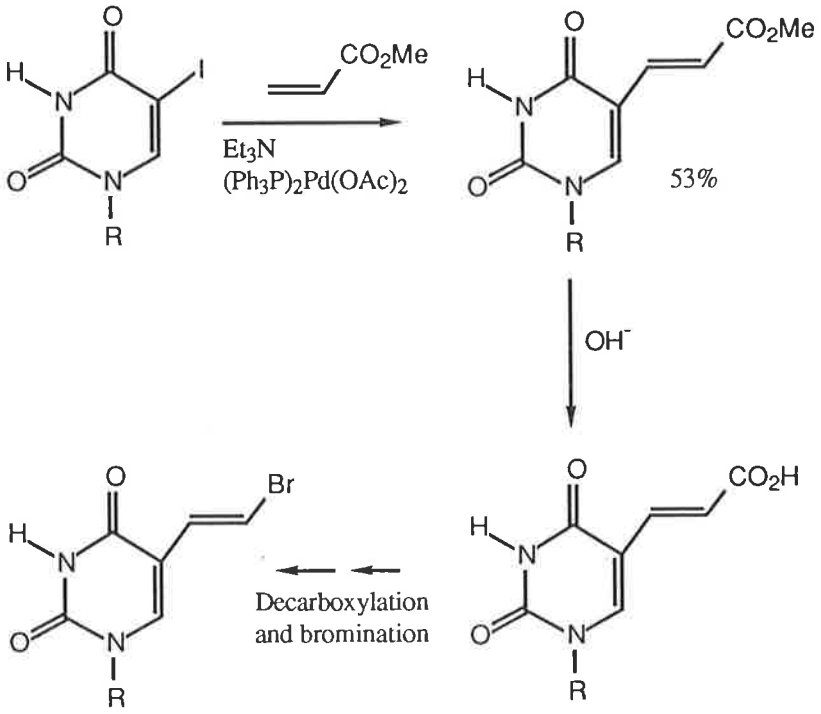
Figure 6



Bergstrom utilized the Heck reaction as a convenient approach to *E*-2-substituted 5-alkenyl-pyrimidine nucleosides.[58] This approach has been subsequently applied to the synthesis of BVDU and related compounds (Scheme 5).[59,60] The process involves initial substitution of the iodo group with an

activated olefin followed by basic hydrolysis, decarboxylation and bromination to give the *E*-2-bromovinyl products.

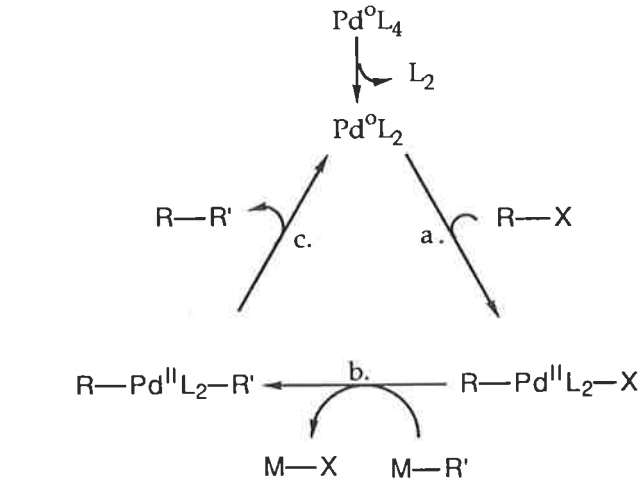
Scheme 5



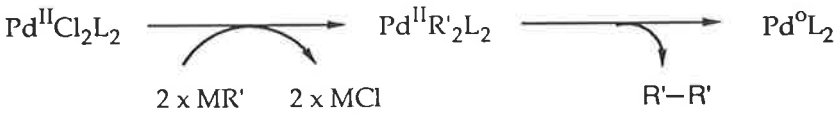
R = 2'-deoxyuridine, BVDU

A more recently developed palladium-catalysed coupling involves the coupling of organohalides or trifluoromethanesulfonates (triflates) with organometallics. A detailed mechanistic discussion is given in Chapter 3, however the process can be summarized by three major steps as outlined in Scheme 6. The intial step, as with the Heck reaction, is oxidative addition of an organohalide (or triflate) to the palladium(0) species to give an organopalladium(II) halide or triflate. Transmetallation with an organic nucleophile effects the formation of the required diorganopalladium(II) complex. Reductive elimination of the two organic units gives the coupled product and regenerates the palladium(0) catalyst.

Scheme 6

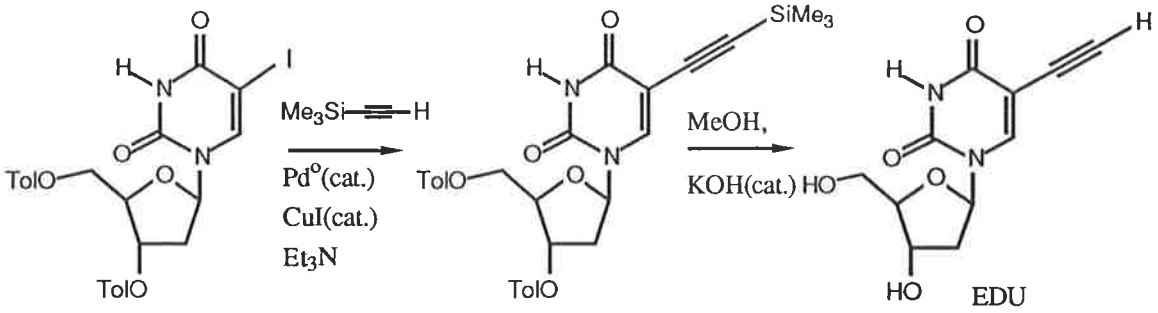


a. Oxidative addition; b. Transmetallation; c. Reductive elimination.
X = halide (I or Br) or trifluoromethanesulfonate (triflate).
Where catalyst is $Pd^{II}Cl_2L_2$ prior activation of the catalyst is achieved as follows:

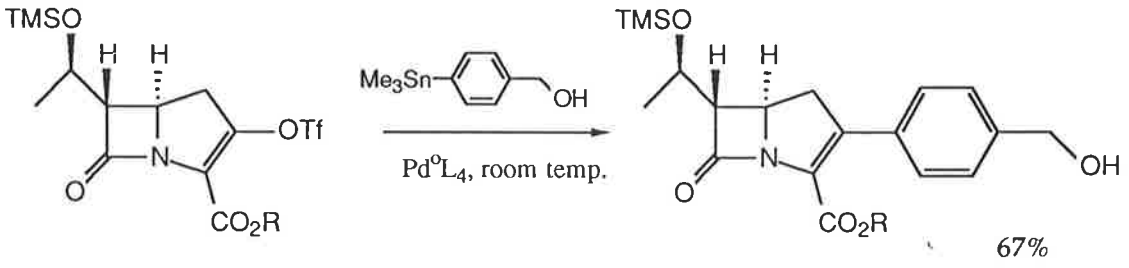


Numerous organometallics can function as the source of organic nucleophiles in the palladium-catalysed coupling processes (e.g. Mg, Cu, B, Zn, Sn, Al, Zr, etc.).[65] Each organometallic reagent has its own individual advantages and disadvantages in terms of the ease of preparation, relative stability, reactivity, stereocontrol and functional group tolerance.[65] Perhaps one of the most important applications of this technique to nucleosides has, up until recently, been the coupling of copper acetylides with 5-iodopyrimidine nucleosides introduced by Robins and Barr.[66-7] This procedure provides an expeditious route to 5-alkynyl nucleosides, including the familiar EDU (Scheme 7). In this process the organometallic is generated *in situ* from the terminal alkyne upon reaction with the triethylamine and copper(I)iodide to give the copper acetylide. Only a catalytic amount of copper(I)iodide is necessary as it is continually regenerated upon transmetallation.

Scheme 7



One of the most significant advances in the palladium-catalysed coupling of organometallics has been the introduction of organostannanes to this process.[65] The palladium-catalysed coupling of organostannanes to organohalides or triflates (The Stille reaction)* is tolerant to a large range of functional groups, frequently gives high yields of product and usually proceeds with complete stereochemical retention.[65] Organostannanes are, on the whole, quite stable to atmospheric air and moisture. Also, a rich and divergent array of synthetic methods are available for the preparation of organostannanes, allowing for the incorporation of organosubunits of virtually unlimited complexity.[65,68] The versatility of this process is exemplified by the increasing application of this process to the synthesis of highly functionalized natural products (eg. eq. 1[69]).



(1)

In terms of the type of chemistry that would allow for the facile substitution of the base ring, palladium assisted coupling of nucleoside and substituent would appear quite attractive. Only most recently has the Stille

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* Although the first report of palladium-catalysed coupling of aryl halides with allyl stannanes was by Kosugi *et al.*[79] in 1977, the enormous contribution of Stille and coworkers in the development and application of this process has lead to his name being recurrently associated with it,[57,80] in view of this precedent and for brevity sake this term is adopted here. Though it should be recognized that a number of groups have been involved in the development of this process.[81]

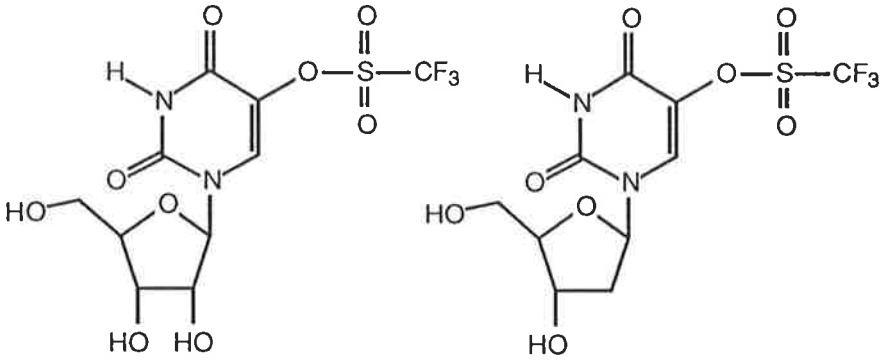
reaction been applied to the elaboration of nucleoside base rings.[32,53-7,70] The substrate of the Stille reaction, in its application to C5 substitution of pyrimidine based nucleosides, has invariably been the 5-iodopyrimidine derivative.§ However an important development in the investigations of Stille and co-workers has been the emergence of alkenyl and aryl triflates as suitable substrates for oxidative addition[71-3] to palladium and hence are subject to the same types of palladium chemistry as are the corresponding halides (usually Br and I).

Whilst the 5-iodouracil nucleosides undergo the Stille reaction quite efficiently and can be used to generate a divergent array of coupled products in moderate to excellent yields this precursor may have some limitations. Firstly, an iodo group on the base ring may limit the chemistry that can be performed on the sugar unit (e.g. radical reactions). Also, certain sugar modifications may not be stable to iodonation of the base (e.g. olefins).

For these reasons it was considered that routes to the preparation of 5-trifluoromethanesulfonyl pyrimidine nucleosides and their effectiveness in palladium-catalysed coupling reactions should be investigated. It was considered that the C5 triflate of pyrimidine nucleosides may be more stable than the corresponding iodides to a number of reaction conditions. Moreover, it was hoped that either the C5 triflates could be prepared from the various sugar modified pyrimidine nucleosides or perhaps more importantly that 5-trifluoromethanesulfonyluridine **7** and the 2'-deoxyuridine equivalent **8**, Fig. 7, may be considered as precursors suitable to both sugar and base ring modification.

§ Other than the reports produced in connection with this work, refs 53& 54.

Figure 7



7 8

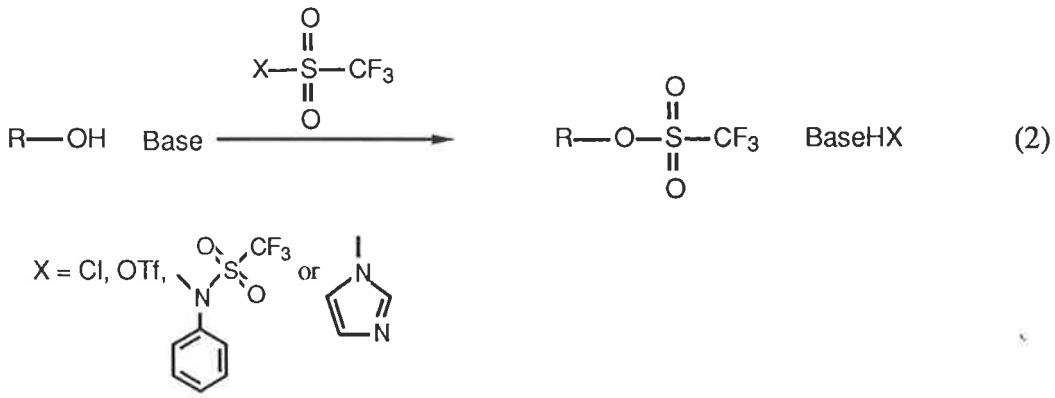
Chapter 2

The Synthesis of 5-Trifluoromethanesulfonyluridine (7) and 5-Trifluoromethanesulfonyl-2'-deoxyuridine (8).

2.1 Introduction

In order that 5-trifluoromethanesulfonyluridine **7** and 5-trifluoromethanesulfonyl-2'-deoxyuridine **8**, Fig. 7 (previous page), be considered as viable precursors in the further elaboration of the base ring, it was necessary to develop short, efficient routes to their preparation.

Triflate moieties are most usually introduced by treatment of an alcohol or an enolizable ketone with a triflating agent in the presence of a base (eq. 2.).[74] Thus in order to introduce a triflate moiety to the C5 centre of uridine in the manner described in eq. 2 intial hydroxylation of uridine to give 5-hydroxyuridine **9** was necessary.



(2)

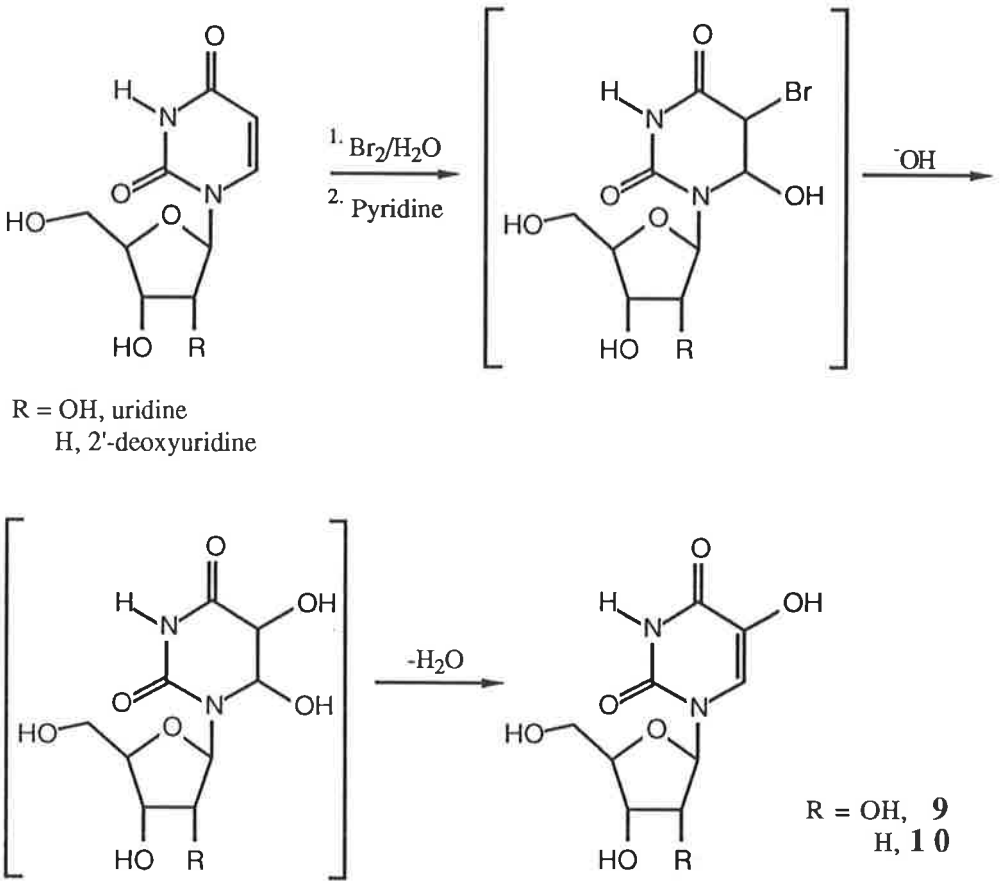
2.2 Results and Discussion

2.2.1 5-Trifluoromethanesulfonyluridine (7)

5-Hydroxyuridine **9** was prepared using a modification of the method originally described by Ueda.[75] According to Ueda's preparation an aqueous solution of uridine was treated intially with bromine (1 equivalent) to give a presumed bromohydrin intermediate,[75-6] which on subsequent treatment with pyridine (3.5 equivalents) and heating for 10 hours on a water bath gave the

desired 5-hydroxyuridine **9** in 40% yield (Scheme 8). The proposed pathway for this reaction involves the substitution of the bromide of the bromohydroxy intermediate to give a diol (possibly via an epoxide) with subsequent elimination of water to give **9** (40%).[75]

Scheme 8



Visser has reported that by decreasing the reaction temperature to 37°C (reducing the amount of decomposition) that **9** could be obtained in a higher yield (60.5%).[77]

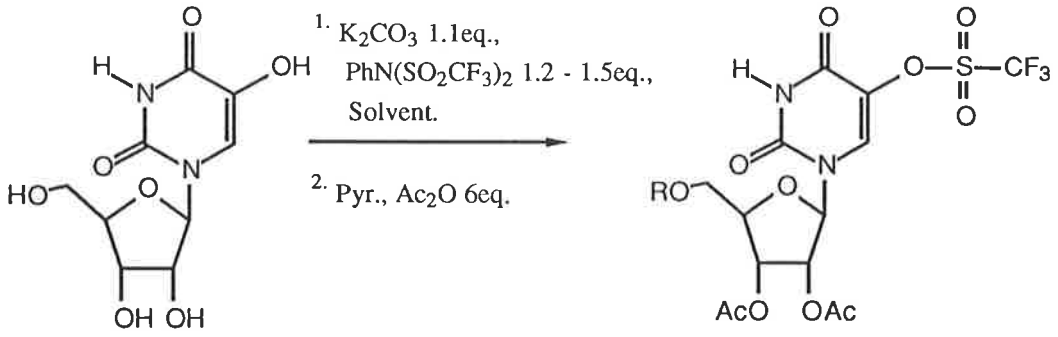
In their preparation of the less stable 5-hydroxy-2'-deoxyuridine **10** from 2'-deoxyuridine Podrebarac and Cheng[78] were able to obtain a moderate yield (47%) of product by performing reactions at room temperature in the presence of a much larger excess of pyridine. In view of these results, 5-hydroxyuridine was prepared at an even lower temperature and larger excess of base than that used by Visser. At room temperature (30°C) and with 8 equivalents of pyridine Uedas method gives **9** in a 77% isolated yield.

In order that the preparation of the C5 triflate of uridine be as short and simple as possible it was preferable to find conditions that would allow for the selective triflation of the enolic C5-hydroxyl group in the presence of the primary and secondary hydroxyl groups of the sugar moiety and the imidyl group of the base ring, so as to avoid the labourious process of selective protection and deprotection.[83]

Whilst there did not appear to be a literature precedent for a selective reaction of this type, the mild triflating agent, N-phenyltriflimide [$PhN(SO_2CF_3)_2$] had been shown previously to be capable of triflating a phenol in methanol in the presence of a mild base, potassium carbonate.[82] This suggested the possibility of triflating the more reactive enol in the presence of the primary and secondary hydroxyl groups of 5-hydroxyuridine.

In order to test this, 5-hydroxyuridine was reacted under similar conditions to those outlined previously for a phenol, with 1.5 equivalents of potassium carbonate and 1.3 equivalents of N-phenyltriflimide in methanol at room temperature (Scheme 9). Monitoring of this reaction mixture by t.l.c. revealed that two products were forming at the same time. Consumption of the 5-hydroxyuridine was complete after five hours. Chromatographic separation of the two products was most readily achieved after acetylation of the sugar hydroxyl groups.

Scheme 9



9

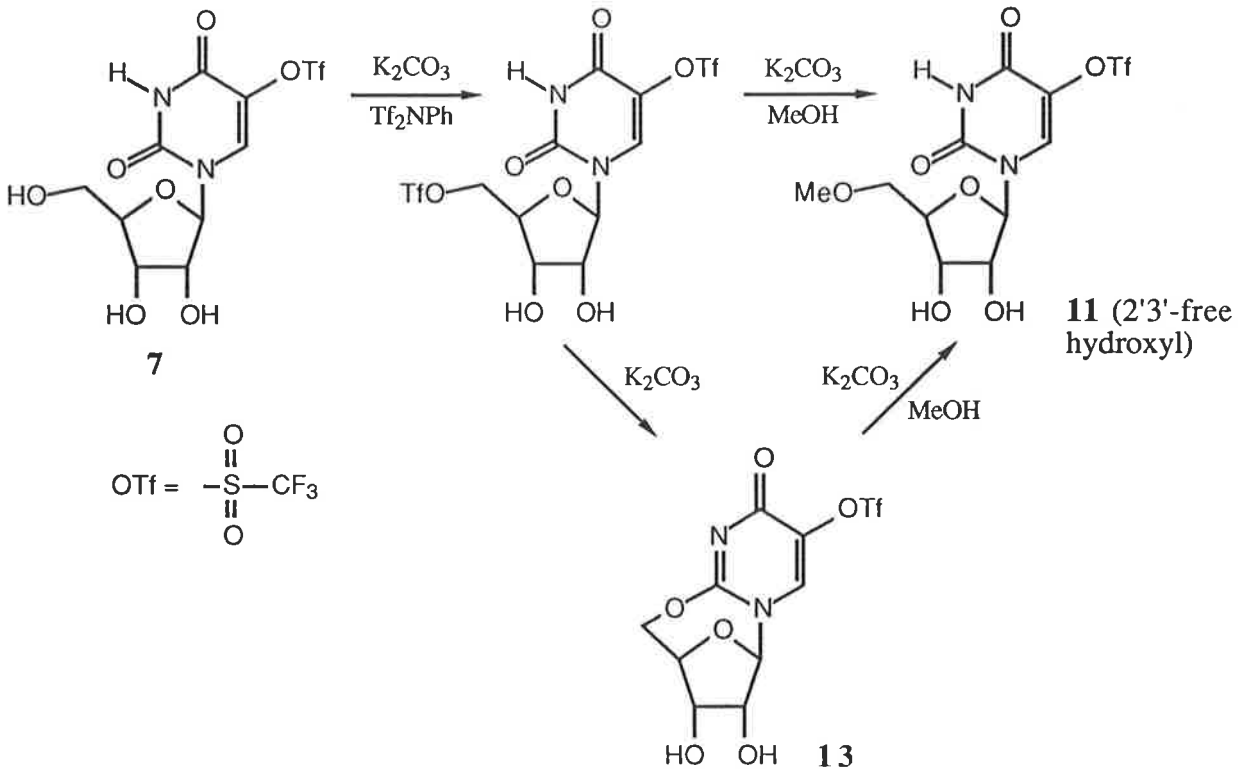
Yield (%) in different solvent systems.

R	Methanol	1,4- Dioxane/ Water 1:1
Ac (**12**)	60	97
Me (**11**)	30	0

The less polar component was determined, spectroscopically, to be the 2',3'-di-*O*-acetyl-5'-methyl ether **11** (R = Me) and the more polar component, the desired, 2',3',5'-tri-*O*-acetyl-5-trifluoromethanesulfonyluridine **12** (R = Ac). The ^1H NMR spectrum of the methyl ether **11** was similar to that of **12** except for the appearance of a singlet at 3.34 ppm and the absence of an acetate resonance at ~2.0 ppm. In addition A small upfield chemical shift in the resonance of the 5' carbon was observed in the ^{13}C NMR spectrum of **11** (59.0ppm) compared to **12** (62.9ppm). Also the FAB mass spectrum showed a molecular ion (M^++H) for **11**.

The methyl ether **11** may have formed by initial triflation of the 5'-hydroxyl which is either displaced by methanol directly, or is initially attacked by an internal nucleophile to form an anhydro species of the type **13** which then undergoes addition of methanol to give **11** (2',3'-free hydroxl) (Scheme 10). The formation of methyl triflate (MeOTf) followed by nucleophilic attack of the 5'-hydroxl, as an alternative was dimissed as both **11** (2',3'-free hydroxl) and **7** were observed by t.l.c. to be forming at the same time, yet no 5'-methyl was observed. Also any MeOTf formed would quickly form dimethylether.

Scheme 10



The formation of the 5'-methylether **11** was readily overcome by replacement of methanol with a mixture 1,4-dioxane and water. The ratio of 1,4-dioxane to water (3:1) was chosen in order to maximise the solubility of the hydrophilic potassium enolate of **7** and the hydrophobic N-phenyltriflimide. The product from this reaction, **7**, could be isolated at this point or the crude product could be acetylated directly to the give **12**. In either case, the product isolated after chromatography was obtained in excellent yield (91 & 97% respectively) as a stable, white solid.

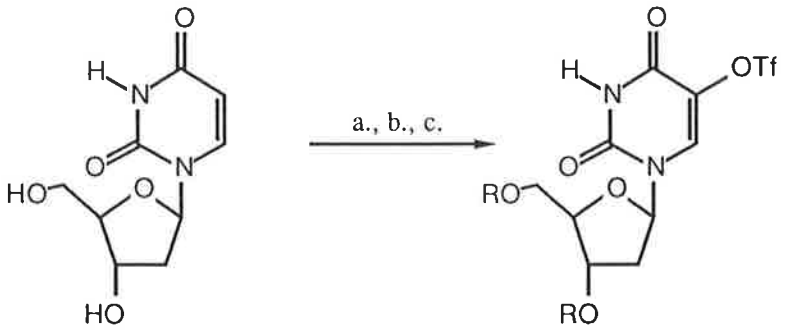
The presence of the triflate group in **11**, **7** and **12** was evidenced by the appearance of a quartet at ~118 ppm (J_{CF} ~ 320 Hz, CF_3) in the ^{13}C NMR spectrum. Regiospecific C5 triflation was evidenced by the down field shift of the C6 proton in the ^1H NMR in **7** (8.2ppm) compared to 5-hydroxyuridine **9** (7.2ppm).

2.2.2 5-Trifluoromethanesulfonyl-2'-deoxyuridine (8)

Many biologically active C5 substituted uracil nucleoside analogues have a 2'-deoxyribose unit, consequently it would be advantageous to have a high yielding preparation of 5-trifluoromethanesulfonyl-2'-deoxyuridine **8**.

2'-Deoxyuridine is a relatively expensive, therefore it was considered important to maximise the yield of the C5 triflate **8** relative to this precursor. The preparation and isolation of 5-hydroxy-2'-deoxyuridine **10** [Scheme 8 or step (a) Scheme 11] gives only a 47% yield of the desired product.[78] Subsequent triflation of the isolated C5-hydroxyl compound **10** [step (b)] gives **8** in a virtually quantitative yield (98%, isolated) as does acetylation [step(c)] of **8** to give **14** (100%, isolated).

Scheme 11



2'-deoxyuridine

a. Br_2/H_2O, pyridine; b. K_2CO_3, Tf_2NPh (H_2O/1,4-dioxane); c. Ac_2O/pyridine.

	R	R'		Yield %
Step a. isolate	H	OH	**10**	47
Step a.+b. isolate	H	OTf	**8**	58
Step a.+b.+c. isolate	Ac	OTf	**14**	56

When the reaction sequence (a),(b) or (a),(b),(c) (Scheme 9) was carried out as a single pot, two step or three step process, compounds **8** and **14** were isolated in 58 and 56% overall yield respectively. However, in these cases, minor amounts of 5-bromo-2'-deoxyuridine, carried over as a by product from step (a), could be detected in the product after intial purification by flash chromatography. This was removed upon a second purification by flash chromatography, or in the case of **8**, by a single recrystallization from isopropanol to give a highly pure,

crystalline product in an overall 52% yield from 2'-deoxyuridine. The single pot, multi-step process represents the most convenient, highest yielding approach to **8** or **14** from the expensive precursor 2'-deoxyuridine.

Convenient methods for the preparation of **8** and **14** from uridine are discussed in Chapter 6.

Chapter 3

Palladium-Catalysed Coupling of 2',3',5'-Tri-*O*-acetyl-5-trifluoromethanesulfonyluridine (12) with Organostannanes (The Stille Reaction).

3.1 Introduction

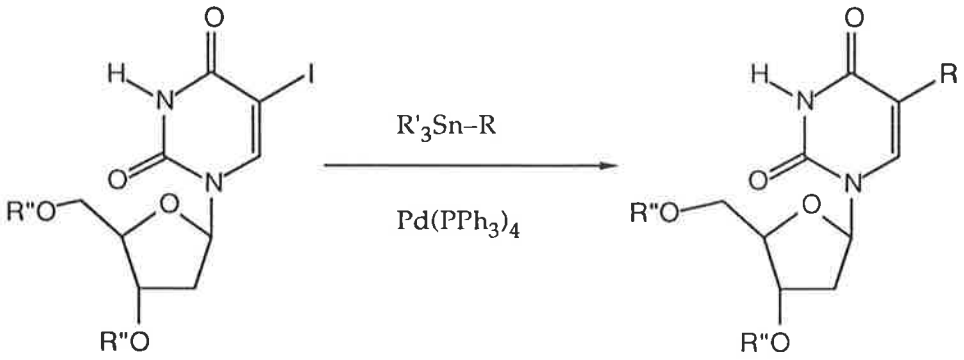
The transition metal-catalysed coupling of organic halides with organometallics has proved to be a very effective means of carbon-carbon bond formation.[66,81,84] In particular, the application of organostannanes to this process (the Stille reaction)[66,81,84] and the emergence of trifluoromethanesulfonates[71-3] (in place of halides) as premier coupling partners has been of enormous importance, for reasons cited earlier (Chapter 1).*

It has been shown that the Stille reaction is able to provide a general procedure for the C5 substitution of pyrimidine nucleosides.[32,53-7,70,87] The earliest reports of this application came from our laboratories.[32,53-5] However, it has since become apparent that a number of research groups have been working in this area simultaneously.[56-7,70,87] Other than the work detailed here all other reports on the application of the Stille reaction to the C5 elaboration of pyrimidine nucleosides have utilized the 5-iodo[32,54-7,70] or 5-bromo[87] nucleoside as the substrate.

It has been demonstrated that palladium assisted coupling of protected 5-iodo-2'-deoxyuridine with variously functionalized alkenylstannanes gives coupled products in high yields (eq. 3).[32] However, aryl groups transfer somewhat slower than alkenyl groups[65] and as a result only moderate yields (40-

* Diazonium salts[85] and phosphates[86] have also been utilized in place of aryl and alkenyl halides but to a much lesser extent.

60%) of aryl coupled products were obtained using the 5-iodouridines as a substrate.[54-5,57] It has been reported that the harsher conditions and/or longer reaction times required in order to drive these reactions to completion has resulted in the competitive decomposition of the iodonucleoside, often reduction of the iodo group was observed.[57,70] This reduction may result from a direct interaction of tin radicals with the iodonucleoside, or alternatively reduction of the nucleoside from a σ-palladium complex is possible but appears less likely because it is not observed for the corresponding triflates where similar palladium intermediates would be involved.



(3)

R = alkenyl 70-90%, aryl 40-60%
R' = *n*-Bu or Me
R" = protecting group

It was considered that the uridine triflate **12** may be more stable to the harsher reaction conditions required to transfer aryl groups, certainly direct reduction of the triflate with tin radicals was not likely to be a problem.

Subsequent to the work presented here Wigernick *et al.*[70] improved the yields obtained for the palladium-catalysed coupling of iodouridine to some heteroaryl stannanes by appropriate choice of catalyst and solvent obtaining yields of 85-90%. Wigernick proposed that either a coordinatively unsaturated catalyst [$PdCl_2(PPh_3)_2$] should be used in a coordinating solvent (tetrahydrofuran or 1,4-dioxane) or a coordinatively saturated catalyst [$Pd(PPh_3)_4$] should be used in a non-coordinating solvent (toluene).[70] Identical reaction conditions as those used by Wigerinck *et al.* were deduced independently by Yamamoto *et al.*[87] for a

single arylstannane bearing a boronic ester group in the *para* position to a 5-bromo pyrimidine nucleoside [i.e. $Pd(PPh_3)_4$, toluene].

3.1.1 Background to the Stille Reaction

The Stille reaction involves the substitution of halides or triflates of organic substrates for one of the organic groups of a tetrasubstituted organostannane in the presence of a catalytic amount of zero valent palladium (eq. 4).

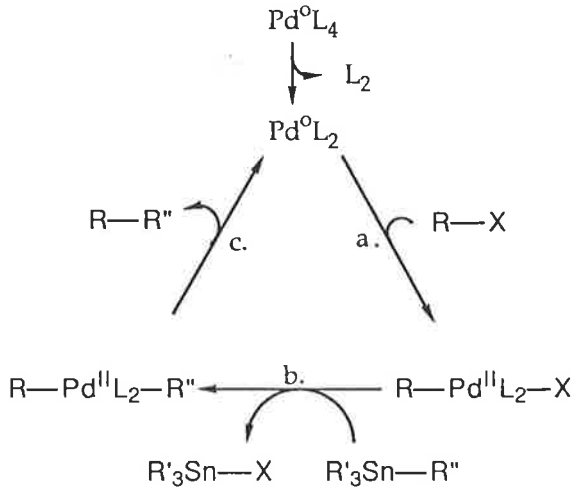
$$RX + R''SnR'_3 \xrightarrow{Pd^0L_4\ (cat.)} RR'' + XSnR'_3 \qquad (4)$$

X = halide or triflate

The rate at which the various groups are transferred from the tin is usually in the order R" = alkynyl > alkenyl > aryl > allyl ~ benzyl > alkyl.[63] Therefore a stannane bearing three alkyl groups (e.g. R' = methyl or *n*-butyl) and one of the more labile groups (e.g. R" = alkenyl) will substitute the more labile group exclusively. It is organostannanes of this type that are most often used in the Stille reaction.

The catalytic cycle devised for the Stille reaction is that depicted in Scheme 12.[63]

Scheme 12



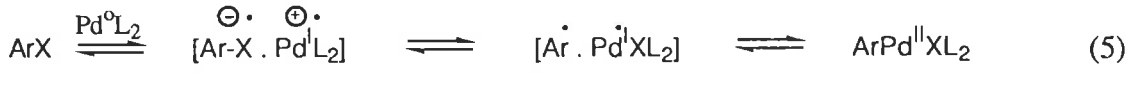
a. Oxidative addition; b. Transmetallation; c. Reductive elimination.
X = halide (I or Br) or trifluoromethanesulfonate (triflate).

Although this cycle is yet to be established, conclusively, the three key steps oxidative addition, transmetallation and reductive elimination are all processes in which palladium is known to partake.[88-93] The three steps are considered in detail below, where each process was studied (by others) in isolation using stoichiometric amounts of palladium.

Oxidative addition:

The oxidative addition of an organic halide or triflate to palladium(0) gives a square-planar σ-palladium(II) complex. The mechanism of oxidative addition varies depending on the nature of the organic substrate.

Where the organic substrate is an aryl halide the order of rates of oxidative addition is invariably X = I > Br >> Cl >> F and electron-withdrawing groups on the aromatic ring promote addition. Two possible mechanisms for oxidative addition are consistent with the available data[90,94-6] (eq's. 5 & 6). In each case the reactive species is the bis-ligated palladium(0) complex (Pd^0L_2).



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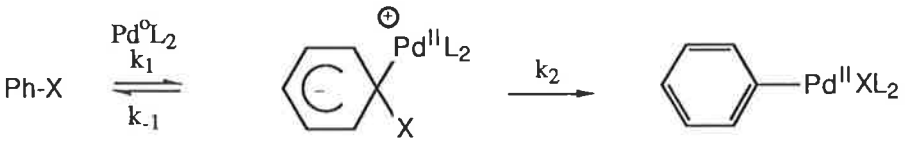

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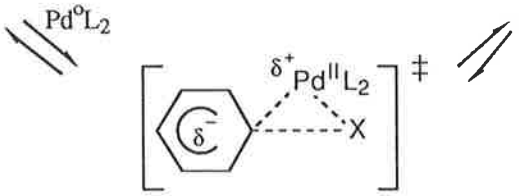


(I)

A single electron transfer process is one proposed pathway (eq. 5), however, no products arising from the presence of free radicals (such as hydrogen abstraction or homocoupling) are observed, at least not in reactions where the ratio of ArX:Pd^0L_4 is 1:1. This would imply that the formation and

collapse of radical ions to give addition products must occur almost exclusively within the solvent cage.[90,95-6]

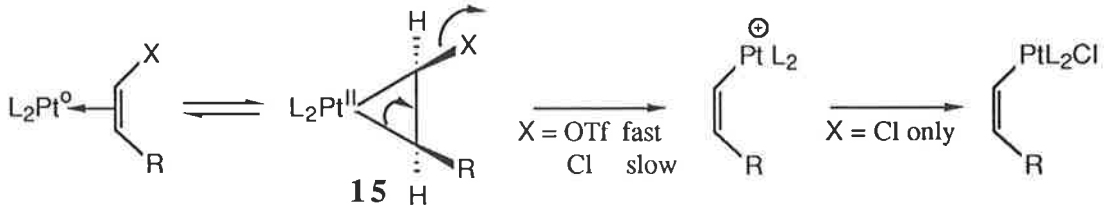
An alternative mechanism, similar to nucleophilic aromatic substitution, has also been postulated (eq. 6).[90,97] If the palladium(0) nucleophile were undergoing a classical nucleophilic aromatic substitution, in which the first step (k_1/k_{-1}) is rate determining, the order of reactivity of the aryl halide would be X = F > Cl > Br > I. If the second step (k_2) were the rate determining step then the order of reactivity would be reversed, namely X = I > Br > Cl > F. Thus in order for a mechanism of oxidative addition to be akin to nucleophilic aromatic substitution k_2 must be rate determining.

Perhaps another possibility is that a concerted process is operating. Where nucleophilic addition of the palladium proceeds with simultaneous transfer of the halide from the aryl group to the palladium via transition state (I). In such a concerted process the transfer of electron density from the palladium to the aryl group may occur slightly ahead of halide departure and thus would be promoted in the presence of electron-withdrawing groups. The order of reactivity X = I > Br >> Cl >> F may result from a combination of the relative leaving group potentials and a more favourable interaction between the palladium and the more electron diffuse halides I and Br.

Of the three mechanisms discussed above the second seems more applicable to aryl triflates, though a completely different mechanism may be operating. Although triflates are very powerful leaving groups the nucleophilic addition of a palladium(0) species to a transient aryl cation or direct attack of the aryl triflate C–O bond is not likely as aryl triflates are very stable.[98] In the case of a mechanism similar to nucleophilic aromatic substitution the C-OTf bond breaking step can not be rate determining as the order for the rates of oxidative addition of ArX to Pd° is X = I > Br ≥ OTf >> Cl, yet the triflate group is a much

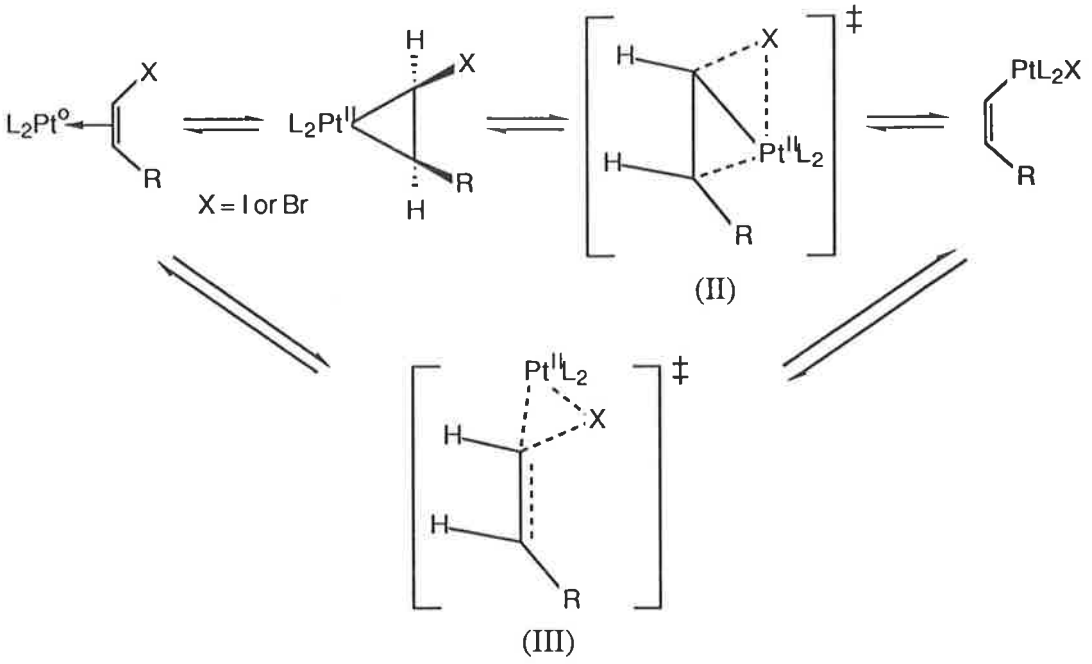
more powerful leaving group than either I or Br. The observed rate order is perhaps consistent with the classical two step aromatic nucleophilic substitution mechanism where the first step is rate determining for the aryltriflate and the second step is rate determining, or a concerted mechanism is operating for the arylhalides.

In the case of alkenyl triflates there is strong evidence to suggest that oxidative addition of platinum(0) phosphine complexes proceeds via a metallacycle (**15**, X = OTf)[99] (eq. 7). It is considered, by analogy, that this could also be the case for palladium.[99] The mechanism is consistent with the stereochemical retention observed for these processes.[71]

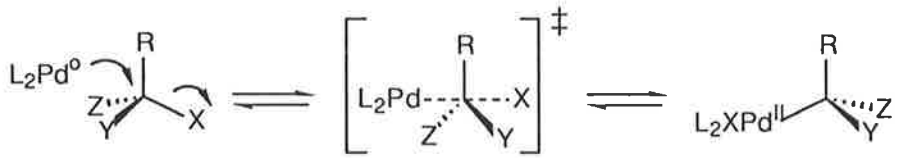


(7)

The rate of oxidative addition of alkenyl halides to platinum is also the reverse of that observed for nucleophilic substitutions and follows the order I > Br > Cl (no reaction for F).[90] For alkenyl chlorides an intermediate similar to that proposed for the alkenyl triflates (**15**, X = Cl) is thought to be occurring, though the overall rate of addition to the platinium is somewhat slower (eq. 7).[90,100] In the case of alkenyl bromides and iodides there is evidence for mechanisms involving favourable interaction between the platinum and leaving group in the transition states (II) & (III) (eq. 8).[90] Again by analogy similar processes are thought to be occurring for palladium.[90] The transition state (III) is similar to that postulated here for oxidative addition of aryl bromides and iodides to $Pd^{\circ}L_2$ ((I), eq. 6)



For oxidative addition of sp^3 centres to $Pd^{\circ}L_2$ the mechanism is analogous to S_N2 nucleophilic substitution (eq. 9).[90] The addition process goes with inversion of stereochemistry and the rate is in the order X = I> Br > Cl > F, R = benzyl ≈ allyl > alkyl.

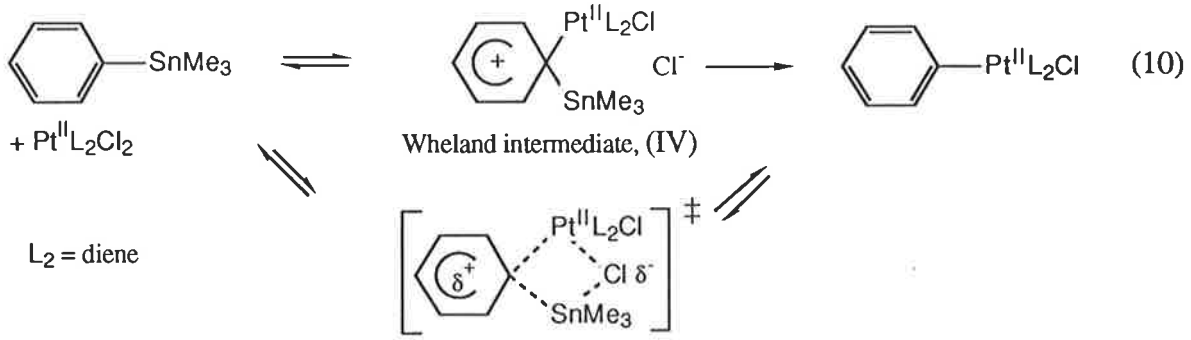


(9)

Transmetallation:

The transfer of the most labile organic substituent from the organostannane to the σ-palladium(II) complex in exchange for a halide (or triflate) is the next major step in the catalytic cycle (Scheme 12). The mechanism of transmetallation is probably the least understood process of the catalytic cycle, though in most cases this step is considered to be rate determining.[80,90]

Eaborn and co-workers[101] have carried out a series of transmetallations of aryltrimethylstannanes to platinum(II)dichloro complexes bearing the chelating diene cyclo-octa-1,5-diene (cod) [i.e. $PtCl_2$ (cod)]. Two possible mechanisms for this process were proposed,[101] both of which are akin to electrophilic aromatic substitution (eq. 10).



(V)

It was considered that the transfer may go by the Wheland intermediate (IV), or through a concerted transition state such as (V), where a substantial amount of positive charge lies on the aromatic ring. These mechanisms were supported by the following observations:

-electron donors on the aromatic ring increased the rate of substitution (stabilizing the positive charge) whilst, in general, electron-acceptors decreased it.[101]

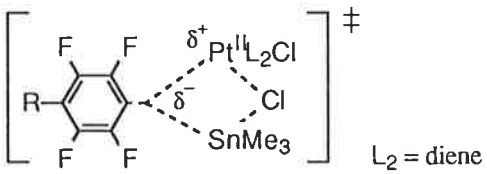
-strong donor ligands (e.g. PR_3) inhibited the reaction by reducing the electrophilicity of the platinum.[102]

-monoarylation is, in general, faster than diarylation [i.e. $PtCl_2(cod)$ is more electrophilic than PtClR(cod)].[101]

-electrophilic substitution of aryl stannanes has been shown to occur with other electrophiles.[101]

Two inexplicable anomalies appeared, however. Firstly, that the exceedingly electron deficient chromium tricarbonyl aryl stannane [η^6-$Cr(CO)_3$-*p*-$MeC_6H_4SnMe_3$] transmetallated quite readily to give only the diaryl complex. This complex does not normally undergo electrophilic substitution.[101] Also, it appeared from the results (although not explicitly stated) that other aryl stannanes that bore electron-withdrawing groups reacted slowly, to give preferably diaryl complexes even when the ratio of PtL_2Cl_2 : $ArSnMe_3$ > 1 : 2.[101] This appears to suggest that stannanes bearing electron-withdrawing groups react fastest with the least electrophilic platinium [i.e. faster with PtClR(cod) than

$PtCl_2(cod)$]. The same observation was made by Deacon *et al.*[103] in their preparation of similar platinium complexes using electron deficient perfluoroaryltrimethylstannanes, where significant quantities and in some cases exclusively diaryl complexes were formed despite the ratio of PtL_2Cl_2 : $ArSnMe_3$ > 1 : 2. The order of reactivity for the transfer of aryl groups was observed to be $C_6F_5SnMe_3$ > *p*-$MeOC_6F_4SnMe_3$ > *p*-$HC_6F_4SnMe_3$. In order to account for this order of reactivity Deacon *et al.*[103] proposed a different transition state (VI).



(VI)

In this case significant negative charge is transferred to the aryl ring via the σ-framework. Stabilization through the σ and not the π-system was proposed in order to account for the reactivity order *p*-$MeOC_6F_4SnMe_3$ > *p*-$HC_6F_4SnMe_3$, where the methoxy group is a σ-acceptor and a π-donor. The mechanism of transmetallation, as it pertains to the Stille reaction, is further considered below in connection with the results obtained in this work ("Results and Discussion", this chapter).

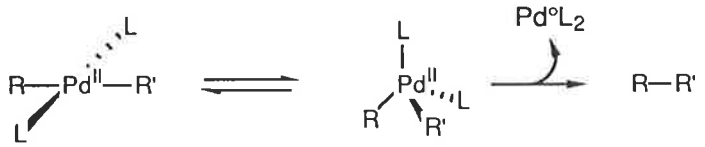
In many Stille couplings involving organo triflates as substrates the addition of lithium chloride is necessary in order to achieve coupling,[71-3,86,104] in others it slightly retards the rate of coupling.[105-6] Stille has shown that in the presence of lithium chloride the product of oxidative addition to aryl or alkenyl triflates is the *trans*–$L_2Pd^{II}RCl$ complex.[72] In the absence of lithium chloride an ion pair ($Pd^{II}L_3^+$ ^-OTf) complex is likely, especially where the ratio of L:Pd is equal to or greater than 3:1.[99,107] The role of lithium chloride in promoting some coupling reactions but retarding others seems a mystery. Although it seems likely that the process of transmetallation is affected.[72,86,104]

In a recent study by Farina and Krishman[105] it was demonstrated that transmetallation is likely to be preceeded by ligand dissociation from the σ-palladium(II) complex, followed by π-complexation of the organostannane via a dissociative mechanism. Since the rate of coupling varied significantly for ligands with different rates of dissociation it was therefore anticipated to be, to some extent, rate determining for the process.

Reductive Elimination:

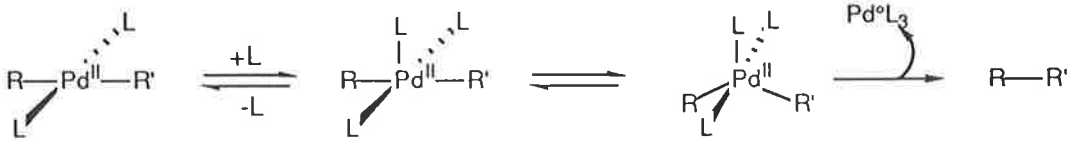
The likely mechanisms of reductive elimination have been extensively studied both for palladium and platinium complexes.[90,97,108]

In order for reductive elimination of two organic groups to occur from a palladium(II) complex the two groups need to be adjacent to each other.[90,108] There are a number of ways in which two organic groups on a *trans*-$PdRR'L_2$ complex can become adjacent to each other so that reductive elimination take place: (i) distortion of a *trans*-square planar complex to a tetrahedral one **16** (eq. 11);[90,108]



square planar tetrahedral, **16** (11)

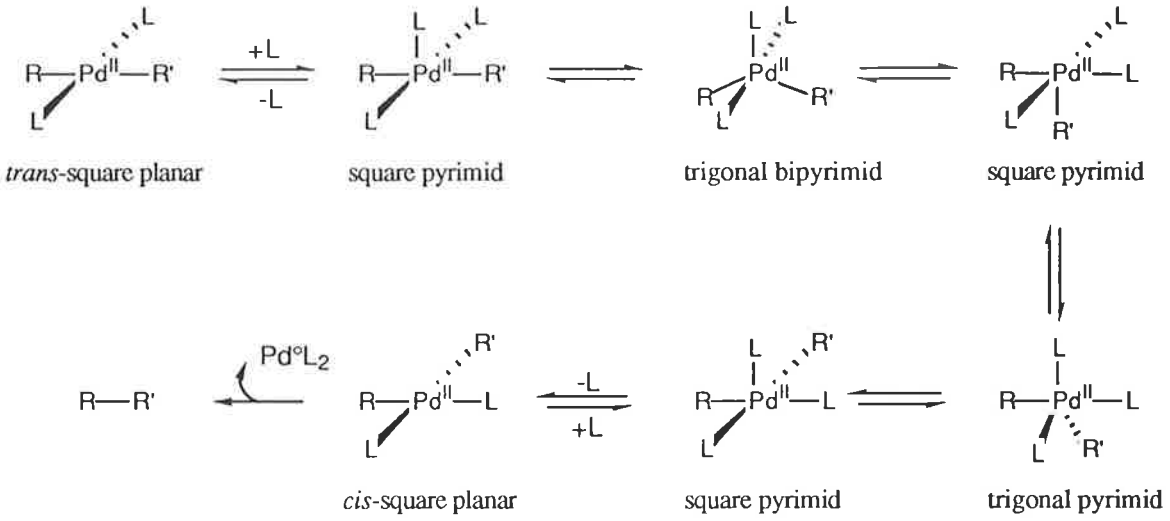
(ii) addition of a ligand to give a pentacoordinate trigonal bipyrimidal complex **17** (eq. 12);[90,108]



trans-square planar square pyrimid trigonal bipyrimid, **17** (12)

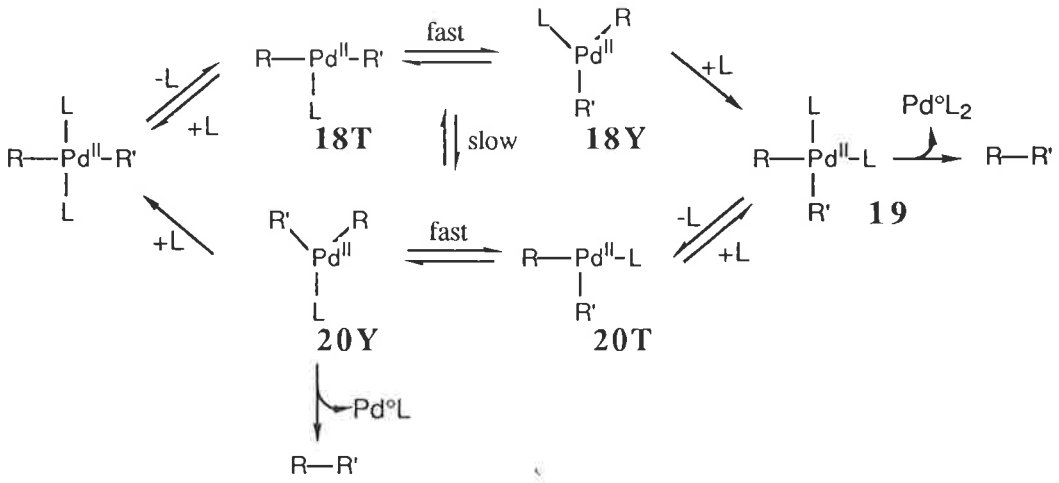
(iii) pseudorotation of a highly fluxional pentacoordinate complex which undergoes ligand dissociation to give a *cis*-square planar complex (associative rearrangement, Scheme 13);[90,109,110]

Scheme 13



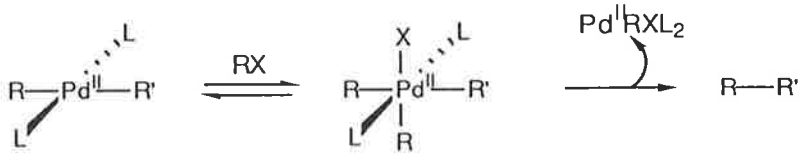
(iv) ligand dissociation to give three coordinate T and/or Y complexes **18T** and/or **18Y** followed by addition of a ligand to give a *cis*-square planar complex **19** (dissociative rearrangement, Scheme 14);[90,108]

Scheme 14



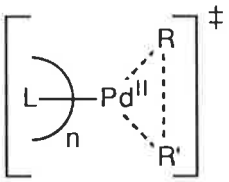
(v) direct rearrangement of **18T** and/or **18Y** to **20T** and/or **20Y** (a high energy process, Scheme 14) is possible, though reductive elimination can only occur from **20Y** as R & R' are too far apart in **18T** & **18Y** and the process is symmetry forbidden for **20T**;[90,111-2]

(vi) oxidative addition of an organic halide (RX) to the *trans* complex to give a palladium(IV) complex **21** which gives a $RPd^{II}XL_2$ upon reductive elimination (eq. 13).[90]



trans-square planar octahedral, **21** (13)

The method by which the two organic groups become adjacent prior to reductive elimination depends on the nature of R, R' and L and the reaction conditions, such as the ratio of Pd:L, the presence of RX and the solvent type (i.e. polarity and ability to coordinate to the metal). No matter what the coordination number around the palladium (3-6) or its oxidation state (II or IV) reductive elimination takes place from a three membered ring transition state (XII) and is promoted for organic groups bearing electron-donating groups and is resisted by those bearing electron-withdrawing groups.[90]



(VII)

n = 1-6

In the case where R and R' are both sp^3 hybridized prior departure of a ligand from the palladium complex is required in order for reductive elimination to occur as *trans* donor ligands inhibit reductive elimination.[90,108] The presence of a coordinating solvent often promotes reductive elimination in such cases, either by aiding the removal of a ligand through solvation or by occupation of the vacant coordination site in the T shaped complex to give a four coordinate complex from which reductive elimination is symmetry allowed.

When one of the organic groups being eliminated is sp^2 hybridized the energy of activation for the reductive elimination process is significantly reduced.[90,108] In these cases reductive elimination is likely to be a lower energy process then dissociative ligand departure from the square planar complex *cis*-R_2PdL_2. Thus, reductive elimination is likely to occur from a four coordinate

complex. This occurs because the product of reductive elimination is able to π-coordinate with the palladium to give a more stable product and is evidence for a late, product like, transition state.[90]

Reductive elimination from a palladium(IV) complex to return a palladium(II) organohalide complex (eq. 13) is very fast ($Pd^{IV} \rightarrow Pd^{II}$ is faster than $Pd^{II} \rightarrow Pd°$).[90] In the Stille reaction the resulting palladium(II) organohalide complex can undergo transmetallation and so the catalytic cycle takes on a slightly different path ($Pd^{IV} \leftrightarrow Pd^{II}$ as opposed to $Pd^{II} \leftrightarrow Pd°$). Such an alternative mechanism is only likely where the energy of activation for reductive elimination ($Pd^{II} \rightarrow Pd°$) is greater than the energy for oxidative addition ($Pd^{II} \rightarrow Pd^{IV}$). This is likely to be the exception rather than the rule but it may explain the presence of homocoupled products in some reactions.[90] This alternate pathway may be most likely for systems involving electron-deficient groups for which the energy requirement for reductive elimination is quite high.

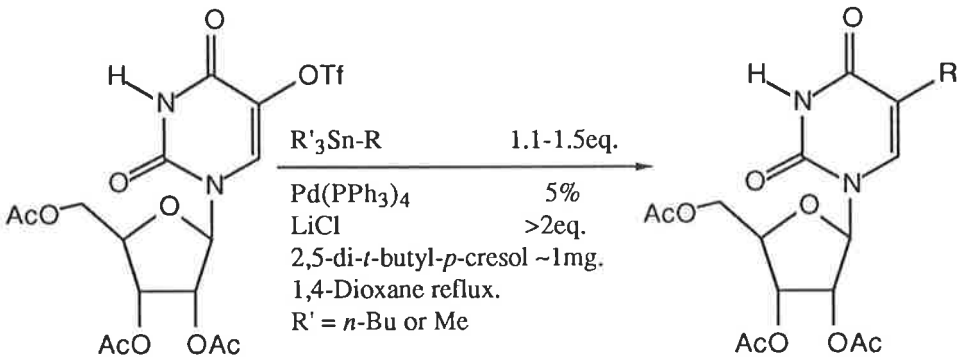
3.2 Results and Discussion

3.2.1 Palladium-Catalysed Coupling of Alkenyltrialkylstannanes with 2',3',5'-Tri-*O*-acetyl-5-trifluoromethanesulfonyluridine (12)

In order to determine the scope and limitations of the Stille reaction of C5-triflated uridines, compound **12** was chosen for an intial methodology study.

A systematic study of the conditions necessary for coupling was undertaken using tri-*n*-butylethenylstannane (R' = *n*-butyl, R = –CH=CH_2, eq. 14), the tetrakis(triphenylphosphine)palladium(0) catalyst [$Pd(PPh_3)_4$] and **12**. The reaction proceeded slowly (15hrs, as monitored by t.l.c.) in the presence of LiCl in THF at reflux (b.p. 67°C) and the desired product **22a** was isolated in a moderate yield (63%). No reaction was observed in the absence of LiCl (1,4-dioxane, reflux). A higher yield of **22a** (75%) was obtained over a shorter reaction time (4hrs)

when the reaction was carried out in 1,4-dioxane at reflux (b.p. 101°C). The optimum yield, however, was obtained when a small amount of radical inhibitor (2,5-di-*t*-butyl-*p*-cresol) was added to give **22a** in high yield (87%).



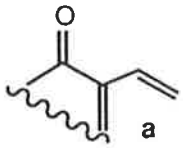
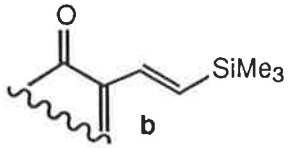
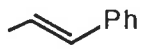
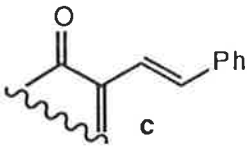
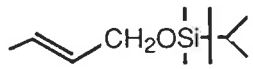
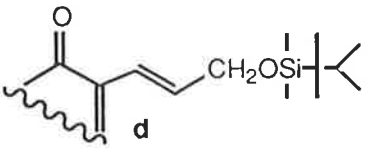
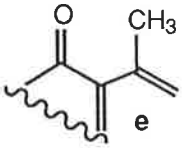
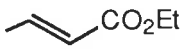
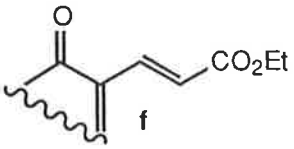
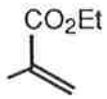
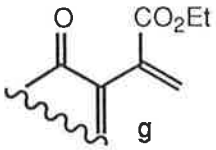
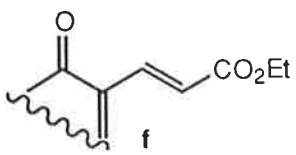
12

R = alkenyl, **22a-g**
aryl, **23a-f**

(14)

All subsequent reactions were carried out using the conditions derived of the simple alkenyl stannane (eq. 14). Reactions were allowed to proceed until analytical t.l.c. indicated complete consumption of **12**, subsequent washings and the purification by flash chromatography gave the desired products as shown in Table 1. The progress of each reaction was monitored by analytical t.l.c. and was "worked up" when consumption of **12** appeared complete. Also reaction mixtures usually remained more or less clear whilst the nucleoside triflate was still present but went very dark upon its complete consumption, presumably as a result of "palladium black" formation.

Table 1: Stille Coupling of **12** With Alkenylstannanes.

Entry	Stannane R'_3SnR [a]	Product **22**	Reaction Time (hrs)	Yield %
1	R = 	 **a**	4	87
2	R = 	 **b**	4	73
3[b]	R = 	 **c**	8	75
4	R = 	 **d**	5	92
5[b]	R = 	 **e**	6	86
6	R = 	 **f**	1	92
7	R = 	(a)  **g**	15	46
		+		
		(b)  **f**		42

[a] R' = *n*-butyl unless otherwise stated. [b] R' = Methyl.

Retention of *trans* stereochemistry for the products **22b-f** was confirmed by the coupling constant $^3J_{HH}$ ~16 Hz in each case.

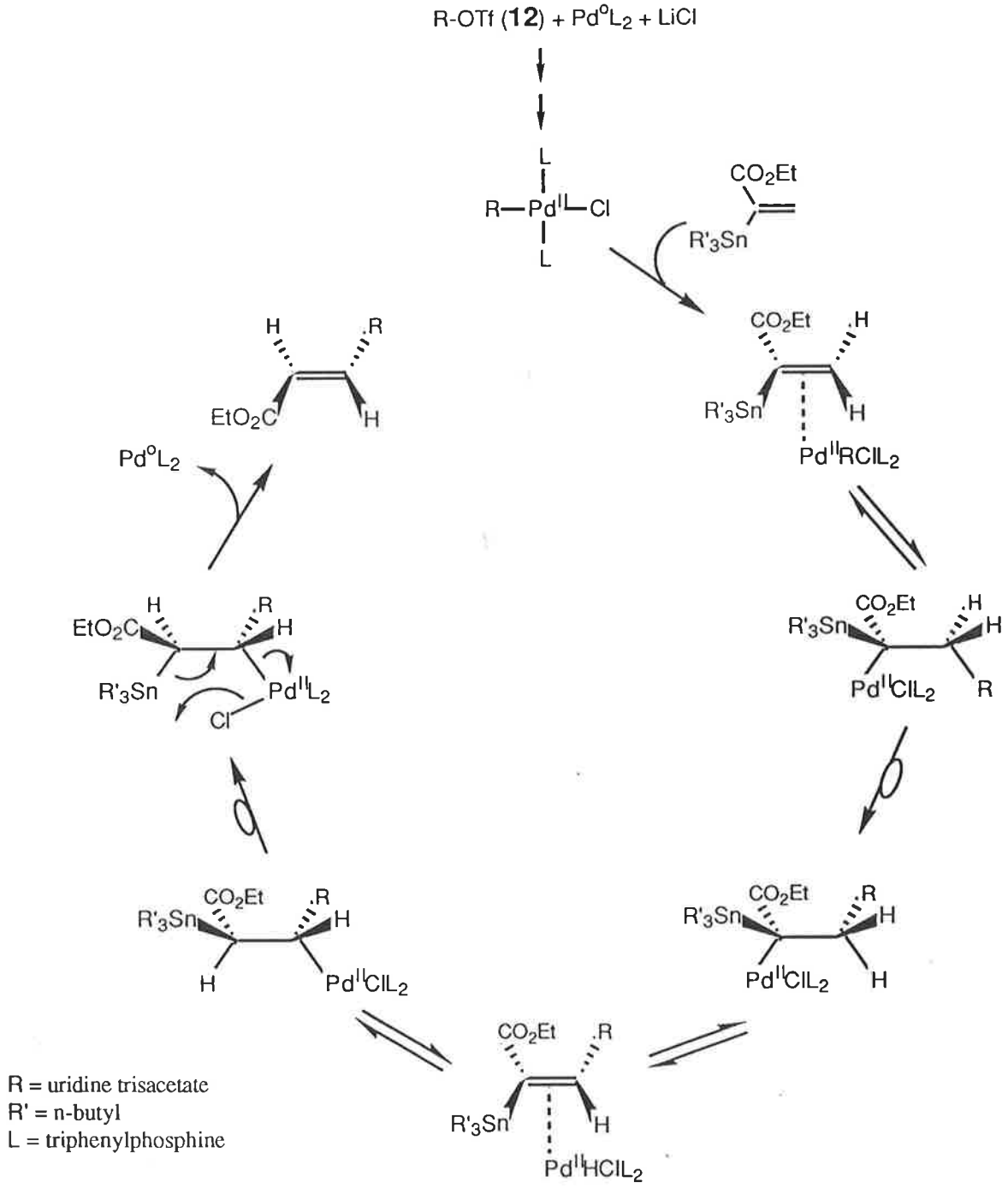
We have shown that other organometallics are capable of leading to *cine* (i.e. C6) substitution of the triflate moiety of a protected C5-trifloxyl uracil, in the absence of a palladium catalyst (see Chapter 7). That only *ipso* (C5) substitution was obtained in the palladium assisted coupling of organostannanes with **12** was confirmed by the appearance of a singlet at δ ~8ppm in the ^{1}H NMR which corresponds to the chemical shift of the C6 proton of similar C5 substituted uridine derivatives, the chemical shift of the C5 proton in the ^{1}H NMR is δ 5.0-6.0ppm.

The rapid consumption of starting material **12** in the formation of **22f** (entry 6) reflects an increase in the rate of transmetallation with increasing electron deficiency of the alkenyl group.[113,114] That this is not reflected in the formation of **22c** (entry 3) may be due to the fact that after 6-7 hrs t.l.c analysis of the reaction mixture indicated that considerable amounts of **12** and that further addition of palladium catalyst and styrenylstannane led to the rapid consumption of the remaining starting material **12**. The reaction conditions for this particular reaction were not optimized.

In the case of entry 7 two regioisomers were formed, one from direct transmetallation (*ipso* substitution) and the other from a Heck type reaction (*cine* substitution). Similar regioisomeric scrambling has been observed for α-styrenylstannanes by Kikukawa *et al.*,[115] for a stannylnorbornene by Stork and Richard[116] and for a γ-stannyl allylglycine by Crisp and Glink.[117]

A mechanism similar to that proposed by Kikukawa *et al.*[115] for the *cine* substitution of α-styrenylstannanes with arenediazonium salts (ArN_2BF_4) under the influence bis(dibenzylideneacetone)palladium(0) as a catalyst is thought to have been operating (Scheme 15).

Scheme 15



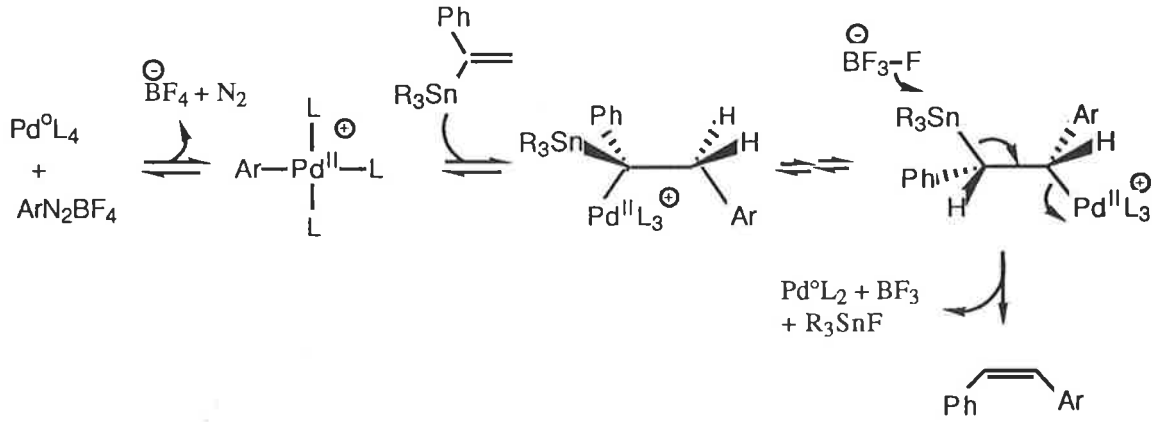
Oxidative addition of the uridine triflate **12** to $Pd(PPh_3)_4$, addition of a chloride to the $[RPd^{II}(PPh_3)_n]^+$ complex and substitution of a ligand to give π-complexed alkenyl stannane would occur as for the direct transmetallation process.[72,105] Hence at this point the alkenyl group can either transmetallate or undergo *syn* addition as in the Heck reaction.* β-Elimination of the palladium-

* For a discussion on the mechanism of the Heck reaction see Chapter 1.

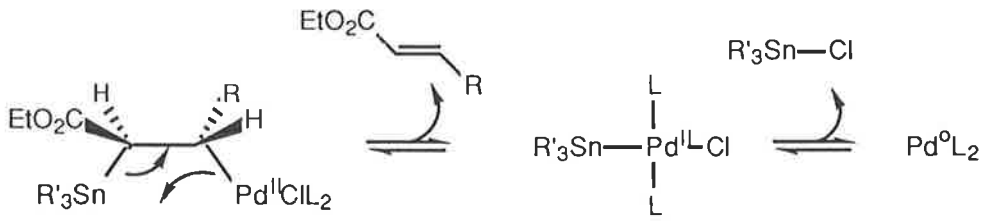
hydride from the most favoured eclipsed conformer (nucleoside and tri-*n*-butylstannane moieties as far from each other as possible) gives the Heck-type product. Intramolecular readdition of the PdHClL in the opposite sense gives a σ-complex capable of reductive elimination of the palladium and tin via a five membered cyclic transition state to give the substituted nucleoside with the observed stereochemistry.

By contrast with the *cine* substitution observed here and in the case of the allylglycine derivative,[117] Kikukawa *et al.*[115] observed predominantly *Z*-configuration in *cine* substituted products. In this latter case *anti*-elimination of the tin and the palladium was proposed (Scheme 16). This is probably due to the halide source (BF_4^-) being independent of the palladium and hence *anti*-elimination is prefered.

Scheme 16



It is also possible in the case of entry 7, that elimination of the palladium and tin is analogous to β-hydride elimination and that subsequent reductive elimination of the tin and chloride from the palladium occurs (eq. 15).

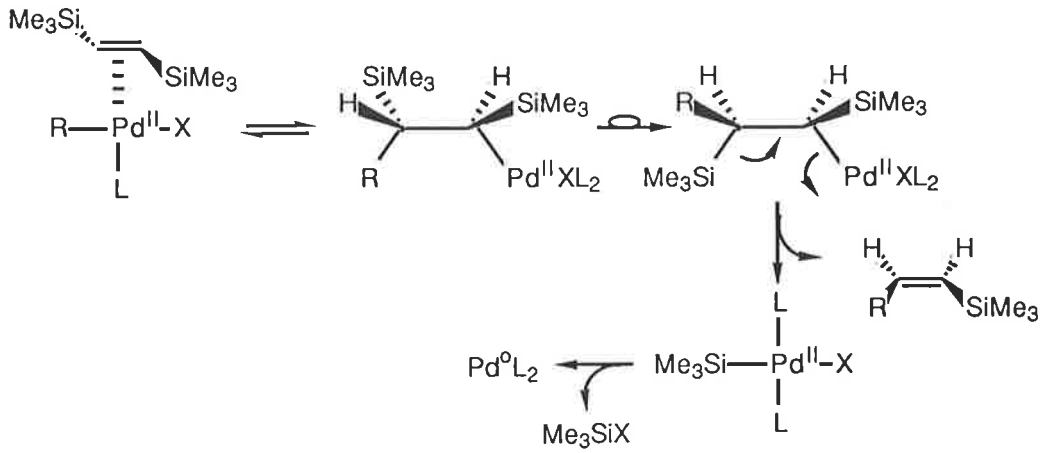


(15)

Hallberg *et al.*[118] preferred this latter sequence of events in explaining their observation of a competitive β-silyl, β-hydride elimination upon addition of an

organopalladium halide complex to *E*-1,2-bis(trimethylsilyl)ethylene (Scheme 17). Although the possibility of a five-membered cyclic transition state was discussed it was tentatively considered less likely than the four-membered ring transition state because the use of different halides did not give rise to different $Me_3SiPdX/HPdX$ ratios. However, this has not been tested for in the case of stannane equivalents. This reaction does appear to require the presence of a halide.[117]

Scheme 17



3.2.2 Palladium-Catalysed Coupling of Aryltrimethylstannanes with 2',3',5'-Tri-*O*-acetyl-5-trifluoromethanesulfonyluridine (12).

The conditions chosen for the coupling of uridine triflate **12** to a series of arylstannanes were identical to those used in the alkenyl series (eq. 14) and similar to those used by Crisp and Macolino[54-5] in coupling arylstannanes to 5-iodouridine. In these cases a coordinatively saturated catalyst $Pd(PPh_3)_4$ was used in conjunction with a coordinating solvent (1,4-dioxane or tetrahydrofuran), such conditions only gave very low yields or no product at all in Wigernick's[70] and Yamamoto's[87] work. This may explain the low yields (40-60%) obtained by Crisp and Macolino in their reactions.

The coupling of the uridine triflate **12** to a series of aryltrimethylstannanes possessing both electron-donating and electron-withdrawing groups is

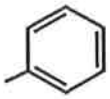
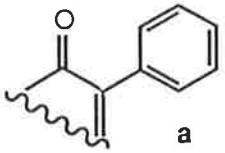
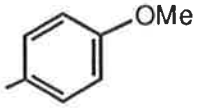
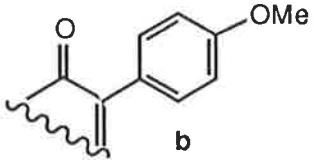
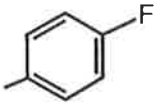
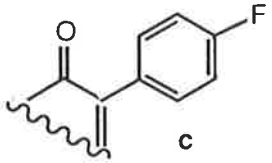
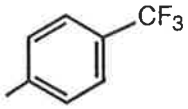
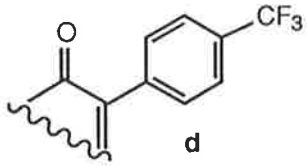
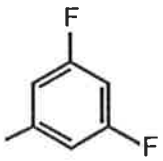
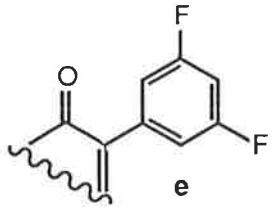
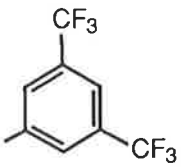
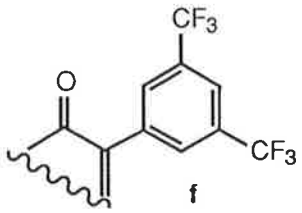
summarized in Table 2. On the basis of the subsequent work of Wigernick[70] low yields of product would have been expected for couplings under these conditions, however, moderate to high yields were achieved for these couplings, often requiring only a short reaction period.

As expected, in no reaction was any reduced product detected. The reaction times and yields reflect an increase in the rate of coupling with an increasing electron-deficiency of the aryl group as indicated by the σ-Hammett values. The necessity for lithium chloride in these couplings was only tested for in the case of entry 4, where no coupling was observed in its absence.

In contrast to the observations of Eaborn *et al.*[101] for the transmetallation of the majority of their aryltrimethylstannanes to platinium(II) complexes, in the case of the Stille reactions conducted in this work it was observed that electron-donors on the aryl group, including π-electron-donors such as the methoxy group, retard coupling and that electron-acceptors promote coupling.* Since transmetallation is most likely to be rate determining these electronic effects should be expressed here. This observation and the anomalies apparent in Eaborn's work (see *Transmetallation*) could be explained if the concerted transition state put forward by Eaborn (V) had essentially the reverse polarity (VIII) (i.e. the same polarity as the the transition state (VI) proposed by Deacon *et al*[103] (VI)) Fig. 8.

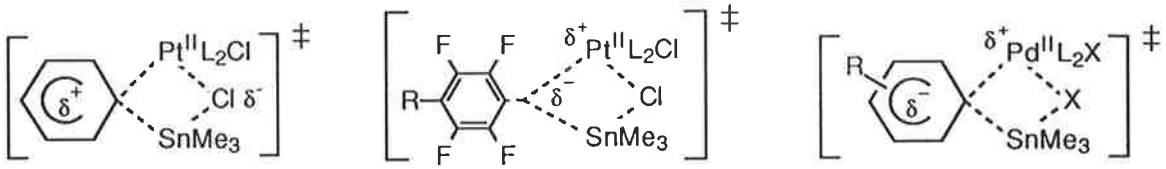
* Beletskaya *et al.*[119,120] have also used a number of aryltrimethylstannanes bearing both electron-withdrawing and donating groups, however, in these cases either all reactions proceeded with such facility that no trends with regards to reactivity and substituent effect were apparent and/or that transmetallation was no longer the rate determining step.

Table 2: Stille Coupling of **12** With Trimethylarylstannanes.

Entry	Stannane Me_3SnR	σ^a	Product **23**	Reaction Time (hrs)	Yield %
1		0.00	 **a**	20	64
2		-0.27	 **b**	28	55
3		+0.06	 **c**	8	89
4		+0.54	 **d**	3	91
5		+0.68	 **e**	5	81
6		+0.86	 **f**	4	85

[a] σ-Hammett values were obtained from ref. 154.

Figure 8



(V) L_2 = diene (VI) L_2 = diene (VIII) L = triphenylposphine

This type of transition state would be further favoured in the Stille reaction as the electron donating triphenylphosphine ligands would reduce the electrophilicity of the catalyst and help stabilize the positive charge developing upon it as the chloride leaves.

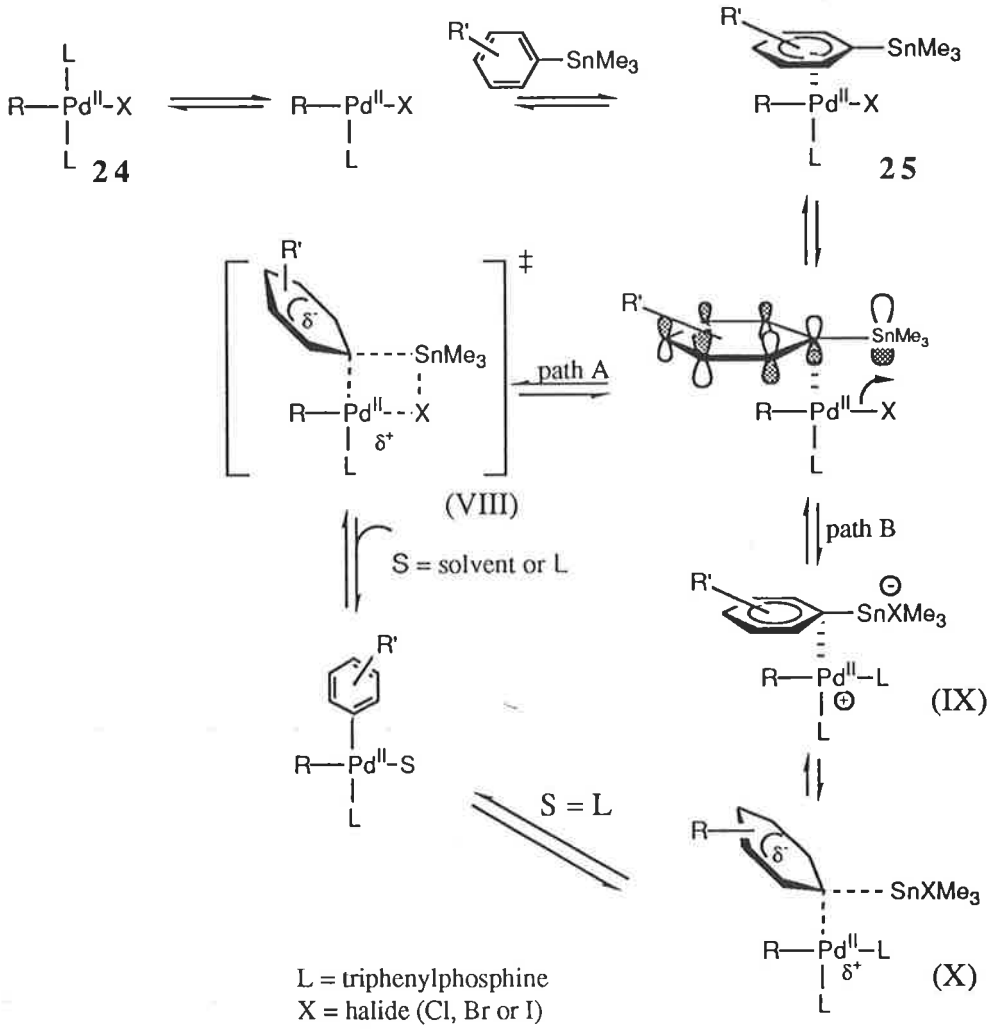
It is important to consider the orbital interactions associated with these transition states. It is not possible for the chloride of the palladium to attack the σ* orbital of the Ar–Sn bond as this would require an Ar–Sn–Cl angle of approximately 180°. The halide may attack the vacant 3d orbital of the tin which is at 90° to the Ar–Sn bond.

By combining the the transition state proposed here (VIII) with the mechanistic arguments devised by Stille *et al.*[72] and by Farina and Krishman[105] (see *Transmetallation* this chapter) the following scenario could be anticipated. The complex resulting from oxidative addition would be of the *trans* configuration[72,90] **24** in Scheme 18. Loss of a ligand from this complex followed by π-complexation as suggested by Farina and Krishman[105] to the arylstannane gives an intermediate **25** geometrically predisposed to form the transition state (VIII) (path A).* In the early stage of this transition state the halide begins to attack the vacant 3d orbital of the tin. The 3d orbital may overlap π-orbitals of the aromatic ring so that electron-acceptors would promote this process and electron-donors would retard it. As the tin takes on the halide it begins to rehybridize, transfering the σ-electrons of the Sn–Ar bond into the π-system of the aryl ring,

* Whilst only alkenyl and allylstannanes were represented in Farina's work,[105] it is anticipated here that aryl stannanes will act in a similar manner.

which is stabilized by electron-acceptors on the ring (path A). At the same time the aryl carbon is rehybridizing and transferring accumulated electron density into the newly forming Ar–Pd σ-bond.

Scheme 18



Alternatively, formation of a distinct pentacoordinate stannate intermediate (IX) could occur (path B), which may be promoted by associative ligand exchange of the halide for a ligand (L). Associative exchange of a halide for phosphine ligand is a known process.[97] In this case transmetallation would be completed via transition state (X).

The involvement of either an insipient or distinct pentacoordinate stannate (path A or B respectively) is perhaps supported by the presumed formation of analogous pentacoordinate silicates in the recently introduced cross

coupling of organosilicates in the presence of a fluoride source[121-4] and the necessity of a halide source in the coupling of aryl and acetylenic copper compounds, where it is expected that the halide gives nucleophilic assistance to the transmetallation process i.e. gives rise to the formation of a $(RCu^{I}X)^{-}$ species (R = aryl or alkynyl, X = halide).[81,125]

A process such as the one just described above could also be used to account for the anomalous transfer of aryl stannanes bearing electron-withdrawing groups in Eaborn's studies[101] (see *Transmetallation* above) A preference for diarylation of $PtCl_2L_2$ is also consistent with the mechanism presented here, as exchange of a halide for an organic group ($PtCl_2L_2 \rightarrow PtClRL_2$) results in a less electron deficient platinium centre, which is therefore less resistant to the development of a positive charge at this centre. This mechanism would also account for the necessity of lithium chloride in Stille couplings where aryl and alkenyl triflates are substrates of oxidative addition,[71-3] i.e. as with the organosilyl and copper compounds organostannanes require, under certain conditions, the nucleophilic assistance of a halide in order to transmetallate.

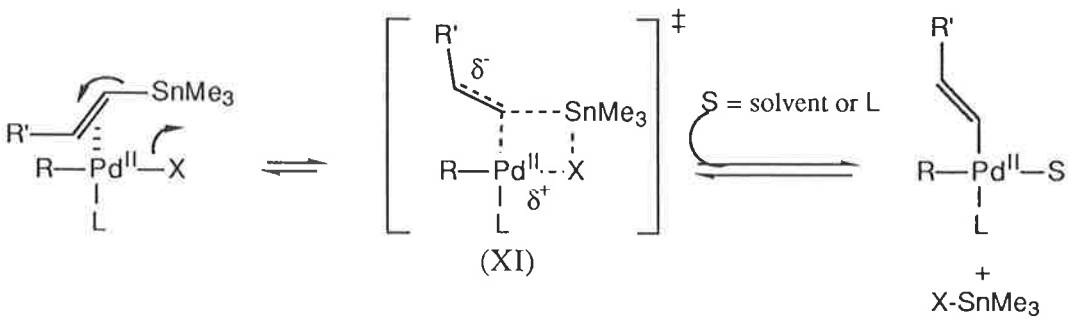
Couplings of aryl or alkenyl triflates that are promoted in the absence of a halide source[80,106] (see *Transmetallation* above) may involve a transmetallation step similar to electrophilic substitution. The absence of a halide on the palladium gives a positively charged palladium(II) species[99,107] of increased electrophilicity which may give rise to a Wheland intermediate similar to that described for platinium(II) complexes (VI) Fig. 7.[101] The removal of the stannonium ion to give the $RArPd^{II}L_2$ complex may take place either by solvation or nucleophilic displacement by a phosphine ligand. The formation of a Wheland intermediate would place opposite electronic demands on the aromatic ring than have been observed here for the Stille reaction. In view of the limited precedent for these couplings and the fact that lithium chloride is often added to reactions involving aryl and alkenyl triflates as a matter of

protocol it is difficult to assess the way in which electronic effects influence reactions that proceed in the absence of a halide. It would appear, however, that the discreet addition of lithium chloride to such reactions may help distinguish between the different mechanisms.

Stille and Scott observed small rate enhancements for the different lithium salts LiX in the order (X = I > Br > Cl).[72] This would appear to support the formation of transition state (VIII), path A. In path B rehybridization of the tin and the aryl carbon via transition state (X) would be expected to be rate determining and is thus not likely to depend on the halide type.

Geometrically, transition states (V) and (VIII) are identical, varying only in polarity and it would appear from the rationalization presented here that depending on the electrophilicity of the transition metal and on whether the aryl group is electron-withdrawing or donating, either could exist.

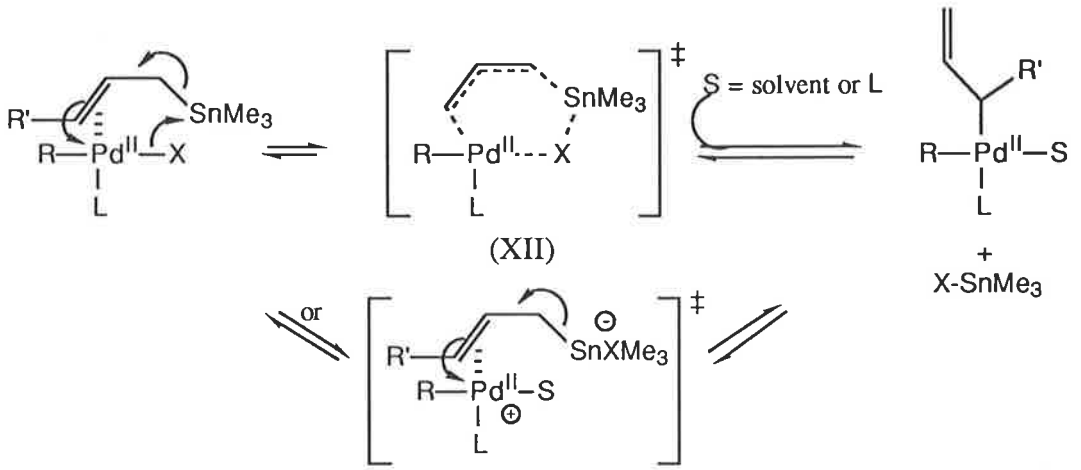
This mechanism can be easily extended to alkenyl systems where a similar observations with respect to substituent effects and halogen requirement have been made. Alkenyl stannanes transfer with complete retention of stereochemistry, this is accounted for on the basis of the mechanism presented here (eq. 16). The order of reactivity alkenyl > aryl would arise from the higher energy required in order to achieve rehybridization in the aromatic ring which leads to a breakdown in aromaticity, i.e. transition state (VIII) is of higher energy than transition state (XI).



(XI)

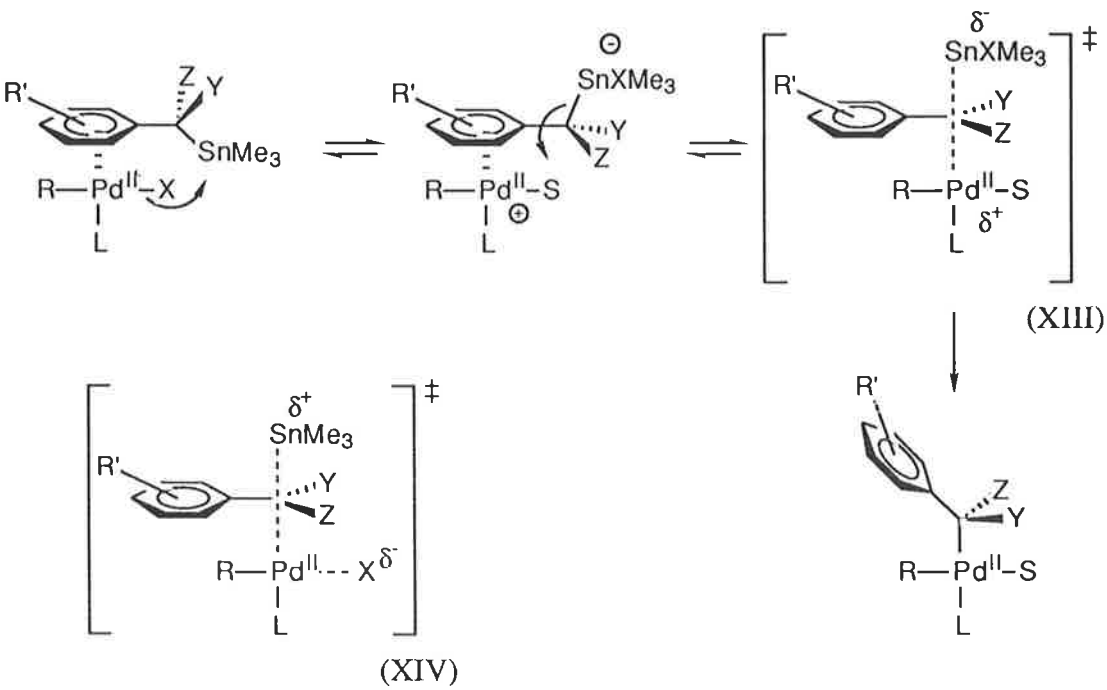
(16)

Allyl stannanes are not as geometrically predisposed to halogen transfer. None-the-less transfer of the allyl group via transition state (XII) or formation of a transient pentacoordinate stannate and subsequent transfer of the allyl group with the allylic rearrangement usually observed[126] are both conceivable (eq. 17).



(17)

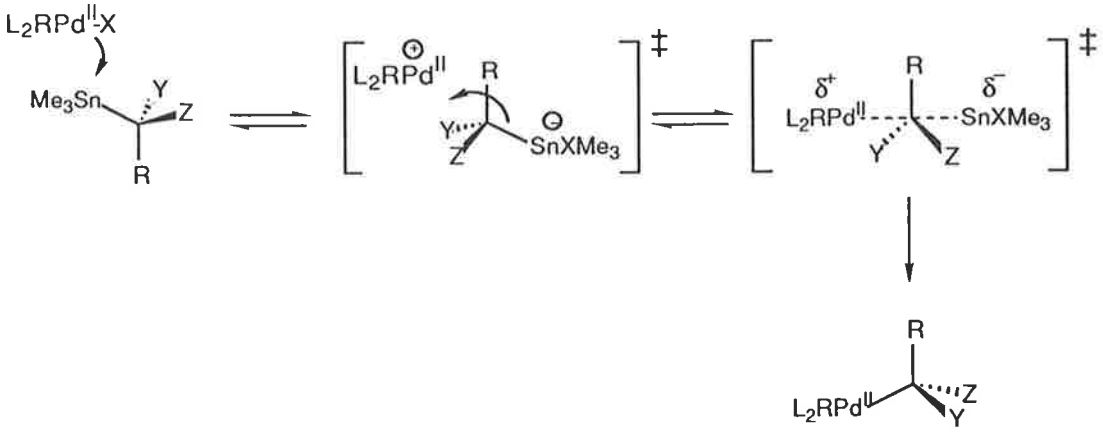
In applying the aforementioned mechanistic considerations to benzyl stannanes the formation of a discrete stannate is necessary in order for the observed inversion of configuration to occur.[127] Hence a transition state of the type (XIII) may occur eq. 18. Stille[127] has already postulated a very similar transition state (XIV).



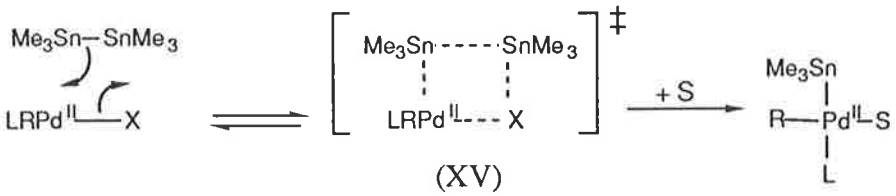
(18)

Stille[127] showed that the rate of transfer increases with increasing electron-withdrawing ability of the aromatic ring, a linear correlation of reaction rate and σ-Hammett values was observed. This data alone may not distinguish between the two transition states (XII) and (XIV) although it would seem more consistent with (XIII) where the transfer of a unit of negative charge through the benzylic carbon is likely to be stabilized in the presence of electron-withdrawing groups. Whilst transition state (XIV) would operate just as, if not more, effectively in the absence of a halide, in (XIII) it would be beneficial. There does not appear to have been a systematic study of the influence of lithium chloride upon the transfer of benzyl groups. In the reports of couplings involving aryl or alkenyl triflates and benzyl stannanes lithium chloride has been added as a matter of protocol.[72] Hence the absolute requirement of lithium chloride in the coupling of either aryl or alkenyl triflates to benzyl stanannes has not been tested.

Alkyl groups may transfer in a similar manner to benzyl groups although prior π-complexation is not possible (eq. 19). Tin transfer from hexamethylditin may occur via a transition state such as (XV), where both 3d orbitals are involved (eq. 20).



(19)



(20)

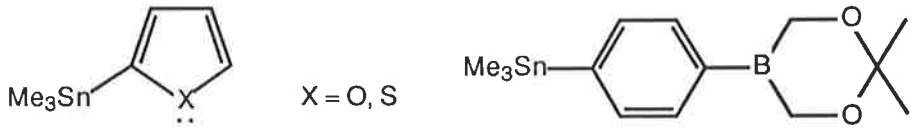
(XV)

On the basis of the above discussion two basic mechanisms appear likely for transmetallation, (i) electrophilic substitution or (ii) halide transfer from the Pd or Pt complex to give a pentacoordinate stannate followed transfer of the most labile group from the stannane to the Pd or Pt complex. Synchronous processes appear possible in most cases depending on the group to be transferred. Conceivably anticipation of the likely mechanism could be based on the substituents born by the group to be transfered from the organostannane, decisions on the choice of catalyst, solvent and the presence of a halide source could be made on the basis of whether they are consistent with the anticipated mechanism. To date the choice of reaction conditions has, in most cases, been based on a trial and error approach, no general set of conditions has been shown to be ideal under all circumstances.[73,97]

The fact that Wigerinck *et al.*[70] and Yamamoto *et al.*[87] observed large rate accelerations using conditions that minimized coordination around the palladium and that high yields over short reaction times were obtained in this work where extensive coordination around the palladium is likely to occur can

perhaps be explained in terms of the mechanistic consideration proposed above. Wigerinck used heteroaryl stannanes of the type shown in Fig. 9, **26**.

Figure 9

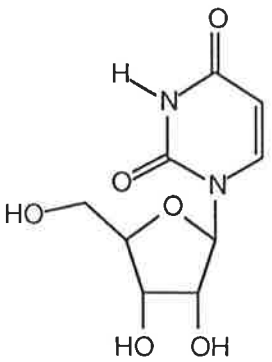


26 **27**

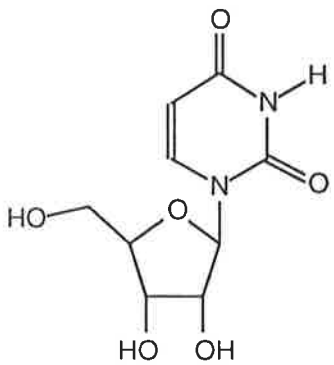
Such furyl and thienyl stannanes would be expected to under undergo electrophilic substitution quite readily in view of the potential of such species to stabilize the presumed Wheland type intermediate or transition state (VI), via lone pair donation from the heteroatom. In Eaborn's work stannanes of this type were the fastest to transfer to the electrophilic platinium complex $Pt(cod)Cl_2$.[101] Yamamoto *et al.*[87] used a phenyltrimethylstannane bearing a boronic ester group in the *para* position **27**, possibly a mechanism akin to electrophilic substitution operates here also. Reducing the level of coordination of electron-donating ligands around the palladium increases its electrophilicity and hence promotes transmetallation via a mechanism akin to electrophilic substitution. The same variation in conditions may aid coupling in the case of **23a** and perhaps **23b** (Table 2, entries 1 and 2), but not those bearing electron-withdrawing groups which may involve an insipient pentacoordinate stannate where the ease of transfer of electron density onto the aryl ring and not the removal of it determines the rate.

Interestingly, by contrast with what has been observed here for aryltrimethylstannanes, Crisp and Macolino[54-5] were able to couple aryl boronic acids bearing powerful electron-donors to sugar protected 5-iodo-2'-uridine, albeit in moderate yield, but were unable to couple those bearing electron-withdrawing groups. This is consistent with a mechanism akin to electrophilic substitution in the case of the aryl boronic acids. The electrophilic substitutions of aryl boronic acids is well precedented.[128]

Not all aryl stannanes attempted coupled to the 5-trifloxyl uridine **12**. Pentafluorophenyltrimethylstannane ($C_6F_5SnMe_3$) did not couple with **12** under the conditions described above. Even after 30hrs only starting material was recovered (73%, the other 27% has presumably decomposed to unknown compounds at the baseline of t.l.c.). The inability to couple this pentafluorophenyl group may result from the formation of a stable nucleoside/pentafluorophenylpalladium(II) intermediate. As cited earlier (see *Reductive Elimination*, this chapter) electron-donating groups promote reductive elimination whilst electron-withdrawing groups retard it. The powerfully electron-withdrawing pentafluorophenyl group may resist reductive elimination entirely in this case. Beleskaya *et al.*[119,120] have also observed this pentafluoroaryltrimethylstannane to be significantly less reactive than other aryltrimethylstannanes in similar coupling reactions, though some coupled products were obtained. Usón *et al.*[129] have isolated *cis*-$Pd(C_4H_8O)_2(C_6F_5)_2$ (C_4H_8O = tetrahydrofuran) and *cis*-$Pd(CO)_2(C_6F_5)_2$ which are quite stable at ambient temperatures.



syn



anti

The biological activity of some pyrimidine nucleosides has been shown to be dependent upon the conformational preference of the base ring with respect to the sugar unit.[130] Both uridine and 2'-deoxyuridine show a preference for the *anti* rather than the *syn* conformation. This presumably arises from a steric interaction between the C2-carbonyl and the 5'-alcohol functionalities in the *syn* conformation.[131,132] Substitution at the C6-position of the pyrimidine base can

lead to a predominance of the *syn* conformation.[132] Previous NMR methods used to establish the *syn* or *anti* conformational preference of pyrimidine nucleosides have included (i) the 3-bond proton-carbon coupling constant, $^{3}J_{C2-H1'}$;[130] (ii) the chemical shift difference between H2'α and H2'β in 2'-deoxyribose nucleosides;[132] and (iii) the chemical difference between C2' and C3' in ribose nucleosides in the ^{13}C NMR spectrum.[131]

For all nucleosides **23a-f** a significant upfield shift of one of the acetate methyl groups was observed in the ^{1}H NMR spectrum (see Experimental). This could be explained where the nucleoside adopts, predominantly, an *anti* conformation. The C5-aromatic substituent would then reside over the C5'-acetate with the methyl group in the shielding region of the aromatic ring, giving rise to the observed upfield shift. It maybe, however, that a positive π-π* stacking interaction exists between the phenyl ring and the acetate groups, aiding conformational preference. Hence, this observation *may* not reflect the situation in the deacetylated nucleosides.

3.2.3 The Attempted Palladium-Catalysed Coupling of Alkylorganostannanes With 2',3',5'-Tri-*O*-Acetyl-5-trifluoromethanesulfonyluridine (12)

Allyl, benzyl and alkyl substituents are known to transfer from an organostannane to the palladium complex much more slowly than do the corresponding alkenyl and aryl subunits.[65] No coupling was observed when allyl and benzyl tri-*n*-butylstannane and tetramethylstannane were reacted with **12** under the conditions already described (eq. 14).

Interestingly, an attempted coupling of allyltri-*n*-butylstannane with **12** in the absence of radical inhibitor lead to the formation of the N3-allylated C5 reduced product **28** in moderate yield (51%) (Scheme 19) . The only other product

isolated from the reaction mixture was a trace amount of monodeacetylated product (<5%).

In devising a possible mechanism to account for this reaction a number of factors were taken into consideration.

-No reaction was observed in the absence of palladium.

-Only starting material was returned when benzyltri-*n*-butylstannane was reacted under the same conditions.

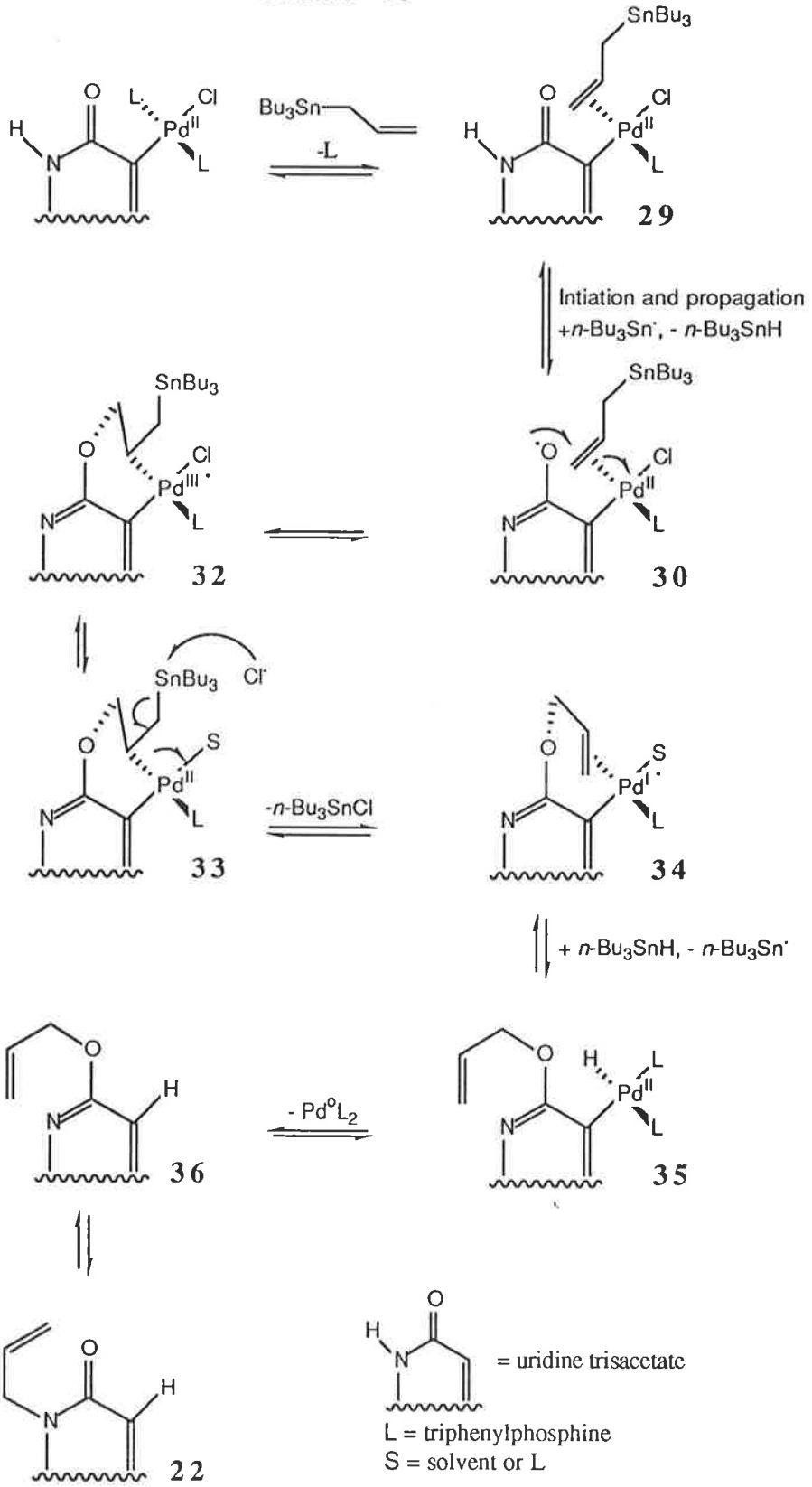
-Allylation at the N3 centre appeared to be concomitant with reduction of the C5 centre, i.e. no N3-allyl C5-trifloxyl product or any simple reduction product was isolated, only **12, 28** and only monodeacetylated **28** were observed by t.l.c. during the progress of the reaction. Furthermore, direct reduction of the C5-trifloxyl centre under the coupling conditions (with or without radical inhibitor) has not been observed in this study.

-This reaction is completely inhibited by the addition of a few crystals of radical inhibitor.

Consequently a unique palladium assisted radical chain process has been postulated (Scheme 19). This mechanism allows for concomitant reduction of the C5 centre and allylation of the N3 centre.

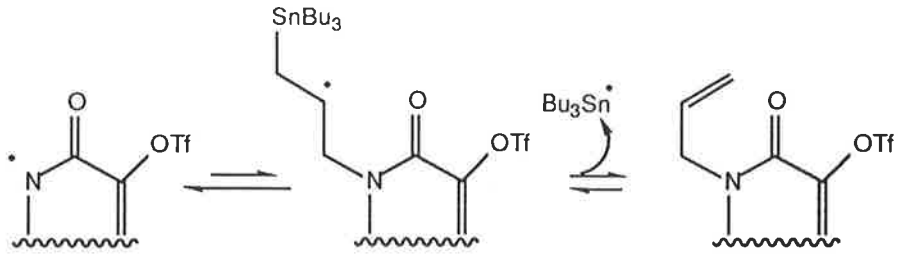
Oxidative addition and triflate/chloride exchange should occur as usual. Since no coupling was observed in the presence of radical inhibitor it is unlikely that transmetallation occurs as reductive elimination would be expected to proceed quite readily for this system. Initial π-complexation of the allyl stannane may occur however, perhaps by dissociative ligand exchange as suggested by Farina and Krishman[105] to give **29**. Radical abstraction of a hydrogen from the the imidyl group by an unknown initiator (probably a tin radical, *n*-$Bu_3Sn^{\cdot}$) gives an imidyl radical **30**.

Scheme 19



Similar formation of an imidyl radical may occur in the absence of C5-palladium(II) group but is of insufficient energy to add to the terminal end of an

allyl stannane to give **31** (eq. 69), or the elimination of an imidyl radical from **31** is very much faster than elimination of a stannyl radical.



31 (21)

The species **30** bears a π-complexed allyl group, such complexation may reduce the energy requirement for addition of an O-centered nucleophilic radical to the terminal end of the electrophilic allyl group, which gives rise to what is perhaps a lower energy palladium(III) radical **32**. Departure of a chlorine radical from **32** returns a palladium(II) species **33**. Attack of the stannane by the chlorine radical promotes elimination to give a palladium(I) radical **34**. A synchronous two electron process similar to that postulated for the formation of **22f** (Scheme 15) could also be evoked for the net reductive elimination of *n*-Bu_3SnCl (**32** to **34**). The palladium(I) radical **34** can abstract a hydrogen from the tin hydride, produced in the formation of **30**, to give the palladium(II) hydride species **35** from which reductive elimination can occur to give the C5-reduced product **36** and regenerating the catalyst (Pd^0L_2). O to N transfer of the allyl group can occur via a thermal polyhetero-Claisen rearrangement or by the palladium-catalysed version of the same to give the observed product **28**.[133,62]

The propagating step in this reaction would presumably involve the tin radical resulting from the formation of **35**, which is capable of abstraction of a hydrogen from the imidyl group **29**→**30**.

Though only speculative this reaction mechanism accounts for all of the observations made in connection with this reaction including, notably, the non-participance of the benzylstannane in this process, where the presumed allylic rearrangement would be much less likely. This process also appears consistent

with the principle of higher energy to lower energy radical formation for radical chain processes, except perhaps for the hydrogen abstraction step **34** to **35** where the equilibrium may lie to the left, however, the subsequent rapid reductive elimination to give **36** becomes the driving force for the process. The palladium assisted reduction of alkenyl and aryl triflates with tri-*n*-butyltin hydride is well precedented but may not involve a single electron process.[135,136]

Chapter 4

Palladium-Catalysed Coupling of Terminal Alkynes to 5-Trifluoromethanesulfonyluridines.

4.1 Introduction

Pyrimidine nucleosides containing a C5 alkynyl group have been shown to express significant anticancer and/or antiviral properties.[28,137-9] This has been, in part, attributed to their ability to act as mechanism based inhibitors of thymidylate synthase.* Also the C5 alkynyl group has been extensively employed as a linker arm for the attachment of fluorescent labels on 2',3'-dideoxynucleotides which are used in modern DNA sequencing techniques.[140-1]

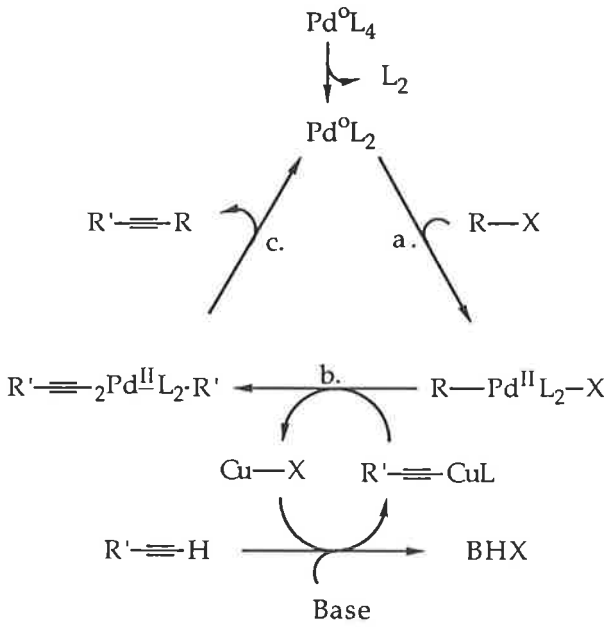
The first reports on the preparation of alkynyl nucleosides involved initial construction of the substituted heterocycle, followed by coupling to the sugar moiety and separation of the resultant anomeric mixture.[46,143] Heck developed a technique whereby terminal alkynes could be coupled to simple alkenyl or aryl halides.[144] Bergstrom subsequently reported that attempts to couple phenylacetylene with 5-chloromercurio or 5-iodouridine in the manner described by Heck returned only starting material or complex mixtures.[145-6]

Sonogashira *et al.*[147] described a modification of Heck's original method in which a co-catalyst, copper(I)iodide, was added in addition to palladium for the coupling of terminal alkynes to aryl and alkenyl iodides. This modification substantially facilitated the coupling process giving good yields of product under milder reaction conditions. The likely catalytic cycle for this process is depicted in Scheme 20. As discussed in Chapter 1 Robins and Barr successfully applied this

* For a discussion on mechanism based inhibition of thymidylate synthase see Chapter 1.

procedure to 5-iodouridine to obtain 5-alkynyluridines in high yield.[66-7] This allowed for ready access 5-alkynyl-2'-deoxyuridines, several expressed appreciable antiviral activity and the corresponding 5'-monophosphates were shown to be mechanism based inhibitors of thymidylate synthase (see Chapter 1).

Scheme 20



a. Oxidative addition; b. Transmetallation; c. Reductive elimination.
X = halide (I or Br) or trifluoromethanesulfonate (triflate).
L = halide.[81]

The 5-alkynyl-2'-deoxyuridines prepared by Robins and Barr bore only simple alkyl chains or an unsubstituted phenyl ring.[47,137] A subsequent structure activity relationship study on 5-alkenyl-2'-deoxuridines revealed that electron-withdrawing, lipophilic groups in the *E*-2 position of the alkenyl substituent enhanced antiviral activity.[27] The effects of lipophilic electron-withdrawing groups on an acetylenic linkage with regards to antiviral activity have not been formally investigated.

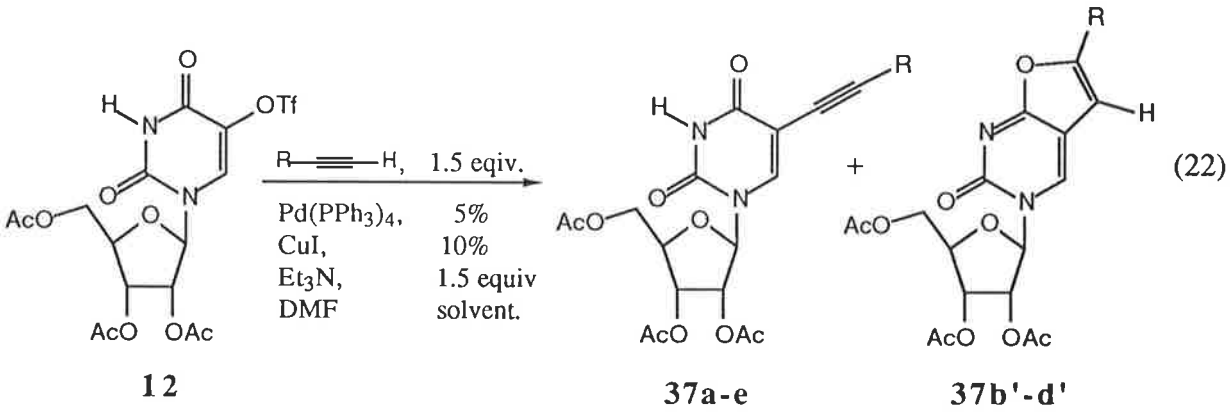
In view of the importance of C5 alkynyl uracil nucleosides and nucleotides the coupling of terminal alkynes to the C5-trifloxyl uridines was considered worthy of investigation. For inclusion in this study a series of fluorinated and trifluoromethylated phenylacetylenes (PAs) were prepared (Chapter 5), so that

their 2'-deoxyuridine adducts could be investigated as potential antiviral/anticancer agents.

4.2 Results and Discussion

4.2.1 Coupling of Terminal Alkynes to 2',3',5'-Tri-*O*-acetyl-5-trifluoromethanesulfonyluridine (12.)

The acetylated uridine triflate **12** was coupled with a range of functionalized terminal alkynes as described in eq. 22. The results of the coupling reactions have been summarized in Table 3.



(22)

Table 3: Coupling of Terminal Alkynes With **12** (eq. 22).

Entry	R	Reaction Conditions		Product (37)	Yield % [a]
		Time (hr)	Temp (°C)		
1.	Me_3Si-	2.5	50	a	85
2.	$HOMe_2C$-	0.5	55	b	90
				b'	3
3.	$CH_3(CH_2)_3$	6.0	90	c	90
				c'	2
4.	Ph	2.0	45	d	93
				d'	5
5.	4-MeOPh	0.5	55	e	97

[a] Isolated.

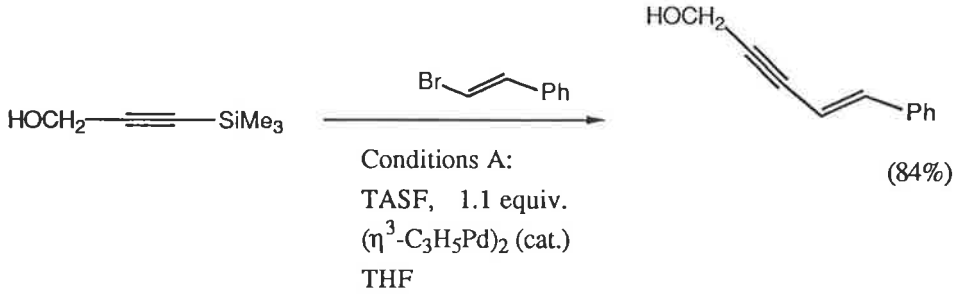
Reactions were monitored by t.l.c. and were "worked up" when it was apparent that all of the C5-trifloxyl uridine had been consumed. In all cases the coupled product, **37** was obtained in high yield. In most cases coupling was

observed at room temperature but it was often very slow, slight elevations in the reaction temperature led to a dramatic increase in the rate of coupling and was, in general, much more convenient. In the case of **37c**, however, no coupling was observed by t.l.c. until the reaction mixture was heated to 90°C.

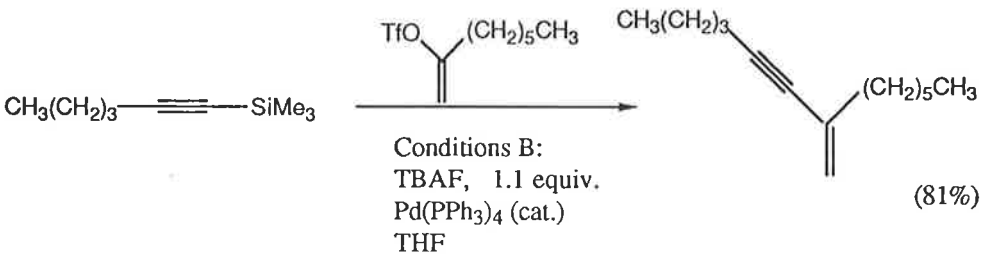
Whilst reactions, generally, proceeded cleanly by t.l.c. in a few cases a fluorescent spot of lower R_f appeared. This by-product was readily separated from the desired product and was shown in each case to be the rearranged product **37'**. In all cases the formation of **37'** was evidenced by an absence of the NH resonance (δ = 9-11ppm) in the ^1H NMR spectrum and the appearance of a vinylic resonance (δ = 6-7ppm) relative to **37**. The formation of this by-product in the coupling of terminal alkynes to 5-iodouridine is well precedented.[66-7] Robins *et al.*[148] recently reported that the use of dimethylformamide (DMF) as a solvent reduces the amount of cyclized product obtained and it is for this reason that DMF was used in all couplings. It has been demonstrated that the product cyclization is catalysed by copper(I)iodide.[67]

4.2.2 Coupling of Fluorinated and Trifluoromethylated Phenylacetylenes to 3',5'-Di-*O*-acetyl-5-trifluoromethanesulfonyl-2'-deoxyuridine (14)

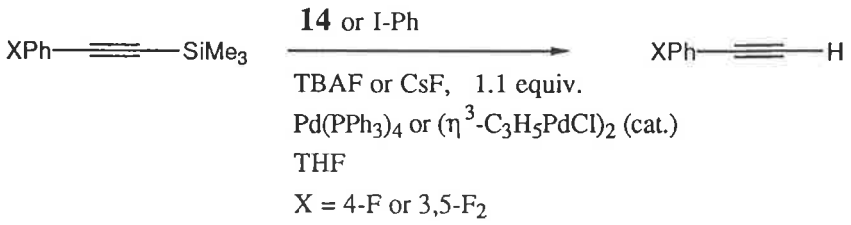
Hiyama *et al.*[121-3] recently reported that trimethylsilylalkynes can be cross coupled with alkenyl and aryl halides (Br or I) or triflates as illustrated by eq. 23 and eq. 24. Since the immediate precursor of the substituted phenylacetylenes (PAs) was the corresponding phenyltrimethylsilylacetylene (PSA) direct coupling of PSA derivatives with the protected 2'-deoxyuridine triflate **14** under the conditions described by Hiyama *et al.* was attempted as described in eq. 25.



(23)[121]



(24)[123]



(25)

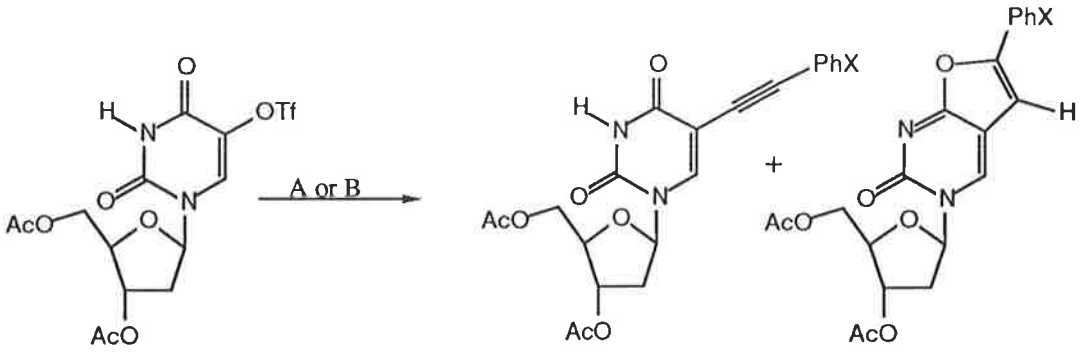
Unfortunately no coupling was observed, only the desilylated phenylacetylene and **14** could be detected after 24hrs by gas-liquid-chromatography (g.l.c.) and t.l.c. respectively under either Conditions A or B (eq.s 23 & 24 respectively) (eq. 25). Reactions were then attempted under a variety of conditions with respect to the rates and order of addition of the various reagents but to no avail. The replacement of either the nucleoside triflate with iodobenzene or of the tetrabutylammonium fluoride with anhydrous cesium fluoride did not alter the outcome eq. 25.

Hiyama *et al.*[124] proposed that transfer of an organic group onto the palladium occurs from a pentacoordinate silicate. It may be that where desilylation is facilitated by the presence of electron-withdrawing groups no, or only a very transient, pentacoordinate silicate is obtained and desilylation occurs rapidly. The resultant acetylide may not then react with the organohalide-palladium complex.

Since in our hands, the PSAs could not be coupled directly, they were desilylated to give the corresponding PAs in high yield (Chapter 5, Table 9).

Coupling of terminal alkynes to the protected C5-trifloxy-2'-deoxyuridine **14** were initially carried out under the conditions described previously in eq. 22 (i.e. conditions A eq. 26). The results of these couplings are summarized in Table 4 entries 1-5.

As expected the trimethylsilylacetylene coupled as effectively to the protected C5-trifloxy-2'-deoxyuridine **14** as it did to the equivalent uridine **12** (compare entries 1 Table 3 and 4). The coupling of the PAs containing fluoro substituents entries 2-5 Table 4 were carried out at room temperature, although the consumption of the starting material **14** (as monitored by t.l.c.) was much slower than anticipated for such activated systems. Also, unlike previous couplings, the further addition of alkyne and in some cases catalyst was necessary in order to ensure the complete consumption of the starting material **14**. In the case entries 2-4 considerable amounts of cyclized product **38'** was obtained. This was attributed to the longer reaction times which, allow for more product to cyclize and the presence of electron withdrawing groups which appear to promote cyclization. A high R_f (t.l.c.) component of the reaction mixtures was also isolated for entries 3 and 4 and determined to be homocoupled alkyne, **39**. The mass balance of homocoupled alkyne plus the expected cross coupled product accounted for all of the terminal alkyne added to the reaction mixture. A similar high R_f component was detected by t.l.c. for entries 2 and 5 (as an intense and faint spot respectively) but in these cases the component was not isolated.



14 **38a-f** **38b'-e'** (26)

A: PhX—≡—H, 1.5 equiv.; $Pd(PPh_3)_4$, 5%; CuI, 10%; Et_3N, 1.5 equiv.; DMF, solvent.

B: PhX—≡—H, 2.0-2.5 equiv.; $Pd(PPh_3)_4$, 10%; CuI, 20%; Et_3N, 1.2 equiv.; DMF, solvent.

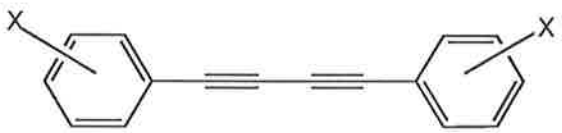
Table 4: Coupling of Trimethysilylacetylene (TMSA) and Substitituted Phenylacetylenes (PA's) With **14** Under Conditions A & B (eq. 26).

Entry	X	Reaction Conditions			Product (38)	Yield % [a]
		Reagents	Time (hr)	Temp. (°C)		
1.	Me_3Si-C≡C- [b]	A	2.5	50	a	87
2.	3,5-CF_3	A[c]	8.0	20	b	51
					b'	21
3.	3,5-F	A[c]	10.0	20	c	31
					c'	60
4.	4-CF_3	A[c]	10.0	20	d	43
					d'	33
5.	4-F	A[c]	8.0	20	e	50
					e'	*
6.	3,5-CF_3	B	4.0	20	b	80
					b'	7
7.	3,5-F	B	2.0	38	c	70
					c'	12
8.	4-CF_3	B	2.0	37	d	82
					d'	*
9.	4-F	B	3.0	38	e	87
					e'	*
10.	F_5	B	5.0	55	f	87

a Isolated yields, except for * – material detected by t.l.c. but not isolated.

b Trimethylsilylacetylene used in reaction (eq. 26) not a substituted phenylacetylene.

c Further addition of PA and catalyst was required in order to achieve complete consumption of **14**.



39

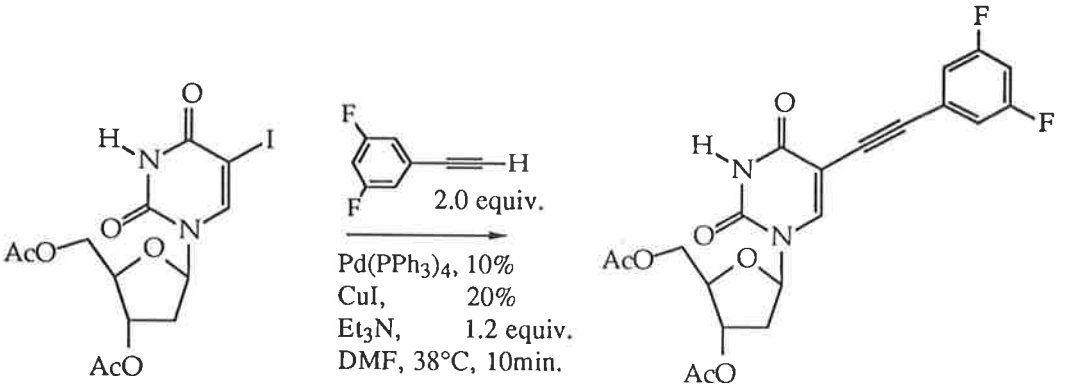
The homocoupling of the alkyne was studied in detail (Chapter 6) so that conditions could be adjusted to minimize this competing reaction. It was found that both catalytic amounts of copper(I), palladium(0) and base were all required in order to achieve homocoupling of terminal alkynes. Homocoupling was promoted in the presence of electron-withdrawing groups on the phenyl ring of PAs. Evidently the process of homocoupling competes with cross coupling not only in the consumption of terminal alkyne but also with regards to access to the catalyst. This may account for the longer reaction times than anticipated in cases where considerable homocoupling was observed.

In order to increase the overall rate of cross-coupling and so decrease the reaction time and therefore the amount of cyclized product formation, the amounts of both catalysts were increased. An increased amount of terminal alkyne was also used so as to avoid any interruption in the cross-coupling reaction due to premature depletion of PA through homocoupling (conditions B, eq. 26). Only in the case of **38b** did these alterations prove significantly effective (entry 6, Table 4). When the amount of catalyst and PA was doubled in the cases **38c-e** only a minor benefit was achieved (these results are not shown). However, when the reactions were carried out under these new conditions at slightly higher temperatures a much better result was obtained. Apparently the process of cross-coupling is much more sensitive to minor temperature changes then are either homocoupling or product cyclization. Again, all excess terminal alkyne could be accounted for as homocoupled product for entries 6-8. Formation of **39** also appeared to be occurring in the case of entries 6 and 9 (as observed by t.l.c.) but again this product was not isolated.

Minimizing the reaction time appeared important in achieving a high ratio of coupled product to cyclized product, the major cause of the extended reaction time has been attributed to a competition for the palladium(0) between the nucleoside triflate and the terminal alkyne. Increasing the competitiveness

of the nucleoside substrate for the palladium(0) should increase the rate of reaction and consequently the yield of desired product. As discussed previously in Chapter 3 (*Oxidative Addition*), aryl iodides undergo oxidative addition to palladium(0) faster than do aryl triflates (Chapter 5). If this were also true of the C5 iodo and trifloxyl uridine than the iodo nucleoside should be a more effective substrate in the coupling of these activated PAs.

In order to test this possibility 3',5'-di-*O*-acetyl-5-iodouridine **40** was reacted with 3,5-difluoro-PA, as this acetylene, even under the best possible conditions described, entry 7 Table 4, gave only a relatively moderate yield of cross coupled product when reacted with **14**. The reaction was carried out under the same conditions as described for the triflate **14** (entry 7, Table 4) as depicted in eq. 27. The reaction was complete within 10 minutes compared with 1hr for **14** (entry 7, Table 14), no cyclized product and only a very small amount of homocoupled product could be detected by t.l.c. The desired product, **38c**, was isolated in a 98% yield.



40 **38c** 98%

(27)

The coupling of the pentafluoro-PA was not attempted till the reaction conditions of the other substituted phenylacetylenes had been optimized, as it was anticipated that the powerfully electron-withdrawing perfluoroaryl group would hypersensitize the alkyne towards homocoupling and the cross coupled product to cyclization. Surprisingly these concerns were quite unfounded, only a small amount of homocoupled material and no cyclized product could be detected in these reactions (t.l.c.). Furthermore, the pentafluoro-PA was the least

reactive of all the aryl acetylenes, requiring a longer reaction time at the same temperature than even the *p*-methoxy-PA in order to couple. None-the-less due to the lack of by-product formation the reaction proceeded smoothly at elevated temperatures to give **38f** in high yield (87%, entry 10, Table 4). In short, the pentafluoro-PA showed opposite reactivity in all processes (cross-coupling, homocoupling and cyclization) for which electron-deficient PAs are activated.

The reduced rate of reaction of the pentafluoro-PA in both homocoupling and cross coupling in comparison to other aryl acetylenes may result from a reduced rate of reductive elimination. As indicated earlier (Chapter 3, *Reductive Elimination*) electron-withdrawing groups inhibit reductive elimination. This is consistent with the observation that pentafluorophenyltrimethyltin did not couple at all with the C5-trifloxyl uridine **12** (Chapter 3). In the case of the pentafluoro-PA, however, the inhibiting effect of the five fluoro groups during the reductive elimination step of the catalytic cycle is, presumably, not as pronounced when it is commuted through an acetylenic linkage.

Possible explanations for why the **38f** resists cyclization are considered in Chapter 5 in connection with the relevant substituent effects.

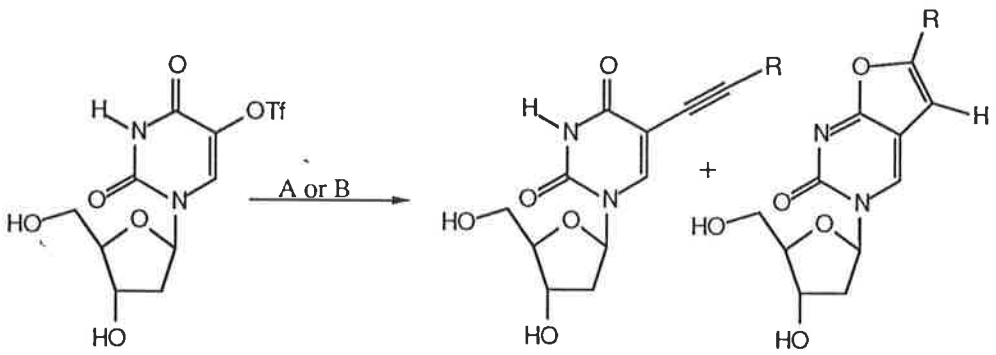
4.2.3 Coupling in the Absence of Sugar Protecting Groups.

5-Trifluoromethanesulfonyl-2'-deoxyuridine **8** and other 5-substituted 2'-deoxyuridines containing the free hydroxyl sugar groups are readily soluble in polar organic solvents and can be chromatographed readily using silica gel. This is in contrast to unprotected 5-trifluoromethanesulfonyluridine **7** which can only be chromatographed using very polar organic solvent systems in normal phase chromatography, which does not allow for effective separation of the various compounds that may result from coupling reactions.

Robins and Barr[66] reported that an attempt to couple 5-iodo-2'-deoxyuridine in the absence of sugar ring protecting groups with 1-hexyne did not proceed and that attempts under more strenuous conditions gave mixtures of products.

Whilst the coupling of 1-hexyne to **8** was not attempted, coupling of other alkynes was readily achieved (eq. 28) and the results have been summarized in Table 5. The unprotected nucleoside **8** appeared only slightly less reactive than the protected nucleoside. Slightly higher reaction temperatures and/or longer reaction times were required in order to achieve coupling in equivalent reactions (compare Table 5 entries 1-6 with Table 4 entries 1&6-10 and compare Table 5 entries 7&8 with Table 3 entries 4&5).

The slight reduction in reactivity of the unprotected nucleoside triflate **8** was sufficient in cases sensitive to homocoupling to have this become the favoured process. Consequently only small amounts of cross coupled material could be observed in these cases (entries 2-4, Table 5).



8 **41a-h** **41b'-e'&g'** (28)

A: R—≡—H, 1.5 equiv.; $Pd(PPh_3)_4$, 5%; CuI, 10%; Et_3N, 1.5 equiv.; DMF, solvent.
B: R—≡—H, 2.0-2.5 equiv.; $Pd(PPh_3)_4$, 10%; CuI, 20%; Et_3N, 1.2 equiv.; DMF, solvent.

Table 5: Coupling of Trimethysilylacetylene (TMSA) and Substitituted Phenylacetylenes (PA's) With **8** (eq. 28).

Entry	X	Reaction Conditions			Product (41)	Yield % [a]
		Reagents[b]	Time (hr)	Temp (°C)		
1.	Me_3 Si-C≡C-	A	2.0	50	**a**	88
2.	3,5-CF_3	B	5.0	30	**b**	*
					b'	*
3.	3,5-F	B	5.0	38	**c**	*
					c'	*
4.	4-CF_3	B	5.0	38	**d**	*
					d'	*
5.	4-F	B	4.0	40	**e**	76
					e'	*
6.	F_5	B	3.0	80	**f**	89
7.	H	A	2.0	50	**g**	88
					g'	*
8.	4-MeO	A	2.0	65	**h**	95

[a] Isolated except, * – small amounts of product or cyclized product observed by t.l.c., in these cases mostly starting material and homocoupled alkyne and **8** were observed (except for entry 7).

[b] For reagents A and B see eq. 28.

The direct coupling of unprotected C5-trifloxy-2'-deoxyuridine **8** with fluoaromatic alkynes gave directly some compounds which could be sent for testing as anticancer or antivirial agents. Nucleosides which were not accessible from direct coupling of the corresponding PA with the unprotected nucleoside **8**, were readily obtained by deacetylation of the corresponding bisacetate, in particular **41b-d**. This deprotection was readily achieved by treatment of compounds **41b-d** with methanolic ammonia to give the free hydroxyl products **41b-d** in high yield (80-99%, see Experimental).

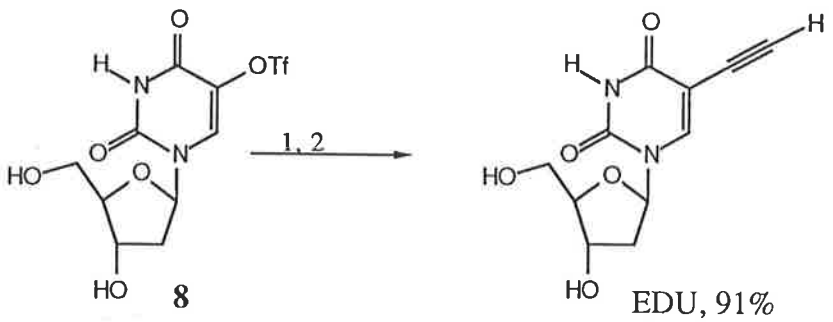
4.2.4 Reverse Coupling: The Coupling of 5-Ethynyl-2'-deoxyuridine to Aryl Bromides

An alternative to the coupling of terminal acetylenes to C5 iodo or trifloxyl uridine would be to couple the 5-ethynyl uridine to either an aryl or alkenyl triflate or halide. This would avoid prior elaboration of the aryl or alkenyl halides or triflates to alkynes in order to achieve coupling to the iodo or trifloxyl

nucleosides. It may also avoid some of the difficulties associated with homocoupling experienced in this work.

5-Ethynyl-2'-deoxyuridine (EDU) is a known compound which exhibits significant antiherpetic activity (Chapter 1). If this terminal alkyne could be coupled to the fluorinated aryl bromides used in the preparation of the corresponding PAs, then the modified nucleosides outlined in Table 5 would be conveniently obtained.

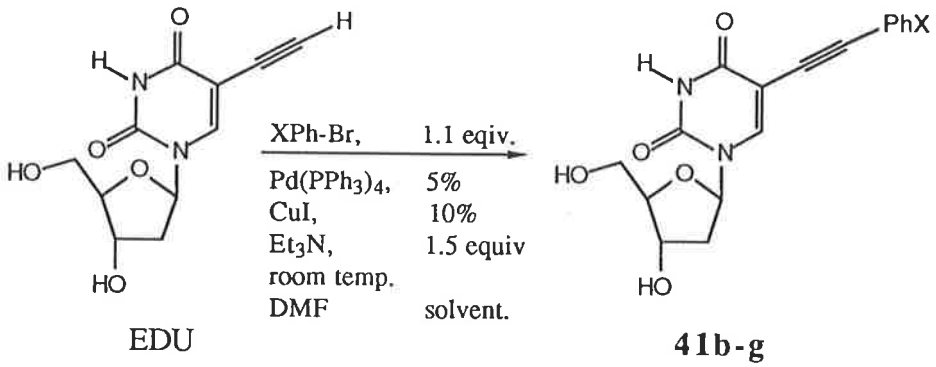
EDU was readily prepared by desilylation of the corresponding trimethylsilyl compound **41a**. The best yield was achieved by treatment of the crude product resulting from the coupling of trimethylsilylacetylene with the unprotected nucleoside triflate 8 (entry 1, Table 5) with tetrabutylammonium fluoride, giving the desired product (EDU) in high yield (91%, eq. 29).



(29)

1. $Me_3Si{-}{\equiv}{-}H$ 1.5 equiv.; $Pd(PPh_3)_4$, 5%; CuI, 10%; Et_3N, 1.5 equiv.; DMF, solvent.
2. TBAF 1 equiv., MeOH

The coupling of EDU with a series of aryl bromides was investigated, reactions were carried out under the conditions depicted in eq. 30 and the results are summarized in Table 6. Only those systems activated towards oxidative addition coupled at all (entries 1-3, Table 6). In the case of bromopentafluorobenzene coupling, again, may have been inhibited by the slow rate of reductive elimination.



(30)

Table 6: Coupling of 5-Ethynyl-2'-deoxyuridine (EDU) to substituted Aryl Bromides (eq. 30).

Entry	X	Reaction Conditions		Product (41)	Yield % [a]
		Time (hr)	Temp (°C)		
1.	3,5-CF_3	4	20	b	49
2.	3,5-F	6	40	c	16
3.	4-CF_3	6	40	d	55
4.	4-F	8	50	e	0
5.	F_5	8	50	f	0
6.	H	18	50	g	0

[a] Isolated.

It was apparent by t.l.c. that the EDU was undergoing competitive decomposition since the U.V. active spot corresponding to EDU disappeared after ~6hrs under the reaction conditions when no coupling took place. Homocoupling of EDU may have been occurring but no new U.V. active spot was observed by t.l.c. (254 and 365nm).

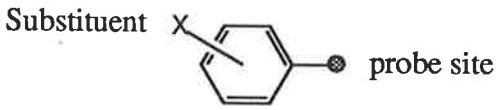
The use of aryl iodides in place of the bromides and perhaps the presence of acetate protecting groups on the the sugar ring of EDU may give more efficient couplings, however, these reactions were not investigated.

Chapter 5

Substituent Effects in the Preparation, Reactivity and Nuclear Magnetic Resonance Spectra of Substituted Phenylacetylenes and Substituted 5-Phenylalkynyl-2'-deoxyuridines.

5.1 Introduction

The quantification of electronic influence upon a molecular framework has lead to the development of a series of very useful, experimentally derived, parameters termed substituent constants (σ). Of particular interest has been the effect of various substituents located at different positions on a benzene derivative upon a probe site. The effect of a substituent upon the probe site is normally given by the linear free energy relationship (eq. 31).[149-51]



$$f(\sigma_{o,m,p}) = \rho\ \sigma_{o,m,p}\,(X) + C \qquad (31)$$

Where f is the effect being measured at the probe site (e.g. reaction rate, ionization energy, NMR chemical shift etc.); the value ρ (constant for a given f) is the susceptibility factor of f to the substituent effects; $\sigma_{o,m,p}$ is the effect of a particular substituent (X) in a given position (*ortho*, *meta* or *para*); and the constant, C, is the measure of the effect, f when X = H since $\sigma_{o,m,p}$ (H) = 0.

When determining $\sigma_{o,m,p}$ values for the first time a *standard reaction series* was used by Hammett,[152,153] namely the pKa values of a series of substituted benzoic acids (i.e. the carboxylic acid was the probe site). In this case ρ was arbitrarily placed at 1.0 and the $\sigma_{o,m,p}$ values determined. Other *standard reaction series* have been developed,[154-6] in order to avoid the problems

sometimes associated with solubility or hydrolysis in the case of some substituted benzoic acids and slightly different σ values have been obtained. Also statistical values of σ have also been determined based upon a number of different experimental determinations of σ values.[156] *Ortho* substituent constants are usually neglected however, as they often reflect proximity effects in addition to electronic influences (e.g. steric effects, hydrogen bonding).[157]

It has long been understood that substituent effects arise from several different properties of the substituent. Considerable effort has been directed at determining and quantifying these different properties and their relative contributions to the overall value of σ. Four basic properties have been generally recognized as contributing to the substituent effect and in many cases a specific σ value has been assigned to this property for a given substituent:[151,158]

(i) the inductive effect, χ (constant σ_χ) where the electronegativity of the substituent leads to a through-sigma-bond charge transfer to the probe.

(ii) the field effect, F (σ_F) where the substituent dipole induces a dipole upon the probe via through space interactions.*

(iii) the resonance effect R (σ_R) where the substituent transfers electron density via conjugation, hyperconjugation, or through a lone pair interaction with the probe.

(iv) the polarizability effect, P (σ_α) where a charge or dipole on the probe can be stabilized by inducing a dipole upon the substituent.

By appropriate use of the parameters σ_F, σ_χ, σ_R and σ_α substituent induced changes at the probe can be analyzed by deriving a multiple linear regression correlation of the form eq. 32.

$$f(\sigma_i) = \sum \rho_i \sigma_i + C \qquad (32)$$

* The field effect is also refered to as the inductive effect in the literature and the corresponding parameter is often written as σ_I but this should not be confused with the electronegative inductive effect χ.

The relative value of ρ_i reflects the contribution of a particular effect F, χ, R or P to the nett effect of the substituent. This value, ρ_i, is likely not only to vary with the nature of the probe, but also the position of the substituent (*o*, *m*, *p*).

Inductive (χ) and polarizability (P) effects are very distance dependant and fall off dramatically after only a couple of bonds.[158-60] Whilst these effects may be relevant for *ortho* substituted benzenes, for the much more studied *meta* and *para* substituted benzenes ρ_χ and ρ_α are usually considered to be negligible. Hence eq. 32 becomes eq. 32a,

$$f(\sigma_{F,R}) = \rho_F \sigma_F + \rho_R \sigma_R + C \qquad (32a)$$

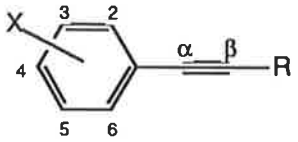
This use of two substituent parameters in deriving a correlation is termed a dual substituent parameter (DSP) analysis.[150] The ratio of ρ_F:ρ_R ($\rho_R/\rho_F = \lambda$) reflects the relative contribution of field and resonance effects experienced by the probe.

Several elegant DSP correlation studies on *p*-substituted phenyltrimethylsilylacetylenes (PSAs) and *p*-substituted phenylacetylenes (PAs) have been reported.[161-3] The substituent effects were measured as a function of the change in ^{1}H, ^{29}Si and ^{13}C NMR chemical shifts ($\Delta\delta$) of the alkynyl unit (probe). Where changes arising anisotropic or solvent effects are absent or negligible the difference in chemical shift ($\Delta\delta$) at infinite dilution gives a precise measure of the change in electron-density (Δq) for systems bearing different substituents.

The λ values obtained from DSP analysis of *p*-substituted phenyltrimethylsilylacetylenes and phenylacetylenes are summarized in Fig. 10, in each case this value for R = H and Si is approximately the same, in particular $\lambda_\beta \sim 0.80$.[161-3] These λ values are significantly higher than those at equivalent sites in styrene

and are reflective of an increased contribution from F (the field effect) and a decreased contribution from R (the resonance effect) in *p*-substituted phenylacetylenes and and phenyltrimethylsilylacetylenes relative to their styrene equivalents.[162-3]

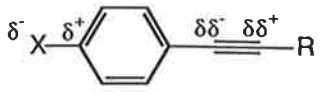
Figure 10



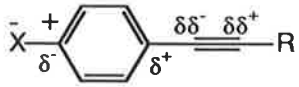
R = H[a]	λ_β	= 0.79	(0.53)[b]
	λ_{C1}	= 0.20	(0.21)
	λ_H	= 0.84, 0.81[c]	(0.55)
R = $SiMe_3$[d]	λ_β	= 0.83	
	λ_{C1}	= 0.24	
	λ_{Si}	= 0.75	

[a] Unless otherwise stated values are from ref. 162.
[b] Values in parenthsis are for styrene.
[c] Value from ref.161.
[d] Values from ref. 163.

The field effect of the substituent upon the acetylene moiety is represented by **42**, a through space polarization of the acetylene results from the dipole of the C-X bond. This dipole will also polarize the π-system of the phenyl ring as depicted by **43**. The polarization of the phenyl π-system leads in turn to the polarization of the acetylene π-system. This latter, secondary type, effect as shown by **43** is termed π-polarization.[162-3]

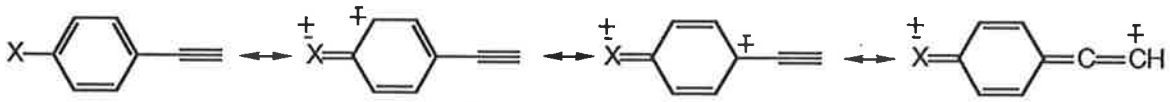


42



43

polarization: ± > δ > δδ



44a 44b 44c 44d

The resonance effect of the substituent upon the acetylene unit is represented by the resonance contributors **44a-d**. There is other theoretical[164] and physiochemical[165] evidence to suggest a reduced contribution from **44d** in PAs relative to the equivalent in styrene. Consequently, for phenylacetylenes, the resonance contribution of the substituent is localized on the phenyl ring leading to an increase in contribution from **44b** and **44c** relative to the styrene equivalents. Contributor **44c** may accentuate π-polarization of the alkynyl unit (**43**) or decrease it depending on whether the values σ_F and σ_R have the same or opposite sign.[162] The major contributor to perturbation of electron density in the acetylene unit of substituted PAs and PSAs is thought to be F induced π-polarization **43**.[162-3]

These previous studies have concentrated primarily on *p*-substituted PAs and PSAs, different λ values could be expected for *m*-substituted and *o*-substituted PAs & PSAs and may involve other substituent effects, although some of the same principles with regard to mechanisms of transmission of effects may still apply, e.g. π-polarization.

In the study presented here a limited number of variously substituted phenyl bromides have been coupled to trimethylsilylacetylene (TMSA) and desilylated, the terminal acetylenes were then coupled to nucleoside triflates as described in Chapter 4. In view of the limited number of aryl bromides used and

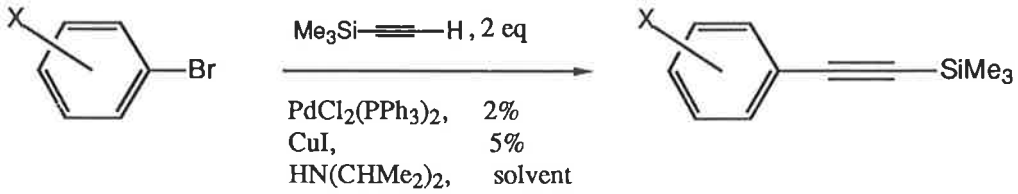
the variation in substituent location (*ortho, meta* or *para*) a precise correlation study was not anticipated. However, a qualitative comparison of substituent parameters (σ-Hammett), NMR chemical shifts and reactivity data may increase our understanding of observed trends in reactivity.

In particular, it was considered that a correlation between substituent σ-Hammett values and $\Delta\delta\ ^{13}C6$ (and therefore the Δq at the C6 centre) of 5-phenylalkynyl-2'-deoxyuridines with various substituents on the phenyl ring may be of use in developing mechanism based inhibitors of enzymes that utilize the C6 centre of uracil based nucleosides in cysteine-thiol Michael additions. Thymidylate synthase is an example of such an enzyme, its significance as a target in anticancer and antiviral chemotherapy is discussed in Chapter 1.

5.2 Results and Discussion

5.2.1 The Coupling of Trimethylsilylacetylene to Arylbromides.

The fluoro-substituted aryl bromides were coupled to trimethylsilylacetylene in the presence of a catalytic amount of bis(triphenylphosphine)palladium dichloride [$Pd(PPh_3)_2Cl_2$] and copper(I)iodide in diisopropylamine (eq. 33). Reactions mixtures were initially stirred at room temperature and monitored by g.l.c.. If no, or only a small amount of, coupled product was observed after 16hrs reaction mixtures were heated to reflux. The the reaction conditions for these couplings are summarized in Table 7. Also included in this table are the Hammett-$\sigma_{o,m,p}$ values for each substituents, where more than one substituent is present the $\sigma_{o,m,p}$ values have been added directly. The change in chemical shift values were obtained from eq. 34.



(33)

$$\Delta\delta\, C_n\,(X) = \delta\, C_n\,(X) - \delta\, C_n\,(H) \qquad (34)$$

Where $\Delta\delta\, C_n\,(X)$ is the change in chemical shift ($\Delta\delta$) for a particular carbon centre (C_n) of a certain substituted PSA. The values $\delta\, C_n\,(X)$ and $\delta\, C_n\,(H)$ are the chemical shifts (δ) at a particular carbon (C_n) for the substituted and unsubstituted PSAs respectivily.

Table 7: Mildest Reaction Conditions for the Coupling of Substituted Aryl Bromides with Trimethysilylacetylene (TMSA) eq. 33.

Entry	X	σ [a]	Reaction Conditions		Yield %[e]	$\Delta\delta$ (PSA) [d]			$\Delta q\pi$ ($\times 10^4$)[b]
			Time (hr)	Temp (°C)		C1	C_α	C_β	C1
1.	F_5	1.80[c]	1.5	84	84[f]	–22.94	–18.21	+16.14	–836
2.	3,5-CF_3	0.86	5	20	98	+ 2.28	– 3.60	+ 5.66	+ 20
3.	3,5-F	0.68	16	20	95	+ 2.77	– 2.71	+ 3.64	+439
4.	4-CF_3	0.54	16	20	97	+ 3.88	– 1.69	+ 4.06	+244
5.	4-F	0.06	1	84	97	– 6.63	– 1.16	+ 0.80	–271
6.	4-OMe	–0.27	1.5	84	87	– 7.97	+ 0.02	– 3.93	–425

[a] All values from reference 154.
[b] All values obtained from reference 166, value for entries 1 & 2 were calculated assuming additivity (see text).
[c] The σ_0 (F) value was obtained from reference 167.
[d] Changes in chemical shift $\Delta\delta$ are relative to unsubstituted PSA for which δ C1 = 123.13ppm, C_α = 105.12ppm and C_β = 94.06ppm. Relative assignment of C_α and C_β chemical shifts was based on the appearance of a satelite doublet for C_α arsing from $^{29}Si/^{13}C$ coupling.
[e] Isolated.
[f] Considerable amounts of homocoupled TMSA was also isolated.

For entries 2-6 there was quite a good correlation between the σ value and the rate of coupling. As discussed earlier (Chapter 3) oxidative addition of an aryl halide is promoted in the presence of electron-withdrawing groups. Transmetallation of the copper(I)trimethylsilylacetylide with the $RPdClL_2$ complex is not likely to be very sensitive to aryl substituent effects.* Reductive elimination is inhibited by electron-deficient groups. Thus the correlation between the rates of coupling and σ values suggests that oxidative addition is rate determining. The slow rate of coupling for entry 1 (Table 7) may once more be

* For a description of the mechanism in the presence of a catalytic amount of copper(I)iodide and $Pd^{II}(PPh_3)_2Cl_2$ see Chapter 4 Scheme 20.

explained in terms of a slow, rate determining, reductive elimination step of the highly electron deficient pentafluorophenyl group.

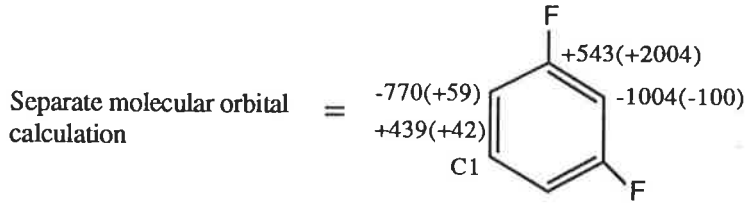
A good correlation also exists between the σ value and the values $\Delta\delta\ ^{13}C_{\alpha}$ & $^{13}C_{\beta}$. A positive σ value gives a positive $\Delta\delta\ ^{13}C_{\beta}$ and a negative $\Delta\delta\ ^{13}C_{\alpha}$, reflecting a nett polarization of the electron density of the alkynyl unit towards the ring, the reverse is true for negative σ values. In the DSP analysis of *p*-substituents it was shown that the effect was commuted primarily through the π-system of the aromatic ring in the form of a π-polarization effect.[162-3]

The relative contribution of the π-polarization effect to the polarization of electrons in the acetylene should be directly related to the charge density of the π-system at C1.[162] For this reason the change in π charge densities at C1 ($\Delta q\pi$ C1, q = 1 = 1 unit of charge) are also given in Table 7. These values were obtained from CNDO/2 molecular orbital calculations performed by Brownlee and Taft[166] for mono and disubstituted benzenes. For entries 3–6 these values were obtained directly from the results reported for the individual molecular orbital calculations. However in the case of entries 1 and 2 the values were determined by assuming additivity of substituent effects upon the $\Delta q\pi$ C1, as the individual calculations were not performed by Brownlee and Taft. This additivity was tested by comparing the values obtained from eq. 35 where the appropriate values of two monofluorobenzenes are added together to give a difluorobenzene. The values obtained from the separate molecular orbital calculation of Brownlee and Taft are given below those from eq. 35.

The values obtained from eq. 35 approximate very well those from the separate CNDO/2 molecular orbital calculation with a largest deviation of only 1.6%. Presumably then the $\Delta q\pi$ and $\Delta q\sigma$ values for the pentafluorophenyl group and the 3,5 CF_3 phenyl group (which were not represented in Brownlee and Taft's work) can be obtained using similar additions. The $\Delta q\pi$ C1 values only roughly

correlate with $\Delta\delta\ ^{13}C1$ value because the $\Delta q\sigma$ C1 values have not been taken into consideration, i.e. the change in chemical shift is going to reflect total ($\sigma + \pi$) electron density changes.

(35)



Other than entry 1, which is discussed below, the largest $\Delta q\pi$ C1 value occurs for entry 3 (Table 7) indicating that in this case π-polarization may be a major contributor to polarization of the alkynyl group. In entry 4 a smaller $\Delta q\pi$ C1 exists but the degree of total polarization of the alkyne is similar, perhaps reflecting an increase in through space contribution from the field effect for this PSA. The reduction in π-polarization and increase in through space contribution appears even more pronounced in entry 2 (Table 7) where there is only a small $\Delta q\pi$ C1 yet a large polarization of the alkyne. In the case of entries 5 and 6 positive F induced π-polarization may occur but may be being countered by resonance effects, to varying degrees in each case, as each has a negative σ_R value but a positive σ_F value (X = OMe: $\sigma_R = -0.41$, $\sigma_F = +0.26$. X = F: $\sigma_R = -0.35$, $\sigma_F = +0.52$).[150]

Once more the pentafluorophenyl group, entry 1 (Table 7) proves to be the anomaly. This time bearing an enormous π-electron density at C1 yet experiencing by far the greatest polarization of electron density towards the ring. In order to resolve this anomaly both the $\Delta q\pi$ and the $\Delta q\sigma$ of all the phenyl carbons of the pentafluorophenyl group were calculated in a similar manner as

described for the 3,5-difluorophenyl group (eq. 35) and in each case the Δq_{Total} determined (Table 8).

Table 8: Changes in Electron Density and ^{13}C Chemical Shift at All Carbons of Pentafluorobenzene Relative to Benzene

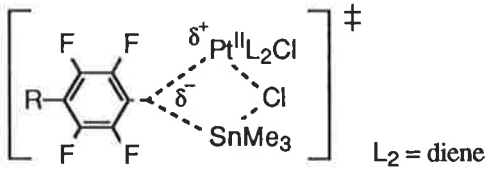
Carbon (C_6F_5)	Δq ($\times 10^4$)[a]			$\Delta\delta$ ppm	$\Delta\delta / \Delta q$
	π	σ	Total ($\pi + \sigma$)		
C1	−836	− 41	− 877	−28.13	320.8
C2,6	− 5	+1991	+1986	+13.2	66.4
C3,5	−723	+1920	+1199	+ 7.7	64.3
C4	−235	+1810	+1575	+13.79	87.5

[a] All values calculated from eq. 35 using $\Delta q\pi$ and δ values from reference 166.

The large negative value for Δq_{Total} Cl is consistent with the large upfield chemical shift observed for this carbon in the ^{13}C NMR spectrum of the pentafluoro-PSA relative to the unsubstituted PSA, as are the large positive values for Δq_{Total} C2-4 where $\Delta\delta$ C2-4 of the pentafluoro-PSA are all large and positive. It should be noted that the $\Delta\delta/\Delta q$ value for a carbon on a benzene ring should be constant and ~200[162,168-9] for where the carbon centre under attention is remote (> 3 bonds) from the substituent. In the case of the pentafluoro-PA C1-4 are either 1 or 2 bonds from a fluoro group, consequently anisotropic effects are likely to be significant and may account for variations of $\Delta\delta/\Delta q$ from 200.

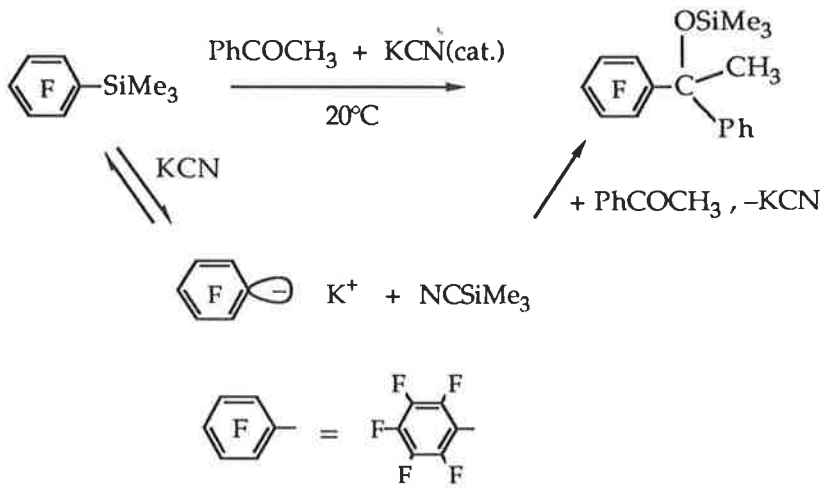
Whilst the Δq_{Total} C1 for the pentafluorophenyl group is quite large and negative this is more then countered by the enormity of the positive Δq_{Total} values for C2–4 resulting in an overall extremely electron deficient phenyl group. Any π-polarization between C1 of the phenyl group and the alkynyl group which may give rise to polarization of the alkyne away from the ring is more than compensated for by the through space field effects (F) of the other positions on the phenyl group. Through-sigma inductive effect (χ) are unlikely to be significant inview of the small negative $\Delta q\sigma$ C1 value obtained.

The excess electron density at C1 of the pentafluorophenyl group must be held very tightly to the phenyl ring since this phenyl group is known to be deactivated towards electrophilic attack. This ability to hold electron density at this centre helps explain the order of reactivity observed by Deacon *et al.*[108] (see Chapter 3) for the facile transmetallation of para substituted perfluorostannanes (X = F > OMe > H) via transition state (VI). Thus, the formation of a negative charge on the sp^2 orbital of C1 is either stabilized via the through space effects of the powerfully electron-withdrawing fluoro substituents.



(VI)

Presumably the methoxy group behaves in a similar manner as the fluoro group but with reduced inductive and increased donor capacity. The donor capacity of the fluoride and the methoxy groups increase the π-electron density at C1 but their inductive capacity immobilizes it to transfer to the platinium and is also able to stabilize formation of negative charge in the σ-system. The ability of the pentafluorophenyl group to stabilize a negative charge on the sp^2 orbital at C1 has also been reflected in the addition of trimethylsilylpentafluorophenyl groups to non-enolizable carbonyls in the presence of catalytic amounts of potassium cyanide (eq. 36[170]).

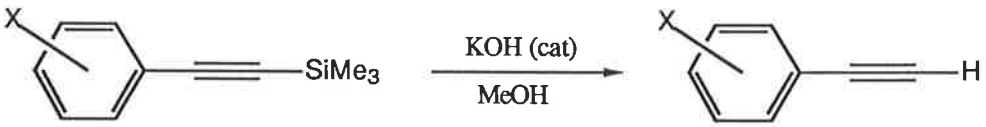


(36)

5.2.2 Desilylation of Phenyltrimethylsilylacetylenes

The desilylation of all PSAs was readily achieved using a catalytic amount of potassium hydroxide (0.05mol%) in methanol (eq. 37). Though care had to be taken in the isolation of these relatively volatile products (see Experimental) all were isolated in high yield. All reactions were carried out at room temperature and monitored every 15 minutes by g.l.c. for the first hour and every half hour thereafter. If no or very little reaction was observed after 2.5 hours the concentration of base was increased (from 0.05 to 1mol%). The rates of desilylation are correlated, qualitatively, with substituent constants (σ) the $\Delta\delta$ of the silyl group (^{29}Si NMR) and of the acetylenic proton (^{1}H NMR), Table 9.

As with oxidative addition, the rate of desilylation was reflected in the corresponding σ values and with the $\Delta\delta$ ^{29}Si of the starting material and $\Delta\delta$ ^{1}H (acetylenic) of the product. In this case the pentafluoro-PSA (entry 1, Table 9) shows the high rate of reaction anticipated for the PSA bearing the most electron-withdrawing phenyl group.



(37)

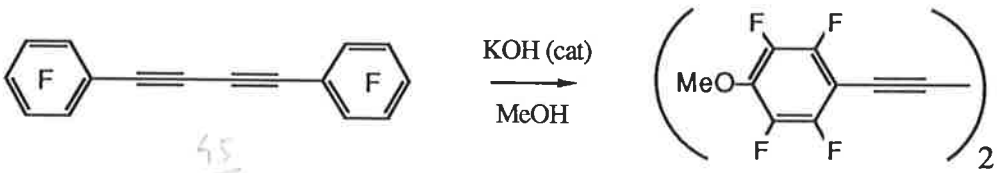
Table 9: Mildest Reaction Conditions for the Desilyation of Substituted PSAs (eq. 37).

Entry	X	σ [a]	Reaction Conditions		Yield %	$\Delta\delta$ ppm [b]	
			Time (hr)	KOH equiv.(mol%)		^{29}Si (PSA)	^{1}H(acetylenic, PA)
1.	F_5	1.80[c]	< 0.5	0.05	86	+ 2.35	+ 0.52
2.	3,5-CF_3	0.86	0.75	0.05	95	+ 0.83	+ 0.27
3.	3,5-F	0.68	1.5	0.05	93	+ 0.19	+ 0.07
4.	4-CF_3	0.54	1.5	0.05	95	+ 0.18	+ 0.12
5.	4-F	0.06	2	1.00	90	- 0.01	- 0.05
6.	4-OMe	-0.27	3	1.00	96	- 1.03	- 0.08

[a] See Table 7 notes **a** and **d**.

[b] The $\Delta\delta$ values are relative to X = H, the δ value for X = H are : δ ^{29}Si = –17.13ppm, δ ^{1}H (acetylenic) = 3.08ppm.

In order to obtain a better appreciation of the rate of desilylation for the pentafluoro-PSA the number of base equivalent was reduced to 0.02mol% and the reaction monitored every 5 minutes. It was apparent, however, that the reaction had terminated in less than 20 minutes at only ninety percent completion. Further addition of a small amount of base allowed the reaction to continue to completion. It was concluded from this observation that competing with the desilylation reaction was the slower consumption of base by nucleophilic substitution of an aryl fluoride. It has been recently reported that di-pentafluorophenyl-butadiyne **45** readily undergoes nucleophilic substitution with methoxide in methanol (eq. 38[171]).



(38)

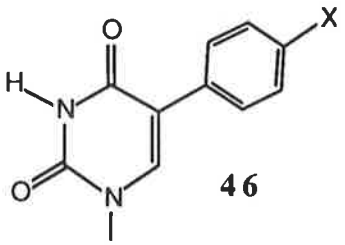
In view of this competing reaction an accurate determination of the rate of desilylation for pentafluoro-PSA would not be possible. Certainly this must be a very fast reaction in order to go to completion in such a relatively short time when the base concentration is continually falling from an already initial low concentration.

5.2.3 ^{13}C NMR Data and Potential Activity of Substituted 5-Phenylethynyl-2'-deoxyuridines.

The coupling of substituted PAs to 5-trifloxy-2'-deoxyuridine **8** & **14** was discussed in Chapter 4. In general, an increase in rate was observed with increasing electron-deficiency of the PAs, this was attributed to the rate determining step of the reaction being the formation of the copper acetylide or the rate of transmetallation. However, for the most electron-deficient PAs, oxidative addition of the nucleoside triflate to the palladium(0) catalyst appears to become, to some extent, the rate determining process, as demonstrated by the

difference in the rates of coupling of the 5-trifloxyl and 5-iodouridines with 3,5-difluorophenylacetylene (Chapter 4).

Mertes *et al.*[172] have previously conducted a correlation study of 5-phenyl-2'-deoxyuridines **46** where the σ values of the *p*-substituents (X) on the phenyl ring were correlated with the change in chemical shift ($\Delta\delta$ ^{1}H & $\Delta\delta$ ^{13}C) for the various centres on the 2,4-dioxopyrimidine base ring.



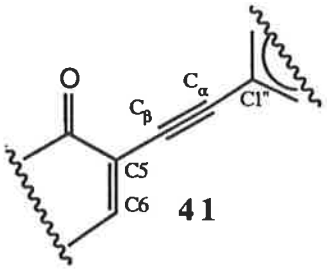
46

2'-deoxyribose

Significant changes in the chemical shift were only observed at the C5 & C6 centres. The remote nature of the substituent (X) relative to C5 & C6 ensures that primarily electronic effects are responsible for changes in chemical shifts and the co-planarity of the two rings facilitates transmission of substituent effects.[172] Consequently good correlations between $\Delta\delta$ and σ-Hammett values were obtained. The effect of a positive σ value was to give a positive $\Delta\delta$ $^{13}C6$ and a negative $\Delta\delta$ $^{13}C5$ (a decrease and increase in electron density respectively) the opposite was true of negative σ values.

In view of the biological significance of 5-alkynyl-2'-deoxyuridines it was considered of interest to investigate the effect of incorporating an alkynyl group between the phenyl and 2,4-dioxopyrimidine rings and any correlation of the change in ^{13}C chemical shifts and σ-Hammett values. For this purpose the $\Delta\delta$ $^{13}C1$, C_{α}, C_{β}, C5, C6 of **41** have been correlated with the σ values of the various substituents (X) (Table 10). Again it was anticipated that $\Delta\delta$ reflects a change in electron-density (Δq) via an inverse relationship.

Table 10: The Changes in ^{13}C Chemical Shift of Substituted 5-Phenylalkynyl-2'-deoxuridines **41** at C1, C_α, C_β, C5 and C6.



41

Entry	Compound **41**	X	σ[a]	Δδ (ppm)					ΔδC6 / ΔδCβ
				C1	C_α	C_β	C5	C6	
1.	**f**	F_5	+1.80	-22.86	-16.33	+12.63	- 1.43	+ 1.81	0.14
2.	**b**	3,5-CF_3	+0.86	+ 2.53	- 3.36	+ 3.13	- 1.35	+ 1.32	0.34
3.	**c**	3,5-F	+0.68	+ 3.40	- 1.57	+ 2.65	- 0.21	+ 1.44	0.54
4.	**d**	4-CF_3	+0.54	+ 4.63	- 0.43	+ 2.97	+ 0.25	+ 1.28	0.43
5.	**e**	4-F	+0.06	- 3.16	+ 0.02	- 0.02	+ 0.88	+ 0.60	–
6.[b]	**g**	H	0.00	121.87	91.33	80.80	98.23	142.65	–
7.	**h**	4-OMe	-0.27	- 7.55	+ 0.8	- 1.42	+ 1.04	- 0.03	–

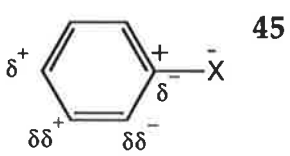
[a] See Table 8 notes **a** and **d** .
[b] Actual chemical shift values are given.

The effect of an acetylene unit between the phenyl and 2,4-dioxopyrimidine ring has been to effectively halve the substituent effect upon Δδ at the C6 centre, i.e. $\Delta\sigma/\Delta\delta\ ^{13}C6$ (**46**) = 3.1 and $\Delta\sigma/\Delta\delta\ ^{13}C6$ (**41**) = 1.6. Since $\Delta\delta\ ^{13}C6$ is directly proportional to Δq C6 the effect of a substituent upon the electron density C6 centre is also halved. Also the $\delta\ ^{13}C6$ (X = H) for **46** = 137.9 ppm where as that for **41g** = 142.7 ppm, this difference reflecting not only electronic effects but also anisotropic However, this last observation does seem to indicate that the PA series is perhaps on the whole more electron withdrawing than the phenyl series. This is also supported by the larger σ-Hammett value associated with a phenylalkynyl group than a phenyl group (σ_p = 0.16 and –0.01 respectively).

As anticipated the pentafluorophenyl group of **41f** gives rise to the largest $\Delta\delta\ ^{13}C6$ and hence the most electron deficient C6 centre, but not as large as may have been expected. The communication of the substituent effects from the alkyne to the C5-C6 double bond for **41f** does not appear to have been as efficient

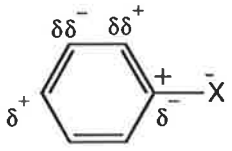
as it has been in the other fluorinated compounds **41b-d**. A measure of the efficiency by which the substituent effect is communicated from the alkynyl bond is given by $\Delta\delta\ ^{13}C6/\Delta\delta\ ^{13}C_{\beta}$, the greater this ratio the more efficient the transfer of the effect. This ratio has been calculated in the case of entries 1-4. An explanation of this difference in ability to transfer the substituent effects has been developed from consideration of the way in which the electron density of the π-orbitals of the alkynyl bond are being perturbed. Although much consideration has been given to the nett effect of different substituents upon an alkynyl group,[161-3,173] no discussion of the effects of different substituents upon the individual π-systems of an alkyne has been attempted.

In the ground state the orthogonal π-systems of an alkyne are considered to overlap forming a single cylindrical cloud of electrons, which gives rise to a ring current and a magnetic field along the length of the triple bond.[174] It may be tempting then to consider that polarization of the alkyne occurs as a single homogeneous cloud of four electrons in the same direction, even when only one of the π-systems is being exposed to the effect, such as in π-polarization of PAs. We can perhaps consider an alkyne to be analogous to benzene, where again a single circular cloud of electrons results from π-overlap of all π-systems. In the case of benzene exposure of one of the π-systems to a strong polarizing effect does not lead to a general shift of the entire "π-cloud" to one end of the benzene ring as depicted by **47** but the polarization of alternate double bonds **48**.[162,166]



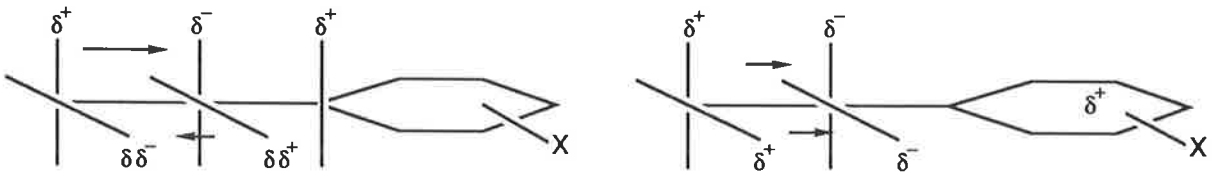
45

47



48

A similar scenario could be envisaged for triple bonds where in the case of π-polarization of a PA, polarization of the double bond in conjugation with the phenyl ring may lead to a smaller polarization of the orthogonal double bond in the opposite direction in an attempt to maintain overlap at each carbon **49**. Where only the field effect is operating both π–systems should experience a similar effect **50**.



π-polarization **49** Through-space polarization **50**

X = electron-withdrawing group.

For same nett polarization: → + ← = → + →

49 **50**

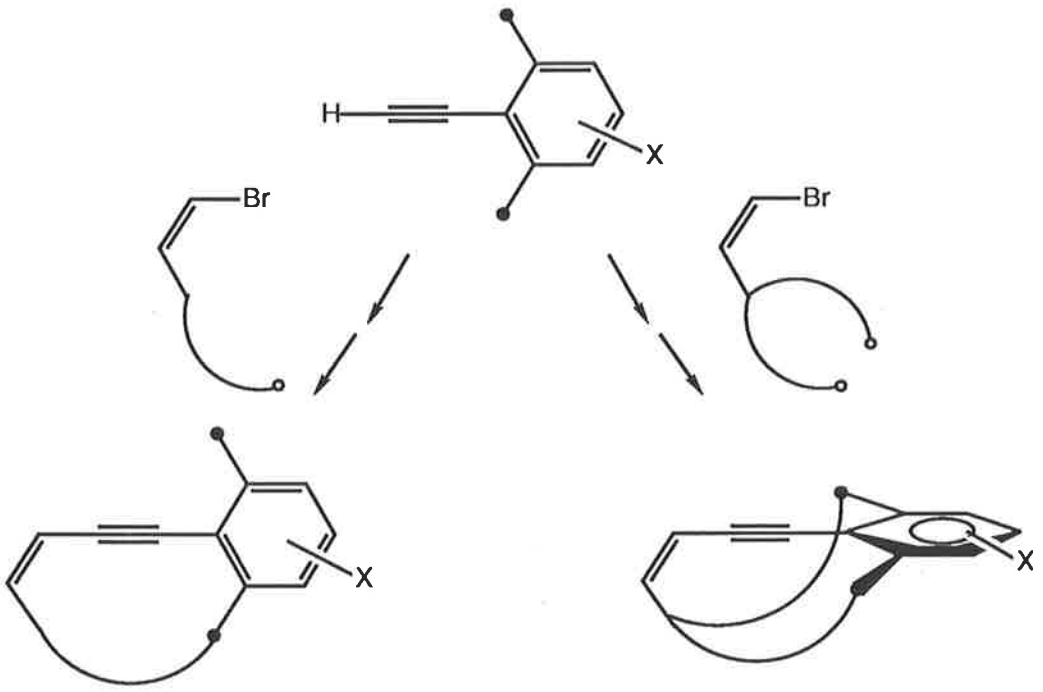
The C5-C6 double bond of the 2,4-dioxopyrimidine ring can only overlap with one π-system of the alkyne at a time. In that the phenyl group prefers to be co-planar, rather than orthogonal, to the uracil ring the C5-C6 double bond will experience a larger effect from **49** than from **50** for the same effect on the alkyne. The coplanar arrangement would be expected to be of lower energy than the orthogonal one in view of the extended conjugation in the former.

The descending order for the relative contribution from the π-polarization effect for electron withdrawing groups has already been established (π-polarization, X = 3,5-F_2 > 4-CF_3 > 3,5-CF_3 >> F_5) (see above, this chapter), based on the above hypothesis the relative contribution of **49** over **50** should follow the same order, and so then should the efficiency of transfer of effects ($\Delta\delta\ ^{13}C6$ / $\Delta\delta\ ^{13}C\beta$). As can be seen from Table 10 the descending order of $\Delta\delta\ ^{13}C6$ / $\Delta\delta\ ^{13}C\beta$ does match that for π-polarization ($\Delta\delta\ ^{13}C6$ / $\Delta\delta\ ^{13}C\beta$ & X = 3,5-F_2 > 4-CF_3 >3,5>CF_3 >> F_5).

Thus, it has been demonstrated that an increase in π-polarization relative to through space polarization leads to an increase in efficiency of transfer of substituent effect C_β to C6. This has been explained by postulating **49** for π-polarization and **50** for through space polarization and assuming a preference for co-planarity of the two rings. However, this correlation between π-polarization and the transfer of substituent effects C_β to C6 may be simply fortuitous. None-the-less, the idea of using an alkene as a probe for detecting different effects in each of the orthogonal π-systems of substituted PAs is quite attractive. This could perhaps be best achieved using rotationally rigid eneynephenyl derivatives as outlined in Scheme 21, where the attachment sites at the phenyl ring *ortho* to the alkyne are as neutral, in terms of substituent effects, as possible whether bound or unbounded to the olefin (e.g. $-CH_2-$ and $-CH_3$). By appropriate use of substituents (X) significant π-polarization of the aryl group and hence the alkyne could be achieved. Differences in the ^{13}C NMR chemical shift between the olefin in **51** as compared to **52** could be used to investigate the postulate **49**.

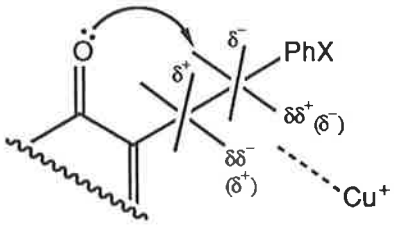
In Chapter 4 it was noted that the presence of electron-withdrawing groups on the aromatic ring increased the level of cyclization of coupled product and that donors reduced it. Yet in the case of the C5 pentafluorophenyl group cyclization was completely inhibited even under the harsher reaction conditions required in order to achieve coupling. These observations could possibly be explained using the above descriptions of alkyne polarization **49** and **50**.

Scheme 21



Co-planar Orthogonal

In the cyclization process the C4 carbonyl lone pair is correctly positioned for attack upon the π-system of the acetylene orthogonal to the plane of the 2,4-dioxopyrimidine ring, where an angle of incidence of approximately 120° is required **53**.[175] The cyclization process is catalysed in the presence of copper(I) so that the π-system undergoing attack is complexed to the copper(I) prior to attack.



53; X = electron-withdrawing group
$\delta\delta^{\pm}$ for π-polarization,
$(\delta^{\pm})$ for through space polarization.

The preference for co-planarity of the two rings may be increased as a result of π-complexation of the orthogonal π-system in view of the reduction in π-bond character complexation is likely to cause. A dominant contribution from π-polarization of the alkyne in **53** polarizes the π-system under attack in the

correct direction for cyclization, though the major contribution to the electrophilicity of this double bond is likely to come from copper complexation. The major influence that electron-withdrawing groups may have on the rate of cyclization is in their polarization of the uncomplexed π-system, leading to an increase in electron-density at C_α which may stabilize the insipient positive charge that develops on this carbon during the cyclization process.

The level of polarization of the alkyne in **41f** is much greater than for any of the other 5-phenylethynyl-2'-deoxuridines and is likely to have equal contributions from each π-system of the triple bond **50**. Presumably the enormity of electron density at C_α for the π-system under nucleophilic attack inhibits cyclization even in the presence of copper(I) complexation. Steric factors are not likely to be important since the angle of incidence of the oxygen is so large (~120°) and the fluorine atom is so small. In the case of **41f** it is the enormity of the polarization, not just the fact that it operates on both π-systems **50**, that is likely inhibit cyclization. Other alkynyl nucleosides may be polarized solely in the manner described by **50** and still show promoted cyclization, so long as copper(I) complexation is able to induce sufficient positive charge at C_α.

What is implicit in the above rationalization is that for the same level of polarization of the triple bond the rate of cyclization should be increased with increasing contribution from **49** relative to **50**. Based on $\Delta\delta\ ^{13}C_\alpha$ & $^{13}C_\beta$ values (Table 10) **41b-d** all have similar levels of nett polarization of the triple bond. An increased contribution from **49** is expected with increase π-polarization, the relative contribution from π-polarization for entries 2-4 has been determined to be X = 3,5-F_2 > 4-CF_3 > 3,5-CF_3 it is perhaps then more than coincidence that the amount of cyclized product isolated (Table 4 entries 2-4) is also in the order X = 3,5-F_2 > 4-CF_3 > 3,5-CF_3.

To date the nucleoside analogues **8** & **41b-f** have been screened as potential anti-HIV agents and anticancer agents, in each case the analogues were inactive and on the whole non-toxic towards cells.* It still remains for these analogues to be tested as antiherpetics, that is as anti-HSV 1 & 2 agents, for which they have been most specifically designed.

The inactivity of these analogues as anti-HIV agents is not too surprising in view of the proposed mechanism of inhibition of HIV by nucleoside analogues as outlined previously (Chapter 1). The mode of action of most anti-HIV nucleoside analogues involves phosphorylation of the 5'-hydroxyl group.[1,5,6] Nucleosides substituted with sizeable groups in the 5-position of the pyrimidine ring are unlikely to undergo phosphorylation by cellular kinases[47] and since HIV does not induce its own kinase, nucleoside derivatives **41b-f** would not be expected to be phosphorylated in the cell and therefore cannot participate in the normal routes of inhibition of HIV. Phosphorylation must be achieved within the cell as nucleoside 5'-phosphates do not normally cross cell membranes very effectively. Furthermore nucleoside analogues which show significant activity against HIV invariably have a C3' centre modified by substitution or deoxygenation.[1,5]

Uridine analogues substituted at the C5 position are often used as anticancer agents, where it is considered that they may act as inhibitors of cellular DNA polymerase or thymidylate synthase or via some other mechanism (Chapter 1). In order for nucleoside analogues to act as inhibitors of either DNA polymerase or thymidylate synthase they are required to undergo intial phosphorylation of the 5'-hydroxyl. Where the C5 substituent is small (e.g. fluoro group) cellular kinases may phosphorylate the nucleoside, whereas in the case of large C5 substituents monophosphorylation can be achieved by viral thymidine kinase. A number of cancers have been shown to be virally induced

* See Appendix at the rear of thesis.

and some induce the manufacture of viral thymidine kinase within the cell (TK^+ cell lines). Some of the most relevant cancer cell lines such as murine leukemia (L1210), human B-cell lymphoblast (Raji) and T-cell lymphoblasts (Molt/4F) which might be expected to display this type of behaviour were not used in the standard NIH screening.

In order for a nucleoside analogue to act most effectively as an antiviral/anticancer agent via mechanism based inhibition of thymidylate synthase a number of criteria need to be met. These are listed below and in each case the relevance of this criterion is defined and the nucleoside analogues **41b-f** that best meets this criterion is discussed:

(i) **Lipophilicity;** so that the nucleoside analogue can cross the cell membrane. **41f** has the highest R_f by t.l.c., polyfluoro compounds are usually quite lipophilic.

(ii) **Selectivity;** inhibits thymidylate synthase of infected cells only. All nucleoside analogues **41b-f** can only be phosphorylated in infected cells where viral thymidine kinase is present (TK^+ cells).[47] Phosphorylation is a prerequisite in thymidylate synthase inhibition.[43,44]

(iii) **Stability;** antiviral agent is not readily metabolized. This factor remains to be determined.

(iv) **Enzyme affinity;** propensity of the substrate to enter the enzymes active site. Thymidylate synthase shows a high affinity towards C5 substituted 2'-deoxyuridylates even when the substituent is quite large.[176] A lipophilic substituent may aid entry into the active site by virtue of a "greasy hole" effect. Thus all analogues **41b-f** meet this criterion **41f** may have an advantage as the substituent is relatively small and lipophilic.

(v) **Enhanced electrophilicity;** mechanism based inhibition of thymidylate synthase requires initial cysteine thiol Michael addition to the C6 centre of the nucleoside (Scheme 22) reducing electron density at this centre may

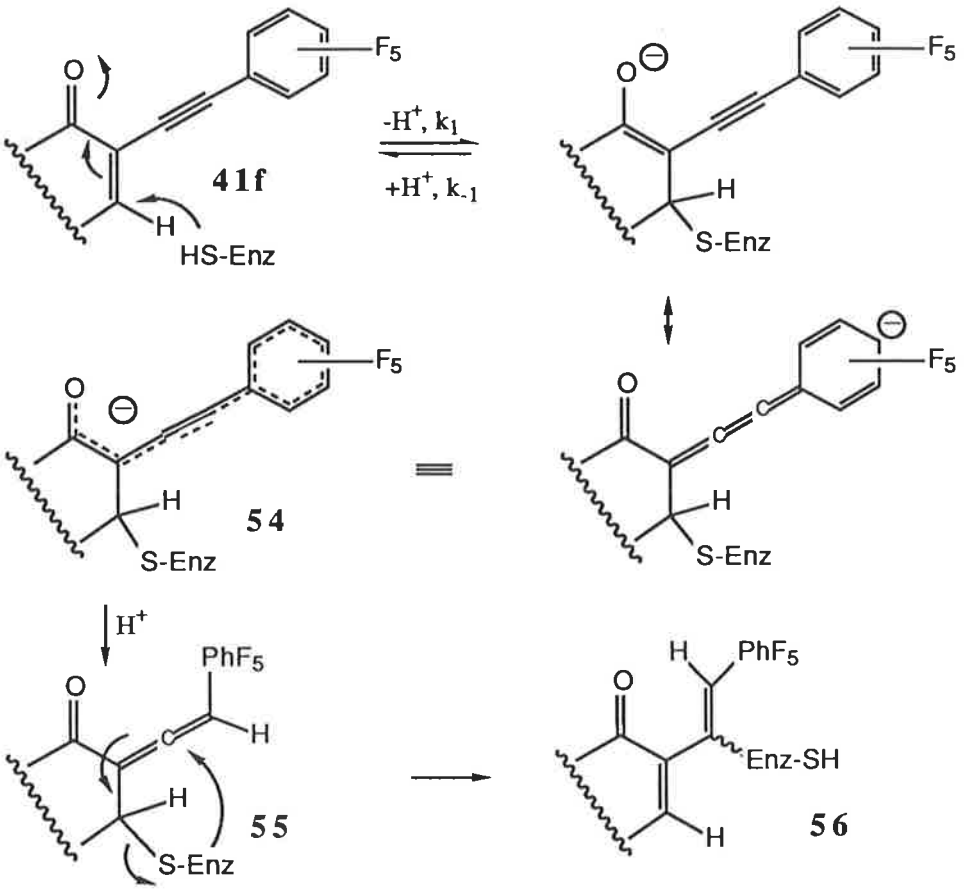
increase rate of inhibition.[43,46-50] Analogue **41f** is the most electron deficient at the C6 centre.

(vi) **Potential for the formation of a reactive intermediate;** enzyme inhibition is best effected if, upon thiol addition, a reactive intermediate is formed that is capable of giving a covalent, irreversibly bound, enzyme/substrate complex. In the case of 5-alkynyl-2'-deoxyuridylates it has been proposed that this intermediate is a transient allene (Chapter 1) which undergoes a second Michael addition by the enzyme with concomitant elimination of the C6 thiol, this process will leave the enzyme covalently bound to the C5-substituent.[47,49] The large positive σ value of **41f** suggests an ability to stabilize the formation of a negative charge resulting from thiol addition **54** (Scheme 22), thus increasing the rate of addition (k_1) and decreasing the rate of elimination (k_{-1}). Protonation of **54** gives the transient allene **55** which undergoes the second Michael addition to give the covalently bound enzyme substrate complex **56**.

On the basis of the above criteria **41f** appears to be the most likely candidate to act as a mechanism based inhibitor of thymidylate synthase. Fortunately this nucleoside analogue is also one of the most accessible as it can be formed by direct coupling of the pentafluoro-PA to the C5-trifloxyl nucleoside without prior protection of the sugar ring and without formation of cyclized by-products.

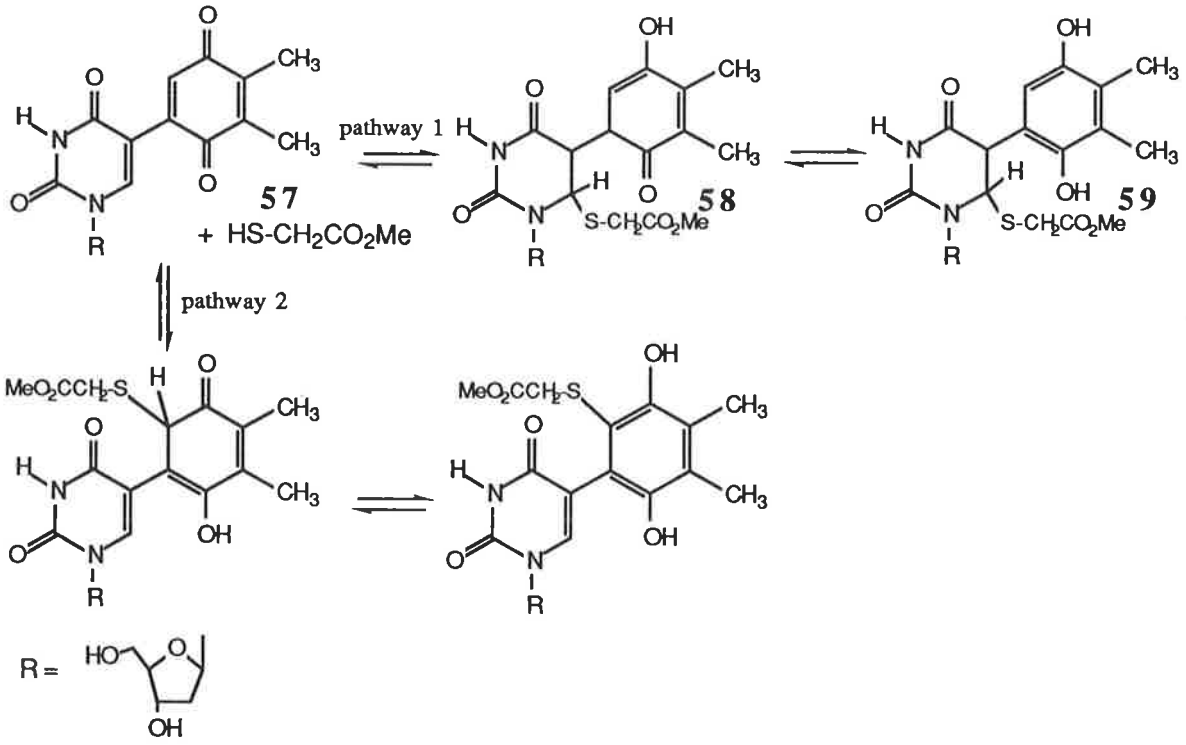
Apart from testing the 5-alkynyl pyrimidine nucleosides prepared here as anti-HSV 1&2 agents in cell cultures, future work may also entail a study of the corresponding monophosphates as inhibitors of pure thymidylate synthase in the presence of the co-factor 5,10-methylene tetrahydrofolic acid, in a similar manner as that previously described.[47]

Scheme 22



Mertes *et al.*[176] have described an attempt to observe an irreversible Michael addition to the C6 centre of the 5-dimethylquinone-2'-deoxyuridine **57** using methyl thioglycolate (Scheme 23). This thiol has a similar pKa value (8.0) to the active-site of thymidylate synthase. Instead of obtaining the C6 thiol adduct **58** which could then aromatize via intramolecular proton shift to give the irreversibly bound enzyme/substrate complex **59** (pathway 1) they obtained addition to the quinone followed by aromatization (pathway 2).

Scheme 23

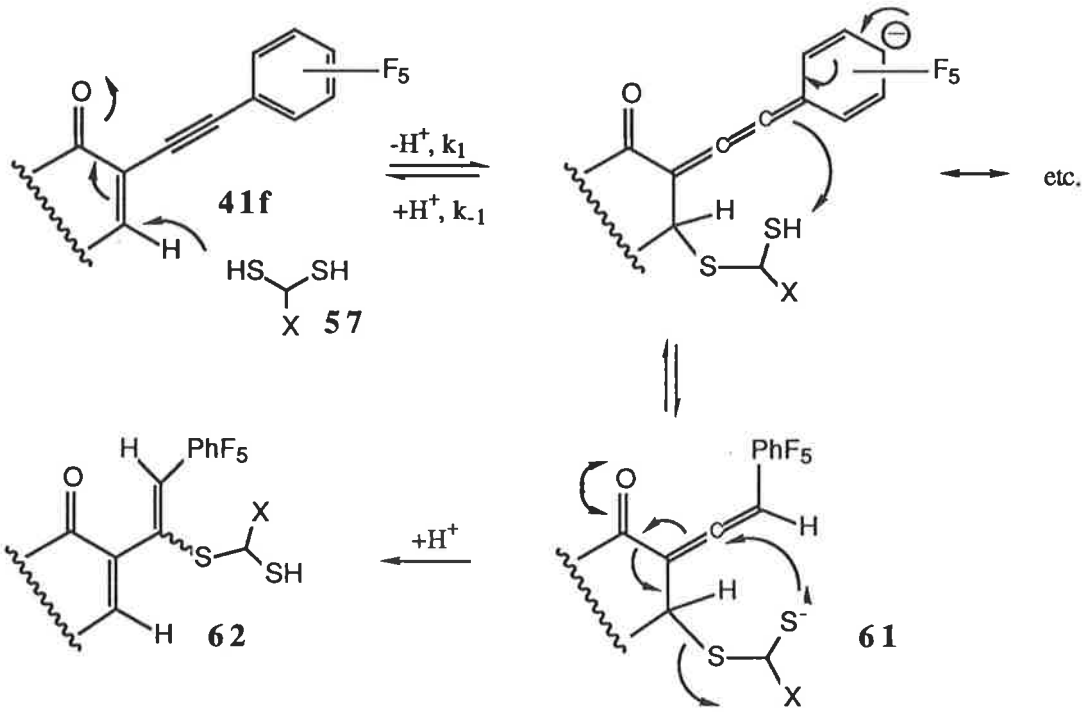


Although the intermediacy of an allene has been proposed in the inhibition of thymidylate synthase there is no evidence that an allene does form or even that the enzyme binds to the C5-substituent of the nucleoside. Indirect evidence for the possibility of such a mechanism could be obtained by appropriate use of thiols in a similar manner to that described by Mertes *et al.*[176] above. In this case the geminal dithiol **60** (Scheme 24) could be used, where the pKa of the dithiol is determined by the nature of the group X. Initial Michael addition is followed by proton transfer from the other thiol to give the allene **61** which is then attacked by the thioxide anion to give the irreversibly bound alkenylthiol-ether **62**.

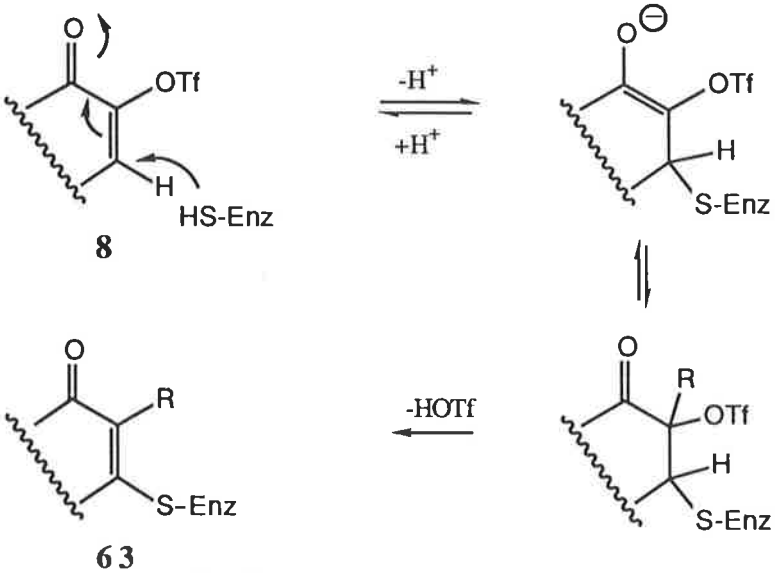
It is perhaps also worth considering the viability of the C5-trifloxy-2'-deoxyuridine derivative **8** itself as an inhibitor of thymidylate synthase (Scheme 25). Cysteine thiol Michael addition to the C6 centre of **8** followed by either C5 protonation or addition to the co-factor (i.e. R = H^+ or N^5,N^{10}-methylene tetrahydrofolic acid) gives an intermediate capable of eliminating triflic acid and

leaving the enzyme, the nucleoside and perhaps the tetrahydrofolic acid bound covalently as either a ternary or binary complex **63**.

Scheme 24



Scheme 25



R = H or N^5,N^{10}-methylenetetrahydrofolate

Chapter 6

The Palladium/Copper-Catalysed Homocoupling of Terminal Alkynes

6.1 Introduction

The head to head, oxidative coupling of terminal alkynes to give 1,3-butadiynes or polynes is a very important synthetic procedure.[177-9] The method reported by Glaser[180] and subsequently modified by others,[178-9] involves the bubbling of air or oxygen through a solution of alkyne and catalytic amounts of copper(I) chloride and ammonium chloride, eq. 39.

$$R\text{-}C{\equiv}CH + 0.5\ O_2 + (CuCl + NH_4Cl)_{cat.} \rightarrow R(C{\equiv}C)_2R + H_2O \quad (39)$$

$$R\text{-}C{\equiv}CH + Cu^{II} \rightarrow R(C{\equiv}C)_2R + 2H^+ + 2\ Cu^{I} \quad (39a)$$

$$Cu^{I} + 0.5\ O_2 + 2H^+ \rightarrow Cu^{II} + H_2O \quad (39b)$$

Copper(II) is the actual oxidant in the homocoupling process, the oxygen merely serves to regenerate the catalyst (eq. 39a and 39b respectively).[177] The stoichiometric use of copper(II) acetate allows for the coupling of alkynes in the absence of oxygen.[177] Since copper(II) is such a mild oxidant the coupling can be achieved in the presence of almost any functional group. Other methods, although somewhat less direct, involving different organometallic derivatives have also been reported.[177]

In Chapter 4 the homocoupling of PAs bearing electron-withdrawing groups proved quite a nuisance in that it competed with the cross-coupling of these PAs to C5-trifloxy-2'-deoxuridines **8** and **14**. A similar homocoupled by-product was also observed during the coupling of pentafluorophenylbromide to trimethylsilylacetylene.

In order to better understand the source of this homocoupled product it was decided that this process should be studied in isolation. This study was not undertaken with the aim of developing a new method for the oxidative coupling of alkynes but merely to determine the requirements of this side reaction with respect to the other reagents present in the cross-coupling reactions.

6.2 Results and Discussion

As with cross-coupling reactions all reactions studied here were carried out under high purity nitrogen in deoxygenated solvent.* A series of reactions were carried out in the same solvent and at the same temperature as used for the cross-coupling of 3,5-difluoro-PA with **14** (entry 7, Tab 4), but with only the terminal alkyne present and no **14**. The results from this study are summarized in Table 11 entries 1-5.

As can be seen from Table 11 homocoupling of the PA was only readily achieved when all three reagents namely palladium, copper and base were present. Although a trace amount of product was observed by t.l.c. for entry 3 only in the case of entry 4, where a deep red/brown solution was observed, was homocoupling readily achieved. In cross-coupling reactions this intense red/brown colour was only observed for reactions where homocoupling was competitive, other reaction mixtures remained only slightly yellow. It should be noted that most cross-coupling reactions turned black, after or near complete consumption of the nucleoside triflate **8** or **14**.

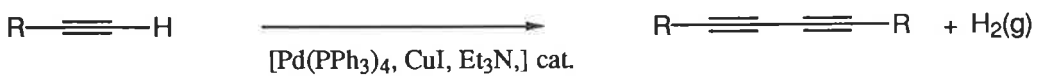
* $N_{2(g)}$, pretreated with "MnO' was bubbled through the solvent for 10 minutes.

Table 11: Homocoupling of Terminal Alkynes.

Entry	R	Reaction Conditions [a,d]					Yield % [b,c]
		$Pd(PPh_3)_4$ (mol%)	CuI (mol%)	Et_3N[g] mol%	Time(hr)	Temp°C	
1.	3,5-F_2-Ph	0	5	110	5	38	0
2.	3,5-F_2-Ph	2	0	0	2	38	0
3.	3,5-F_2-Ph	2	0	110	3	38	*
4.	3,5-F_2-Ph	2	5	110	0.5	38	99
5.	Ph	2	5	110	12	20	82
6.	4-CF_3-Ph	2	5	5	12	20	54[h]
7.	Me_3 Si-	2 [f]	5	#	5	50	91
8.[e]	Me_3 Si-	2 [f]	5	#	5	84	22

a Entries 1-6 were carried out in DMF.
b Isolated yields, except for * – small amount of product detected by t.l.c. only.
c For entries 5 and 6 reactions were not monitored by g.l.c.as solvent (DMF) peak obscured the starting material peak.
d Entry 7: pentafluorobromobenzene 80 mol% was also present.
e Reaction mixture was initially heated at 50°C for 4 hours but no product could be detected by t.l.c., temperature was increased to 84°C for 16 hours. Consumption of TMSA could not be monitored.
f Same result was obtained using $(PPh_3)_2PdCl_2$ (2 mol%).
g # – Diisopropylamine as solvent.
h Some terminal alkyne still detectable by t.l.c..

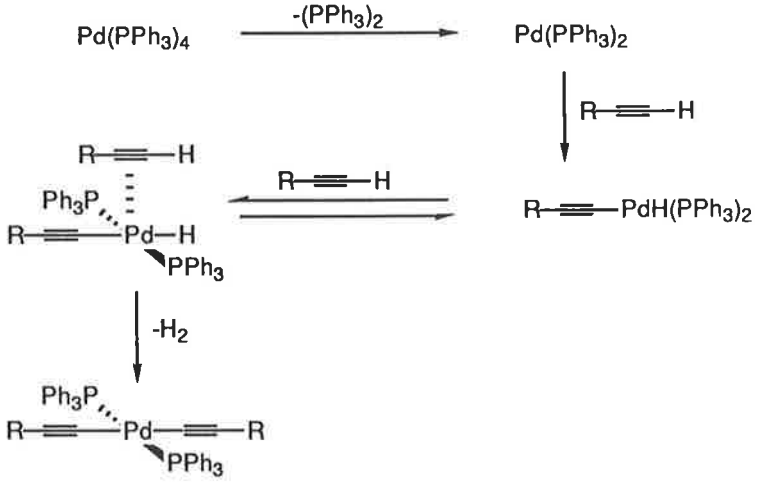
The homocoupling reaction is not restricted to activated alkynes as shown by entry 5, Table 11 which demonstrates that in the absence of the nucleoside triflate unactivated PAs can also undergo homocoupling. Entry 6, Table 11 demonstrates that whilst homocoupling can be achieved in the presence of only a catalytic amount of base, the lower base concentration leads to a lower rate of reaction (this reaction mixture was dark green in colour). Unlike the Glaser-type couplings which involve stoichiometric amounts of oxidant, this homocoupling process which is catalytic in all reagents other than the alkyne must involve the formation of molecular hydrogen (eq. 40).



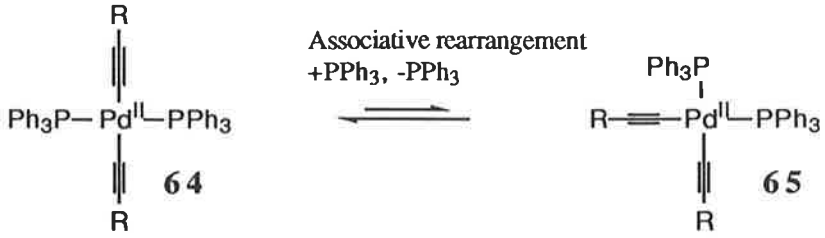
(40)

Nelson *et al.*[181] reported in 1974 that $Pd(PPh_3)_4$ reacts with terminal alkynes to yield stable *trans*-bisacetylidepalladium(II) complexes $[(PPh_3)_2Pd(C{\equiv}CR)_2]$. No reductive elimination of a homocoupled product was observed. A mechanism for the formation of a $(PPh_3)_2Pd(C{\equiv}CR)_2$ complex was proposed as outlined in (Scheme 26). The oxidative addition of terminal alkynes to Pd^0L_2 is perhaps favoured in the presence of electron-withdrawing groups as with aryl and alkenyl halides (see Chapter 5, *Oxidative Addition*). Thus, in the case of entries 2 and 3 of Table 11 a bisacetylide complex should form, which is perhaps evidenced by the formation of a trace amount of homocoupled product in the case of entry 3. Assuming that the bisacetylide complex does form in the absence of base, as in Nelsons work, the formation of the trace amount of homocoupled material in entry 3 but not 2 (Table 11) may be due to a promotion of reductive elimination by the base.

Scheme 26

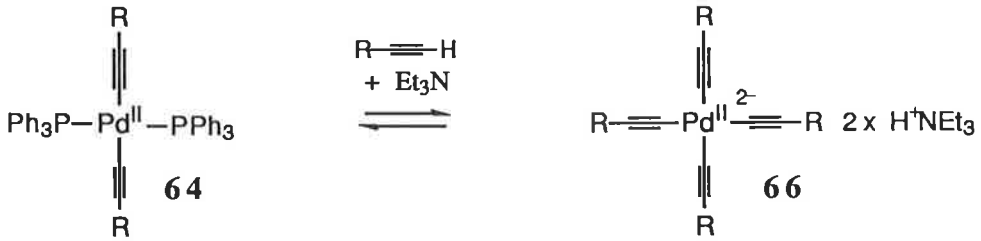


It is known that to achieve reductive elimination from palladium complexes that the two groups to be eliminated must be *cis* to each other (see Chapter 3, *Reductive Elimination*). Isomerization of the intially formed *trans* complex **64** to the *cis* complex **65** (eq. 41) would be a disfavoured process as it places the two large triphenylphosphine ligands *cis* to each other increasing steric interactions.

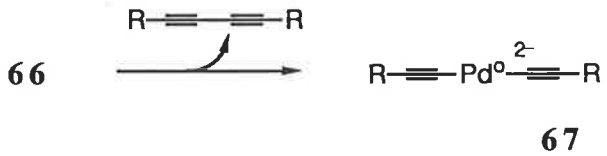


(41)

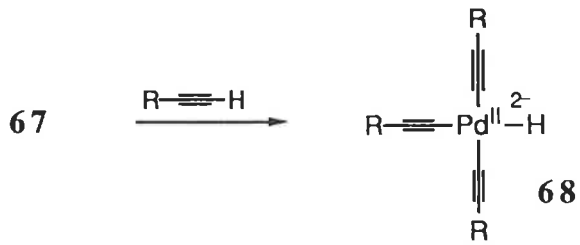
The requirement for a *cis* arrangement would be met should the two triphenylphosphine groups of the presumed bisacetylide complex be replaced with two more acetylides to form the tetrakisacetylidepalladium(II) anionic complex **66**. (eq. 42). Reductive elimination from this complex would give a bisacetylidepalladium(0) anionic complex **67** (eq. 43). This complex may then undergo oxidative addition of another terminal alkyne to give **68** (eq. 44), where electron-donating groups (acetylide anions) on the palladium promote the oxidative addition (see Chapter 5, *Oxidative Addition*).



(42)

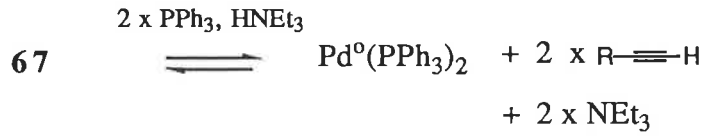


(43)



(44)

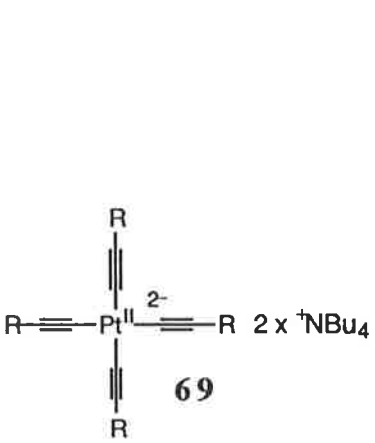
or



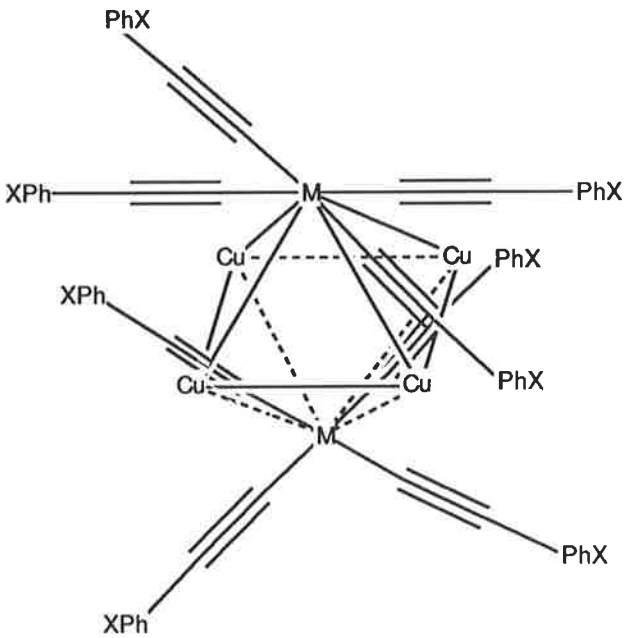
(45)

Alternatively it may undergo ligand substitution to give $Pd^{\circ}(PPh_3)_2$, in either case the catalytic cycle is perpetuated. The proposed formation of the

tetrakisacetylide complex is possible since the known tetrakisacetylideplatinium anionic complex **69** has been isolated and characterized.[182]



69



M = Pd, **70**
Pt, **71**

The role then of the copper(I)iodide in dramatically enhancing the rate of homocoupling may be due to the formation of a slightly different complex such as the hexanuclear cluster **70.** Analogous platinium cluster **71** has recently been isolated.[182] These platinium complexes were described as dark garnet crystals which may explain the intense red/brown colouration of reaction mixtures involving rapid homocoupling in this work.

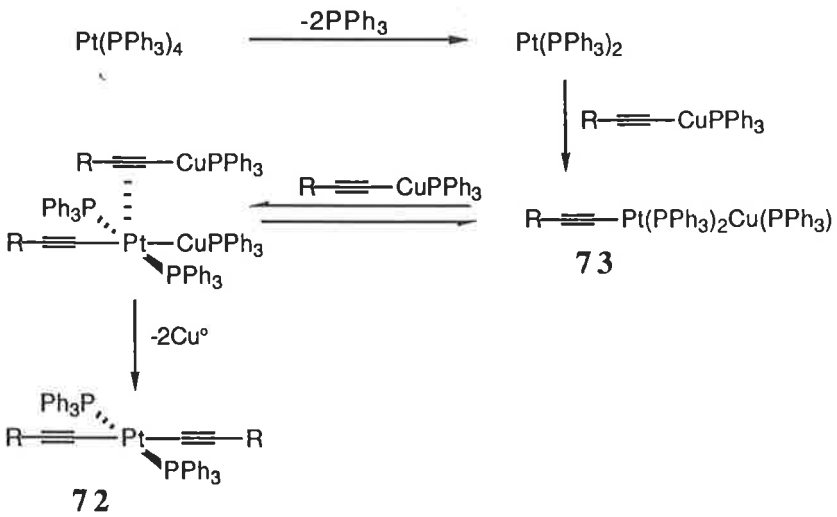
The palladium/copper cluster **70** may be the catalyst involved in all steps. It may assist in more than just easy access to *cis* substituted isomers, as electron transfers between copper and palladium may provide for low energy pathways between the different oxidation states. The homocoupling of arylcopper(I) compounds to give a biaryls and free copper(0) has been related to the existence of preformed arylcopper(I) clusters.[128]

The dependance of this process on the electron-deficiency of the alkyne and the concentration of base are consistent with the involvement of a copper

acetylide species whose formation is promoted in the presence of electron-withdrawing groups and at higher base concentrations.

Similar formation of homocoupled alkynes has been observed upon attempted palladium assisted cross couplings of organic halides with stoichiometric amounts of preformed copper(I) acetylides. In the absence of oxygen this would necessitate the nett reduction of Cu^{I} to Cu^{0}. Conceivably this process need not involve palladium, however, attempted cross coupling of MeI and Ph-C≡C-Cu in the presence a catalytic amount of $Pd(PPh_3)_4$ gave $(Ph\text{-}C{\equiv}C)_2$ (54%) and Ph-C≡C-Me (46%), whereas in the absence of palladium neither product was obtained even under more strenuous conditions.[81,183] Abu Salah and Bruce[189] have described the preparation of platinium(II) bisacetylide complexes **72** from a reaction of platinium(0) complexes with copper(I)acetylides (Scheme 27). It was presumed that intial oxidative addition of the copper acetylide platinium(0) complex occured to give an intermediate of the type **73**. This is a similar mechanism as proposed by Nelson *et al.*[181] for the formation of bisacetylides of palladium from terminal acetylenes (see Scheme 26). Presumably an analogous process could occur for palladium and copper(I)acetylides as that described by Abu Salah and Bruce for platinium.

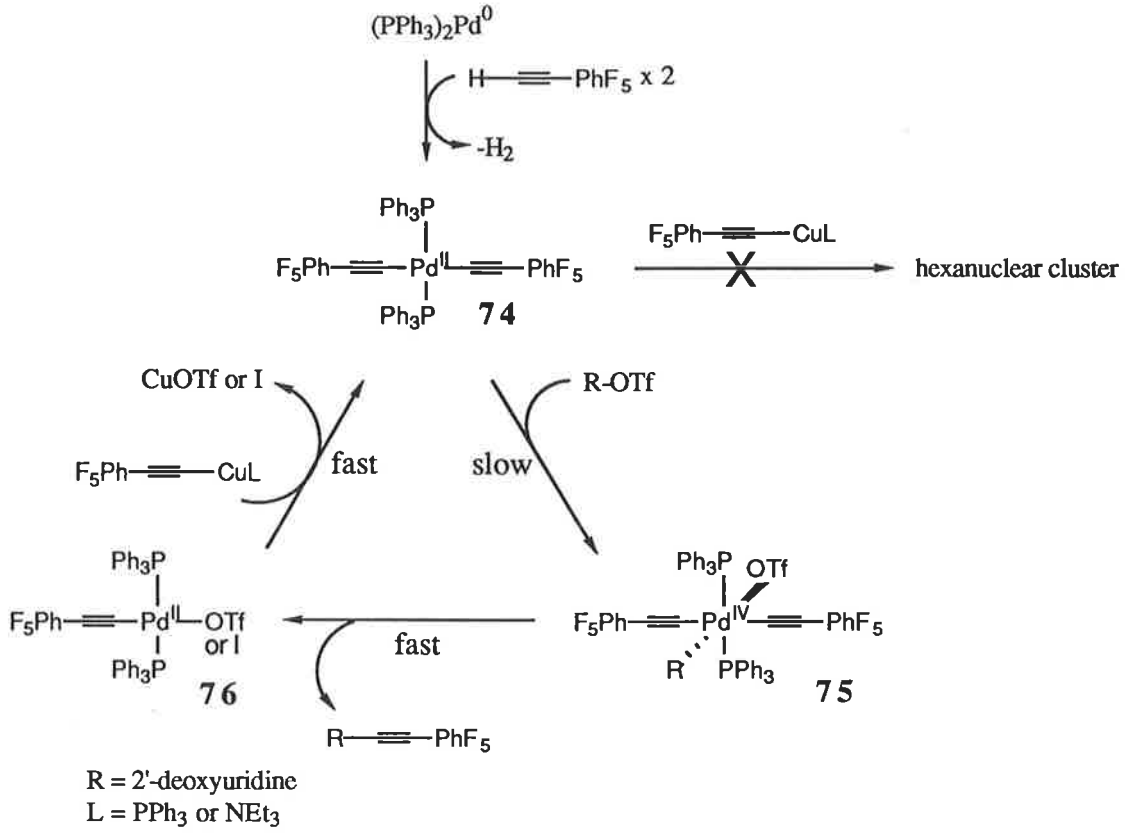
Scheme 27



In reactions where there is a competition between cross-coupling of the nucleoside triflate **14** and homocoupling of some of the more electron-deficient PAs there is, presumably, a competition between **14** and the PA for oxidative addition to the palladium(0). The most electron-deficient PA is the pentafluoro-PA, yet only a trace of homocoupling was observed for this PA in its cross-coupling with **14** and **8**, even under the harsher conditions employed (Chapter 4,entry 10, Table 4, entry 8 Table 5). If we assume that the formation of $(C_6F_5C{\equiv}C)_2Pd^{II}(PPh_3)_2$ **74** is very fast, in accordance with the electron-deficiency of the system, then for some reason the formation of the cluster **70** (PhX = PhF_5) must be inhibited as the colour of the solution remains clear. Perhaps other copper acetylide clusters of the type $(Cu\text{-}C{\equiv}C\text{-}C_6F_5)_n$ form preferentially from which homcoupling does not occur or occurs very slowly. The slow rate of cross-coupling may then be explained in terms of the larger energy required for the nucleoside triflate to oxidatively add to **74** to give a palladium(IV) species **75** from which cross-coupling can be achieved (Scheme 28). Reductive elimination from **75** gives **76** which can transmetallate to return **74**.

This is an alternative explanation to the one given in Chapter 4 where the slow rate of cross coupling was explained in terms of a slow reductive elimination of the highly electron-deficient alkyne from the palladium(II) complex of the usual catalytic cycle (Chapter 4 Scheme 20; R = 2'-deoxyuridine, R' = PhF_5, X = OTf). Though in order for the catalytic cycle in Scheme 20 to be operative oxidative addition of the terminal alkyne ($C_6F_5C{\equiv}CH$) has to be much slower than for the nucleoside triflate. Based on what was observed for other PAs this seems counter to the trend. However, if oxidative addition of $C_6F_5C{\equiv}CH$ to $Pd(PPh_3)_4$ is in some way inhibited then the lack of homocoupling could be explained. In terms of the slow rate of reductive elimination from the Pd^{II} complex in Scheme 20.

Scheme 28

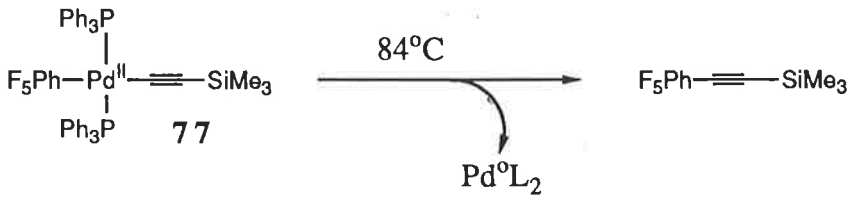


For entry 7 (Table 11) all the ingredients for a cross-coupling reaction were present. At room temperature no reaction was observed, although after 5hrs at 50°C a virtually quantitative yield (92%) of homocoupled trimethylsilylacetylene was isolated. Yet, as was observed in Chapter 5 (entry 1, Table 7), heating this solution directly to 84°C gives, after 4 hours, a high yield (84%) of cross-coupled product as well as a considerable quantity of homo-coupled material.

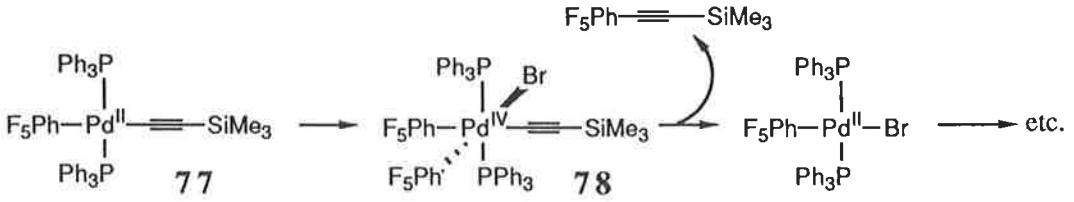
As discussed previously (Chapter 5) oxidative addition of the pentafluorobromobenzene to $Pd(PPh_3)_2$ should occur quite readily, as should transmetallation of a copper acetylide (Scheme 20) but reductive elimination of a highly electron deficient group may be the cause of the slow rate of cross-coupling. At temperatures above 50°C homocoupling of TMSA was observed. Assuming that at this temperature the pentafluorophenyl group remains bound to the palladium then homocoupling of TMSA from this complex may involve

different oxidation states than that postulated in Scheme 20 for the PAs. Evidence that the pentafluorophenyl group may remain bound to the catalyst comes from the fact that there is a difference in the rate of homocoupling in its presence (compare entries 7&8, Table 11). In what way the pentafluorophenyl group on the palladium promotes homocoupling remains unclear, posibly it promotes the formation of other polynuclear clusters. The use of colour as an indicator of homocoupling was not possible as all solutions involving the coupling to arylbromides to TMSA went black rapidly.

At 84°C cross-coupling occurs as the predominant process. Perhaps at this temperature an energy threshold is surpassed that allows for direct reductive elimination of the pentafluorophenyl group and the TMSA to form the corresponding pentafluoro-PSA (eq. 46). Alternatively the energy threshold that is surpassed may be for the oxidative addition of the pentafluorobromobenzene to the palladium(II) complex **77** to give a palladium(IV) complex **78** as depicted in eq. 47. If this latter mechanism were operating the use of the corresponding pentafluoro-iodobenzene should allow for cross-coupling at lower temperatures. In fact Wen *et al.*[171] have reported that pentafluoroiodobenzene couples to TMSA in Et_3N in the presence of a catalytic amount of $Pd(PPh_3)_2Cl_2$ and CuI at 35°C (91%). No homocoupled material was reported.



(46)



(47)

6.3 Summary

In summary then the following catalytic cycles for the various coupling reactions have been proposed. For the cross coupling of the nucleoside triflates **12, 14,** and **8** with terminal alkynes, it seems reasonable that most of these coupling would proceed via the cycle depicted in Scheme 20 (Chapter 4) where an increase in reaction rate with increasing electron-deficiency of the alkyne is consistent with copper acetylide formation or transmetallation being rate determining. Although, in the case of PAs bearing electron-withdrawing groups the rate of copper acetylide formation and/or transmetallation may be so fast that oxidative addition of the nucleoside triflate may also become at least partially rate determining. In the case of the highly electron-deficient pentafluoro-PA reductive elimination maybe the rate determining step.

An alternative mechanism for the pentafluoro-PA has been postulated based on the anticipated fast rate of bis(pentafluorophenylacetylide)palladium(II) complex **74** formation (Scheme 28). In this case the slow rate of overall reaction was attributed to the slow formation of a palladium(IV) complex **75**. The fact that almost no homocoupling and no intense colouration of the reaction mixture were observed suggests that the palladium/copper hexanuclear cluster **70** (PhX = PhF_5), thought to be responsible for both, was not formed. However, a satisfactory explanation for why such a cluster does not form in this case, but does for other electron-deficient PAs has not been derived. However, it is not unusual for pentafluorophenyl derivatives to run counter to observed trends. It may be that other clusters involving only copper form $(Cu\text{-}C{\equiv}C\text{-}C_6F_5)_n$ or that the bisacetylide palladium(II) complex **74** does not form, in which case the absence of homocoupling would be accounted for and the slow rate of cross-coupling with **8** and **14** results from the slow rate of reductive elimination from the diorganopalladium(II) complex (Scheme 20).

The cross-coupling of phenyl bromides with TMSA went smoothly in nearly all cases (entries 2 to 6, Table 7, Chapter 5). It was likely to have proceeded via the cycle depicted in Scheme 20 (Chapter 4) where oxidative addition was rate determining as the reaction rate increased with increasing electron-deficiency of the aryl halide. The pentafluorobromobenzene proved an exception as it only proceeded at high temperature and with considerable TMSA homocoupled by-product formation (entry 1, Table 7). In fact, under certain conditions, such as entry 7, Table 11, only homocoupled TMSA was obtained. Homocoupling appeared to be promoted by the presence of the pentafluorobromobenzene and the catalytic cycle for this process may again involve intermediates, such as polynuclear clusters.

Chapter 7

Approaches to Sugar Modified 5-Trifluoromethanesulfonylpyrimidine nucleosides.

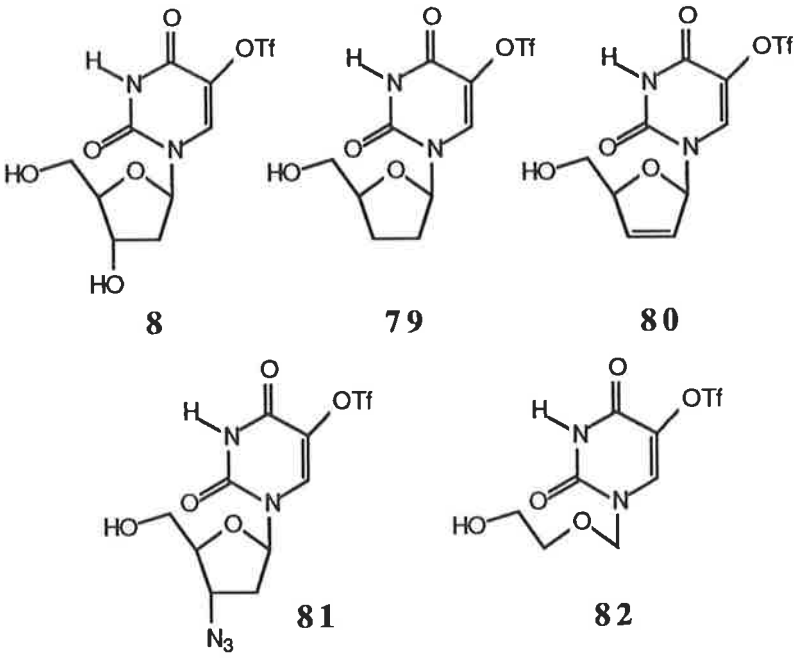
7.1 Introduction

A number of sugar ring modifications have been identified as extremely fruitful in the preparation of nucleoside analogues as highly potent and selective antiviral/anticancer agents. The combination of both sugar and base elaboration has sometimes lead to an amplification of either (or both) the selectivity and potency of the nucleoside analogue, however, only a few studies of this type have been reported.[20,21,190-3] Thus identification of convenient routes to sugar modified nucleosides activated towards base ring elaboration was considered necessary in order to to provide ready access to a wide variety of compounds with potential biological activity.

Having developed a simple, high yielding route to C5-trifloxyl uridine **7** and explored its potential as a convenient substrate for palladium assisted substitution, it only remained to identify convenient routes to sugar modified analogues of this triflate.

Four important sugar modified analogues of C5-trifloxyl uridine were targeted, as shown in Fig. 11 (**8** to **81**). Although 2'-deoxyuridine triflate **8** has been prepared by C5 hydroxylation of 2'-deoxyuridine and subsequent triflation as described in Chapter 2, an alternative to this procedure was sought in view of the high cost of 2'-deoxyuridine. Uridine is considerably less expensive than 2'-deoxyuridine and undergoes C5 hydroxylation in much higher yield.

Figure 11



The 2',3'-dideoxy, the 2',3'-dideoxydidehydro and the 3'-azido-2',3'-dideoxyuridine triflates (**79, 80 & 81** respectively) all represent important classes of sugar modified uracil nucleosides activated towards C5 substitution. All three of these sugar modifications have been shown to endow a number of pyrimidine nucleoside analogues with significant anti-HIV activity. Furthermore it was considered that approaches towards the acyclic uridine triflate analogue **82** from uracil should be sought. There are only a few reports of the antiviral/anticancer activity of the C5-substituted derivatives of these nucleoside analogues **79-82**.[1,20,190-1] Yet significant anti-HIV and antiherpetic activity has been observed for other base ring modified pyrimidine type nucleosides.[20,190]

In principle the preparation of the uridine analogues bearing both a C5 triflate and a modified sugar moiety could be achieved in one of four ways:

1. Uridine → sugar modification → hydroxylation → triflation
2. Uridine → hydroxylation → sugar modification → triflation
3. Uridine → hydroxylation → triflation → sugar modification

4. Uridine → sugar modification

hydroxylation triflation

i.e. Hydroxylation and/or triflation could be carried out on an intermediate of the sugar modification.

Each of the possible routes, 1-4, appeared to have its own merits. In the case of (1) direct application of literature methods for sugar modification of uridine could be applied or alternatively commercially available sugar modified nucleosides could be utilized. However, hydroxylation via the method described in Chapter 2 involved the use of molecular bromine (Br_2), the use of this reagent might not be compatible with all sugar modifications (e.g. olefins). Also a decrease in yield of C5-hydroxyl product was observed in going from uridine to 2'-deoxyuridine as a substrate, further sugar modification, in particular, further deoxygenation may have an even greater effect on the yield. Hydroxylation of uridine has been refined in this present work (Chapter 2) to give a good yield of 5-hydroxyuridine **9** (77%), thus it may be advantageous to perform sugar modifications on this precursor using routes (2-4).

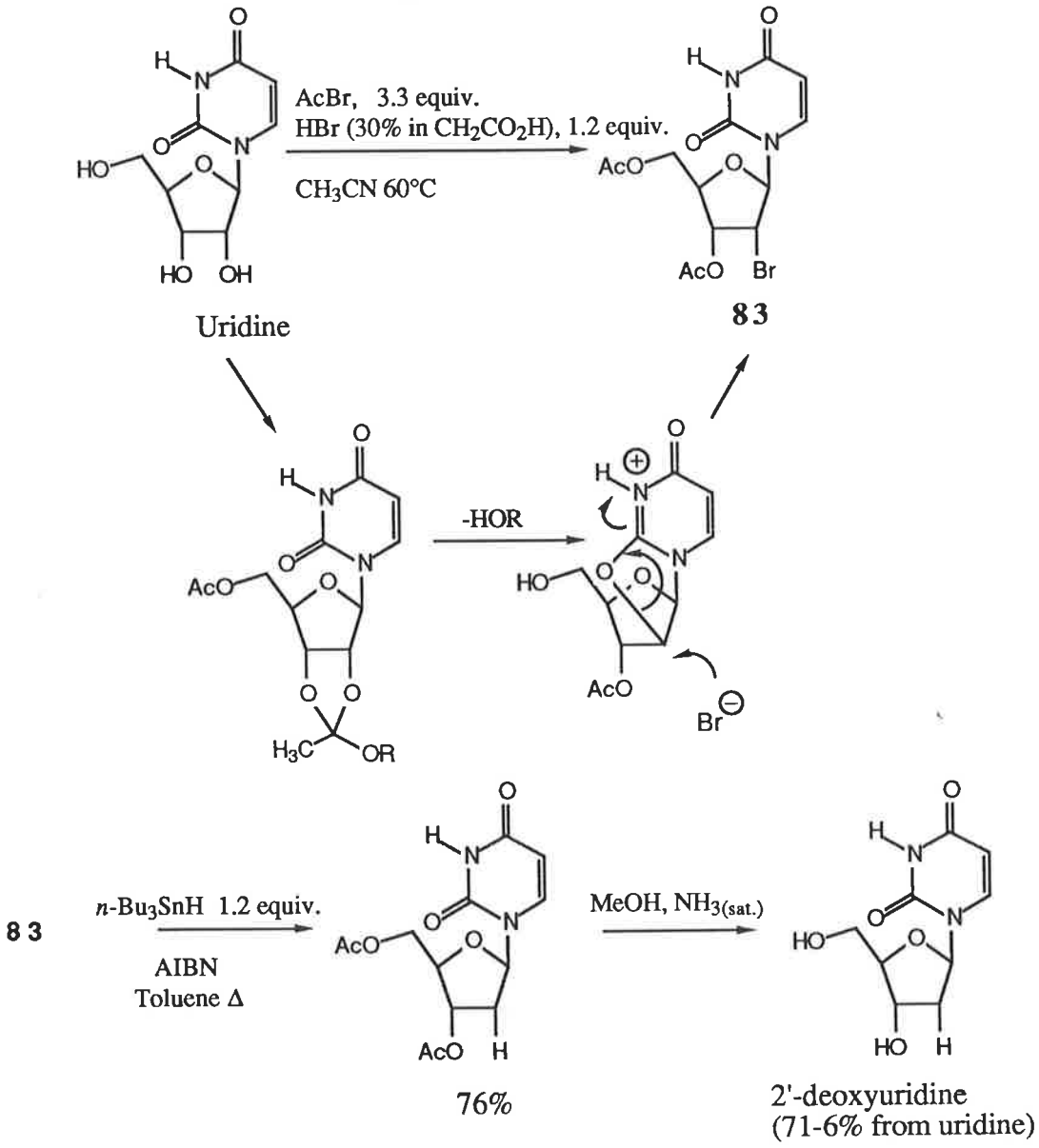
The C5-triflate of uridine **7** has also been obtained in good yield (75%, from uridine). The triflate group may be stable to a variety of reaction conditions and could prove to be a suitable substrate to sugar modification (route 3). The ability of the C5 triflate group to survive typical reactions used to modify sugar groups would be of inherent interest, since only a limited number of reactions have been reported on multifunctional molecules containing a alkenyl or aryltriflate. Route (4) may act as a compromise between the other three.

7.2 Results and Discussion

7.2.1 2'-Deoxy-5-trifluoromethanesulfonyluridine (8) from Uridine.

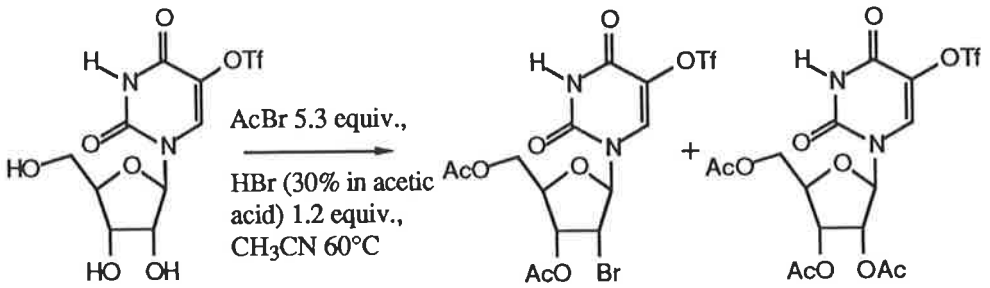
Triflate **8** has been prepared from commercially available 2'-deoxyuridine in 55% yield (Chapter 2). However, a convenient synthesis of 2'-deoxyuridine from uridine has been reported[194] (Scheme 29). In this literature procedure uridine was treated with acetyl bromide (3.3 equiv.) and HBr (30% in acetic acid, 1.2 equiv.) at 55-60° in acetonitrile to give 3',5'-di-*O*-acetyl-2'-bromo-2'-deoxyuridine **83** in excellent yield (> 95%).

Scheme 29[194]



The regio and stereochemical control of the bromide addition results from anchimeric assistance of the base ring, where 1,5-cyclization is preferable to 1,6 and bromide addition occurs via an S_N2 displacement of the C2 oxygen. Reductive dehalogenation using tri-*n*-butyltin hydride and deacetylation of the crude product in methanolic ammonia gave 2'-deoxyuridine in a reported 71-76% yield from uridine. This suggests that the preparation of **8** from uridine could be achieved in an overall 39-41% yield based on route 1.

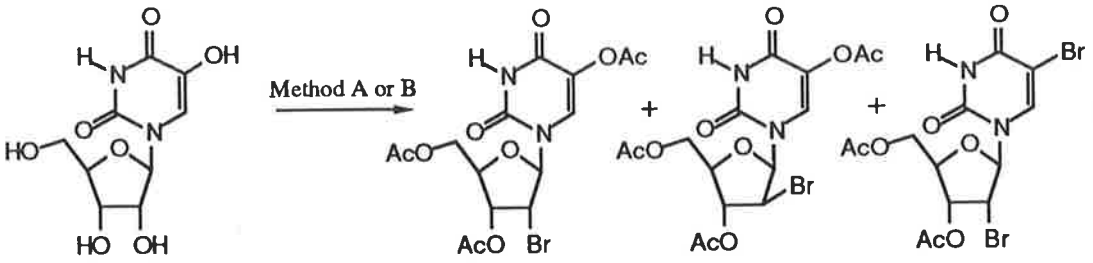
An attempt to prepare the 2'-deoxyuridine triflate **8** from C5-trifloxyl uridine **7** (i.e. route 3) was attempted. Treatment of **7** with acetyl bromide (5 equiv.) HBr (30% in acetic acid, 1.2 equiv.) 60°C in acetonitrile (eq. 48) gave only the trisacetate **12**. Consequently the 2'-deoxy product **8** was not available via route 3. The role of the C5-trifloxyl group in inhibiting the incorporation of a bromide at the 2'-centre is a mystery.



7 0% **12** ~72% (48)

An alternative procedure based on route 2 was also investigated (eq. 49). Treatment of 5-hydroxyuridine with acetyl bromide (5.3 equiv.) and HBr (30% in acetic acid, 1.2 equiv.) at 60°C in acetonitrile, gave the desired 3',5'-di-*O*-acetyl-2'-bromo-5-acetoxyuridine **84** (76%), some 2'β-bromo product **85** (5%) and some 5-bromo product **86** (16%) (Method A, eq. 49).

The 5-bromo derivative **86** may result from initial 1,4-addition of HBr to the α,β-unsaturated carbonyl of the base ring, followed by protonation and substitution of the acetoxy group with a bromide and subsequent elimination of HBr (Scheme 30).

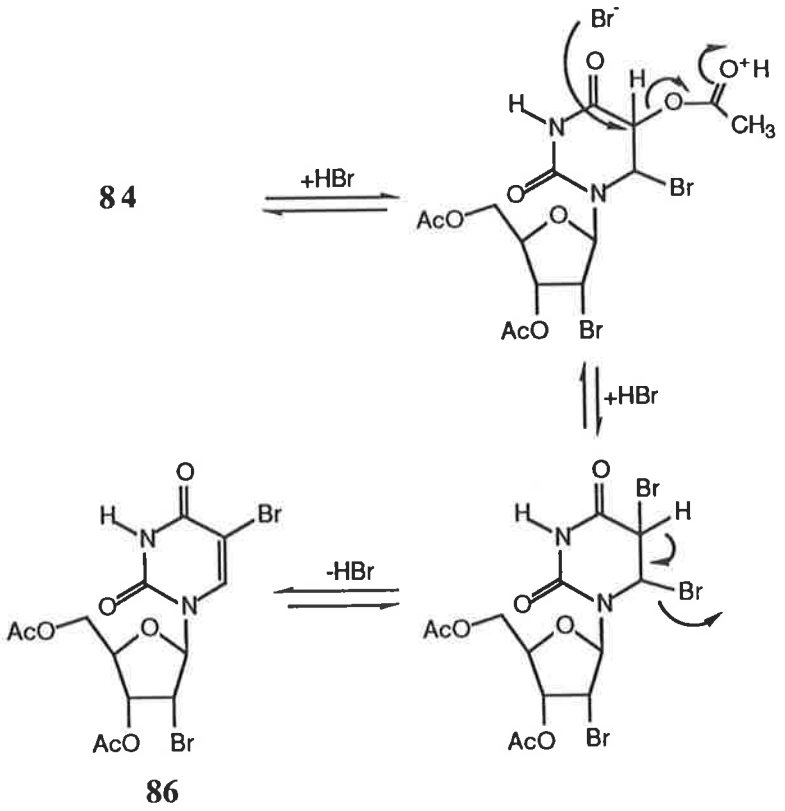


(49)

9	84	85	86
	Method A: 76%	5%	16%
	Method B: ~90-95%	~5-10%	0%

Method A: AcBr 5.3 equiv., HBr (30% in acetic acid) 1.2 equiv.,CH_3CN 60°C
Method B:AcBr. 8.0 equiv., CH_3CN 60°C

Scheme 30



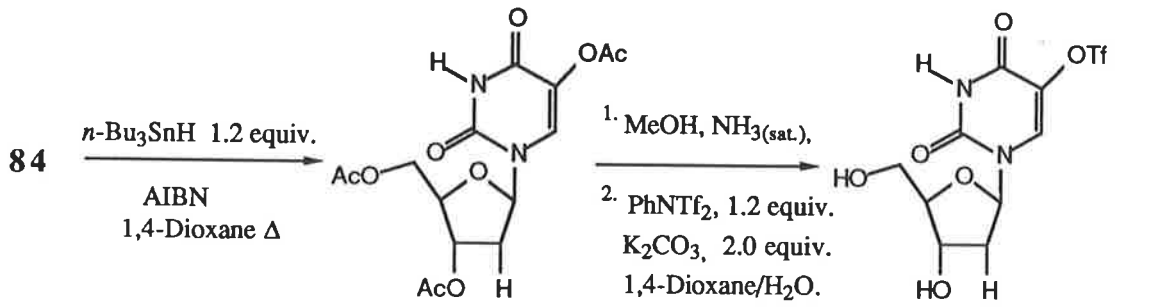
86

Formation of **86** could be avoided by use of a larger excess of acetyl bromide (8.0 equiv.) and no HBr, to give **84** (90%) and a minor amount of **85** (6%) (Method B, eq. 49).

Reductive dehalogenation of **84** with tri-*n*-butyltin hydride gave 3',5'-di-*O*-acetyl-5-acetoxyuridine (**87**, 76%) (Scheme 31). 1,4-Dioxane and not toluene was used as the solvent in this reaction in view of the poor solubility of **84** in toluene.

Deacetylation and triflation of **87** as a one pot two step process gave **8** in a 94% yield (Scheme 31). In this way **8** was obtained from uridine in an overall 55% yield. This is a similar yield to that obtained from the more expensive precursor 2'-deoxyuridine.

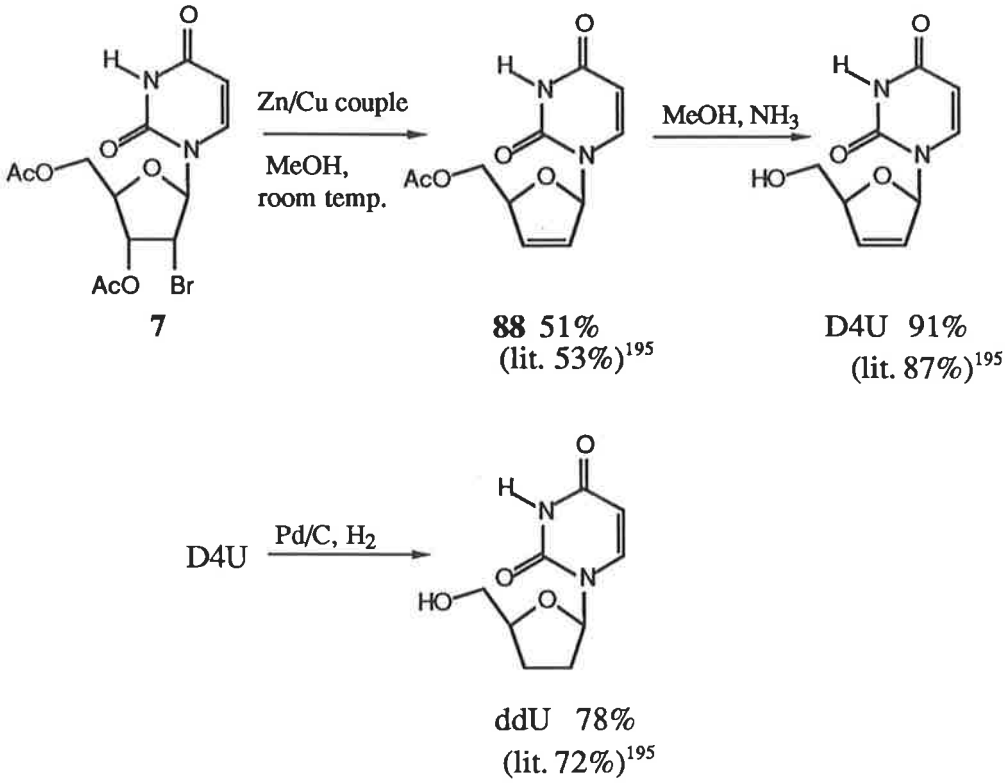
Scheme 31



7.2.2 2',3'-Dideoxy-5-trifluoromethanesulfonyluridine (79).

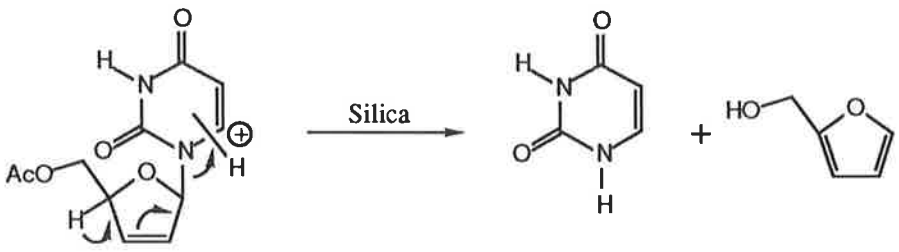
Initial attempts to prepare **79** were based on route 1 where sugar modification preceeds C5 hydroxylation and triflation. 2',3'-Dideoxyuridine (ddU) was prepared from uridine in accordance with literature procedures.[195] Again the 2'-bromo-di-*O*-acetyl product **83** was utilized. Elimination of the bromo and acetate groups of **83** to give the olefin **88**, deacetylation to give D4U and palladium on charcoal reduction to yield ddU all proceeded without difficulty (Scheme 50). In the case where intermediates were isolated, similar yields to those obtained in the literature were achieved. Confirmation of the structure of the product was based on the similar physical and spectral data obtained to that reported in the literature.

Scheme 32



Yield from uridine (1.0 x 0.51 x 0.91 x 0.78) x100 = 36%
from single pot four step process = 57%

The highest yields of ddU were obtained when the reaction was carried out as a single pot, four step process. The increase in yield for the single pot process was attributed, primarily, to an avoidance of a loss of product during chromatography of the crude **88**. The slightly acidic silica appears to have promoted cleavage of the glycosidic linkage which is facilitated by the presence of the double bond (eq. 50).[196] Some uracil was isolated during chromatography but was not detected by t.l.c. of the crude mixture of **88**.



(50)

88

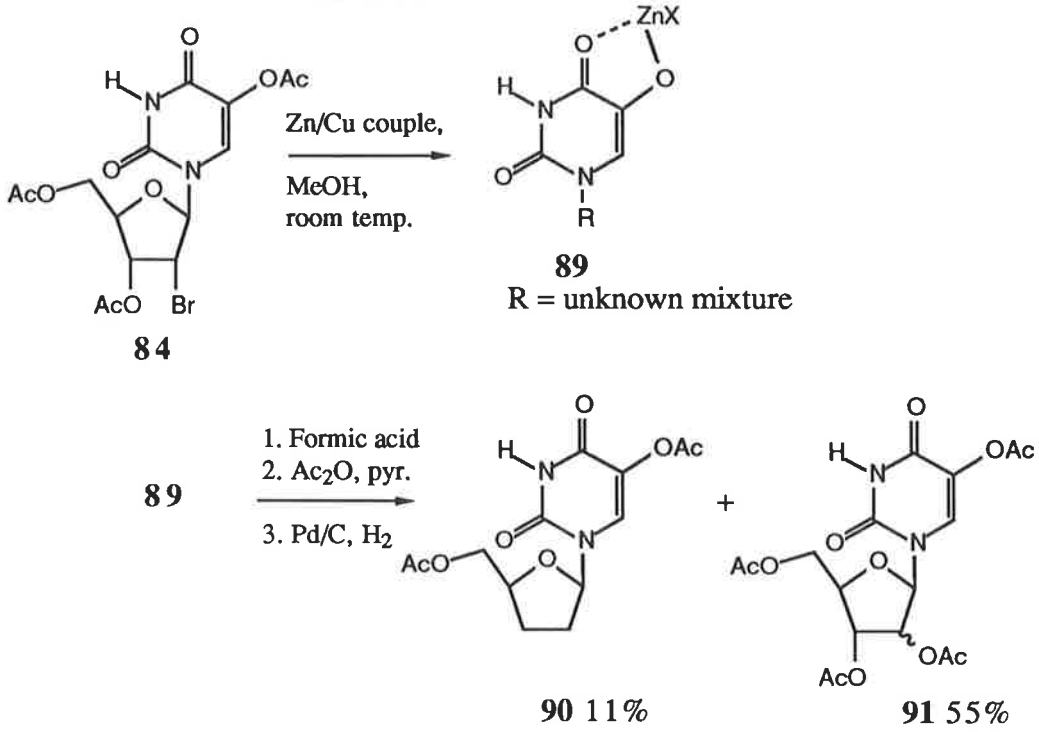
A number of attempts at C5-hydroxylation of ddU under the conditions described for 2'-deoxyuridine and uridine were made at several different

temperatures (4, 20, 30, 37°C). Analysis of reaction mixtures (t.l.c.) indicated complete decomposition of the ddU as no U.V. active material could be detected. As anticipated, further sugar modification of uridine leads to an increased tendency to decomposition under hydroxylation conditions.

It was considered that 2'-bromo'-3',5'-di-*O*-acetyl-5-acetoxyuridine **84** may provide an alternative pathway (i.e. route 2) where hydroxylation of sugar modified uridine is avoided. However treatment of **84** with zinc/copper couple did not give the desired product (Scheme 33). Analysis of the reaction mixture by t.l.c. revealed only a highly fluorescent base line spot (at 254nm) plus two very faint spots of higher R_f which did not correspond to starting material. The reaction mixture was filtered and a U.V. spectrum of the filtrate was run. Two maxima, one at 310nm and the other at 208nm, predominated. This corresponded almost exactly with the U.V. spectrum of 5-hydroxyuridine **9** with excess potassium carbonate (310 & 206nm) (**9** at neutral pH absorbs 278 & 207nm). Presumably a zinc enolate **89** had formed during the elimination reaction. This implies deacetylation of the C5 acetoxy group. Gonzalez *et al.*[197] have reported the selective deacetylation of phenyl acetates in the presence of other primary and secondary acetates using caytalytic amounts of zinc/copper couple (~10mol%) in methanol.

In order to further investigate the nature of this proposed zinc enolate **87**, formic acid was added to the filtrate from the zinc/copper couple reaction until the solution was slightly acidic whereupon a white precipitate formed. Analysis of the slightly acidic solution by t.l.c. revealed a large increase in intensity of the previously faint spots and complete diappearance of the fluorescent base line spot. The slurry was filtered and the filtrate concentrated and purged of all formic acid.

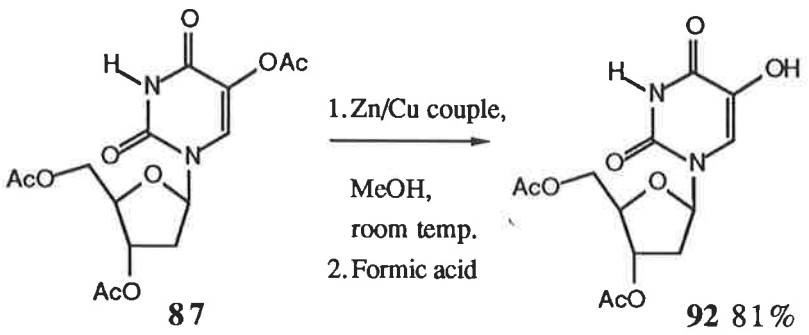
Scheme 33



To ensure the product would be amenable and stable to chromatography the residue was acetylated (free hydroxy products, in particular the 5-hydroxyl nucleosides tend to bind very strongly to silica) and hydrogenated (to avoid deglycosylation) (Scheme 33). After chromatography two products were isolated the desired 5'-*O*-acetyl-2' 3'-dideoxy-5-acetoxyuridine (**90**, 11%) and 2',3',5'-tri-*O*-acetyl-5-acetoxyuridine (**91**, 55%). The structural determination of **90** was based on the disappearance of the downfield resonances (in the ^{1}H NMR spectrum) corresponding to the 2' and 3' hydrogens geminal to an hydroxyl function (4.48 and 4.89 ppm respectively) and the appearance of several upfield multiplets (1.74–2.40 ppm) corresponding to the 2' and 3' methylene groups of **84**. Also only two acetate resonances (2.34 & 2.04 ppm) were apparent in the ^{1}H NMR. A M^++H peak for **84** in the FAB mass spectrum was obtained. In the case of **91** four acetate methyl resonances were observed in the ^{1}H NMR and the coupling for the 1', 2' and 3' protons were very similar to that of other 2'α acetylated uridine analogues, however, no definite stereochemistry has been assigned. A M^++H peak was also obtained in the FAB mass spectrum **91**. The source of this 2'-acetoxy group is uncertain and was not investigated.

The attempts to improve the yield of the elimination product **90** by variations of the reaction conditions were not successful. Neither elimination nor deacetylation was observed when alternative, aprotic solvents, such as THF and 1,4-dioxane were used. The addition of 0.5 - 1.0 equiv. of formic acid to the original reaction mixture (methanol as solvent) resulted in a much slower reaction and the formation of many products as evidenced by t.l.c. (products were not isolated).

The presumed C5 zinc enolate formation was studied in isolation using the trisacetate obtained previously **87**, resulting from reductive dehalogenation of **89**. Addition of zinc/copper couple (5 equiv.) to a methanolic solution of **87** gave, after 1hr, complete enolate formation (by t.l.c.) (eq. 80). The mixture was filtered to remove excess zinc/copper couple and the filtrate acidified using formic acid (5 equiv.), concentrated and diluted with ethyl acetate and filtered. Evaporation of all the solvent and formic acid gave a solid foam. This residue was pure 3',5'-di-*O*-acetyl-5-hydroxy-2'-deoxyuridine (**92**, 81%) as shown by its ^{1}H NMR spectrum.



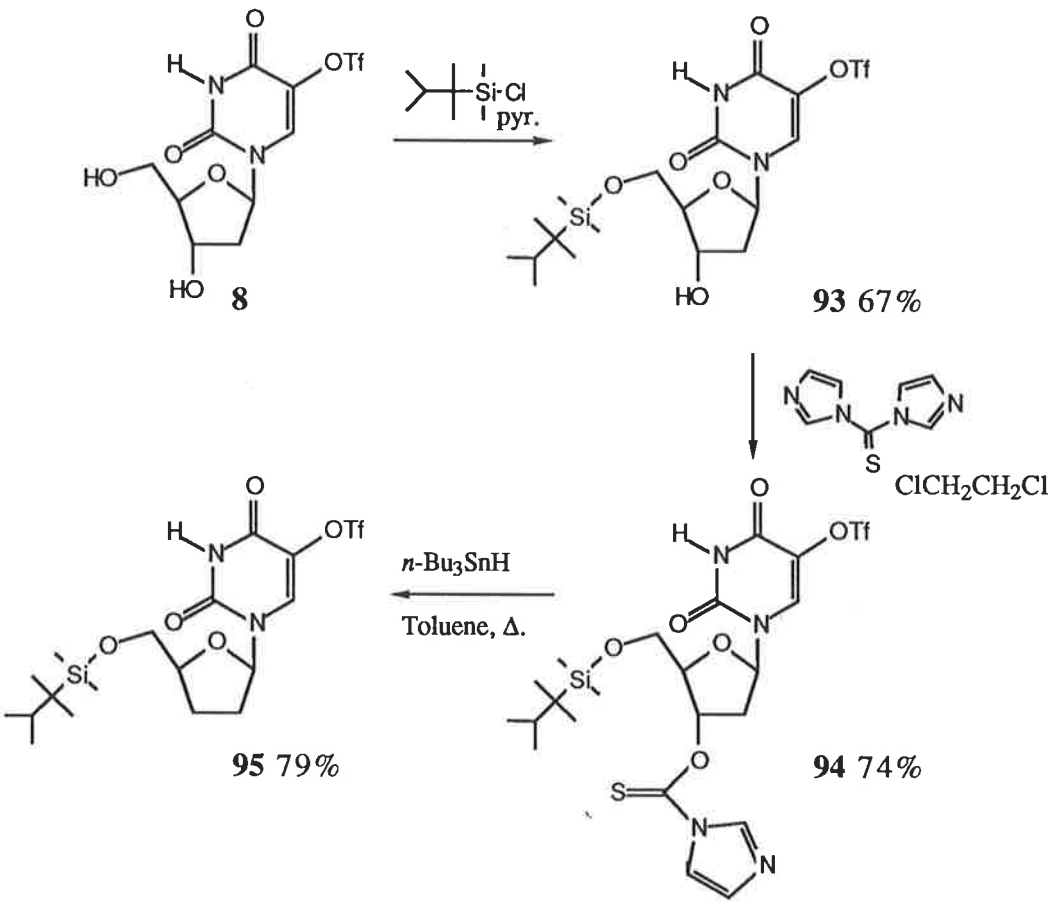
87 **92** 81%

(51)

No such selective deacetylation could be observed (by t.l.c.) using catalytic amounts of mild or strong base ($KHCO_3$, K_2CO_3, DBU, KOH) in methanol but rather, just slow or fast (respectively) deacetylation at two or more centres simultaneously.

Returning to the formation of the 2',3'-dideoxyuridine triflate **79**, the 2',3'-bromoacetate **83** was abandoned as a precursor and a new approach sought. 2'-Deoxygenation of the 2'-deoxyuridine triflate **8**, readily available in a 55% overall yield from uridine, via the Barton-McCombie method, seemed an attractive alternative (route 4). Selective silylation of the 5'-hydroxyl group of **8** was achieved only in a moderate yield (65%) using thexyldimethylsilyl chloride (Scheme 34),* more complex solvent/base systems involving imidazole and/or dimethylaminopyridine (DMAP) in DMF[177] gave lower yields.

Scheme 34



Treatment of **93** with 1,1'-thiocarbonyldiimidazole gave the 3'-thiocarbonate **94** (74%). Radical deoxygenation of **94** with tri-*n*-butyltin hydride and 2,2'-azobis(2-methylpropionitrile) (AIBN) in refluxing toluene gave 5'-*O*-[(dimethyl-2-(2,3-dimethybutyl)silyl)]-2',3'-dideoxy-5-trifluoromethanesulfonyluridine **95** (79%).

---

* Kelvin *et al.*[142] have investigated the selective protection of nucleoside sugar moieties where, under similar conditions, selective silylation of uridine was acheived in a 83% yield using *t*-BuMe$_2$SiCl.

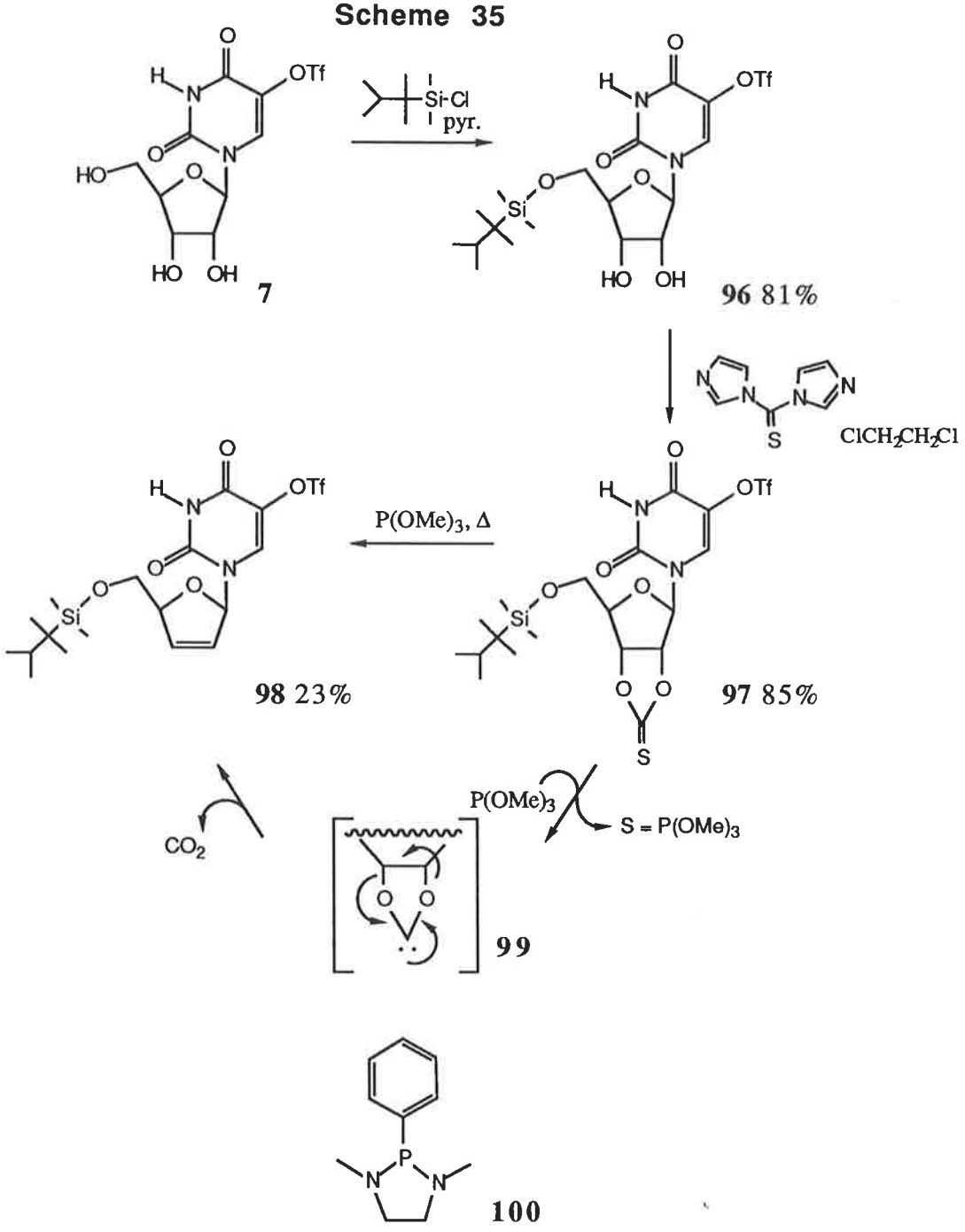
This represents an overall yield of 21% for **95** from uridine over 7 steps. Whilst **95** was not desilylated it should be noted that desilylation of the over silylated by product of **93** yielded **8** quantitatively.

7.2.3 2',3'-Dideoxydidehydro-5-trifluoromethanesulfonyluridine (80).

The most attractive precursor to the formation of **80** was considered to be uridine triflate **7** (route 3) as a number of methods exist for the conversion of vicinal diols to olefins.[199-205] The application of the Corey-Winter reaction to nucleosides has been shown to be a convenient method for olefin formation.[199] Application of this methodology to **7** involved initial silylation of the 5'-hydroxyl to give **96** which was treated with 1,1'-thiocarbonyldiimidazole to give thiocarbonate **97** (Scheme 35). Thermolysis of **97** in trimethylphosphite to give the olefin **98** is expected to proceed via a carbene intermediate **99**. The low yield of **98** (21%) obtained was attributed to competitive decomposition of the C5-trifloxyl pyrimidine base ring. Similar thermolysis of a thiocarbonate of uridine in the absence of a triflate group has been reported to proceed in a 80% yield.[199]

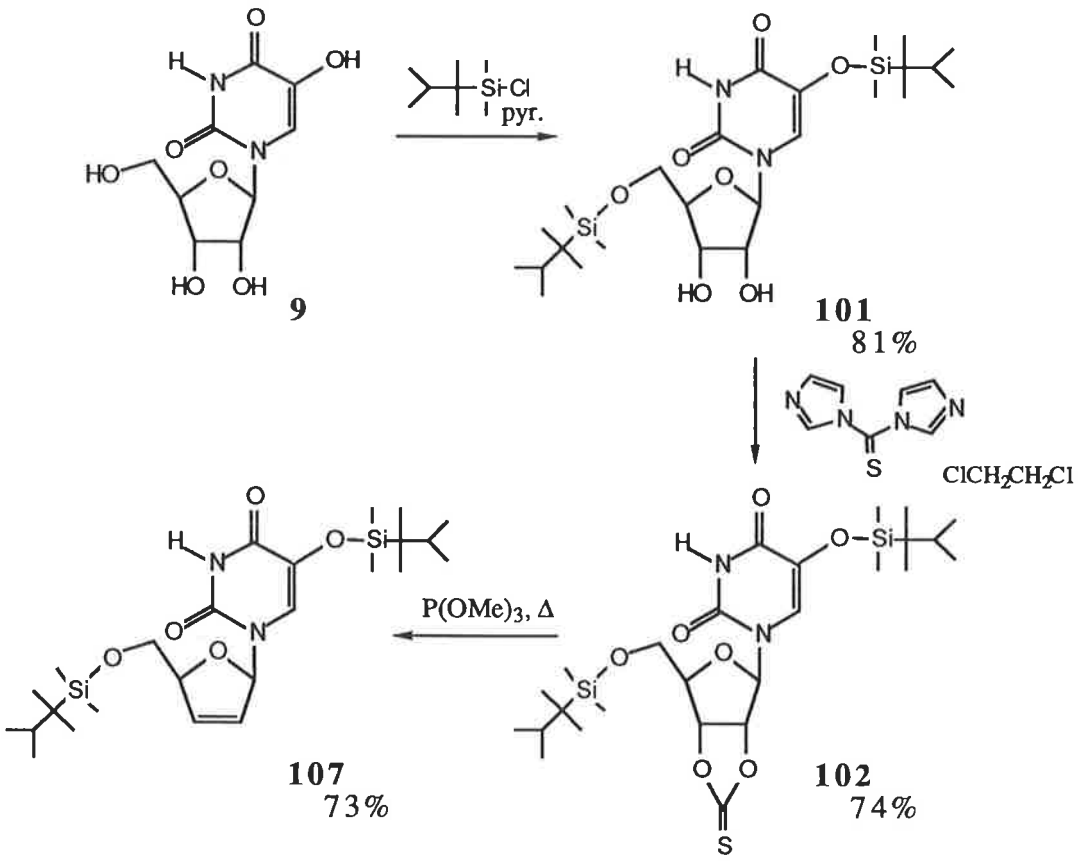
In an attempt to reduce competitive decomposition the alternative phosphorus reagent, 1,3-dimethyl-2-phenyl-1,3-diazaphospholidine **100** was utilized. Corey and Hopkins[204] had previously demonstrated that **100** leads to fragmentation of thiocarbonates to olefins under considerably milder conditions (20-40°C) than trimethylphosphite achieving olefin formation at much lower temperatures. However treatment of **97** with **100** at room temperature lead to decomposition of **97**, no observable product formation was detected by t.l.c., in fact only very little U.V active material was observed.

Scheme 35

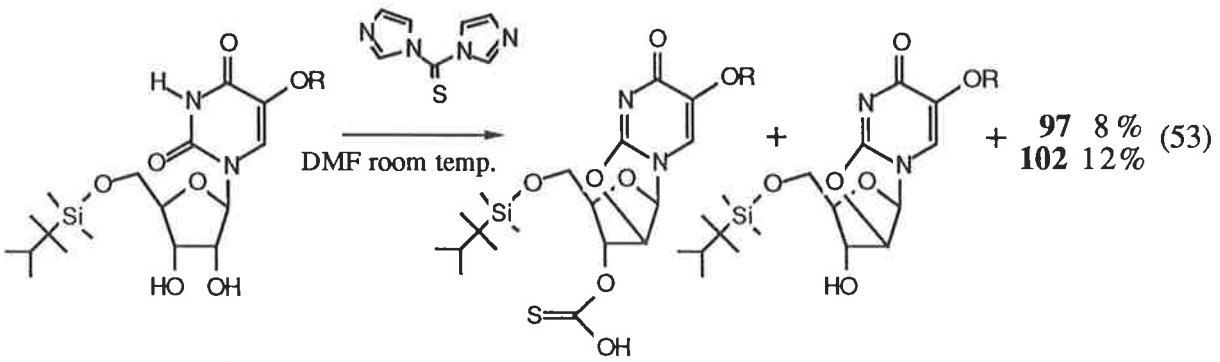


An alternative approach was to introduce the 2',3'-olefinic group prior to triflation of the C5-hydroxyl group of 5-hydroxyuridine **9** (route 4). Selective silylation of the 5 and 5'-hydroxyl groups of **9** gave **101** in moderate yield (65%), (Scheme 36). Again thiocarbonate formation proceeded efficiently in 1,2 dichloroethane giving **102** in good yield (74%).

Scheme 36



In an attempt to further improve the yield of this reaction dimethylformamide (DMF) was used as a solvent. DMF has not been demonstrated previously to be a superior solvent but has been used before for some purine based nucleosides.[198] Different products were isolated from this reaction mixture. It appears that in DMF the formation of the 2,2'-anhydro-nucleosides **103** and **104** were favoured (eq. 53). This was also found to be the case for the uridine triflate **96**. A similar scenario was also observed for the

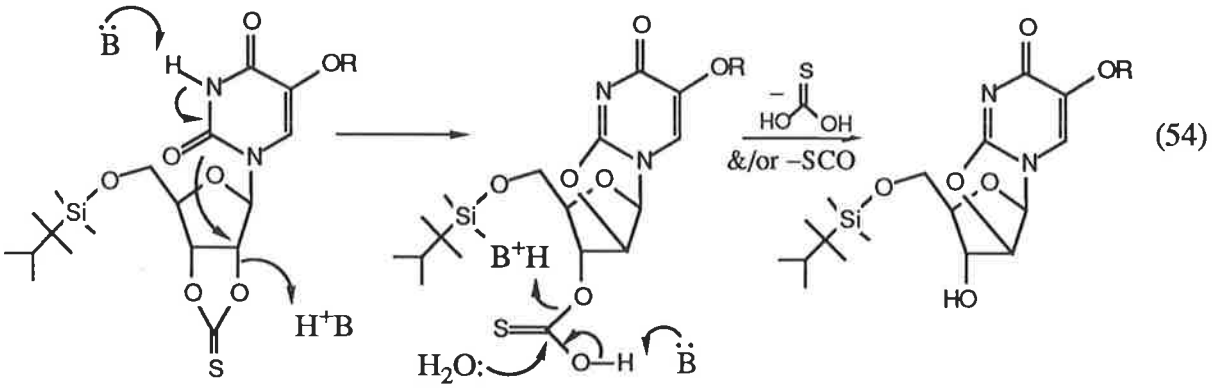


101, R = -Si(t-Bu)Me2 **103**, R = -Si(t-Bu)Me2 11% **104**, R = -Si(t-Bu)Me2 63%

96, R = Tf **105**, R = Tf 7% **106**, R = Tf 55%

5'-protected, C5-trifloxyl uridine **96** where in DMF the major product was **106** with smaller amounts **97** and **105** also being isolated.

The formation of **104** would appear to have resulted from initial formation of **102**, which then underwent base catalysed rearrangement to give **103** followed by hydrolysis upon work up, or direct loss of SCO, to give **104** as the major product (eq. 54). Such a process would be promoted in polar solvents such as DMF where ionic intermediates would be stabilized.



(54)

102, R = –Si(t-Bu)Me₂ **103**, R = –Si(t-Bu)Me₂ **104**, R = –Si(t-Bu)Me₂

97, R = Tf **105**, R = Tf **106**, R = Tf

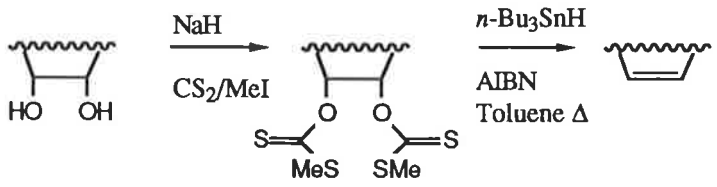
Nevertheless, having achieved a satisfactory yield of **102** (74%) in 1,2-dichloroethane thermolysis of **102** in trimethylphosphite was attempted and gave **107** in a good yield (73%) (Scheme 36). To date deprotection and selective triflation of **107** have not been attempted. However, the efficient formation of **107** from thermolysis of **102** suggests that this may be a suitable route to 2',3'-dideoxydidehydro-5-trifluoromethanesulfonyluridine **80**.

The formation of a 2',3'-olefin prior to triflation has the added advantage of also providing a precursor to the 2',3'-saturated equivalent via palladium on charcoal reduction, which can be utilized in the formation of **79**.

Radical deoxygenation of **94** to give **95** demonstrated the stability of the triflate moiety to radical processes. Radical deoxygenation of bis-xanthates has been demonstrated by Barton *et al.*[205] as providing a route to olefins (Scheme 37).

Such an approach may be applicable to the 5'-*O*-protected trifloxyuridine **96** to give **98**, providing an alternative means of converting **7** into the dideoxy-didehydrouridine triflate (route 3).

Scheme 37

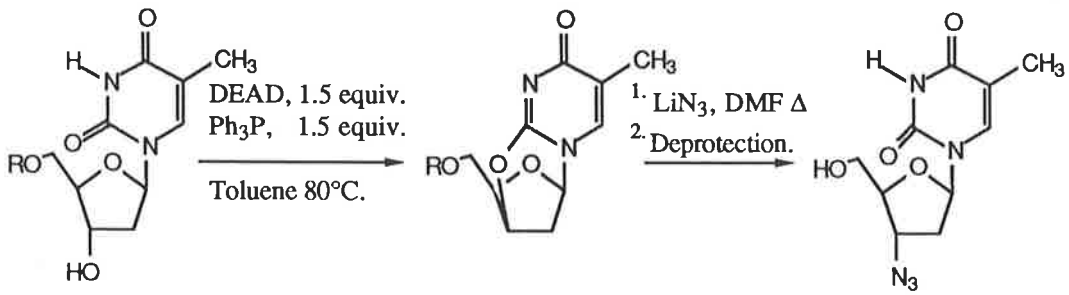


7.2.4 3'α-Azido-2',3'-dideoxydidehydro-5-trifluoromethanesulfonyluridine (81).

As with 2'-dideoxy nucleosides, 2',3'-substituted nucleosides are also of great interest as potential antiviral/anticancer agents especially as anti-HIV agents. Incorporation of an α-azido group at the 3'-centre has been shown to be very effective in the development of anti-HIV agents (e.g. AZT).

Stereospecific substitution of a hydroxyl group of the sugar moiety in uridine based nucleosides with an azide has been perhaps most efficiently realized via an initial intramolecular Mitsunobu reaction to achieve 2,3'-cyclization followed by an intermolecular S_N2 displacement with lithium azide (Scheme 38).[206-8]

Scheme 38



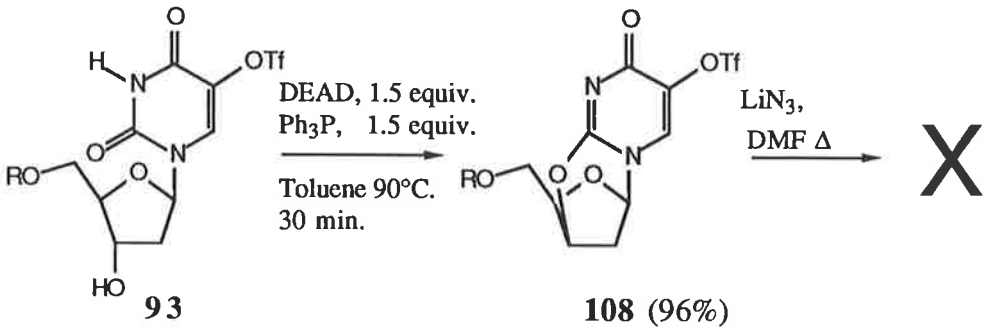
R = protecting groups

AZT
73% from thymidine.[207]

Treatment of **93** with triphenylphosphine and diethyl azodicarboxylate in toluene gave the 2,3'-anhydro derivative **108** in an excellent yield (96%) (Scheme

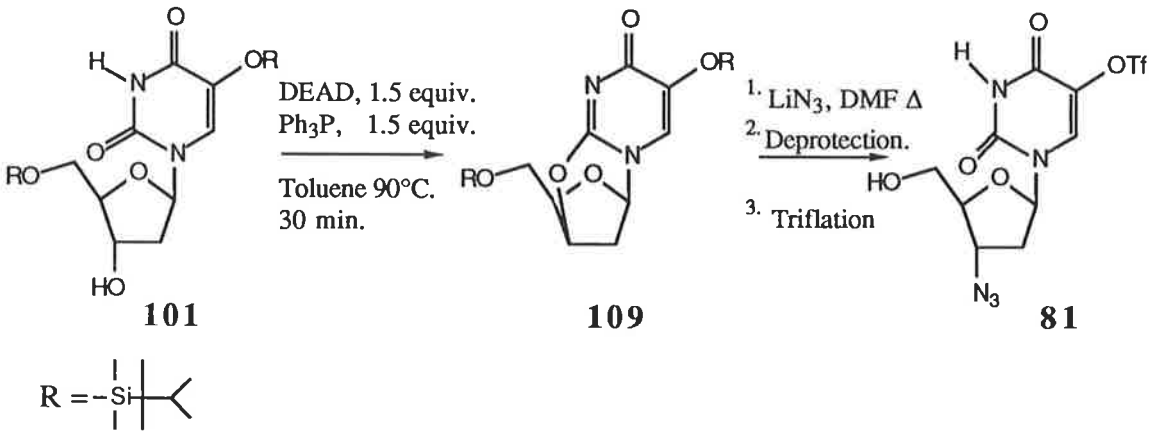
39). However, despite numerous attempts under a range of conditions, including the use of catalytic[206] and stoichiometric amounts of mild acids (benzoic acid and ammonium chloride), only decomposition of starting material **108** was observed and no formation of an azido nucleoside was detected.

Scheme 39



93 **108** (96%)

Scheme 40



101 **109** **81**

Again the triflate moiety appears to have activated the base ring towards decomposition at high temperature in the presence of a nucleophile. An alternative approach which could be utilized in the future maybe to again utilize the 5,5'-disilyl ether, this time of 2'-deoxy-5-hydroxyuridine, as the precursor to sugar modification (route 2) (Scheme 40). The corresponding 2, 3'-anhydride **109** is likely to be less prone to base ring decomposition.

In any case it would be of interest to investigate the palladium-catalysed coupling of the 2,3'-anhydro derivative **108**. A recent study on the anti-HIV/anticancer activity of 2,5'-anhydrido pyrimidine nucleoside analogues demonstated that whilst some of these derivatives were somewhat less potent

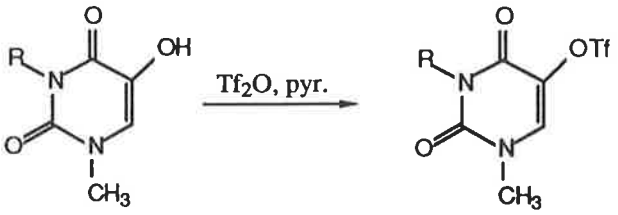
than their parent, nonanhydro derivatives, the 2,5'-anhydro derivatives were significantly less toxic.[209] No reports on the antiviral/anticancer activity of 2,3'-anhydro nucleoside derivatives could be found.

7.2.5 1-[(2–Hydroxyethoxy)methyl]-5-trifluoromethansulfonyluridine (82).

Perhaps one of the most important discoveries of recent times, as regards the development of antiviral nucleosides, has been the advent of HEPT and related compounds as highly selective inhibitors of HIV-1 (see Chapter 1). These agents are perhaps indicative of the potential benefits to be gained from combined sugar and base ring modifications. They are also illustrative of the fact that not all significant sugar modifications are most readily derived from the parent nucleoside. The formation of a glycosidic linkage between the base ring and the sugar analogue is important in the development of both acyclic and carbocyclic sugar mimics, amongst others.

The C5 substituted uracils are also of biological interest[210-1] themselves and are often readily incorporated into DNA.[212] Thus, preparation of 5-trifluoromethanesulfonyluracil **110** would allow for the C5 substitution of uracil and for access to other sugar modified nucleosides which are activated towards base ring substitution.

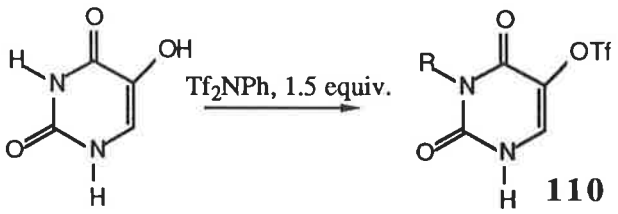
Hirota *et al.*[213] prepared 1,2-dimethyl and 1-methyl-5-trifluoromethane-sulfonyluracil from triflic anhydride and pyridine (eq. 55). They demonstrated the ability of these N-protected C5-trifloxyl uracil derivatives to undergo C5 palladium assisted substitution with activated olefins (Heck reaction) and terminal alkynes. Farina and Hauk[57] have recently reported the application of the Stille reaction to 5-iodouracil.



R = H, 54%
= CH_3, 99%

(55)

In view of the difficulties anticipated in trying to remove N-methyl protecting groups it was considered that 5-hydroxyuracil (isobarbituric acid) should be triflated directly. Under identical conditions used for the preparation of the corresponding uridine triflate the uracil triflate was only obtained in low yield (eq. 56). Some improvement in yield was realized in using pyridine instead of potassium carbonate in a water/1,4-dioxane mix to give **110** in moderate yield (62%).



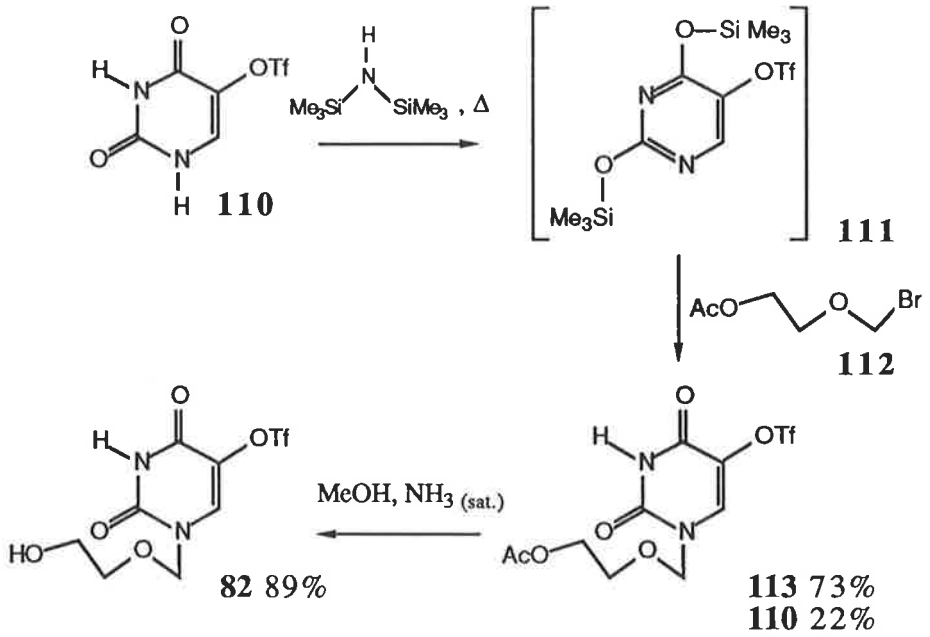
110

(56)

Conditions	Yield%
Tf_2NPh 1.3 equiv., K_2CO_3, 1.5 equiv., 1,4-dioxane/water 3:1.	41
Tf_2NPh 1.6 equiv.	62

The acyclic 5-trifloxyuridine derivative **82** was prepared in a similar manner as that previously described for other acyclic nucleoside analogues.[191] Silylation of **110** to give **111** activates the base ring to *in situ* coupling to the acyclic sugar mimic (2-acetoxyethoxy)methyl bromide **112** to give **113** (Scheme 41). The product was isolated in good yield (73%) with nearly all the remaining starting **110** material being returned (22%). Deacetylation of **113** gave the desired product 1-[(2-hydroxyethoxy)methyl]-5-trifluoromethanesulfonyluracil **82** in an 89% yield.

Scheme 41

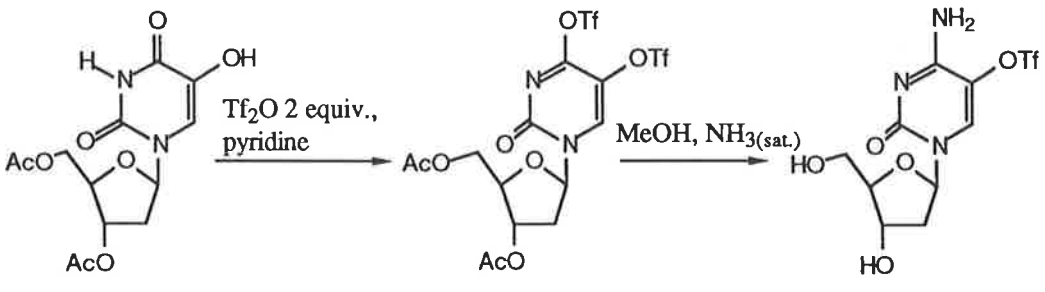


110 **111** **112** **82** 89% **113** 73% **110** 22%

7.3 Other Possibilities

Some of the results generated within this work have lead to the formulation of possible new efficient strategies for base and sugar ring modification. For instance, the highly selective deacetylation of the trisacetate **87** to give **92** could be utilized in a C4 substitution/C5 triflation process. Treatment of **92** with triflic anhydride (2 equiv.) in pyridine would be expected to give the ditriflate **114**. The 4-trifloxyl group should be labile to mild nucleophiles (e.g. H_2S + base, NEt_3 etc.) and in this way give C4 substituted pyrimidine nucleosides or the cytosine derivative **115** activated to C5 substitution can be accessed (Scheme 42).

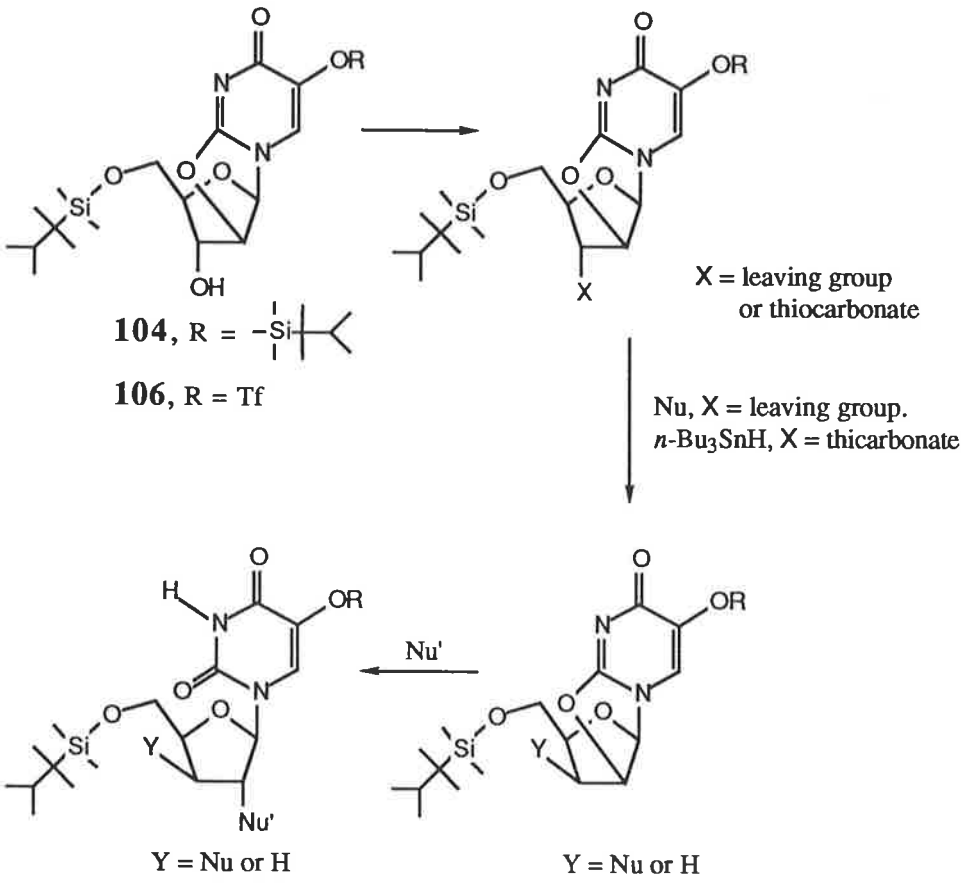
Scheme 42



92 **114** **115**

The accidental formation of the 2,2'-anhydrides **104** and **106** could be utilized in the preparation of 2'α substitution and 3'β substituted or deoxygenated product (Scheme 43).

Scheme 43



104, R = $-Si(t\text{-}Bu)Me_2$

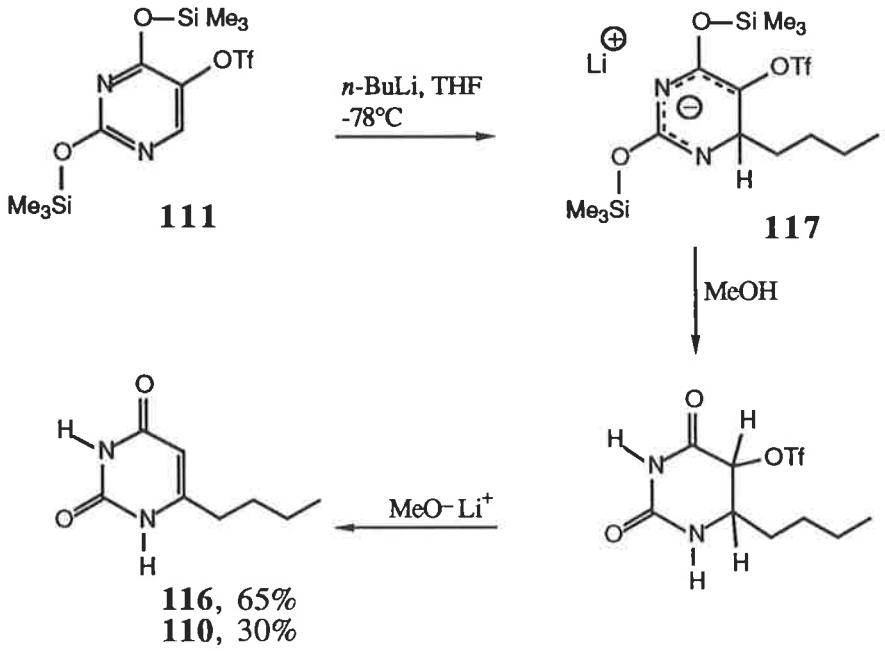
106, R = Tf

Higher yields of **104** and **106** are anticipated for reactions at higher temperatures as its formation is likely to have resulted from the decomposition of the other thiocarbonates isolated (eq. 54).

In contrast with what was observed for the palladium assisted *ipso* (C5) substituion of the uridrine triflates **8, 12** and **14** with organo stannanes and cuprates *cine* (C6) substitution of the uracil triflate **110** was observed when the silyl protected derivative **111** was treated with *n*-butyllithium in THF at –78°C, giving 6-*n*-butyluracil **116** (65%, Scheme 44). Since starting material **110** still persisted (30%) it was concluded that competitive desilylation of **111** by *n*-butyllithium was occurring. *Cine* substitution was evidenced by the presence of a singlet at 5.14ppm, intergrating to 1 hydrogen, in the ^1H NMR; a typical δ value

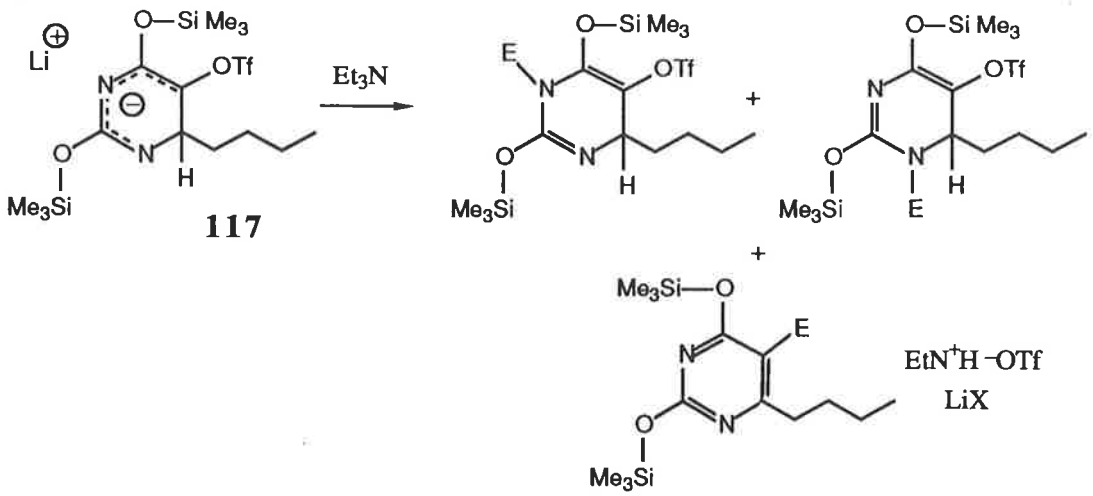
for the C5-H of uracil The C6-H resonance is usually between 7.5 & 8.5ppm and was not observed for **116**.

Scheme 44



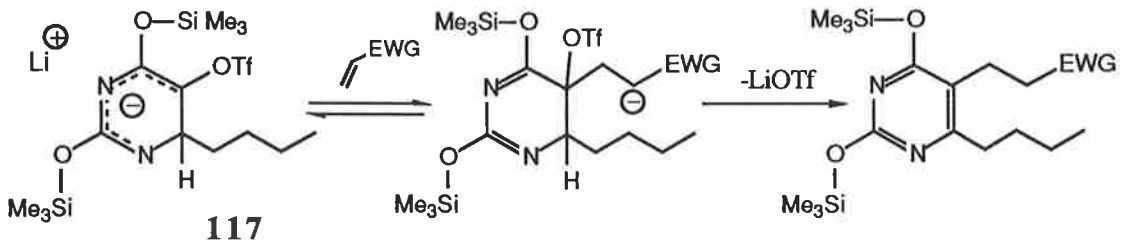
The *cine* substitution of the uracil triflate to give the C6-butyl product presumably involves initial addition of *n*-butyllithium to the C6 centre to give a resonance stabilized N-centered anion **117**. Addition of a protic solvent upon workup allows for C5 protonation and elimination of the triflate to give **116** (Scheme 44). This mechanism is similar to other addition elimination sequences of C5 or C6-halouracils and uridines,[214-5] such as that discussed previously for C5 hydroxylation[77] (see Chapter 2, Scheme 8) and the proposed mechanism for the possible inhibition of thymidylate synthase by a 5-trifloxyl uridylate (Chapter 5, Scheme 25).

Treatment of **117** with an electrophile rather than a proton source may lead to addition at either a N or C centre, the ratio depending upon the nature of the electrophile. Only in the event of C5 alkylation would the triflate be able to eliminate, especially in the presence of acid scavengers (eq. 57).



(57)

Where the electrophile is an activated double bond and undergoes reversible addition to either the C5 or a N centre, only in the event of C5 alkylation is the resulting anion able to deprotonate and eliminate the triflate group to give a stable product (eq. 58). The elimination of the triflate group would then be both the driving force and the regiochemical directing force for the process.

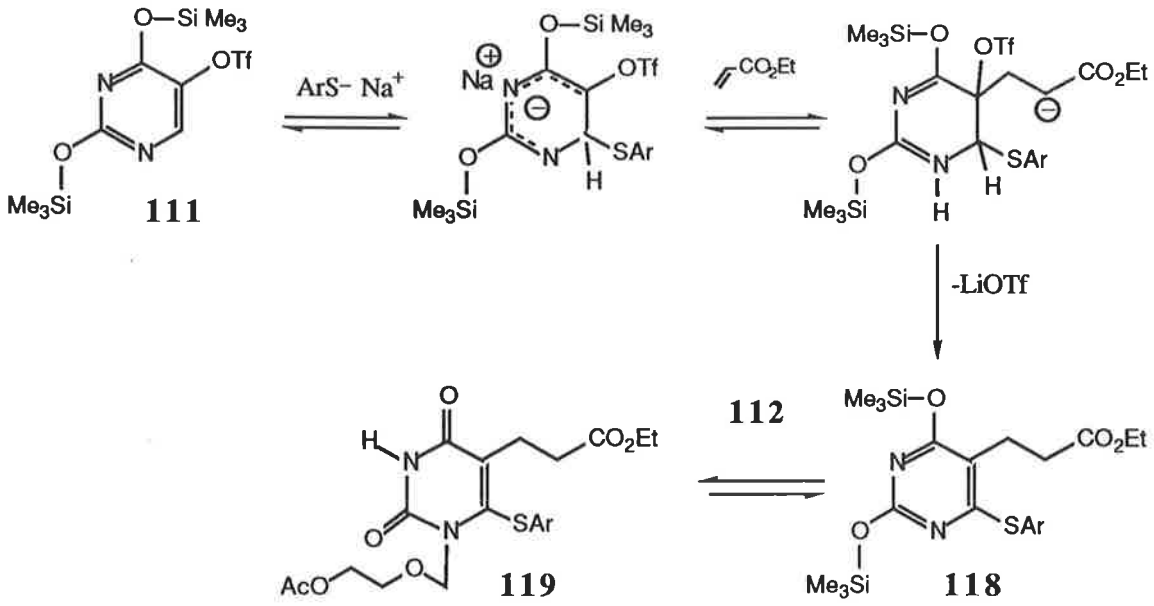


(58)

Such a process could perhaps be extended to where both the nucleophilic and electrophilic additions are reversible and the whole process is driven by the elimination of the triflate to give a disubstituted uracil (e.g. **118**, Scheme 45). Finally treatment of **118** with **112** would give the acetate protected HEPT type derivative **119**. In this way N1, C5 and C6 substitution could be achieved in a single step, providing perhaps an expeditious route to HEPT derivatives.*

* Existing methods for the preparation of HEPT derivatives involves intial N1 alkylation followed by the sequential lithation and electrophilic addition to both the C5 and C6 centres.[20,21]

Scheme 45



7.4 Conclusion.

Convenient routes to a number of sugar modified uridine triflates have been identified. Of the routes intially proposed route 1 seems very limited as sugar modification has a detrimental affect on the yield of C5-hydroxylation consequently route 4 is also less viable. Routes 2&3 have proven most successful, though some limitations of route 3 have been encountered. Route 2 may be the most general. Whilst not all nucleosides analogues targeted were successfully prepared in good yields, likely routes to their preparation have been identified.

Chapter 8

Summary

The preparation of C5-trifloxyl uridine derivatives **7** & **8** was achieved in good yields by selective triflation of the corresponding 5-hydroxyuridine derivatives **9** & **10** (respectively). The sugar protected equivalents of **7** & **8**, i.e. **12** & **14** (respectively) were employed in palladium assisted coupling processes with aryl and alkenyl stannanes and terminal alkynes (copper(I)iodide co-catalyst) giving coupled products in good to excellent yields. In the coupling of the terminal alkynes with **8** it was demonstrated that the presence of free hydroxyl groups only slightly reduces the rate of reaction.

In the coupling of **12** with aryl and alkenyl stannanes an increase in rate was observed with an increase in electron-deficiency of the aryl or alkenyl group. This order of reactivity has been explained in terms of the mechanism proposed for the transmetallation step (Chapter 3). A similar order of reactivity was observed for the coupling of terminal alkynes to **12**, **14** & **8**, though in this case the order of reactivity may also reflect the rate of copper acetylide formation.

In the coupling of **12** with ethyl 2-tri-*n*-butyl-stannylpropenoate (entry 7, Table 1) a significant quantity of the product isolated was the *cine* substitution product **22f** (~50%). The formation of this product was readily explained in terms of the addition/destannylation process intially described by Kikukawa *et al.*[115] The fact that the opposite stereochemistry was obtained to that observed by Kikukawa *et al.* has been explained by proposing a *syn* elimination process involving an intramolecular halogen transfer from the palladium to the tin as opposed to the *anti* elimination process proposed by Kikukawa *et al.* where the halogen transfer occurs in a intermolecular fashion from borontetrafluoride to the tin (Schemes 15 & 16, Chapter 3).

A unique palladium assisted radical chain process has been devised in order to explain the concomitant N-allylation C5-reduction process observed for the attempted palladium assisted coupling of **12** & allyltri-*n*-butylstananne (Scheme 19, Chapter 3). This process is of intrinsic interest as it appears to involve the promotion of nucleophilic radicals to olefins by π-complexation to palladium(II), which may increase the electrophilicity of the double bond and stabilize an alkyl radical intermediate via a Pd^{II} to Pd^{III} transfer. The use transition metals as catalysts in radical reactions is worthy of further investigation.

Several 5-phenylethynyl-2'-deoxyuridine derivatives of potential biological interest have been prepared **41b-f**. The preparation, and potential antiherpetic activity of these compounds has been discussed in terms of the observed effects of the various phenyl substituents. Whilst these compounds have proved to be inactive against the AIDS viri HIV 1 & 2 and as anticancer agents this can perhaps be explained by an inability of these compounds to be phoshporylated within the cell (Chapter 5). It is hoped that the monophosphate derivatives of **41b-f** may act as inhibitors of thymidylate synthase. It is also anticipated that such compounds maybe useful as mechanistic probes in determining the likely mechanism of thymidylate synthase inhibition.

In the coupling of electron-deficient phenylacetylenes (PAs) to **14** & **8** considerable amounts of homocoupled PA was observed as a by-product. This appears to involve the intial addition of two terminal alkynes to the $Pd(PPh_3)_4$ to form a bisacetylidepalladium(II) complex which may form a metal cluster **70**, with the copper acetylide present, from which reductive elimination is promoted.

Modification of the nucleoside sugar ring has been of enormous importance in the development of antiviral and anticancer nucleoside

analogues. Expeditious routes to a number of sugar modified C5-trifloxyl uridine derivatives have been identified. Also a number of important discoveries were made in connection with these investigations which could be utilized in the development of new approaches to the elaboration of the sugar and base ring of pyrimidine nucleosides.

Thus, 5-trifluoromethanesulfonyluridine **7** and 5-trifluoromethanesulfonyl-2'-deoxyuridine **8** have been shown to be accessible and highly efficient precursors to the palladium assisted C5-substitution of uridine derivatives. These C5-trifloxyl derivatives and their C5-hydroxyl precursors have also proven to be suitable substrates to a number of important sugar manipulations.

Experimental

Melting points were recorded on a Reichhert hot stage apparatus and are uncorrected. Proton and carbon NMR spectra were recorded on a Brüker CXP-300, a Brüker ACP-300 or a Varian WP-80 spectrometer. $CDCl_3$ was used as a solvent unless otherwise stated. Electron Impact mass spectra were recorded on a AEI-GEC MS 3074 machine at 70eV and Fast Atom Bombardment mass spectra on a VG ZAB 2HF mass spectrometer. Optical rotations were measured on a Perkin Elmer 141 polarimeter. Infrared spectra were recorded on a Hitachi 270-30 spectrophotometer. Ultraviolet spectra were recorded on a PYE UNICAM SP 8-100 spectropotometer. Gas-liquid-chromatography was performed on a DANI 8510 Chromatograph.

1,4-Dioxane was freshly distilled from potassium under nitrogen. Tetrahydrofuran was freshly distilled from sodium under nitrogen. Dimethylformamide (DMF) was distilled from calcium hydride (~80°C, ~20mmHg) and stored over 4Å molecular sieves. Acetonitrile and hexamethyldisilazane were refluxed over and then distilled from calcium hydride under nitrogen and stored over 4Å molecular sieves. Analytical thin-layer-chromatograhy was carried out on Merck aluminium sheets precoated with Kieselgel 60 F_{254} and visualized using a 254nm lamp. Column chromatography was carried out using Kieselgel 60 (230-400 mesh).

The following compounds were synthesized according to literature procedures: 5-hydroxy-2'-deoxyuridine,[78] tri-*n*-butylethenylstannane,[216] *E*-1-trimethyl-silyl-2-tri-*n*-butylstannylethene,[217] *E*-tri-*n*-butyl(2-phenylethenyl) stannane,[127] ethyl *E*-3-tri-*n*-butylstannylpropenoate,[218] ethyl 2-tri-*n*-butyl-stannyl-propenoate,[218] 2-trimethylstannylpropene,[219] dimethyl-2,3-dimethylbutyl[(3-tri-*n*-butylstannyl)-2-propenyl)oxy] silane,[127] trimethylphenylstannane,[220] 4-(trimethylstannyl) anisole,[221] 4-(trimethylstannyl)fluorobenzene,[222] 4-(trimethyl-

stannyl)-α,α,α-trifluoromethyl-benzene,[223] 5-(trimethylstannyl)-1,3-difluoro-benzene,[222] 5-(trimethylstannyl)-1,3-bis(α,α,α-trifluoromethyl)benzene,[222] tetrakis(triphenylphosphine)palladium,[224] dichlorobis(triphenylphosphine)-palladium,[225] zinc/copper couple,[226] 1,3-dimethyl-2-phenyl-1,3-diaza-phospholidine (**100**),[184] 5-hydroxy-2'- deoxyuridine (**10**),[78] ,3'-dideoxydide-hydrouridine (ddU),[195] (2-acetoxyethoxy)methyl bromide (**112**),[191] N-phenyltriflimide.[188]

The following abbreviations have been used in defining peak shape for the various spectra: ^{1}H & ^{13}C NMR; br = broad, d = doublet, m = multiplet, q = quartet, s = singlet, t = triplet. IR & UV; br = broad, m = medium, w = weak, s = sharp, sh = shoulder, str = strong. Unless otherwise stated J = ^{3}J for ^{1}H NMR and ^{1}J for ^{13}C NMR.

Chapter 2:

5-Hydroxyuridine (9).

To a stirring solution of uridine (10g, 41.7mmol) in water (150ml) bromine was added until an amber colour persisted. Excess bromine was removed by bubbling nitrogen through the solution until it became completely clear. Pyridine (20ml) was added to the solution and the reaction mixture stirred at 30°C overnight. The solution was concentrated to give a honey like syrup which was azeotroped with ethanol (3 x 150ml) and triturated in boiling ethanol (50ml) to form a white precipitate. The suspension was allowed to cool to room temperature and filtered and washed with cold ethanol (20ml) to give **9** (8.2g, 77%) as a white solid m.p. = 236-238°C (lit.[77] = 238-240°C, rapid heating).

5-Trifluoromethanesulfonyluridine (7).

N-phenyltriflimide (4.78g, 13.38mmol) was added to a solution of 5-hydroxyuridine **9** (2.90g, 11.15mmol) and K_2CO_3 (1.69g, 12.26mmol) in water/1,4-dioxane (1:4, 200ml). The intially cloudy solution became clear after stirring at

room temperature overnight. Analytical t.l.c. ($CHCl_3$/methanol 3:1) revealed that all the 5-hydroxyuridine had been consumed. The solution was evaporated to dryness under vacuum at room temperature, giving a white foam. Silica (~10g) and methanol (50ml) were added to the residue and the resulting slurry evaporated under vacuum to give a free flowing powder which was subjected to flash chromatography through a short column of t.l.c. silica (6.5cm wide, 3.5 cm high). The column was eluted sequentially with hexanes/ethyl acetate 1:1 (200ml), ethyl acetate (200ml), ethyl acetate /methanol 9:1 (200ml), ethyl acetate /methanol 8:2 (500ml). 5-Trifluoromethanesulfonyluridine **7** (3.98g, 91%) was obtained as a white solid; m.p. = 177-80 °C. ^{1}H NMR ($DMSOd_6$) δ 11.02 (br s, 1 H, NH), 8.20 (s, 1 H, H-6), 5.32 (d, $J_{H\text{-}1',2'}$ = 3.8 Hz, 1 H, H-1'), 4.63 (d, J = 5.1 Hz, 1 H, C2' or 3'-OH), 4.53 (t, J = 4.4 Hz, 1 H, C5,-OH), 4.11 (d, J = 5.2 Hz, 1 H, C2' or 3'-OH), 3.55 (m, 2 H, H-2',3'), 3.47 (m, 1 H, H-4'), 3.29-3.13 (ABMX, J_{AB} = 12.2 Hz, J_{AB} ~ 1 Hz, J_{BM} ~ 2.5 Hz, $J_{AX} = J_{BX}$ = 4.4 Hz, 2 H, H-5'). ^{13}C NMR (20.1 MHz) δ 158.1 (C4), 149.5 (C2), 135.2 (C6), 126.8 (C5), 89.5 (C1'), 85.1 (C4'), 74.8(C3'), 69.4 (C2'), 60.1 (C5'). FABMS m/z 393 (M^++H, <5%). IR (KBr) ν_{max} 3412 cm^{-1} (br str, OH), 2952 (str, C-H), 1710 (br str, C=O).

2',3',5'-Tri-*O*-acetyl-5-trifluoromethanesulfonyluridine (12).

5-Hydroxyuridine **9** (0.838g, 3.22mmol) was treated with N-phenyltriflimide (1.38g, 3.87mmol) and K_2CO_3 (0.433g, 3.38mmol) in a similar manner as that described for the preparation of **7** above, except that, after evaporation of the reaction mixture to give crude **7** as a white foam, pyridine 20ml and acetic anhydride 2.2ml (2.4 g, 23.5mmol) were added and the clear pale yellow solution was stirred at room temperature for 20hrs under nitrogen. The solution was evaporated under vacuum at room temperature and the residue taken up in ethyl acetate in (40ml), washed twice with 10% aqueous citric acid (30ml) and saturated NaCl(aq.) 30ml, dried over $MgSO_4$ and evaporated under vacuum. The resultant honey like residue (2.8g) was chromatographed (5cm flash column hexanes/ethyl acetate, 1:1) to give **12** as a white solid upon removal

of all traces of solvent (1.63g, 97%), m.p. = 63-4°C. α_D = -12.11 ($CHCl_3$, c = 1.04). ^1H NMR δ 7.76 (s , 1 H, H-6), 5.98 (d, J = 5.1 Hz, 1 H, H-1'), 5.27 (dd, $J_{1'2'}$ = 5 Hz, $J_{2'3'}$ = 5.0 Hz, 1 H, H-2'), 5.23 (dd, $J_{4'3'}$ = 5.0 Hz, $J_{2'3'}$ = 5.0 Hz 1 H, H-3'), 4.39-4.23 (m, 3 H, H-4',5'), 2.09, 2.05 & 2.02 (3 x s, 9 H, 3 x CH_3CO_2). ^{13}C NMR (20.1 MHz) δ 170.5 & 169.9 (3 x $CH_3\underline{C}O_2$), 156.9 (C4), 149.2 (C2), 133.6 (C6), 127.2 (C5), 116.5 (q, J_{CF} = 322 Hz, CF_3), 87.9 (C1'), 83.0 (C4'), 73.0 (C2'), 69.8 (C3'), 62.9 (C5'), 20.0 (3 x $\underline{C}H_3CO_2$). FABMS m/z 519 (M^++H, <5%). IR ($CHCl_3$) ν_{max} 3525 cm^{-1} (m, NH), 3016 (m, CH), 1750 (str br, C=O), 1658 (w, C=O), 1238 (m, SO_3), 1136 (str, SO_3). UV ($CHCl_3$) λ_{max} 265.5 nm (ε 7,402). Anal found: C, 36.97; H, 4.37; N, 5.29. Calcd for $C_{16}H_{17}N_2O_{12}SF_3$: C, 37.07; H, 3.31; N, 5.40.

2',3'-Tri-*O*-acetyl-5'-*O*-methyl-5-trifluoromethanesulfonyluridine (11) and 2',3',5'-Tri-*O*-acetyl-5-trifluoromethanesulfonyluridine (12).

5-Hydroxyuridine **9** (270mg, 1.09mmol) was treated in the same manner as described for **12** above, except using methanol in place the 1,4-dioxane/water mix. Two products were isolated after chromatography as solid foams (**11,** 160mg, 30%; **12**, 338mg, 60%) **11**: ^1H NMR δ 7.78 (s , 1 H, H-6), 6.02 (d, J = 5.1 Hz, 1 H, H-1'), 5.31 (m, 2 H, H-2',3'), 4.36 (m, 3 H, H-4',5'), 3.34 (s, 3H, OCH_3), 2.12 & 2.09 (2 x s, 2 x 3 H, 2 x CH_3CO_2). ^{13}C NMR (20.1 MHz) δ 169.7 & 168.9 (3 x $CH_3\underline{C}O_2$), 157.4 (C4), 149.1 (C2), 134.1 (C6), 126.9 (C5), 116.7 (q, J_{CF} = 322 Hz, CF_3), 88.1 (C1'), 83.2 (C4'), 73.5 (C2'), 69.6 (C3'), 59.0 (C5'), 38.7 (OCH_3), 20.0 (3 x $\underline{C}H_3CO_2$). FABMS m/z 491 (M^++H, <5%). IR ($CHCl_3$) ν_{max} 3527 cm^{-1} (m, NH), 3008 (m, CH), 1756 (str br, C=O), 1650 (w, C=C), 1240 (m, SO_3), 1136 (str, SO_3).

5-Trifluoromethanesulfonyl-2'-deoxyuridine (8).

Method A (Step b, Scheme 11): Potassium carbonate (0.7g, 5.0mmol) was added to a suspension of 5-hydroxy-2'-deoxyuridine **10** (1.12g, 4.6mmol) in water (12ml) to give a clear, orange solution. 1,4-Dioxane (26ml) followed by a solution of N-phenyltriflimide (2.1g, 5.9mmol) in 1,4-dioxane (10ml) were added and the solution left to stir overnight. The reaction mixture was evaporated and the

residue purified by flash chromatography hexanes 60% : ethyl acetate 35% : methanol 5%) to give **8** (1.7g, 98%) as a white solid.

Method B (Step a + b Scheme 11): Bromine was added slowly to a rapidly stirring solution of 2'-deoxyuridine (4.5g, 19.7mmol) in water until a brown colour just persists. Excess bromine was removed by bubbling nitrogen through the solution until it was completely clear. Pyridine (100ml) was then added to the solution and the resulting reaction mixture allowed to stand overnight. The solution was evaporated to give a honey coloured syrup, after several azeotropes with ethanol the residue solidified to give crude 5-hydroxy-2'-deoxyuridine **10** as a tan solid.

Water (40ml) was added to the crude **10** to give a tan slurry, subsequent addition of K_2CO_3 (6g, 434mmol) gave a clear, dark red solution. 1,4-Dioxane (60ml) and a solution of N-phenyltriflimide (8.5g, 23.7mmol) in 1,4-dioxane (60ml) were added giving a white suspension in a dark red solution. After stirring overnight the reaction mixture was light brown and clear.

The solution was neutralized by addition of citric acid and the solvent removed. The resultant light brown foam was dissolved in methanol (80ml) and filtered to remove insoluble salts. Silica (~3-5g) was added to the filtrate and the slurry evaporated to dryness and subjected to flash chromatography [hexanes : ethyl acetate : methanol 60 : 38:2 (500ml) & 60:35:5 (500ml), through a 4.5cm x 16cm column of silica, flow rate of 15ml/min] all fractions containing **8** were combined and evaporated to give only a slightly impure product (4.3g, 58% from 2'-deoxyuridine, containing some 5-bromo-2'-deoxyuridine, of higher R_f by t.l.c.). Recrystalization from isopropanol (~10-15ml) gives **8** as a highly pure white crystaline solid (3.8g, 52% from 2'-deoxyuridine).

Method C (Chapter 7, Scheme 31): Saturated methanolic ammonia (20ml) was added to **87** (2.1g, 5.7mmol) and the solution allowed to stand overnight. The reaction mixture was then concentrated and treated in a similar manner as that described for **10** in Method A (assuming 100% product formation in this step). The product, **8**, was obtained as a white solid after flash chromatography (2.0g, 94%); m.p. = 185-6°C. α_D = + 27.3 (MeOH, c = 0.48). ^{1}H NMR ($CDCl_3$/$DMSOd_6$)δ 11.47 (br s, 1 H, NH), 8.54 (s , 1 H, H-6), 6.10 (t, J = 6.10 Hz, 1 H, H-1'), 4.69 (br d, J = 4.11 Hz, 1 H, 3'-OH), 4.47(br t, J = 4.20 Hz, 1 H, 5'-OH), 4.26 (m, 1 H, H-3'), 3.80 (m, 1 H, H-4'), 3.62 (m, 2 H, H-5'), 2.22 (m, 1 H, H-2'$_\beta$), 2.03 (m, 1 H, H-2'$_\alpha$). FABMS m/z 377 (M^++H, 5%), 260 (20), 117 (100). IR (nujoll mull) ν_{max} 3628 cm^{-1} (m, NH), 3456 (br, OH), 1728 (str br, C=O), 1684 (str, C=O),1239 (m, SO_3). UV (MeOH) λ_{max} 269.0 nm (ε 1,100), 207.5 (1,002). Found: C, 31.85; H, 2.86; N, 7.32. Calcd for $C_{16}H_{11}N_2SO_8F_3$: C, 31.92; H, 2.95; N, 7.44.

3', 5'-Di-*O*-acetyl-5-trifluoromethanesulfonyl-2'-deoxyuridine (14).

Method A' (Step c, Scheme 11):

Acetic anhydride (4ml, 40mmol) was added to a solution of **8** (2.3g, 6.1mmol) in pyridine (20ml) and allowed to stand overnight. The solution was concentrated and dissolved in ethyl acetate (30ml), washed with 10% citric acid (aq., 30ml) and water (2 x 20ml) and dried over $MgSO_4$. The solution was evaporated onto silica (~2g) and subjected to flash chromatography (hexanes/ethyl acetate 3:2) giving **14** (2.8g, 100%) as a solid foam.

Method B' (Step a+b+c, Scheme 11): 2'-Deoxyuridine (2.0g, 8.8m mol) was treated as in the preparation of **8** Method B except that after filtration to remove insoluble salts the filtrate was evaporated and treated as in Method A' assuming 100% conversion in Method B. The product **14** (2.2g, 55%) was again obtained as a solid foam; α_D = + 4.17 (MeOH, c = 0.48). ^{1}H NMR δ 10.13 (br s, 1 H, NH), 7.84 (s , 1 H, H-6), 6.17 (dd, J_{trans} = 8.10 Hz, J_{cis} = 5.4 Hz, 1 H, H-1'), 5.15 (m, 1 H, H-3'), 4.40-4.18 (m, 3 H, H-4',5'), 2.59 (m, 1 H, H-2'$_\beta$), 2.11 (m, 1 H, H-2'$_\alpha$), 2.05 & 2.04 (2 x s,

6H, 2 x CH_3CO_2). ^{13}C NMR δ 170.3 (CH_3CO_2), 156.8 (C4), 148.8 (C2), 133.2 (C6), 126.8 (C5), 116.3 (q, J_{CF} = 322 Hz, CF_3), 86.1 (C1'), 83.0 (C4'), 74.0 (C3'), 63.6 (C5'), 38.1 (C2'), 20.6 & 20.4 (2 x $\underline{C}H_3CO_2$). EIMS m/z 461 (M^++H, <5%), 401 (3), 201 (100). IR ($CHCl_3$) ν_{max} 3400 cm^{-1} (m, NH), 3016 (m, CH), 1734 (str br, C=O), 1661 (w, C=O), 1240 (m, SO_3), 1198 (str, SO_3). UV ($CHCl_3$) λ_{max} 268 nm (ε 1,785). HREIMS found: 461.0491. Calcd for $C_{14}H_{16}N_2O_{10}SF_3$: 461.0477.

Chapter 3:

2',3',5'-Tri-*O*-Acetyl-5-ethenyluridine (22a).

Tri-*n*-butylethenylstannane (73mg, 0.23mmol) and 2,5-di-*t*-butyl-*p*-cresol (~1mg) were added to a solution of 2',3',5'-tri-*O*-acetyl-5-trifluoromethane-sulfonyluridine **12** (102mg, 0.19 mmol), $Pd(Ph_3)_4$ (11mg, 0.01 mmol) and lithium chloride (37mg, 0.87mmol) in 1,4-dioxane 4ml under nitrogen. The clear pale orange solution was heated to reflux. After 4hrs the reaction mixture turned black and analytical t.l.c. (hexanes/ethyl acetate, 1:1) revealed that all **12** had been consumed. The reaction mixture was filtered through celite and evaporated to dryness under vacuum. The residue was taken up in dichloromethane 25ml and washed with 30% aqueous potassium fluoride and water, dried over $MgSO_4$ and evaporated under vacuum. The residue was chromatographed (hexanes/ethyl acetate, 4:3) and gave 2',3',5'-tri-*O*-acetyl-5-ethenyluridine **22a** as a white foam (67.6mg, 87%). α_D = - 21.74 (CH_2Cl_2, c = 0.68). ^1H NMR δ 9.55 (br s, 1 H, NH) 7.39 (s , 1 H, H-6), 6.34 (X of ABX, J_{XA} = 17.6 Hz, J_{XB} = 11.3 Hz, 1 H, $C\underline{H_X}$=CH_AH_B), 6.05 (d, J = 5.0 Hz, 1 H, H-1'), 5.88 (A of ABX, J_{AX} =17.6 Hz, J_{AB} = 1.1 Hz, 1 H, CH_X=$C\underline{H_A}H_B$), 5.30 (m, 2 H, H-2'&3'), 5.20 (B of ABX, J_{BX} = 11.3 Hz, J_{BA} = 1.1 Hz, 1 H, CH_X=$CH_A\underline{H_B}$), 4.31 (m, 3 H, H-4',5'), 2.08, 2.07 & 2.03 (3 x s, 9 H, 3 x CH_3CO_2). ^{13}C NMR (20.1 MHz) δ 170.3 & 169.9 (3 x $CH_3\underline{C}O_2$), 161.9 (C4), 149.9 (C2), 135.6 (C6), 127.7 ($\underline{C}H$=CH_2), 116.6 (CH=$\underline{C}H_2$), 113.7 (C5), 87.2 (C1'), 80.0 (C4'), 72.8 (C2'), 70.2 (C3'), 63.2 (C5'), 20.6 & 20.3 (3 x $\underline{C}H_3CO_2$). FABMS m/z 397 (M^++H, <5%). IR ($CHCl_3$) ν_{max} 3400 cm^{-1} (m, NH), 3012 (m, CH), 1750 (str br, C=O), 1704 (str, C=O).

UV ($CHCl_3$) λ_{max} 278 nm (ε 5,269), 248.8 (5,475). HREIMS found: 368.0878. Calcd for $C_{15}H_{16}N_2O_9$ (M^+-C_2H_4): 368.0856.

Compounds **22b-g** and **23a-f** were prepared in a similar manner as that described for **22a** above, varying only in the reaction time and temperature and the product yield obtained as detailed in Tables 1 and 2 (Chapter 3).

2',3',5'-Tri-*O*-Acetyl-5-[*E*-2-(trimethylsilyl)ethenyl]uridine (22b).

73%. m.p. = 48-50 °C. α_D = - 51.62 (CH_2Cl_2, c = 0.66). ^1H NMR δ 9.21 (br s, 1 H, NH) 7.49 (s , 1 H, H-6), 6.67 (d, J = 16.23 Hz, 1 H, C<u>H</u>=CHSiMe$_3$), 6.52 (d, J = 16.2 Hz, 1 H, CH=C<u>H</u>SiMe$_3$) 6.14 (d, J = 5.1 Hz, 1 H, H-1'), 5.37 (m, 2 H, H-2'&3'), 4.39 (m, 3 H, H-4',5'), 2.15, 2.14 & 2.11 (3 x s, 9 H, 3 x CH_3CO_2), 0.12 (s, 9 H, SiMe$_3$). ^{13}C NMR (20.1 MHz) δ 170.0 & 169.5 (3 x $CH_3\underline{C}O_2$), 162.0 (C4), 149.7 (C2), 135.3 (C6), 133.6 (CH=<u>C</u>HSiMe$_3$) 131.7 (<u>C</u>H=CHSiMe$_3$), 114.3 (C5), 86.9 (C1'), 79.9 (C4'), 72.5 (C2'), 70.0 (C3'), 63.0 (C5'), 20.4 & 20.0 (3 x $\underline{C}H_3CO_2$), 1.72 (SiMe$_3$). EIMS m/z 468 (M^+, <5%), 453 (M^+-CH_3, 100). IR ($CHCl_3$) ν_{max} 3396 cm^{-1} (m, NH), 3028 (m, CH), 2956 (m, CH), 1750 (str br, C=O), 1714 (w, C=O). UV ($CHCl_3$) λ_{max} 294 nm (ε 9,026), 244 (11,919). HREIMS found: 468.1563. Calcd for $C_{20}H_{28}N_2O_9Si$: 468.1584.

2',3',5'-Tri-*O*-acetyl-5-(*E*-2-phenylethenyl)uridine (22c).

75%. m.p. = 79-80 °C. α_D = - 66.73 (CH_2Cl_2, c = 0.50). ^1H NMR δ 9.47 (br s, 1 H, NH) 7.48 (s , 1 H, H-6), 7.38-7.15 (m, 6 H, ArH & C=C<u>H</u>Ph), 6.73 (d, J = 16.4 Hz, 1 H, C<u>H</u>=CHPh), 6.07 (d, J = 5.2 Hz, 1 H, H-1'), 5.33-5.29 (m, 2 H, H-2'&3'), 4.35-4.29 (m, 3 H, H-4',5'), 2.07 & 2.04 (2 x s, 9 H, 3 x CH_3CO_2). EIMS m/z 472 (M^+, <5%). IR ($CHCl_3$) ν_{max} 3395 cm^{-1} (m, NH), 3040 (m, ArH), 2952 (w, CH), 1748 (str br, C=O), 1714 (w, C=O), 1605 (w, C=C). UV ($CHCl_3$) λ_{max} 314 nm (ε 9,244), 259 (9,702). HREIMS found: 472.1480. Calcd for $C_{23}H_{24}N_2O_9$: 472.1481.

2',3',5'-Tri-*O*-acetyl-5-{1-*E*-[3-(dimethyl-2,3-dimetylbutyl)silyloxy]propenyl}uridine (22d).

92%. α_D = - 33.85 (CH_2Cl_2, c = 0.91). ^{1}H NMR δ 9.07 (br s, 1 H, NH) 7.25 (s , 1 H, H-6), 6.52 (dt, J_d = 15.8 Hz, J_t = 4.4 Hz, 1 H, CH=C<u>H</u>CH_2O), 6.16 (bd, J = 15.8 Hz, 1 H, CH=C<u>H</u>CH_2O), 5.98 (d, J = 5.4 Hz, 1 H, H-1'), 5.23 (m, 2 H, H-2'&3'), 4.23 (m, 3 H, H-4',5'), 4.02 (bs, 2 H, CH=CHC<u>H$_2$</u>O), 2.02, 1.99 & 1.97 (3 x s, 9 H, 3 x CH_3CO_2), 1.52-0.75 (19 H, $SiMe_2CMe_2CHMe_2$). IR ($CHCl_3$) ν_{max} 3390 cm^{-1} (m, NH), 3030 (m, CH), 2960 (m, CH), 1748 (str br, C=O), 1714 (w, C=O). UV ($CHCl_3$) λ_{max} 290 nm (ε 7.258), 243 (8,210).

2',3',5'-Tri-*O*-acetyl-5-(2-propenyl)uridine (22e).

86%. α_D = - 33.70 (CH_2Cl_2, c = 0.61). ^{1}H NMR δ 9.23 (br s, 1 H, NH) 7.34 (s , 1 H, H-6), 6.12 (d, J = 5.0 Hz, 1 H, H-1'), 5.60 (d, J = 1.0 Hz, 1 H, MeC=C<u>H</u>), 5.36 (m, 2 H, H-2'&3'), 5.15 (d, J = 1.0 Hz, 1 H, MeC=C<u>H</u>), 4.36 (m, 3 H, H-4',5'), 2.13, 2.11 & 2.08 (3 x s, 9 H, 3 x CH_3CO_2), 1.99 (s, 3 H, C<u>H$_3$</u>C=CH_2). EIMS m/z 410 (M^+, 5%), 350 (12), 259 (57), 97 (100). IR ($CHCl_3$) ν_{max} 3396 cm^{-1} (m, NH), 3020 (m, CH), 1750 (str br, C=O), 1704 (w, C=O). UV ($CHCl_3$) λ_{max} 273 nm (ε 6,223), 234 (4,407). HREIMS found: 368.0878. Calcd for $C_{15}H_{16}N_2O_9$ (M^+-C_2H_4): 368.0856.

2',3',5'-Tri-*O*-acetyl-5-(ethyl-1-*E*-propenoate)uridine (22f).

92%. m.p. = 59-60 °C. α_D = - 61.50 (CH_2Cl_2, c = 0.61). ^{1}H NMR δ 9.68 (br s, 1 H, NH) 7.68 (s , 1 H, H-6), 7.20 (d J = 15.8 Hz, 1 H, C<u>H</u>=CHCO_2Et), 6.89 (d, J = 15.8 Hz, 1 H, CH=C<u>H</u>CO_2Et), 6.12 (d, J = 5.0 Hz, 1 H, H-1'), 5.36-5.25 (m, 2 H, H-2'&3'), 4.39-4.27 (m, 3 H, H-4',5'), 4.14 (q, J = 7.1 Hz, 2 H, CO_2C<u>H$_2$</u>CH_3), 2.12, 2.06 & 2.04 (3 x s, 9 H, 3 x CH_3CO_2),1.23 (t, J = 7.1 Hz, 3 H, CO_2CH_2C<u>H$_3$</u>). EIMS m/z 468 (M^+, 5%), 350 (12), 259 (57), 97 (100). IR ($CHCl_3$) ν_{max} 3392 cm^{-1} (m, NH), 3028 (m, CH), 1750 (str br, C=O), 1710 (w, C=O), 1464 (str, C=C). UV ($CHCl_3$) λ_{max} 298 nm (ε 7,449), 238 (5,273). HREIMS found: 468.1395. Calcd for $C_{20}H_{24}N_2O_{11}$: 468.1380.

2',3',5'-Tri-*O*-acetyl-5-(ethyl-2-propenoate)uridine (22g).

46%. α_D = - 33.08 (CH_2Cl_2, c = 0.13). ^1H NMR δ 8.35 (br s, 1 H, NH) 7.71 (s , 1 H, H-6), 6.43 (d, J = 0.9 Hz, 1 H, $EtO_2CC=C\underline{H}$), 6.15 (d, J = 0.9 Hz, 1 H, $EtO_2CC=C\underline{H}$), 6.04 (d, J = 5.0 Hz, 1 H, H-1'), 5.30 (m, 2 H, H-2'&3'), 4.28 (m, 3 H, H-4',5'), 4.17 (q, J = 7.1 Hz, 2 H, $CO_2C\underline{H}_2CH_3$), 2.07, 2.04 & 2.03 (3 x s, 9 H, 3 x CH_3CO_2), 1.24 (t, J = 7.1 Hz, 3 H, $CO_2CH_2C\underline{H}_3$). FABMS m/z 469 (M^++H, <5%), 259 (70). IR ($CHCl_3$) ν_{max} 3390 cm^{-1} (m, NH), 3090 (m, CH), 1750 (str br, C=O), 1722 (w, C=O). UV ($CHCl_3$) λ_{max} 271.0 nm (ε 8,388).

22f (42%) was also isolated (see above for physical data).

2',3',5'-Tri-*O*-acetyl-5-phenyluridine (23a).

64%. m.p. = 66-7 °C. α_D = -80.82 (CH_2Cl_2, c = 0.48). ^1H NMR δ 9.42 (br s, 1 H, NH) 7.49 (s , 1 H, H-6), 7.45-7.36 (m, 2 H, ArH), 7.34-7.28 (m, 3 H, ArH), 6.11 (d, J = 5.6 Hz, 1 H, H-1'), 5.35 (dd, J = 2 x 5.6, 1 H, H-2'), 5.28 (m, 1 H, H-3'), 4.29 (br s, 3 H, H-4',5'), 2.06 & 2.04 (2 x s, 6 H, CH_3CO_2C-2'&3'), 1.62 (s, 3 H, CH_3CO_2C-5'). ^{13}C NMR (20.1 MHz) δ 170.0 & 169.6 (3 x $CH_3\underline{C}O_2$), 161.9 (C4), 150.9 (C2), 136.4 (C6), 134.0 (ArC), 128.6 (ArC), 128.1 (ArC), 116.2 (C5), 86.9 (C1'), 80.0 (C4'), 72.5 (C2'), 70.0 (C3'), 62.9 (C5'), 20.1 (3 x $\underline{C}H_3CO_2$) EIMS m/z 446 (M^+, <5%). IR ($CHCl_3$) ν_{max} 3395 cm^{-1} (m, NH), 3032 (m, ArH), 1750 (str br, C=O), 1718 (str, C=O), 1694 (w, C=O). UV ($CHCl_3$) λ_{max} 282.8 nm (ε 9,518), 241.9 (9,508). Anal found: C, 56.16; H, 5.17; N, 5.99. Calcd for $C_{21}H_{22}N_2O_9$: C,56.50; H, 4.97; N, 6.27.

2',3',5'-Tri-*O*-acetyl-5-(4-methoxyphenyl)uridine (23b).

55%. m.p. = 65-7 °C. α_D = -66.66 (CH_2Cl_2, c = 0.27). ^1H NMR δ 8.98 (br s, 1 H, NH) 7.42 (s , 1 H, H-6), 7.36 (d, J = 9 Hz, 2 H, ArH), 6.65 (d, J = 9 Hz, 2 H, ArH), 6.11 (d, J = 5.7 Hz, 1 H, H-1'), 5.34 (dd, J = 2 x 5.8, 1 H, H-2'), 5.28 (m, 1 H, H-3'), 4.24 (br s, 3 H, H-4',5'), 3.75 (s, 3 H, OCH_3), 2.07 & 2.06 (2 x s, 6 H, CH_3CO_2C-2'&3'), 1.86 (s, 3 H, CH_3CO_2C-5'). EIMS m/z 476 (M^+, <5%). IR ($CHCl_3$) ν_{max} 3405 cm^{-1} (m, NH), 3040 (m, ArH), 2940 (m, CH) 1780 (str, C=O), 1750 (str, C=O), 1710 (str, C=O), 1612

(m, C=C). UV (CH_2Cl_2) λ_{max} 282.8 nm (ε 9,518), 241.9 (9,508). HREIMS found: 476.1417. Calcd for $C_{22}H_{24}N_2O_{10}$: 476.1430.

2',3',5'-Tri-*O*-acetyl-5-(4-fluorophenyl)uridine (23c).

89%. m.p. = 69-70 °C. α_D = -56.98 ($CHCl_3$, c = 0.70). ^1H NMR δ 9.49 (br s, 1 H, NH) 7.53 (s , 1 H, H-6), 7.47 (dd, J = 8.6 & 5.4 Hz, 2 H, ArH), 7.08 (dd, 2 x J = 8.6 Hz, 2 H, ArH), 6.15 (d, J = 5.6 Hz, 1 H, H-1'), 5.42 (dd, J = 2 x 5.8 Hz, 1 H, H-2'), 5.33 (m, 1 H, H-3'), 4.37 (br s, 3 H, H-4',5'), 2.14 & 2.12 (2 x s, 6 H, CH_3CO_2C-2'&3'), 1.92 (s, 3 H, CH_3CO_2C-5'). ^{19}F NMR (282.4 MHz, $CDCl_3$) δ 36.42 (s, ArF). EIMS m/z 464 (M^+, <5%). IR ($CHCl_3$) ν_{max} 3400 cm^{-1} (m, NH), 3030 (m, ArH), 2950 (m, CH), 1750 (str, C=O), 1720 (str, C=O), 1692 (str, C=O), 1604 (m, C=C). UV (CH_2Cl_2) λ_{max} 279.0 nm (ε 7,905), 236.0 (7,360). HREIMS found: 464.1224. Calcd for $C_{21}H_{21}N_2O_9F$: 464.1231.

2',3',5'-Tri-*O*-acetyl-5-(3,5-difluorophenyl)uridine (23d).

81%. m.p. = 74-5 °C. α_D = -58.35 ($CHCl_3$, c = 0.67). ^1H NMR δ 9.34 (br s, 1 H, NH) 7.57 (s , 1 H, H-6), 7.03 (dd, J = 8.9 & 2.2 Hz, 2 H, ArH), 6.73 (tt, J = 8.8 & 2.2 Hz, 2 H, ArH), 6.07 (d, J = 5.6 Hz, 1 H, H-1'), 5.34 (dd, J = 2 x 5.7 Hz, 1 H, H-2'), 5.27 (m, 1 H, H-3'), 4.39-4.26 (m, 3 H, H-4',5'), 2.07 & 2.05 (2 x s, 6 H, CH_3CO_2C-2'&3'), 1.94 (s, 3 H, CH_3CO_2C-5'). ^{19}F NMR (282.4 MHz, $CDCl_3$) δ 32.55 (s, ArF). EIMS m/z 482 (M^+, <5%). IR ($CHCl_3$) ν_{max} 3392 cm^{-1} (m, NH), 3012 (m, ArH), 2940 (m, CH), 1750 (str, C=O), 1725 (str, C=O), 1698 (str, C=O), 1624 (m, C=C). UV (CH_2Cl_2) λ_{max} 281.3 nm (ε 11,123), 239.0 (9,980). HREIMS found: 482.1123. Calcd for $C_{21}H_{20}N_2O_9F_2$: 482.1136.

2',3',5'-Tri-*O*-acetyl-5-[4-(α,α,α-trifluoromethyl)phenyl]uridine (23e).

91%. m.p. = 84-5 °C. α_D = -48.40 ($CHCl_3$, c = 0.58). ^1H NMR δ 9.43 (br s, 1 H, NH), 7.57 (s, 4 H, ArH), 7.56 (s , 1 H, H-6), 6.05 (d, J = 5.5 Hz, 1 H, H-1'), 5.36 (dd, J = 2 x 5.7 Hz, 1 H, H-2'), 5.27 (m, 1 H, H-3'), 4.34-4.29 (m, 3 H, H-4',5'), 2.10 & 2.07 (2 x s, 6 H, CH_3CO_2C-2'&3'), 1.84 (s, 3 H, CH_3CO_2C-5'). ^{19}F NMR (282.4 MHz, $CDCl_3$) δ 13.94 (s, $ArCF_3$). FABMS m/z 515 (M^++H, <5%). IR (KBr disc) ν_{max} 3440 cm^{-1} (m,

NH), 3080 (m, ArH), 2960 (m, CH), 1752 (str, C=O), 1726 (str, C=O), 1698 (str, C=O), 1618 (w, C=C). UV (CH_2Cl_2) λ_{max} 281.3 nm (ε 11,123), 239.0 (9,980). Anal found: C, 51.41; H, 4.04; N, 5.44. Calcd for $C_{22}H_{21}N_2O_9F_3$: C, 51.37; H, 4.11; N, 5.45.

2',3',5'-Tri-*O*-acetyl-5-[3,5-bis(α,α,α-trifluoromethyl)phenyl]uridine (23g) .

85%. m.p. = 137-9 °C. α_D = -63.32 ($CHCl_3$, c = 0.69). ^{1}H NMR δ 9.50 (br s, 1 H, NH), 7.94 (s, 2 H, ArH), 7.78 (s, 1 H, ArH), 7.64 (s , 1 H, H-6), 6.07 (d, J = 5.5 Hz, 1 H, H-1'), 5.34 (dd, J = 2 x 5.7 Hz, 1 H, H-2'), 5.27 (m, 1 H, H-3'), 4.342-4.22 (m, 3 H, H-4',5'), 2.08 & 2.05 (2 x s, 6 H, CH_3CO_2C-2'&3'), 1.88 (s, 3 H, CH_3CO_2C-5'). ^{19}F NMR (282.4 MHz, $CDCl_3$) δ 13.78 (s, $ArCF_3$). FABMS m/z 523 (M^+-CH_2CO_2, <5%). IR (KBr disc) ν_{max} 3440 cm^{-1} (m, NH), 3080 (m, ArH), 2960 (m, CH), 1752 (str, C=O), 1726 (str, C=O), 1698 (str, C=O), 1618 (w, C=C),. UV (CH_2Cl_2) λ_{max} 278.9 nm (ε 10,181), 247.0 (7,142). Anal found: C, 47.33; H, 3.59; N, 4.81. Calcd for $C_{23}H_{20}N_2O_9F_6$: C, 47.33; H, 3.59; N, 4.76.

2',3',5'-Tri-*O*-acetyl-N3-allyluridine (28).

Tri-*n*-butylallylstannane (222mg, 0.67mmol) was added to a solution of 2',3',5'-tri-*O*-acetyl-5-trifluoromethanesulfonyluridine **12** (174mg, 0.34 mmol), $Pd(Ph_3)_4$ (17mg, 0.016 mmol) and lithium chloride (30mg, 0.71mmol) in 1,4-dioxane (4ml) under N_2. The clear pale orange solution was heated to reflux. After 14hrs the reaction mixture turned black and analytical t.l.c. (hexanes/ethyl acetate, 1:1) revealed that **12** had been consumed. The reaction mixture was filtered through celite and evaporated to dryness under vacuum. The residue was taken up in dichloromethane (25ml) and washed with 30% aqueous potassium fluoride and water, dried over $MgSO_4$ and evaporated under vacuum. The residue was chromatographed (hexanes/ethyl acetate, 4:3) and gave 2',3',5'-tri-*O*-acetyl-N3-allyluridine **28** (65.6mg, 47%) as a white foam. ^{1}H NMR δ 7.31 (d, J = 8.1 Hz, 1 H, H-6), 5.94 (d, J = 4.5 Hz, 1 H, H-1'), 5.86-5.73 (m, 1 H, $CH_2C\underline{H}=CH_2$), 5.76 (d, J = 8.1 Hz, 1 H, H-5), 5.32-5.05 (m, 4 H, H-2',3' & $CH=C\underline{H_2}$), 4.46 (m, 2 H, $NC\underline{H_2}CH=CH_2$), 4.29 (m, 3 H, H-4',5'), 2.07, 2.03 & 2.01 (3 x s, 9 H, 3 x CH_3CO_2). ^{13}C

NMR (20.1 MHz) δ 170.2 & 169.7 (3 x $CH_3\underline{C}O_2$), 164.5 (C4), 150.6 (C2), 137.4 (C6), 131.3 ($\underline{C}H=CH_2$), 118.1 ($CH=\underline{C}H_2$), 102.8 (C5), 88.7 (C1'), 79.7 (C4'), 72.9 (C2'), 69.8 (C3'), 62.8 (C5'), 43.0 ($N\underline{C}H_2CH=CH_2$), 20.6 & 20.3 (3 x $\underline{C}H_3CO_2$). EIMS m/z 410 (M^+, <5%). IR ($CHCl_3$) ν_{max} 3004 cm^{-1} (m, CH), 2952 (m, CH), 1750 (str br, C=O), 1714 (str, C=O), 1674 (str, C=O), 1458 (str, C=C), 1430 (str, C=O). HREIMS found: 4101335. Calcd for $C_{15}H_{16}N_2O_9$: 4101343.

Another minor product of lower R_f was isolated that gave a ^1H NMR consistant with monodeacetylated **28** (10mg, 8%). ^1H NMR δ 7.27 (d, J = 7.9 Hz, 1 H, H-6), 5.90 (d, J = 4.3 Hz, 1 H, H-1'), 5.91-5.77 (m, 1 H, $CH_2C\underline{H}=CH_2$), 5.79 (d, J = 8.0 Hz, 1 H, H-5), 5.34-5.10 (m, 4 H, H-2',3' & $CH=C\underline{H}_2$), 4.51 (m, 2 H, $NC\underline{H}_2CH=CH_2$), 4.32-3.91 (m, 3 H, H-4',5'), 2.00 & 1.99 (2 x s, 6 H, 2 x CH_3CO_2).

Chapter 4:

2',3',5'-Tri-*O*-acetyl-5-[2-(trimethylsilyl)ethynyl]uridine (37a).

A stream of nitrogen was passed through a solution of 2',3',5'-tri-*O*-acetyl-5-trifluoromethanesulfonyluridine **12** (100mg, 0.19mmol) and $Pd(PPh_3)_4$ (11mg, 0.01mmol) in dimethylformamide DMF (2.0ml) for 10 mins. To the resultant orange solution triethylamine (56μl, 0.38mmol), trimethylsilylacetylene (37mg, 0.38mmol) and copper(I)iodide (3.30mg, 0.02mmol) were added. The solution was heated to 45°C and the progress of the reaction monitored by t.l.c. (65% hexane, 30% ethyl acetate, 4% methanol, 1% formic acid). After 2.5hrs the consumption of starting material was complete and the reaction mixture went from dark orange to black. The solvent was evaporated under vacuum to give a black resin which was dissolved in dichloromethane and activated charcoal added. After stirring for 1hr the suspension was filtered through celite and the tan solution evaporated on to silica (~200mg) and chromatographed (65% hexane, 35% ethyl acetate). 2',3',5'-tri-*O*-acetyl-5-[-2-(trimethylsilyl)ethynyl]-uridine **37a** (75mg, 85%) was obtained as a clear solid foam; m.p. = 192-5°C α_D = -69.52 ($CHCl_3$, 0.63). ^1H NMR δ 9.53 (br s, 1 H, NH), 8.68 (s , 1 H, H-6), 6.05 (d, J = 4.6 Hz, 1 H, H-1'), 5.25 (m, 2 H, H-2',3'), 4.29 (m, 2 H, H-4',5'), 2.12, 2.04,2.02 (3 x s, 3

x 3 H, 3 x CO_2CH_3), 0.12 [s, 9 H, $Si(CH_3)_3$]. EIMS m/z 408 (M^+, 8%) 451 (25), 446 (27). IR ($CHCl_3$) ν_{max} 3395 cm^{-1} (m, NH), 2960 (m, CH), 1752 (str br, C=O), 1722 (br, C=O), 1626 (m, C=C). HREIMS found: 466.1422. Calcd for $C_{20}H_{26}N_2O_9$: 466.1408.

Compounds **37b-e** were prepared in a similar manner as that described for **37a** above, varying only in the reaction time and temperature and the product yield obtained as detailed in Table 13. In the cases of **37b-d** a small amount of by-product was also isolated (**37b'-d'**, respectively).

2',3',5'-Tri-*O*-acetyl-5-(2-hydroxy-2-methylpropynyl)uridine (37b).

90%. α_D = - 46.1 (MeOH,. c = 0.59). ^1H NMR δ 9.66 (br s, 1 H, NH), 7.65 (s , 1 H, H-6), 6.02 (d, J = 4.60 Hz, 1 H, H-1'), 5.30 (m, 2 H, H-3', 2'), 4.30 (m, 3 H, H-4', 5'), 2.15, 2.11, 2.05 (3 x s, 9 H, 3 x CH_3CO_2), 1.53 (s, 6 H, $C(C\underline{H_3})_2OH$). EIMS m/z 452 (M^+, 5%), 259 (100). IR ($CHCl_3$) ν_{max} 3392 cm^{-1} (m, NH), 3004 (m, CH), 1748 (str br, C=O), 1730 (br, C=O), 1630 (m, C=C). UV (MeOH) λ_{max} 285 nm (ε 11,054), 227 (9,752), 204 (8,679). HREIMS found: 452.1442. Calcd for $C_{20}H_{24}N_2O_{10}$: 452.1431.

6-(1-Hydroxy-1-methylethyl)-3-(2,3,5-tri-*O*-acetyl-β-D-*erythro*-pentofuranosyl)-furano[2,3-*d*]pyrimidin-2-one (37b').

3%. ^1H NMR δ 8.10 (s , 1 H, H-6), 6.24 (s, 1 H, -$\underline{H}C{=}CO(CH_3)_2OH$), 6.17 (d, J = 5.5 Hz, 1 H, H-1'), 5.35 (m, 1 H, H-2'), 5.23 (m, 1 H, H-3'), 4.42 (m, 3 H, H-4', 5'), 2.15, 2.05, 2.04 (3 x s, 9 H, 3 x CH_3CO_2), 1.54, 1.53 (2 x s, 2 x 3 H, $C(C\underline{H_3})_2OH$). EIMS m/z 452 (M^+, 12%), 259 (100). HREIMS found: 452.1438. Calcd for $C_{20}H_{24}N_2O_{10}$: 452.1431.

2',3',5'-Tri-*O*-acetyl-5-hexynyluridine (37d).

90%. α_D = - 45.4 (MeOH,. c = 0.50). ^1H NMR δ 9.12 (br s, 1 H, NH), 7.62 (s , 1 H, H-6), 6.07 (d, J = 4.60 Hz, 1 H, H-1'), 5.29 (m, 2 H, H-3', 2'), 4.32 (m, 3 H, H-4', 5'), 2.35 (t, J = 7.0, 2 H, $C{\equiv}CCH_2$), 2.17, 2.13, 2.09 (3 x s, 3 x 3 H, 3 x CH_3CO_2), 1.5 (m, 2 H, $C{\equiv}CCH_2C\underline{H_2}$), 1.38 (m, 2 H, $C\underline{H_2}CH_3$), 0.87 (t, J = 7.3 Hz, 3 H, CH_3). EIMS m/z 450

(M^+, <5%), 259 (100). IR ($CHCl_3$) ν_{max} 3392 cm^{-1} (m, NH), 2956 (m, CH), 1750 (str br, C=O), 1730 (br, C=O), 1630 (m, C=C). UV (MeOH) λ_{max} 282 nm (ε 10,197), 229 (9,198), 204 (9,450). HREIMS found: 450.1662. Calcd for $C_{20}H_{24}N_2O_{10}$: 450.1659.

6-*n*-Butyl-3-(2,3,5-tri-*O*-acetyl-β-D-*erythro*-pentofuranosyl)-furano[2,3-*d*]pyrimidin-2-one (37c').

2%. ^1H NMR δ 7.61 (s , 1 H, H-6), 6.04 (d, J = 4.6 Hz, 1 H, H-1'), 5.26 (m, 2 H, H-3', 2'), 4.32 (m, 3 H, H-4', 5'), 2.32 (t, J = 7.0, 2 H, C=CCH_2), 2.17, 2.13, 2.09 (3 x s, 3 x 3 H, 3 x CH_3CO_2), 1.52 (m, 2 H, C=CCH_2CH_2), 1.36 (m, 2 H, C$H_2$$CH_3$), 0.87 (t, J = 7.3 Hz, 3 H, CH_3). EIMS m/z 450 (M^+, <5%), 259 (100). HREIMS found: 450.1663. Calcd for $C_{20}H_{24}N_2O_{10}$: 450.1659.

2',3',5'-Tri-*O*-acetyl-5-[2-phenylethynyl]uridine (37d).

93%. m.p. = 67 - 70 °C. α_D = -90.1 (MeOH,. c = 1.14). ^1H NMR δ 9.82 (br s, 1 H, NH), 7.85 (s , 1 H, H-6), 7.47 (m, 2 H, ArH), 7.32 (m, 3 H, ArH), 6.13 (d, J = 4.53 Hz, 1 H, H-1'), 5.39 (m, 2 H, H-3', 2'), 4.39 (m, 3 H, H-4', 5'), 2.21 (s, 3 H, CH_3CO_2), 2.12 (s, 6 H, 2 x CH_3CO_2). EIMS m/z 470 (M^+, 8%), 259 (100). IR ($CHCl_3$) ν_{max} 3400 cm^{-1} (m, NH), 3020 (m br, ArH), 1755 (str br, C=O), 1714 (br, C=O), 1615 (m, C=C). UV (CH_2Cl_2) λ_{max} 307 nm (ε 17,512), 278 (12,746), 264 (14,518), 207 (23,894). HREIMS found: 470.1322. Calcd for $C_{23}H_{22}N_2O_9$: 470.1325.

6-Phenyl-3-(2,3,5-tri-*O*-acetyl-β-D-*erythro*-pentofuranosyl)-furano[2,3-*d*]pyrimidin-2-one (37d').

5%. m.p. = 137-9 °C. ^1H NMR δ 8.25 (s , 1 H, H-6), 7.77 (m, 2 H, ArH), 7.47 (m, 3 H, ArH), 6.71 (s, 1H, HC=COPh), 6.27 (d, J = 3.6 Hz, 1 H, H-1'), 5.47 (t, J = 4.5 Hz, 1 H, H-2'), 5.34 (dd, $J_{3',2'}$ = 4.6 Hz, $J_{3',4'}$ = 5.6 Hz, 1 H, H-3'), 4.16 (br s, 3 H, H-4',5'), 4.39 (m, 3 H, H-4', 5'), 2.20, 2.17, 2.14 (3 x s, 3 x 3 H, 3 x CH_3CO_2). EIMS m/z 470 (M^+, 30%), 291 (30), 259 (100). HREIMS found: 470.1307. Calcd for $C_{23}H_{22}N_2O_9$: 470.1325.

2',3',5'-Tri-*O*-acetyl-5-[2-(4-methoxyphenyl)ethynyl]uridine (37e).

97%. α_D = -94.4 (MeOH,. c = 0.45). ^{1}H NMR δ 9.30 (br s, 1 H, NH), 7.75 (s , 1 H, H-6), 7.35 (d, J = 8.7 Hz, 2 H, ArH), (d, J = 8.7 Hz, 2 H, ArH), 6.07 (d, J = 3.4 Hz, 1 H, H-1'), 5.30 (m, 2 H, H-3', 2'), 4.31 (m, 3 H, H-4', 5'), 3.75 (s, 3 H, CH_3), 2.13, 2.07, 2.05 (3 x s, 3 x 3 H, 3 x CH_3CO_2). EIMS m/z 500 (M^+, 10%), 440 (28), 259 (100). IR ($CHCl_3$) ν_{max} 3410 cm^{-1} (m, NH), 3020 (m br, ArH), 1752 (str br, C=O), 1710 (br, C=O), 1610 (m, C=C). UV (CH_2Cl_2) λ_{max} 318 nm (ε 13,444), 266 (16,411). HREIMS found: 500.1442. Calcd for $C_{24}H_{24}N_2O_{10}$: 500.1431.

Attempted Coupling of 4-Fluorophenyltrimethylsilylacetylene (Table 7, entry 5) with 3',5'-Di-*O*-acetyl-5-trifluoromethanesulfonyl-2'-deoxyuridine (14).

To a solution of 3',5'-di-*O*-acetyl-5-trifluoromethanesulfonyl-2'-deoxyuridine **14** (100mg, 0.22mmol) and $Pd(PPh_3)_4$ (12.5mg, 0.01mmol) in THF (2.0ml) 4-fluorophenyltrimethylsilylacetylene (58mg,0.30mmol) and tetrabutylammoniumfluoride TBAF (0.25mmol, 250μl of a 1M solution) were added. After stirring at room temperature for 24hrs only starting material **14** was detected by t.l.c., analysis by g.l.c. revealed that all 4-fluorophenyl-2-trimethylsilylacetylene had been desilylated to give 4-fluorophenylacetylene. A similar result was obtained when iodobenzene was used in place of **14** or when anhydrous CsF was used instead of TBAF or when $(\eta^3\text{-}C_3H_5PdCl)_2$ was used in place of $Pd(PPh_3)_4$. As was the case when all these reagents were replaced simultaneously.

3',5'-Di-*O*-acetyl-5-[2-(trimethylsilyl)ethynyl]-2'-deoxyuridine (38a).

Conditions A (eq. 26): A stream of nitrogen was passed through a solution of 3',5'-di-*O*-acetyl-5-trifluoromethanesulfonyl-2'-deoxyuridine **14** (100mg, 0.22mmol) and $Pd(PPh_3)_4$ (12.5mg, 0.01mmol) in DMF (2.0ml) for 10 mins. To the resultant orange solution triethylamine (47μl, 0.33mmol), trimethylsilylacetylene (33mg, 0.33mmol) and copper(I)iodide (3.4mg, 0.02mmol) were added. The solution was heated to 50°C and the progress of the reaction

monitored by t.l.c. (70% hexane, 26% ethyl acetate, 3% methanol, 1% formic acid). After 2.5hrs the consumption of starting material was complete and the reaction mixture went from dark orange to black. The solvent was evaporated under vacuum to give a black resin which was dissolved in dichloromethane (5ml). Activated charcoal was added and the slurry stirred for 1hr the suspension was filtered through celite and the tan solution evaporated on to silica (~200mg) and chromatographed (hexane/ethyl acetate 7:3). 3',5'-Di-*O*-acetyl-5-[2-(trimethylsilyl)ethynyl]-2'-deoxyuridine **38a** (78mg, 87%) was obtained as a clear solid foam; m.p. = 181-2°C (lit.[67] =178-9°C). ^{1}H NMR δ 9.70 (br s, 1 H, NH), 8.10 (s , 1 H, H-6), 5.90 (d, J = 4.60 Hz, 1 H, H-1'), 5.25 (m, 1 H, H-3'), 4.33 (m, 3 H, H-4', 5'), 2.19, 2.17 (2 x s, 6 H, 2 x CH_3CO_2) 0.22 [s, 9 H, $Si(CH_3)_3$]. EIMS m/z 408 (M^+, 8%), 393 (<1), 279 (100). HREIMS found: 408.1337. Calcd for $C_{20}H_{26}N_2O_9$: 408.1321.

Compounds **38b-e** were prepared in a similar manner as that described for **38a** above (i.e. Conditions A eq. 26), varying only in the reaction time and temperature and the product yield obtained as detailed in Table 4. In the cases **38b-e** considerable amounts of by-product was also isolated (**38b'-e'**, respectively). In the cases of **38b** & **c** 1,4-bis-(3,5-difluorophenyl)-1,3-butadiyne **39 (X = 3,5-F_2)** and 1,4-bis-(4-α,α,α-trifluoromethylphenyl)-1,3-butadiyne **39 (X = 4-CF_3)** were isolated (respectively) in sufficient quantities as to account the mass balance of all phenylacetylene intially added (see below for physical data).

3',5'-Di-*O*-acetyl-5-{2-[3,5-bis(α,α,α-trifluoromethyl)phenyl]ethynyl}-2'-deoxyuridine (38b).

51%. m.p. = 179.0-180.0 °C. α_D = -18.25 (MeOH,. c = 2.00). ^{1}H NMR δ 10.07 (br s, 1 H, NH), 7.91 (s , 1 H, H-6) 7.88 (s, 2 H, ArH), 7.75 (s, 1 H, ArH), 6.25 (dd, J_{trans} = 7.35 Hz, J_{cis} = 6.12 Hz, 1 H, H-1'), 5.21 (m, 1 H, H-3'), 4.35 (m, 3 H, H-4',5'), 2.57 (m, 1 H, H-2'$_\beta$), 2.18 (m, 1 H, H-2'$_\alpha$), 2.10 & 2.07 (2 x s, 6 H, 2 x CH_3CO_2). EIMS m/z 548 (M^+, <5%), 529 (<5) 348 (30) 81 (100). IR ($CHCl_3$) ν_{max} 3388 cm^{-1} (m, NH), 3084 (m, ArH), 2956 (m, CH), 2240 (w, C≡C), 1720 (str br, C=O), 1632 (sh, C=C). UV

(MeOH) λ_{max} 312 nm (ε 23,358), 241 (10,206). HREIMS found: 548.1059. Calcd for $C_{23}H_{18}N_2O_7F_6$: 548.1059.

6-[3,5-Bis(α,α,α-trifluoromethyl)phenyl]-3-(2,5-di-*O*-acetyl-2-deoxy-β-D-*erythro*-pentofuranosyl)-furano[2,3-*d*]pyrimidin-2-one (38b').

21%. ^{1}H NMR δ 8.44 (s , 1 H, H-6), 7.28 (t , J = 3.4 Hz, 2 H, ArH), 6.85 (m, 1 H, ArH), 6.83 (s, 1 H, C=COAr), 6.32 (dd, J = 7.28 Hz, J = 5.95 Hz, 1 H, H-1'), 5.26 (m, 1 H, H-3'), 4.38 (m, 3 H, H-4',5'), 2.99 (m, 1 H, H-2'$_\beta$), 2.11 (m, 1 H, H-2'$_\alpha$), 2.14 & 2.02 (2 x s, 6 H, 2 x CH_3CO_2). EIMS m/z 548 (M^+, <5%). HREIMS found: 548.1057. Calcd for $C_{23}H_{18}N_2O_7F_6$: 548.1059.

3',5'-Di-*O*-acetyl-5-[2-(3,5-difluorophenyl)ethynyl]-2'-deoxyuridine (38c).

31%. m.p. = 186.0-186.5 °C. α_D = -21.0 (MeOH,. c = 1.69). ^{1}H NMR δ 9.59 (br s, 1 H, NH), 7.85 (s , 1 H, H-6), 6.94 (m, 2 H, ArH), 6.75 (m, 1 H, ArH), 6.25 (dd, J_{trans} = 7.50 Hz, J_{cis} = 5.91 Hz, 1 H, H-1'), 5.18 (m, 1 H, H-3'), 4.33 (m, 3 H, H-4',5'), 2.51 (m, 1 H, H-2'$_\beta$), 2.17 (m, 1 H, H-2'$_\alpha$), 2.10 & 2.06 (2 x s, 6 H, 2 x CH_3CO_2). EIMS m/z 448 (M^+, <5%), 259 (7). IR ($CHCl_3$) ν_{max} 3388 cm^{-1} (m, NH), 3013 (m, ArH), 1714 (str br, C=O), 1614 (sh, C=O), 1590 (sh, C=C). UV (MeOH) λ_{max} 307 nm (ε 14,729), 277 (10,744), 264 (10,696), 207 (23,894). HREIMS found: 448.1070. Calcd for $C_{21}H_{18}N_2O_7F_2$: 448.1082.

6-(3,5-Difluorophenyl)-3-(3,5-di-*O*-acetyl-2-deoxy-β-D-*erythro*-pentofuranosyl)-furano[2,3-*d*]pyrimidin-2-one (38c').

50%. HREIMS found: 448.1080. Calcd for $C_{21}H_{18}N_2O_7F_2$: 448.1082.

3',5'-Di-*O*-acetyl-5-{2-[4-(α,α,α-trifluoromethyl)phenyl]ethynyl}-2'-deoxyuridine (38d).

43%. m.p. = 194.0-196.0 °C. α_D = -23.26 (MeOH,. c = 1.44). ^{1}H NMR δ 10.17 (br s, 1 H, NH), 7.94 (s , 1 H, H-6) 7.60 (s, 4 H, ArH), 6.33 (t, J = 7.30 Hz, 1 H, H-1'), 5.27 (m, 1 H, H-3'), 4.33 (m, 3H, H-4',5'), 2.61 (m, 1 H, H-2'$_\beta$), 2.29 (m, 1 H, H-2'$_\alpha$),

2.17 & 2.13 (2 x s, 6 H, 2 x CH_3CO_2). EIMS m/z 480 (M^+, <5%), 446 (<5) 280 (30) 81 (100). IR ($CHCl_3$) ν_{max} 3400 cm^{-1} (m, NH), 3010 (m, ArH), 2996 (m, CH), 1730 (str br, C=O), 1620 (sh, C=C). UV (MeOH) λ_{max} 310 nm (ε 21,085), 241 (44,400). HREIMS found: 480.1159. Calcd for $C_{22}H_{19}N_2O_7F_3$: 480.1144.

6-[4-(α,α,α-trifluoromethyl)phenyl]-3-(3,5-di-*O*-acetyl-2-deoxy-β-D-*erythro*-pentofuranosyl)-furano[2,3-*d*]pyrimidin-2-one (38d').

33%. HREIMS found: 480.1150. Calcd for $C_{22}H_{19}N_2O_7F_3$: 480.1144.

3',5'-Di-*O*-acetyl-5-[2-(4-fluorophenyl)ethynyl]-2'-deoxyuridine (38e).

50%. m.p. = 156.0-158.0 °C. α_D = -26.20 (MeOH,. c = 1.00). ^1H NMR δ 10.03 (br s, 1 H, NH), 7.93 (s , 1 H, H-6), 7.50 (dd, J_{HH} = 6.67 Hz, $^4J_{HF}$ = 2.08 Hz, 2 H, ArH), 7.03 (t, J_{HH} = $^3J_{HF}$ = 8.72, 2 H, ArH), 6.34 (dd, J_{trans} = 7.56 Hz, J_{cis} = 6.06 Hz, 1 H, H-1'), 5.26 (m, 1 H, H-3'), 4.32 (m, 3H, H-4',5'), 2.60 (m, 1 H, H-2'β), 2.27 (m, 1 H, H-2'α), 2.16 & 2.12 (2 x s, 6 H, 2 x CH_3CO_2). ^{13}C NMR δ 170.32 & 170.08 ($CH_3\underline{C}O_2$), 162.46 (d, J_{CF} = 250.0 Hz, ArC4), 161.31 (C4), 149.31 (C2), 141.35 (C6), 133.40 (d, $^3J_{CF}$ = 8.53 Hz, ArC2), 118.30 (d, $^4J_{CF}$ = 3.47 Hz, ArC1), 115.49 (d, $^2J_{CF}$ = 22.04 Hz, ArC3), 100.43 (C5), 92.38 (C_α), 85.38 (C1'), 82.38 (C4'), 79.83 (C_β), 73.85 (C3'), 63.66 (C5'), 37.93 (C2'), 20.67 & 20.58 ($\underline{C}H_3CO_2$). EIMS m/z 430 (M^+, 10%), 230 (100). IR ($CHCl_3$) ν_{max} 3392cm^{-1} (m, NH), 3010 (m, ArH), 1704 (str br, C=O), 1630 (sh, C=C). UV (CH_2Cl_2) λ_{max} 307 nm (ε 14,878), 278 (10,922), 263 (12,588), 249 (10,986). HREIMS found: 430.1181. Calcd for $C_{21}H_{19}N_2O_7F$: 430.1176.

Conditions B eq. 54: Compounds **38b-e** were also prepared in accordance with Conditions B eq. 26 as was **38f**, varying only in reaction time and temperature and the product yield obtained (Table 4), as exemplified for the preparation of **38f** described below.

3',5'-Di-*O*-acetyl-5-[2-(pentafluorophenyl)ethynyl]-2'-deoxyuridine (38f).

A stream of nitrogen was passed through a solution of 3',5'-di-*O*-acetyl-5-trifluoromethanesulfonyl-2'-deoxyuridine **14** (100mg, 0.22mmol) and $Pd(PPh_3)_4$ (25mg, 0.02mmol) in DMF (2.0ml) for 10 mins. To the resultant orange solution triethylamine (34µl, 0.24mmol), pentafluorophenylacetylene (84.5mg, 0.44mmol) and copper(I)iodide (7.1mg, 0.05mmol) were added. The reaction mixture was intially stirred at room temperature for 2.0hrs, after which time no product could be detected by t.l.c. (70% hexane, 26% ethyl acetate, 3% methanol, 1% formic acid), and then at 45°C for 3hrs after which time only a trace of product was detected. The reaction mixture was then heated to 55°C and the consumtion of **14** was complete after 5hrs, at which time the orange solution turned black. The solvent was evaporated under vacuum to give a black resin which was dissolved in dichloromethane (5ml). Activated charcoal was added and the slurry stirred for 1hr. The suspension was filtered through celite and the tan filtrate evaporated on to silica (~200mg) and chromatographed (hexanes/ethyl acetate 7:3). 3',5'-Di-*O*-acetyl-5-[2-(pentafluorophenyl)ethynyl]-2'-deoxyuridine **38f** (95mg, 87%) was obtained as a white solid; m.p. = 209.0-210.0 °C. α_D = -37.88 (MeOH,. c = 0.33). ^{1}H NMR δ 9.84 (br s, 1 H, NH), 8.02 (s , 1 H, H-6), 6.33 (dd, J_{trans} = 7.61 Hz, J_{cis} = 5.81 Hz, 1 H, H-1'), 5.26 (m, 1 H, H-3'), 4.35 (m, 3H, H-4',5'), 2.63 (m, 1 H, H-2'$_\beta$), 2.30 (m, 1 H, H-2'$_\alpha$), 2.16 & 2.13 (2 x s, 6 H, 2 x CH_3CO_2). ^{13}C NMR δ 170.40 & 170.12 ($CH_3\underline{C}O_2$), 160.54 (C4), 149.12 (C2), 142.88 (C6), 99.33 (C5), 92.10 (C_β), 85.73 (C1'), 82.75 (C4'), 73.91 (C3'&C_α), 63.67 (C5'), 38.32 (C2'), 20.77 & 20.55 ($\underline{C}H_3CO_2$). EIMS m/z 502 (M^+, 40%), 428 (28), 301 (100). IR ($CHCl_3$) ν_{max} 3388 cm^{-1} (m, NH), 2250 (w, C≡C), 1710 (str br, C=O), 1626 (sh, C=C). UV (MeOH) λ_{max} 305 nm (ε 19,489), 217 (13,819). HREIMS found: 502.0818. Calcd for $C_{21}H_{15}N_2O_7F_5$: 502.0799.

For **38b-e** only a small amount of by-product **38b'-e'** was detected, no such by-product was detected in the case of **38f**. The yields for **38b-e** under Conditions B eq. 26 were 80, 70, 82 & 87% respectively (see above for physical data).

Compound **38c** was also prepared from 3',5'-di-*O*-acetyl-5-iodo-2'-deoxyuridine **40** (100mg, 0.23mmol) in a similar manner as that described for **38f** (from **14**) above, except that the reaction was worked up after only 10min. Work up and purification was carried out as described for **38f** above giving **38c** (100mg, 98%) as a white solid.

5-[2-(Trimethylsilyl)ethynyl]-2'-deoxyuridine (41a).

A stream of nitrogen was passed through a solution of 5-trifluoromethanesulfonyl-2'-deoxyuridine **8** (200mg, 0.53mol) and $Pd(PPh_3)_4$ (30mg, 0.03mmol) in DMF (4.0ml) for 10 mins. To the resultant orange solution triethylamine (1.15µl, 0.80mmol), trimethylsilylacetylene (78mg, 0.80mmol) and copper(I)iodide (8.0mg, 0.05mmol) were added. The solution was heated to 50°C and the progress of the reaction monitored by t.l.c. (60% hexane, 35% ethyl acetate, 4% methanol, 1% formic acid). After 2.0hrs the consumption of starting material was complete and the reaction mixture went from dark orange to black. The solvent was evaporated under vacuum to give a black resin which was dissolved in methanol (5ml). Activated charcoal was added and the slurry stirred for 1hr the suspension was filtered through celite and the tan solution evaporated on to silica (~400mg) and chromatographed (65% hexane, 35% ethyl acetate). 5-[2-(Trimethylsilyl)ethynyl]-2'-deoxyuridine **41a** (151mg, 87%) was obtained as a white solid; m.p. = 162-5°C. α_D = + 6.32 (MeOH,. c = 1.90). ^{1}H NMR δ 10.70 (br s, 1 H, NH), 8.15 (s , 1 H, H-6), 6.10 (t, J = 6.36 Hz, 1 H, H-1'), 4.64 (br s, 1 H, 3'-OH), 4.39 (br s, 1 H, 5'-OH), 4.10 (m, 1 H, H-3'), 3.68 (m, 1 H, H-4'), 3.57 (m, 2 H, H-5'), 2.16 (m, 1 H, H-2'$_\beta$), 1.98 (m, 1 H, H-2'$_\alpha$), 0.24 [s, 9 H, $Si(CH_3)_3$]. EIMS m/z 324 (M^+, <1%). UV (MeOH) λ_{max} 295 nm (ε 2,842), 233.2 (2,619), 203.6 (2,116).

An attempt to prepare compounds **41b-h** in a similar manner to that described for **41a** above was successful only for compounds **41e-h**, variations in the reaction conditions and the product yields obtained are detailed in Table 5 and eq. 28. In the cases **41b-d** only trace amounts of of product and by-product

(**41b'-d'**, respectively) and considerable amounts of starting material **8** were observed by t.l.c..

5-[2-(4-Fluorophenyl)ethynyl]-2'-deoxyuridine (41e).

76%. m.p. = 213-214°C. α_D = -4.1 (MeOH,. c = 1.60). ^{1}H NMR ($CDCl_3$/$DMSOd_6$) δ 10.98 (br s, 1 H, NH), 8.33 (s , 1 H, H-6), 7.48 (dd, J_{HH} = 8.68 Hz, $^4J_{HF}$ = 5.70 Hz, 2 H, ArH), 7.03 (t, J_{HH} = $^3J_{HF}$ = 8.72, 2 H, ArH) 6.29 (t, J = 6.27 Hz, 1 H, H-1'), 4.75 (br s, 1 H, OH), 4.46 (br m, 2 H, OH & H3'), 3.98 (m, 1 H, H-4'), 3.83 (m, 2 H, H-5'), 2.40 (m, 1 H, H-2'$_\beta$), 2.25 (m, 1 H, H-2'$_\alpha$). ^{13}C NMR ($CDCl_3$/$DMSOd_6$) δ 162.11 (d, J_{CF} = 250.0 Hz, ArC4), 161.82 (C4), 149.29 (C2), 143.25 (C6), 133.20 (d, $^3J_{CF}$ = 8.53 Hz, ArC2), 118.70 (d, $^4J_{CF}$ = 3.47 Hz, ArC1), 115.24 (d, $^2J_{CF}$ = 22.30 Hz, ArC3), 99.11 (C5), 91.35 (C_α), 87.37 (C1'), 85.38 (C4'), 80.68 (C_β), 70.18 (C3'), 61.21 (C5'), 40.78 (C2'). FABMS m/z 347 (M^++H, <5%). IR (nujol mull) ν_{max} 3456 cm^{-1} (m, NH), 3288 (br, OH), 3024 (ArH), 1696 (str , C=O). UV (MeOH) λ_{max} 301 nm (ε 14,212), 275 (12,983), 262 (13,805), 219 (9,376), 204 (13,650). Anal found: C, 58.75; H, 4.37; N, 8.08. Calcd for $C_{17}H_{15}N_2O_5F$: C, 58.94; H, 4.33; N, 8.09.

5-[2-(Pentafluorophenyl)ethynyl]-2'-deoxyuridine (41f).

89%. m.p. = 302-305°C. α_D = -2.03 (MeOH,. c = 0.64). ^{1}H NMR ($CDCl_3$/$DMSOd_6$) δ 11.58 (br s, 1 H, NH), 8.56 (s , 1 H, H-6), 6.27 (t, J = 6.21 Hz, 1 H, H-1'), 5.09 (br s, 1 H, OH), 4.83 (br m, 1 H, OH), 4.44 (m, 1 H, H-3'), 3.98 (m, 1 H, H-4'), 3.78 (m, 2 H, H-5'), 2.39 (m, 1 H, H-2'$_\beta$), 2.21 (m, 1 H, H-2'$_\alpha$). ^{13}C NMR ($CDCl_3$/$DMSOd_6$) δ 160.67 (C4), 148.69 (C2), 145.81 (dm, J_{CF} = 256.6 Hz, ArC2), 144.46 (C6), 140.11 (dm, J_{CF} = 256.6 Hz, ArC4), 136.49 (dm, J_{CF} = 249.1 Hz, ArC3), 99.01 (m, ArC1), 96.80 (C5), 93.43 (C_β), 87.11 (C1'), 84.77 (C4'), 75.00 (C_α), 69.43 (C3'), 60.89 (C5'), 40.43 (C2'). FABMS m/z 419 (M^++H, <5%), 302 (100), 117 (80). IR (nujol mull) ν_{max} 3470 cm^{-1} (m, NH), 3168 (br, OH), 2224 (C≡C), 1680 (br, C=O) (UV) λ_{max} 303 nm (ε 12,540), 280 (11,890), 204 (23,408). Anal found: C, 48.57; H, 2.71; N, 6.74. Calcd for $C_{17}H_{11}N_2O_5F_5$: C, 48.80; H, 2.63; N, 6.69.

5-(2-Phenylethynyl)-2'-deoxyuridine (41g).

88%. m.p. = 175 - 177°C (lit.[67] 174 - 176°C) ^{1}H NMR ($CDCl_3$/$DMSOd_6$) δ 11.08 (br s, 1 H, NH), 8.02 (s , 1 H, H-6), 7.07 (m, 3 H, ArH), 6.92 (m, 2 H, ArH) 5.90 (t, J = 6.00 Hz, 1 H, H-1'), 4.72 (d, J = 4.11 Hz, 1 H, 3'-OH), 4.55 (t, J = 4.41, 1 H, 5'-OH), 4.01 (m, 1 H, H-3'), 3.55 (m, 1 H, H-4'), 3.40 (m, 2 H, H-5'), 1.94 (m, 1 H, H-2'β), 1.78 (m, 1 H, H-2'$_\alpha$). ^{13}C NMR ($CDCl_3$/$DMSOd_6$) δ 161.13 (C4), 148.70 (C2), 142.65 (C6), 130.46 (ArC2), 127.40 (ArC4), 127.33 (ArC3) 121.87 (ArC1), 98.23 (C5), 91.33 (C_α), 86.88 (C1'), 84.31 (C4'), 80.80 (C_β), 79.49 (C3'), 60.36 (C5'), 40.20 (C2').

5-[2-(4-Methoxyphenyl)ethynyl]-2'-deoxyuridine (41h).

95%. m.p. = 175.0 - 176.0°C. ^{1}H NMR ($CDCl_3$/$DMSOd_6$) δ 10.94 (br s, 1 H, NH), 8.05 (s , 1 H, H-6), 7.14 (d, J = 8.01 Hz, 2 H, ArH), 7.03 (t, J = 8.00, 2 H, ArH) 6.00 (t, J = 5.90 Hz, 1 H, H-1'), 4.66 (br s, 1 H, 5'-OH), 4.44 (br s, 1 H, 3'-OH), 4,16 (br m, 1 H, 3'-H), 3.68 (m, 1 H, H-4'), 3.53 (m, 2 H, H-5'), 2.12 (m, 1 H, H-2'β), 1.95 (m, 1 H, H-2'$_\alpha$). ^{13}C NMR ($CDCl_3$/$DMSOd_6$) δ 161.84 (C4), 159.07 (ArC4), 149.2 (C2), 142.62 (C6), 132.53 (ArC2), 114.32 (ArC1), 113.34 (ArC3), 99.27 (C5), 92.13 (C_α), 87.22 (C1'), 84.92 (C4'), 79.38 (C_β), 70.02 (C3'), 60.95 (C5'), 40.56 (C2'). FABMS m/z 359 (M^++H, <5%). IR (nujol mull) ν_{max} 3400 cm^{-1} (m br, NH), 3248 (br, OH), 1696 (br str , C=O), 1604 (s, C=C).

Deacetylation:

5-{2-[3,5-Bis(α,α,α-trifluoromethyl)phenyl]ethynyl}-2'-deoxyuridine (41b).

3',5'-Di-*O*-acetyl-5-{2-[3,5-bis(α,α,α-trifluoromethyl)phenyl]ethynyl}-2'-deoxyuridine **38b** (87mg, 0.16mmol) was added to a saturated solution of methanolic ammonia (5ml). The resulting solution was stirred for 6hrs, at which point t.l.c. analysis revealed the consumption of **38b** to be complete. Silica (~200mg) was added, the slurry evaporated to dryness and subjected to fash chromatography (hexanes 70% : ethyl acetate 25% : methanol 5%) giving **41b** as a white solid (68mg, 90%). A larger stock of **41b** (135mg) was recrystalized from isopropanol (~0.5ml) to give **41b** as a micro-crystaline solid (114mg, 84%

recovery); m.p. = 247-250 °C. α_D = -8.0 (MeOH,. c = 0.50). ^{1}H NMR ($CDCl_3$/$DMSOd_6$) δ 11.25 (br s, 1 H, NH), 8.37 (s , 1 H, H-6), 7.56 (s, 2 H, ArH), 7.40 (s, 1 H, ArH), 5.90 (t, J = 7.56 Hz, 1 H, H-1'), 4.70 (m, 1 H, OH), 4.55 (m, 3H, OH), 4.04 (m, 1 H, H-3'), 3.59 (m, 1 H, H-4'), 3.40 (m, 2 H, H-5'), 1.98 (m, 1 H, H-2'$_\beta$), 1.76 (m, 1 H, H-2'$_\alpha$). ^{13}C NMR ($CDCl_3$/$DMSOd_6$) δ 160.80 (C4), 148.51 (C2), 143.97 (C6), 130.39 (q, $^2J_{CF}$ = 34.2, ArC3), 130.22 (ArC2), 124.46 (ArC1), 121.9 (q, J_{CF} = 272.5, CF_3), 119.99 (ArC4), 96.88 (C5), 87.97 (C_α), 86.87 (C1'), 84.73 (C_β), 74.44 (C4'), 69.20 (C3'), 60.12 (C5'), 40.15 (C2'). FABMS m/z (M^++H, <5%). IR (nujol mull) ν_{max} 3440 cm^{-1} (m, NH), 3210 (br, OH), 3050 (ArH), 1730 (str , C=O), 1656 (sh, C=C). UV (MeOH) λ_{max} 312 nm (ε 24,274), 203 (41,115). Anal found: C, 49.22; H, 3.18; N, 6.22. Calcd for $C_{19}H_{14}N_2O_5F_6$: C, 49.14; H, 3.03; N, 6.03.

Compounds **41c** and **41d** were prepared in a similar manner as that described for **41b** in yields of 99 & 84% respectively. In each case the yield is for the product isolated by chromatography. High purity samples were obtained from recrystalization in isopranol/water (9:1) (~80% recovery).

5-[2-(3,5-Difluorophenyl)ethynyl]-2'-deoxyuridine (41c).

99%. m.p. = 199-200°C. α_D = -7.2 (MeOH, c = 0.90). ^{1}H NMR ($CDCl_3$/$DMSOd_6$) δ 8.25 (s , 1 H, H-6), 6.91 (m, 2 H, ArH), 6.55 (m, 1 H, ArH) 6.07 (dd, J_{trans} = 11.2 Hz, J_{cis} = 6.12 Hz, 1 H, H-1'), 4.77 (br s, 1 H, OH), 4.52 (br s, 1 H, OH), 4.21 (m, 1 H, H-3'), 3.75 (m, 1 H, H-4'), 3.57 (m, 2 H, H-5'), 2.13 (m, 1 H, H-2'$_\beta$), 1.98 (m, 1 H, H-2'$_\alpha$). ^{13}C NMR ($CDCl_3$/$DMSOd_6$) δ 162.05 (dd, J_{CF} = 248.8 Hz, $^3J_{CF}$ = 13.3, ArC3), 161.47 (C4), 149.08 (C2), 144.09 (C6), 125.27 (t, $^3J_{CF}$ = 12.0 Hz, ArC1), 113.87 (d, $^2J_{CF}$ = 26.56 Hz, ArC3), 103.79 (t, $^2J_{CF}$ = 25.43 Hz, ArC4), 98.02 (C5), 89.76 (C_α), 87.34 (C1'), 85.12 (C4'), 83.45 (C_β), 69.88 (C3'), 60.86 (C5'), 40.84 (C2'). FABMS m/z 365 (M^++H, <5%). IR (nujol mull) ν_{max} 3456 cm^{-1} (m, NH), 3200 (br, OH), 3064 (ArH), 1688 (str, C=O), 1588 (m, C=C). UV (MeOH) λ_{max} 307 nm (ε 25,025), 270 (17690), 264 (14,706), 204 (21,913). Anal found: C, 39.93; H, 3.70; N, 6.20. Calcd for $C_{17}H_{14}N_2O_5F_2$: C, 40.11; H, 3.87; N, 6.24.

5-{2-[4-(α,α,α-Trifluoromethyl)pheny])ethynyl}-2'-deoxyuridine (41d).

84%. m.p. = 192.5-195.0 °C. α_D = -7.84 (MeOH,. c = 1.30). ^{1}H NMR ($CDCl_3$/$DMSOd_6$) δ 10.96 (br s, 1 H, NH), 8.21 (s , 1 H, H-6), 7.39 (d, J = 7.95 Hz, 2 H, ArH), 7.34 (d, J = 7.95 Hz, 2 H, ArH), 6.07 (t, J = 6,42 Hz, 1 H, H-1'), 4.62 (m, 1 H, OH), 4.35 (m, 1 H, OH), 4.25 (m, 1 H, H-3'), 3.77 (m, 1 H, H-4'), 3.62 (m, 2 H, H-5'), 2.17 (m, 1 H, H-2'$_\beta$), 1.96 (m, 1 H, H-2'$_\alpha$). ^{13}C NMR ($CDCl_3$/$DMSOd_6$) δ 161.64 (C4), 149.19 (C2), 143.92 (C6), 131.40 (ArC2), 129.20 (q, $^2J_{CF}$ = 34.2, ArC4), 126.52 (ArC1), 124.75 (q, $^3J_{CF}$ = 3.5 Hz, ArC3), 123.47 (q, J_{CF} = 272.9, CF_3), 98.48 (C5), 90.90 (C_α), 87.41 (C1'), 85.34 (C4'), 83.77 (C_β), 70.05 (C3'), 61.07 (C5'), 40.87 (C2'). FABMS m/z 397 (M^++H, <5%). IR (nujol mull) ν_{max} 3400-3150 cm^{-1} (v br str, NH & OH), 3050 (ArH), 1702 (str, C=O). UV (MeOH) λ_{max} 309 nm (ε 36,234), 222.0 (24,275), 203 (29,264). Anal found: C, 54.47; H, 3.87; N, 6.99. Calcd for $C_{18}H_{15}N_2O_5F_3$: C, 54.55; H, 3.78; N, 7.07.

5-Ethynyl-2'-deoxyuridine (EDU).

5-Trifluoromethanesulfonyl-2'-deoxyuridine **8** (2.1g, 4.56mmol) was converted to crude 5-[2-(trimethylsilyl)ethynyl]-2'-deoxyuridine **41a** in the same manner as that described above. Removal of the decolourizing charcoal by filtration through celite gave crude **41a** dissolved in methanol (15ml). Tetrabutylammoniumfluoride (5ml of 1M solution, 5.0mmol) was added, after 30min all of the crude **41a** was consumed, as shown by t.l.c. (60% hexane, 35% ethyl acetate, 4% methanol, 1% formic acid). Silica (~10g) was added to the reaction mixture and the slurry evaporated to dryness and subjected to flash chromatograhy (hexanes 65%: ethyl acetate 30%: methanol 5%) to give EDU as a white solid (1.05g 91%) (recryst. ethyl acetate/hexanes); m.p. = 200-2°C (lit.[143] = 197-9°C). α_D = + 25.0 (MeOH,. c = 0.70). ^{1}H NMR δ 10.99 (br s, 1 H, NH), 8.13 (s , 1 H, H-6), 6.00 (t, J = 6.33 Hz, 1 H, H-1'), 4.64 (d, J = 4.30 Hz, 1 H, 3'-OH), 4.39 (t, J = 4.76 Hz, 1 H, 5'-OH), 4.17 (m, 1 H, H-3'), 3.71 (m, 1 H, H-4'), 3.54 (m, 2 H, H-5'), 2.97

(s, 1 H, C≡CH), 2.13 (m, 1 H, H-2'β), 1.92 (m, 1 H, H-2'α). UV (MeOH) λ_{max} 287.8 nm (ε 12,559), 225.7 (11,953), 204.2 (8,064).

Reverse Couplings:

5-{2-[3,5-Bis(α,α,α-trifluoromethyl)phenyl]ethynyl}-2'-deoxyuridine (41b).

Copper(I)iodide (6.5mg,0.04mmol) was added to a solution of 3,5-bis(α,α,α-trifluoromethyl)bromobenzene (140mg, 0.48mmol), 5-ethynyl-2'-deoxuridine (EDU, 100mg, 0.40mmol), $Pd(PPh_3)_4$ (23mg, 0.02mmol) and triethylamine (44mg, 0.44mmol) in deoxygenated DMF (2ml). The solution went very dark within a few minutes. After 30min stirring at room temperature the consumption of EDU was complete. The reaction mixture was evaporated to dryness and the residue dissolved in methanol (10ml). Decolourizing charcoal was added and the slurry stirred for 15min, filtered through celite and evaporated on to silica (~200mg). The residue was subjected to flash chromatography (hexanes 70% : ethyl acetate 25% : methanol 5%) giving **41b** as a white solid (67mg, 49%). As mentioned above larger stock of **41b** (135mg) was recrystalized from isopropanol (~0.5ml) to give **41b** as a micro-crystaline solid (114mg, 84% recovery).

Compounds **41c** and **41d** were prepared in a similar manner as that described for **41b**, variations in reaction times and yields are recorded in Table 6. In each case the yield is for the product isolated by chromatography. As mentioned above high purity samples were obtained by recrystalization from isopranol/water (9:1) (~80% recovery). See above for physical data on compounds **41b-d**. Attempts to prepare compounds **41e-g** by a similar route as that just descibed for **41b-d** were unsuccessful (see also Table 6, entries 4-6 for variations in conditions). In each case the starting material, EDU, was consumed slowly as observed by t.l.c., although no product was observed.

Chapter 5:

4-Fluorophenyltrimethylsilylacetylene (entry 5, Table 7).

A stream of nitrogen was passed through a suspension of $Pd(PPh_3)_2Cl_2$ (50mg, 0.172mmol) in diisopropylamine (14ml). To this suspension 4-bromofluorobenzene (1.5g, 8.6mmol), triphenylphosphine (225mg, 0.86mmol) and copper(I)iodide (70mg, 0.43mmol) were added to give an orange suspension. Finally trimethylsilylacetylene (1.0g, 10.32mmol) was added over 30min, during this addition the solution changed colour from orange to brown to black. The reaction was monitored by g.l.c., after 16hrs at room temperature only a small amount of product and mostly starting material could be observed. The reaction mixture was heated to reflux for 3 hours after which time no starting material and only product could be observed. The solution was evaporated under vacuum at room temperature to give a black paste. This residue was suspended in hexanes (~20ml) and filtered through celite to remove catalyst and inorganic salts. The resultant tan solution was again evaporated at room temperature under vacuum to give a tan oil which was distilled using a kugelrohr to give 4–fluorophenyltrimethylsilylacetylene (1.60g, 97%) as a clear oil; b.p. = 80°C (0.1 mmHg, block). ^{1}H NMR δ 7.48 (dd, J_{HH} = 8.72 Hz, $^4J_{HF}$ = 5.73 Hz, 2 H, ArH), 6.94 (t, J_{HH} = 8.70, $^3J_{HF}$ = 8.70, 2 H, ArH), 0.24 [s, 9 H, $Si(CH_3)_3$]. ^{13}C NMR δ 161.79 (d, J_{CF} = 246.6 Hz, C4), 132.95 (C2), 117.20 (d, $^2J_{CF}$ = 22.2 Hz, C3), 116.50 (d, $^4J_{CF}$ = 2.8 Hz, C1), 103.96 (C_α), 93.83 (C_β), -0.10 [$Si(CH_3)_3$]. ^{29}Si NMR δ -17.62 ppm. EIMS m/z 192 (M^+, 15%), 177 (100). IR (film) ν_{max} 2960 cm^{-1} (m, CH), 2160 (str sh, C≡C), 1600 (str sh, C=C). HREIMS found: 192.07762. Calcd for $C_{11}H_{13}SiF$: 192.077057.

Other phenyltrimethylsilylacetylene (Table 7, entries 1-4 & 6) were prepared in a similar manner as that described for 4-fluorophenyltrimethylsilyl-acetylene above, varying only in the reaction time and temperature and the product yield obtained as detailed in Table 7.

Pentafluorophenyltrimethylsilylacetylene (entry 1, Table 7).

b.p. = 50 °C, 0.1 mmHg (block) (lit.[186] = 97°C, 13mmHg). ^{1}H NMR δ 0.29 [s, $Si(CH_3)_3$]. ^{13}C NMR δ 147.73 (d of m, J_{CF} = 253.5 Hz, C2), 142.01 (d of m, $^{3}J_{CF}$ = 261.4 Hz, C4), 137.91 (d of m, J_{CF} = 253.6 Hz, C3), 109.29 (C_β), 100.19 (dt, $^{2}J_{CF}$ = 18.4 Hz, $^{4}J_{CF}$ = 4.0 Hz, C1), 86.92 (C_α), -0.83 [$Si(CH_3)_3$]. ^{29}Si NMR δ -15.38 ppm. EIMS m/z 210 (M^+, 10%), 249 (100). HREIMS found: 264.0397. Calcd for $C_{11}H_9SiF_5$: 264.0393.

3,5-Bis-(α,α,α-trifluoromethyl)phenyltrimethylsilylacetylene (entry 2, Table 7).

98% m.p. = 48-9°C. ^{1}H NMR δ 7.69 (s, 2 H, ArH), 7.59 (s, 1 H, ArH), 0.11 [s, 9 H, $Si(CH_3)_3$]. ^{13}C NMR δ 131.84 (C2), 131.77 (q, $^{2}J_{CF}$ = 32.90 Hz, C3), 125.41 (C1), 122.89 (q, J_{CF} = 272.47 Hz, CF_3), 101.46 (C_α), 98.72 (C_β), -1.60 [$Si(CH_3)_3$]. ^{29}Si NMR δ -16.30 ppm. EIMS m/z 310 (M^+, 100%), 295 (45). IR (film) ν_{max} 3025 cm^{-1} (str sh, ArH), 2956 (m, CH), 2148 (str sh, C≡C), 1616 (C=C). HREIMS found: 310.0792. Calcd for $C_{13}H_{12}SiF_6$: 310.0791.

3,5-Difluorophenyltrimethylsilylacetylene (entry 3, Table 7).

95%. b.p. = 130 °C, 18 mmHg (block). ^{1}H NMR δ 7.26 (m, 2 H, ArH), 6.94 (m, 1 H, ArH), 0.27 [s, 9 H, $Si(CH_3)_3$]. ^{13}C NMR δ 162.70 (dd, $^{1}J_{CF}$ = 249.0, $^{3}J_{CF}$ = 13.3 Hz, C3), 125.90 (t, $^{3}J_{CF}$ = 11.4 Hz, C1), 114.81 (dd, $^{4}J_{CF}$ = 8.52, $^{2}J_{CF}$ = 26.34 Hz, C2), 104.61 (t, $^{2}J_{CF}$ = 25.1 Hz, C4), 102.41 (t, $^{4}J_{CF}$ = 3.55 Hz, C_α), 96.70 (C_β), -0.35 [$Si(CH_3)_3$]. ^{29}Si NMR δ -16.94 ppm. EIMS m/z 210 (M^+, 18%), 195 (100). IR (film) ν_{max} 2960 cm^{-1} (m, CH), 2164 (str sh, C≡C), 1616 (C=C), 1588 (str sh, C=C). HREIMS found: 210.0684. Calcd for $C_{11}H_{12}SiF_2$: 210.0673.

4-(α,α,α-trifluoromethyl)trimethylsilylphenylacetylene (entry 4, Table 7).

97%. b.p. = 150 °C, 20 mmHg (block). ^{1}H NMR δ 7.59 (s, 4 H, ArH), 0.21 [s, 9 H, $Si(CH_3)_3$]. ^{13}C NMR δ 132.19 (C2), 130.20 (q, $^{2}J_{CF}$ = 32.8 Hz, C4), 127.01 (C1), 125.16 (C3), 123.93 (q, J_{CF} = 272.4 Hz, CF_3), 103.43 (C_α), 97.12 (C_β), -0.28 [$Si(CH_3)_3$].

^{29}Si NMR δ -16.95 ppm. EIMS m/z 242 (M^+, 18%), 227 (100). HREIMS found: 242.0740. Calcd for $C_{12}H_{13}SiF_3$: 242.0738.

4-Methoxyphenyltrimethylsilylacetylene (entry 1, Table 7).

87%. b.p. = 130 °C, 0.5 mmHg (block). ^1H NMR δ 7.38 (d, J = 8.79, 2 H, ArH), 6.79 (s, J = 8.80, 2 H, ArH), 03.77 (s, 3H, CH_3), .22 [s, 9 H, $Si(CH_3)_3$]. ^{13}C NMR δ 159.67 (C4), 133.43 (C2), 115.17 (C1), 113.75 (C3), 105.14 (C_α), 88.13 (C_β) [$Si(CH_3)_3$]. ^{29}Si NMR δ -18.16 ppm. EIMS m/z 204 (M^+, 27%), 189 (100). HREIMS found: 204.0978 Calcd for $C_{12}H_{16}SiO$: 204.0970. For comparable spectral data see ref. 163.

Desilylation:

4-Fluorophenylacetylene (entry, Table 9).

4-Fluorophenyltrimethylsilylacetylene (1g, 8.3mmol) was added to a solution of KOH (0.23mg, $4.2x10^{-3}$mmol, 0.05 mol%) in methanol (10ml). The reaction was monitored by g.l.c., no product formation was evident after 3hrs. A further 4.5mg (0.97mol%) of KOH methanol (1.1ml) was added to this solution. After standing at room temperature for 1hr the consumption of starting material and the formation of product was complete. The reaction mixture was diluted with water (20ml) to give a milky emulsion which was extracted with ether (2x15 ml). The ether solution was dried over $MgSO_4$ and the ether removed by distillation at ambient pressure, where the external temperature did not exceed 60°C. The resultant oil was distilled using a kugelrohr to give 4-fluorophenylacetylene (896mg, 90%) as a clear oil; b.p. = 62°C, 28.0mmHg (block) (lit.[187] = 52°C, 30mmHg). ^1H NMR δ 7.48 (dd, J_{HH} = 8.67 Hz, $^4J_{HF}$ = 5.40 Hz, 2 H, ArH), 6.94 (t, J_{HH} = 8.69, $^3J_{HF}$ = 8.69, 2 H, ArH), 3.03 (s, 1 H, C≡CH). ^{13}C NMR δ 162.88 (d, J_{CF} = 250.3 Hz, C4), 134.15 (C2), 118.29 (C1), 115.66 (d, $^2J_{CF}$ = 23.47 Hz, C3), 82.69 (C_α), 77.11 (C_β). EIMS m/z 120 (M^+, 70%), 75 (100). HREIMS found: 120.0375. Calcd for C_8H_5F: 120.0375.

Other phenylacetylenes (Table 9, entries 1-4 & 6) were prepared in a similar manner as that described for 4-fluorophenylacetylene above, varying only in the reaction time , KOH equivalents and the product yield obtained as detailed in Table 9.

Pentafluorophenylacetylene (entry 1, Table 9).

b.p. = 80°C, 20mmHg (block) (lit.[186] = 129-131°C). 1H NMR 3.59 (s, 1 H, C≡CH). ^{13}C NMR δ 147.94 (dm, J_{CF} = 265.9 Hz, C2), 142.06 (dm, $^{3}J_{CF}$ = 253.6 Hz, C4), 137.91 (dm, J_{CF} = 250.7 Hz, C3), 98.89 (t, $^{2}J_{CF}$ = 16.8 Hz, C1), 89.77 (C_β), 67.45 (C_α).

3,5-Bis-(α,α,α-trifluoromethyl)phenylacetylene (entry 2, Table 9).

95% b.p. = 90°C, 20mmHg (block) (lit.[187] = 74°C, 60mmHg). ^{1}H NMR δ 8.04 (s, 1 H, ArH), 8.01 (s, 2 H, ArH), 3.35 (s, 2 H, C≡CH). EIMS m/z 238 (M^+, 100%), 219 (57), 169 (53). IR (film) ν_{max} 3308 cm^{-1} (str sh, C≡C-H), 2996 (w, ArH), 2124 (w, C≡C), 1616 (m, C=C). HREIMS found: 238.0225. Calcd for $C_{10}H_4F_6$: 238.0217.

4-(α,α,α-trifluoromethyl)phenylacetylene (entry 4, Table 9).

95% b.p. = 70°C, 20mmHg (block) (lit.[186] = 56°C, 31mmHg). ^{1}H NMR δ 7.59 (s, 4 H, ArH), 3.20 (s, 1 H, C≡CH). ^{13}C NMR δ 132.35 (C2), 129.07 (q, $^{2}J_{CF}$ = 32.0 Hz, C4), 126.01 (C1), 124.65 (C3), 123.89 (q, J_{CF} = 271.3 Hz, CF_3), 82.11 (C_β), 79.54 (C_α). EIMS m/z 170 (M^+, 100%). IR (film) ν_{max} 3304 cm^{-1} (str sh, C≡C-H), 3010 (w, ArH), 1614 (m, C=C). HREIMS found: 170.0336. Calcd for $C_9H_5F_3$: 170.3043.

3,5-Difluorophenylacetylene (entry 3, Table 9).

93% b.p. = 80°C, 30mmHg (block). ^{1}H NMR δ 7.02 (dd, J = 4.5&2.2 Hz, 2 H, ArH), 6.85 (tt, J = 9.0&2.2 Hz, 1 H, ArH), 3.15 (s, 1 H, -C≡CH). ^{13}C NMR δ 162.62 (dd, J_{CF} = 249.0, $^{3}J_{CF}$ = 13.5 Hz, C3), 125.21 (m, C1), 115.15 (dd, $^{4}J_{CF}$ = 8.45, $^{2}J_{CF}$ = 26.41 Hz, C2), 105.13 (t, $^{2}J_{CF}$ = 25.7 Hz, C4), 79.25 (C_α&C_β). EIMS m/z 138 (M^+, 100%). IR (film) ν_{max} 3304cm^{-1} (str sh, C≡C-H), 3088 (w, ArH), 1678 (w, C=C), 1620

(str, C=C), 1588 (str sh, C=C). HREIMS found: 138.0276. Calcd for $C_8H_4SiF_2$: 138.0281.

4-Methoxyphenylacetylene (entry 6, Table 9).

96%. b.p. = 90°C, 4mmHg (block) (lit.[162] = 73-4°C, 2mmHg). ^{1}H NMR δ 7.43 (d, J = 8.83 Hz, 2 H, ArH), 6.83 (d J = 8.79 Hz, 2 H, ArH), 3.78 (s, 3 H, CH_3) 3.00 (s, 1 H, C≡CH). ^{13}C NMR δ 159.80 (C4), 133.47 (C2), 114.02 (C1), 113.80 (C3), 83.57 (C_α), 75.77 (C_β). EIMS m/z 132 (M^+, 100%). HREIMS found: 132.0581. Calcd for C_9H_8O: 132.0575.

Chapter 6:

1,4-Bis-(3,5-difluorophenyl)-1,3-butadiyne [39 (X = 3,5-F_2), entries 2-4, Table 11].

3,5-Difluorophenylacetylene (100mg, 0.36mmol) was added to a solution of $Pd(PPh_3)_4$ (17mg, 0.015mmol) in deoxygenated DMF (1ml). The yellow solution was allowed to stir at 38°C for 2hrs, after which time no product formation could be observed by t.l.c. (hexanes). Triethylamine (116μl, 0.80mmol) was added and a slight colour change, yellow to orange was observed. After stirring at 38°C for 3hrs a small amount of product could be observed by t.l.c.. The addition of copper(I)iodide (6mg, 0.037mmol) gave immediately a deep red/brown solution, significant product formation was evident by t.l.c. after only 30min. The solution was evaporated and the residue dissolved in hexanes (5ml) and the resulting slurry filtered through celite. Silica (~200mg) was added to the filtrate, the slurry evaporated and the residue subjected to flash chromatography (hexanes). The product, 1,4-bis-(3,5-difluorophenyl)-1,3-butadiyne **39 (X = 3,5 -F_2)** was obtained as a colourless, highly crystaline solid (98mg, 99%). This product was unstable at ambient temperature and turned brown overnight; m.p. = 152-3°C. ^{1}H NMR δ 7.01 (m, 4 H, ArH), 6.66 (m, 2H, ArH). ^{13}C NMR δ 162.3 (dd, J_{CF} = 249.9 Hz, $^3J_{CF}$ = 12.9 Hz, CF), 123.9 (t, $^3J_{CF}$ = 11.6 Hz, ArC), 115.5 (m, ArC), 105.9 (t, $^2J_{CF}$ = 25.4 Hz, ArC), 79.9 (m, Ar-C≡C-), 74.9 (s, Ar-C≡C-). EIMS m/z 274 (M^+, 100%), 255 (M^+-F,

10), 137 (M^+-$C_8H_3F_2$, 15). IR (film) ν_{max} 3088cm^{-1} (str, ArH), 1644 (C=C). HREIMS found: 274.0398. Calcd for $C_{16}H_6F_4$: 274.0405.

1,4-Bis-(4-α,α,α-trifluoromethylphenyl)-1,3-butadiyne [39 (X = 4-CF_3), entry 6, Table 11].

Copper(I)iodide (4.8mg, 0.03mmol) was added to a solution of 4-trifluoromethylphenylacetylene (100mg, 0.59mmol), triethylamine (4.5µl, 0.03mmol) and $Pd(PPh_3)_4$ (13.6mg, 0.012mmol) in DMF (1ml). Over a period of ~0.5hrs at room temperature the solution went from clear orange to very dark green. The solution was allowed to stir at room temperature. After 2hrs some product could be detected by t.l.c.. The solution was allowed to stand overnight. The reaction mixture was worked up and the product purified in the sme manner as outlined above (entry 2-4, Table 11). 1,4-Bis-(4-α,α,α-trifluoromethylphenyl)-1,3-butadiyne **39 (X = 4-CF_3)** was obtained as a colourless highly crystaline product (54mg, 54%); m.p. = 164-5°C. ^{1}H NMR δ 7.62 (m, ArH). ^{13}C NMR δ 132.7, 125.4, 80.9 (Ar-C≡C-), 75.6 (Ar-C≡C-). EIMS m/z 338 (M^+, 100%), 319 (M^+-F, 10), 169 (M^+-$C_9H_4F_3$, 1). IR (film) ν_{max} 2926cm^{-1} (str, ArH), 1648 (C=C). HREIMS found: 338.0524. Calcd for $C_{18}H_8F_6$: 338.0530.

1,4-Diphenyl-1,3-butadiyne [39 (X = H), entry 5, Table 11]

Copper(I)iodide (16mg, 0.10mmol) was added to a solution of phenylacetylene (200mg, 1.96mmol), triethylamine (566µl, 3.92mmol) and $Pd(PPh_3)_4$ (45 mg, 0.04mmol) in THF (2ml). The initially orange solution went red/brown after ~5mins. The solution was allowed to stand at room temperature over night. The reaction mixture was worked up and purified as outlined above (entry 2-4, Table 11). 1,4-Diphenylbutadiyne was obtained as a highly crystaline solid (160mg, 82%); m.p. = 85-6 °C (lit.[185] = 86-7 °C). ^{1}H NMR δ 7.58 (m, 4 H, ArH), 7.28 (m, 6 H, ArH). ^{13}C NMR δ 132.5, 129.4, 128.6, 121.8 (ArC), 81.7 (Ar-C≡C-), 74.1 (Ar-C≡C-).

Bis-1,4-(trimethylsilyl)-1,3-butadiyne.

Method A (entry 7, Table 11): Copper(I)iodide (20mg, 0.13mmol) was added to a solution of pentafluorobromobenzene (600mg, 2.43mmol), trimethylsilylacetylene (282mg, 3.0mmol) and $Pd(PPh_3)_4$ (56mg, 0.05mmol) in deoxygenated diisopropylamine (10ml) to give a very dark (black) solution. The reaction mixture was stirred at 50°C for 5hrs and worked up and purified as outlined above (entry 2-4, Table 11). The isolated bis-1,4-(trimethylsilyl)-1,3-butadiyne was further purified by sublimation (110°C, 0.1mmHg) to give a crystaline product (268mg, 91%).

Method B (entry 8, Table 11): Copper(I)iodide (41mg, 0.26 m mol) was added to a solution of trimethylsilylacetylene (800mg, 5.1mmol) and $Pd(PPh_3)_4$ (115mg, 0.10mmol) in deoxygenated diisopropylamine (10ml) to give a black solution. The reaction mixture was stirred at 50°C for 4hrs, no product was observed by t.l.c. (hexanes).

The reaction mixture was heated at reflux for 6hrs, worked up and the product purified as outlined above (entry 2-4, Table 11). Pure bis-1,4-(trimethysilyl)-1,3-butadiyne was obtained as a crystaline solid (110mg, 22%); m.p. = 113-4°C (lit.[185] = 113°C). ^{13}C NMR δ 88.0(Si-C≡C-), 85.6 (Si-C≡C-), -0.5 ($-Si(CH_3)_3$). IR ($CHCl_3$) ν_{max} 2960 (C-H), 2900 (m s, C-H), 2064 (str s, C≡C).

Chapter 7:

1-(2-Bromo-2-deoxy-3,5-di-*O*-acetylribosyl)-5-acetoxyuracil (84).

Acetyl bromide (14.0ml, 189mmol) was added dropwise over 30min to a suspension of 5-hydroxyuridine **9** (6.2g, 23.6mmol) in acetonitrile (150ml) at 60°C. After the addition was complete the amber solution was allowed to cool and concentrated under vacuum. The residue was dissolved in dichloromethane (50ml), washed with water (3 x 50ml) and dried over $MgSO_4$. Toluene (50ml) was added, the slurry evaporated and subjected to high vacuum (0.001mmHg) for

6 hrs to remove all traces of acetic acid giving **84** and its β-bromo **85** isomer as a beige solid (10.58g, 23.6mmol, 100%). Flash chromatography (hexane 70% : ethyl acetate 38% : methanol 2%) of 600mg of this crude product gave 548mg of **84** and 38mg of its β-isomer **85** (i.e. ~9α : 1β) as white solids; **84**: m.p. = 184-5°C. ^{1}H NMR δ 11.41 (br s, 1 H, NH), 7.38 (s, 1 H, H-6), 5.98 (d, $J_{H\text{-}1',2'}$ = 5.1 Hz, 1 H, H-1'), 4.89 (dd, $J_{H\text{-}3',2'}$ = 5.4 Hz, $J_{H\text{-}3',4'}$ = 2.8 Hz, 1 H, H-3'), 4.48 (dd, $J_{H\text{-}2',1'}$ = $J_{H\text{-}2',3'}$ = 5.3 Hz, 1 H, H-2'), 4.19 (m, 1 H, H-4'), 4.15 (m, 2 H, H-5'), 2.05, 1.95, 1.90 (3 x s, 3 x 3 H, 3 x CH_3CO_2). FABMS m/z 449&451 (d, M^++H, <5%). Anal found: C, 52.96; H, 3.72; N, 7.33. Calcd. for $C_{15}H_{17}N_2O_9Br$: C, 52.75; H, 3.85; N, 7.69.

85: ^{1}H NMR δ 11.54 (br s, 1 H, NH), 7.41 (s, 1 H, H-6), 6.12 (d, $J_{H\text{-}1',2'}$ = 5.1 Hz, 1 H, H-1'), 4.91 (m, 1 H, H-3'), 4.30 (m, 1 H, H-2'), 4.21 (m, 1 H, H-4'), 4.15 (m, 2 H, H-5'), 2.03, 1.98, 1.91 (3 x s, 3 x 3 H, 3 x CH_3CO_2). FABMS m/z 449&451 (d, M^++H, <5%).

3',5'-Di-*O*-acetyl-5-acetoxy-2'-deoxyuridine (87).

Tri-*n*-butyltinhydride (3.7g, 30.0mmol) was added to a solution of crude 1-(2-bromo-2-deoxy-3, 5-di-*O*-acetylribosyl)-5-acetoxyuracil **84** (9.0g, 20.0mmol) and 2,2'-azobis-(2-methylpropionitrile) (AIBN, 0.5g) in 1,4-dioxane (150ml) and the solution heated to reflux. After 1.5hrs all of the starting material **84** had been consumed and a single new product obtained by t.l.c. (hexanes/ethyl acetate 1:1). The solution was allowed to cool and was evaporated under vacuum. The residue was dissolved in ethyl acetate (50ml) and washed with 10% KF(aq.) (2x50ml), water (50ml) and dried over $MgSO_4$. The organic phase was evaporated and the residue purified by flash chomatography (hexane 50% : ethyl acetate 45% : methanol 5%) giving **87** (5.62g, 76%) as a solid foam; m.p. = 64-5°C. ^{1}H NMR δ 10.13 (br s, 1 H, NH), 7.39 (s, 1 H, H-6), 6.07 (t, $J_{H\text{-}1',2'}$ = 7.0 Hz, 1 H, H-1'), 5.01 (br s, 1 H, H-3'), 4.19-4.05 (br m, 3 H, H-4'&5'), 2.32 (m, 1 H, H-2'$_\beta$), 2.06 (s, 3 H, C5-O_2CCH_3), 2.05 (m, 1 H, H-2'$_\alpha$), 1.90, 1.89 (2 x s, 2 x 3 H, 2 x CH_3CO_2). FABMS m/z 371 (M^++H, <5%).

5'-*O*-Acetyl-5-acetoxy-2',3'-dideoxyuridine (90):

Zinc/copper couple (3.5g, 53.4mmol) was added to a solution of **84** (3.0g, 6.68mmol) in methanol (25ml) and the slurry stirred at room temperature for 1.5hrs. After which time only a bright green baseline spot was detectable by t.l.c. as well as two other faint spots of high R_f (hexane/ethyl acetate 1:1), all starting material had been consumed. The reaction mixture was filtered and formic acid was added until a pH of 4.5-5.5 was obtained and the solution stirred at room temperature for 2.5hrs. The resulting slurry was concentrated to half its volume and filtered to remove insoluble salts.

The filtrate was concentrated to give a solid foam. This residue was dissolved in pyridine (10ml) and acetic anhydride (2ml) was added and the solution allowed to stir overnight. Two intense spots were evident by t.l.c. (hexanes/ethyl acetate 1:1). The reaction mixture was concentrated and the residue dissolved in ethyl acetate (30ml) and washed with 10% citric acid (aq., 30ml) and water (30ml). The organic phase was dried over $MgSO_4$ and concentrated under vacuum to gice a foam.

The foam was dissolved in a water/ethanol mix (1:9), 5% palladium on charcoal (500mg) was added and the slurry stirred overnight under an atmosphere of H_2(g). The reaction mixture was filtered through celite and concentrated to dryness. The residue was subjected to flash chromatography (hexanes/ethyl acetate 3:2) to give **90** (0.24g, 11%) and 2',3',5'-tri-*O*-acetyl-5-acetoxyuridine **91** (1.52g, 55%) as foams; ^{1}H NMR δ 10.09 (br s, 1 H, NH), 7.67 (s, 1 H, H-6), 5.96 (m, 1 H, H-1'), 4.25 (m, 3 H, H-4'&5'), 2.40 (m, 1 H, H-2'$_\beta$), 2.34 (s, 3 H, C5-O_2CCH_3), 2.04 (s, 3 H, C5'-O_2CCH_3). 2.02 (m, 2 H, H-2'$_\alpha$&3'$_\beta$), 1.74 (m, 1 H, H-3'$_\alpha$). FABMS m/z 313 (M^++H, <5%).

3',5'-Di-*O*-acetyl-5-hydroxy-2'-deoxyuridine (92):

Zinc/copper couple (1.0g, 15.0mmol) was added to a solution of **87** (1.4g, 3.78mmol) in methanol (20ml) and the slurry stirred at room temperature for 5hrs. The reaction mixture filtered, formic acid (3ml) was added and the solution stirred for 2hrs. The solution was concentrated to ~5ml and diluted with ethyl acetate (20ml). The filtrate was evaporated and purged of all formic acid under high vacuum (0.001mmHg) to give **92** (1.0g, 81%) as a solid foam which was pure by ^{1}H NMR; m.p. = 71-4 °C. ^{1}H NMR δ 10.75 (br s, 1 H, NH), 7.21 (s, 1 H, H-6), 6.32 (m, 1 H, H-1'), 5.18 (br s, 1 H, H-3'), 4.28 - 4.18 (br m, 3 H, H-4'&5'), 2.42 (m, 1 H, H-2'$_\beta$), 2.05 (m, 1 H, H-2'$_\alpha$), 2.13, 2.06 (2 x s, 2 x 3 H, 2 x CH_3CO_2). ^{13}C NMR δ 170.5 (CO_2), 161.1 (C4), 149.1 (C2), 133.6 (C5), 118.4 (C6), 84.8 (C1'), 82.0 (C4'), 74.1 (C3'), 63.8 (C5'), 37.03 (C2'), 20.8, 20.2 (2 x $\underline{C}H_3CO_2$). FABMS m/z 329 (M^++H, <5%).

5'-*O*-{[Dimethyl-2-(2,3-dimethylbutyl)]silyl}-5-trifluoromethanesulfonyl-2'-deoxyuridine (93):

Dimethylthexylsilyl chloride (421mg, 2.35 m mol) was added dropwise over 1hr to a solution of **8** (630mg, 1.68mmol) in pyridine 5ml and the reaction mixture stirred at room temperature overnight. The solution was concentrated and dissolved in dichloromethane (50ml) washed with 10% citric acid (aq., 50ml), water (2 x 50ml) and dried over $MgSO_4$. Silica (~1g) was added to the organic phase and the slurry evaporated to dryness and subjected to flash chromatography (hexanes/ethyl acetate 7:3). The desired monosilylated product **93** (583mg, 67%) was isolated as a crystaline white solid and some disilylated by-product (243mg, 22%) as a solid foam; **93**: m.p. = 155-7 °C. ^{1}H NMR δ 10.25 (br s, 1 H, NH), 8.49 (s, 1 H, H-6), 6.18 (t, $J_{H\text{-}1',2'}$ = 5.8 Hz, 1 H, H-1'), 4.45 (m, 1 H, H-3'), 3.97-3.75 (m, 3 H, H-4',5'), 2.45 - 2.15 (m, 1 H, H-2'), 1.65 - -0.04 [19 H, $Si(CH_3)_2C(CH_3)_2CH(CH_3)_2$]. ^{13}C NMR δ 156.8 (C4), 148.6 (C2), 135.0 (C6), 136.5 (C5), 118.5 (q, J_{CF} = 321.0 Hz, CF_3), 87.6 (C1'), 86.5 (C4'), 70.57 (C3'), 64.0 (C5'), 33.2 (C2',3'), 33.9 - -0.99 [$Si(CH_3)_2C(CH_3)_2CH(CH_3)_2$]. FABMS m/z 519 ($M^+$+H, <5%). IR ($CHCl_3$) ν_{max} 2960 cm^{-1} (m, C-H), 1710 (str br, C=O), 1136 (str, SO_3CF_3)

5'-*O*-{[Dimethyl-2-(2,3-dimethylbutyl)]silyl}-3'-*O*-(1-imidazolthiocarbonyl)-5-trifluoromethanesulfonyl-2'-deoxyuridine (94):

A solution of **93** (100mg, 0.19mmol) and 1,1'-thiocarbonyldiimidazole (69mg, 0.38mmol) in dry 1,2-dichloroethane (3ml) was stirred for 7hrs at room temperature. Silica (~ 200mg) was added to the solution, the solvent evaporated and the residue subjected to flash chromatography (hexanes/ethyl acetate 3:2). The product **94** (102mg, 85%) was obtained as a solid foam; ^{1}H NMR δ 9.12 (br s, 1 H, NH), 8.43 (s, 1 H, N=CH-N), 7.95 (s, 1 H, H-6), 7.63 (br s, 1 H, CH=C<u>H</u>-N-C=S), 7.06 (br s, 1 H, C<u>H</u>=CH-N-C=S), 6.32 (dd, $J_{H\text{-}1',2'\alpha\beta}$ = 8.9&5.2 Hz, 1 H, H-1'), 5.90 (d, J_{H}-3',2' = 5.7 Hz, 1 H, H-3'), 4.43 (br s, 1 H, H-4'), 4.03 (ABX, J_{AB} = 11.4 Hz, J_{AX} = 1.5 Hz, J_{BX} = 0.9 Hz, 2 H, H-5'), 2.83&2.26 (2 x m, 2 x H, H-2'), 1.65 - -0.16 [19 H, $Si(CH_3)_2C(CH_3)_2CH(CH_3)_2$]. ^{13}C NMR δ 182.8 (C=S), 157.2 (C4), 149.2 (C2), 136.8 (N-CH=N), 132.9 (C6), 131.9 (C5), 130.8 (CH=<u>C</u>H-N-C=S), 118.5 (q, J_{CF} = 32.0 Hz, CF_3), 118.4 (N-<u>C</u>H=CH-N-C=S), 86.2 (C1'), 865.4 (C4'), 84.2 (C3'), 63.56 (C5'), 38.8 (C2'), 33.82 - -4.45 [$Si(CH_3)_2C(CH_3)_2CH(CH_3)_2$]. FABMS m/z 629 ($M^+$+H, <5%). IR ($CHCl_3$) ν_{max} 3390 cm^{-1} (w, NH), 2960 (m, C-H), 1712 (str br, C=O), 1196 (str, SO_3).

5-*O*-{[Dimethyl-2-(2,3-dimethylbutyl)]silyl}-2',3'-dideoxy-5-trifluoromethanesulfonyluridine (95):

A solution of **94** (70mg, 0.11mmol), 2,2'-azobis-(2-methylpropionitrile) (AIBN, ~1mg), and tri-*n*-butyltin hydride (47 mg, 0.16mg) in toluene (1ml) was refluxed for 1hr. The solution was evaporated and dissolved into dichroromethane (10ml), washed with 10% KF(aq.) (2 x 10ml) and water (10ml) and dried over $MgSO_4$. Silica (~200mg) was added to the organic phase which was then concentrated and the residue subjected to flash chromatography (hexane/ethyl acetate 9:1) giving **95** (44mg, 79%) as a viscous resin; ^{1}H NMR δ 8.97 (br s, 1 H, NH), 8.08 (s , 1 H, H-6), 5.92 (m, 1 H, H-1'), 4.18 (m, 1 H, H-4'), 3.96&3.68 (2 x m, 2 x 1 H, H-5'), 2.43 (m, 1 H, H-2'β), 2.12 - 1.89 (m, 3H, H2'α & 2 x H-3'), 1.65 - -0.04 [19 H, $Si(CH_3)_2C(CH_3)_2CH(CH_3)_2$]. ^{13}C NMR δ 157.2 (C4), 148.3

(C2), 134.3 (C6), 131.6 (C5), 87.6 (C1'), 82.5 (C4'), 64.0 (C5'), 33.2 (C3'), 25.0 (C3'), 33.9 - -0.99 [$Si(CH_3)_2C(CH_3)_2CH(CH_3)_2$]. FABMS m/z 503 ($M^+$+H, <5%). IR ($CHCl_3$) ν_{max} 3380 cm^{-1} (w, NH), 2952 (str, C-H), 1706 (str br, C=O), 1430 (m, C=C).

5'-*O*-{[Dimethyl-2-(2,3-dimethylbutyl)]silyl}-5-trifluoromethanesulfonyluridine (96):

Dimethylthexylsilyl chloride (1.8g, 10.0mmol) was added dropwise over 2hrs to a solution of **7** (2.0g, 5.1 m mol) in pyridine 20ml and the mixture stirred for 50hrs at room temperature. The solution was concentrated, residue dissolved in ethyl acetate (50ml), washed with 10% citric acid (aq., 50ml) and water (2 x 50ml) and dried over $MgSO_4$. Silica (~3g) was added to the solution, the slurry evaporated to dryness and subjected to flash chromatography (hexanes/ethyl acetate 7:3). The desired monosilylated product **96** (2.2g, 81%) was obtained as a white solid; m.p. = 144-7 °C. ^1H NMR ($CDCl_3$/$DMSOd_6$) δ 7.41 (s, 1 H, H-6), 5.32 (d, $J_{H\text{-}1',2'}$ = 3.3 Hz, 1 H, H-1'), 4.62, 4.34 (2 x br s, 2 x 1 H, C2' & 3'-OH), 3.50 (m, 3 H, H-2',3', 4'), 3.25 (ABX, 2 H, H-5'), 1.10 - -0.04 [18 H, $Si(CH_3)_2C(CH_3)_2CH(CH_3)_2$]. ^{13}C NMR ($CDCl_3$/$DMSOd_6$) δ 156.6 (C4), 148.5 (C2), 132.7 (C6), 125.7 (C5), 88.3 (C1'), 84.4 (C4'), 74.2 (C3'), 69.4 (C2'), 61.8 (C5'), 32.9 - 4.41 [$Si(CH_3)_2C(CH_3)_2CH(CH_3)_2$]. FABMS m/z 535 ($M^+$+H, <5%). IR ($CHCl_3$) ν_{max} 3412 cm^{-1} (br str, OH), 2952 (str, C-H), 1710 (br str, C=O).

5'-*O*-{[Dimethyl-2-(2,3-dimethylbutyl)]silyl}-2',3'-*O*-thiocarbonyl-5-trifluoromethanesulfonyluridine (97).

A solution of **96** (500mg, 0.94mmol) in ethyl acetate (1.5ml) was diluted with 1,2–dichloroethane (12ml) and 1,1'-thiocarbonylimidazole (260mg, 1.5mmol) was added, the resulting solution was stirred at room temperature for 3hrs. Silica (~1.5g) was added and the solvent removed. The residue was purified by flash chromatography (hexanes/ethyl acetate 1:1) to give **97** (410mg, 76%) as a white solid; m.p. = 90-91 °C. ^1H NMR δ 7.73 (s, 1 H, H-6), 5.84 (d, $J_{H\text{-}1',2'}$ = 1.6 Hz, 1 H, H-1'), 5.61 (dd, $J_{H\text{-}2',1'}$ = 1.7 Hz, $J_{H\text{-}2',3'}$ = 7.5 Hz, 1 H, H-2'), 5.42 (dd,

$J_{H-3',2'}$ = 7.4 Hz, $J_{H-3',4'}$ = 2.9 Hz, 1 H, H-3'), 4.59 (dd, $J_{H-3',4'}$ = 3.1 Hz, $J_{H-4',5'}$ = 4.0 Hz, 1 H, H-4'), 3.85 (d, J = 4.2 Hz, 2 H, H-5'), 1.58 - -0.08 [19 H, $Si(CH_3)_2$-$C(CH_3)_2CH(CH_3)_2$]. ^{13}C NMR δ 189.3 (C=S), 157.4 (C4), 148.6 (C2), 134.9 (C6), 126.7 (C5), 118.1 (q, J_{CF} = 321 Hz, CF_3), 94.0 (C1'), 88.3 (C4'), 87.6 (C2',3'), 62.2 (C5'), 33.9 - -3.7 [$Si(CH_3)_2C(CH_3)_2CH(CH_3)_2$]. FABMS m/z 577 ($M^+$+H, <5%). IR ($CHCl_3$) ν_{max} 2948 (str, C-H), 1720 (str br, C=O&C=S).

5-*O*-{[Dimethyl-2-(2,3-dimethylbutyl)]silyl}-2',3'-dideoxydidehydro-5-trifluoromethanesulfonyluridine (98).

A solution of **97** (200mg, 0.35mmol) in trimethylphosphite (1.5ml) was refluxed for 1hr, after which time t.l.c. revealed complete consumption of **97**. The solution was evaporated at room temperature and dissolved in dichloromethane. Silica (~500mg) was added to the solution, the slurry evaporated and the residue subjected to flash chromatography (hexanes/ethyl acetate 85:15) giving **98** (40mg, 23%) as a viscous resin; ^1H NMR δ 7.96 (s, 1 H, H-6), 6.90 (m, 1 H, H-1'), 6.33 (dm, $J_{H-2',3'}$ = 5.0 Hz,1 H, H-2'), 5.9 (br d, $J_{H-2',3'}$ = 5.0 Hz, 1 H, H-3'H), 4.90 (br s, 1 H, H-4'), 3.8 (m, 2 H, H-5'), 1.60 - -0.07 [19 H, $Si(CH_3)_2C(CH_3)_2CH(CH_3)_2$]. ^{13}C NMR δ 157.2 (C4), 149.7 (C2), 135.6 (C6), 132.1 (C5), 125.4 (C2',3'), 118.5 (q, J = 321.0 Hz, CF_3), 91.8 (C1'), 87.9 (C4'), 64.3 (C5'), 33.2 (C2',3'), 33.9 - -3.7 [$Si(CH_3)_2C(CH_3)_2CH(CH_3)_2$]. FABMS m/z 501 ($M^+$+H, <5%). IR ($CHCl_3$) ν_{max} 3410 cm^{-1} (br w, NH), 2952 (str, C-H), 2864 (m, C-H), 1724 (m, C=O), 1678 (str, C=C).

5'-*O*-{[Dimethyl-2-(2,3-dimethylbutyl)]silyl}-5-{[dimethyl-2-(2,3-dimethylbutyl)]siloxy}uridine (101).

Dimethylsilylthexyl chloride (4.1g, 23.1mmol) was added dropwise over 2hrs to solution of 5-hydroxyuridine **9** (2.0g, 7.7mmol) in pyridine (30ml) and the reaction mixture allowed to stir at room temperature for 50hrs. The solution was concentrated, dissolved in ethyl acetate (70ml), washed in 10% citric acid (aq., 50ml) and water (2 x 50ml) and dried over $MgSO_4$. Silcia (~3g) was added to the

organic phase and the slurry evaporated to dryness and purified by flash chromatography (hexanes/ethyl acetate 7:3). The desired disilylated product **101** (2.7g, 65%) was isolated as a solid foam; ^{1}H NMR δ 7.08 (s, 1 H, H-6), 5.84 (t, $J_{H-1',2'}$ = 4.3 Hz, 1 H, H-1'), 5.21 (br s, 2 H, 2 x OH), 4.08 (br s, 3 H, H-2',3',4'), 3.74 (ABX, 2 H, H-5'), 1.66 to -0.08 [38 H, 2 x $Si(CH_3)_2C(CH_3)_2CH(CH_3)_2$]. ^{13}C NMR δ 160.9 (C4), 150.3 (C2), 132.3 (C6), 124.5 (C5), 89.3 (C1'), 85.2 (C4'), 75.0 (C2'), 70.5 (C3'), 62.9 (C5'), 33.9 - -3.62 [2 x $Si(CH_3)_2C(CH_3)_2CH(CH_3)_2$]. IR ($CHCl_3$) ν_{max} 3400 cm^{-1} (br str, OH/NH), 2956 (str, C-H), 1632 (m, C=C). HREIMS Found: 516.2632. Calcd for $C_{23}H_{43}Si_2N_2O_7$ (M^+-CH_3CH_2): 516.2608

5'-*O*-{[Dimethyl-2-(2,3-dimethylbutyl)]silyl}-2',3'-*O*-thiocarbonyl-5-{[dimethyl-2-(2,3-dimethylbutyl)]siloxy}uridine (102)

A solution of **101** (1.5g, 2.8mmol) and 1,1'-thiocarbonyldiimadazole (997mg, 5.6mmol) in 1,2-dichloromethane (20ml) was stirred at room temperature for 4hrs. Silica (~2-3g) was added, the slurry concentrated and the residue subjected to flash chromatography (hexanes/ethyl acetate 3:2). The product **102** (1.2g, 74%) was obtained as a white solid; m.p. = 116-7 °C. ^{1}H NMR δ 10.63 (br s, 1 H, NH), 6.82 (s, 1 H, H-6), 5.80 (d, $J_{H-1',2'}$ = 7.32 Hz, 1 H, H-1'), 5.50 (br s, 1 H, H-3'), 5.43 (m, 1 H, H-2'), 4.27 (m, 1 H, H-4'), 3.85 (m, 2 H, H-5'), 1.63 - -0.04 [38 H, 2 x $Si(CH_3)_2C(CH_3)_2CH(CH_3)_2$]. ^{13}C NMR δ 189.3 (C=S), 160.9 (C4), 149.2 (C2), 131.7 (C5), 128.3 (C6), 94.8 , 88.2, 87.7, 85.8 (C1'-4'), 62.3 (C5'), 36.3 - -3.8 [2 x $Si(CH_3)_2C(CH_3)_2CH(CH_3)_2$]. FABMS m/z 587 ($M^+$+H, <5%). IR ($CHCl_3$) ν_{max} 2960 cm^{-1} (str, C-H), 1706 (br str, C=O&C=S). UV λ_{max} 273.8 nm (ε 9,872), 233.2 (19,034). Anal found: C, 52.97; H, 8.10; N, 4.57. Calcd. for $C_{26}H_{46}Si_2N_2O_7S$: C, 53.21; H, 7.89; N, 4.77.

5-*O*-{[Dimethyl-2-(2,3-dimethylbutyl)]silyl}-2,2'-anhydro-3'-*O*-thiocarboxy-5-trifluoromethanesulfonyluridine (105) and 5-*O*-{[Dimethyl-2-(2,3-dimethylbutyl)]silyl}-2,2'-anhydro-5-trifluoromethanesulfonyluridine (106)

A solution of **96** (200mg, 0.37mmol) and 1,1'-thiocarbonyldiimadazole (79mg, 0.44mmol) in DMF (3ml) was stirred at room temperature for 40hrs. The reaction mixture was concentrared and the residue dissolved in dichloromethane (20ml). Silica (~1g) was added, the slurry concentrated and the residue subjected to flash chromatography (hexanes/ethyl acetate 3:2). Three products were isolated from the column (**106**, 107mg, 55%; **105**, 15mg, 7%; **97**, 20mg, 8%) as a white solids: **106**; m.p. =249-50°C. ^{1}H NMR δ 7.96 (s, 1 H, H-6), 6.37 (d, $J_{H\text{-}1',2'}$ = 5.8 Hz, 1 H, H-1'), 5.51 (d, $J_{H\text{-}2'.1'}$ = 6.0 Hz, 1 H, H-2'), 5.22 (br s, 1 H, OH), 4.58 (br s, 1 H, H-3'), 4.26 (m, 1 H, H-4'), 3.55 (m, 2 H, H-5'), 1.54 - -0.03 [19 H, $Si(CH_3)_2C(CH_3)_2CH(CH_3)_2$]. ^{13}C NMR δ 165.3 (C4), 158.8 (C2), 133.2 (C5), 129.4 (C6), 118.4 (q, J_{CF} = 320.7 Hz, CF_3), 91.35, 90.9, 89.0, 75.1 (C1'-4'), 62.1 (C5'), 33.9 - -3.7 [$Si(CH_3)_2C(CH_3)_2CH(CH_3)_2$]. UV (MeOH) λ_{max} 256.5 nm (ε 5,785), 224.6 (7,402), 205.5 (5,765).

105; m.p. =254-5°C. ^{1}H NMR δ 7.60 (s, 1 H, H-6), 6.45 (d, $J_{H\text{-}1',2'}$ = 5.9 Hz, 1 H, H-1'), 5.83 (br s, 1 H, H-3'), 5.73 (d, $J_{H\text{-}2'.1'}$ = 5.9 Hz, 1 H, H-2'), 4.56 (br s, 1 H, H-4'), 3.85-3.60 (m, 2 H, H-5'), 1.58 - -0.03 [19 H, $Si(CH_3)_2C(CH_3)_2CH(CH_3)_2$]. FABMS m/z 577 ($M^+$+H, <5%). IR ($CHCl_3$) ν_{max} 2948 cm^{-1} (str, C-H), 1720 (str br, C=O&C=S). UV (MeOH) λ_{max} 255.2 nm (ε 2,803), 224.4 (3,856), 204.9 (4,992).

97: see above.

5-*O*-{[Dimethyl-2-(2,3-dimethylbutyl)]silyl}-2,2'-anhydro-3'-*O*-thiocarboxy-5-{[dimethyl-2-(2,3-dimethylbutyl)]siloxy}uridine (103) and 5-*O*-{[Dimethyl-2-(2,3-dimethylbutyl)]silyl}-2,2'-anhydro-5-{[dimethyl-2-(2,3-dimethylbutyl)]siloxy}-uridine (104)

Compounds **103** and **104** were prepared from **101** (2.5g, 4.60mmol) in a similar manner as that described for **105** and **106** above. Purification by chromatography (hexanes/ethyl acetate 4:1) gave three products (**104**, 1.5g, 63%; **103**, 297mg, 11%; **102**, 323mg, 12%) as white solids: **104**; m.p. = 194-5°C. ^{1}H NMR δ

6.93 (s, 1 H, H-6), 6.17 (d, $J_{H-1',2'}$ = 5.6 Hz, 1 H, H-1'), 6.05 (br s, 1 H, OH), 5.41 (d, $J_{H-2',1'}$ = 5.5 Hz, 1 H, H-2'), 4.52 (br s, 1 H, H-3'), 4.23 (m, 2 H, H-4'), 3.44 (m, 2 H, H-5'), 1.63 - -0.05 [38 H, 2 x $Si(CH_3)_2C(CH_3)_2CH(CH_3)_2$]. ^{13}C NMR δ 167.0 (C4), 156.6 (C2), 138.3 (C5), 119.7 (C6), 90.7, 89.9, 88.7 (C1',2',4'), 74.9 (C3'), 62.2 (C5'), 33.8 - -3.7 [2 x $Si(CH_3)_2C(CH_3)_2CH(CH_3)_2$]. EIMS m/z 511 ($M^+$-$CH_3$, <5%). IR ($CHCl_3$) ν_{max} 3312 cm^{-1} (br m, OH), 2956 (str, C-H), 1632 (br str, C=O&C=S), 1574 (str, C=N), 1494 (str, C=C). UV λ_{max} 264.5 nm (ε 6,003), 206.8 (5,763). Anal found: C, 56.96; H, 9.07; N, 5.21. Calcd. for $C_{25}H_{46}Si_2N_2O_6$: C, 57.00; H, 8.80; N, 5.32.

103; m.p. = 198-9 °C. ^1H NMR δ 6.86 (s, 1 H, H-6), 6.22 (d, $J_{H-1',2'}$ = 5.9 Hz, 1 H, H-1'), 5.81 (s, 1 H, H-3'), 5.48 (d, $J_{2',1'}$ = 5.9 Hz, 1 H, H-2'), 4.45 (t, $J_{4',5'}$ = 5.4 Hz, 1 H, H-4'), 3.53 (m, 2 H, H-5'), 1.64 - -0.01 [38 H, $Si(CH_3)_2C(CH_3)_2CH(CH_3)_2$]. ^{13}C NMR δ 191,3 (C=S), 168.1 (C4), 156.2 (C2), 138.8 (C5), 118.5 (C6), 94.8, 88.2, 87.7, 85.8 (C1',2',3',4'), 62.4 (C5'), 34.0 - -6.5 [$Si(CH_3)_2C(CH_3)_2CH(CH_3)_2$]. FABMS m/z 587 ($M^+$+H, <5%). IR ($CHCl_3$) ν_{max} 2956 cm^{-1} (str, C-H), 1636 (br str, C=O&C=S), 1582 (C=N). UV λ_{max} 264.0 nm (ε 6,817), 233.8 (6,567). Anal found: C, 53.31; H, 8.18; N, 4.78. Calcd. for $C_{26}H_{46}Si_2N_2O_7S$: C, 53.21; H, 7.89; N, 4.77.

102: see above.

5'-*O*-{[Dimethyl-2-(2,3-dimethylbutyl)]silyl}-2',3'-dideoxdidehydro-5-[(dimethyl-2-(2,3-dimethylbutyl))silyloxy]uridine (107).

A solution of **102** (450mg, 1.3mmol) in trimethylphosphite (2ml) was refluxed for 3hrs at which time t.l.c. indicated very nearly complete consumption **102**. The solution was allowed to cool, the solvent evaporated and the residue dissolved in dichloromethane (5ml). Silica (~1g) was added, the slurry concentrated and the residue subjected to flash chromatography (hexanes/ethyl acetate 9:1) giving **107** (285mg, 73%) as a viscous resin; m.p. = 116-7 °C. ^1H NMR δ 8.54 (br s, 1 H, NH), 6.96 (s, 1 H, H-6), 6.79 (s, 1 H, H-1'), 6.40 (d, $J_{H-2',3'}$ = 6.0 Hz, 1 H, H-3'), 5.78 (d, $J_{H-2',3'}$ = 5.9 Hz, 1 H, H-2'), 4.80 (br s, 1 H, H-4'), 3.80 - 3.51 (ABX, 2 H, H-5'), 1.71 - -0.04 [38 H, 2 x $Si(CH_3)_2C(CH_3)_2CH(CH_3)_2$]. ^{13}C NMR δ 160.3 (C4), 149.5 (C2), 135.7 (C5), 132.1 (C6), 125.3 (C3'), 124.5 (C2'), 90.3 (C1'), 86.70 (C4'), 65.31

(C5'), 34.1 - -8.5 [2 x $Si(CH_3)_2C(CH_3)_2CH(CH_3)_2$]. FABMS m/z 511 ($M^+$+H, <5%). IR ($CHCl_3$) ν_{max} 2960 cm^{-1} (m, C-H), 1724 (br str, C=O), 1682 (str, C=O).

5-*O*-{[Dimethyl-2-(2,3-dimethylbutyl)]silyl}-2,3'-anhydro-5-trifluoromethanesulfonyl-2'-deoxyuridine (108).

A slurry of **93** (430mg, 0.83mmol) in toluene (3ml) was heated to 90°C to give a clear solution. Triphenylphosphine (230mg, 0.88mmol) was added and the temperature maintained at 90°C during the dropwise addition of diethylazodicarboxylate (DEAD, 160mg, 0.91mmol) over 2 minutes. After heating for a further 1hr the solution was cooled and the solvent removed. The residue was dissolved in ethyl acetate (10ml), silica (~1g) was added and the slurry concentrated and subjected to flash chromatography (hexanes 68% : ethyl acetate 30% : methanol 2%). The product **108** (400mg, 96%) was isolated as a white solid; m.p. = 192-3 °C. ^1H NMR δ 7.53 (s, 1 H, H-6), 5.79 (m, 1 H, H-1'), 5.20 (br s, 1 H, H-3'), 4.25 (m, 1 H, H-4'), 3.81-3.62 (m, 2 H, H-5'), 2.84-2.45 (m, 2 H, H-2'), 1.58 - -0.05 [19 H, $Si(CH_3)_2C(CH_3)_2CH(CH_3)_2$]. ^{13}C NMR δ 164.06 (C4), 152.87 (C2), 133.05 (C6), 132.25 (C5), 118.42 (q, J_{CF} = 320.6 Hz, CF_3), 88.87 (C1'), 86.07 (C4'), 77.66 (C3'), 60.67 (C5'), 34.05 (C2'), 33.34 - -3.67 [$Si(CH_3)_2C(CH_3)_2CH(CH_3)_2$]. EIMS m/z 500 ($M^+$, <5%), 424 (80). IR ($CHCl_3$) ν_{max} 2968 cm^{-1} (m, CH), 1666 (str br, C=O), 1536 (str, C=O), 1468 (m, C=C), 1430 (m, C-O), 1196 (str, SO_3). UV (MeOH) λ_{max} 256.2 nm (ε 6,105), 256.2 (7,756), 206.0 (8,697). HREIMS found: 500.1220. Calcd for $C_{18}H_{27}SiN_2O_7SF_3$: 500.1260.

5-Trifluoromethanesulfonyluracil (110).

N-Phenyltriflimide (251mg, 0.70m mol) was added to a solution of isobarbituric acid 75mg, 0.59mmol) in pyridine (2ml) and allowed to stir overnight. The reaction mixture was concentrated onto silica (~0.5g) and subjected to flash chromatography (hexanes/ethyl acetate 1:1) giving **110** (95mg, 62% as a crystaline solid; m.p. = 268-70 °C. ^1H NMR δ 11.80 (br s, 1 H, NH), 11 41 (v br s, 1 H, NH), 7.97 (s, 1 H, H-6). ^{13}C NMR δ 157.3 (C4), 149.2 (C2), 134.4 (C6), 125.2

(C5), 117.2 (q, J_{CF} = 320.8 Hz, CF_3). EIMS m/z 260 (M^+, <5%), 128 (M^++H-SO_2CF_3, 100%). Anal found: C, 22.43; H, 1.05; N, 10.41. Calcd. for $C_5H_3N_2O_5SF_3$: C, 23.07; H, 1.15; N, 10.77 (Unfortunately a more accurate value for C could not be obtained).

1-[(2-Acetoxyethoxy)methyl]-5-trifluoromethanesulfonyluracil (113):

A suspension of **110** (200mg, 0.77mmol) and ammonium sulfate (~0.5mg) in dry hexamethydisilazane (7ml) was heated to reflux under nitrogen until a clear solution was obtained (~1hr). The solution was evaporated to dryness at room temperature, with the careful exclusion of atmospheric moisture, to give an amber oil. Freshly distilled, dry acetonitrile (10ml) was added to the oil and the solution cooled to 0°C. A solution of (2-acetoxyethoxyl)methyl bromide (227 mg, 1.15 m mol) in the same acetonitrile (8ml) was added dropwise over 1hr and the reaction mixture left to stand at room temperature for 48hrs. The solution was then evaporated onto silica and subjected to flash chromatography (hexane/ethyl acetate 1:1). Both the product **113** (211mg, 73%) and some starting material **110** (44mg, 22%) were isolated. The product **113** was isolated as a slightly tan, viscous oil; ^{1}H NMR δ 10.29 (br s, 1 H, NH), 7.68 (s, 1 H, H-6), 5.18 (s, 1 H, OCH_2N), 4.17 (m, 2 H, CH_2OAc), 3.76 (m, 2 H, $OC\underline{H}_2CH_2$), 2.00 (s, 3 H, CH_3CO_2). ^{13}C NMR δ 170.0 (CO_2), 157.4 (C4), 149.7 (C2), 136.8 (C6), 129.5 (C5), 118.1 (q, J_{CF} = 320.6 Hz, CF_3), 77.0 (OCH_2N), 67.9 (CH_2OAc), 62.73 ($O\underline{C}H_2CH_2$), 20.7 ($\underline{C}H_3CO_2$). EIMS m/z 376 (M^+, <5%). IR ($CHCl_3$) ν_{max} 3380 cm^{-1} (w, NH), 3020 (m, C-H), 1718 (str, C=O), 1196 (str, SO_3). HREIMS found: 376.0175. Calcd for $C_{18}H_{27}SiN_2O_7SF_3$: 376.0188.

1-[(Hydroxyethoxy) methyl]-5-trifluoromethanesulfonyluracil (82).

A saturated methanolic ammonia solution (10ml) was added to **113** (150mg, 0.40mmol) and the solution left standing for 6hrs. Silica (~200mg) was added and the solution evaporated to dryness and subjected to flash chromatography (hexanes 60% : ethyl acetate 38% : methanol 2%) giving **82** (116mg, 87%) as a white solid; ^{1}H NMR δ 7.67 (s, 1 H, H-6), 5.24 (s, 1 H, OCH_2N),

3.75 (m, 2 H, CH_2OAc), 3.76 (m, 4 H, OCH_2CH_2), 1.7 (br s, 1 H, OH). EIMS m/z 335 (M^+, <5%). IR ($CHCl_3$) ν_{max} 3380 cm^{-1} (w, NH), 2956 (m, C-H), 1701 (str, C=O), 1198 (str, SO_3).

6-Butyluracil (116):

A suspension of 5-triflouromethanesulfonyluracil **110** (200mg, 0.77mmol) in hexamethyldisilazane (7ml) was heated at reflux under nitrogen until all the **110** had dissolved (~1hr). The solution was allowed to cool and was evaporated to dryness at room temperature with the careful exclusion of atmospheric moisture. The residue was dissolved in dry tetrahydrofuran (5ml) and cooled to –78°C. A 2.5 M solution of *n*-butyllithium in hexane (0.28ml, 0.77mmol) was added and the solutions allowed to warm to room temperature overnight. The reaction mixture was quenched with methanol (5ml) and silica (0.5g) was added and the slurry evaporated to dryness. The residue was subjected to flash chromatography (hexanes 60% : ethyl acetate 38% : methanol 2%) to give **116** (84mg, 65%) and the starting material **110** (60mg, 30%); m.p. = >300 °C. ^1H NMR ($CDCl_3/DMSOd_6$) δ 10.39 (v br s, 1 H, N1H), 9.99 (br s, 1 H, N3H), 5.14 (s, 1 H, H-5), 2.27 (t, J = 7.74 Hz, 1H, C6-CH_2-), 1.37 (p, J = 7.2 Hz, 2 H, C6-CH_2-$C\underline{H}_2$-), 1.18 (h, J = 7.5 Hz, 2 H, $C\underline{H}_2$-CH_3), 0.74 (t, J = 7.5 Hz, CH_3). ^{13}C NMR ($CDCl_3/DMSOd_6$) δ 161.1 (C4), 150.0 (C2), 134.6 (C6), 98.9 (C5), 28.8 (C6-CH_2-), 26.3 (C6-CH_2-$\underline{C}H_2$-), 21.7 (CH_3). HREIMS found: 168.0888. Calcd for $C_8H_{12}N_2O_2$: 168.0898.

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Appendix

Results from the screening of compounds **8** and **41b-f** for anti-HIV and anticancer activity as performed by:

The National institutes of Health
National Cancer Institute
Bethesda, Maryland 208932
U.S.A.

5-Trifluoromethanesulfonyl-2'-deoxyuridine (**8**)

5-{2-[3,5-Bis(α,α,α-trifluoromethyl)phenyl]ethynyl}-2'-deoxyuridine (**41b**)

5-(3,5-Difluorophenylethynyl)-2'-deoxyuridine (**41c**)

5-[2-(4-α,α,α-Trifluoromethylphenyl)ethynyl]-2'-deoxyuridine (**41d**)

5-[2-(4-Fluorophenyl)ethynyl]-2'-deoxyuridine (**41e**)

5-[2-(Pentafluorophenyl)ethynyl]-2'-deoxyuridine (**41f**)

The NCI *In Vitro* Anti-AIDS Drug Discovery Program

The *In Vitro* Testing Results Form provided to you has three sections:

The Sample and Test Identification Section specifies which of your compounds was tested (NSC), our computer's code for you (Supplier Code), the identification number you provided for the compound (COMI), the actual experiment number from which the results were taken (Plate), which of our laboratories performed the experiment (Lab), when the test was performed (Test Date), the date the report was printed (Report Date), and which cell line was used in the test (Cell Line). The QNS (sample quantity sufficiency) and Material Classification (MC) codes provide NCI staff with important administrative information.

The Graphics Results Summary Section displays a plot of the $\log_{10}$ of your sample's concentrations (as ug/mL or molar) against the measured test values expressed as a percentage of the uninfected, untreated control values. The solid line connecting the diamond symbols depicts the percentage of surviving HIV-infected cells treated with your sample (at the indicated concentration) relative to uninfected, untreated controls. This line expresses the *in vitro* anti-HIV activity of your sample. The dashed line connecting the triangular symbols depicts the percentage of surviving uninfected cells treated with your sample relative to the same uninfected, untreated controls. This line expresses the *in vitro* growth inhibitory properties of your sample. The viral cytopathic effect in this particular experiment is indicated by a dotted reference line. This line shows the extent of destruction of cells by the virus in the absence of treatment and is used as a quality control parameter. Survival values of this parameter less then 50% are considered acceptable in the current protocol. The percent of protection has been calculated from the data and is presented on the right side of the graph.

The Tabular Dose Response Data and Status Section provides a listing of the numerical data plotted in the graphics section. Approximate values for 50% effective concentration (EC_{50}), 50% inhibitory concentration (IC_{50}), and Therapeutic Index ($TI = IC_{50}/EC_{50}$) have been calculated for each test and are provided for your information. The NCI staff determination of the activity of your compound is printed in the lower left-hand corner.

NATIONAL CANCER INSTITUTE

Anti-HIV Drug Testing System

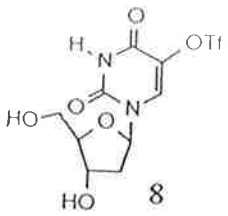
The procedure* used in the National Cancer Institute's test for agents active against Human Immunodeficiency Virus (HIV) is designed to detect agents acting at any stage of the virus reproductive cycle. The assay basically involves the killing of T4 lymphocytes by HIV. Small amounts of HIV are added to cells, and a complete cycle of virus reproduction is necessary to obtain the required cell killing. Agents that interact with virions, cells, or virus gene-products to interfere with viral activities will protect cells from cytolysis. The system is automated in several features to accommodate large numbers of candidate agents and is generally designed to detect anti-HIV activity. However, compounds that degenerate or are rapidly metabolized in the culture conditions may not show activity in this screen. All tests are compared with at least one positive (e.g., AZT-treated) control done at the same time under identical conditions.

The Procedure:

1. Candidate agent is dissolved in dimethyl sulfoxide (unless otherwise instructed) then diluted 1:100 in cell culture medium before preparing serial half-$\log_{10}$ dilutions. T4 lymphocytes (CEM cell line) are added and after a brief interval HIV-1 is added, resulting in a 1:200 final dilution of the compound. Uninfected cells with the compound serve as a toxicity control, and infected and uninfected cells without the compound serve as basic controls.
2. Cultures are incubated at 37° in a 5% carbon dioxide atmosphere for 6 days.
3. The tetrazolium salt, XTT, is added to all wells, and cultures are incubated to allow formazan color development by viable cells.
4. Individual wells are analyzed spectrophotometrically to quantitate formazan production, and in addition are viewed microscopically for detection of viable cells and confirmation of protective activity.
5. Drug-treated virus-infected cells are compared with drug-treated noninfected cells and with other appropriate controls (untreated infected and untreated noninfected cells, drug-containing wells without cells, etc.) on the same plate.
6. Data are reviewed in comparison with other tests done at the same time and a determination about activity is made.

* Weislow, O.W., Kiser, R., Fine, D., Bader, J., Shoemaker, R.H., Boyd, M.R.: New soluble-formazan assay for HIV-1 cytopathic effects: application to high-flux screening of synthetic and natural products for AIDS-antiviral activity. J. Natl. Cancer Inst. 81:577-586, 1989.

NATIONAL CANCER INSTITUTE

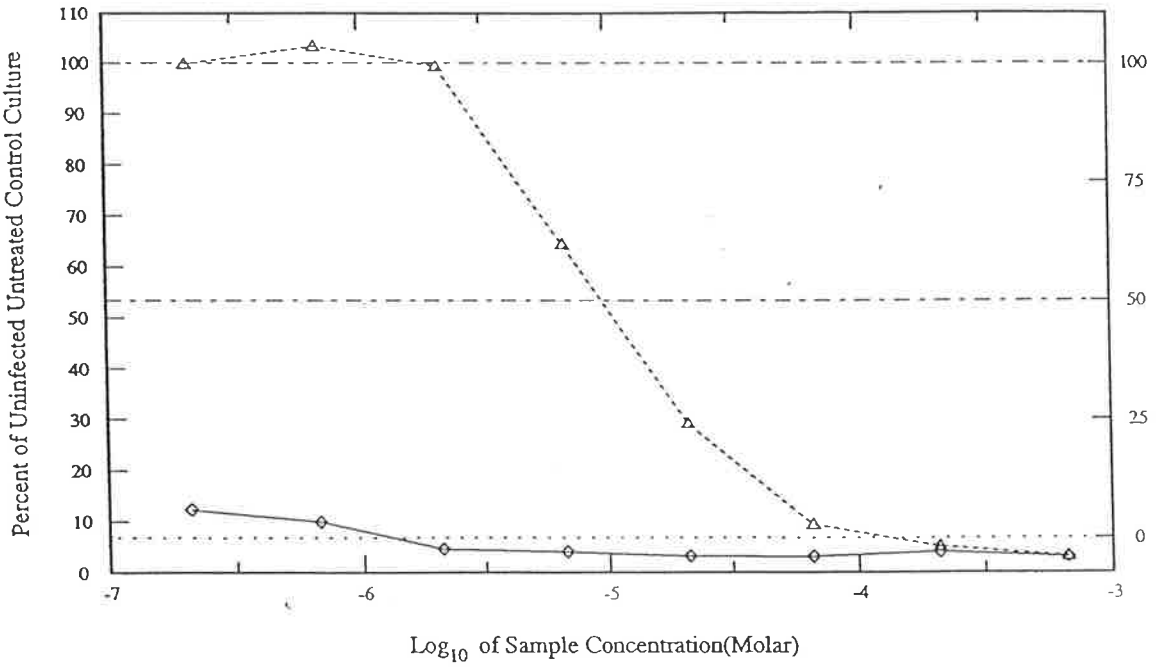


8

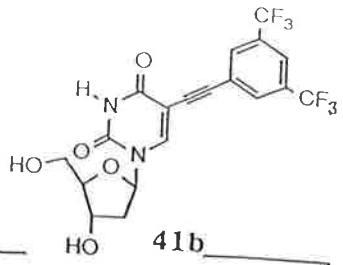
National Cancer Institute
Developmental Therapeutics Program
In-Vitro Testing Results

NSC: 642901-O/ 1	Plate: 9316	Lab: 9N	Assay:
Test Date: June 14, 1991	SSPL: V19U		COMI:
Report Date: July 1, 1991	Cell Line: CEM-IW		Solvent: DMSO < .25%

IN VITRO ANTI-HIV DRUG SCREENING RESULTS



SUMMARY		DOSE	INFECTED RESPONSE	UNINFECTED RESP
Index	Concentration	(Molar)	Percent of Control	Percent of Contr
IC50 (Molar)	1.10×10^{-5}	2.10×10^{-7}	12.28	99.74
EC50 (Molar)		6.70×10^{-7}	9.96	103.29
TI50 (IC/EC)		2.10×10^{-6}	4.55	99.25
Conclusion		6.70×10^{-6}	4.01	64.30
		2.10×10^{-5}	3.15	29.19
		6.60×10^{-5}	3.07	9.42
INACTIVE		2.10×10^{-4}	4.07	5.12
		6.60×10^{-4}	3.07	3.23

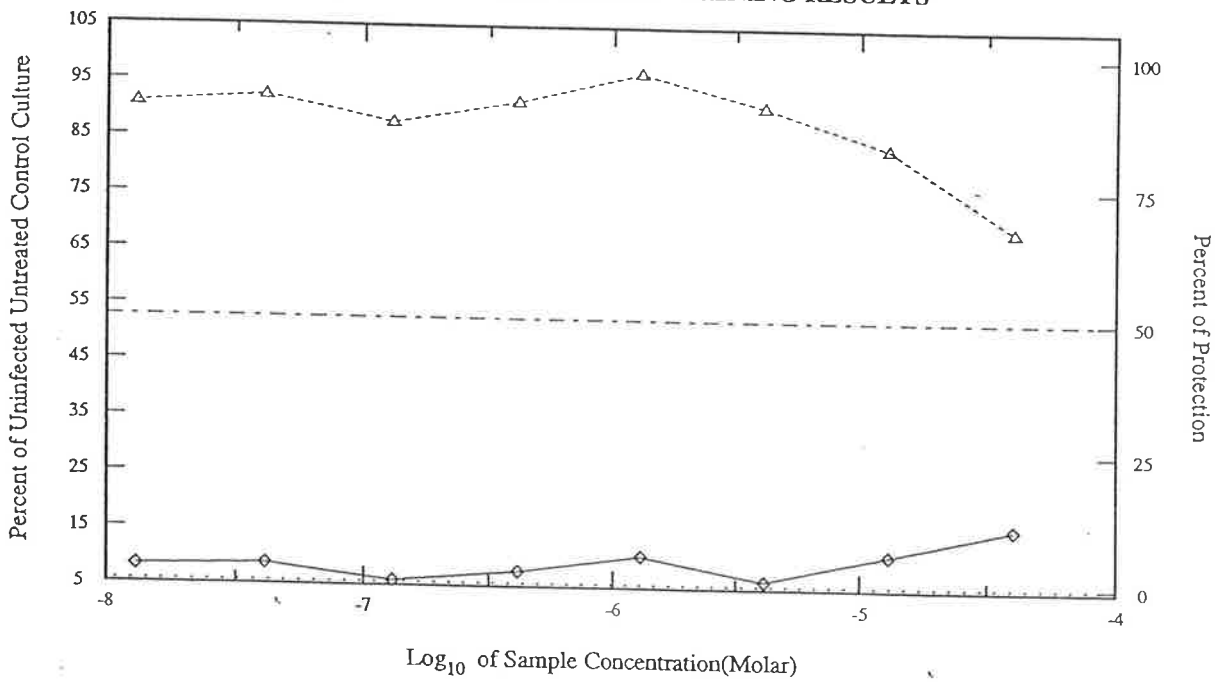


41b

National Cancer Institute
Developmental Therapeutics Program
In-Vitro Testing Results

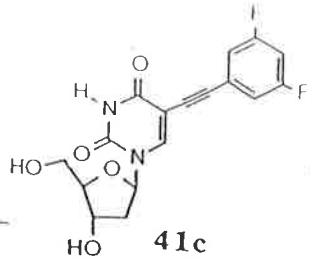
NSC: - 645986-R/ 1	Plate: 3366	Lab: 9N	Assay: Primary Screen
Test Date: October 29, 1991	SSPL: V19U		COMI:
Report Date: November 18, 1991	Cell Line: CEM-IW		Solvent: DMSO < .25%

IN VITRO ANTI-HIV DRUG SCREENING RESULTS



SUMMARY		DOSE	INFECTED RESPONSE	UNINFECTED RESPONSE
Index	Concentration	(Molar)	Percent of Control	Percent of Control
IC50 (Molar)	$>4.00 \times 10^{-5}$	1.30×10^{-8}	8.22	90.97
EC50 (Molar)		4.10×10^{-8}	8.63	92.37
TI50 (IC/EC)		1.30×10^{-7}	5.84	87.74
Conclusion		4.10×10^{-7}	7.71	91.61
		1.30×10^{-6}	10.63	96.94
		4.10×10^{-6}	6.44	91.10
		1.30×10^{-5}	11.14	83.74
		4.00×10^{-5}	16.00	69.20

INACTIVE



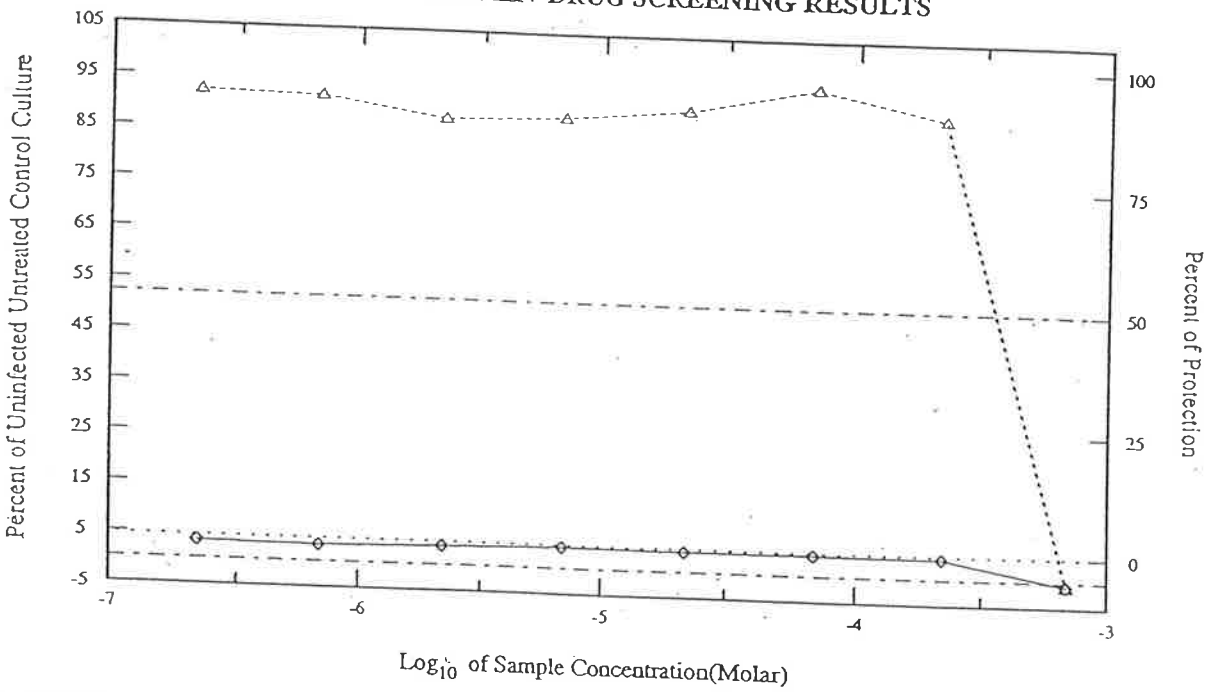
41c

NCI DRUG INFORMATION SYSTEM

National Cancer Institute
Developmental Therapeutics Program
In-Vitro Testing Results

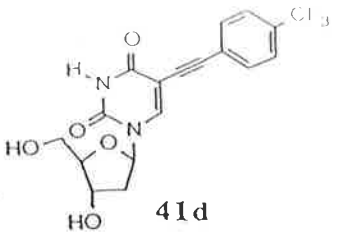
NSC: 648476-X/1	Plate: 5390	Lab: 9N	Assay: Primary Screen
Test Date: January 17, 1992	SSPL: V19U		COMI:
Report Date: February 3, 1992	Cell Line: CEM-IW		Solvent: DMSO < 25%

IN VITRO ANTI-HIV DRUG SCREENING RESULTS



0%, 50% Reference Lines — Viral Cytopathic Effect — Infected Treated Culture — Uninfected Treated Culture

SUMMARY		DOSE	INFECTED RESPONSE	UNINFECTED RESPONSE
Index	Concentration	(Molar)	Percent of Control	Percent of Control
IC50 (Molar)	3.60×10^{-4}	2.20×10^{-7}	3.46	92.04
EC50 (Molar)		6.90×10^{-7}	3.25	91.53
TI50 (IC/EC)		2.20×10^{-6}	3.67	87.69
Conclusion		6.90×10^{-6}	4.15	88.54
INACTIVE		2.20×10^{-5}	4.06	90.59
		6.90×10^{-5}	4.10	95.55
		2.20×10^{-4}	4.06	89.99
		6.90×10^{-4}	-0.77	-0.85

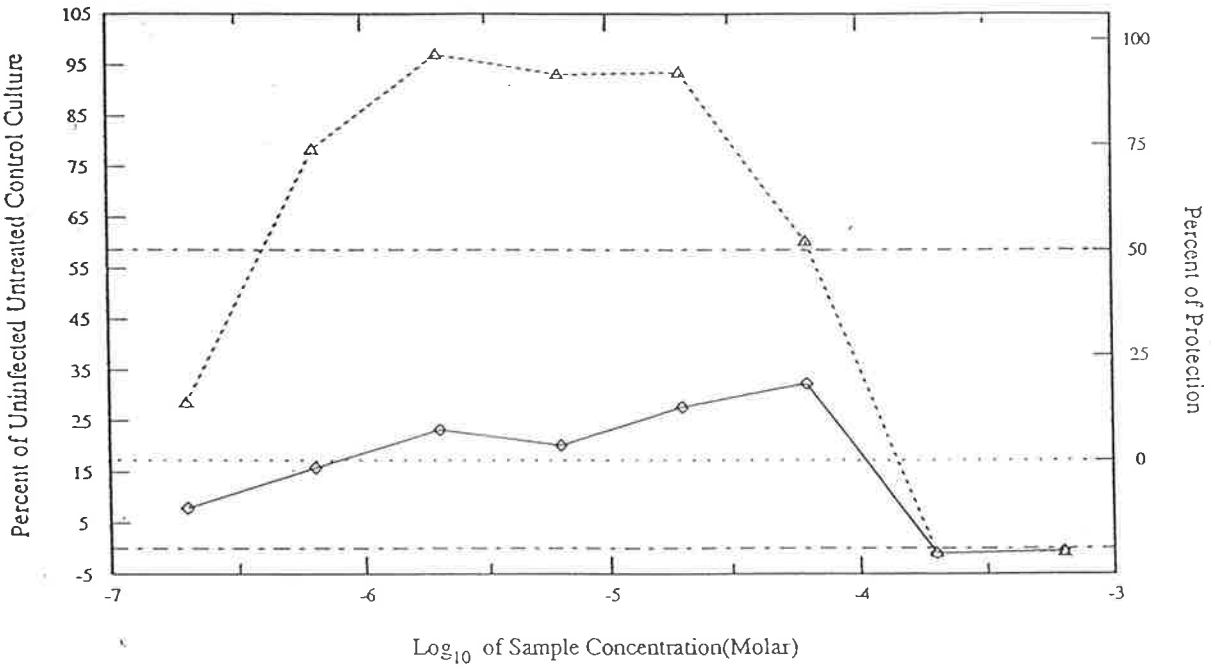


41d

National Cancer Institute
Developmental Therapeutics Program
In-Vitro Testing Results

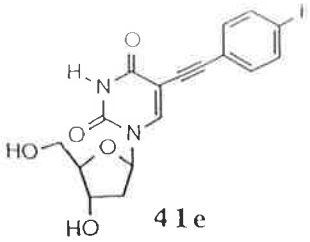
NSC: 648048-J/ 1	Plate: 4927	Lab: 9N	Assay: Primary Screen
Test Date: December 31, 1991	SSPL: V19U		COMI:
Report Date: January 10, 1992	Cell Line: CEM-IW		Solvent: DMSO < .25%

IN VITRO ANTI-HIV DRUG SCREENING RESULTS



0%, 50% Reference Lines — Viral Cytopathic Effect — Infected Treated Culture — Uninfected Treated Culture

SUMMARY		DOSE	INFECTED RESPONSE	UNINFECTED RESPONSE
Index	Concentration	(Molar)	Percent of Control	Percent of Control
IC50 (Molar)		2.00×10^{-7}	7.88	28.58
EC50 (Molar)		6.30×10^{-7}	15.81	78.34
TI50 (IC/EC)		2.00×10^{-6}	23.30	96.95
Conclusion		6.30×10^{-6}	20.20	93.07
		2.00×10^{-5}	27.74	93.49
		6.30×10^{-5}	32.34	60.32
		2.00×10^{-4}	-0.95	-0.89
		6.30×10^{-4}	-0.48	-0.72

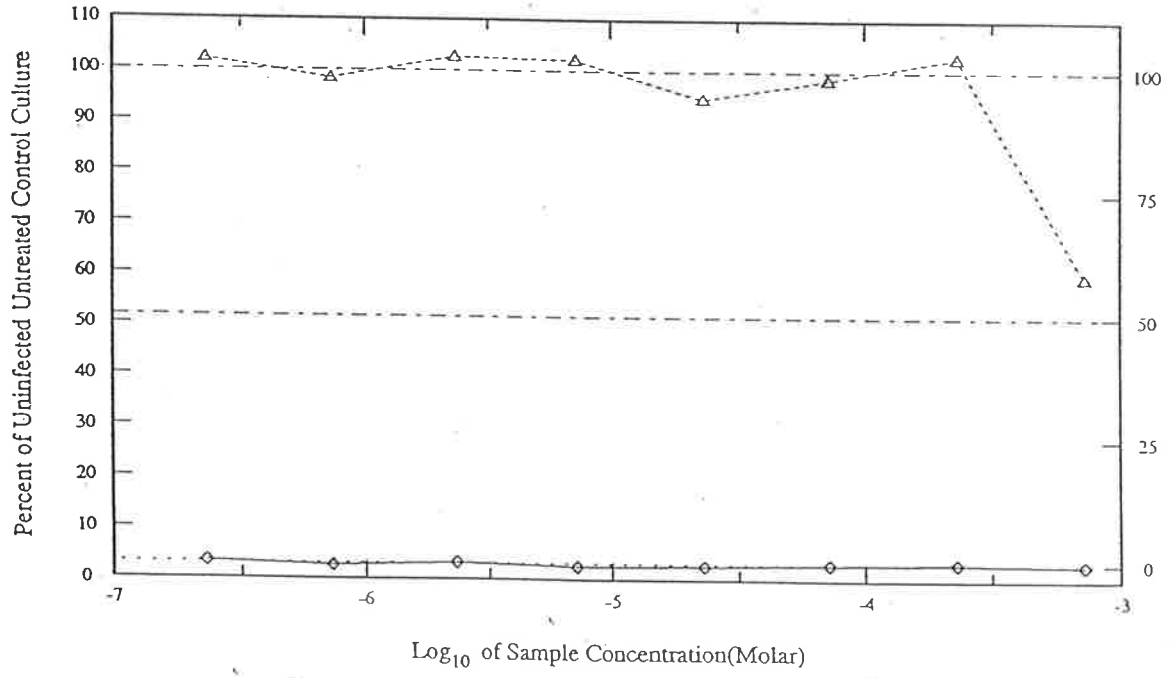


41e

National Cancer Institute
Developmental Therapeutics Program
In-Vitro Testing Results

NSC: 648477-Y/1	Plate: 5415	Lab: 9N	Assay: Primary Screen
Test Date: January 17, 1992	SSPL: V19U		COMI:
Report Date: February 3, 1992	Cell Line: CEM-IW		Solvent: DMSO < .25%

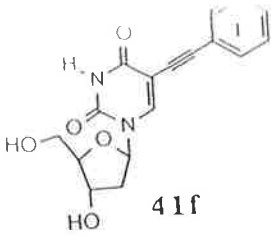
IN VITRO ANTI-HIV DRUG SCREENING RESULTS



0%, 50% Reference Lines — Viral Cytopathic Effect · · · · · · · · · · Infected Treated Culture ——— Uninfected Treated Culture - - - - - - - -

SUMMARY		DOSE	INFECTED RESPONSE	UNINFECTED RESPO
Index	Concentration	(Molar)	Percent of Control	Percent of Control
IC50 (Molar)	$>7.20 \times 10^{-4}$	2.30×10^{-7}	3.37	102.03
EC50 (Molar)		7.30×10^{-7}	2.64	98.30
TI50 (IC/EC)		2.30×10^{-6}	3.21	102.60
Conclusion		7.20×10^{-6}	2.56	102.11
		2.30×10^{-5}	2.68	94.56
		7.20×10^{-5}	3.08	98.38
		2.30×10^{-4}	3.29	102.68
		7.20×10^{-4}	2.96	59.66

INACTIVE



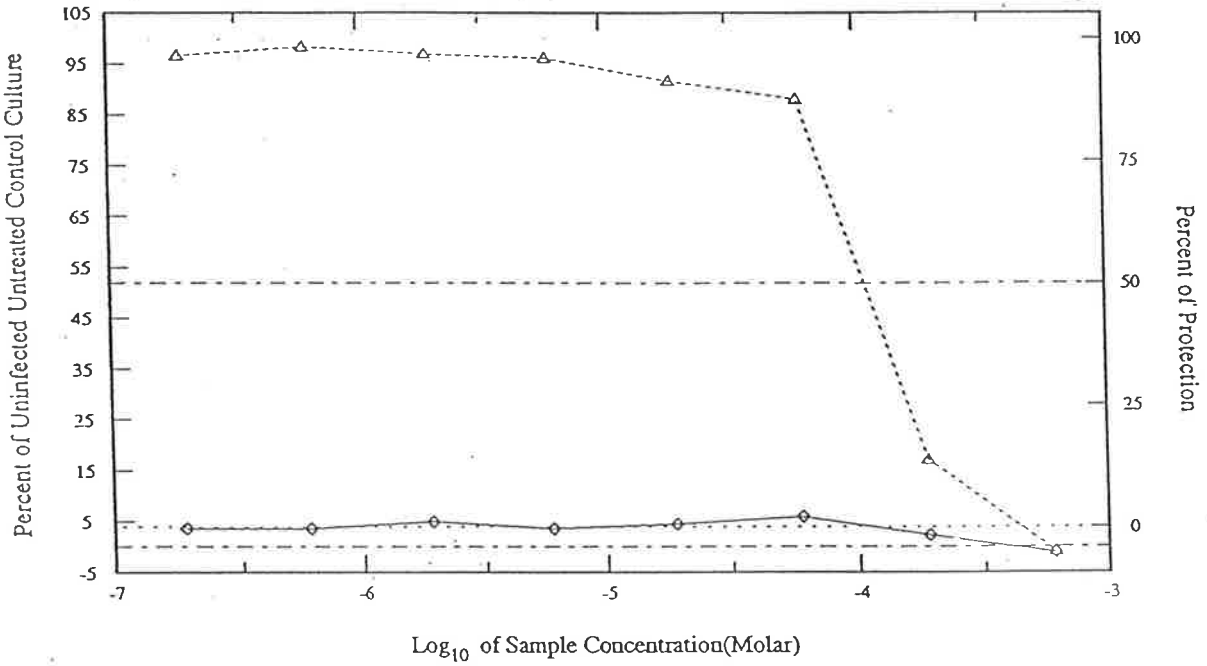
41f

NCI DRUG INFORMATION SYSTEM

National Cancer Institute
Developmental Therapeutics Program
In-Vitro Testing Results

NSC: 648478-Z/1	Plate: 5416	Lab: 9N	Assay: Primary Screen
Test Date: January 17, 1992	SSPL: V19U		COMI:
Report Date: February 3, 1992	Cell Line: CEM-IW		Solvent: DMSO < .25%

IN VITRO ANTI-HIV DRUG SCREENING RESULTS



SUMMARY		DOSE	INFECTED RESPONSE	UNINFECTED RESPONSE
Index	Concentration	(Molar)	Percent of Control	Percent of Control
IC50 (Molar)	1.10×10^{-4}	1.90×10^{-7}	3.58	96.63
EC50 (Molar)		6.00×10^{-7}	3.58	98.37
TI50 (IC/EC)		1.90×10^{-6}	4.96	96.91
Conclusion		6.00×10^{-6}	3.58	96.15
		1.90×10^{-5}	4.48	91.63
		6.00×10^{-5}	5.87	88.02
		1.90×10^{-4}	2.29	17.15
		6.00×10^{-4}	-1.15	0.90

INACTIVE

SCREENING DATA REPORT COMPONENTS

The Calculated Measurement of Effect: Percentage Growth (PG)

The measured effect of the compound on a cell line is currently calculated according to one or the other of the following two expressions:

If $(\text{Mean OD}_{test} - \text{Mean OD}_{tzero}) \geq 0$, then

$$PG = 100 \times (\text{Mean OD}_{test} - \text{Mean OD}_{tzero})/(\text{Mean OD}_{ctrl} - \text{Mean OD}_{tzero})$$

If $(\text{Mean OD}_{test} - \text{Mean OD}_{tzero}) < 0$, then

$$PG = 100 \times (\text{Mean OD}_{test} - \text{Mean OD}_{tzero})/\text{Mean OD}_{tzero}$$

Where:

Mean OD_{tzero} = The average of optical density measurements of SRB-derived color just before exposure of cells to the test compound.

Mean OD_{test} = The average of optical density measurements of SRB-derived color after 48 hours' exposure of cells to the test compound.

Mean OD_{ctrl} = The average of optical density measurements of SRB-derived color after 48 hours with no exposure of cells to the test compound.

The Data Sheet:

This page of the data package presents the experimental data collected against each cell line. The first two columns describe the subpanel (e.g., leukemia) and cell line (e.g., CCRF-CEM) involved. The next two columns list the Mean OD_{tzero} and Mean OD_{ctrl}; the next five columns list the Mean OD_{test} for each of five different concentrations. Each concentration is expressed as the $\log_{10}$ (molar or μg/ml). The next five columns list the calculated PGs for each concentration. The response parameters GI50, TGI, and LC50 are interpolated values representing the concentrations at which the PG is +50, 0, and −50, respectively. Sometimes these response parameters cannot be obtained by interpolation. If, for instance, all of the PGs in a given row exceed +50, then none of the three parameters can be obtained by interpolation. In such a case, the value given for each response parameter is the highest concentration tested and is preceded by a ">" sign. This practice is extended similarly to the other possible situations where a response parameter cannot be obtained by interpolation.

Dose-Response Curves:

The dose-response curve page of the data package is created by plotting the PGs against the $\log_{10}$ of the corresponding concentration for every cell line. The cell line curves are grouped by subpanel. Horizontal lines are provided at the PG values of +50, 0, and −50. The concentrations corresponding to points where the curves cross these lines are the GI50, TGI, and LC50, respectively.

The Mean Graphs:

Mean graphs facilitate visual scanning of data for potential patterns of selectivity for particular cell lines or for particular subpanels with respect to a selected response parameter. Differences in apparent selectivity patterns may occur for the same compound against the same cell lines when different parameters are compared. The mean graphs page of the data package shows mean graphs at each of the principal response parameters: GI50, TGI, and LC50. Bars extending to the right represent sensitivity of the cell line to the test agent in excess of the average sensitivity of all tested cell lines. Since the bar scale is logarithmic, a bar 2 units

Dose-Response Matrix Analysis

D_{GI50}, D_{TGI}, D_{LC50}, D_H, and MGD_H

Measures of Subpanel Selectivity

The values of D_{GI50}, D_{TGI}, and D_{LC50} displayed near the bottom of the dose-response matrix are measures of subpanel selectivity based on the response parameters GI50, TGI, and LC50. These values identify whether subpanel-selective effects occur at a high (LC50 or TGI) or moderate (GI50) effect level. Computer simulations suggest that a value of D_{GI50}, D_{TGI}, or $D_{LC50} \geq 50$ is statistically significant. The concentration at which the maximum selective effect occurred is given in parentheses, ( ), next to the corresponding D_{GI50}, D_{TGI}, or D_{LC50} value.

To calculate D_{GI50}, we first calculate the percentage of cell lines that achieve GI50 for each subpanel. We do this separately for each concentration. We then calculate, separately for each of the 5 concentrations, 3 differences: (1) the highest subpanel percentage minus the percentage of the remaining cell lines, (2) the percentage for the 2 highest subpanels, taken together, minus the percentage for the remaining cell lines, and (3) the average of the 3 highest subpanel percentages minus the percentage for the remaining cell lines. D_{GI50} is the largest of these 15 differences. The values D_{TGI} and D_{LC50} are the analogous maximum differences relating to TGI and LC50.

The value of D_H at the bottom of the Dose-Response Matrix provides a more general measure of selective effect. The D_H value is given primarily as a means of assigning relative scores of selectivity to the compounds, and the practical significance of this value will be determined empirically. However, computer simulations suggest that values of $D_H \geq 75$ are statistically significant, and in this case, a dot is printed immediately to the left of the disease label for the selectively sensitive subpanel(s). The concentration at which the maximum selective effect occurred is given in parentheses, ( ), next to the corresponding D_H value.

To calculate D_H, each cell line is ranked 1 to N (where N is the total number of cell line tests which satisfy quality control criteria) from least to most sensitive, by PG value. This is done separately for each concentration. At each concentration, a mean rank is calculated for each subpanel by averaging the individual cell line ranks, and the subpanels are ordered by sensitivity as measured by mean rank. At each of the 5 concentrations, we calculate the 3 mean rank differences: the mean rank for the k (where k = 1, 2, 3) most sensitive subpanels, taken together, minus the mean rank of the remaining cell lines. D_H is the largest of these 15 differences, multiplied by 200/N, so that its range is 0-100.

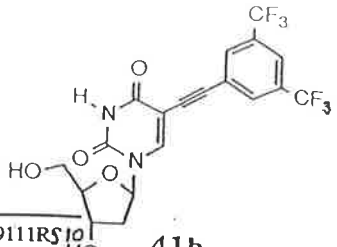
The maximum of D_{GI50}, D_{TGI}, and D_{LC50} is used to determine whether subpanel-selective cytotoxicity is occurring most markedly at the GI50, the TGI, or the LC50 level, and thus to determine which of the three corresponding mean graphs to display. The MGD_H value is a measure of subpanel selectivity similar to D_H, but relating to this chosen mean graph. Each cell line is ranked, from least to most sensitive, by the mean graph bar extension. MGD_H is the maximum difference in mean rank between the most and least sensitive subpanels, where we maximize over the choice of 1, 2, or 3 subpanels to be the most sensitive, and multiply by 200/N, just as in the calculation of D_H. The MGD_H value is given primarily as a means of assigning relative scores to the mean graphs of the compounds. However, computer simulations indicate that values exceeding 75 are statistically significant, and in these cases the most sensitive subpanels are marked with a dot to the right of their names.

to the right implies the compound achieved the response parameter (e.g., GI50) for the cell line at a concentration one-hundredth the mean concentration required over all cell lines, and thus the cell line is unusually sensitive to that compound. Bars extending to the left correspondingly imply sensitivity less than the mean. If, for a particular drug and cell line, it was not possible to determine the desired response parameter by interpolation, the bar length shown is either the highest concentration tested (and the listed $\log_{10}$ of the response parameter will be preceded by a ">") or the lowest concentration tested (and the listed $\log_{10}$ will be preceded by a "<").

The values at either limit (> or <) are also calculated in the mean used for the meangraph. Therefore, the mean used in the meangraph may not be the actual mean of the GI50, for instance. For this reason, we shall refer to this value as the MG_MID (for meangraph midpoint).

The Dose-Response Matrix:

The dose-response matrix combines some qualities of the dose-response curve with some qualities of the mean graph. Selective effects at the cell line or subpanel levels are visualized as in the mean graph, however, different levels of effect are also depicted simultaneously, as in dose response curves. Each column of the matrix corresponds to the drug effect at one of the five concentration levels, and each row corresponds to the effect against each cell line. Thus, each block within a row depicts the effect of a given concentration against a given cell line. The shading given the block depends on the value of the PG for the given concentration against the given cell line in comparison with the corresponding values for the GI50, TGI, and LC50. If the $PG > +50$, the block is white. If the $PG < -50$, the block is black. Two intermediate shades of gray are used if $+50 > PG > 0$ or if $0 > PG > -50$. Blocks for missing values have a period (.) in the center.



41b

NCI Developmental Therapeutics Program
Dose Response Matrix

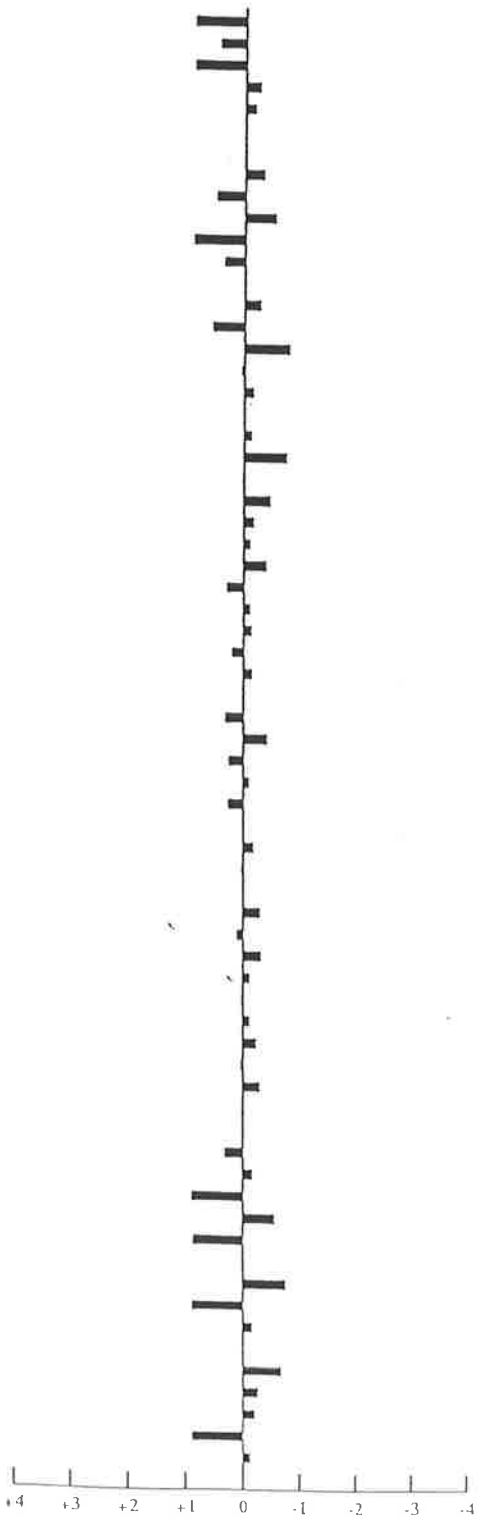
NSC: 645986-R/1	Exp. ID: 9111RS10
Test Date: November 19, 1991	Stain: PROTEIN-51
Report Date: February 11, 1992	SSPL: V19U

Log_{10} Concentration (Molar)

PG (-4.0)	-4.0	-5.0	-6.0	-7.0	-8.0
72					
41					
64					
10					
18					
.	•	•	•	•	•
6					
27					
47					
58					
19					
46					
-5					
37					
8					
-13					
16					
1					
-1					
-93					
20					
17					
6					
12					
-67					
6					
11					
21					
29					
5					
36					
26					
29					
.	•	•	•	•	•
6					
14					
-87					
36					
-79					
-18					
13					
14					
-46					
23					
.	•				
30					
25					
15					
67					
14					
49					
12					
53					
18					
.	•	•	•	•	•
-19					
17					
16					
70					
17					

Panel/Cell Line	Log_{10} GI50
Leukemia	
CCRF-CEM	> -4.00
HL-60(TB)	-4.44
K-562	> -4.00
MOLT-4	-5.14
RPMI-8226	-5.06
SR	
Non-Small Cell Lung Cancer	
A549/ATCC	-5.21
EKVX	-4.39
HOP-18	-5.42
HOP-62	> -4.00
HOP-92	-4.52
NCI-H226	
NCI-H23	-5.16
NCI-H322M	-4.34
NCI-H460	-5.67
NCI-H522	-4.85
LXFL 529	-5.04
Small Cell Lung Cancer	
DMS 114	-5.01
DMS 273	-5.64
Colon Cancer	
COLO 205	-5.34
DLD-1	-5.06
HCC-2998	-4.99
HCT-116	-5.28
HCT-15	-4.59
HT29	-4.99
KM12	-5.02
KM20L2	-4.69
SW-620	-5.04
CNS Cancer	
SF-268	-4.57
SF-295	-5.30
SF-539	-4.63
SNB-19	-4.99
SNB-75	-4.62
SNB-78	
U251	-5.07
XF 498	-4.87
Melanoma	
LOX IMVI	-5.19
MALME-3M	-4.78
M14	-5.21
M19-MEL	-5.01
SK-MEL-2	-4.87
SK-MEL-28	-5.00
SK-MEL-5	-5.12
UACC-257	-4.85
UACC-62	-5.18
Ovarian Cancer	
IGROV1	-4.88
OVCAR-3	-4.57
OVCAR-4	-5.06
OVCAR-5	> -4.00
OVCAR-8	-5.44
SK-OV-3	-4.03
Renal Cancer	
786-0	-5.64
A498	> -4.00
ACHN	-5.06
CAKI-1	
RXF-393	-5.57
RXF-631	-5.16
SN12C	-5.11
TK-10	> -4.00
UO-31	-5.03

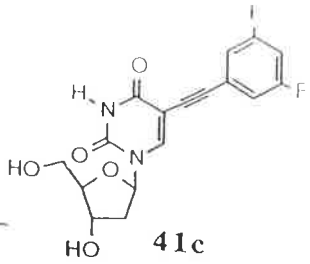
GI50



PG > GI50
PG ≤ GI50

D_{GI50} = 5.0 (-5.0)

MG_MID GI50 = -4.89



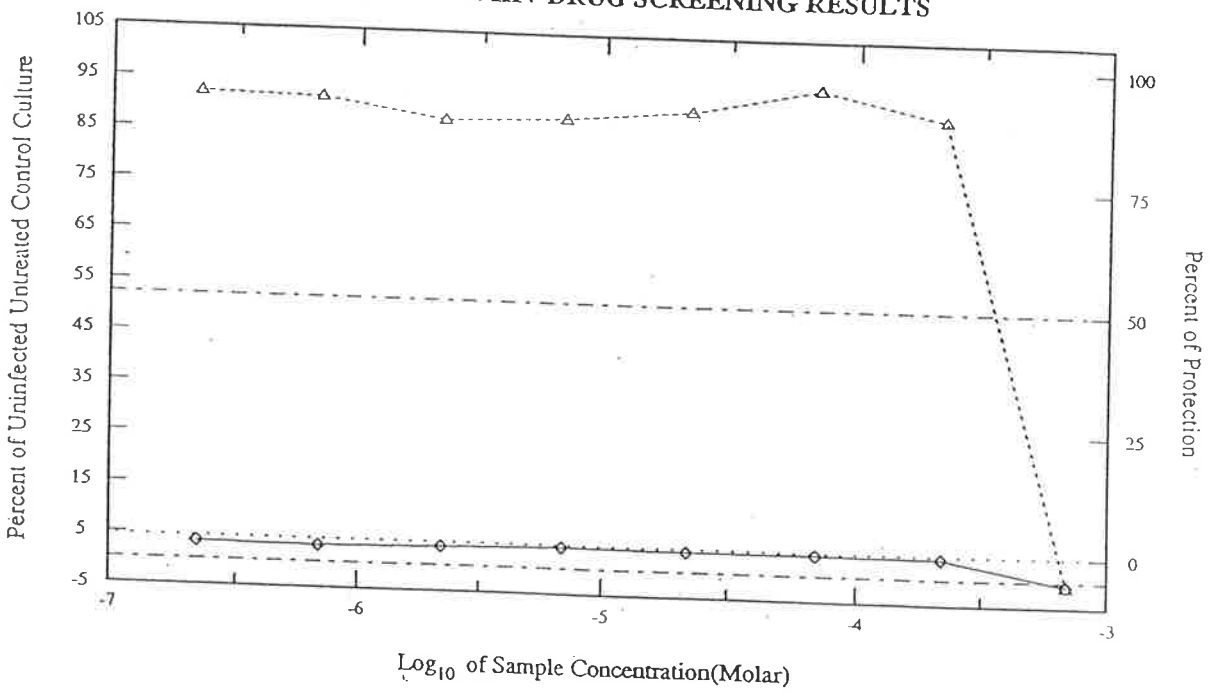
41c

NCI DRUG INFORMATION SYSTEM

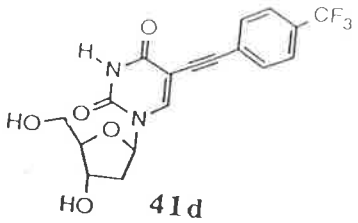
National Cancer Institute
Developmental Therapeutics Program
In-Vitro Testing Results

NSC: 648476-X/1	Plate: 5390	Lab: 9N	Assay: Primary Screen
Test Date: January 17, 1992	SSPL: V19U		COMI:
Report Date: February 3, 1992	Cell Line: CEM-IW		Solvent: DMSO < .25%

IN VITRO ANTI-HIV DRUG SCREENING RESULTS



SUMMARY		DOSE	INFECTED RESPONSE	UNINFECTED RESPONSE
Index	Concentration	(Molar)	Percent of Control	Percent of Control
IC50 (Molar)	3.60×10^{-4}	2.20×10^{-7}	3.46	92.04
EC50 (Molar)		6.90×10^{-7}	3.25	91.53
TI50 (IC/EC)		2.20×10^{-6}	3.67	87.69
Conclusion		6.90×10^{-6}	4.15	88.54
INACTIVE		2.20×10^{-5}	4.06	90.59
		6.90×10^{-5}	4.10	95.55
		2.20×10^{-4}	4.06	89.99
		6.90×10^{-4}	-0.77	0.85



41d

NCI Developmental Therapeutics Program
Dose Response Matrix

NSC: 648048 -J/1	Exp. ID: 9201NS53
Test Date: January 6, 1992	Stain: PROTEIN-51
Report Date: April 15, 1992	SSPL: V19U

Log_{10} Concentration (Molar)						Panel/Cell Line	Log_{10} GI50	GI50
PG (-4.0)	-4.0	-5.0	-6.0	-7.0	-8.0			
89						Leukemia		
95						CCRF-CEM	> -4.00	
85						HL-60(TB)	> -4.00	
75						K-562	> -4.00	
74						MOLT-4	> -4.00	
85						RPMI-8226	> -4.00	
						SR	> -4.00	
87						Non-Small Cell Lung Cancer		
41						A549/ATCC	> -4.00	
51						EKVX	-4.20	
88						HOP-18	> -4.00	
23						HOP-62	> -4.00	
88						HOP-92	-4.62	
78						NCI-H226	> -4.00	
56						NCI-H23	> -4.00	
86						NCI-H322M	> -4.00	
88						NCI-H460	> -4.00	
88						NCI-H522	> -4.00	
						LXFL 529	> -4.00	
73						Small Cell Lung Cancer		
89						DMS 114	> -4.00	
						DMS 273	> -4.00	
100						Colon Cancer		
90						COLO 205	> -4.00	
89						DLD-1	> -4.00	
85						HCC-2998	> -4.00	
100						HCT-116	> -4.00	
100						HCT-15	> -4.00	
78						HT29	> -4.00	
109						KM12	> -4.00	
95						KM20L2	> -4.00	
						SW-620	> -4.00	
60						CNS Cancer		
97						SF-268	> -4.00	
81						SF-295	> -4.00	
90						SF-539	> -4.00	
42						SNB-19	> -4.00	
	•	•	•	•	•	SNB-75	-4.21	
94						SNB-78		
102						U251	> -4.00	
						XF 498	> -4.00	
93						Melanoma		
65						LOX IMVI	> -4.00	
105						MALME-3M	> -4.00	
82						M14	> -4.00	
84						M19-MEL	> -4.00	
70						SK-MEL-2	> -4.00	
102						SK-MEL-28	> -4.00	
91						SK-MEL-5	> -4.00	
85						UACC-257	> -4.00	
						UACC-62	> -4.00	
95						Ovarian Cancer		
93						IGROV1	> -4.00	
68						OVCAR-3	> -4.00	
93						OVCAR-4	> -4.00	
95						OVCAR-5	> -4.00	
77						OVCAR-8	> -4.00	
						SK-OV-3	> -4.00	
100						Renal Cancer		
	•	•	•	•	•	786-0	> -4.00	
91						A498		
	•	•	•	•	•	ACHN	> -4.00	
96						CAKI-1		
124						RXF-393	> -4.00	
90						RXF-631	> -4.00	
97						SN12C	> -4.00	
113						TK-10	> -4.00	
						UO-31	> -4.00	

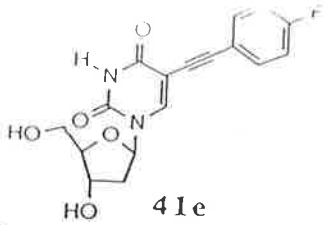
+4 +3 +2 +1 0 -1 -2 -3 -4

PG > GI50

PG < GI50

D_{GI50} = 17.0 (4.0)

MG_MID GI50 -5.02



41e

NCI Developmental Therapeutics Program
Dose Response Matrix

	NSC: 648477 -Y/1	Exp. ID: 9201SR01
	Test Date: January 6, 1992	Stain: PROTEIN-51
	Report Date: May 30, 1992	SSPL: V19U

Log_{10} Concentration (Molar)

PG (-4.0)	-4.0	-5.0	-6.0	-7.0	-8.0
114					
111	•	•	•	•	•
117					
100					
104					
88					
72					
63					
86					
79	•	•	•	•	•
84					
86					
87					
83					
93					
82					
89					
95					
88					
91					
90	•	•	•	•	•
103					
95					
97					
109					
87					
74					
93					
100					
57					
65					
102					
93					
98					
77					
89					
69					
83					
103					
76					
82					
161					
148					
74					
32					
93					
88					
107					
100					
102	•	•	•	•	•
86					
82	•	•	•	•	•
99					
93	•	•	•	•	•

Panel/Cell Line	Log_{10} GI50
Leukemia	
CCRF-CEM	> -4.00
HL-60(TB)	
K-562	> -4.00
MOLT-4	> -4.00
RPMI-8226	> -4.00
SR	> -4.00
Non-Small Cell Lung Cancer	
A549/ATCC	> -4.00
EKVX	> -4.00
HOP-18	> -4.00
HOP-62	> -4.00
HOP-92	
NCI-H226	> -4.00
NCI-H23	> -4.00
NCI-H322M	> -4.00
NCI-H460	> -4.00
NCI-H522	> -4.00
LXFL 529	> -4.00
Small Cell Lung Cancer	
DMS 114	> -4.00
DMS 273	> -4.00
Colon Cancer	
COLO 205	> -4.00
DLD-1	> -4.00
HCC-2998	> -4.00
HCT-116	
HCT-15	> -4.00
HT29	> -4.00
KM12	> -4.00
KM20L2	> -4.00
SW-620	> -4.00
CNS Cancer	
SF-268	> -4.00
SF-295	> -4.00
SF-539	> -4.00
SNB-19	> -4.00
SNB-75	> -4.00
SNB-78	> -4.00
U251	> -4.00
XF 498	> -4.00
Melanoma	
LOX IMVI	> -4.00
MALME-3M	> -4.00
M14	> -4.00
M19-MEL	> -4.00
SK-MEL-2	> -4.00
SK-MEL-28	> -4.00
SK-MEL-5	> -4.00
UACC-257	> -4.00
UACC-62	> -4.00
Ovarian Cancer	
IGROV1	> -4.00
OVCAR-3	> -4.00
OVCAR-4	-4.59
OVCAR-5	> -4.00
OVCAR-8	> -4.00
SK-OV-3	> -4.00
Renal Cancer	
786-0	> -4.00
A498	
ACHN	> -4.00
CAKI-1	> -4.00
RXF-393	
RXF-631	> -4.00
SN12C	> -4.00
TK-10	
UO-31	> -4.00

GI50

-4 -3 -2 -1 0 -1 -2

PG > GI50

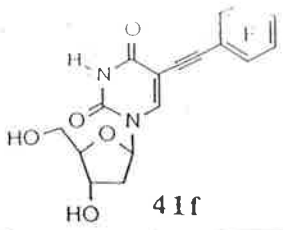
PG < GI50

D_{GI50} = 170 (-4.0)

D_{TGI} = 00 (00)

MG_MID GI50 = -5.03

Delta = 0.53



41f

NCI Developmental Therapeutics Program Dose Response Matrix	NSC: 648478 -Z/1	Exp. ID: 9201SR01
	Test Date: January 6, 1992	Stain: PROTEIN-51
	Report Date: May 30, 1992	SSPL: V19U

Log_{10} Concentration (Molar)

PG (-4.0)	-4.0	-5.0	-6.0	-7.0	-8.0	Panel/Cell Line	Log_{10} GI50
						Leukemia	
85	•	•	•	•	•	CCRF-CEM	> -4.00
.						HL-60(TB)	
87						K-562	> -4.00
96						MOLT-4	> -4.00
96						RPMI-8226	> -4.00
88						SR	> -4.00
						Non-Small Cell Lung Cancer	
89						A549/ATCC	> -4.00
95						EKVX	> -4.00
27	▨	•	•			HOP-18	
77						HOP-62	> -4.00
.	•	•	•	•	•	HOP-92	
104						NCI-H226	> -4.00
96						NCI-H23	> -4.00
81						NCI-H322M	> -4.00
95						NCI-H460	> -4.00
.	•					NCI-H522	> -5.00
90						LXFL 529	> -4.00
						Small Cell Lung Cancer	
74						DMS 114	> -4.00
88						DMS 273	> -4.00
						Colon Cancer	
101						COLO 205	> -4.00
93						DLD-1	> -4.00
98						HCC-2998	> -4.00
91						HCT-116	> -4.00
90						HCT-15	> -4.00
100						HT29	> -4.00
88						KM12	> -4.00
102						KM20L2	> -4.00
140						SW-620	> -4.00
						CNS Cancer	
83						SF-268	> -4.00
73						SF-295	> -4.00
97						SF-539	> -4.00
97		•	•			SNB-19	> -4.00
80						SNB-75	> -4.00
.	•	•	•	•	•	SNB-78	
96						U251	> -4.00
103						XF 498	> -4.00
						Melanoma	
88						LOX IMVI	> -4.00
81						MALME-3M	> -4.00
84						M14	> -4.00
80						M19-MEL	> -4.00
92						SK-MEL-2	> -4.00
99						SK-MEL-28	> -4.00
77						SK-MEL-5	> -4.00
76						UACC-257	> -4.00
114						UACC-62	> -4.00
						Ovarian Cancer	
91					▨	IGROV1	
94						OVCAR-3	> -4.00
68						OVCAR-4	> -4.00
110						OVCAR-5	> -4.00
87						OVCAR-8	> -4.00
91						SK-OV-3	> -4.00
						Renal Cancer	
98						786-0	> -4.00
.	•	•	•	•	•	A498	
106						ACHN	> -4.00
87						CAKI-1	> -4.00
.	•	•	•	•	•	RXF-393	
88						RXF-631	> -4.00
97						SN12C	> -4.00
.	•	•	•	•	•	TK-10	
[illegible]		•	•			UO-31	[illegible]

GI50

-4 -3 -2 -1 0 -1 -2 -3

PG > GI50

PG < GI50

D_{GI50} = 170 (5.0)

D_{TGI} = 00.00 ( )

MG_MID GI50 = -4.02

Delta = 0.07