NICKEL PEROXIDE AS A CHEMICAL MODEL FOR THE PEPTIDYLGLYCINE α-AMIDATING MONOOXYGENASE ENZYME

A Thesis
Submitted in Partial Fulfilment of the
Requirements for the Degree
of
Master of Science

by

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June, 1992

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STATEMENT

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ACKNOWLEDGEMENTS

I would like to thank my supervisor Dr. Christopher J. Easton for his assistance during the course of this work and in the preparation of this manuscript. I enjoyed the project immensely as it is in an area of chemistry in which I have a keen interest.

Mr. L. Paltridge, Mr. T. Blumenthal, Mr. P. Clements, Mr. J. Cameron, Mr. P. McClarin, Mr. K. Newton and Ms. M. Smith also deserve special thanks. Without their assistance much that we take for granted would be impossible.

Thanks to the members of lab. 6 both past and present whose zest for life and sense of humour have helped make the time spent there enjoyable. Many thanks also to fellow members of Dr. Eastons' research group for their thoughts and comments.

I would like to acknowledge receipt of a Commonwealth Postgraduate Research Scholarship.

Finally, I would like to thank my Grandma, Peter (great friend and now husband) and Magoo as well as both our families for their help and support. Having written his Ph.D. thesis, Peter has been exceptionally patient and helpful while I've been writing my thesis. Now I look forward to helping him with his work while we await the arrival of our first child.

ABSTRACT

With a view to elucidating details of the mechanisms of the reactions catalysed by the enzyme Peptidylglycine α-Amidating Monooxygenase (PAM), nickel peroxide was investigated as a potential chemical model.

Initial studies on reactions of *N*-benzoylamino acid methyl esters with nickel peroxide were carried out and evidence for the mechanisms of the reactions were derived from product studies which provide supporting evidence for proposed intermediates, and by the measurement of deuterium isotope effects. The results indicate that the reaction of the amino acid derivatives with nickel peroxide proceeds *via* mechanisms that are analogous to those proposed for the PAM enzyme.

The relative rates of reaction of *N*-benzoylamino acid methyl esters with nickel peroxide were measured and a selectivity for reaction of glycine derivatives was observed analogous to that shown by the PAM enzyme. This selectivity is attributed to the stability of the intermediate α-centred radicals formed by hydrogen atom transfer and to the preferential binding of the more hydrophilic, less substituted amino acid derivative to the nickel peroxide.

The contrasting effects of different *C*- and *N*-terminal substituents on the reaction pathways and products of the reaction of amino acid derivatives with nickel peroxide were investigated. An *N*-benzoylamino acid derivative possessing a *C*-terminal carboxylic acid was found to bind

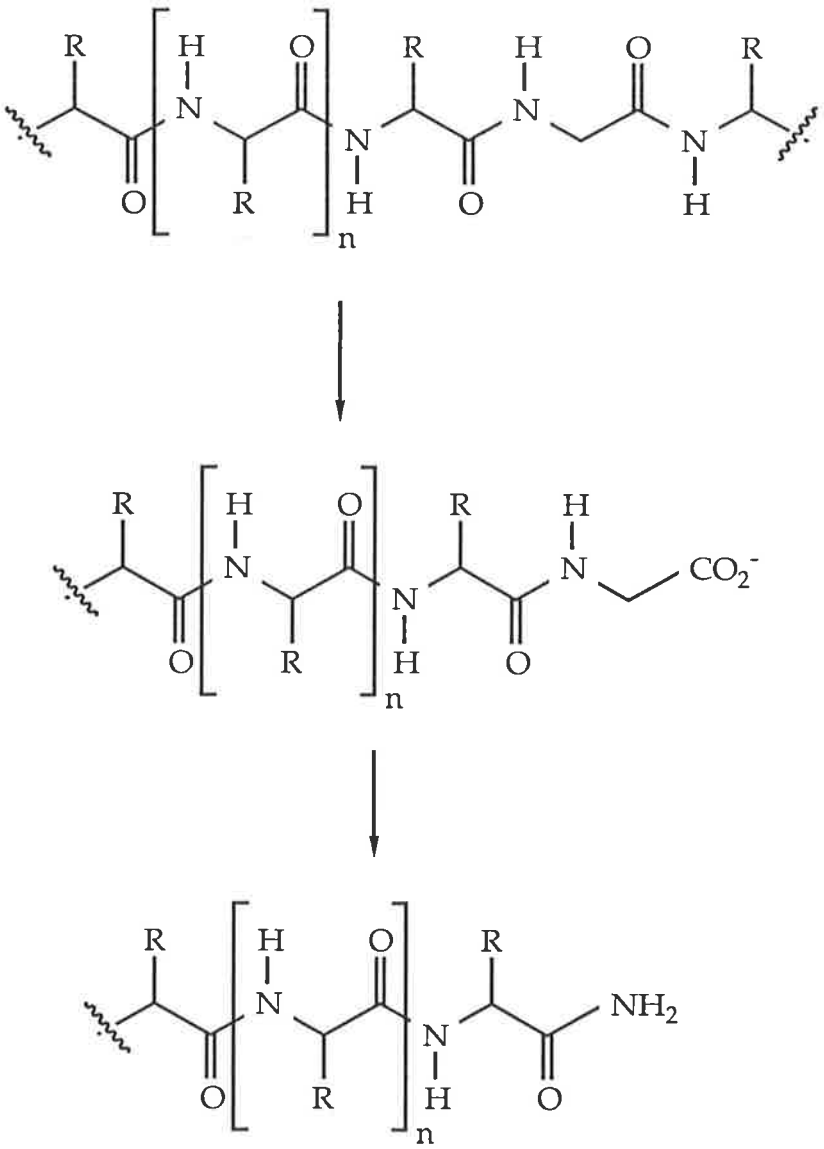
to nickel peroxide in preference to one possessing a *C*-terminal amide, which in turn binds in preference to the corresponding *C*-terminal methyl ester derivative. The *N*-phthaloyl substituent was found to be less activating than the *N*-benzoyl substituent.

The reactions of a series of *N*-benzoyl- and *N*-phthaloyl-dipeptide methyl esters with nickel peroxide were studied. In the case of *N*-benzoyldipeptide methyl esters preferential reaction of glycine was observed regardless of whether glycine was the *C*- or *N*-terminal residue of the dipeptide, whereas in the case of the *N*-phthaloyldipeptide methyl esters the *N*-phthaloyl substituent was found to disfavour reaction of an *N*-terminal residue to such an extent that preferential reaction of *C*-terminal non-glycine residues were observed even when the *N*-terminal residue was glycine. This property of the *N*-phthaloyl group enabled the synthesis of several α,β-dehydrodipeptides without affecting the stereochemical integrity of the *N*-terminal amino acid.

INTRODUCTION

Many naturally occuring biologically active peptides in mammalian and invertebrate neural and endocrine systems have a primary amide functionality at the carboxyl terminus.[1-10] Nervous systems as primitive as that of the sea anemone contain α-amidated peptides, and it has been suggested that this modification may be a very early specialization for use of peptides in communication between cells.[6,11] α-Amidated peptides elicit a large variety of physiological effects.[12,13] In mammals numerous α-amidated peptides occur for example, in the pituitary (*eg.*, vasopressin, oxytocin), the hypothalamus (*eg.*, thyrotropin releasing hormone, growth hormone releasing hormone), the gut (*eg.*, gastrins) and substance P from the spinal ganglia.[14-22] Generally the presence of the α-amide moiety is required for full biological activity however it also contributes to the biological stability.[2,3,5,6,13,23]

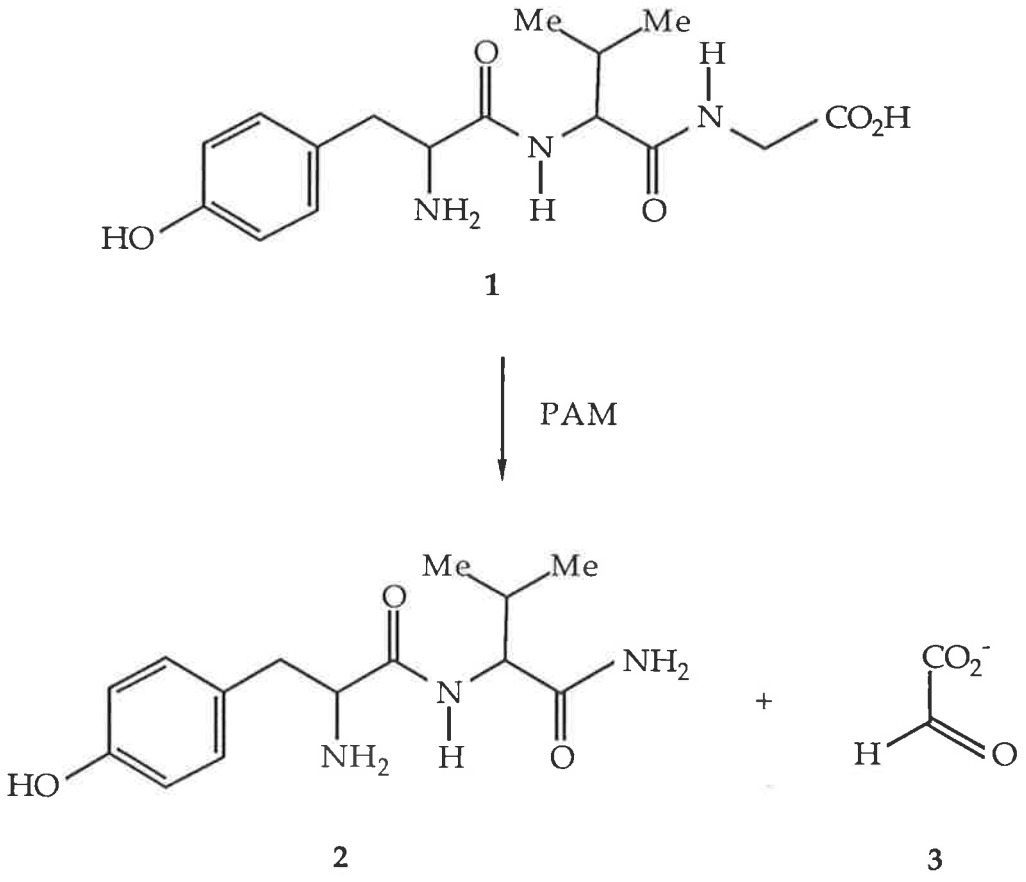
These bioactive α-amidated peptides are derived from larger precursor proteins in a two-step posttranslational processing sequence. Firstly, specific peptidase-catalysed hydrolyses[24-26] result in the *C*-terminal glycine extended peptides as the penultimate hormones.[1,5,8,27-30] These prohormones are then converted to the α-amides by an enzyme known as Peptidylglycine α-Amidating Monooxygenase or PAM (EC 1.14.17.3) (Scheme 1).[5,6,13,31-38]



Scheme 1

Because the PAM enzyme is central to the biosynthesis of a wide variety of neurohormones and is present in almost all neural tissue,[13,38-43] both its biochemical role and mechanism of action are under active investigation. Most of the work relating to the PAM enzyme has been published in the last 3-4 years. It is hoped that information about its mechanism and mode of operation may be useful in the design of inhibitors or orally active prodrugs.

The PAM enzyme was first isolated from the porcine pituitary by Bradbury and coworkers in 1982.[1] They demonstrated that it was capable of cleaving the two terminal carbons of a synthetic tripeptide, *D*-tyrosyl-*L*-valylglycine (**1**) to form a dipeptide amide, *D*-tyrosyl-*L*-valinamide (**2**). Subsequent experiments[34] with ^{15}N- and ^{14}C-labelled glycine residues showed that the terminal amide nitrogen of **2** originates from **1** and indicated that the other product was glyoxylate (**3**) (Scheme 2). It has subsequently been shown that for maximal activity the PAM enzyme requires enzyme bound copper ions, molecular oxygen and ascorbate.[1,8,18,38,44-47]



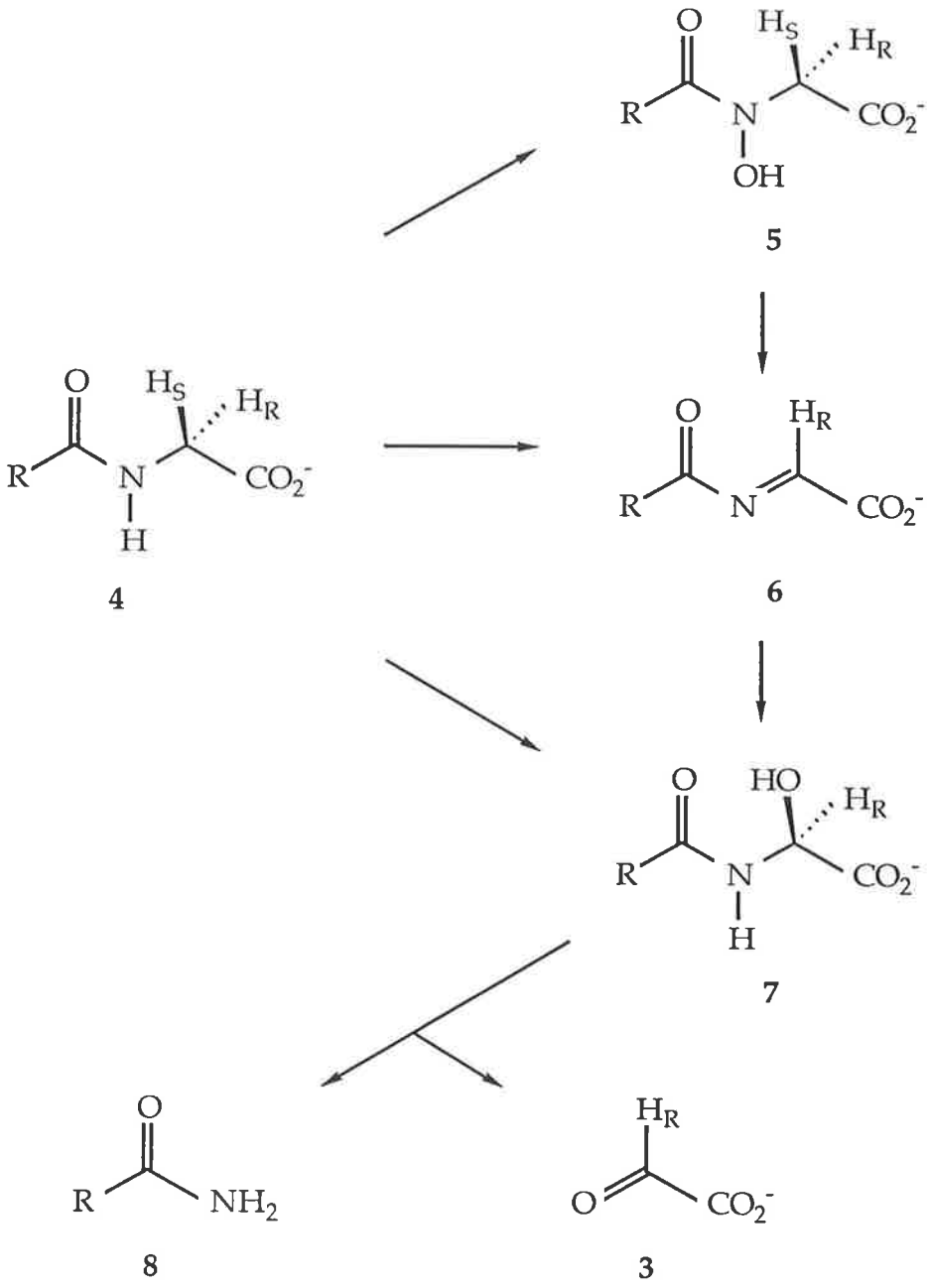
Scheme 2

Since its discovery the PAM enzyme has been found to be present in a host of mammalian tissues.[6,18,35,36,42,48-53] It has also been observed in and isolated from various sources including frogs (*Xenopus laevis*),[6,40,54-58] and can be produced in cell culture.[67,68] There exists both a soluble form,[35-38,40] and a membrane bound form.[37,38] Complementary DNAs encoding PAM have been cloned from bovine pituitary, frog skin and rat atrium.[41,57,58,61,62] Multiple forms of PAM with similar activities co-occur[38,58] and are thought to be members of a family of closely related enzymes with differing substrate specificities.[18]

In 1988 Ramer and coworkers investigated the stereochemistry of the oxidation of *D*-tyrosyl-*L*-valylglycine (**1**) to form the dipeptide amide, *D*-tyrosyl-*L*-valinamide (**2**) by the PAM enzyme (Scheme 2).[34,63] Using stereospecifically α-^3H-labelled glycine residues they showed that the enzyme oxidizes **1** with specific loss of the *pro-S* hydrogen of the glycyl residue, the *pro-R* hydrogen being retained in the glyoxylate (**3**). This is consistent with the ability of PAM to convert peptides terminating in *D*-alanine but not *L*-alanine residues.[7,8,45]

There are a number of mechanisms that have been proposed for the PAM-catalysed oxidation. The mechanism initially suggested by Bradbury and coworkers[1] begins with dehydrogenation of the *C*-terminal glycine-bearing peptide **4** to an *N*-acylimine **6** (Scheme 3). This would then spontaneously add water[64,65] to form **7**, which would cleave to the peptide amide **8** and glyoxylate (**3**). A more recent and generally accepted mechanism involves direct hydroxylation of the α-carbon of **4** to give **7**;[29,31,47,66-68] this is supported both by the cofactor requirements of PAM, by analogy with dopamine-β-hydroxylase,[69,70] and by its ability to transform glyoxylic acid phenylhydrazone to oxalic acid

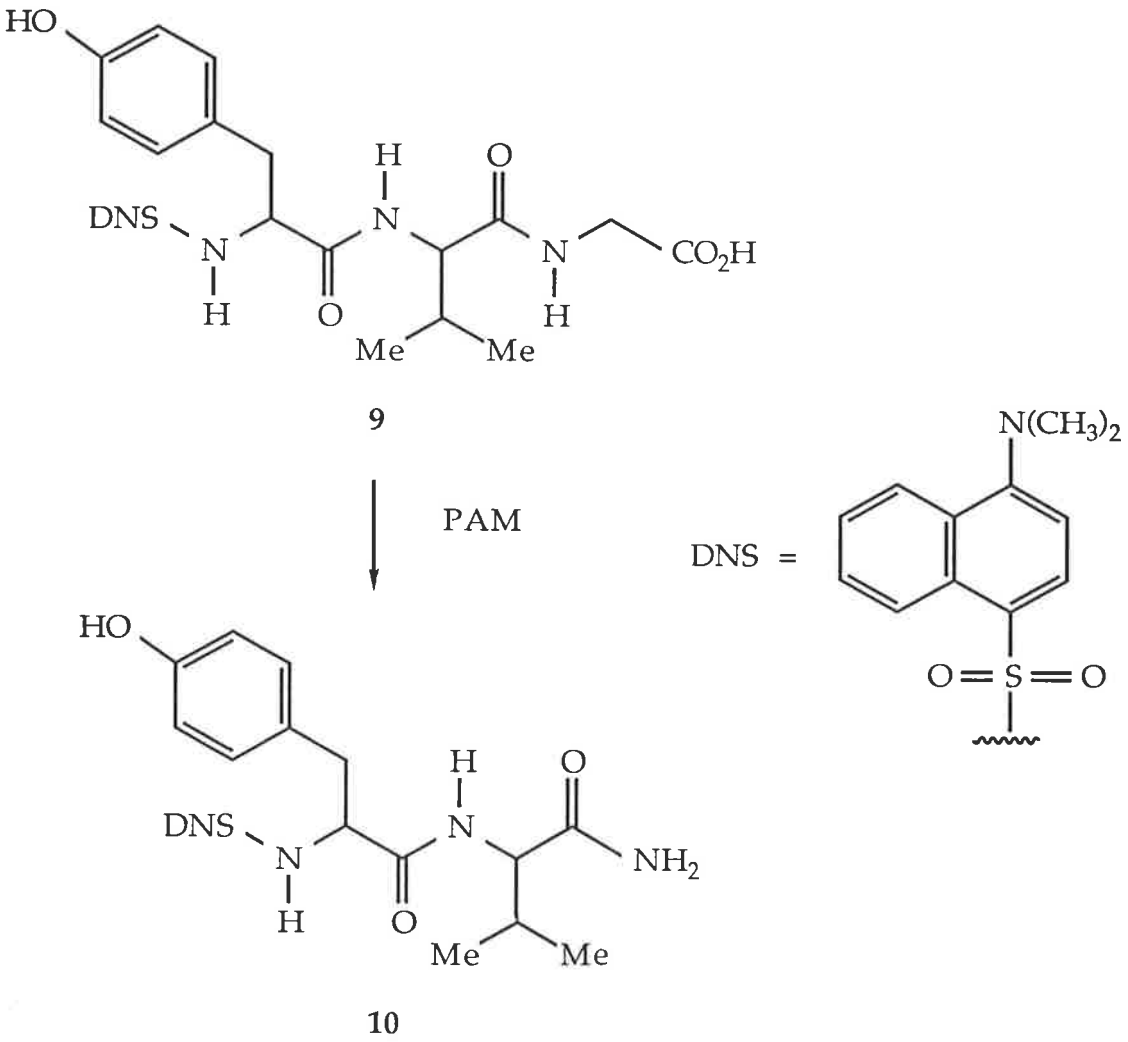
phenylhydrazide.[68] Further evidence for direct hydroxylation has been provided by the detection of incorporation of ^{18}O-labelled oxygen in reactions catalysed by the PAM enzyme.[31] The latter mechanism involving direct hydroxylation is the more favoured hypothesis at present although there are still those who prefer the hypothesis of α-C-N bond dehydrogenation followed by hydrolysis.[71] Another, although less likely possibility is *N*-hydroxylation to generate **5** followed by transformation to **6** and **7**. A chemical precedent for this sequence exists in the oxidation of *N*-aroylglycines with lead tetraacetate.[72]



Scheme 3

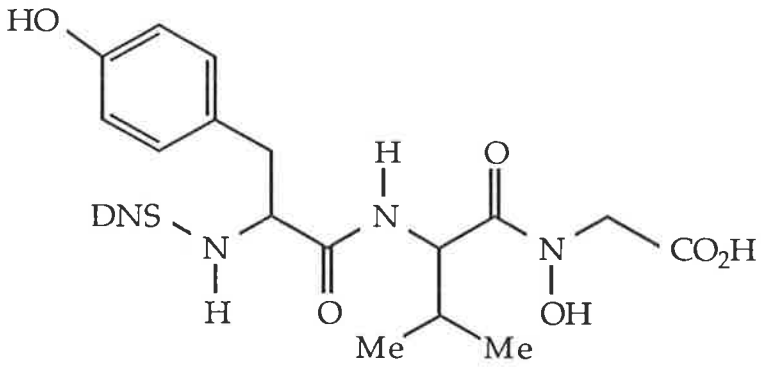
In an attempt to determine whether the mechanism of the C-N bond cleavage catalysed by the PAM enzyme involves either an *N*- or α-hydroxyglycine intermediate, Young and Tamburini[66] synthesized both the *N*- and α-hydroxyglycine analogues **11** and **12** of *N*-dansyltyrosylvalylglycine (**9**) and investigated the ability of the PAM enzyme to convert either **9** or its analogues **11** and **12** to *N*-

dansyltyrosylvalinamide (**10**). Although **9** was readily converted to **10** (Scheme 4), they found that neither the *N*-hydroxyglycine derivative **11**, nor one of the diastereomers of the α-hydroxyglycine derivative of **12**[a] were metabolised. The other diastereomer of **12** was readily converted to **10** by the PAM enzyme at a pH ranging from 4 - 9.



Scheme 4

[a] The absolute configuration of the diastereomers of the α-hydroxyglycine derivative was not assigned.



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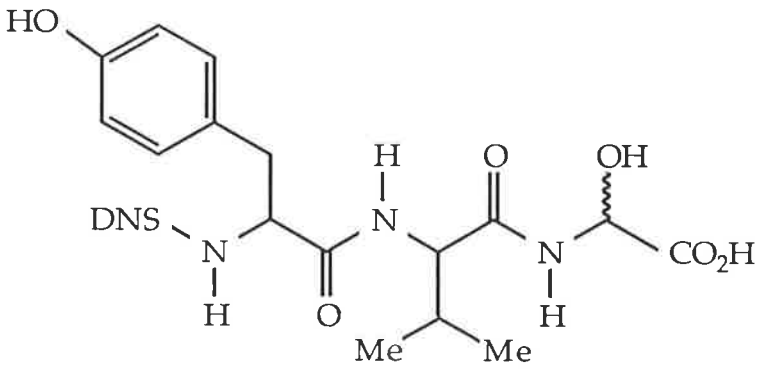

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Metabolism of **9** by the PAM enzyme to give **10** was found to be inhibited by 1,10-phenanthroline,[47,66] a metal chelator which is thought to interfere with molecular oxygen binding and activation, whereas formation of **10** from the diastereomer of **12** reported to undergo conversion above, was found to be independent of ascorbate and unaffected by 1,10-phenanthroline.

The results described above implicate one diastereoisomer of the α-hydroxyglycine derivative **12** as an intermediate in the reaction and show that the catalytic steps involving copper ion, molecular oxygen and ascorbate, precede the steps involved in the enzymic conversion of **12** to **10**.

Pertinent to this observation is the recent discovery that the PAM enzyme is not a single enzyme as first thought, but that it contains two protein subunits with separable activities:[30,32,67,73,74] Peptidyl α-Hydroxylating Monooxygenase (PHM) and Peptidylhydroxyglycine α-Amidating Lyase (PAL), the latter of which has also been referred to as α-Hydroxyglycine Amidating Dealkylase (HGAD).[73] The first subunit, PHM oxidizes a *C*-terminal glycine residue to form an intermediate α-hydroxyglycine peptide in a process which requires enzyme bound copper ion, molecular oxygen and ascorbate. The second subunit PAL catalyses the conversion of the intermediate peptidyl α-hydroxyglycine to the α-amidated peptide and glyoxylic acid at neutral and acidic pH with no cofactor requirements. Conversion of peptidyl α-hydroxyglycine to the α-amidated product occurs spontaneously at alkaline pH.

As mentioned earlier much of the work relating to the PAM enzyme has been done in the past three to four years. To date the smallest non-peptide substrates shown to be accepted by the PAM enzyme are hippuric acid and several ring substituted derivatives.[30] The PAM enzyme catalyses the conversion of these to form the corresponding benzamides and glyoxylic acid. Thus the PAM enzyme is highly substrate specific with apparently only the acylglycine structure required for recognition.

Many investigations are currently underway by biochemists and organic chemists to try and mimic the reaction catalysed by the PAM enzyme. Recently several papers have been published, mainly during the course of this work, reporting non-enzymic models for the PAM enzyme.[29,65,71,75,76] Among these are three copper mediated models, which are based on coordination of copper (I) or copper (II) to the peptides.

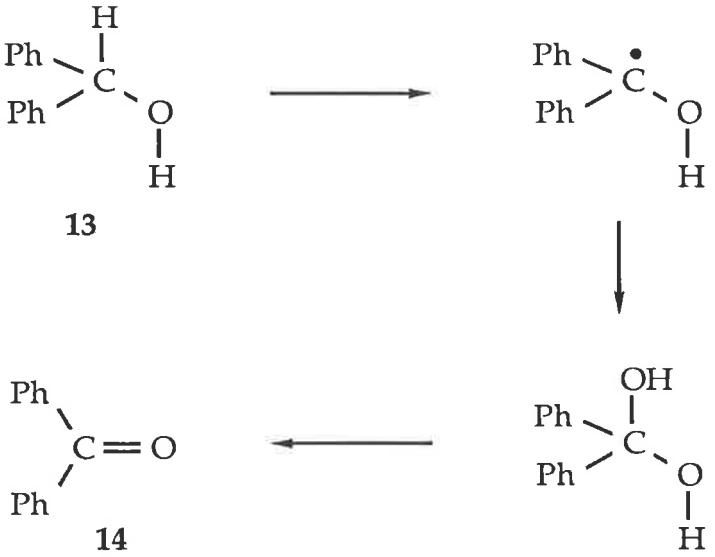
These copper complexes of peptide derivatives in the presence of an oxidant undergo reaction to form C-terminal amides *via* similar intermediates to those proposed for reactions catalysed by the PAM enzyme. Their analogy to the PAM enzyme also includes the use of copper. These systems are similar to others previously reported involving nickel(II)-, cobalt(II)- and iron(II)- peptide complexes,[77] although the latter were not proposed as enzyme mimics.

It is hoped that by finding suitable non-enzymic models for the PAM enzyme and investigating their reactions with different substrates, the results will provide more information about the mechanism of action of the enzyme. Such information would allow the design of potential inhibitors orally active prodrugs which could then be tested using the non-enzymic model. It would also give an indication of the possible use of the enzyme in synthetic methods. Enzymes in synthetic organic chemistry[78] are becoming increasingly popular primarily because they can catalyse reactions under mild conditions and functionalise non-activated positions with a high degree of regioselectivity. Conversely, enzyme models may offer advantages in synthesis.

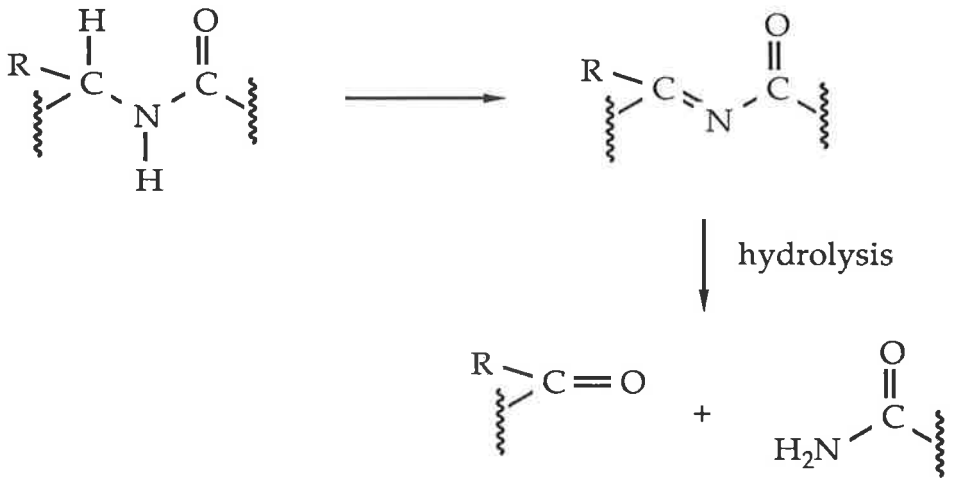
Like other enzymes requiring a metal cofactor the mechanism of reaction of the substrates of the PAM enzyme is thought to involve single electron transfer *i.e.* free radical reactions. For the ease of isolation of products, the study of reactions of derivatised amino acids and peptides in organic solvents is preferred, but in order to have an effective potential mimic for the PAM enzyme we required a free radical oxidant that would work in aqueous solvents with underivatised amino acids and peptides, as well as organic solvents with derivatised amino acids and peptides. This thesis examines nickel peroxide as a possible non-enzymic model for the

PAM enzyme. Nickel peroxide was chosen as it is a known free radical oxidant that can be used in both organic and aqueous solvents.[79-82] It is easily and cheaply prepared by treatment of an aqueous solution of nickel sulphate with an alkaline solution of sodium hypochlorite.[83] The activity of the nickel peroxide is determined by iodometry and is generally about 0.35×10^{-2} g.-atom of available oxygen per gram. Another advantage with using nickel peroxide is that it is easily recovered from reactions and regenerated for further use by treatment with an alkaline solution of sodium hypochlorite. The mechanism of reaction of nickel peroxide has been explained in terms of its characteristic ability to abstract hydrogen atoms and act as a source of hydroxyl radicals.

There are many publications[81,83-89] and at least one comprehensive review[90] on the oxidation of organic compounds with nickel peroxide, however the action of nickel peroxide on the α-carbon-nitrogen (α-C-N) bond in an amino acid or peptide has not been reported. By analogy with a mechanism postulated for the oxidation of diphenylcarbinol (benzhydril) (**13**) to benzophenone (**14**) by nickel peroxide (Scheme 5),[90] it seemed likely that nickel peroxide would induce the oxidative dehydrogenation of an amino acid or peptide, with subsequent hydrolysis (Scheme 6). Such a process would have analogy with reactions catalysed by PAM.



Scheme 5

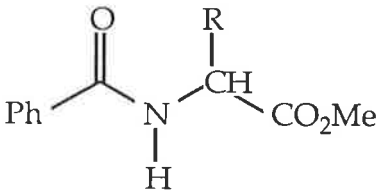


Scheme 6

CHAPTER 1

Reactions of Amino Acid Derivatives with Nickel Peroxide.

In order to investigate the mechanism of reaction of amino acid derivatives with nickel peroxide, reactions of *N*-benzoylglycine methyl ester (**15a**), *N*-benzoyl-*D,L*-alanine methyl ester (**15b**) and *N*-benzoyl-*D,L*-valine methyl ester (**15c**) with nickel peroxide were studied. Each of the amino acid derivatives was dissolved in dry distilled benzene, at 0.05 Molar, to which 2.6 mole equivalents of nickel peroxide was added. The mixtures were then heated at reflux under nitrogen for 1 hour, after which the hot reaction mixtures were filtered and concentrated.

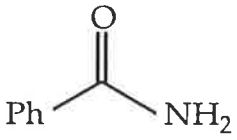


15

a) R = H
b) R = Me
c) R = $CH(Me)_2$

Treatment of *N*-benzoylglycine methyl ester (**15a**) with nickel peroxide produced benzamide (**16**). This was isolated from the reaction mixture in 39% yield, together with 51% unreacted starting material **15a**, by chromatography on silica after removal of the solvent benzene. The identity of benzamide (**16**) was confirmed by comparison of the melting

point and the ^{13}C n.m.r. spectrum with that of an authentic sample purchased from Aldrich Chemical Company.



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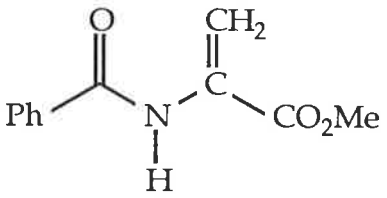


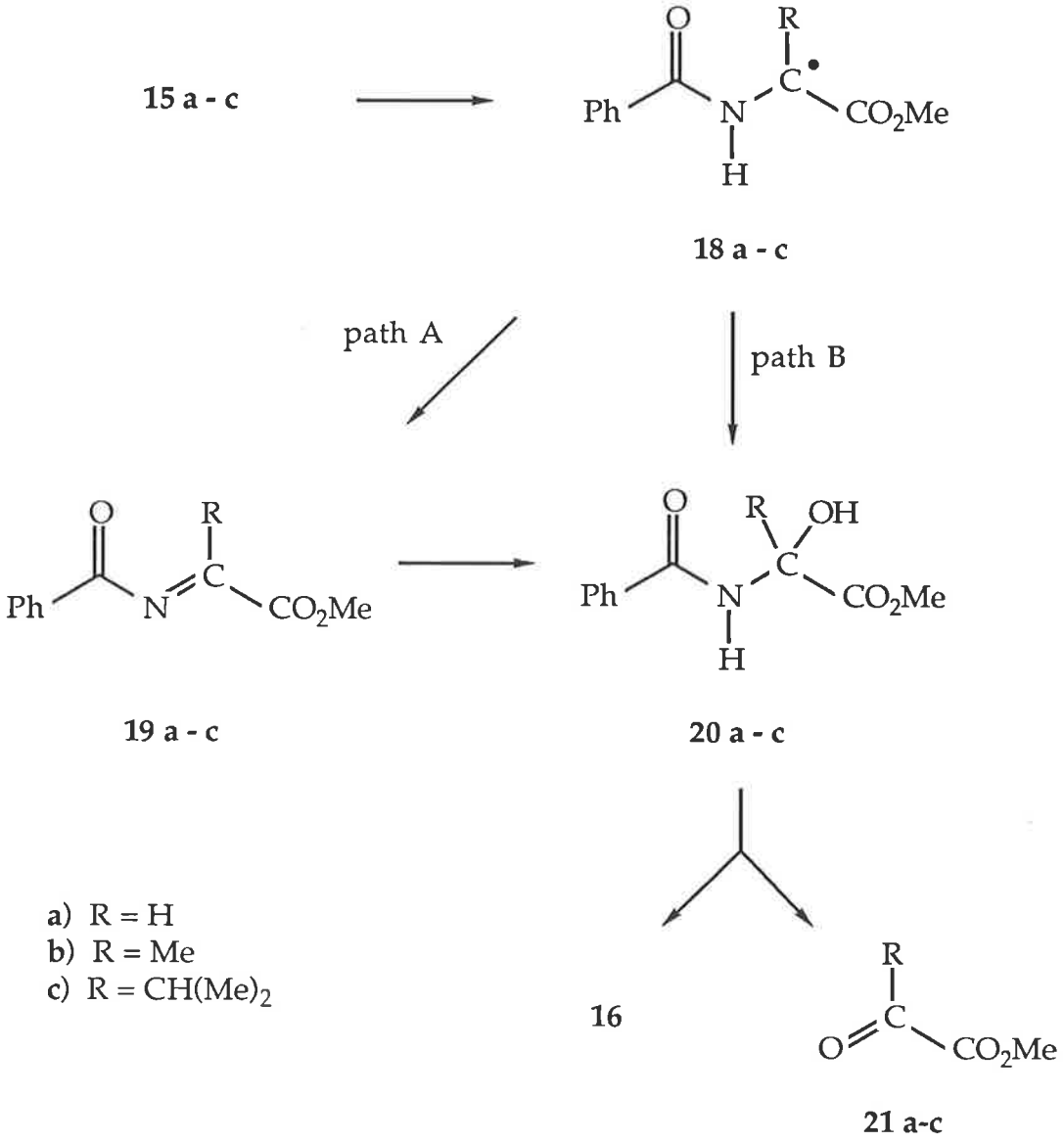
17

Treatment of *N*-benzoyl-*D,L*-alanine methyl ester (**15b**) with nickel peroxide afforded **16** and methyl 2-benzamidopropenoate (**17**). The formation of **17** was confirmed by the characteristic resonances[91,92] of the olefinic hydrogens at δ 6.00 (d, J = 1.3 Hz, 1H) and δ 6.80 (s, 1H) in the ^1H n.m.r. spectrum of the reaction mixture. Both **16** and **17** were isolated from the crude reaction mixture by chromatography on silica, in yields of 13% and 9%, respectively, together with 69% unreacted starting material **15b**.

Treatment of *N*-benzoyl-*D,L*-valine methyl ester (**15c**) with nickel peroxide afforded a trace amount of **16**, detected by H.P.L.C. (high performance liquid chromatography) analysis of the reaction mixture. Unreacted starting material **15c** was subsequently isolated from the reaction mixture in 93% yield, by chromatography on silica. Repetition of the experiment with 10 equivalents of nickel peroxide and refluxing over 48 hours, resulted in the isolation of **16** and unreacted starting material **15c**, in yields of 14% and 12%, respectively, after chromatography on silica. The filtered reaction mixture showed some discolouration, indicating

decomposition had occurred. In the three systems studied, no reaction occurred in the absence of nickel peroxide.

Based on reports of the oxidative action of nickel peroxide in terms of its ability to act as both a hydrogen abstractor and a source of hydroxyl radicals, as discussed in the Introduction, reasonable mechanisms for the reactions of **15a-c** with nickel peroxide to form **16** are shown in Scheme 7.



Scheme 7

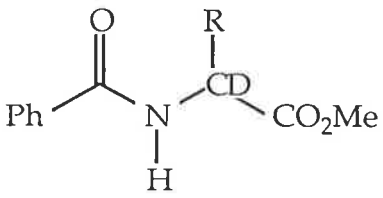
The initial step in the reactions involves the formation of the α-carbon-centered captodative radicals **18a-c**, by hydrogen atom transfer from the corresponding amino acid derivatives **15a-c**. The α-centered radicals **18a-c** can then form the corresponding *N*-acylimines **19a-c** by a second hydrogen atom transfer, followed by addition of water to give the corresponding α-hydroxy amino acid derivatives **20a-c** (path A). Alternatively the radicals **18a-c** can combine with hydroxyl radicals from nickel peroxide to give the corresponding α-hydroxy amino acid derivatives **20a-c** directly (path B). Subsequent hydrolysis of **20a-c** affords **16** and the methyl glyoxlates **21a-c**. Nickel peroxide is known to be a hydrate, and can provide water for the hydrolysis. The methyl glyoxylates **21a-c** are not isolated from their respective reaction mixtures, presumably because they react further with the nickel peroxide.

The production of the α,β-dehydroamino acid derivative **17** in the reaction of the alanine derivative **15b** with nickel peroxide can be attributed to tautomerization of the intermediate *N*-acylimine **19b**.# Treatment of **17** with nickel peroxide in refluxing benzene, under nitrogen overnight, afforded **16**. The identity of **16** was confirmed by comparison of the H.P.L.C. trace of the crude reaction mixture, with that of an authentic sample of **16**. The formation of **16** from **17** can be rationalised simply by tautomerization of **17** to the intermediate *N*-acylimine **19b**, which is then hydrolysed (Scheme 8). Hence **17** can be considered as a transient intermediate in the reaction of **15b** with nickel peroxide, formed by a diversion from the pathway proposed in Scheme 7. Formation of **17** in the reaction implies that the tautomerization of the intermediate *N*-acylimine **19b** to give **17** competes with the hydrolysis of **19b** to give **16**.

#Alternatively, the dehydroamino acid derivative **17** may result from dehydration of the hydroxyamino acid derivative **20b**.

Scheme 8

To seek support for the mechanism proposed in Scheme 7, *N*-benzoyl-α,α-dideuterioglycine methyl ester (**22a**) was treated with nickel peroxide in refluxing benzene, under nitrogen. The deuterium content of the starting material **22a** was established at 80% dideuteriated and 18% monodeuteriated by mass spectrometry. After 1 hour the hot reaction mixture was filtered and concentrated, and both benzamide (**16**) and unreacted starting material **22a** were recovered, in yields of 19% and 70%, respectively, by chromatography on silica. A mass spectrum of the recovered, unreacted starting material **22a** established the deuterium content at 82% dideuteriated and 13% monodeuteriated. Hence the deuterium in the deuteriated glycine derivative **22a** was not being exchanged under the reaction conditions.



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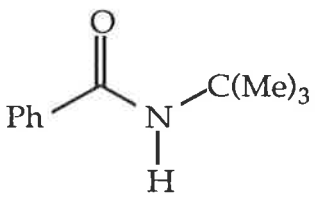


23

a) R = D
b) R = Me

The relative rate of reaction of the glycine derivative **15a** compared to its deuteriated analogue **22a** with nickel peroxide was determined in a competitive experiment. A solution of the amino acid derivatives **15a** and

22a was treated with nickel peroxide in refluxing benzene, under nitrogen. *N-tert*-Butylbenzamide (**23**) was found to be unreactive under these conditions and was used as an internal standard to measure the extent of reaction. Aliquots were removed from the reaction at intervals and filtered while hot. ^{1}H n.m.r. Spectroscopy was used to measure the ratio of the amino acid derivatives **15a** and **22a**, from which a value for the ratio of their rates of reaction could be measured (Equation 1). The competitive experiment showed that the unlabelled glycine derivative **15a** reacted 2.9 ± 0.5 times as fast as the deuteriated glycine derivative **22a**.

$$k_{15a} / k_{22a} = \ln ([15a]_{t=1} / [15a]_{t=0}) / \ln ([22a]_{t=1} / [22a]_{t=0}) \qquad \textbf{Equation 1}$$

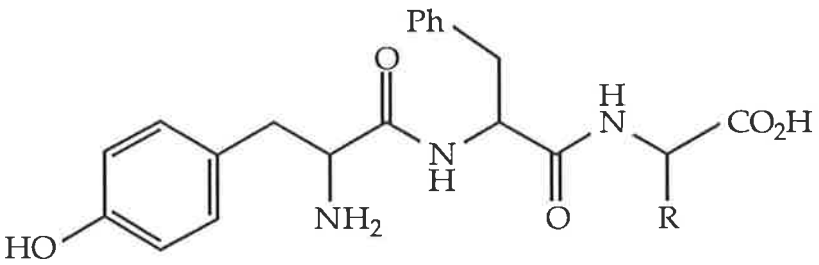
The relative rate of reaction of the alanine derivative **15b** compared to *N*-benzoyl-*D,L*-α-deuterioalanine methyl ester (**22b**) with nickel peroxide was also determined in a competitive experiment analogous to the one above. The unlabelled alanine derivative **15b** was found to react approximately 3 times as fast as the deuteriated alanine derivative **22b**.

The deuterium isotope effects of approximately 3 observed in the competitive reactions of the amino acid derivatives **15a** and **22a**, and **15b** and **22b** is consistent with the mechanisms shown in Scheme 7, in which α-carbon-hydrogen bond homolysis is a rate determining step in the reactions of **15a-c** with nickel peroxide. The value of these deuterium isotope effects is consistent with other deuterium isotope effects observed for abstraction of the α-hydrogen in reactions of amino acid derivatives under free radical conditions.[93]

The mechanisms for reaction of the amino acid derivatives **15a-c** with nickel peroxide to produce benzamide (**16**), proposed above (Scheme

through integration of the methyl and methylene signals

7), are analogous to mechanisms proposed for the reaction of substrates of PAM to produce *C*-terminal amides (Scheme 3). As discussed in the Introduction, PAM has been found to be selective for *C*-terminal glycine extended precursors. The only other reported[46] reactive substrate is *D*-tyrosylphenylalanyl-*D*-alanine (**24b**), containing a *D*-alanine residue at the *C*-terminus, but the rate at which **24b** reacts is much less than that for the corresponding *C*-terminal glycine derivative **24a**.



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a) R = H
b) R = Me

Qualitative comparison of the yields of products from the reactions of the amino acid derivatives **15a-c** with nickel peroxide (Table 1), also indicates a selectivity for reaction of the glycine derivative **15a** in preference to the alanine derivative **15b**, which in turn is more reactive than the valine derivative **15c**. To examine the extent and cause of this selectivity, the relative rates of reaction of the amino acid derivatives **15a-c** with nickel peroxide were determined in a series of competitive experiments. Typically a solution of two of the amino acid derivatives **15a-c**, each at 0.025 Molar in benzene, was treated with nickel peroxide at reflux under nitrogen, in the presence of *N-tert*-butylbenzamide (**23**) as an internal standard. Aliquots were removed from the reaction at intervals and filtered while hot. Both ^{1}H n.m.r. spectroscopic and H.P.L.C.

techniques were used to measure the ratios of the amino acid derivatives **15a-c**. From the variation in these ratios, as a function of the extent of reaction, the relative rates of reaction of **15a-c** could be calculated (Equation 1). The results are given in Table 2. Good correlation was found between the results of analysis of experiments by the two techniques, however, the error in measurement was large when the difference in rates was large, in the comparison between **15a** and **15b**.

Table 1. Qualitative comparison of the extent of reaction of the amino acid derivatives 15a-c at 0.05 M in benzene, with 2.6 mole equivalents of nickel peroxide.

Substrate	% Unreacted starting material	Products 16 (%)	17 (%)
N-Benzoylglycine methyl ester (**15a**)	51	39	
N-Benzoyl-*D,L*-alanine methyl ester (**15b**)	69	13	9
N-Benzoyl-*D,L*-valine methyl ester (**15c**)	93	trace	

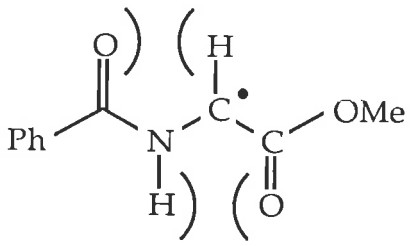
The relative rates of reaction of the amino acid derivatives **15a-c** listed below (Table 2), confirm the qualitative comparison made earlier, and clearly indicate that the glycine derivative **15a** reacts faster than the alanine derivative **15b**, which in turn reacts faster than the valine derivative **15c**.

Table 2. Quantitative comparison of the relative rates of reaction of the amino acid derivatives 15a-c, each at 0.025 M in benzene, with nickel peroxide.

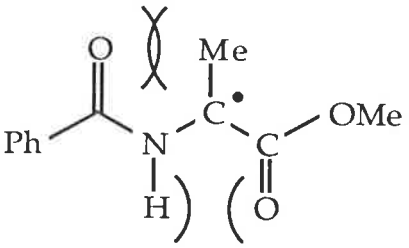
Substrate	Relative Rate of Reaction
N-Benzoylglycine methyl ester (**15a**)	10.0 ± 2.5
N-Benzoyl-*D,L*-alanine methyl ester (**15b**)	1.0[a]
N-Benzoyl-*D,L*-valine methyl ester (**15c**)	0.14 ± 0.03

[a] Assigned as unity

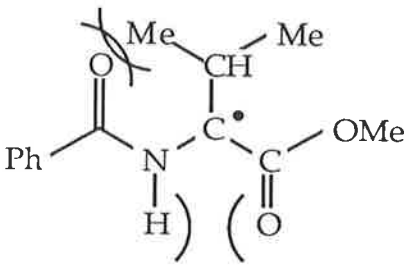
There are two possible factors which contribute to this selectivity, one is based on the relative ease of formation of the α-centered radicals **18a-c**. Since a rate determining step of the reaction has been shown by the isotope experiments to be α-hydrogen atom transfer, the ease of formation of the radicals **18a-c** is reflected in the relative rates of reaction of the corresponding amino acid derivatives **15a-c**. Therefore the rate of formation of the α-centered radical **18a** from the glycine derivative **15a** is faster than the rate of formation of **18b** from **15b**, which is in turn faster than the rate of formation of **18c** from **15c**. This implies that the radical **18a** is more stable than **18b**, which in turn is more stable than **18c**. The particular stability of the radical **18a** can be attributed to the favourable geometry of planar conformations where there is maximum overlap of orbitals necessary for stabilization (Figure 1). The radical **18b** is destabilized with respect to the radical **18a** by the non-bonding interactions associated with planar conformations of **18b**. The radical **18c** is even less stable owing to even more severe non-bonding interactions. This order of reactivity has been observed in other free radical reactions of amino acid derivatives.[93,94]



18a



18b



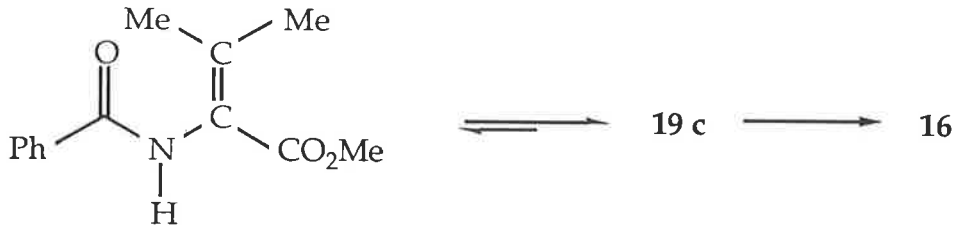
18c

Figure 1. Non-bonding interactions associated with planar conformations of the radicals **18a - c**.

The other factor which probably contributes to the selectivity involves the relative ease of binding of the amino acid derivatives **15a-c** to the surface of the nickel peroxide in the heterogeneous reaction mixture. The nickel peroxide hydrate will have a relatively polar surface, in contrast with the nonpolar enviroment of the solvent, benzene. The glycine derivative **15a** will be less soluble than the alanine derivative **15b** in benzene, and will therefore be more likely to bind to the nickel peroxide. Similarly **15b** will be less soluble in benzene than the valine derivative **15c** and so will bind selectively to the nickel peroxide.

Based on the formation of **17** in the reaction of **15b** with nickel peroxide, it is reasonable to propose that methyl 2-benzamido-3-

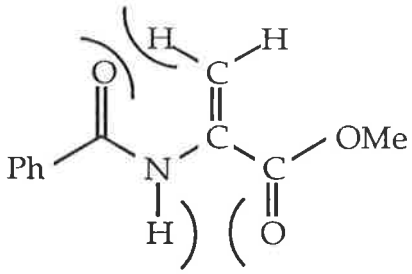
methylbut-2-enoate (**25**) would be formed in the reaction of the valine derivative **15c** with nickel peroxide. Treatment of **25** with nickel peroxide in refluxing benzene under nitrogen, overnight resulted in the formation of **16**, which was detected by H.P.L.C analysis of the crude reaction mixture. Benzamide (**16**) was subsequently isolated from the reaction mixture in 11% yield, by chromatography on silica. Formation of **16** is proposed to arise from tautomerization of **25** to the intermediate *N*-acylimine **19c**. Subsequent hydrolysis of **19c** affords **16** as shown in Scheme 9.



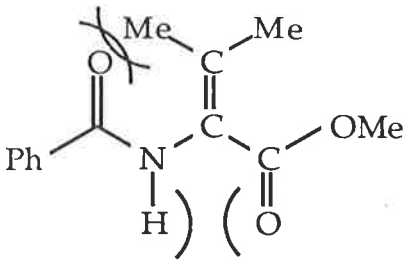
25

Scheme 9

Presumably, the formation of **25** is not observed in the reaction of **15c** with nickel peroxide, because the rate of reaction of **25** is faster than its rate of formation. To be observed the rate of reaction of the α,β-dehydroalanine derivative **17** must be slower than its rate of formation. As discussed above the reaction of **15b** to form **17**, is faster than the reaction of **15c**. The rate of reaction of **25** is probably faster than that of **17**, because steric interactions associated with **25** are more severe (Figure 2). This is consistent with the detection of **17** in the reaction of **15b**, but not of **25** in the reaction of **15c**.



17



25

Figure 2. Non-bonding interactions associated with planar conformations of the α,β-dehydroamino acid derivatives **17** and **25**.

The effect of change in substrate concentration, and change in temperature on the relative rates of reaction of the amino acid derivatives **15a-c** with nickel peroxide was also investigated. The relative rate of reaction of the glycine derivative **15a** compared to the alanine derivative **15b**, each at 0.0025 Molar in refluxing benzene, with nickel peroxide, was measured using the method described above. In a similar study the relative rate of reaction of **15b** compared to the valine derivative **15c**, each at 0.0025 Molar with nickel peroxide was determined. The relative rate of reaction of **15a** compared to **15b**, each at 0.025 Molar, in benzene at room temperature, with nickel peroxide was also measured. The results of these studies are given in Table 3, along with the results from the studies with the amino acids **15a-c** at 0.025 Molar in refluxing benzene. A comparison of the relative reactivity of the alanine derivative **15b** and the valine derivative **15c** at room temperature was not attempted because the absolute rate of reaction of **15c** under those conditions was too slow.

Table 3. Concentration and temperature studies of the reactions of the amino acid derivatives 15a-c in benzene, with nickel peroxide.

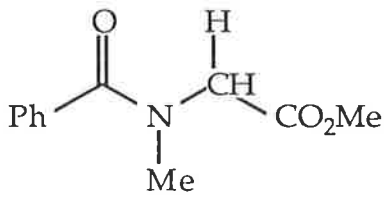
Substrate	Relative Rate of Reaction		
	80° C		20° C
	0.025 M.	0.0025 M.	0.025 M.
N-Benzoylglycine methyl ester (**15a**)	10.0 ± 2.5	4.5 ± 0.4	4.0 ± 0.5
N-Benzoyl-*D,L*-alanine methyl ester (**15b**)	1.0[a]	1.0[a]	1.0[a]
N-Benzoyl-*D,L*-valine methyl ester (**15c**)	0.14 ± 0.03	0.43 ± 0.03	-

[a] Assigned as unity

The results show that a decrease in the concentration of each substrate in the reaction leads to a decrease in the selectivity for reaction of the glycine derivative **15a** compared to the alanine derivative **15b**, and the selectivity for reaction of **15b** compared to the valine derivative **15c**. This decrease in selectivity can be attributed to the decreased nickel affinity of the more hydrophilic amino acid at lower concentrations in benzene. Hence **15a** does not bind to the nickel peroxide as fast or as selectively, and so the relative rate of reaction of **15a** compared to **15b** decreases. Similarly there is a decrease in affinity to the nickel of **15b** compared to **15c**, and so a drop in the selectivity for reaction of **15b** is also observed. The decrease in selectivity from 10 observed for the reaction of **15a** compared to **15b** at 80° C, to 4 when the temperature of the reaction is decreased to 20° C, at first appears unusual. It is presumably a result of competition between the factors affecting the reactivity of **15a** and **15b**. The reaction at 20° C is performed over 7 days, whereas the reaction at 80° C is performed in 4 hours. The lower temperature may allow pre-equilibration of binding of

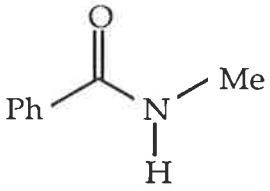
15a and **15b** to the nickel peroxide before hydrogen abstraction. Thus it is likely from these results that binding of the amino acid derivatives **15a-c** to the nickel peroxide is also one of the rate determining steps in their reactions.

In Scheme 7, the reaction of the amino acid derivatives **15a-c** with nickel peroxide was proposed to go *via* an *N*-acylimine (path A), or directly *via* an α-hydroxy intermediate (path B). Evidence for path A is provided by the formation of the α,β-dehydroamino acid derivative **17** in the reaction of the alanine derivative **15b** with nickel peroxide, which was rationalised as occurring by tautomerization of the intermediate *N*-acylimine **19b**. The reaction of *N*-benzoylsarcosine methyl ester (**26**) with nickel peroxide was studied to seek evidence for direct formation of an α-hydroxy intermediate (path B), as the sarcosine derivative **26** is unable to form an *N*-acylimine.



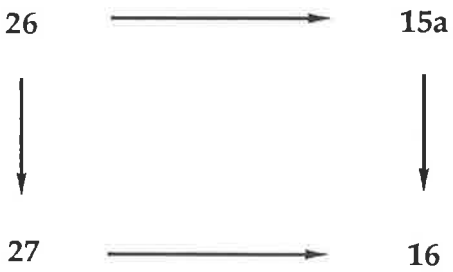
26

Treatment of *N*-benzoylsarcosine methyl ester (**26**) in refluxing benzene for 4 hours, with 2 mole equivalents of nickel peroxide, afforded *N*-methylbenzamide (**27**) and benzamide (**16**). The identities of **27** and **16** were confirmed by comparison of the H.P.L.C. trace of the reaction mixture with those of authentic samples of **27** and **16**. Both **27** and **16** were subsequently isolated by chromatography on silica, in yields of 24% and 7% repectively, together with 29% unreacted starting material **26**.



27

In the reaction of **26** with nickel peroxide, the formation of benzamide (**16**) can be rationalised by two routes (Scheme 10). One involves *N*-demethylation of **26** to give the glycine derivative **15a**, which then reacts further with nickel peroxide to produce **16**, as shown previously (Scheme 7). The other route is *via* loss of the methoxycarbonylmethyl group of **26** to produce *N*-methylbenzamide (**27**) which then reacts further with nickel peroxide to produce **16**.



Scheme 10

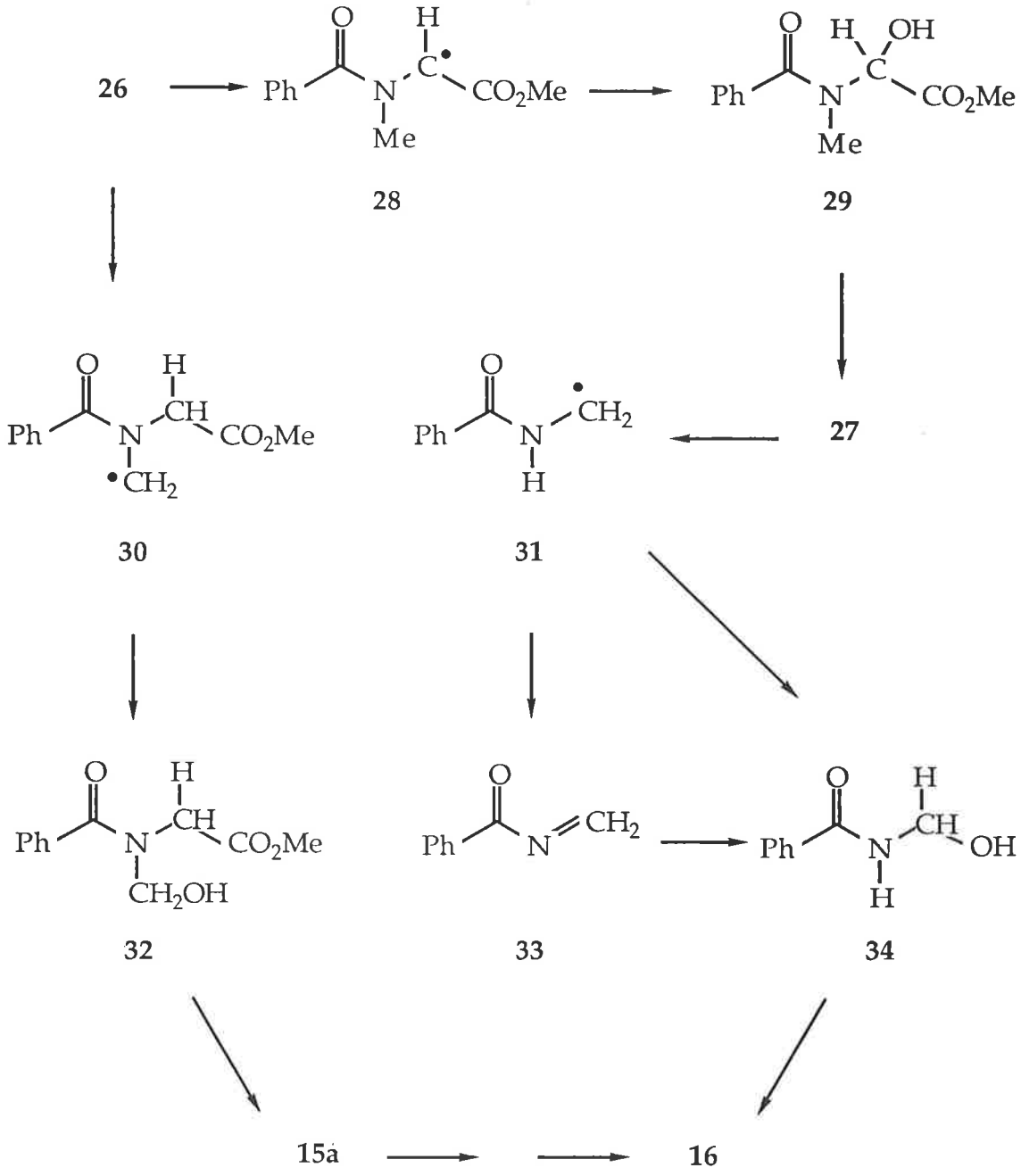
To examine the latter proposal, the reaction of *N*-methylbenzamide (**27**) with nickel peroxide, in refluxing benzene was investigated. Analysis of the reaction mixture by H.P.L.C. confirmed the formation of **16**. The reaction mixture was chromatographed on silica and **16** was recovered in 22% yield. Unreacted *N*-methylbenzamide (**27**) was also recovered in 56% yield.

The relative rate of reaction of **26** was determined in a competitive experiment with the alanine derivative **15b**. A solution of the alanine derivative **15b** and the sarcosine derivative **26** at 0.025 Molar in refluxing benzene, was treated with nickel peroxide in the presence of *N-tert*-butylbenzamide (**23**) as internal standard. Aliquots were removed from the reaction at intervals, filtered and analysed by H.P.L.C. chromatography. The results show that the sarcosine derivative **26** reacts 1.3 ± 0.2 times slower than the alanine derivative **15b**, and is therefore approximately thirteen times less reactive than the glycine derivative **15a** under these conditions.

The relative rate of reaction of *N*-methylbenzamide (**27**) compared to the glycine derivative **15a**, was also determined by way of a competitive experiment between **27** and the alanine derivative **15b**, analogous to the one described above. *N*-Methylbenzamide (**27**) was found to react 1.1 ± 0.2 times faster than the alanine derivative **15b**, and hence is approximately nine times less reactive than the glycine derivative **15a**.

The relative rates of reaction of **26** and **27**, compared to **15a** do not indicate whether **16** is formed from **26** only *via* **27**, or also *via* **15a** (Scheme 10). If the reaction of **26** with nickel peroxide to form **16** is *via* formation of the glycine derivative **15a**, on the basis of the substantially slower relative rate of reaction of **26** compared to **15a**, **15a** would only be formed as a transient intermediate in the reaction and would not be expected to be produced in significant quantities. Thus the fact that formation of **15a** was not observed in the reaction of **26** with nickel peroxide, does not preclude the possibility of reaction of **26** to give **16** *via* **15a**.

In line with the mechanisms proposed for the reaction of nickel peroxide with the amino acid derivatives **15a-c** (Scheme 7), the mechanisms proposed for the formation of **27** and **16** in the reaction of **26** with nickel peroxide are shown in Scheme 11.



Scheme 11

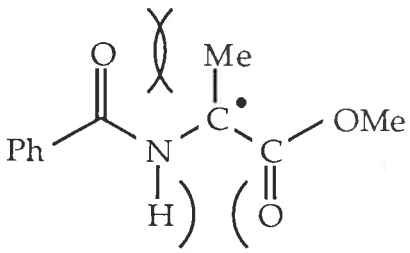
The mechanism for the *N*-demethylation reaction of the sarcosine derivative **26** with nickel peroxide, to form benzamide (**16**) *via* the glycine derivative **15a**, can be rationalised as follows. The initial step in the reaction is hydrogen atom transfer from the *N*-methyl group of **26** to form the primary radical **30**. The radical **30** is unable to form an *N*-acylimine, however it can combine with a hydroxyl radical from nickel peroxide to form the hydroxy derivative **32**. Hydrolysis of **32** leads to the formation of the glycine derivative **15a**, which will then react with the nickel peroxide further to form **16** as illustrated previously (Scheme 7).

The reaction of the sarcosine derivative **26** with nickel peroxide to form *N*-methylbenzamide (**27**), is proposed to go *via* hydrogen abstraction at the α-position in line with the mechanisms already proposed (Scheme 7). The initial step in the reaction of **26** with nickel peroxide is formation of the α-centered radical **28** by hydrogen atom transfer from **26** (Scheme 11). The radical **28** is unable to form an intermediate *N*-acylimine, therefore the reaction is proposed to proceed *via* the α-hydroxy intermediate **29**, formed by the radical **28** combining with a hydroxyl radical from nickel peroxide. Subsequent hydrolysis of **29** affords **27**.

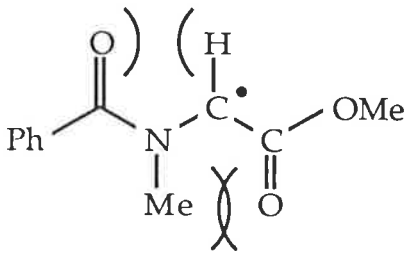
The formation of **16** by further reaction of **27** with nickel peroxide, can also be rationalised as shown in Scheme 11. The initial step is formation of the primary radical **31** by hydrogen atom transfer from **27**. In line with the mechanisms previously proposed (Scheme 7), the radical **31** can then form either the *N*-acylimine **33**, which can add water to form the hydroxy derivative **34**, or it can form **34** directly by combining with a hydroxyl radical from nickel peroxide. Subsequent hydrolysis of **34** afffords **16**. The formation of *N*-methylbenzamide (**27**) in the reaction of **26** with nickel peroxide is a clear indication that reaction is proceeding *via*

direct hydroxylation. On this basis the reactions of the amino acid derivatives **15a-c** with nickel peroxide are probably proceeding *via* direct hydroxylation (Scheme 7, path B) as well as *via* imine formation (Scheme 7, path A). The direct hydroxylation mechanism proposed for reaction of **26** with nickel peroxide is analogous to the more generally accepted mechanism for PAM which involves direct hydroxylation.

The slower relative rate of reaction of **26** compared to **15a** is consistent with the hypothesis that the rate of hydrogen atom transfer from amino acid derivatives is affected by the extent of nonbonding interactions in the product radicals. The degree of non-bonding interactions associated with planar conformations of **28** is similar to those in **18b** (Figure 3). The radical **28** will be destabilized relative to **18a** due to the steric interactions between the *N*-methyl substituent and the ester moiety. The slower relative rate of reaction of **26** compared to **15a** may also reflect the ease of binding to the nickel peroxide, of **26** compared to **15a**. The presence of the *N*-methyl substituent in **26** may hinder binding to nickel peroxide *via* the electrons of the amide moiety.



18b



28

Figure 3. Non-bonding interactions associated with planar conformations of the radicals **18b** and **28**.

The slower relative rate of reaction of **27** compared with **15a** indicates that the methoxycarbonyl group in conjunction with the benzamido group facilitates reaction, presumably due to the relative ease of formation of the more stable captodative radical **18a**. In the case of the reaction of **27** with nickel peroxide, the primary radical **31** is only stabilized by interaction with the electrons of the amide. The extent of binding to nickel peroxide of **27** may also be less than that of **15a** due to the interaction of the methoxycarbonyl group of **15a** with the nickel peroxide.

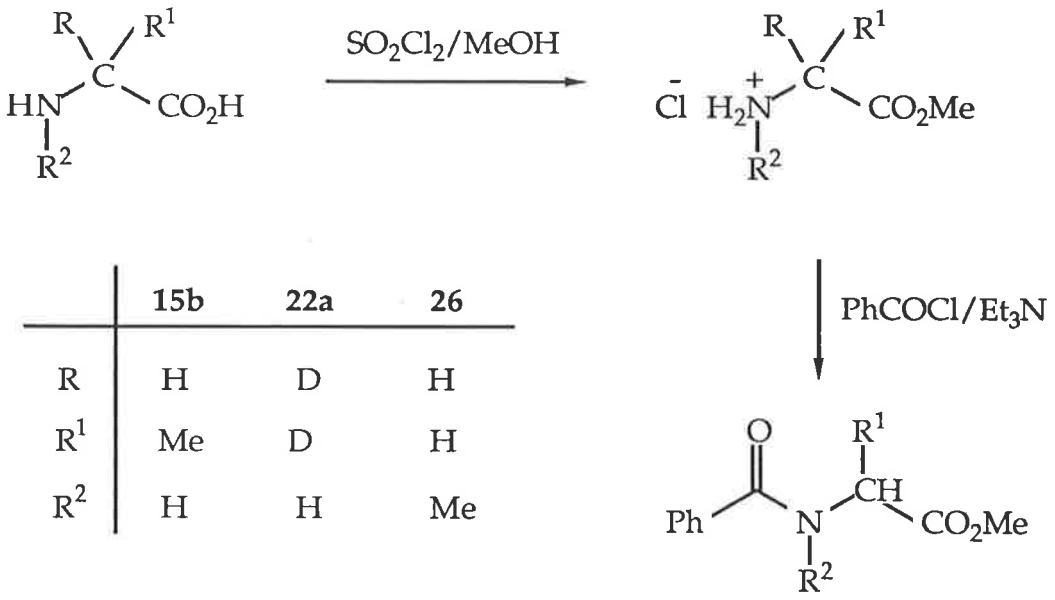
In summary, the results discussed in this Chapter illustrate that nickel peroxide causes the hydrolytic cleavage of the amino acid derivatives **15a-c** to produce benzamide (**16**), *via* a mechanism that is similar to that proposed for the PAM enzyme. Nickel peroxide also exhibits a selectivity for reaction of the glycine derivative **15a**, compared to the alanine and valine derivatives **15b** and **15c** respectively, which is analogous to the substrate specificity observed for the PAM enzyme. This selectivity for reaction of glycine by nickel peroxide has not been reported as being observed in other papers on non-enzymic models for the PAM enzyme published to date. Based on these results and the similarities of the mechanisms proposed for nickel peroxide and for the PAM enzyme, nickel peroxide would appear to be a good chemical model for the PAM enzyme. Factors affecting the selectivity for nickel peroxide may also reflect factors contributing to the substrate selectivity of the PAM enzyme, for example, the preferential binding of glycine derivatives and the relative ease of formation of glycyl radicals. Thus it is possible that the naturally occuring substrates of the PAM enzyme are synthesized with a glycine residue at the *C*-terminus because it is so easily removed by oxidation.

Synthesis of Substrates.

The *N*-benzoylamino acid methyl esters **15a, 15b, 22a, 26** and *N*-methylbenzamide (**27**) required for the studies in this Chapter were synthesized as described below. Samples of the *N*-benzoylamino acid methyl esters **15c, 17, 22b, 25**, and *N-tert*-butylbenzamide (**23**) were available from previous work.

The *N*-benzoylamino acid methyl esters **15b, 22a** and **26** were prepared from *D,L*-alanine, α,α-dideuterioglycine and sarcosine respectively, as shown in Scheme 12. Hydrochloride salts of the methyl esters of the amino acids were obtained by addition of the appropriate amino acids to methanol which had been pretreated with thionyl chloride. The *N*-benzoylamino acid methyl esters were subsequently prepared by treatment of the appropriate hydrochloride salts of the amino acid methyl esters with benzoyl chloride under basic conditions.

N-Benzoylglycine methyl ester (**15a**) was prepared from commercially available hippuric acid *via* the esterification procedure described above.



	15b	22a	26
R	H	D	H
R^1	Me	D	H
R^2	H	H	Me

Scheme 12

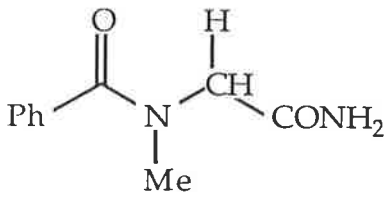
α,α-Dideuterioglycine used in the preparation of *N*-benzoyl-α,α-dideuterioglycine methyl ester (**22a**) described above, was prepared by treatment of glycine with acetic anhydride and deuterium oxide.[95]

N-Methylbenzamide (**27**) was prepared by treatment of a solution of methylamine with benzoyl chloride under basic conditions.

CHAPTER 2

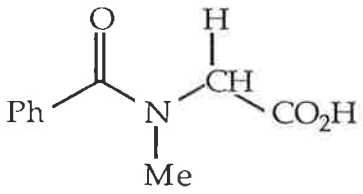
The Effects of Different *C*- and *N*-Terminal Protecting Groups on the Reactions of Amino Acid Derivatives with Nickel Peroxide.

The results described in Chapter 1 show that nickel peroxide reacts with *N*-benzoylamino acid methyl ester derivatives in a manner analogous to that of the PAM enzyme. Nickel peroxide also exhibits a specificity for reaction of glycine similar to that of the PAM enzyme. This Chapter looks at the effects on the reaction pathways and products when different *C*- and *N*-terminal protected amino acid derivatives are treated with nickel peroxide.



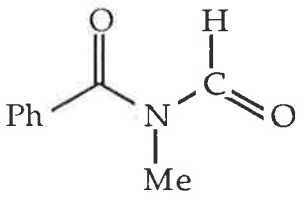
35

A solution of *N*-benzoylsarcosinamide (**35**) in refluxing benzene was treated with nickel peroxide. After 2 hours the reaction mixture was filtered and concentrated. Purification of the reaction mixture by chromatography on silica resulted in *N*-methylbenzamide (**27**) and benzamide (**16**) being isolated in 11% and 7% yields, respectively. Unreacted *N*-benzoylsarcosinamide (**35**) was also recovered from the reaction mixture in 42% yield.



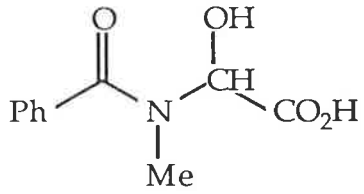
36

A solution of *N*-benzoylsarcosine (**36**) in refluxing benzene was treated with 2.6 mole equivalents of nickel peroxide as described for **35**. ^{1}H n.m.r. Spectroscopic analysis of the crude reaction mixture indicated the formation of *N*-methylbenzamide (**27**), benzamide (**16**) and a third product identified as *N*-formyl-*N*-methylbenzamide (**37**). The reaction mixture was chromatographed on silica, and **27**, **16** and **37** were isolated in yields of 9%, 10% and 5%, respectively. The identification of **37** was made on the basis of ^{1}H and ^{13}C n.m.r. spectroscopy, mass spectrometry and accurate mass determination.



37

The mechanisms for reaction of **35** and **36** to give **27** and **16** are likely to be analogous to the mechanisms proposed for reaction of *N*-benzoylsarcosine methyl ester (**26**) with nickel peroxide to give **27** and **16** (Scheme 11). The formation of **37** is unique to reaction of **36** with nickel peroxide. The formation of **37** in the reaction of **36** with nickel peroxide, can be attributed to the oxidative decarboxylation of the intermediate α-hydroxy derivative **38**.



38

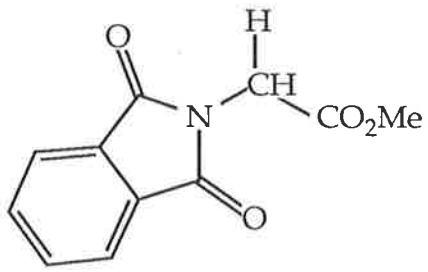
It has been previously reported that α-hydroxy acids upon reaction with nickel peroxide in organic and aqueous solvents, undergo oxidative decarboxylation resulting in the formation of the corresponding carbonyl compound and carbon dioxide.[96]

Nickel peroxide has not been reported as being able to catalyse the hydrolytic cleavage of esters to the corresponding carboxylic acids, hence products of type **37** are not seen in reactions of the various *N*-benzoylamino acid methyl ester derivatives discussed in Chapter 1.

When the relative rates of reaction of **15a-c**, **26**, and **27** with nickel peroxide were measured, in each case the relative loss of the substrates from the benzene solutions was comparable throughout the reaction. In contrast, when the relative rates of reaction of **35** and **36**, compared to **26**, were investigated, analysis of the initial samples taken from the reaction mixtures after the addition of the nickel peroxide, showed that **35** and **36** were selectively adsorbed onto the nickel peroxide, although the yields of products at that stage were not enough to account for the loss of the starting materials. The relative rate of reaction of **35** compared to **36**, was also investigated. Analysis of the initial samples taken from the reaction mixtures after the addition of the nickel peroxide showed that **36** was selectively adsorbed onto the nickel peroxide in preference to **35**, although again the yields of products at that stage were not enough to account for

the loss of the starting materials. Thus, the relative rates of reaction of **35** and **36** compared to **26** could not be determined, but it is evident that **35** and **36** bind selectively to nickel peroxide, in preference to **26**.

The results from the reactions of the sarcosine derivatives **26, 35** and **36** indicate that nickel peroxide is capable of catalysing the hydrolytic cleavage of amino acid derivatives where the carboxyl group is free or protected, either as the ester or the amide. The results also show that **36** has a greater affinity for the nickel peroxide than **35**, and both bind more selectively when compared to **26**. This indicates that the free carboxylic acid group of **36** binds to the nickel peroxide more readily than the amide group of **35**, which in turn binds more readily than the methoxycarbonyl group of **26**. This is analogous to the specificity for binding of substrates exhibited by the PAM enzyme in that it too is specific in binding the free *C*-terminal carboxyl group of its known substrates.

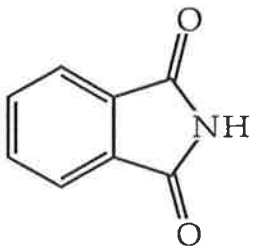


39

A solution of *N*-phthaloylglycine methyl ester (**39**) in refluxing benzene was treated with 5 mole equivalents of nickel peroxide. After 5 hours the reaction mixture was filtered and concentrated. High field ^1H n.m.r. spectroscopic analysis of the reaction mixture showed only signals

corresponding to those of the starting material **39**, which was subsequently isolated in 66% yield by chromatography on silica.

The expected product from the reaction of **39** with nickel peroxide, based on the proposed mechanisms (Scheme 7), would be phthalimide (**40**). If formation of **40** had occurred it is likely that due to its low solubility in benzene it would have bound preferentially to the nickel peroxide, in which case it would have been removed from the reaction at the filtration step.#

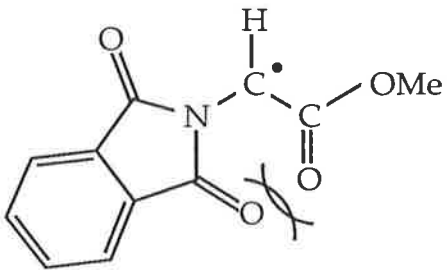


40

The relative rate of reaction of *N*-phthaloylglycine methyl ester (**39**) and *N*-benzoylglycine methyl ester (**15a**), was determined by way of a competitive experiment between **39** and the *N*-benzoylalanine derivative **15b**. A solution of the glycine derivative **39** and the alanine derivative **15b**, each at 0.026 Molar in refluxing benzene, was treated with nickel peroxide in the presence of *N*-*tert*-butylbenzamide (**23**). Aliquots were removed from the reaction at intervals, filtered and analysed by ^{1}H n.m.r. spectroscopy. The results show that the *N*-phthaloylglycine derivative **39** reacts 3.5 ± 0.5 times slower than the *N*-benzoylalanine derivative **15b**, and is therefore approximately thirty five times less reactive than the corresponding *N*-benzoylglycine derivative **15a**.

#All attempts to elute organic material that may have been bound to the nickel were unsuccessful

The relative rate of reaction of **15a** compared to **39** indicates that *N*-benzoyl-substituted amino acid residues are more reactive than those with an *N*-phthaloyl substituent, and that the α-centered radical **18a** is formed in preference to the α-centered radical **41**. The greater rate of reaction of **15a** compared to **39** can be explained in terms of electron delocalization and the non-bonding interactions associated with the intermediate radicals **18a** and **41** (Figure 4), and differences in the ability of **15a** and **39** to bind to the nickel peroxide.



41

Figure 4. Non-bonding interactions associated with planar conformations of the radical **41**.

With respect to the delocalization of the unpaired spin density in the intermediate radicals **18a** and **41**, the *N*-benzoyl substituent of **18a** would be expected to afford greater delocalization than the *N*-phthaloyl substituent of **41**. This is because of the greater availability of the electrons on the amide which are involved in resonance with one carbonyl group, compared to those on the imide which are involved in resonance with two carbonyl groups (Figure 5). Thus the presence of the *N*-phthaloyl substituent is observed to "deactivate" the α-centre of the adjacent amino acid towards reaction. The contrasting effects of *N*-phthaloyl and *N*-benzoyl substituents have been observed previously in the reactions of *N*-

phthaloyl and *N*-acyl substituted α-amino acids with *N*-bromosuccinimide.[97]

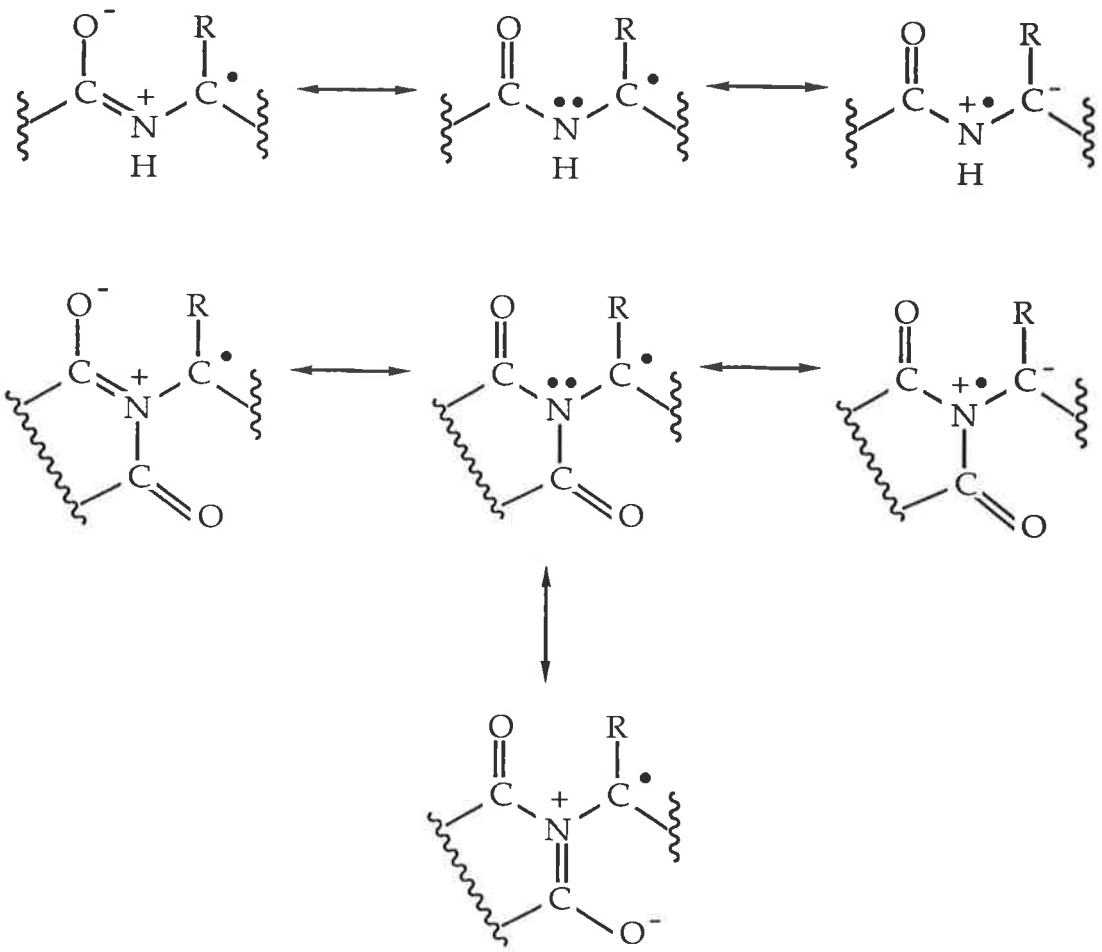
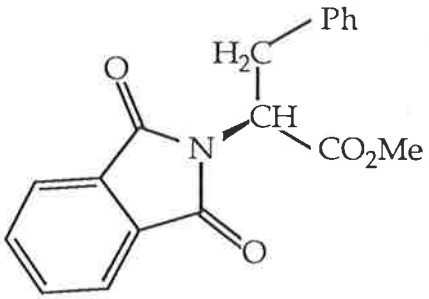


Figure 5. Resonance stabilization of acylamino and diacylamino substituted radicals.

With respect to the difference in the ability of **15b** and **39** to bind to the nickel peroxide, the binding of amino acid derivatives to nickel peroxide is thought to be in part, *via* the electrons on nitrogen of the amino acid residues. The close proximity of the carbonyl moieties of the *N*-phthaloyl group around the nitrogen of **39** is likely to hinder binding of

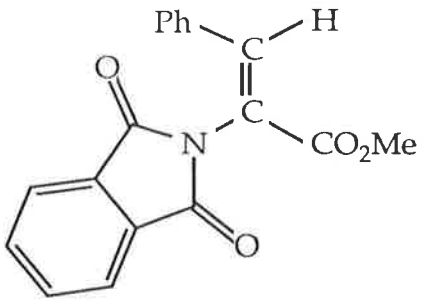
39 to the nickel peroxide more so than the *N*-benzoyl group of **15a** and **15b**. On this basis alone we would expect to observe a slower rate of reaction of **39** when compared with **15b**.

The reactions of *N*-phthaloyl-*L*-phenylalanine methyl ester (**42**) and *N*-benzoyl-*L*-phenylalanine methyl ester (**44**) with nickel peroxide were also investigated.



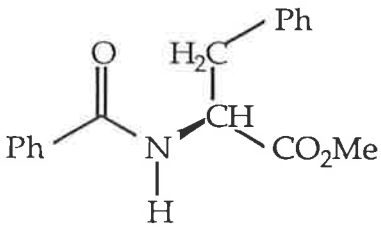
42

N-Phthaloyl-*L*-phenylalanine methyl ester (**42**) was treated with nickel peroxide in refluxing benzene. After 48 hours the hot reaction mixture was filtered, concentrated and purified by chromatography on silica. *N*-Phthaloyl-α,β-dehydrophenylalanine methyl ester (**43**) was isolated in 4% yield. Unreacted *N*-phthaloyl-*L*-phenylalanine methyl ester (**42**) was also recovered from the reaction mixture in 80% yield. The identity of **43** was established by ^{1}H n.m.r. spectroscopy, mass spectrometry and accurate mass determination, as well as through comparison with an authentic sample.[98]



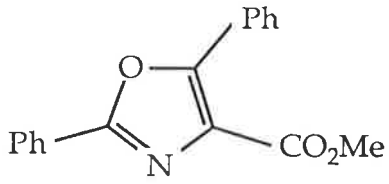
43

The ^1H n.m.r. spectrum of **43** showed a single resonance, with integration equivalent to one hydrogen, at δ 8.13 attributable to the vinyl proton of the phenylalanine residue. As only one ^1H n.m.r. resonance for the vinyl proton was detected, presumably only one isomer is present. The isomer of **43** isolated from the reaction was assigned the *Z*-configuration based on the fact that the *Z*-configuration is known to be favoured by α,β-dehydroamino acids.[99-103]



44

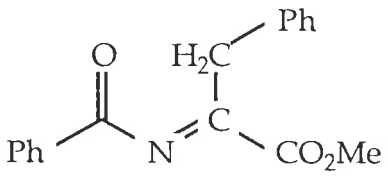
N-Benzoyl-*L*-phenylalanine methyl ester (**44**) was treated with nickel peroxide in refluxing benzene as described for **42**. Benzamide (**16**) and a second product determined to be methyl 2,5-diphenyloxazole-4-carboxylate (**45**) were isolated in yields of 19% and 12% respectively, by chromatography on silica. Unreacted *N*-benzoyl-*L*-phenylalanine methyl ester (**44**) was also recovered from the reaction mixture in 22% yield. The physical characteristics of **45** were found to be consistent with those previously reported.[104]



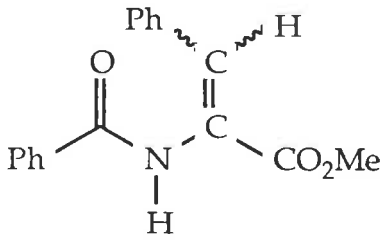
45

The relative rate of reaction of **44** compared to **42** was established in a direct comparison. A solution of **44** and **42** each at 0.026 Molar in refluxing benzene, was treated with nickel peroxide in the presence of *N*-*tert*-butylbenzamide (**23**). Aliquots were removed from the reaction at intervals, filtered, concentrated and analysed by ^1H n.m.r. spectroscopy. As expected the results showed that the *N*-benzoylphenylalanine derivative **44** reacted 4.5 ± 0.5 times faster than the *N*-phthaloyl substituted analogue **42**.

The mechanisms for reaction of **44** with nickel peroxide to produce benzamide (**16**), are likely to be analogous to those discussed for the reaction of **15a-c** (Scheme 7). The formation of the oxazole **45**, in the reaction of **44** with nickel peroxide may be attributed to subsequent oxidation of the either the intermediate *N*-acylimine derivative **46** or the tautomeric α,β-dehydrophenylalanine derivative **47**. Oxidative cyclizations of substrates are known to be effected by nickel peroxide.[89,105,106]

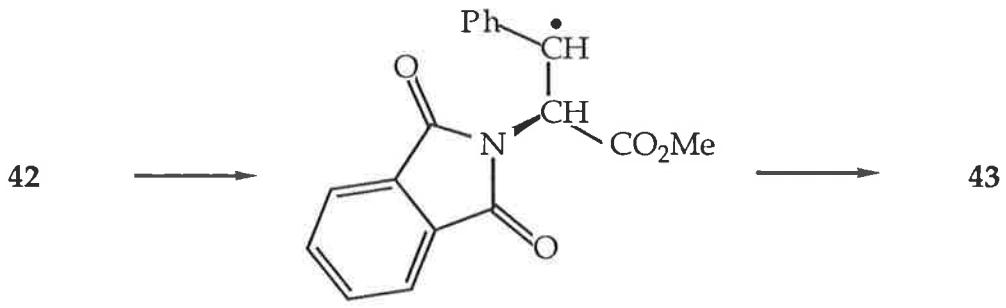


46



47

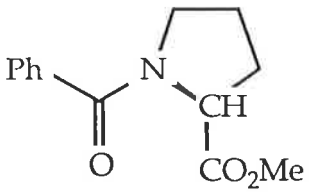
By analogy with the radical reactions of *N*-phthaloylphenylalanine derivatives with *N*-bromosuccinimide (NBS),[107] the reaction of **42** with nickel peroxide probably occurs at the β-position to give the benzylic radical **48** (Scheme 13). This would explain why the reaction of the corresponding *N*-benzoyl-*L*-phenylalanine derivative **44** with nickel peroxide is only 4.5 times faster than that of the corresponding *N*-phthaloyl-*L*-phenylalanine derivative **42**, whereas, the relative rate of formation of the α-centred radical **18a** from the *N*-benzoylglycine derivative **15a**, is at least thirty five times faster than the formation of the α-centred radical **41** from the corresponding *N*-phthaloylglycine derivative **39**. The benzylic radical **48** can then lose a hydrogen atom to form *N*-phthaloyl-α,β-dehydrophenylalanine methyl ester (**43**) (Scheme 13).



48

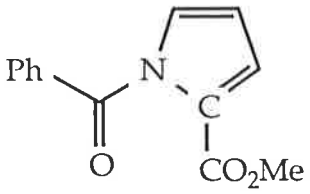
Scheme 13

The reaction of *N*-benzoyl-*L*-proline methyl ester (**49**) with nickel peroxide was also investigated.



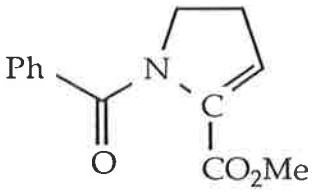
49

N-Benzoyl-*L*-proline methyl ester (**49**) was treated with nickel peroxide in refluxing benzene as described above for **42**. The reaction mixture was purified by chromatography on silica. Benzamide (**16**) and a second product determined to be *N*-benzoyl-2-methoxycarbonylpyrrole (**50**) were isolated in yields of 6% and 5%, respectively. Unreacted *N*-benzoyl-*L*-proline methyl ester (**49**) was also recovered from the reaction mixture in 57% yield. The spectral characteristics of **50** were found to be consistent with those previously reported.[108]

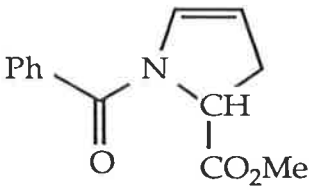


50

The pathways for the formation of **16** from **49**, are likely to be analogous to those for the reaction of the sarcosine derivative **26** with nickel peroxide, to give **16**. The formation of the pyrrole **50** may be attributed to the subsequent oxidation of either of the intermediate *N*-acylenamines **51** or **52**. Nickel peroxide has been reported as being able to catalyse the aromatisation of cyclic compounds *via* oxidative dehydrogenation.[109]



51



52

In summary, the selectivity for binding to the nickel peroxide observed for the sarcosine derivatives **26, 35** and **36** is also analogous to that exhibited by PAM, with the free carboxyl group of **36** showing greater affinity for the nickel peroxide than the amide of **35**, which in turn binds more selectively than the methoxycarbonyl group of **26**.

The influence of *N*-phthaloyl and *N*-benzoyl substituents on the reactivity of amino acid derivatives with nickel peroxide was also determined. Compared to the *N*-benzoyl substituent, the *N*-phthaloyl substituent deactivates the α-centre of the adjacent amino acid to reaction with nickel peroxide. The formation of *N*-phthaloyl-α,β-dehydrophenylalanine methyl ester (**43**), methyl 2,5-diphenyloxazole-4-carboxylate (**45**) and *N*-benzoyl-2-methoxycarbonylpyrrole (**50**) in the reactions of *N*-phthaloyl-*L*-phenylalanine methyl ester (**42**), *N*-benzoyl-*L*-phenylalanine methyl ester (**44**) and *N*-benzoyl-*L*-proline methyl ester (**49**) respectively, with nickel peroxide are consistent with oxidative cyclization and dehydrogenation reactions known to be effected by nickel peroxide.

Synthesis of Substrates.

N-Benzoylsarcosinamide (**35**) was prepared from *N*-benzoylsarcosine methyl ester (**26**), by bubbling ammonia through a cooled solution of **26** in methanol. The *N*-benzoylsarcosinamide (**35**) was subsequently isolated and fully characterised.

N-Benzoylsarcosine (**36**) was prepared by treatment of sarcosine with benzoyl chloride under basic conditions.

The *N*-benzoylamino acid methyl esters **44**, and **49** required for the studies in this Chapter were synthesized from *L*-phenylalanine and *L*-proline respectively, using the method described in Chapter 1 for the preparation of *N*-benzoylamino acid methyl esters.

N-Phthaloylglycine methyl ester (**39**) was prepared using commercially available *N*-phthaloylglycine *via* the esterification procedure described in Chapter 1.

N-Phthaloyl-*L*-phenylalanine required for the synthesis of *N*-phthaloyl-*L*-phenylalanine methyl ester (**42**) was prepared by an established procedure[110] which involved heating a mixture of phthalic anhydride and *L*-phenylalanine to 150-160° C and stirring the melt with in that temperature range for 30 minutes. *N*-Phthaloyl-*L*-phenylalanine methyl ester (**42**) was subsequently prepared from the *N*-phthaloyl-*L*-phenylalanine *via* the esterification procedure described in Chapter 1.

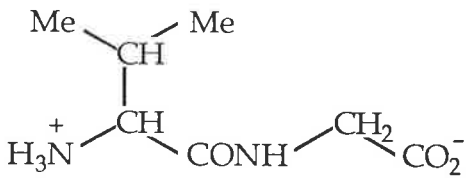
CHAPTER 3

Reactions of Underivatised and Derivatised Dipeptides with Nickel Peroxide.

On the basis of the results obtained in Chapters 1 and 2, it was anticipated that nickel peroxide could be used as a chemical model for the PAM enzyme. In this Chapter reactions of various dipeptides were investigated to see whether those factors affecting the reactivity in simple amino acid derivatives would also affect the reactivity of small peptides.

3.1 Reactions of Underivatised Dipeptides with Nickel Peroxide.

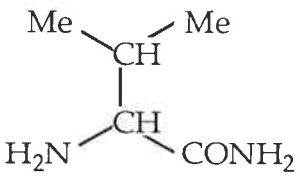
As discussed in the Introduction nickel peroxide can be used in both organic and aqueous solvents. To see if the analogy between nickel peroxide and PAM could be extended further, the reactions of nickel peroxide with underivatised dipeptides in solution were investigated.



53

A solution of *L*-valylglycine (53) at pH 8 in water was treated with 2 mole equivalents of nickel peroxide. The nickel peroxide in the reaction

mixture changed from the characteristic black colour to an olive green. The reaction mixture was filtered, and the pink coloured filtrate freeze dried *in vacuo*. Analysis of the residue by ^{1}H n.m.r. spectroscopy showed a mixture of products and unreacted starting material **53**. The major product, *L*-valinamide (**54**) was subsequently isolated in 21% yield. The identity of **54** was confirmed by comparison of the ^{1}H n.m.r. spectrum and the reverse phase high performance liquid chromatography (R.P. H.P.L.C.) trace with that of an authentic sample.[b]



54

The mechanisms for formation of *L*-valinamide (**54**) in the reaction of **53** with nickel peroxide are likely to be analogous to those proposed previously (Scheme 7). However the complexity of the ^{1}H n.m.r. spectrum of the reaction mixture indicated that apart from the reaction to produce **54**, nickel peroxide is reacting with **53** resulting in the formation of other products.

Reactions of substrates with nickel peroxide are known to be affected by the basicity of the reaction mixture. The reaction of **53** described above was repeated using a solution of sodium hydroxide in water with a pH of 14. Comparison of the ^{1}H n.m.r. spectrum of the reaction mixture with the ^{1}H n.m.r. spectrum for the reaction carried out at pH 8 described above, indicated that the same products were being formed.

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[b] The hydrochloride salt of *L*-valinamide was purchased from aldrich and converted to the free base.

Several other dipeptides were also treated with nickel peroxide and the filtrates from all the reactions were brightly coloured, as in the case of **53** above and all had complex ^{1}H n.m.r. spectra and R.P. H.P.L.C. traces. The brightly coloured filtrates from the reactions correspond to the formation of complexes involving nickel in some form. The formation of similarly coloured complexes has been reported previously, with respect to catalysing the hydrolysis of dipeptides and peptides using nickel and other metal oxides.[111] Peptide complexes involving transition-metal salts, for example cobalt (II), iron (II) and copper (I) and (II), have also been shown to react with molecular oxygen in an autocatalytic process resulting in the oxidation of the peptide ligands.[77]

The results described above show nickel peroxide can selectively oxidize the *C*-terminal glycine residue of **53** to produce **54** in reasonable yield, in a manner analogous to that of the PAM enzyme. However, competing reactions catalysed by coordination of the peptides to the nickel are occurring, resulting in the formation of other products *via* other mechanisms. Based on these results, a number of control experiments were carried out to ensure that the reactions discussed in Chapters 1 and 2 were in fact unique to nickel peroxide. *N*-Benzoylglycine methyl ester (**15a**) was treated with the two types of nickel oxide labelled here as nickel oxide (A)[c] which is black in colour and nickel oxide (B)[d] which is olive green in colour, that have been shown to catalyse the hydrolysis of underivatised dipeptides in aqueous solution, and also the nickel sulphate hexahydrate used to make nickel peroxide.

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[c] Nickel oxide black is prepared by the careful decomposition of nickel carbonate at 250-300° C.

[d] Nickel oxide II has a distictive olive green colour and is less dense than nickel oxide black.

N-Benzoylglycine methyl ester (**15a**) was treated with nickel oxide (A) (black), at reflux in benzene for 2 hours. The reaction mixture was filtered while hot, concentrated and analysed by H.P.L.C. chromatography. Only unreacted starting material was detected. Unreacted starting material **15a** was subsequently recovered from the reaction mixture in quantitative yield, by chromatography on silica. Analogous results were obtained when **15a** was treated with nickel oxide (B) (olive green) and nickel sulphate hexahydrate. Thus the reactions described in Chapters 1 and 2 are unique to the action of nickel peroxide.

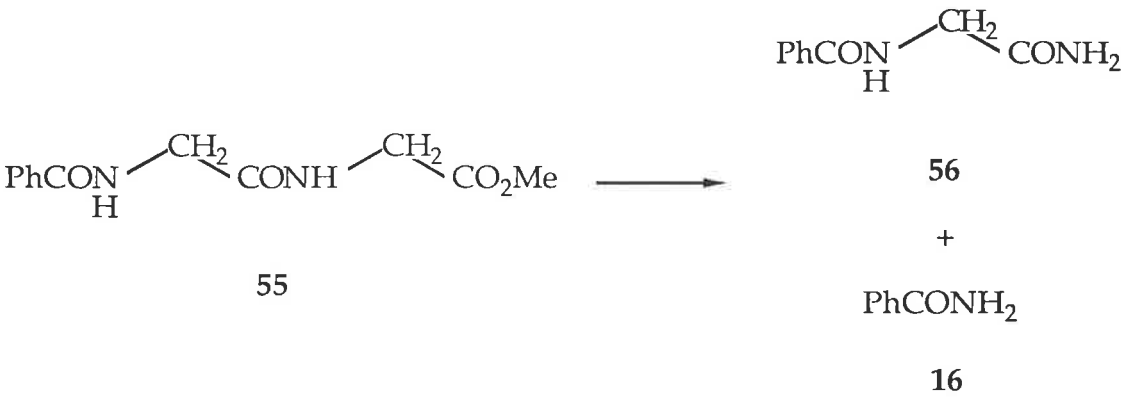
In summary, the results described in this section showed that although nickel peroxide was capable of catalysing the C-terminal oxidation of *L*-valylglycine (**53**) to *L*-valinamide (**54**), there was a problem with competing side reactions forming other products. However, control experiments determined that the reactions discussed in previous Chapters were in fact unique to the action of nickel peroxide.

Due to the problems associated with the formation of other products in the investigation of reactions of underivatised peptides with nickel peroxide described above, it was decided to concentrate further in studies on the reactions of derivatised dipeptides with nickel peroxide in organic solvent.

3.2 Reactions of *N*-Benzoyldipeptide Methyl Esters with Nickel Peroxide.

N-Benzoylglycylglycine methyl ester (**55**) was treated with 2 mole equivalents of nickel peroxide in refluxing benzene for 2.25 hours. Both

N-benzoylglycinamide (**56**) and benzamide (**16**) were isolated from the reaction mixture, in yields of 6% and 33% respectively, by chromatography on silica (Scheme 14). The identity of **56** was confirmed by comparison of the ^1H n.m.r. spectrum with that of an independently synthesized sample. Unreacted *N*-benzoylglycylglycine methyl ester (**55**) was also recovered from the reaction mixture in 40% yield.



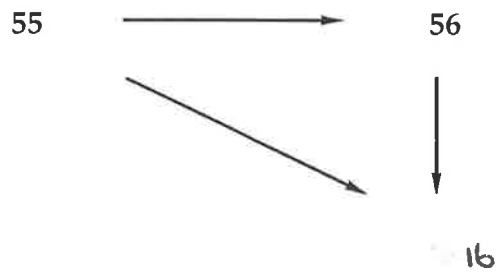
Scheme 14

The production of *N*-benzoylglycinamide (**56**) and benzamide (**16**) in the reaction of *N*-benzoylglycylglycine methyl ester (**55**) with nickel peroxide can be rationalised in terms of the mechanisms proposed in Chapter 1.

At first glance the yields of **16** and **56** appear to suggest that preferential hydrogen atom transfer from the α-centre of the *N*-terminal glycine residue to produce the α-centred radical **57** has occurred. This result is not unlikely based on the results discussed in Chapter 2 which indicated that *N*-benzoylsarcosinamide (**35**) had a greater affinity for nickel peroxide than the corresponding methyl ester derivative **26**. The formation of the α-centred radical **57** would lead to the direct formation of **16**.

57

Alternatively, it is also likely that **16** could also be produced by further reaction of *N*-benzoylglycinamide (**56**) with nickel peroxide. This is based on the analogy with the reaction of *N*-benzoylsarcosine methyl ester (**26**) with nickel peroxide to produce *N*-methylbenzamide (**27**), which was shown to react further with nickel peroxide to produce **16** and also the reaction of *N*-benzoylsarcosinamide (**35**) with nickel peroxide discussed in Chapter 2. Thus the formation of benzamide (**16**) can occur *via* two possible routes as shown in Scheme 15.

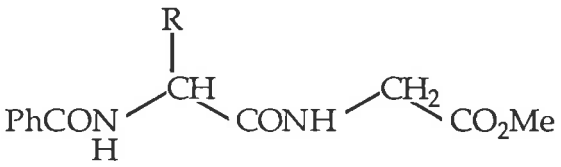


Scheme 15

The results obtained in the study of the relative rates of reaction of the *N*-benzoyl methyl ester derivatives of glycine **15a**, alanine **15b** and valine **15c** with nickel peroxide, discussed in Chapter 1, indicated selective reaction of the glycine derivative **15a**. On that basis and based on the results discussed above, it was anticipated that in reactions of nickel peroxide with derivatised dipeptides, the regioselective reaction of a

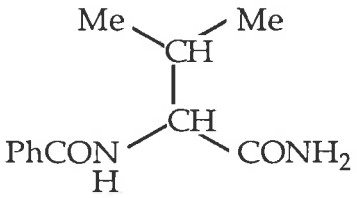
glycine residue in a dipeptide could be best effected when the second residue possessed a large α-substituent.

The dipeptides that were initially chosen for study were *N*-benzoyl-*D,L*-valylglycine methyl ester (**58**) and *N*-benzoyl-*D,L*-leucylglycine methyl ester (**59**).



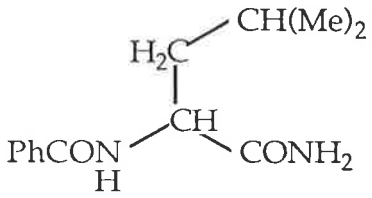
	58	**59**
R	$CHMe_2$	CH_2CHMe_2

Treatment of *N*-benzoyl-*D,L*-valylglycine methyl ester (**58**) with nickel peroxide afforded *N*-benzoyl-*D,L*-valinamide (**60**) which was isolated from the reaction mixture in a 27% yield, by chromatography on silica. The identity of **60** was confirmed by comparison with an independently synthesized sample. The physical and spectral characteristics of **60** were also found to be consistent with those previously reported.[112] Unreacted *N*-benzoyl-*D,L*-valylglycine methyl ester (**58**) was also isolated from the reaction mixture in 60% yield.



60

Treatment of *N*-benzoyl-*D,L*-leucylglycine methyl ester (**59**) with nickel peroxide afforded *N*-benzoyl-*D,L*-leucinamide (**61**) which was isolated from the reaction mixture in 37% yield, by chromatography on silica. The physical and spectral characteristics of **61** were found to be consistent with those previously reported.[112] Unreacted *N*-benzoyl-*D,L*-leucylglycine methyl ester (**59**) was also isolated from the reaction mixture in 20% yield.



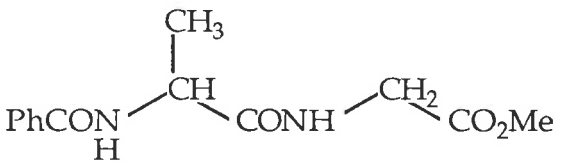
61

The formation of *N*-benzoyl-*D,L*-valinamide (**60**) and *N*-benzoyl-*D,L*-leucinamide (**61**) in the reactions of the dipeptides **58** and **59** respectively, with nickel peroxide described above are consistent with the results expected. The mechanisms proposed for the formation of **60** and **61** are analogous to those described in Chapter 1. The large α-substituents of the *N*-terminal valine and leucine residues of the dipeptides **58** and **59** respectively, act to disfavour reaction at the α-carbon of those residues. This is due in part to the unfavourable steric interactions associated with the planar conformations of the α-centred radicals that would result from reaction at those residues. The steric interactions associated with the α-centred glycyl radicals formed by initial reaction of the *C*-terminal glycine residues of **58** and **59** are much less severe. The way in which the dipeptides **58** and **59** bind to the nickel peroxide is also likely to contribute to the selective reaction of the glycine residue in each case. It is likely that the dipeptides **58** and **59** do not bind to the nickel peroxide in such a way

that reaction occurs at the α-centre of the corresponding *N*-terminal residues, as the large α-substituents of the *N*-terminal residues of the dipeptides **58** and **59** would interfere with that binding.

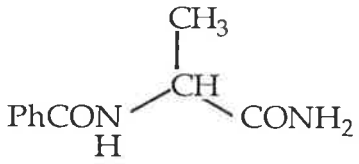
The extent to which the large α-substituents of the valine and leucine residues disfavour reaction at the α-carbon of the *N*-terminal residues of the dipeptides **58** and **59** respectively, also accounts for the relative stability of **60** and **61** to further reaction with nickel peroxide and hence the fact that benzamide (**16**) was not detected in either reaction.

Reaction of *N*-benzoyl-*D,L*-alanylglycine methyl ester (**62**) with nickel peroxide was also investigated to determine whether the selectivity for reaction of a glycine residue could be effected in the presence of an alanine residue.



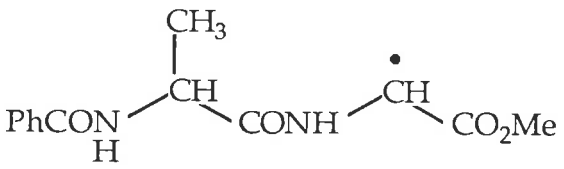
62

Treatment of *N*-benzoyl-*D,L*-alanylglycine methyl ester (**62**) with 2 mole equivalents of nickel peroxide at reflux in benzene for 2 hours afforded *N*-benzoyl-*D,L*-alaninamide (**63**) which was isolated from the reaction mixture in 20% yield by chromatography on silica. The physical and spectral characteristics of **63** were found to be consistent with those previously reported.[113] Unreacted *N*-benzoyl-*D,L*-alanylglycine methyl ester (**62**) was also isolated from the reaction mixture in 69% yield. Benzamide (**16**) was not detected in the reaction.



63

The formation of *N*-benzoyl-*D,L*-alaninamide (**63**) in the reaction of **62** with nickel peroxide is consistent with the result expected and can be rationalised in terms of the selective reaction of the *C*-terminal glycine residue of **62** to form the α-centred glycyl radical **64**. The radical **64** then reacts further to form **63** *via* mechanisms discussed previously in Chapter 1.

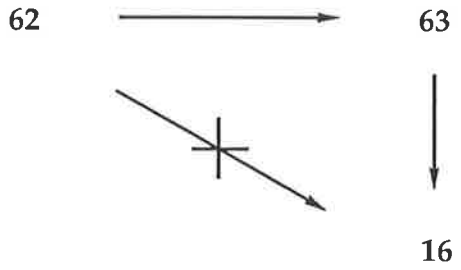


64

The reaction of *N*-benzoyl-*D,L*-alanylglycine methyl ester (**62**) with nickel peroxide described above, was repeated using 4 mole equivalents of nickel peroxide and the reaction was heated at 65-70° C in benzene overnight. Purification of the reaction mixture by chromatography on silica resulted in the isolation of benzamide (**16**) in 79% yield. Neither **63** nor unreacted starting material **62** were detected in the reaction mixture.

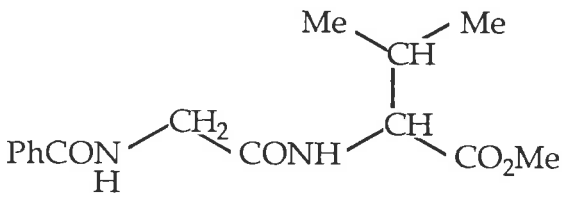
The more vigorous conditions and longer reaction time resulted in the exclusive formation of benzamide (**16**). Based on the initial reaction in which *N*-benzoyl-*D,L*-alaninamide (**63**) is the only detectable product, the formation of benzamide (**16**) under the more vigorous reaction conditions can be attributed to the direct result of further reaction of **63** with nickel

peroxide, as opposed to initial reaction at the α-carbon of the *N*-terminal alanine residue of **62** (Scheme 16).



Scheme 16

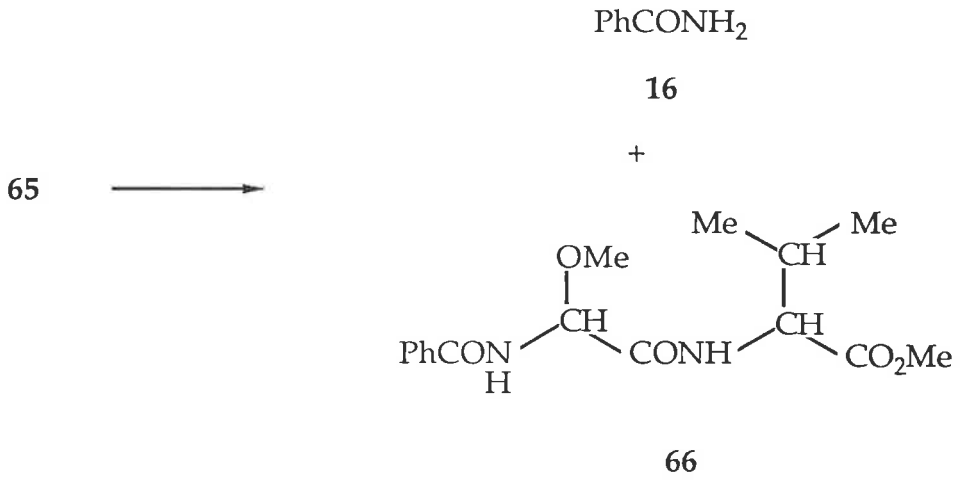
The reaction with nickel peroxide of an *N*-benzoyldipeptide methyl ester in which the glycine residue is the *N*-terminal residue was also investigated.



65

N-Benzoylglycyl-*D,L*-valine methyl ester (**65**) was treated with 6 mole equivalents of nickel peroxide at 60-65° C in benzene overnight. The reaction mixture was purified by chromatography on silica to afford benzamide (**16**) in 19% yield, as the major product. A second product identified as *N*-benzoyl-α-methoxyglycyl-*D,L*-valine methyl ester (**66**) was isolated in 10% yield (Scheme 17). The spectral characteristics of **66** were found to be consistent with those previously reported.[114] Unreacted *N*-

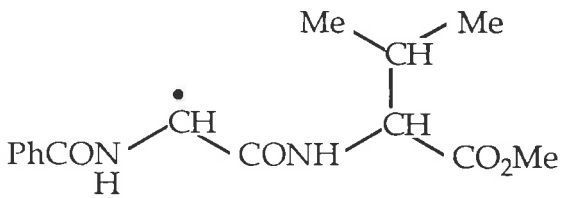
benzoylglycyl-*D,L*-valine methyl ester (**65**) was also recovered from the reaction mixture in 12% yield.



66

Scheme 17

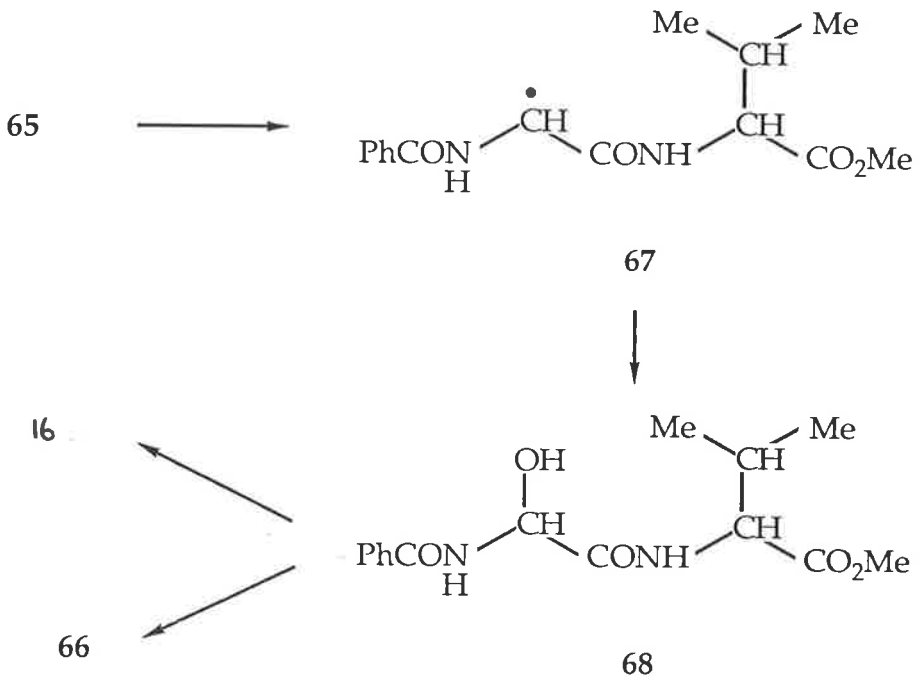
As expected on the basis of the selective reaction of glycine residues observed previously, the formation of *N*-benzoylglycinamide (**56**) was not detected in the reaction of **65** with nickel peroxide. Thus in line with mechanisms proposed previously, the initial step in the reaction of **65** with nickel peroxide is likely to be hydrogen atom transfer from the *N*-terminal glycine residue to give the corresponding α-centred glycyl radical **67**.



67

The radical **67** then combines with a hydroxyl radical from the nickel peroxide to form the α-hydroxyglycyl derivative **68**. This can then react further to form benzamide (**16**) (Scheme 18). Formation of the α-

methoxyglycyl derivative **66** is thought to be the result of the α-hydroxyglycyl derivative **68** coming into contact with methanol, most likely at the stage of chromatography. α-Hydroxyamino acid derivatives are known to be unstable and are often characterised as their α-methoxy derivatives. It is interesting to speculate that it may be possible to trap the proposed α-hydroxyamino acid intermediates in the reactions catalysed by PAM as the corresponding α-methoxy derivatives.



Scheme 18

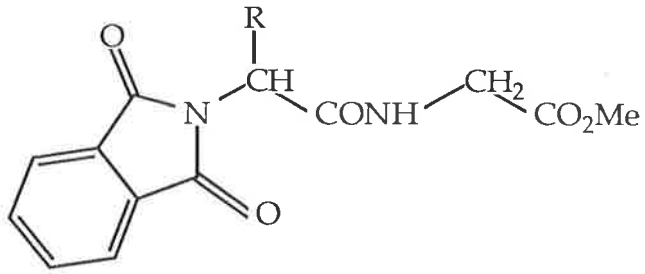
In summary, the results discussed in this section demonstrate that selective reaction of a glycine residue in a dipeptide can be effected by nickel peroxide. However, preferential reaction of a glycine residue in the dipeptides studied was found to occur regardless of whether the glycine residue was the *C*- or *N*-terminal residue. The observed preferential reaction of the glycine residues in the *N*-benzoyldipeptide derivatives can

be attributed partly to the stability of the intermediate α-centred glycyl radicals, which adopt planar conformations that are relatively free of non-bonding interactions. It can also be attributed in part to the fact that the large α-substituents on the second residue in the dipeptides discussed will interfere with binding of the dipeptide in such a way that reaction cannot occur at the α-centre of the residue possessing the large α-substituent.

3.3 Reactions of *N*-Phthaloyldipeptide Methyl Esters with Nickel Peroxide.

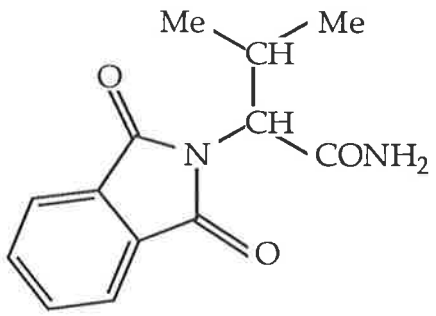
From the results discussed in Chapter 2 it is obvious that different protecting groups can affect the reactivity of an amino acid residue. In particular, the *N*-phthaloyl substituent appeared to deactivate reaction at the α-centre of the substituted amino acid residue. The reactions of a similar series of *N*-phthaloyl-protected dipeptide methyl esters with nickel peroxide were investigated to try to enhance the reactivity of the *C*-terminal residue by using the *N*-phthaloyl group to disfavour reaction at the *N*-terminal residue, particularly in the cases where the *N*-terminal residue is glycine. The presence of the *N*-phthaloyl group should also prevent further reaction of the product amides with nickel peroxide.

Initially reactions of *N*-phthaloyl-*D,L*-valylglycine methyl ester (**69**), *N*-phthaloyl-*L*-leucylglycine methyl ester (**70**) and *N*-phthaloyl-*D,L*-alanylglycine methyl ester (**71**) with nickel peroxide were investigated.



	69	70	71
R	$CH(Me)_2$	$CH_2CH(Me)_2$	Me

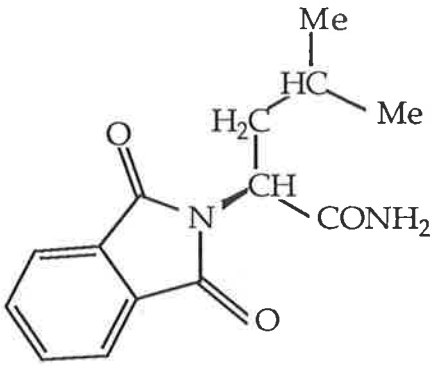
Treatment of *N*-phthaloyl-*D,L*-valylglycine methyl ester (**69**) with nickel peroxide afforded *N*-phthaloyl-*D,L*-valinamide (**72**), which was isolated from the reaction mixture in 55% yield, by chromatography on silica. The physical and spectral characteristics of **72** were found to be consistent with those previously reported.[115] Unreacted *N*-phthaloyl-*D,L*-valylglycine methyl ester (**69**) was also isolated from the reaction mixture in 32% yield.



72

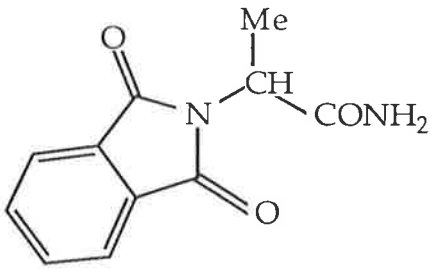
Treatment of *N*-phthaloyl-*L*-leucylglycine methyl ester (**70**) with nickel peroxide afforded *N*-phthaloyl-*L*-leucinamide (**73**), which was isolated from the reaction mixture in 41% yield, by chromatography on silica. The physical and spectral characteristics of **73** were found to be

consistent with those previously reported for the *L*-isomer.[116] Thus as expected, no racemization at the α-carbon of the *N*-terminal leucine residue of **70** occurred during the reaction. Unreacted *N*-phthaloyl-*L*-leucylglycine methyl ester (**70**) was also isolated from the reaction mixture in 26% yield.



73

Similar treatment of *N*-phthaloyl-*D,L*-alanylglycine methyl ester (**71**) with nickel peroxide afforded *N*-phthaloyl-*D,L*-alaninamide (**74**), which was isolated from the reaction mixture in 43% yield, by chromatography on silica. The physical and spectral characteristics of **74** were found to be consistent with those previously reported.[115] Unreacted *N*-phthaloyl-*D,L*-alanylglycine methyl ester (**71**) was also isolated from the reaction mixture in 42% yield.



74

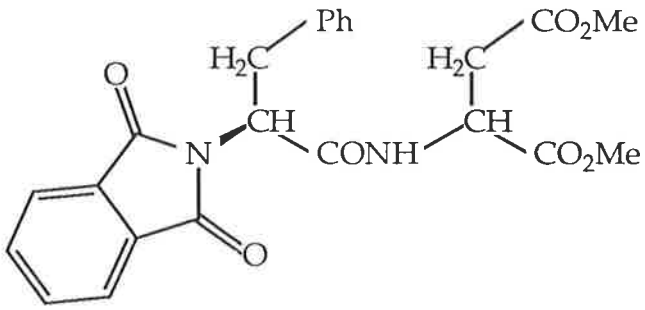
The formation of **72, 73** and **74** in the reactions of **69, 70** and **71** with nickel peroxide respectively, are consistent with the results expected. The mechanisms for formation of **72, 73** and **74** are analogous to those described previously (Scheme 7).

The reaction of *N*-phthaloyl-*D,L*-alanylglycine methyl ester (**71**) with nickel peroxide results in the formation of *N*-phthaloyl-*D,L*-alaninamide (**74**). From the combined yields of unreacted **71** and **74** recovered from the reaction mixture it is unlikely that any further reaction of **74** has occurred. This is unlike the reaction of *N*-benzoyl-*D,L*-alanylglycine methyl ester (**62**) with nickel peroxide (section 3.2), where the production of benzamide (**16**) occurs as a direct result of further reaction of *N*-benzoyl-*D,L*-alaninamide (**63**) with nickel peroxide.

In order to examine the effect on the relative rate of reaction of the binding to nickel peroxide of the substrate, the relative rate of reaction of *N*-phthaloyl-*D,L*-valylglycine methyl ester (**69**) compared to *N*-phthaloyl-*D,L*-alanylglycine methyl ester (**71**) was determined in a direct comparison. Since initial formation of an α-centred glycyl radical occurs in the reactions of both **69** and **71** with nickel peroxide, any difference in their relative rates of reaction can be attributed to the differences in their ability to bind to the nickel peroxide. A solution of **69** and **71** each at 0.026 Molar in refluxing benzene, was treated with nickel peroxide in the presence of *N-tert*-butylbenzamide (**23**). Aliquots were removed from the reaction at intervals, filtered, concentrated and analysed by ^{1}H n.m.r. spectroscopy. The results showed that the *N*-phthaloyl-*D,L*-alanylglycine derivative **71** reacted 1.7 ± 0.4 times faster than the *N*-phthaloyl-*D,L*-valylglycine derivative **69**.

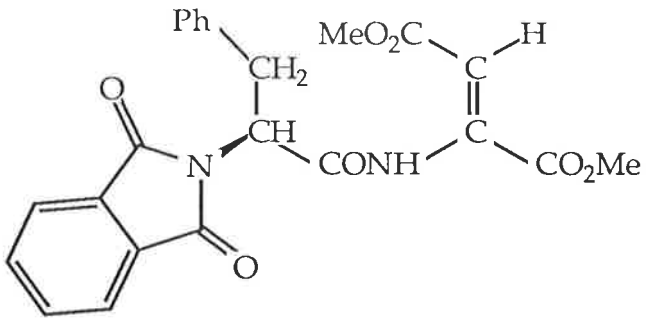
This result is consistent with the observations made earlier that the more hydrophilic, less substituted amino acid derivative will bind selectively to the nickel peroxide. In this case the alanylglycine derivative **71** binds to the nickel peroxide in preference to the valylglycine derivative **69** resulting in a slightly greater rate of reaction.

The reactions, with nickel peroxide, of a number of *N*-phthaloyldipeptide methyl esters which had *C*-terminal amino acid residues other than glycine were also investigated, in order to determine whether the extent to which the *N*-phthaloyl substituent disfavours reaction of an *N*-terminal residue would result in the preferential reaction of a *C*-terminal non-glycine residue.

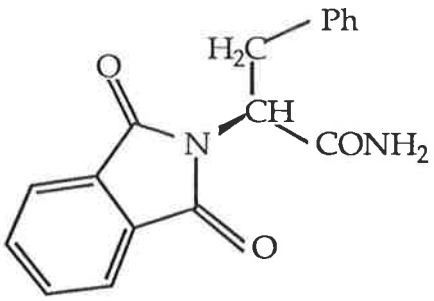


75

Treatment of *N*-phthaloyl-*L*-phenylalanyl-*D,L*-aspartate dimethyl ester (**75**) with nickel peroxide afforded *N*-phthaloyl-*L*-phenylalanyl-α,β-didehydroaspartate dimethyl ester (**76**) as the major product and *N*-phthaloyl-*L*-phenylalaninamide (**77**). Both the α,β-didehydroderivative **76** and the amide **77** were isolated from the reaction mixture in yields of 17% and 6% respectively, by chromatography on silica. Unreacted *N*-phthaloyl-*L*-phenylalanyl-*D,L*-aspartate dimethyl ester (**75**) was also isolated from the reaction mixture in 37% yield.



76



77

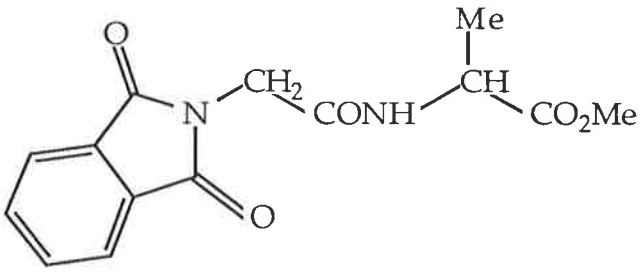
The identity of **76** was confirmed by ^{1}H and ^{13}C n.m.r. and I.R. spectroscopy, mass spectrometry and accurate mass determination as well as microanalysis. In the ^{1}H n.m.r. spectrum of **76** there is a single resonance at δ 5.58 with integration equivalent to one hydrogen corresponding to the olefinic hydrogen of the α,β-didehydroaspartate residue of **76**. The evidence obtained is consistent with the formation of a single isomer, which has been assigned as the *L*-phenylalanyl-(*Z*)-α,β-didehydroaspartate isomer of **76**. This assignment is made on the basis that as mentioned previously in Chapter 2, α,β-dehydroamino acids are known to favour the *Z* configuration.[99-103]

The identity of **77** was also determined by ^{1}H and ^{13}C n.m.r. and I.R. spectroscopy, mass spectrometry and accurate mass determination. The physical characteristics of **77** were found to be consistent with those previously reported for the *L*-isomer.[116] Mechanisms for the formation of **76** and **77** in the reaction of **75** with nickel peroxide are likely to be analogous to those previously reported.

There was no significant difference in the yields of the products **76** and **77** when the individual *L,D*- and *L,L*-diastereomers of **75** were treated

with nickel peroxide under the same conditions as those described above for the reaction of **75** with nickel peroxide. Therefore, there is no preference for abstraction of the α-hydrogen of a particular diastereoisomer of **75** based on steric grounds.

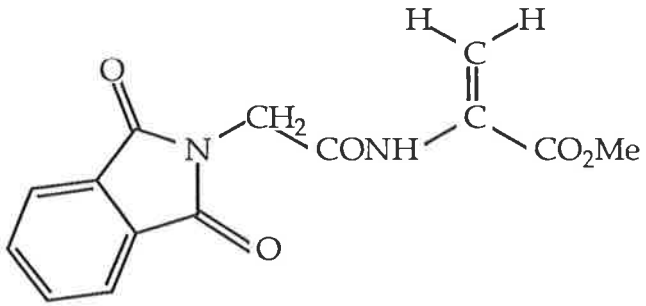
In the reaction of **75** with nickel peroxide described above preferential reaction of the *C*-terminal residue is observed. This is due in part to the deactivating effect of the *N*-phthaloyl substituent on the *N*-terminal residue. Based on this result the reactions of a number of *N*-phthaloyldipeptide methyl esters, possessing *N*-terminal glycine residues, with nickel peroxide were investigated.



78

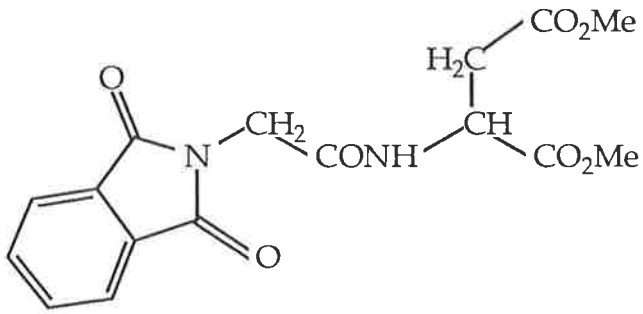
Treatment of *N*-phthaloylglycyl-*D,L*-alanine methyl ester (**78**) with nickel peroxide under the same conditions as those described for *N*-phthaloyl-*D,L*-valylglycine methyl ester (**69**), afforded *N*-phthaloylglycyl-α,β-dehydroalanine methyl ester (**79**). A high field ^{1}H n.m.r. spectrum of the crude reaction mixture showed only signals corresponding to unreacted starting material **78** and to the α,β-dehydroalanine derivative **79**. The α,β-dehydroalanine derivative **79** was subsequently isolated from the crude reaction mixture in 7% yield, by chromatography on silica.

Unreacted *N*-phthaloylglycyl-*D,L*-alanine methyl ester (**78**) was also isolated from the reaction mixture in 23% yield.



79

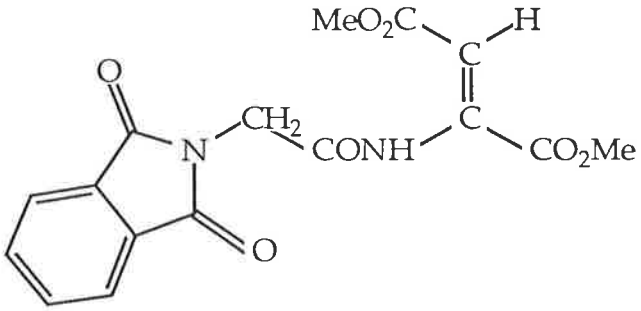
The identity of **79** was confirmed by ^{1}H and ^{13}C n.m.r. and I.R. spectroscopy, mass spectrometry and accurate mass determination. The resonances and their associated coupling constants of the olefinic hydrogens of the α,β-dehydroalanine residue of **79** at δ 5.92 (d, J = 1.3 Hz, 1H) and δ 6.58 (s, 1H) in the ^{1}H n.m.r. spectrum are consistent with those expected for an *N*-acyl substituted α,β-dehydroalanine derivative.[91,92,117]



80

Similarly treatment of *N*-phthaloylglycyl-*D,L*-aspartate dimethyl ester (**80**) with nickel peroxide, afforded *N*-phthaloylglycyl-*(Z)*-α,β-didehydroaspartate dimethyl ester (**81**). A high field ^{1}H n.m.r. spectrum of

the reaction mixture showed only signals corresponding to unreacted starting material **80** and a single isomer of the α,β-didehydroaspartate derivative **81**. The α,β-didehydroaspartate derivative **81** was subsequently isolated from the reaction mixture in 54% yield, by chromatography on silica. Unreacted *N*-phthaloylglycyl-*D,L*-aspartate dimethyl ester (**80**) was also isolated from the reaction mixture in 36% yield.



81

The ^{1}H n.m.r. spectrum of **81** showed a single resonance, with integration equivalent to one hydrogen, at δ 5.60 attributable to the vinyl proton of the aspartate residue. The absence of coupling associated with the resonance at δ 5.60, and other spectral details are consistent with the formation of only one isomer which has been previously assigned as the *Z* isomer.[97]

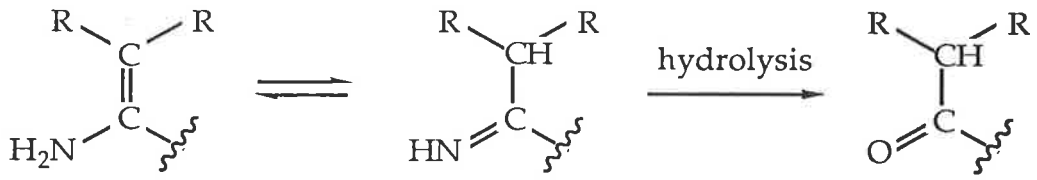
The mechanisms for formation of the α,β-dehydroamino acid derivatives **76, 79** and **81** in the reactions described above are likely to be analogous to that proposed for the formation of the α,β-dehydroalanine derivative **17** shown in Scheme 8. The results of the reactions of **78** and **80** with nickel peroxide described above indicate that it is possible to effect selective reaction at the *C*-terminus of an *N*-phthaloyldipeptide methyl

ester in the presence of an *N*-terminal glycine residue. However a qualitative comparison of the yields of the α,β-dehydroamino acid derivatives **76, 79** and **81** from the reactions of **75, 78** and **80** respectively, with nickel peroxide indicates that the yield of the α,β-dehydroamino acid derivatives is improved where they result in extended conjugation. Therefore, the better yield of the α,β-didehydroaspartate derivative **81** compared to **76** and **79** is likely to result from the extended conjugation within the α,β-didehydroaspartate residue of **83**, resulting in its greater stability and hence resistance to further reaction.

The reaction of *N*-phthaloylglycyl-*D,L*-aspartate dimethyl ester **(81)** with nickel peroxide illustrates to some extent the possible potential of this method to produce selected *C*-terminal α,β-dehydroamino acid residues in dipeptides, even in extremely hindered situations such as that of the reaction of *N*-phthaloyl-*L*-phenylalanyl-*D,L*-aspartate dimethyl ester **(75)**, while maintaining the stereochemical integrity of the *N*-terminal amino acid residue.

α,β-Dehydroamino acids are components of a large number of natural products and dehydropeptides have been isolated from bacteria,[118,119] fungi[120,121] and marine organisms.[122-125] They are also known inhibitors of pyridoxal phosphate dependant enzymes.[126,127] The formation of dehydropeptides by sequential synthesis from dehydroamino acids is difficult, particularly because the formation of the peptide bond at the *N*-terminus of the α,β-dehydroamino acid requires nucleophilic attack of the amino group at an activated carboxylic acid group. The amino group of an α,β-dehydroamino acid is a particularly poor nucleophile as the electrons are involved in delocalization with the double bond. The

resulting imine tautomer **82** is also susceptible to hydrolysis resulting in the formation of the corresponding carbonyl compound **83** (Scheme 19) .



82 **83**

Scheme 19

Using nickel peroxide to produce α,β-dehydroamino acid derivatives as described above would eliminate this problem as the α,β-dehydroamino acid is at the *C*-terminus of a dipeptide. Subsequent deprotection of the resulting α,β-dehydrodipeptides by known methods, would then allow them to be incorporated into larger peptides. From the results described so far, it would appear that nickel peroxide can be used to produce selected *C*-terminal α,β-dehydrodipeptides, particularly where the formation of the α,β-dehydroamino acid results in extended conjugation within the α,β-dehydroamino acid residue. Also by using the *N*-phthaloyl group, which is known to deactivate the adjacent amino acid to reaction with nickel peroxide, the stereochemical integrity of the *N*-terminal amino acid should be able to be preserved. Other advantages are the ease of purification of the reactions, and the fact that both the nickel peroxide and the recovered unreacted starting material are recyclable.

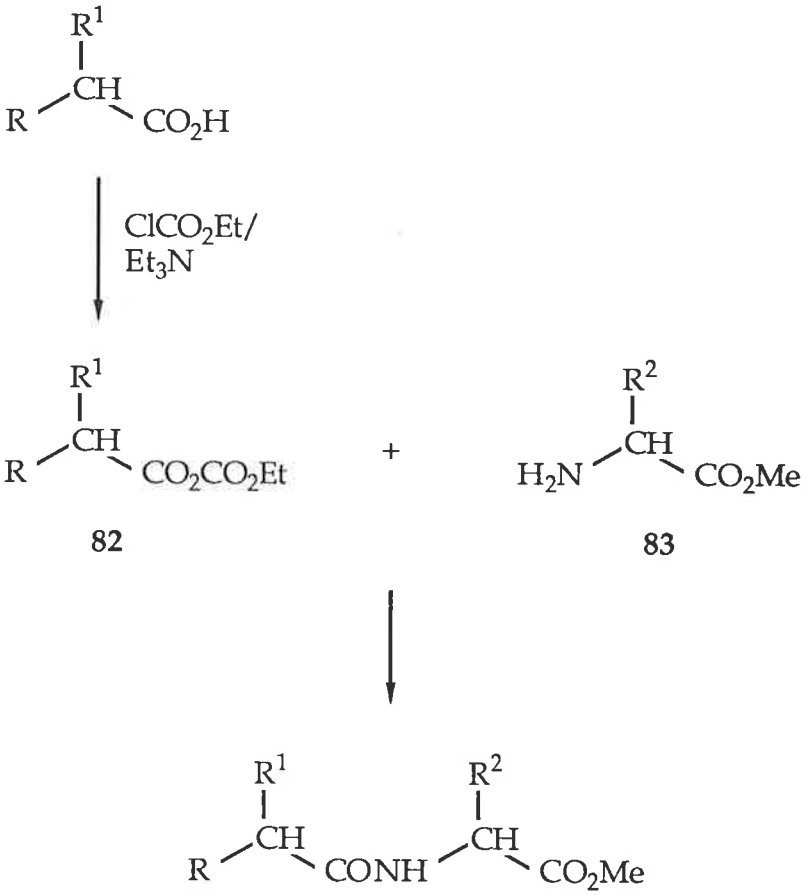
As with all the reactions of substrates with nickel peroxide discussed in this thesis, the nickel peroxide recovered from the reaction mixtures discussed in this Chapter was found to retain some activity by iodometry,

in some cases up to half. Extending the reaction time in these cases did not greatly effect the yields of products. Using large excesses of nickel peroxide also did not greatly increase the yields of products.

In summary, the results of the reactions of *N*-phthaloyldipeptide methyl esters discussed in section 3.3 show that the *N*-phthaloyl group can be used to effect selective reaction of *C*-terminal glycine and non-glycine residues of *N*-phthaloyldipeptide methyl esters even when the *N*-terminal amino acid is a glycine residue. These results illustrate the effectiveness with which the *N*-phthaloyl group deactivates the α-centre of the adjacent amino acid residue towards reaction with nickel peroxide. This "deactivating" effect exhibited by the *N*-phthaloyl group enabled the production of selected *C*-terminal α,β-dehydrodipeptide derivatives while maintaining the stereochemical integrity of the *N*-terminal residue.

Synthesis of Substrates.

The *N*-benzoyl- and the *N*-phthaloyl-dipeptide derivatives required for the studies in this Chapter were synthesized as described below.



	55	75	78
R	PhCONH	PhthN	PhthN
R^1	H	CH_2Ph	H
R^2	H	CH_2CO_2Me	Me

Scheme 20

The coupling procedure for the dipeptides, shown in Scheme 20, involved reaction of the carboxyl group of the appropriate *N*-terminal amino acid residue with ethyl chloroformate under basic conditions, presumably to form the mixed anhydride **82**. A suspension of the methyl ester derivative **83** of the appropriate *C*-terminal amino acid residue in triethylamine was then introduced to the reaction.

N-Benzoylglycylglycine methyl ester (**55**) was prepared using the coupling procedure described above from commercially available hippuric acid and glycine methyl ester prepared as described in Chapter 1.

N-Phthaloylglycyl-*D,L*-alanine methyl ester (**78**) was prepared by coupling commercially available *N*-phthaloylglycine with *D,L*-alanine methyl ester using the coupling procedure described above. *N*-phthaloylglycyl-*D,L*-alanine methyl ester (**78**) was subsequently isolated and fully characterised.

N-Phthaloyl-*L*-phenylalanine required for the synthesis of *N*-phthaloyl-*L*-phenylalanine-*D,L*-aspartate dimethyl ester (**75**) was prepared as described in Chapter 2. The *N*-phthaloyl-*L*-phenylalanine was then coupled to the *D,L*-aspartate dimethyl ester using the coupling procedure described above. The resulting *N*-phthaloyl-*L*-phenylalanine-*D,L*-aspartate dimethyl ester (**75**) was subsequently isolated and the two diastereomers separated by fractional recrystallisation from methanol. Both diastereomers of **75** were fully characterised. Crystal samples of the first isomer to crystallise out of methanol submitted for X-ray analysis were subsequently found to be of insufficient size for a complete structure solution.

Authentic samples of *N*-benzoylglycinamide **(56)** and *N*-benzoyl-*D,L*-valinamide **(60)** were prepared by treatment of glycinamide and *D,L*-valinamide respectively, with benzoyl chloride under basic conditions.

Samples of the *N*-benzoyl- and the *N*-phthaloyl-dipeptide dervatives **58, 59, 62, 65, 69, 70, 71** and **80** were available from previous work.

CONCLUSION

The work described in this thesis involved the study of reactions of amino acid derivatives with nickel peroxide with the primary aim of establishing that nickel peroxide is a reasonable chemical model for the Peptidylglycine α-Amidating Monooxygenase enzyme.

The work described in Chapter 1 of this thesis involved the study of the reactions of *N*-benzoylamino acid methyl esters with nickel peroxide. The product studies and observed deuterium isotope effects provided evidence to support the mechanisms proposed for the reaction of nickel peroxide with the *N*-benzoylamino acid methyl esters, which involved initial formation of an α-carbon-centred captodative radical with subsequent formation of *N*-acylimine and/or α-hydroxyamino acid intermediates These mechanisms are analogous to those proposed for the reaction of substrates with the PAM enzyme.

A thorough study of the relative rates of reaction of amino acid derivatives with nickel peroxide at various concentrations and temperatures was done. The preferential reactivity of glycine derivatives observed was attributed in part to the effect of nonbonding interactions on planar conformations of the α-centred captodative radicals and also to the preferential binding to nickel peroxide of the more hydrophilic, less substituted amino acid derivatives. In Chapter 2, which looked at the effects of differing *C*- and *N*-terminal substituents on the reaction pathways and products upon the reaction of amino acid derivatives with nickel peroxide, an amino acid derivative possessing a free *C*-terminal

carboxylic acid was found to bind to nickel peroxide in preference to one with a *C*-terminal amide, which in turn bound to nickel peroxide in preference to the corresponding *C*-terminal methyl ester derivative. These results are analogous to the substrate specificity exhibited by the PAM which shows a preference for binding the free *C*-terminal glycine residue. A large α-substituent on the residue of the substrate to undergo reaction will inhibit binding to the active site of the enzyme in such a way that the crucial elements within the active site cannot interact closely enough with the residue of the substrate for reaction to occur.

Chapter 3 deals with the reactions of a series of *N*-benzoyl- and *N*-phthaloyl-dipeptide methyl esters with nickel peroxide. Preferential reaction of glycine residues in *N*-benzoyldipeptide methyl esters was demonstrated and was found to be independent of the position of the glycine residue in the dipeptide. The *N*-phthaloyl substituent was found to disfavour reaction of an *N*-terminal residue in *N*-phthaloyldipeptide methyl esters to such an extent that preferential reaction of *C*-terminal non-glycine residues were observed even in the presence of *N*-terminal glycine residues. This effect of the *N*-phthaloyl substituent was utilised to enable the synthesis of several *C*-terminal α,β-dehydrodipeptides, while maintaining the stereochemical integrity of the *N*-terminal amino acid.

Thus, in nickel peroxide we have a practical *in vitro* model for the terminal amidation reaction the characteristics of which closely mimic the characteristics of the enzymic peptide amidation by the PAM enzyme in that it is a free radical process which proceeds *via* an α-hydroxyamino acid intermediate or an *N*-acylimine, and it is selective for reaction of glycine.

The advantages of using nickel peroxide are numerous. It is easily and cheaply prepared, easily removed from reaction mixtures and can be regenerated. The reaction mixtures are easily purified and both unreacted starting material and nickel peroxide can be recycled. Utilising the effect of the *N*-phthaloyl substituent, selected *C*-terminal α,β-dehydrodipeptides can be synthesized without affecting the stereochemical integrity of the *N*-terminal amino acid. The advantage synthesizing α,β-dehydroamino acids in this way is the fact that they are at the *C*-terminus of a dipeptide, which once deprotected can be incorporated directly into a larger peptide without the usual problems. The aspect of nickel peroxide as a model for the PAM enzyme and its ability to be used to synthesize *C*-terminal α,β-dehydrodipeptides should prove to be interesting areas for further investigation.

EXPERIMENTAL

General

Melting points were measured using a Kofler hot-stage melting point apparatus under a Reichert microscope and are uncorrected.

Elemental analyses were carried out by the Canadian Microanalyical Service Ltd., New Westminster, Canada.

Infrared spectra were recorded on a Jasco IRA-1 spectrometer. Mass spectra were recorded on a AEI MS-3010 spectrometer. Only the major fragments are quoted with their relative abundances shown in parentheses.

^{1}H n.m.r. Spectra were recorded on a Varian T-60 or either a Bruker CXP-300 or ACP-300 spectrometer. Unless otherwise stated, ^{1}H n.m.r. spectra were recorded as dilute solutions in deuterochloroform using tetramethylsilane as an internal standard.

^{13}C n.m.r. Spectra were recorded on a Bruker CXP-300 or ACP-300 spectrometer. They were determined in deuterochloroform using tetramethylsilane as an internal reference.

High performance liquid chromatographic analyses were performed on a Waters Model 501 Solvent Delivery System and a U6K Injector with a

Waters Model 481 absobance detector, using a μ-Bond Radial-Pak cartridge (10 cm x 8 mm). Column eluates were monitored at 265 nm.

Chromatography was carried out on a Chromatotron 7924T (Harrison Research, Palo Alto/TC Research, Norwich), using Merck silica gel 60 PF_{254}. Unless otherwise stated ethyl acetate and light petroleum were used as eluants.

All solvents were purified by standard procedures. Light petroleum refers to the fraction of b.p. 55-65° C. Unless otherwise stated all organic solutions were dried with anhydrous magnesium sulphate.

Benzene was dried by refluxing over calcium chloride in an inert atmosphere for 48 hours, then distilled and stored over sodium wire.

Acetic anhydride was purified by stirring over magnesium (1 g / 100 ml) at 60° C, under nitrogen for 5 days. The acetic anhydride was then distilled *in vacuo* (b.p. 54° C at 25 mm Hg).

Nickel peroxide was removed from the reaction mixtures by filtering the reaction mixtures while hot, through kenite (diatomaceous earth), and subsequently washing with dry distilled chloroform.

Work Described in Chapter 1.

Nickel Peroxide.

A mixture of 300 ml of 6% sodium hypochlorite solution and 42 g sodium hydroxide was added dropwise to 130 g. of nickel sulphate hexahydrate (Fluka A.G.) in 300 ml water, and stirred for 0.5 hours at 20° C. The resulting black nickel peroxide was collected by filtration, washed with water to remove active chlorine, and the resulting cake crushed to a powder then dried over anhydrous calcium chloride under reduced pressure for several days.

Determination of available oxygen by iodometry:

Nickel peroxide (0.2 g) was added to 36% acetic acid (20 ml) containing potassium iodide (2 - 3 g), entirely dissolved, and allowed to stand for 10 minutes under nitrogen. The liberated iodine was titrated by 0.1 N sodium thiosulphate solution. Available oxygen content is calculated as follows:

$$\frac{Na_2S_2O_3 \text{ (ml)}}{1000 \times \text{peroxide (g)} \times 10 \times 2} = \text{g-atom oxygen / g. nickel peroxide}$$

The average oxygen content was found to be between

2.8 and 3.0×10^{-3} g-atom oxygen / g. nickel peroxide

N-Benzoylglycine Methyl Ester (15a).

Hippuric acid (Ajax) (5.0 g, 28.0 mmol) was dissolved in a cooled solution of anhydrous methanol (100 ml), to which thionyl chloride (2.5 ml, 33.0 mmol) had been added, and stirred overnight. The solvent was removed *in vacuo* to give the crude *N*-benzoylglycine methyl ester (**15a**). Recrystallisation from ethyl acetate/light petroleum afforded pure *N*-benzoylglycine methyl ester (**15a**) as colourless needles.

Yield 4.9 g, 91%;
m.p. 80-82° C (lit.,[128] 82-83° C);
^{1}H n.m.r. (300 MHz) δ 3.80 (s, 3H), 4.25 (d, J = 5.0 Hz, 2H), 6.73 (br s, 1H), 7.26-7.5 (m, 3H), 7.80-7.84 (m, 2H).

Reaction of *N*-Benzoylglycine Methyl Ester (15a) with Nickel Peroxide.

N-Benzoylglycine methyl ester (**15a**) (200 mg, 1.0 mmol) in benzene (20 ml) was treated with 2.6 mole equivalents of nickel peroxide at reflux under nitrogen for 1 hour. The reaction mixture was filtered while hot, concentrated and then chromatographed on silica (PF_{254}, chromatotron). Products were eluted in the following order;

Unreacted starting material **15a**
Yield 103 mg, 51%.
m.p. 81-83° C (lit.,[128] 82-83° C);

Benzamide (16) recrystallised from ethyl acetate as colourless needles.
Yield 49 mg, 39%;

m.p. 130-132° C (lit.,[129] 128° C);

^{13}C n.m.r. (300 MHz) δ 127.27, 128.56, 131.87, 169.58.

The ^{13}C n.m.r. of benzamide reported above is consistent with that obtained using an authentic sample of benzamide.

<u>*N*-Benzoyl-*D,L*-alanine Methyl Ester (15b).</u>

D,L-Alanine (BDH) (5.0 g, 56 mmol) was dissolved in a cooled solution of anhydrous methanol (250 ml), to which thionyl chloride (25 ml, 0.3 mol) had been added, and stirred overnight. The solvent was removed *in vacuo* to give the crude alanine methyl ester hydrochloride. The salt was dissolved in ethyl acetate (250 ml) and triethylamine (25 ml, 178 mmol). Benzoyl chloride (6.5 ml, 56 mmol) was added dropwise, and the solution stirred for 5 hours on completion of addition. Water (250 ml) was added and the solution stirred overnight. The organic layer was separated. the aqueous layer was further extracted with ethyl acetate (3 x 150 ml), the extracts combined, dried over anhydrous magnesium sulphate, filtered and the solvent removed *in vacuo*. Recrystallisation from ethyl acetate/light petroleum afforded *N*-benzoyl-*D,L*-alanine methyl ester (**15b**) as colourless needles.

Yield 4.9 g, 42%;

m.p. 84-85° C (lit.,[130] 82° C);

^{1}H n.m.r. (300 MHz) δ 1.53 (d, J = 7.2 Hz, 3H), 3.79 (s, 3H), 4.81 (p, J = 7.2 Hz, 1H), 6.72 (br d, J = 7.1 Hz, 1H), 7.4-7.6 (m, 3H), 7.7-7.9 (m, 2H).

Reaction of *N*-Benzoyl-*D,L*-alanine Methyl Ester (15b) with Nickel Peroxide.

N-Benzoyl-*D,L*-alanine methyl ester (**15b**) (200 mg, 0.96 mmol) was treated with 2.6 mole equivalents of nickel peroxide under conditions described above for the reaction of *N*-benzoylglycine methyl ester (**15a**) with nickel peroxide. The reaction mixture was chromatographed on silica (PF_{254}, chromatotron). The products were eluted in the following order:

Methyl 2-benzamidopropenoate (**17**) recrystallised from ethyl acetate/light petroleum as colourless crystals;
Yield 18 mg, 9%;
m.p. 149-154° C;
^{1}H n.m.r. (300 MHz) δ 3.89 (s, 3H), 6.00 (d, *J* = 1.3 Hz, 1H), 6.80 (s, 1H), 7.45-7.58 (m, 3H), 7.82-7.91 (m, 2H), 8.54 (br s, 1H).
The ^{1}H n.m.r. spectral characteristics for **17** were found to be consistent with those previously reported.[91,92]

Unreacted starting material **15b**
Yield 137 mg, 69%.
m.p. 82-84° C (lit.,[130] 82° C);
^{1}H n.m.r. (300 MHz) δ 1.53 (d, *J* = 7.2 Hz, 3H), 3.80 (s, 3H), 4.82 (p, *J* = 7.1 Hz, 1H), 6.73 (br d, *J* = 7.0 Hz, 1H), 7.42-7.54 (m, 3H), 7.79-7.86 (m, 2H).

Benzamide (**16**) recrystallised from ethyl acetate as colourless needles.
Yield 15 mg, 13%;
m.p. 128-130° C (lit.,[129] 128° C).

Reaction of *N*-Benzoyl-*D,L*-valine Methyl Ester (15c) with Nickel Peroxide.

N-Benzoyl-*D,L*-valine methyl ester (**15c**) (200 mg, 0.85 mmol) was treated with 2.6 mole equivalents of nickel peroxide in refluxing benzene under conditions described above for the reaction of *N*-benzoylglycine methyl ester (**15a**) with nickel peroxide. H.P.L.C. Analysis of the filtered reaction mixture detected a trace amount of benzamide (**16**). Unreacted strating material (**15c**) was recovered in 93% yield by chromatography on silica (PF_{254}, chromatotron).

The reaction was repeated using *N*-benzoyl-*D,L*-valine methyl ester (**15c**) (100 mg, 0.42 mmol) and 10 mole equivalents of nickel peroxide in refluxing benzene for 48 hours. The reaction mixture was filtered, concentrated and chromatographed on silica (PF_{254}, chromatotron). Products were eluted in the following order;

Unreacted starting material (**15c**)
Yield 25 mg, 25%;
m.p. 85-87° C (lit.,[131] 86° C);
^{1}H n.m.r. (300 MHz) δ 1.00 (t, J = 7.0 Hz, 6H), 2.23-2.34 (m, 1H), 3.78 (s, 3H), 4.79 (dd, J_{vic} = 4.8 Hz, J_{NH} = 8.0 Hz, 1H), 6.64 (br d, J = 8.1 Hz, 1H), 7.4-7.6 (m, 3H), 7.7-7.9 (m, 2H).

Benzamide (**16**) recrystallised from ethyl acetate as colourless needles.
Yield 7 mg, 14%;
m.p. 126-128° C (lit.,[129] 128° C).

Reaction of Methyl 2-benzamidopropenoate (17) with Nickel Peroxide.

Methyl 2-benzamidopropenoate (**17**) (20.0 mg, 0.085 mmol) in benzene (10.0 ml) was treated with 2.6 mole equivalents of nickel peroxide at reflux under nitrogen overnight. The reaction mixture filtered while hot, concentrated and analysed by T.L.C. (Thin Layer Chromatography) and H.P.L.C.. The presence of benzamide (**16**) was confirmed by comparison of the H.P.L.C. trace with that of an authentic sample of benzamide (**16**).

α,α-Dideuterioglycine.

Glycine (Sigma) (0.6 g, 8.0 mmol) was heated at reflux in deuterium oxide (10.0 ml, 0.5 mol), under nitrogen for 20 minutes, then concentrated to dryness under reduced pressure. This procedure was repeated twice.

The deuterium oxide pretreated glycine (0.6 g, 8.0 mmol) from above, was heated at reflux with deuterium oxide (6.0 ml, 0.3 mol) in acetic anhydride (57.0 ml, 0.56 mol), under nitrogen, for 10 minutes. More deuterium oxide (6.5 ml, 0.33 mol) was carefully added, the solution cooled, and the solvent removed *in vacuo*. The residue was heated at reflux in hydrochloric acid (6 Molar, 30 ml) for 90 minutes, concentrated *in vacuo* to give a white solid. The crude α,α-dideuterioglycine was dried over anhydrous calcium chloride and used without further characterisation or purification.

N-Benzoyl-α,α-dideuterioglycine Methyl Ester (22a).

α,α-Dideuterioglycine (0.52 g, 6.8 mmol) prepared as described above, was dissolved in anhydrous methanol (20 ml) which had been pretreated with thionyl chloride (1.6 ml, 22.0 mmol), and the solution left to stir overnight. The solvent was removed *in vacuo* to give the crude α,α-dideuterioglycine methyl ester as the hydrochloride salt. This was suspended in ethyl acetate (20 ml) and triethylamine (1.9 ml, 13.6 mmol) was added dropwise with stirring. Benzoyl chloride (0.8 ml, 6.8 mmol) was added dropwise and the suspension stirred for 4 hours. Water was added to and the solution stirred overnight. The organic layer was separated, the aqueous layer extracted with ethyl acetate (3 x 20 ml), the extracts combined, dried, filtered, and concentrated under reduced pressure. The residue was purified by chromatography on silica (PF_{254}, chromatotron). Recrystallisation of the crude product from ethyl acetate/light petroleum afforded pure *N*-benzoyl-α,α-dideuterioglycine methyl ester (**22a**) as colourless crystals. Deuterium content was established at 80% dideuterated, and 18% monodeuterated by mass spectrometry.

Yield 0.64 g, 48%;

m.p. 79-81.5° C (lit.,[128] 82-83° C);

^{1}H n.m.r. (300 MHz) δ 3.75 (s, 3H), 7.08 (br s, 1H), 7.37-7.52 (m, 3H), 7.79-7.83 (m, 2H);

Mass spectrum: *m/z* 195 (M^+, 11%), 136 (15), 105 (100), 77 (46).

Reaction of *N*-Benzoyl-α,α-dideuterioglycine Methyl Ester (22a) with Nickel Peroxide.

N-Benzoyl-α,α-dideuterioglycine methyl ester (**22a**) (50 mg, 0.25 mmol) in benzene (10 ml), was treated with 2.6 mole equivalents of nickel peroxide at reflux under nitrogen for 1 hour. The reaction mixture was filtered while hot, concentrated and chromatographed on silica (PF_{254}, chromatotron). Products were eluted in the following order;

Unreacted starting material **22a**
Yield 35 mg, 70%;
m.p. 80-82° C (lit.,[128] 82-83° C).
The deuterium content of the recovered unreacted starting material **22a** was established at 82% dideuterated, and 13% monodeuterated by mass spectrometry.

Benzamide (16)
Yield 6 mg, 19%;
m.p. 128-129.5° C (lit.,[129] 128° C).

Relative rate of reaction of *N*-Benzoylglycine Methyl Ester (15a) and *N*-Benzoyl-α,α-dideuterioglycine Methyl Ester (22a), with Nickel Peroxide.

A mixture of *N*-benzoylglycine methyl ester (**15a**) (50 mg, 0.26 mmol), *N*-benzoyl-α,α-dideuterioglycine methyl ester (**22a**) (50 mg, 0.26 mmol) with *N-tert*-butylbenzamide (**23**) (25 mg, 0.14 mmol) as an internal standard, in benzene (10 ml), was treated with 2.6 mole equivalents of nickel peroxide (based on the number of moles of the amino acid

derivatives) at reflux, under nitrogen. Aliquots were taken before the nickel peroxide was added and further samples were taken throughout the course of the reaction. These were filtered while hot to remove the nickel peroxide, concentrated, dissolved in deuterochloroform and analysed by ^1H n.m.r. spectroscopy. The initial and final ratios of the amino acid derivatives **15a** and **22a** were determined by (a) manual integration of definitive peaks *ie* the peak height x width (at half the height), (b) by weight after photocopying the trace and cutting out the corresponding peaks, and (c) by machine integration. The areas of the two substrates in the product mixtures were corrected using the internal standard. The relative rate of reaction was calculated using Equation 1 and is shown in Table 6. The number of samples in the sample population is N and the standard deviation of the sample population (σ) is depicted as the error shown.

TABLE 6

Reaction Mixture		Temp. °C	Conc. Molar	% Substrate remaining		N	k_Y/k_X	k_X/k_Y
X	Y			X	Y			
15a	**22a**	80	0.025	20	58	16	0.34 ± 0.01	2.9 ± 0.5

Relative rate of reaction of *N*-Benzoyl-*D,L*-alanine Methyl Ester (15b) and *N*-Benzoyl-*D,L*-α-deuterioalanine Methyl Ester (22b), with Nickel Peroxide.

A mixture of *N*-benzoyl-*D,L*-alanine methyl ester (**15b**) (50 mg, 0.26 mmol), *N*-benzoyl-*D,L*-α-deuterioglycine methyl ester (**22b**) (50 mg, 0.26 mmol) with *N-tert*-butylbenzamide (**23**) (25 mg, 0.14 mmol) as an internal standard was treated with nickel peroxide under conditions described above for the comparison of the relative rate of reaction of **15a** and **22a**. The relative rate of reaction of **15b** and **22b** was calculated to be approximately 3.

Reaction of Methyl 2-Benzamido-3-methylbut-2-enoate (25) with Nickel Peroxide.

Methyl 2-benzamido-3-methylbut-2-enoate (**25**) (200 mg, 0.85 mmol) in benzene (20 ml) was treated with 2.6 mole equivalents of nickel peroxide at reflux under nitrogen overnight. The reaction mixture filtered while hot, concentrated and chromatographed on silica (PF_{254}, chromatotron). Products were eluted in the following order;

Unreacted starting material **25**

Yield 22 mg, 11%;

m.p. 135-137° C (lit.,[132] 137-137.5° C);

^{1}H n.m.r. (300 MHz) δ 1.89 (s, 3H), 2.22 (s, 3H), 3.74 (s, 3H), 7.3-7.6 (m, 3H), 7.7-7.9 (m, 2H).

Benzamide (16).

Yield 10 mg, 10%;

m.p. 128-130° C (lit.,[129] 128° C).

Relative rate of reaction of (i) *N*-Benzoylglycine Methyl Ester (15a) and *N*-Benzoyl-*D,L*-alanine Methyl Ester (15b); and (ii) 15b and *N*-Benzoyl-*D,L*-valine Methyl Ester (15c), each at 0.025 Molar in benzene, at reflux with Nickel Peroxide.

The relative rates of reaction of **15a**, **15b** and **15c** each at 0.025 Molar in benzene, at reflux with nickel peroxide were determined using the method described above for the relative rate of reaction of *N*-benzoylglycine methyl ester (**15a**) and *N*-benzoyl-α,α-dideuterioglycine methyl ester (**22a**). Samples were analysed by ^{1}H n.m.r. spectroscopy and H.P.L.C.. The identification of product peaks was made using authentic samples of **15a**, **15b** and **15c**. The relative rates of reaction are listed in Table 7.

Relative rate of reaction of (i) *N*-Benzoylglycine Methyl Ester (15a) and *N*-Benzoyl-*D,L*-alanine Methyl Ester (15b); and (ii) 15b and *N*-Benzoyl-*D,L*-valine Methyl Ester (15c), each at 0.0025 Molar in benzene, at reflux with Nickel Peroxide.

The relative rates of reaction of **15a**, **15b** and **15c** each at 0.0025 Molar in benzene at reflux, with nickel peroxide were determined using the method described above for the relative rate of reaction of *N*-benzoylglycine methyl ester (**15a**) and *N*-benzoyl-α,α-dideuterioglycine methyl ester (**22a**). Samples were analysed by ^{1}H n.m.r. spectroscopy and

H.P.L.C.. The identification of product peaks was made using authentic samples of **15a, 15b** and **15c**. The relative rates of reaction are listed in Table 7.

Relative rate of reaction of *N*-Benzoylglycine Methyl Ester (15a) and *N*-Benzoyl-*D,L*-alanine Methyl Ester (15b), each at 0.025 Molar in benzene, at 20º C with Nickel Peroxide.

The relative rates of reaction of **15a** and **15b** each at 0.025 Molar in benzene at 20º Cwith nickel peroxide, were determined using the method described above for the relative rate of reaction of *N*-benzoylglycine methyl ester (**15a**) and *N*-benzoyl-α,α-dideuterioglycine methyl ester (**22a**). Samples were analysed by H.P.L.C.. The identification of product peaks were made using authentic samples of **15a** and **15b**. The relative rate of reaction is listed in Table 7.

TABLE 7

Reaction Mixture		Temp. ºC	Conc. Molar	% Substrate remaining		N[a]	k_Y/k_X	k_X/k_Y
X	Y			X	Y			
15a	**15b**	80	0.025	20	86	15	0.1 ± 0.03	10 ± 2.5
15b	**15c**	80	0.025	27	84	16	0.14 ± 0.03	7 ± 1.5
15a	**15b**	80	0.0025	16	67	10	0.22 ± 0.02	4.5 ± 0.5
15b	**15c**	80	0.0025	37	66	14	0.43 ± 0.03	2.3 ± 1.5
15a	**15b**	20	0.025	35	77	15	0.25 ± 0.03	4.0 ± 0.5

[a] N is the number of samples in the sample population.

N-Benzoylsarcosine Methyl Ester (26).

N-Benzoylsarcosine methyl ester (**26**) was prepared from sarcosine (Merck) (3.0 g, 34.0 mmol) as described above for *N*-benzoyl-*D,L*-alanine methyl ester (**15b**). The crude product was purified by chromatography on silica (PF_{254}, chromatotron) affording *N*-benzoylsarcosine methyl ester (**26**) as a colourless oil. The spectral characteristics of **26** were found to be consistent with those previously reported.[133]

Yield 4.8 g, 69%;

^{1}H n.m.r. (60 MHz) δ 3.02 (s, 3H), 3.73 (s, 3H), 4.20 (s, 2H), 7.2-7.6 (m, 5H).

Reaction of *N*-Benzoylsarcosine Methyl Ester (26) with Nickel Peroxide.

N-Benzoylsarcosine methyl ester (**26**) (100 mg, 0.5 mmol) in benzene (20 ml) was treated with 2 mole equivalents of nickel peroxide at reflux under nitrogen for 4 hours. The reaction mixture was filtered while hot, concentrated and chromatographed on silica (PF_{254}, chromatotron). Products were eluted in the following order;

Unreacted starting material **26**

Yield 29 mg, 29%;

^{1}H n.m.r. (300 MHz) δ 3.00 (s, 3H), 3.75 (s, 3H), 4.19 (s, 2H), 7.22-7.64 (m, 5H).

***N*-Methylbenzamide** (**27**) recrystallised from ethyl acetate/light petroleum as colourless needles.

Yield 16 mg, 24%;

m.p. 75-77° C (lit.,[134] 75° C).

^{1}H n.m.r. (300 MHz) δ 3.02 (d, *J* = 4.9 Hz, 3H), 6.16 (br s, 1H), 7.29-7.56 (m, 3H), 7.71-7.79 (m, 2H).

Benzamide (16)

Yield 4 mg, 7%;

m.p. 126-128° C (lit.,[129] 128° C).

N-Methylbenzamide (27).

Potassium hydroxide (7.9 g, 0.14 mol), was added in portions to a solution of methylamine (40%/water), (60 ml, 0.08 mol). Benzoyl chloride (10.0 g, 0.07 mol) was added dropwise to the cooled solution. On completion of addition the reaction mixture was stirred at room temperature for a further 3 hours. The reaction mixture was then acidified with concentrated hydrochloric acid and the crude product collected by vacuum filtration. Recrystallisation from ethyl acetate/light petroleum afforded *N*-methylbenzamide (**27**) as colourless crystals.

Yield 7.1 g, 74%;

m.p. 75-77° C (lit.,[134] 75° C);

^{1}H n.m.r. (300 MHz) δ 3.00 (d, *J* = 4.8 Hz, 3H), 6.46 (br s, 1H), 7.37-7.52 (m, 3H), 7.75-7.80 (m, 2H).

Reaction *N*-Methylbenzamide (27) with Nickel Peroxide.

N-Methylbenzamide (**27**) (100 mg, 0.7 mmol) in benzene (20 ml), was treated with 2.6 mole equivalents of nickel peroxide at reflux under

nitrogen for 4 hours. The reaction mixture was filtered while hot, concentrated and chromatographed on silica (PF_{254}, chromatotron). Products were eluted in the following order;

Unreacted starting material **27**

Yield 56 mg, 56%;

m.p. 74-76° C (lit.,[134] 75° C).

Benzamide (16)

Yield 20 mg, 22%;

m.p. 127-128.5° C (lit.,[129] 128° C).

Relative rate of reaction of (i) *N*-Benzoylsarcosine Methyl Ester (26) and *N*-Benzoyl-*D,L*-alanine Methyl Ester (15b); and (ii) *N*-Methylbenzamide (27) and *N*-Benzoyl-*D,L*-alanine Methyl Ester (15b), each at 0.0025 Molar in benzene, at reflux with Nickel Peroxide.

The relative rates of reaction of **26, 27** and **15b** were determined using the method described above for the relative rate of reaction of *N*-benzoylglycine methyl ester (**15a**) and *N*-benzoyl-α,α-dideuterioglycine methyl ester (**22a**). Samples were analysed by ^1H n.m.r. spectroscopy and H.P.L.C.. The identification of product peaks was made using authentic samples of **26, 27** and **15b**. The relative rates of reaction are listed in Table 8.

TABLE 8

Reaction Mixture		Temp. °C	Conc. Molar	% Substrate remaining		N[a]	k_Y/k_X	k_X/k_Y
X	Y			X	Y			
15b	**26**	80	0.025	50	59	14	0.77 ± 0.1	1.3 ± 0.2
27	**15b**	80	0.025	53	57	12	0.9 ± 0.2	1.1 ± 0.2

[a] N is the number of samples in the sample population.

Work Described in Chapter 2

N-Benzoylsarcosinamide (35).

Gaseous ammonia was bubbled through a cooled solution of *N*-benzoylsarcosine methyl ester (**26**) in methanol until the solution was saturated. The resulting mixture was stirred under anhydrous conditions for 3 days. The reaction mixture was concentrated *in vacuo* to yield crude *N*-benzoylsarcosinamide (**35**) as a white solid. Recrystallisation from ethyl acetate/dichloromethane afforded pure *N*-benzoylsarcosinamide (**35**) as colourless needles.

Yield 0.63g, 68%;

m.p. 122-125° C;

^{1}H n.m.r. (300 MHz) δ 3.09 (s, 3H), 4.16 (br. s, 2H), 5.90 (br s, 1H), 6.62 (br s, 1H), 7.43 (br. s, 5H);

^{13}C n.m.r. (300 MHz) δ 39.04, 52.02, 127.31, 128.49, 130.31, 172.41 , 171.10 ;

I.R. (NaCl, nujol) ν_{max} 3392, 3212, 1676, 1624, 1466, 1398, 1380, 1308, 1262, 1076, 976, 726, 704 cm^{-1};

Mass spectrum: *m/z* 192 (M^+, 4%), 191 (7), 175 (5), 148 (39), 105 (100), 77 (6);

Accurate mass: *m/z* 192.09065 (M^+), Calc. for $C_{10}H_{12}N_2O_2$ 192.08987;

Found; C, 62.16; H, 6.34; N, 14.55. Calc. for $C_{10}H_{12}N_2O_2$: C, 62.48; H, 6.29; N, 14.57; O, 16.65%.

Reaction of *N*-Benzoylsarcosinamide (35) with Nickel Peroxide.

N-Benzoylsarcosinamide (35) (50 mg, 0.26 mmol) in benzene (20 ml), was treated with 2.6 mole equivalents of nickel peroxide at reflux under nitrogen for 2 hours. The reaction mixture was filtered while hot, concentrated and chromatographed on silica (PF_{254}, chromatotron). Products were eluted in the following order;

N-Methylbenzamide (27)

Yield 4 mg, 11%;

m.p. 74-76° C (lit.,[134] 75° C);

^{1}H n.m.r. (300 MHz) δ 3.00 (d, *J* = 4.8 Hz,3H), 6.18 (br s, 1H), 7.39-7.53 (m, 3H), 7.74-7.79 (m, 2H).

Benzamide (16)

Yield 2 mg, 7%;

m.p. 127-129° C (lit.,[129] 128° C);

^{1}H n.m.r. (300 MHz).δ 5.80 (br s, 1H), 6.12 (br s, 1H), 7.42-7.64 (m, 3H), 7.79-7.84 (m, 2H).

Unreacted starting material **35**

Yield 21 mg, 42%;

m.p. 123-125° C;

^{1}H n.m.r. (300 MHz) δ 3.09 (s, 3H), 4.16 (br. s, 2H), 5.96 (br s, 1H), 6.60 (br s, 1H), 7.44 (br. s, 5H).

N-Benzoylsarcosine (36).

Sarcosine (Merck) (5.0 g, 56 mmol) was added to 50% aqueous ethyl acetate (50 ml), containing 1.1 equivalents of potassium carbonate (8.5 g, 61 mmol). Benzoyl chloride (7.9 g, 56 mmol) was added dropwise to the solution. On completion of addition the reaction mixture was stirred for a further 2 hours. The aqueous and organic phases were separated. The aqueous phase was washed with ethyl acetate, acidified with concentrated hydrochloric acid, and the product extracted into ethyl acetate (3 x 50 ml). The organic phase was dried, filtered, and concentrated under reduced pressure. The crude product was recrystallised from ethyl acetate to afford *N*-benzoylsarcosine (**36**) as colourless crystals.

Yield 6.4 g, 55%;
m.p. 103-107° C (lit.,[135] 104-107° C);
^{1}H n.m.r. (300 MHz) δ 3.06 (s, 3H), 4.30 (s, 2H), 7.37-7.49 (m, 5H), 10.54 (s, 1H).

Reaction of *N*-Benzoylsarcosine (36) with Nickel Peroxide.

N-Benzoylsarcosine (**36**) (200 mg, 0.1 mmol) in benzene (40 ml), was treated with 2.6 mole equivalents of nickel peroxide at reflux under nitrogen for 2 hours. The reaction mixture was filtered while hot, concentrated and chromatographed on silica (PF_{254}, chromatotron). Products were eluted in the following order;

***N*-Formyl-*N*-methylbenzamide** (**37**), isolated as a colourless oil.
Yield 7 mg, 5%;

^{1}H n.m.r. (300 MHz) δ 3.28 (s, 3H), 7.46-7.60 (m, 5H), 8.99 (s, 1H);

^{13}C n.m.r. (300 MHz) δ 27.50, 128.84, 128.96, 132.21, 164.43, 172.59;

Mass spectrum: *m/z* 135 (M^+ - CO, 24%), 134 (M^+ - CHO, 9), 106 (11), 105 (100), 77 (93);

Accurate mass: *m/z* 135.06800 (M^+ - CO), Calc. for C_8H_9NO 135.068413.

***N*-Methylbenzamide (27)** recrystallised from ethyl acetate/light petroleum as colourless crystals.

Yield 14 mg, 9%;

m.p. 74-76° C (lit.,[134] 75° C);

^{1}H n.m.r. (300 MHz) δ 3.02 (d *J* = 4.9 Hz, 3H), 6.16 (br s, 1H), 7.26-7.52 (m, 3H), 7.74-7.78 (m, 2H).

Benzamide (16)

Yield 12 mg, 10%;

m.p. 127-129° C (lit.,[129] 128° C).

N-Phthaloylglycine Methyl Ester (39).

N-Phthaloylglycine methyl ester (**39**) was prepared from commercially available *N*-phthaloylglycine (1.0 g, 4.9 mmol) as described above for *N*-benzoylglycine methyl ester (**15a**). Recrystallisation from methanol afforded *N*-phthaloylglycine methyl ester (**39**) as colourless crystals.

Yield 0.87 g, 81%;

m.p. 114-116° C (lit.,[136] 116° C);

^{1}H n.m.r. (300 MHz) δ 3.77 (s, 3H), 4.46 (s, 2H), 7.74-7.79 (m, 2H), 7.86-7.91 (m, 2H).

Reaction of *N*-Phthaloylglycine Methyl Ester (39) with Nickel Peroxide.

N-Phthaloylglycine methyl ester (**39**) (50 mg, 0.23 mmol) in benzene (10 ml), was treated with 5 mole equivalents of nickel peroxide at reflux under nitrogen for 5 hours. The reaction mixture was filtered while hot, concentrated and analysed by ^{1}H n.m.r. spectroscopy. The ^{1}H n.m.r. spectrum of the crude reaction mixture showed only signals corresponding to those of the starting material **39**. The reaction mixture was chromatographed on silica (PF_{254}, chromatotron) and unreacted starting material **39** was recovered.

Yield 33 mg, 66%;
m.p. 113-115° C (lit.,[136] 116° C);
^{1}H n.m.r. (300 MHz) δ 3.76 (s, 3H), 4.46 (s, 2H), 7.73-7.77 (m, 2H), 7.87-7.90 (m, 2H).

Relative rate of reaction of *N*-Phthaloylglycine Methyl Ester (39) and *N*-Benzoyl-*D,L*-alanine Methyl Ester (15b), each at 0.025 Molar in benzene, at reflux with Nickel Peroxide.

The relative rates of reaction of **39** and **15b** were determined using the method described above for the relative rate of reaction of *N*-benzoylglycine methyl ester (**15a**) and *N*-benzoyl-α,α-dideuterioglycine methyl ester (**22a**). Samples were analysed by H.P.L.C.. The identification

of product peaks was made using authentic samples of **39** and **15b**. The relative rate of reaction is listed in Table 9.

TABLE 9

Reaction Mixture		Temp. °C	Conc. Molar	% Substrate remaining		N[a]	k_Y/k_X	k_X/k_Y
X	Y			X	Y			
15b	**39**	80	0.0025	63	85	8	0.28 ± 0.03	3.5 ± 0.5

[a] N is the number of samples in the sample population.

<u>*N*-Phthaloyl-*L*-phenylalanine.</u>

L-Phenylalanine (Sigma) (10.0 g, 68 mmol) and 1 mole equivalent of phthalic anhydride (Merck) (11.1 g, 68 mmol) were heated to 150-165° C. The resulting melt was stirred for 30 minutes within that temperature range. After cooling to room temperature the resulting solid was dissolved in methanol (80 ml) and then poured into water (600 ml) to effect crystallisation of *N*-phthaloyl-*L*-phenylalanine as a white solid. This was used without further purification.

Yield 18.96 g, 95%;

m.p. 182-184° C (lit.,[137] 180-182° C);

^{1}H n.m.r. (60 MHz) δ 3.60 (d, *J* = 8.0 Hz, 2H), 5.25 (t, *J* = 8.0 Hz, 1H), 7.2 (s, 5H), 7.7 (m, 4H), 7.82 (br s, 1H);

$[\alpha]_D^{26}$ -210° (*c* 0.01, ethanol).

<u>*N*-Phthaloyl-*L*-Phenylalanine Methyl Ester (42).</u>

N-Phthaloyl-*L*-phenylalanine (**42**) (5.0 g, 16.9 mmol) was dissolved in a cooled solution of methanol (60 ml) which had been pretreated with thionyl chloride (2.4 g, 20.3 mmol). The mixture was stirred under anhydrous conditions overnight at room temperature. The solvent was removed *in vacuo* and recrystallisation of the residue from methanol afforded *N*-phthaloyl-*L*-phenylalanine methyl ester (**42**) as colourless crystals. The spectral characteristics of **42** were found to be consistent with those previously reported.[113]

Yield 4.69g, 90%;

m.p. 126-127.5° C;

^{1}H n.m.r. (60 MHz) δ 3.60 (m, 2H), 3.80 (s, 3H), 5.20 (dd, J_{vic} = 6 Hz, J_{NH} = 10 Hz, 1H), 7.2 (m, 5H), 7.6-7.8 (m, 4H).

<u>Reaction of *N*-Phthaloyl-*L*-phenylalanine Methyl Ester (42) with Nickel Peroxide.</u>

N-Phthaloyl-*L*-phenylalanine methyl ester (**42**) (50 mg, 0.16 mmol) in benzene (5 ml) was treated with 4 mole equivalents of nickel peroxide at reflux under nitrogen overnight. The reaction mixture was filtered while hot, concentrated and chromatographed on silica (PF_{254}, chromatotron). Products eluted in the following order;

Unreacted starting material **42**

Yield 40 mg, 80%;

^{1}H n.m.r. (300 MHz) δ 3.50-3.64 (m, 2H), 3.78 (s, 3H), 5.16 (dd, J_{vic} = 5.5 Hz, J_{NH} = 11.0 Hz, 1H), 7.1-7.2 (m, 5H), 7.66-7.71 (m, 2H), 7.74-7.80 (m, 2H).

***N*-Phthaloyl-α,β-dehydrophenylalanine methyl ester (43)**

Yield 2 mg, 4%;

^{1}H n.m.r. (300 MHz) δ 3.84 (s 3H), 7.16-7.50 (m, 5H), 7.76-7.82 (m, 2H), 7.91-7.96 (m, 2H), 8.13 (s, 1H);

Mass spectrum: *m/z* 307 (M^+, 100%), 248 (44), 152 (23), 105 (67);

Accurate mass: *m/z* 307.09509 (M^+), Calc. for $C_{18}H_{13}NO_4$ 307.08446.

The spectral characteristics of **43** were found to be consistent with those previously reported for the *Z*-isomer.[98]

Reaction of *N*-Benzoyl-*L*-phenylalanine Methyl Ester (44) with Nickel Peroxide.

N-Benzoyl-*L*-phenylalanine methyl ester (**44**) (100 mg, 0.35 mmol) in benzene (10 ml) was treated with 10 mole equivalents of nickel peroxide at reflux under nitrogen overnight. The reaction mixture was filtered while hot, concentrated and chromatographed on silica (PF_{254}, chromatotron). Products were eluted in the following order;

Methyl-2,5-diphenyloxazole-4-carboxylate (**45**) recrystallised from ethyl acetate/light petroleum a colourless crystals.

Yield 12 mg, 12%;

m.p. 84-86° C (lit.,[138] 85.5-86° C);

^{1}H n.m.r. (300 MHz) δ 3.99 (s, 3H), 7.46-7.54 (m, 6H), 8.13-8.18 (m, 4H);

Mass spectrum: *m/z* 279 (M^+, 77%), 248 (16), 165 (7), 139.5 (7), 105 (100), 77 (61).

Unreacted starting material **44**

Yield 22 mg, 22%;

m.p. 85-86° C (lit.,[139] 83.6-84.6° C);

^{1}H n.m.r. (300 MHz) δ 3.23 (dd, J_{vic} = 5.7 Hz, J_{gem} = 13.8 Hz, 1H), 3.28 (dd, J_{vic} = 5.7 Hz, J_{gem} = 13.8 Hz, 1H), 3.74 (s, 3H), 5.08 (dt, J_t = 5.7 Hz, J_d = 7.4 Hz, 1H), 6.70 (br d, J = 7.5 Hz, 1H), 7.10-7.31 (m, 5H), 7.34-7.50 (m,3H), 7.70-7.80 (m, 2H).

Benzamide (16)

Yield 8 mg, 19%;

m.p. 126-128° C (lit.,[129] 128° C);

^{13}C n.m.r. (300 MHz) δ 127.32, 128.62, 132.01, 159.97.

Relative rate of reaction of *N*-Phthaloyl-*L*-phenylalanine Methyl Ester (42) and *N*-Benzoyl-*L*-phenylalanine Methyl Ester (44), each at 0.025 Molar in benzene, at reflux with Nickel Peroxide.

The relative rates of reaction of **42** and **44** were determined using the method described above for the relative rate of reaction of *N*-benzoylglycine methyl ester (**15a**) and *N*-benzoyl-α,α-dideuterioglycine methyl ester (**22a**). Samples were analysed by ^{1}H.n.m.r.. The identification of product peaks was made using authentic samples of **42** and **44**. The relative rate of reaction is listed in Table 10.

TABLE 10								
Reaction Mixture		Temp. °C	Conc. Molar	% Substrate remaining		N[a]	k_Y/k_X	k_X/k_Y
X	Y			X	Y			
42	**44**	80	0.025	85	57	8	4.5 ± 0.5	0.22 ± 0.02
[a] N is the number of samples in the sample population.								

<u>*N*-Benzoyl-*L*-proline Methyl Ester (49).</u>

N-Benzoyl-*L*-proline methyl ester (**49**) was prepared from *L*-proline (Sigma) (3.0 g, 26 mmol) as described above for *N*-benzoyl-*D,L*-alanine methyl ester (**15b**). Recrystallisation from ethyl acetate/light petroleum afforded *N*-benzoyl-*L*-proline methyl ester (**49**) as colourless crystals.

Yield 4.53 g, 75%;

m.p. 93-93.5° C (lit.,[140] 89-89.5° C);

^{1}H n.m.r. (300 MHz) δ 1.86-1.96 (m, 1H), 1.97-2.08 (m, 2H), 2.21-2.38 (m, 1H), 3.50-3.57 (m, 1H), 3.61-3.69 (m, 1H) 3.78 (s, 3H), 4.66 (dd, J_{vic} = 4.9 Hz, J_{gem} = 8.1 Hz, 1H), 7.36-7.46 (m, 3H), 7.55-7.60 (m, 2H).

<u>Reaction of *N*-Benzoyl-*L*-proline Methyl Ester (49) with Nickel Peroxide.</u>

N-Benzoyl-*L*-proline methyl ester (**49**) (200 mg, 0.86 mmol) in benzene (40 ml) was treated with 5 mole equivalents of nickel peroxide at

reflux under nitrogen overnight. The reaction mixture was filtered, concentrated and chromatographed on silica (PF_{254}, chromatotron). Products eluted in the following order;

N-Benzoyl-2-methoxycarbonylpyrrole (50)

Yield 10 mg, 5%;

^{1}H n.m.r. (300 MHz) δ 3.85 (s, 3H), 6.26 (dd, J_{vic} = 2.6 Hz, J_{vic} = 6.1 Hz, 1H), 6.91-6.93 (m, 1H), 6.94-6.96 (m, 1H), 7.21-7.72 (m, 5H);

Mass spectrum: *m/z* 229 (M^+, 11%), 174 (18), 105 (100), 77(6).

The spectral characteristics of **50** were found to be consistent with those previously reported.[141]

Unreacted starting material **49**

Yield 115 mg, 57%;

m.p. 89-91° C (lit.,[140] 89-89.5° C);

^{1}H n.m.r. (300 MHz) δ 1.81-1.90 (m, 1H), 1.92-2.05 (m, 2H), 2.26-2.35 (m, 1H), 3.47-3.54 (m, 1H), 3.57-3.67 (m, 1H) 3.75 (s, 3H), 4.65 (dd, J_{vic} = 5.1 Hz, J_{vic} = 8.2 Hz, 1H), 7.35-7.44 (m, 3H), 7.54-7.57 (m, 2H).

Benzamide (16)

Yield 6 mg, 6%;

m.p. 128-130° C (lit.,[129] 128° C);

^{13}C n.m.r. (300 MHz) δ 127.27, 128.61, 132.00, 169.471.

Work Described in Chapter 3.

Reaction of *L*-Valylglycine (53) with Nickel Peroxide.

A solution of *L*-valylglycine (**53**) (Sigma) (100 mg, 0.57 mmol) at pH 8 in water (10 ml) at 80° C was treated with 2 mole equivalents of nickel peroxide. When the nickel peroxide in the reaction mixture changed from the characteristic black colour to an olive green the reaction mixture was filtered using a H.P.L.C. pre-filter. The pink coloured filtrate was basified with sodium hydroxide solution, and extracted with dichloromethane, ethyl acetate and finally ether. The organic phase was dried, filtered, and concentrated to afford *L*-valinamide (**54**) as a white solid.

Yield 14 mg, 21%;

^{1}H n.m.r. (300 MHz) (D_2O) δ 0.89 (apparent t, J = 7.0 Hz, 6H), 1.87 (septet, J = 7.0 Hz, 1H), 3.16 (d, J = 6.0 Hz, 1H);

The spectral characteristics of **54** were found to be consistent with those obtained using an authentic sample of *L*-valinamide.

Reaction of *N*-Benzoylglycine Methyl Ester (15a) with Nickel Oxide (A) (black).

N-Benzoylglycine methyl ester (**15a**) (100 mg, 0.5 mmol) in benzene (20 ml) was heated at reflux with 2.6 mole equivalents of nickel oxide (A) (black) for 1 hour. The reaction mixture was filtered and concentrated *in vacuo*. Analysis of the reaction mixture by T.L.C., and ^{1}H n.m.r.

spectroscopy showed only unreacted starting material **15a**. Subsequent purification of the reaction mixture by chromatography on silica (PF_{254}, chromatotron) resulted in unreacted starting material **15a** being recovered in quantitative yield.

m.p. 81-83° C (lit.,[128] 82-83° C).

Reaction of *N*-Benzoylglycine Methyl Ester (15a) with Nickel Oxide (B) (olive green).

N-Benzoylglycine methyl ester (**15a**) (100 mg, 0.5 mmol) in benzene (20 ml) was heated at reflux with 2.6 mole equivalents of nickel oxide (B) (olive green) for 1 hour. The reaction mixture was filtered and concentrated *in vacuo*. Analysis of the reaction mixture by T.L.C. and ^1H n.m.r. spectroscopy showed only unreacted starting material **15a**. Purification of the reaction mixture by chromatography on silica (PF_{254}, chromatotron) resulted in unreacted starting material **15a** being recovered in quantitative yield.

m.p. 80-82° C (lit.,[128] 82-83° C).

Reaction of *N*-Benzoylglycine Methyl Ester (15a) with Nickel Sulphate Hexahydrate.

N-Benzoylglycine methyl ester (**15a**) (100 mg, 0.5 mmol) in benzene (20 ml) was heated at reflux with 2.6 mole equivalents of nickel sulphate hexahydrate (Fluka A. G.) for 1 hour. The reaction mixture was filtered, concentrated *in vacuo*. Analysis of the reaction mixture by T.L.C., and ^1H n.m.r. spectroscopy showed only unreacted starting material **15a**.

Purification of the reaction mixture by chromatography on silica (PF_{254}, chromatotron) resulted in unreacted starting material **15a** being recovered in quantitative yield.
m.p. 81-83° C (lit.,[128] 82-83° C).

Glycine Methyl Ester Hydrochloride.

Glycine (Sigma) (5.0 g, 66 mmol) was added to anhydrous methanol (200 ml), which had been pretreated with thionyl chloride (32 g, 70 mmol). The resulting solution was stirred overnight under anhydrous conditions (calcium chloride guard tube). The reaction mixture was concentrated *in vacuo* and recrystallisation of the crude product from dichloromethane/methanol afforded the hydrochloride salt of glycine methyl ester as white needles.

Yield 7.57 g, 91%;
m.p. 172-175° C (dec.) (lit.,[142] 175° C, (dec)).

***N*-Benzoylglycylglycine Methyl Ester (55).**

N-Benzoylglycylglycine methyl ester (**55**) was prepared by coupling commercially available hippuric acid to glycine methyl ester hydrochloride, prepared as described above, using the following procedure. Ethyl chloroformate (2.7 ml, 27.9 mmol) was added dropwise to a cooled (-7 - -5° C) and stirred solution of hippuric acid (5 g, 27.9 mmol) in freshly distilled THF (100 ml) containing triethylamine (3.9 ml, 27.9 mmol). After 20 minutes a suspension of the hydrochloride salt of glycine

methyl ester (3.5 g, 27.9 mmol), in THF (75 ml) containing triethylamine (4.7 ml, 33.5 mmol), was added and the resulting mixture left to stir at 0° C for 2 hours, and at room temperature overnight under nitrogen. The reaction mixture was concentrated to dryness and the residue dissolved in ethyl acetate and water. The organic phase was separated and washed successively with 5% aqueous sodium bicarbonate (x2), 10% hydrochloric acid, and water. The organic phase was then dried over sodium sulphate, filtered, and concentrated under reduced pressure. Recrystallisation of the crude product from dichloromethane/light petroleum gave pure *N*-benzoylglycylglycine methyl ester (**55**) as colourless crystals.

Yield 2.57 g, 37%;

m.p. 79-81° C (lit.,[143] 78-82° C);

^{1}H n.m.r. (300 MHz) δ 3.74 (s, 3H), 4.06 (d, *J* = 5.5 Hz, 2H), 4.21 (d, *J* = 5.3 Hz, 2H), 7.16 (br t, *J* = 5.6 Hz, 1H), 7.37 (br t, *J* = 5.5 Hz, 1H), 7.40-7.54 (m, 3H), 7.81-7.85 (m, 2H).

<u>*N*-Benzoylglycinamide (56).</u>

Glycinamide (Sigma) (2.0 g, 18 mmol) dissolved in iced water (10 ml), to which potassium carbonate (3.0 g) had been added. Benzoyl chloride (0.76 ml, 6.6 mmol) was added dropwise with cooling over 15 minutes, and the mixture stirred at room temperature for 2 hours. The crude product was collected by vacuum filtration and dried over anhydrous calcium chloride. Recrystallisation from methanol afforded *N*-benzoylglycinamide (**56**) as colourless crystals.

Yield 1.85 g, 57%;

m.p. 182-184° C (lit[128]., 182-183° C);

^{1}H n.m.r. (300 MHz) ($CDCL_3/CD_3OD$) δ 4.07 (s, 2H), 7.43-7.57 (m, 3H), 7.85-7.87 (m, 2H).

Reaction of *N*-Benzoylglycylglycine Methyl Ester (55) with Nickel Peroxide.

N-Benzoylglycylglycine methyl ester (**55**) (50 mg, 0.2 mmol) in benzene (20 ml) was treated with 2 mole equivalents of nickel peroxide at reflux under nitrogen for 2.25 hours. The reaction mixture was filtered, concentrated and chromatographed on silica (PF_{254}, chromatotron). Products eluted in the following order;

Benzamide (16)

Yield 8 mg, 33%;

m.p. 126-128° C (lit.,[129] 128° C);

^{1}H n.m.r. (300 MHz) δ 5.71 (br s, 1H), 6.07 (br s, 1H), 7.43-7.06 (m, 3H), 7.80-7.84 (m, 2H);

Unreacted starting material **55**

Yield 24 mg, 40%;

m.p. 79-82° C (lit.,[143] 78-82° C);

^{1}H n.m.r. (300 MHz) δ 3.75 (s, 3H), 4.08 (d, J = 5.4 Hz, 2H), 4.21 (d, J = 5.3 Hz, 2H), 6.94 (br t, J = 5.4 Hz, 1H), 7.19 (br t, J = 5.5 Hz, 1H), 7.40-7.55 (m, 3H), 7.80-7.85 (m, 2H).

***N*-Benzoylglycinamide (56)**

Yield 2 mg, 6%;

m.p. 181-183° C (lit[128]., 182-183° C);

^{1}H n.m.r. (300 MHz) ($CDCL_3/CD_3OD$) δ 4.07 (s, 2H), 7.42-7.55 (m, 3H), 7.83-7.87 (m, 2H).

The ^{1}H n.m.r. spectral characteristics of **56** were found to be consistent with those of an independantly synthesized sample of *N*-benzoylglycinamide (**56**).

<u>*N*-Benzoyl-*D,L*-valinamide (60).</u>

N-Benzoyl-*D,L*-valinamide (**60**) was prepared from the hydrochloride salt of *D,L*-valinamide (Sigma) (1.0 g, 6.6 mmol) as described above for *N*-benzoylglycinamide (**56**). Recrystallisation of the crude product from ethyl acetate/light petroleum afforded *N*-benzoyl-*D,L*-valinamide (**60**) as colourless crystals. The physical and spectral characteristics of **60** were found to be consistent with those previously reported.[111]

Yield 1.4 g, 95%;

m.p. 220-222° C (lit.,[112] 220-221° C);

^{1}H n.m.r. (300 MHz) ($CDCl_3/CD_3OD$) δ 1.04 (apparent t, *J* = 6.3 Hz, 6H), 2.18 (septet, *J* = 6.9 Hz, 1H), 4.45 (d, *J* = 7.3 Hz, 1H), 7.44-7.60 (m, 3H), 7.82-7.85 (m, 2H).

<u>Reaction of *N*-Benzoyl-*D,L*-valylglycine Methyl Ester (58) with Nickel Peroxide.</u>

N-Benzoyl-*D,L*-valylglycine methyl ester (**58**) (100 mg, 0.34 mmol) in benzene (10 ml) was treated with 4 mole equivalents of nickel peroxide

at reflux under nitrogen for 2 hours. The reaction mixture was filtered, concentrated and chromatographed on silica (PF_{254}, chromatotron). Products eluted in the following order;

Unreacted starting material **58**

Yield 60 mg, 60%;

m.p. 157-159° C;

^{1}H n.m.r. (300 MHz) δ 1.00 (d, J = 7.4 Hz, 3H), 1.04 (d, J = 7.4 Hz, 3H), 2.3 (m, 1H), 3.71 (s, 3H), 3.93 (dd, J_{vic} = 5.8 Hz, J_{gem} = 17.0 Hz, 1H), 4.12 (dd, J_{vic} = 6.0 Hz, J_{gem} = 16.9 Hz, 1H), 4.70 (dd, J_{vic} = 7.9 Hz, J_{NH} = 8.4 Hz, 1H),, 7.17-7.40 (m, 3H), 7.48-7.72 (m, 2H), 7.82 (d, J = 8.2 Hz, 2H).

The spectral characteristics of **58** were found to be consistent with those previously reported.[114]

***N*-Benzoyl-*D,L*-valinamide** (**60**) recrystallised from ethyl acetate/light petroleum as colourless crystals.

Yield 20 mg, 27%;

m.p. 221-223° C (lit.,[112] 220-221° C);

^{1}H n.m.r. (300 MHz) ($CDCL_3/CD_3OD$) δ 1.05 (apparent t, J = 6.4 Hz, 6H), 2.15-2.22 (m, 1H), 4.45 (d, J = 7.3 Hz, 1H), 7.44-7.60 (m, 3H), 7.82-7.86 (m, 2H).

The physical and spectral characteristics of **60** were found to be consistent with those of an independantly synthesized sample of **60**.

Reaction of *N*-Benzoyl-*D,L*-leucylglycine Methyl Ester (59) with Nickel Peroxide.

N-Benzoyl-*D,L*-leucylglycine methyl ester (**59**) (50 mg, 0.16 mmol) in benzene (10 ml) was treated with 4 mole equivalents of nickel peroxide

and heated at 65-70° C under nitrogen overnight. The reaction mixture was filtered, concentrated and chromatographed on silica (PF_{254}, chromatotron). Products eluted in the following order;

Unreacted starting material **59**

Yield 10 mg, 20%;

m.p. 173-175° C (lit.,[145] 176° C);

^{1}H n.m.r. (300 MHz) δ 0.93 (t, J = 5.5 Hz, 6H), 1.64-1.79 (m, 3H), 3.70 (s, 3H), 3.98 (J_{NH} = 5.5 Hz, J_{AB} = 18.0 Hz, 2H), 4.79-4.87 (m, 1H), 7.24 (br d, J = 8.4 Hz, 2H), 7.35-7.52 (m, 3H), 7.79-7.87 (m, 2H).

***N*-Benzoyl-*D,L*-leucinamide (61)** recrystallised from ethyl acetate and light petroleum as colourless crystals.

Yield 14 mg, 37%;

m.p. 169-171° C. (lit.,[112] 171-172° C);

^{1}H n.m.r. (300 MHz) ~~δ 0.98 (dd, J_{vic} = 1.9 Hz, J_{gem} = 6.1 Hz, 6H)~~, 1.66-1.83 (m, 3H), 4.72 (dd, J_{vic} = 5.6 Hz, J_{gem} = 8.0 Hz, 1H), 5.49 (br s, 1H), 6.33 (br s, 1H), 6.67 (d, J = 8.0 Hz, 1H), 7.40-7.56 (m, 3H), 7.78-7.83 (m, 2H).

Reaction of *N*-Benzoyl-*D,L*-alanylglycine Methyl Ester (62) with 2 mole equivalents of Nickel Peroxide.

N-Benzoyl-*D,L*-alanylglycine methyl ester (**62**) (100 mg, 0.19 mmol) in benzene (20 ml) was treated with 2 mole equivalents of nickel peroxide and heated at reflux under nitrogen for 2 hours. The reaction mixture was filtered while hot, concentrated and chromatographed on silica (PF_{254}, chromatotron). Products eluted in the following order

Unreacted starting material **62**

Yield 69 mg, 69%;

m.p. 107-109° C (lit.,[145] 110° C);

^{1}H n.m.r. (300 MHz) δ 1.49 (d, J = 7.0 Hz, 3H), 3.68 (s, 3H), 4.01 (dd, J_{vic} = 2.2 Hz, J_{gem} = 5.6 Hz, 2H), 4.88 (pentet, J = 7.1 Hz, 1H), 7.34-7.50 (m, 3H), 7.61, (br d, J = 7.5 Hz, 1H), 7.77 (br t, J = 5.5 Hz, 1H), 7.81-7.84 (m, 2H).

***N*-Benzoyl-*D,L*-alanamide (63)** recrystallised from ethyl acetate/light petroleum as colourless crystals.

Yield 17 mg, 20%;

m.p. 230-232° C (lit.,[113] 231-233° C);

^{1}H n.m.r. (300 MHz) ($CDCl_3$/CD_3OD) δ 1.47 (d, J = 7.2 Hz, 3H), 4.49 (q, J = 7.2 Hz, 1H), 7.46-7.61 (m, 3H), 7.79-7.82 (m, 2H).

Reaction of *N*-Benzoyl-*D,L*-alanylglycine Methyl Ester (62) with 4 mole equivalents of Nickel Peroxide.

N-Benzoyl-*D,L*-alanylglycine methyl ester (**62**) (50 mg, 0.19 mmol) in benzene (3 ml) was treated with 4 mole equivalents of nickel peroxide and heated at 65-70° C under nitrogen overnight. The reaction mixture was filtered, concentrated and chromatographed on silica (PF_{254}, chromatotron). Products eluted in the following order

Benzamide (16)

Yield 18 mg, 79%;

m.p. 129-131° C (lit.,[129] 128° C);

^{1}H n.m.r. (300 MHz) δ 5.63 (br s, 1H), 6.04 (br s, 1H), 7.42-7.61 (m, 3H), 7.80-7.85 (m, 2H);

^{13}C n.m.r. (75 MHz) δ 127.33, 128.65, 132.05, 169.03.

Reaction of *N*-Benzoylglycyl-*D,L*-valine Methyl Ester (65) with Nickel Peroxide.

N-Benzoylglycyl-*D,L*-valine methyl ester (**65**) (50 mg, 0.17 mmol) in benzene (4 ml) was treated with 2 mole equivalents of nickel peroxide and heated at reflux under nitrogen overnight. The reaction mixture was filtered, concentrated and chromatographed on silica (PF_{254}, chromatotron). Products eluted in the following order;

Unreacted starting material **65**

Yield 6 mg,12%;

m.p. 143-145° C (lit.,[146] 145° C);

^{1}H n.m.r. (300 MHz) δ 0.92 (d, J = 6.9 Hz, 3H), 0.95 (d, J = 6.8 Hz, 3H), 2.13-2.25 (m, 1H), 3.72 (s, 3H), 4.26 (d, J = 5.1 Hz, 2H), 4.54 (dd, J_{vic} = 5.2 Hz, J_{NH} = 8.6 Hz, 1H), 7.35 (br d, J = 8.5 Hz, 1H), 7.43-7.51 (m, 3H), 7.53 (br t, J = 5.0 Hz, 1H), 7.81-7.87 (m, 2H).

Benzamide (16)

Yield 4 mg, 19%;

m.p. 126-128° C (lit.,[129] 128° C);

^{1}H n.m.r. (300 MHz) δ 5.84 (br s, 1H), 6.05 (br s, 1H), 7.41-7.57 (m, 3H), 7.80-7.84 (m. 2H).

***N*-Benzoyl-methoxyglycyl-*D,L*-valine methyl ester (66)**

Yield 6 mg, 10%;

^{1}H n.m.r. (300 MHz) δ 0.95 (m, 6H), 2.23 (m, 1H), 3.56 (s, 3H), 3.77 (s, 3H), 4.51 (dd, J_{vic} = 5 Hz, J_{gem} = 8 Hz, 1H), 5.33 (d, J = 6 Hz, 0.5H), 5.40 (d, J = 6 Hz, 0.5H), 7.4-7.5 (m, 5H), 7.6 (m, 2H);

Mass spectrum: *m/z* 322 (M^+, 2%), 263 (5), 192 (3), 164 (72), 105 (100), 77 (81).

The spectral characteristics of **66** were found to be consistent with those previously reported.[114]

Reaction of *N*-Phthaloyl-*D,L*-valylglycine Methyl Ester (69) with Nickel Peroxide.

N-Phthaloyl-*D,L*-valylglycine methyl ester (**69**) (50 mg, 0.16 mmol) in benzene (10 ml) was treated with 6 mole equivalents of nickel peroxide at 55-60° C under nitrogen overnight. The reaction mixture was filtered, concentrated and chromatographed on silica (PF_{254}, chromatotron). Products eluted in the following order;

Unreacted starting material **69**

Yield 16 mg, 32%;

m.p. 118-120° C (lit.,[147] 119-120° C);

^{1}H n.m.r. (300 MHz) δ 0.87 (d, J = 6.6 Hz, 3H), 1.13 (d, J = 6.6 Hz, 3H), 1.89 (m, 1H), ~~(dq, J_q = 6.6 Hz, J_d = 11.4 Hz, 1H)~~, 3.72 (s, 3H), 4.06 (d, J = 5.6 Hz, 2H), 4.47 (d, J = 11.4 Hz, 1H), 6.68 (br t, J = 5.4 Hz, 1H), 7.70-7.80 (m, 2H), 7.83-7.90 (m, 2H).

***N*-Phthaloyl-*D,L*-valinamide** (**72**) recrystallised from ethanol as colourless needles.

Yield 21 mg, 55%;

m.p. 180-181° C (lit.,[115] 186-187° C);

^{1}H n.m.r. (300 MHz) δ 0.86 (d, J = 6.6 Hz, 3H), 1.14 (d, J = 6.6 Hz, 3H), 2.83 (dh, J_h = 6.6, J_d = 11.2 Hz, 1H), 4.40 (d, J = 11.2 Hz, 1H), 5.97 (br s, 1H), 6.90 (br s, 1H), 7.74-7.79 (m, 2H), 7.84-7.90 (m, 2H).

Reaction of *N*-Phthaloyl-*L*-leucylglycine Methyl Ester (70) with Nickel Peroxide.

N-Phthaloyl-*L*-leucylglycine methyl ester (**70**) (50 mg, 0.15 mmol) in benzene (3 ml) was treated with 6 mole equivalents of nickel peroxide and heated at reflux under nitrogen for 2 hours. The reaction mixture was filtered, concentrated and chromatographed on silica (PF_{254}, chromatotron). Products eluted in the following order;

Unreacted starting material **70**

Yield 13 mg, 26%;

m.p. 105-107° C (lit.,[147] 106-107° C);

^{1}H n.m.r. (300 MHz) δ 0.93 (d, J = 6.5 Hz, 3H), 0.95 (d, J = 6.5 Hz, 3H), ~~δ 0.94 (dd, J_{vic} = 1.5 Hz, J_{gem} = 6.5 Hz, 6H)~~, 1.83-1.93 (m, 1H), 2.38 (dd, J_{vic} = 4.4 Hz, J_{gem} = 11.3 Hz, 1H), 2.42 (dd, J_{vic} = 4.4 Hz, J_{gem} = 11.3 Hz, 1H), 3.74 (s, 3H), 4.05 (dd, J_{vic} = 1.5 Hz, J_{gem} = 5.0 Hz, 2H), 4.97 (dd, J_{vic} = 5.0 Hz, J_{gem} = 11.3 Hz, 1H), 6.77 (br t, J = 11.2 Hz, 1H), 7.72-7.79 (m, 2H), 7.84-7.91 (m, 2H).

***N*-Phthaloyl-*L*-leucinamide** (**73**) recrystallised from ethanol as colourless needles.

Yield 16 mg, 41%;

m.p. 169-171° C (lit.,[116] 173-174° C);

^{1}H n.m.r. (300 MHz) δ 0.92 (dd, J_{vic} = 1.3 Hz, J_{gem} = 6.7 Hz, 6H), 1.79-1.89 (m, 1H), 2.36 (dd, J_{vic} = 4.4 Hz, J_{gem} = 11.3 Hz, 1H), 2.41 (dd, J_{vic} = 4.4 Hz, J_{gem} = 11.3 Hz, 1H), 4.89 and 4.95 (d and d, J = 4.9 Hz, 1H), ~~4.91 (dd, J_{vic} = 4.9, J_{gem} = 11.3 Hz, 1H)~~, 5.81 (br s, 1H), 6.23 (br s, 1H), 7.72-7.90 (m, 4H);

Reaction of *N*-Phthaloyl-*D,L*-alanylglycine Methyl Ester (71) with Nickel Peroxide.

N-Phthaloyl-*D,L*-alanylglycine methyl ester (**71**) (100 mg, 0.34 mmol) in benzene (20 ml) was treated with 2 mole equivalents of nickel peroxide and heated at reflux under nitrogen for 5 hours. The reaction mixture was filtered, concentrated and chromatographed on silica (PF_{254}, chromatotron). Products eluted in the following order;

Unreacted starting material **71**

Yield 8 mg, 42%;

m.p. 143-145° C (lit.,[148] 145-147° C);

^{1}H n.m.r. (300 MHz) δ 1.74 (d, *J* = 7.4 Hz, 3H), 3.72 (s, 3H), 4.07 (d, *J* = 5.0 Hz, 2H), 4.99 (q, *J* = 7.4 Hz, 1H), 6.70 (br d, *J* = 5.0 Hz, 1H), 7.71-7.79 (m, 2H), 7.82-7.87 (m, 2H).

***N*-Phthaloyl-*D,L*-alanamide** (**74**) recrystallised from ethanol as colourless crystals.

Yield 43 mg, 43%;

m.p. 217-219° C (lit.,[115] 212-213° C);

^{1}H n.m.r. (300 MHz) ($CDCL_3/CD_3OD$) δ 1.71 (d, *J* = 7.3 Hz, 3H), 4.93 (q, *J* = 7.3, Hz, 1H), 7.75-7.81 (m, 2H), 7.84-7.90 (m, 2H).

Relative rate of reaction of *N*-Phthaloyl-*D,L*-valylglycine Methyl Ester (69) and *N*-Phthaloyl-*D,L*-alanylglycine Methyl Ester (71), each at 0.025 Molar in benzene, at reflux with Nickel Peroxide.

The relative rates of reaction of **69** and **71** were determined using the method described above for the relative rate of reaction of *N*-benzoylglycine methyl ester (**15a**) and *N*-benzoyl-α,α-dideuterioglycine methyl ester (**22a**). Samples were analysed by N.M.R. ~~H.P.L.C.~~. The identification of product peaks was made using authentic samples of **69** and **71**. The relative rate of reaction is listed in Table 9.

TABLE 9

Reaction Mixture		Temp. °C	Conc. Molar	% Substrate remaining		N[a]	k_Y/k_X	k_X/k_Y
X	Y			X	Y			
71	**69**	80	0.0025	36	75	8	0.58 ± 0.15	1.7 ± 0.4

[a] N is the number of samples in the sample population.

<u>*D,L*-Aspartate Dimethyl Ester Hydrochloride.</u>

D,L-Aspartate dimethyl ester hydrochloride was prepared from *D,L*-aspartate (5.0 g, 37 mmol) as described for glycine methyl ester hydrochloride. The crude product recrystallised from dichloromethane/methanol as white needles.

Yield 7.1g, 95%.

N-Phthaloyl-L-phenylalanine-D,L-aspartate Dimethyl Ester (75)

N-Phthaloyl-*L*-phenylalanyl-*D,L*-aspartate dimethyl ester **(75)** was prepared by coupling *N*-phthaloyl-*L*-phenylalanine (prepared as described previously) to *D,L*-aspartate dimethyl ester hydrochloride using the procedure described previously for the preparation of *N*-benzoylglycylglycine methyl ester **(55)**. The crude product was chromatographed on silica (PF_{254}, chromatotron) to give pure *N*-phthaloyl-*L*-phenylalanyl-*D,L*-aspartate dimethyl ester **(75)** as a colourless oil.

Yield 6.58 g, 88 % (based on both diastereomers);

The two diastereomers were separated by fractional recrystallisation from methanol.

1st diastereomer to crystallise out of methanol;

m.p. 110-115° C.;

^{1}H n.m.r. (300 MHz) δ 2.92 (dd, J_{vic} = 4.4 Hz, J_{gem} = 17.2 Hz, 1H), 2.98 (dd, J_{vic} = 4.4 Hz, J_{gem} = 17.2 Hz, 1H), 3.53-3.62 (m, 2H), 3.68 (s, 3H), 3.71 (s, 3H), 4.85 (dt, J_t = 4.3 Hz, J_d = 7.9 Hz, 1H), 5.14 (dd, J_{vic} = 7.3 Hz, J_{gem} = 9.3 Hz, 1H), 7.09 (br d, J = 10.5 Hz, 1H), 7.12-7.19 (m, 5H), 7.37-7.73 (m, 2H), 7.76-7.83 (m, 2H);

^{13}C n.m.r. (300 MHz) δ 34.58, 35.77, 48.89, 52.04, 52.83, 55.07, 123.445, 126.84, 128.57, 128.89, 131.34, 134.24, 136.59, 167.76, 168.30, 170.79, 171.36.;

I.R. (NaCl, nujol) υ_{max} 3525, 3370, 3028, 2950, 2248, 1780, 1710, 1620, 1524, 1443, 1386, 1220, 1100, 1000, 918, 880, 800, 720, 700 cm^{-1};

Mass spectrum: *m/z* 438 (M^+, 19%), 438 (1), 437 (4), 436 (2), 370 (1), 292 (5), 291 (7), 278 (8), 277 (11), 251 (31), 250 (100), 249 (57), 233 (12), 232 (76), 160 (40), 132 (15), 131 (76), 130 (12);

Accurate mass: *m/z* 438.14411 (M^+), Calc. for $C_{23}H_{22}N_2O_7$ 438.14270;

Found; C, 63.11; H, 5.08; N, 6.45. Calc. for $C_{23}H_{22}N_2O_7$: C, 63.00; H, 5.06; N, 6.39; O, 25.54%.

2nd diastereomer to crystallise out of methanol;

m.p. 94-97° C.;

^{1}H n.m.r. (300 MHz) δ 2.94 (dd, J_{vic} = 4.4 Hz, J_{gem} = 17.3 Hz, 2H), 2.99 (dd, J_{vic} = 4.4 Hz, J_{gem} = 17.3 Hz, 2H), 3.44-3.53 (m, 2H), 3.63 (s, 3H), 3.73 (s, 3H), 4.88 (dt, J_t = 4.4 Hz, J_d = 7.9 Hz, 1H), 5.15 (dd, J_{vic} = 5.8 Hz, J_{NH} = 10.6 Hz, 1H), 7.08-7.18 (m, 6H), 7.66-7.72 (m, 2H), 7.74-7.79 (m, 2H);

^{13}C n.m.r. (300 MHz) δ 34.69, 35.68, 48.84, 52.00, 52.92, 55.21, 123.48, 126.94, 128.59, 128.93, 131.37, 134.27, 136.50, 167.69, 168.07, 170.78, 171.47;

I.R. (NaCl, nujol) υ_{max} 3525, 3370, 3028, 2950, 2248, 1780, 1710, 1620, 1524, 1443, 1386, 1220, 1100, 1000, 918, 880, 800, 720, 700 cm^{-1};

Mass spectrum: *m/z* 438 (M^+, 19%), 438 (1), 437 (4), 436 (2), 370 (1), 292 (5), 291 (7), 278 (8), 277 (11), 251 (31), 250 (100), 249 (57), 233 (12), 232 (76), 160 (40), 132 (15), 131 (76), 130 (12);

Accurate mass: *m/z* 438.14411 (M^+), Calc. for $C_{23}H_{22}N_2O_7$, 438.14270;

Found; C, 62.64; H, 5.14; N, 6.34. Calc. for $C_{23}H_{22}N_2O_7$: C, 63.00; H, 5.06; N, 6.39; O, 25.54%.

Reaction of *N*-Phthaloyl-*L*-phenylalanyl-*D,L*-aspartate Dimethyl Ester (75) with Nickel Peroxide.

N-Phthaloyl-*L*-phenylalanyl-*D,L*-aspartate dimethyl ester (**75**) (30 mg, 0.09 mmol) in benzene (5 ml) was treated with 4 mole equivalents of nickel peroxide and heated at reflux under nitrogen overnight. The reaction mixture was filtered, concentrated and chromatographed on silica (PF_{254}, chromatotron). Products eluted in the following order;

***N*-Phthaloyl-*L*-phenylalanyl-α,β-didehydroaspartate dimethyl ester (76)** isolated as a colourless oil.

Yield 5mg, 17%;

^{1}H n.m.r. (300 MHz) δ 3.61 (d, *J* = 8.7 Hz, 2H), 3.63 (s, 3H), 3.86 (s, 3H), 5.26 (t, *J* = 8.3 Hz, 1H), 5.58 (s, 1H), 7.19 (s, 5H), 7.67-7.72 (m, 2H), 7.76-7.80 (m, 2H), 10.75 (br s, 1H);

^{13}C n.m.r. (300 MHz) δ 33.96, 51.99, 53.16, 55.00, 103.29, 123.60, 127.03, 128.61, 128.86, 131.32, 134.3, 136.02, 142.84, 163.78, 166.77, 167.40, 167.96;

I.R. (Thin Film, $CHCL_3$) ν_{max} 3320, 3288, 3028, 2952, 2256, 1800, 1740, 1710, 1660, 1500, 1480, 1438, 1400, 1396, 1310, 1240, 1200, 1145, 1115, 1100, 1040, 980 cm^{-1}.

Mass spectrum: *m/z* 436 (M^+, 4%), 406 (0.6), 405 (2), 404 (1), 378 (5), 377 (14), 345 (2), 317 (1), 287 (4), 251 (21), 250 (100), 249 (67), 232 (28), 230 (8), 229 (12), 174 (6), 160 (4), 147 (6);

Accurate mass: *m/z* 436.12755 (M^+), Calc. for $C_{23}H_{20}N_2O_5$, 436.12705;

Found; C, 62.3; H, 4.67; N, 6.31. Calc. for $C_{14}H_{12}N_2O_5$: C, 63.3; H, 4.62; N, 6.42; O, 25.66%.

Unreacted starting material **75**

Yield 11mg, 37%;

^{1}H n.m.r. (300 MHz) δ 2.95 (qq, *J* = 4.6 Hz, *J* = 17.0 Hz, 2H), 3.52-3.59 (m, 2H), 3.62 (s, 1.5H), 3.66 (s, 1.5H), 3.70 (s, 1.5H), 3.71 (s, 1.5H), 4.84-4.91 (m, 1H), 5.12-5.18 (m, 1H), 7.10-7.19 (m, 6H), 7.65-7.71 (m, 2H), 7.73-7.79 (m, 2H).

***N*-Phthaloyl-*L*-phenylalanamide** (77) recrystallised from ethanol as colourless crystals.

Yield 4 mg, 6%;

m.p. 228-230° C (lit.,[116] 229-230° C);

^{1}H n.m.r. (300 MHz) δ 3.56 (d, J = 8.8 Hz, 2H), 5.13 (dd, J_{vic} = 7.7 Hz, J_{vic} = 9.1 Hz, 1H), 5.50 (br s, 1H), 6.12 (br s, 1H), 7.19 (m, 5H), 7.68-7.74 (m, 2H), 7.76-7.81 (m, 2H);

Mass spectrum. *m/z* 294 (M^+, 39%), 292 (4), 278 (7), 277 (15), 251 (22), 250 (100), 249 (72), 233 (17), 232 (78), 160 (33), 147 (78);

Accurate mass: *m/z* 294.10178 (M^+), Calc. for $C_{17}H_{14}N_2O_3$, 294.10044.

<u>*D,L*-Alanine Methyl Ester Hydrochloride.</u>

D,L-Alanine methyl ester methyl ester hydrochloride was prepared from *D,L*-alanine (Sigma) (10.0 g, 236 mmol) as described previously for glycine methyl ester hydrochloride. The crude product was recrystallised from dichloromethane/methanol as a white solid.

Yield 10.5 g, 90%;

<u>*N*-Phthaloylglycyl-*D,L*-alanine Methyl Ester (78)</u>

N-Phthaloylglycyl-*D,L*-alanine methyl ester (78) was prepared by coupling commercially available *N*-phthaloylglycine (10 g, 0.05 mol) to the hydrochloride salt of *D,L*-alanine methyl ester (6.8 g, 0.05 mol) (prepared as described above) using procedure described above for the preparation of *N*-benzoylglycylglycine methyl ester (55). The crude product was recrystallised from ethyl acetate/light petroleum to give *N*-phthaloylglycyl-*D,L*-alanine methyl ester (78) as a white solid.

Yield 3.4 g, 24 %;

m.p. 177-179° C;

^{1}H n.m.r. (300 MHz) δ 1.43 (d, *J* = 7.1 Hz, 3H), 3.75 (s, 3H), 4.36 (d, *J* = 16.1 Hz, 1H), 4.40 (d, *J* = 16.1 Hz, 1H), 4.60 (pentet, *J* = 7.1 Hz, 1H), 6.56 (br d, *J* = 6.7 Hz, 1H), 7.71-7.77 (m, 2H), 7.85-7.91 (m, 2H);

I.R. (NaCl, nujol) ν_{max} 3288, 3088, 1776, 1732, 1658, 1562, 1420, 1342, 1320, 1226, 1194, 1148, 1116, 1088, 1072, 1020, 996, 954, 854, 764, 740, 716 cm^{-1};

Found; C, 57.70; H, 4.74; N, 9.60. Calc. for $C_{14}H_{14}N_2O_5$: C, 57.93; H, 4.86; N, 9.65; O, 27.56%.

Reaction of *N*-Phthaloylglycyl-*D,L*-alanine Methyl Ester (78) with Nickel Peroxide.

N-Phthaloylglycyl-*D,L*-alanine methyl ester (**78**) (100 mg, 0.34 mmol) in benzene (20 ml) was treated with 8 mole equivalents of nickel peroxide at reflux under nitrogen for 5 hours. The reaction mixture was filtered, concentrated and chromatographed on silica (PF_{254}, chromatotron). Products eluted in the following order;

***N*-Phthaloylglycyl-α,β-dehydroalanine methyl ester (79)** recrystallised from ethyl acetate/light petroleum as colourless needles.

Yield 7 mg, 7%;

m.p. 229-231° C;

^{1}H n.m.r. (300 MHz) δ 3.86 (s, 3H), 4.48 (s, 2H), 5.92 (d, *J* = 1.2 Hz, 1H), 6.58 (s, 1H), 7.74-7.79 (m, 2H), 7.88-7.93 (m, 2H), 8.05 (br s, 1H);

^{13}C n.m.r. (300 MHz) δ 41.32, 53.17, 109.80, 123.75, 130.37, 131.92, 134.35, 164.31, 164.58, 167.68;

I.R. (Thin Film, $CDCL_3$) ν_{max} 3425, 1776, 1724, 1620, 1520, 1422, 1394 cm^{-1};

Mass spectrum. *m/z* 288 (M^+, 6%), 188 (10), 160 (100), 146 (3), 132 (8), 105 (25), 77 (22);

Accurate mass: *m/z* 288.074621 (M^+), Calc. for $C_{14}H_{12}N_2O_5$, 288.07379;

Found; C, 60.06; H, 4.98; N, 8.75. Calc. for $C_{14}H_{12}N_2O_5$: C, 60.75; H, 5.10; N, 8.86; O, 25.29%.

Unreacted starting material **78**

Yield 23 mg, 23%;

m.p. 176-178° C;

^{1}H n.m.r. (300 MHz) δ 1.42 (d, *J* = 7.1 Hz, 3H), 3.75 (s, 3H), 4.37 (d, *J* = 16.1 Hz, 1H), 4.44 (d, *J* = 16.1 Hz, 1H), 4.61 (pentet, *J* = 7.2 Hz, 1H), 6.59 (br d, *J* = 7.0 Hz, 1H), 7.72-7.77 (m, 2H), 7.85-7.90 (m, 2H);

Reaction of *N*-Phthaloylglycyl-*D,L*-aspartate Dimethyl Ester (80) with Nickel Peroxide.

N-Phthaloylglycyl-*D,L*-aspartate dimethyl ester (**80**) (30 mg, 0.09 mmol) in benzene (5 ml) was treated with 4 mole equivalents of nickel peroxide and heated at reflux under nitrogen overnight. The reaction mixture was filtered, concentrated and chromatographed on silica (PF_{254}, chromatotron). Products eluted in the following order;

***N*-Phthaloylglycyl-α,β-dehydroaspartate dimethyl ester (81)** recrystallised from ethyl acetate/light petroleum as colourless needles.

Yield 16 mg, 54%;

m.p. 179-181° C;

^{1}H n.m.r. (300 MHz) δ 3.76 (s, 3H), 3.82 (s, 3H), 4.53 (s, 2H), 5.60 (s, 1H), 7.74-7.78 (m, 2H), 7.88-7.92 (m, 2H), 10.54 (br s, 1H);

^{13}C n.m.r. (300 MHz) δ 29.72, 40.71, 52.10, 53.15, 102.97, 123.77, 131.94, 134.35, 142.78, 163.62, 164.40, 167.33, 168.18;

I.R. (Thin Film, $CHCL_3$) ν_{max} 3288, 2952, 2260, 1788, 1728, 1694, 1640, 1438, 1420, 1396, 1290 cm^{-1};

Mass spectrum: *m/z* 346 (M^+, 5%), 315 (9), 288 (72), 256 (5), 186 (24), 161 (39),160 (100);

Accurate mass: *m/z* 346.07912 (M^+),Calc. for $C_{16}H_{14}N_2O_7$, 346.08010.

The ^1H n.m.r. spectral characteristics of **81** were found to be consistent with those previously reported.[97]

Unreacted starting material **80**

Yield 11mg, 36%.

m.p. 175-177° C;

^1H n.m.r. (300 MHz) δ 2.95 (dd, J_{vic} = 4.5 Hz, J_{gem} = 17.4 Hz, 1H), 3.00 (dd, J_{vic} = 4.5 Hz, J_{gem} = 17.4 Hz, 1H), 3.70 (s, 3H), 3.77 (s, 3H), 4.45 (d, J = 16.1 Hz, 1H), 4.85 (dt, J_t = 4.3 Hz, J_d = 7.8 Hz, 1H), 6.81 (br d, J = 7.6 Hz, 1H), 7.71-7.77 (m, 2H), 7.85-7.92 (m, 2H);

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