Radical Functionalisation of Amino Acid Derivatives

A Thesis

Submitted Towards the

Degree of

Doctor of Philosophy

by

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STATEMENT

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other institution, and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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ABSTRACT

Carbamate derivatives of glycine, alanine and serine have been shown to react by *N*-methylation, without racemisation, on treatment with *tert*-butyl perbenzoate in the presence of cupric octanoate. The selective reaction of *N-tert*-butoxycarbonylglycine methyl ester, in preference to the corresponding alanine and valine derivatives, indicates that the relative reactivity of these substrates is determined by the comparative ease of their complexation to the copper. *N*-Methylation is thought to proceed either through electron transfer from the substrate to cupric ion, followed by combination with methyl radical, or through complexation between the substrate and a methyl-copper(III) species.

The relative reactivity of *N*-protected glycine methyl esters exhibited in hydrogen transfer reactions involving *tert*-butyl perbenzoate, nickel peroxide and *N*-bromosuccinimide has been shown to be markedly affected by the electronic nature of the amine protecting group. Relative rate constants obtained from competitive reactions between *N*-protected glycine methyl esters and *N*-bromosuccinimide indicate a direct correlation between the relative rates of reaction and the pK_a values for the carboxylic acids corresponding to the *N*-acyl substituents of the glycine derivatives. The relative reactivity of the glycine derivatives was attributed to the ability of the *N*-acyl substituents to donate electrons towards the stabilisation of an electron deficient site, developed in the transition state, at the site of hydrogen abstraction. This relative reactivity was reflected in reactions of hippuric acid and *N*-pentafluorobenzoylglycine with peptidylglycine α–amidating monooxygenase (PAM), and gave a preliminary indication that the *N*-acyl substituent of *N*-pentafluorobenzoylglycine has an inhibitory effect on the reaction catalysed by this enzyme.

Relative rates of reaction of modified substrates of *(S)*-phenylalanine ammonia-lyase (PAL) were found not to reflect the relative acidities of the corresponding β–protons. This suggests that β–hydrogen abstraction is not the rate determining step in the reaction catalysed by PAL.

The *(2R,3S)* and *(2R,3R)* stereoisomers of 2,3-dideuteriophenylalanine have been prepared and reacted with PAL. Both gave the product *trans*-cinnamic acid with no deuterium content at the 2-position and approximately 25% deuterium content at the 3-position. Treatment of unlabelled *(R)*-phenylalanine with PAL in deuteriated buffer gave *trans*-cinnamic acid with approximately 25% deuterium content at the 2-position and 50% deuterium content at the 3-position. These results indicate that a minor pathway for reaction of *(R)*-phenylalanine with the enzyme may be attributed, at least in part, to proton exchange. It was therefore not possible to obtain accurate data to distinguish between isomerisation to *(S)*-phenylalanine, before elimination, and synperiplanar elimination as the minor reaction pathway for *(R)*-phenylalanine, both previously suggested.

INTRODUCTION

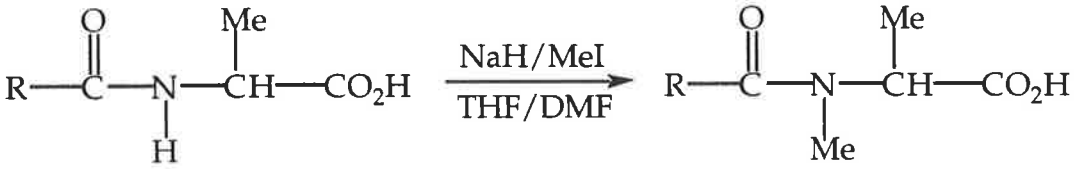
Today, more than 500 naturally occurring amino acids have been identified, of which approximately 240 occur free in nature.[1] They occur in nature as the fundamental constituents of all proteins, peptides and many other natural products, and provide the raw materials from which a large number of biologically important primary and secondary metabolites are constructed.[2] Given their widespread natural occurrence and physiological activity, the synthesis of biologically active amino acids and peptides is of continuing interest, especially in the pharmaceutical,[3] agricultural,[4] and food industries.[5] In addition, their production has opened routes to the synthesis of unnatural analogues which have played a significant role towards the understanding of biological systems. Their study has led to the synthesis of amino acids and derivatives having enzyme inhibiting, anti-metabolite, protease resistant and unique conformation inducing properties.[6] More significant is the direct impact amino acid derivatives have on society. For example, unusual amino acids such as those incorporating the β–amino hydroxy unit are present in biologically active compounds such as Taxol,[7] which is well known for its potent anti-cancer properties, and various hydroxyethylene dipeptide isosteres[8] have been found to be active against the human immunodeficiency virus type-1 (HIV-1), the virus responsible for AIDS.[9]

Another group of unusual amino acids are the *N*-alkylamino acids.[10] Specifically, *N*-methylamino acids are constituents of several naturally occurring peptide antibiotics, most often derived from mono-amino, mono-carboxylic acids.[11] Examples of these antibiotics include the Enniatins,[12] which incorporate *(S)*-*N*-methylisoleucine and *(S)*-*N*-methylvaline, and the Actinomycins,[13] which incorporate sarcosine, *(S)*-*N*-methylalanine, *(S)*-*N*-methylvaline and *(S)*-*N*-methylisoleucine. *N*-Methylamino acids also occur frequently in biological material, such as *(S)*-*N*-methyltryptophane found in the seeds of *Abrus precatorius*,[14] and *(R)*-*N*-methyltyrosine found in cabbage tree bark.[15]

N-Methylated analogues of biologically active peptides are reported to exhibit enhanced potencies or prolonged activities.[16] The interaction between enzyme receptor sites and substrate peptides is modified by incorporation of *N*-methylamino acid residues in the substrate, as a consequence of the loss of hydrogen bonding on *N*-methylation.[17] Thus, the study of *N*-methylamino acids could lead to the production of analogues with higher potencies and prolonged activities.

The spatial structure of peptide systems containing *N*-methylamino acids is also of interest to organic chemists.[18] Such structural details play an important part in the biological activity of many peptides and proteins. A knowledge of the conformational states of simple peptides has served as a key to the spatial arrangement of more complex, naturally occurring proteins. This knowledge has proved useful in understanding the differences in the biological activities of the more complex, naturally occurring proteins.[19] As a result of their structural significance in biological processes, and their occurrence in a series of antibiotics, intense interest has focussed on the synthesis of *N*-methylamino acids since these compounds are sometimes unavailable from natural sources in quantities sufficient for thorough structural and biological testing.

N-Methylamino acid analogues of biologically active peptides are usually prepared by peptide synthesis, using *N*-methylamino acid derivatives in addition to normal protected amino acids as building blocks. In a previously reported procedure for the synthesis of *N*-methylamino acid derivatives, Coggins and Benoiton[17] showed that *N*-acetyl, *N*-benzoyl, and *N*-carbobenzoxy derivatives of aliphatic amino acids gave the corresponding optically active *N*-methylamino acid methyl esters, on treatment with sodium hydride and methyl iodide. The amino acid derivatives (1a), (1b) and (1c) gave the *N*-methylated products (2a), (2b) and (2c) in yields of 57%, 77% and 65%, respectively (Scheme 1). However, with many amino acid derivatives, *N*-methylation according to this procedure is complicated by racemisation and alkylation at the α–position.[20] Consequently, *N*-methylation with methyl iodide and silver oxide was developed as a better



(1) (2)

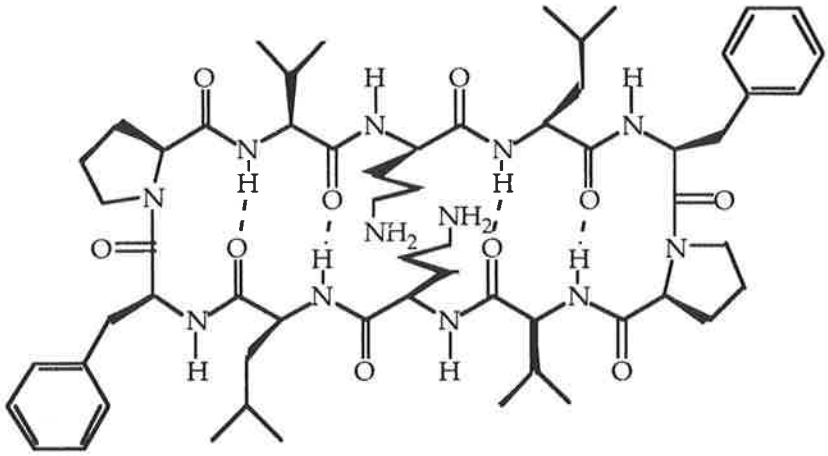
a) R = Ph
b) R = Me
c) R = $PhCH_2O$

Scheme 1

synthetic route to *N*-methylamino acids,[21] but limitations to this procedure exist. Other functional groups which may be present in the amino acid derivative are also susceptible to methylation by this procedure. In addition, competing processes may occur, as in the elimination reaction of the serine derivative shown in Scheme 2, to give only an unsaturated amino acid product. There have also been problems associated with the work up procedure, specifically in the removal of the silver oxide residue.[20]

Scheme 2

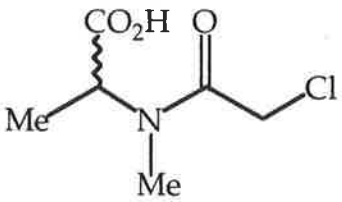
The modification of various peptides has been possible by selective methylation on the amido nitrogen of the parent peptide molecule.[22] For example, *N*-methylation of solvent exposed amide linkages[23] and the quaternisation of primary amino groups have been successfully applied to the preparation of analogues of gramicidin *S* (3), an antibiotic cyclic decapeptide.[24] Here, the rate of *N*-methylation of amino nitrogens which are exposed to solvent



(3)

is much higher than that of intramolecularly hydrogen bonded amino nitrogens. This selective methylation using a solution of methyl iodide and silver oxide in dimethylformamide was found to be useful for synthesising new peptide analogues of this type.[24]

Another possible route to optically active *N*-alkylamino acids is through the enzyme-catalysed enantioselective hydrolysis of appropriate racemic *N*-acylated *N*-alkylamino acids. For example, in order to prepare enantiomerically pure



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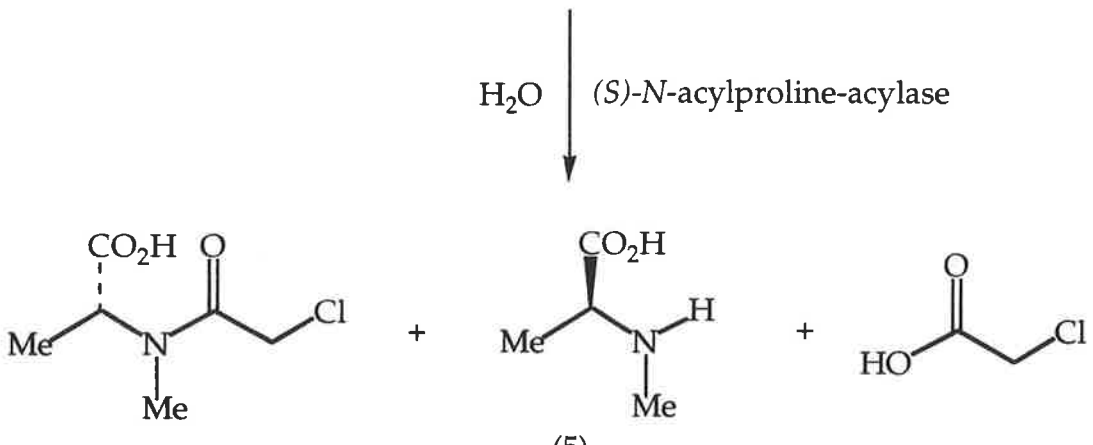
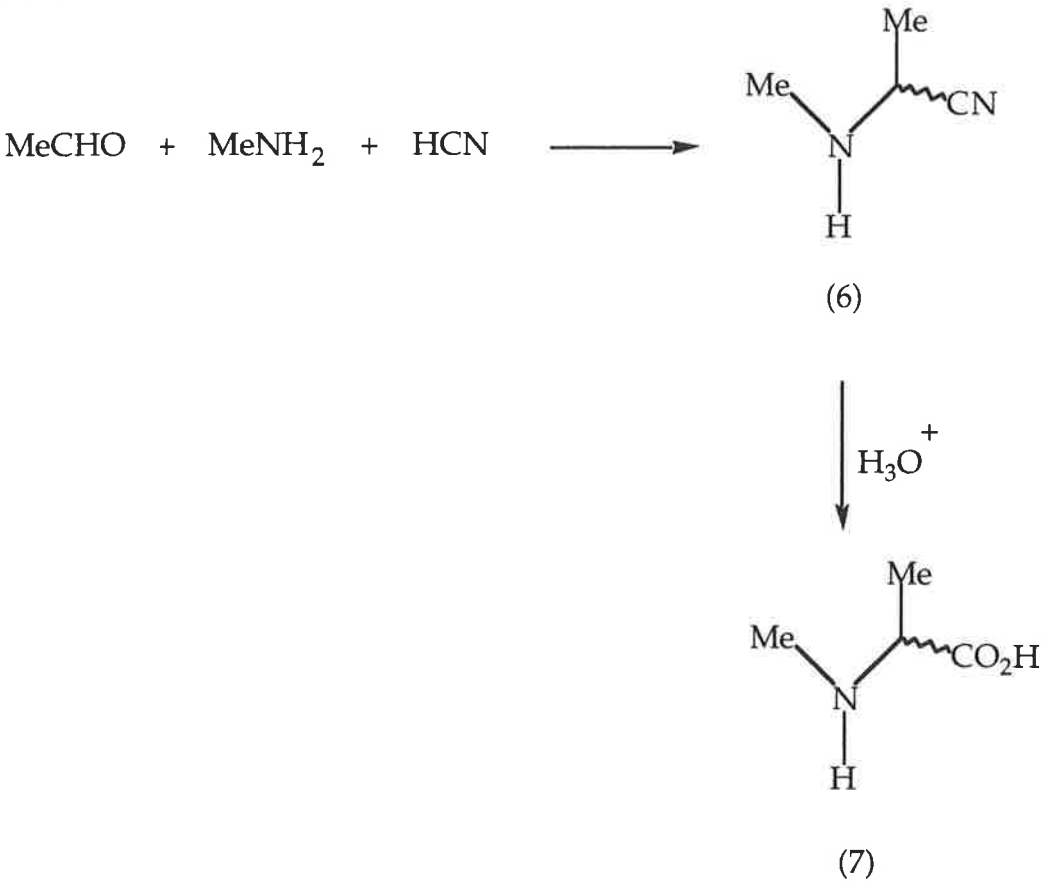


(5)

Scheme 3

(S)-*N*-methylalanine (5), racemic *N*-chloroacetyl-*N*-methylalanine (4) was subjected to cleavage by *(S)*-*N*-acylproline-acylase (Scheme 3).[25] The precursor (4) can be prepared as a racemate from (7) *via* the *N*-alkylaminonitrile (6) (Scheme 4). However, with increasing length of the *N*-alkyl residue, and of the alkyl residue of the amino acid side chain, the activity of this enzyme decreases. Alkyl substituents in the α–position of a chain length of more than two carbons, or branched residues, cause a total loss of activity. In these cases the substituents apparently block access to the active site of *(S)*-*N*-acylproline-acylase. Furthermore, the substrate spectrum of this enzyme is restricted,[25] and like any resolution, a disadvantage of the method is that the maximum chemical yield of any one enantiomer is 50%.

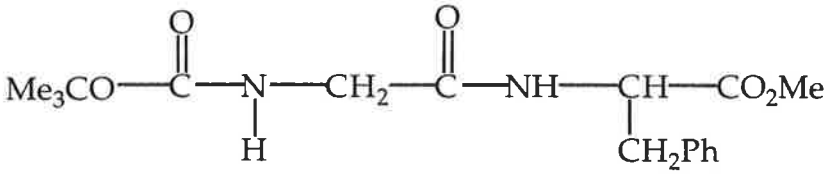


Scheme 4

Recently, Dorow and Gingrich[26] developed a method for producing *N*-methyl-α–amino acids from the readily accessible α–azido acids by reductive alkylation using dimethylbromoborane. Acids, amides and remotely placed

ethers were found to be stable under the reaction conditions, while esters were cleaved. The reaction, facilitated by complexation of the borane to the carboxyl group, affords the *N*-methyl-α–amino acid derivatives as the hydrogen bromide salts, and with very high enantiomeric excess in some cases. This method is therefore useful for the production of various optically active *N*-alkyl derivatives.

A few years ago, a novel synthesis of the *N*-methylamino acid derivative (9) was observed by Peters.[27] When the dipeptide derivative (8) was treated with *tert*-butyl perbenzoate and cupric octanoate, the *N*-methylated product (9) was isolated from the reaction mixture in 41% yield.[27]



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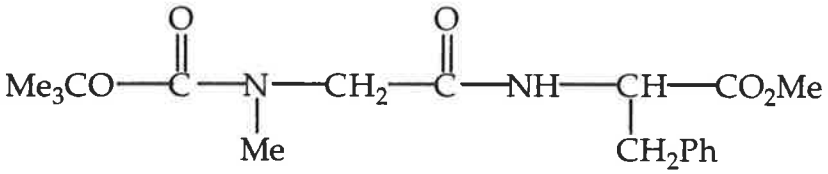
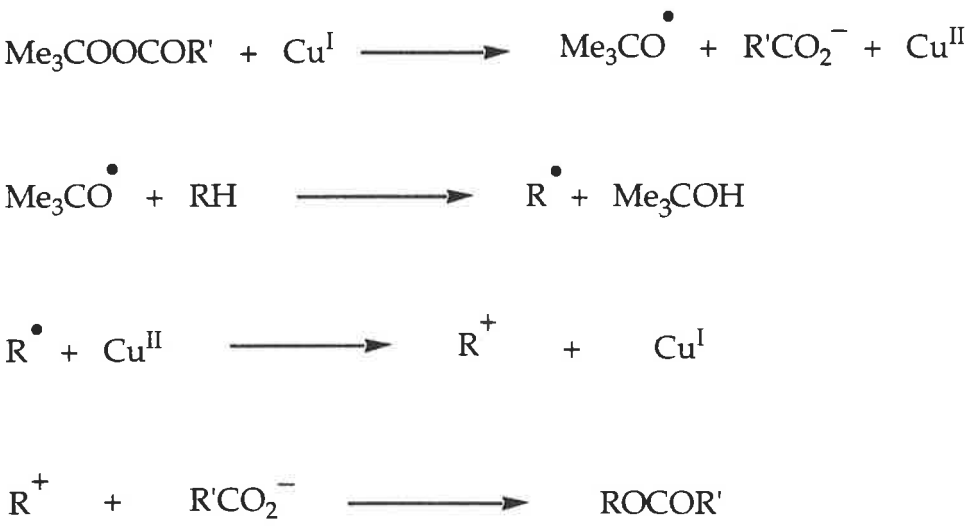


(9)

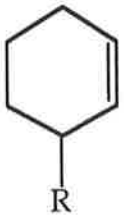
Interestingly, formation of this *N*-methylated product (9) is not consistent with the general reaction pathway. Copper-catalysed reactions of peresters with organic substrates are usually used for direct substitution of the acyloxy functional group in place of hydrogen attached to carbon.[28] The generally accepted mechanism for this process is as shown in Scheme 5.[29] Electron transfer from

cuprous ion to the perester affords cupric ion, the carboxylate and *tert*-butoxy radical. Subsequent hydrogen transfer from the substrate to *tert*-butoxy radical affords the substrate radical, the stability of which determines the regioselectivity of the hydrogen transfer. The substrate radical reacts by electron transfer to cupric ion and, finally, the carboxylate is incorporated at the site of hydrogen abstraction.



Scheme 5

Examples of the acyloxylation procedure include reaction of olefinic hydrocarbons to produce the allyl-substituted derivatives. Cyclohexene (10a) underwent acyloxylation on treatment with *tert*-butyl peracetate and *tert*-butyl perbenzoate, to give the corresponding esters (10b) and (10c), in yields of 95% and 71%, respectively.[30] Functionalisation of the β–lactams (11a) and (12a), each at the *C*-4 position, has also been reported to afford the products (11b,c) and (12b,c), respectively.[30] The β–lactams (11a) and (12a) react *via* the corresponding radicals (13) and (14) because of the stabilising effect of the adjacent amide nitrogen. Consistent with this general reaction pathway, Peters[27] reported that treatment of *N*-benzoylglycine methyl ester (15) with *tert*-butyl perbenzoate in the presence of cupric octanoate, afforded the corresponding α–benzoyloxyglycine derivative (16), in 67% yield (Scheme 6).



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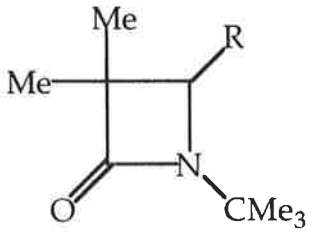
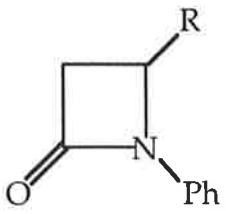
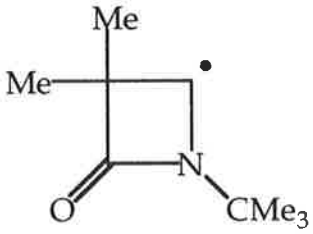


(11)



(12)

a) R = H
b) R = OCOMe
c) R = OCOPh



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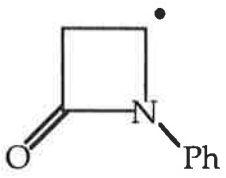
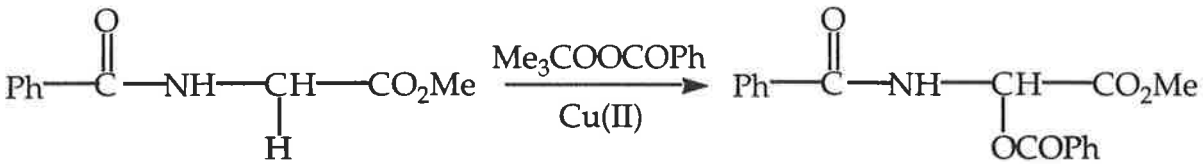


(14)

It is clear that the reaction of the dipeptide (8) to afford the *N*-methyl derivative (9) is in direct contrast to the reactions of the substrates (10a-12a) and (15) to give the corresponding acyloxy-substituted derivatives (10b-12b) and (16). Due to this apparent anomaly, the mechanism, scope and generality of this *N*-methylation procedure was investigated, the results of which are presented in Chapter 1 of the Results and Discussion of this thesis.



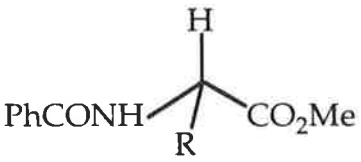
(15) (16)

Scheme 6

Furthermore, due to the immense interest in the production of optically active *N*-methylamino acids generated by the need for these units in biologically active compounds, the potential of this *N*-methylation reaction as a convenient synthetic pathway for the formation of *N*-methylamino acids was examined.

One approach to the synthesis of unnatural α–amino acids involves the elaboration of proteinogenic α–amino acids through side chain modification. Complementary to the side chain modification of amino acids through the manipulation of pre-existing functionality, is the regioselective free radical modification of proteinogenic amino acids. In radical reactions where a hydrogen is replaced by a functional group, the regioselectivity is determined in the hydrogen transfer step, and is affected by a number of factors.[31] These include radical stability, steric effects and polar effects.

Radical stability is important when there is extensive carbon-hydrogen bond breaking, and consequently significant radical character in the transition state. However, predicting the relative stability of radical intermediates may not always be easy. By way of illustration, consider the preferential reactivity of glycine residues in free radical reactions of proteins, peptides, and other amino acid derivatives. This result has been attributed to selective hydrogen abstraction from the α–carbon of the glycine moieties.[32] However, this selectivity is contrary to the expectation that tertiary radicals should be formed in preference to secondary ones.[33] Treatment of the derivatives of glycine (17a), alanine (18a) and valine (19a) with *N*-bromosuccinimide (NBS) afforded the brominated derivatives (17b), (18b) and (19b), respectively. By examining the relative rates of reaction of these amino acid derivatives with NBS, Easton and Hay[34] showed that the secondary radical (21) is marginally more stable than the tertiary radical (22), and radicals (20) and (21) are both more stable than the radical (22). The peculiar stability of the radical (20) has been attributed to a particularly favourable geometry. That is, the captodative[35] radicals (20), (21) and (22) are stabilised by overlap of their semi-occupied p-orbitals with the π–orbitals of the amido and methoxycarbonyl substituents. Maximum overlap occurs when the radical



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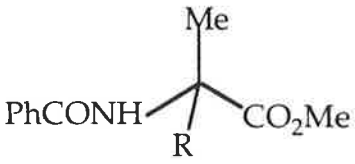

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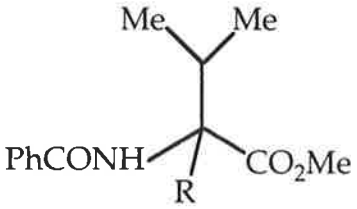
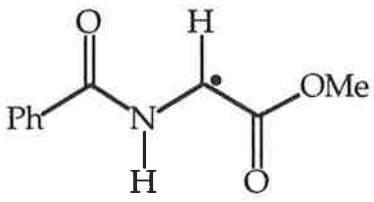


(19)

a) R = H
b) R = Br

intermediates (20), (21) and (22) adopt planar conformations. Non-bonding interactions associated with planar conformations of the intermediate radical (21), and to a greater extent in the radical (22), results in these species being less stable than the intermediate (20) (Fig. 1). These destabilising influences outweigh



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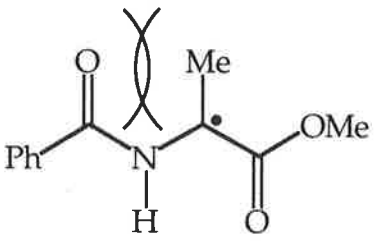

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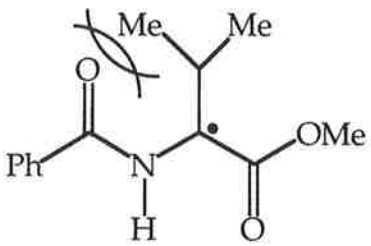
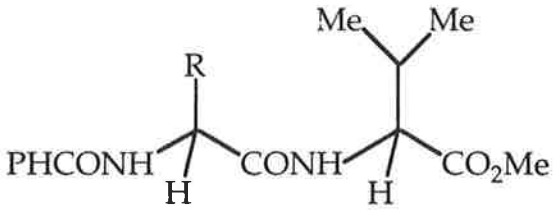


(22)

Fig. 1 Non-bonding interactions associated with planar conformations of the radicals (20), (21) and (22).

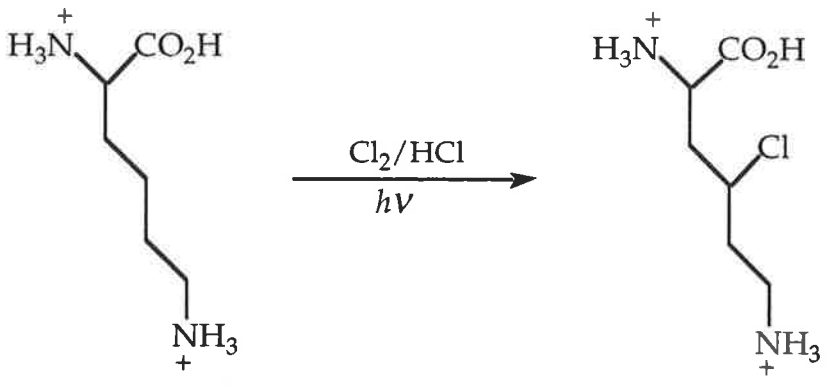
the normal thermodynamic preference for the production of tertiary radicals. Accordingly, treatment of the dipeptide (23a) gives the bromide (23b) in high yield.[34]



(23)

a) R = H
b) R = Br

Polar effects have been used to explain the regioselectivity observed in several radical processes in biological systems. Polar effects refer to the activating or deactivating effects brought on by partial positive or negative charge development in the transition state of an atom transfer reaction.[31,36] The most stable transition state is one in which the developing charge is extensively delocalised. For example, polar effects were used to explain the regioselectivity observed by Kollonitsch[37] in the chlorination of lysine (24) (Scheme 7).



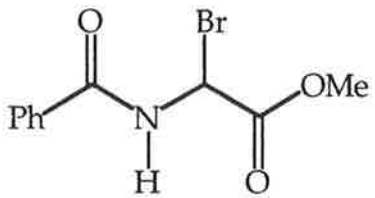
(24) (25)

Scheme 7

Ultraviolet irradiation of a concentrated hydrochloric acid solution of lysine (24), with the concurrent introduction of chlorine gas, led to the formation of γ–chlorolysine (25). Hydrogen abstraction by the electrophilic chlorine atom creates a partial positive charge in the transition state, at the site of hydrogen abstraction. The α– and ε–aminium groups and the carboxyl group are inductively electron withdrawing, and therefore deactivate adjacent positions to attack by the electrophilic chlorine radical. Consequently, reaction occurs at the γ–carbon, rather than at the α– or ε–carbons.

The widespread application of radical reactions to the functionalisation of amino acids is limited by the virtual insolubility of amino acids in the organic solvents commonly used in such processes. This problem can be overcome by suitable protection of the amino acids, however such protection has been shown to markedly affect the regioselectivity of radical reactions.

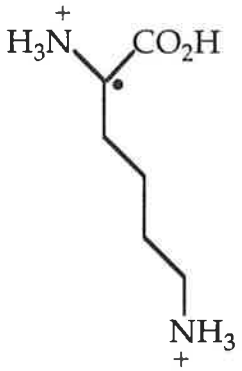
By way of illustration, consider hydrogen transfer reactions of *N*-acyl-α–amino acid derivatives. These derivatives generally favour formation of the corresponding α–centred captodative radicals.[34,38-40] Consistent with this general trend, reactions of *N*-acyl-α–amino acid derivatives with NBS proceed through α–carbon centred radical intermediates. For example, treatment of *N*-benzoylglycine methyl ester (15) with NBS affords the α–brominated derivative (26), *via* the stable radical intermediate (20).[41] In contrast, radical



(26)

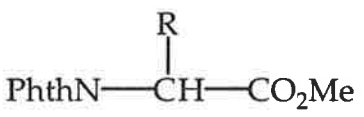
functionalisation of the unprotected amino acid (24) does not occur at the α–position.[37] In combination with the polar effect discussed above in the chlorination of lysine (24), the lack of reaction occurring at the α–position may

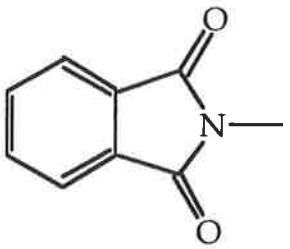
also be attributed to the instability of the α–carbon centred radical (27). The electrons on the amide nitrogen of the *N*-acyl derivative (15) are readily available to contribute towards the stabilisation of the α–carbon centred radical intermediate (20), through a dative effect. However, the electrons on the α–amino nitrogen of the radical intermediate (27) are not available for delocalisation, and cannot stabilise the α–carbon centered radical (27) in the same way. Kollonitsch,[37] therefore, utilised the instability of the radical intermediate (27), as well as polar effects to affect side chain functionalisation of lysine (24).



(27)

Studies have shown that *N*-phthaloyl-protected α–amino acid derivatives are also deactivated towards hydrogen abstraction at the α–position, due to steric and electronic factors. For example, reaction of *N*-phthaloylglycine methyl ester (28a) with NBS to afford the brominated derivative (28b) is considerably less





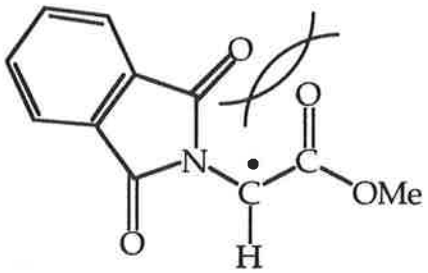
= Phth

(28)

a) R = H
b) R = Br

efficient than reaction of the *N*-acylglycine methyl ester (15) with NBS.[41] This may be attributed to the relative stability and ease of formation of the corresponding α–carbon centred radicals (20) and (29). Whereas acyl-amino substituted radicals are stabilised by resonance, there is less delocalisation of unpaired spin density to the α–carbon by a phthalimido substituent.[42] This is partly a result of steric effects which arise from interactions between the methoxycarbonyl and phthalimido substituents which prevent the intermediate radical (29) from adopting planar conformations in which there is the greatest delocalisation of the unpaired spin density (Fig. 2). Maximum delocalisation occurs when there is maximum overlap of the semi-occupied p-orbital with the electrons on the nitrogen and the π–system of the *N*-protecting group. Steric interactions between the methoxycarbonyl and phthalimido substituents in the radical (29) are greater than those between the methoxycarbonyl and benzamido substituents in the radical intermediate (20).[41] In addition, the *N*-phthaloyl protecting group is deactivating towards hydrogen abstraction at the α–centre because of its greater electron demand which is, presumably, associated with its additional inductively electron withdrawing carbonyl group. As a result, the *N*-benzoyl group has a greater capacity to delocalise electrons towards the stabilisation of the intermediate radical (20).

This deactivating effect has also been used to affect the bromination of *N*-phthaloylvaline methyl ester (30a) and *N*-phthaloylphenylalanine methyl ester

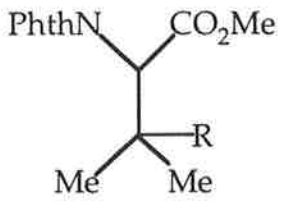


PhthN—CH—CO_2Me

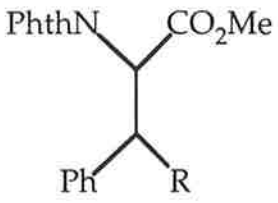
(29)

Fig. 2. Non-bonding interactions associated with planar conformations of the radical (29).

(31a). Treatment of the *N*-phthaloyl-protected derivatives (30a) and (31a) with NBS results in regiospecific formation of the β–bromides (30b) and (31b), respectively.[42] Further, hydrogen abstraction from the tertiary and benzylic centres of the *N*-phthaloyl derivatives of leucine (32a) and homophenylalanine (33a), respectively, takes place in preference to hydrogen abstraction at the α–centres. Subsequent bromine transfer to the γ–centres has been shown to yield the corresponding γ–bromides (32b) and (33b).[43]

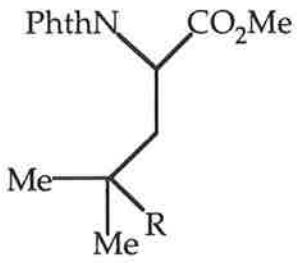


(30)

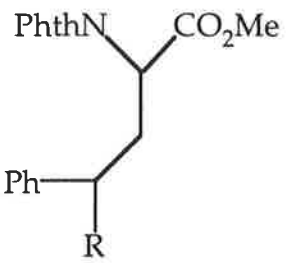


(31)

a) R = H
b) R = Br



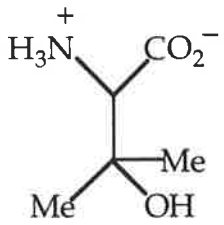
(32)



(33)

It is evident from the free radical reactions of the *N*-phthaloyl-protected amino acid derivatives (30a), (31a), (32a) and (33a), that side chain functionalisation of *N*-phthaloyl substituted amino acids takes place *via* the most stable side chain radical. This type of regioselectivity is also observed in biological systems. For example, β–hydroxyvaline (34) is a naturally occurring amino acid

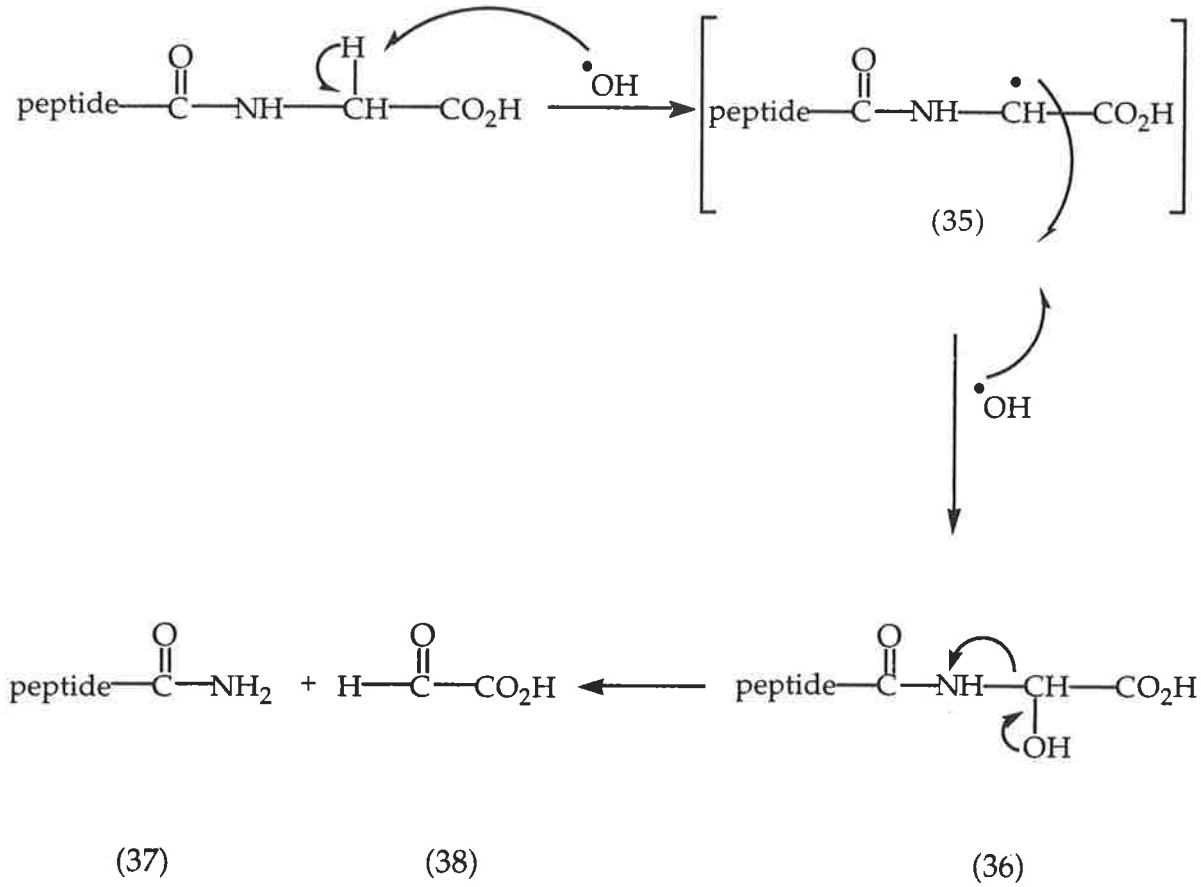
thought to be a result of the enzymatic oxidation of valine. Evidence exists for the oxidation being radical in nature and it is possible that the observed regioselectivity is a reflection of polar effects.[44]



(34)

The study of biochemical reactions can provide information as to the possible use of a particular enzyme in synthetic organic chemistry. Enzymes are becoming increasingly popular in this field[45] because they can catalyse reactions under mild conditions and functionalise non-activated positions in the substrate with a high degree of regioselectivity. To obtain a proper understanding of the mechanism of a biochemical transformation, a precise knowledge of the chemistry of each discrete step is needed. With the aid of analogues of natural amino acids, it has become possible to follow their biochemical fate and consequently gain insight into reaction mechanisms. The elucidation of such enzyme mechanisms can provide information that may allow the design of potential inhibitors and eventually the production of orally active pharmaceuticals. The study of model chemical systems has also been shown to be a useful approach to the study of enzyme mechanisms.[46-48] For example, Kizer and co-workers[47] have proposed the following mechanism to explain the oxidation of histidine containing peptides to peptide amides in the presence of copper and ascorbate. Initially, copper ions in solution catalyse the reduction of molecular oxygen by ascorbate to produce hydrogen peroxide. Cupric ion coordinates to the peptide substrate and is reduced *in situ* by ascorbate to form cuprous ion. The reduced, coordinated copper reacts with hydrogen peroxide in a Fenton-type reaction,[49] producing hydroxy radicals *in situ*. The hydroxy radicals

abstract a hydrogen from the α–position of the substrate generating a carbon-centred radical intermediate (35) (Scheme 8). The intermediate (35) then reacts with either a second hydroxy radical or molecular oxygen to afford the unstable α–hydroxy intermediate (36), which undergoes rapid hydrolysis to produce the corresponding amide product (37), and glyoxylate (38). Many characteristics of this non-enzymatic amidating system have been shown to mimic the features displayed in the reaction catalysed by the enzyme, peptidylglycine α–amidating monooxygenase (PAM).[47]



Scheme 8

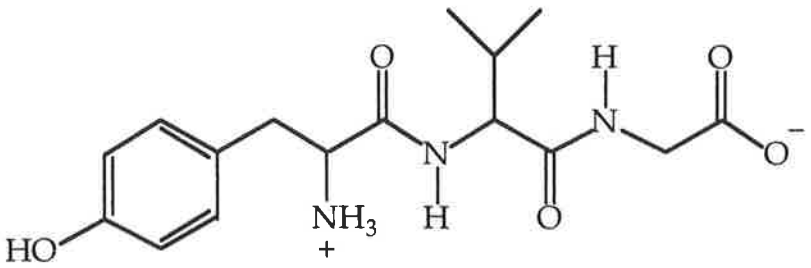
The studies discussed above show that the regioselectivity of radical reactions of amino acid derivatives is markedly affected by the nature of the amine protecting group. This was exemplified in the radical bromination reactions of the *N*-benzoylglycine derivative (15) and the *N*-phthaloyl-protected

amino acid derivatives (30a)-(33a) with NBS, and in the chlorination of the unprotected amino acid (24). The selectivity of radical functionalisation observed in these systems was attributed, in part, to the electron density on the corresponding α–nitrogen. As explained previously, the α–carbon centred radical intermediates generated in reactions of *N*-acyl-α–amino acid derivatives with NBS are stabilised by a dative effect, as the electrons on the nitrogen are available for delocalisation. The ability of the nitrogen to datively stabilise α–carbon centred radical intermediates generated in reactions of *N*-phthaloyl-protected amino acid derivatives is diminished, partly because the electrons on the nitrogen are delocalised over two carbonyl groups, and therefore cannot contribute towards the stabilisation of the radical to the same extent. An extreme example was illustrated in the chlorination of the unprotected amino acid (24), where the availability of electrons on the α–amino nitrogen of lysine (24) is diminished, such that no dative stabilisation is possible, and so functionalisation occurs at the γ–position in preference to the α–position. Given the importance of the dative effect of the α–nitrogen on the stabilisation of α–carbon centred radical intermediates, it was thought that this effect could be exploited in reactions involving the PAM enzyme, which are thought to proceed through the intermediacy of an α–carbon centred radical.

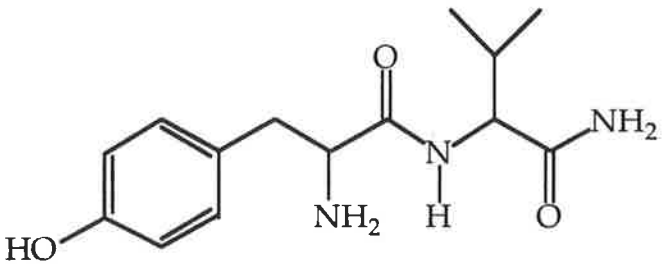
A striking feature, necessary for the bioactivity of approximately 50% of known peptide neurohormones, is the presence of an α–amide group on the carboxy terminal end of the polypeptide chain.[50-52] In most cases, the presence of this structure is essential for biological activity. The study into the mechanism of the PAM-catalysed oxidative cleavage of carboxy-terminal glycine extended precursors, the process responsible for the bioactivation of so many peptide hormones, has therefore been of great interest.[53]

The PAM enzyme was first isolated from the porcine pituitary by Bradbury and co-workers in 1982.[54] They demonstrated that it was capable of cleaving the two terminal carbons of the synthetic tripeptide, *(R)*-tyrosyl-*(S)*-valylglycine (39) to form the dipeptide amide, *(R)*-tyrosyl-*(S)*-valinamide (40). Subsequent

experiments[55] with labelled glycine residues showed that the terminal amide nitrogen of (40) originates from (39) and indicated that the other product was glyoxylate (38) (Scheme 9). It has subsequently been shown that PAM is bi-functional, and for maximum activity requires bound copper ions, molecular oxygen and ascorbate.[54,56]



(39)



+ (38)

(40)

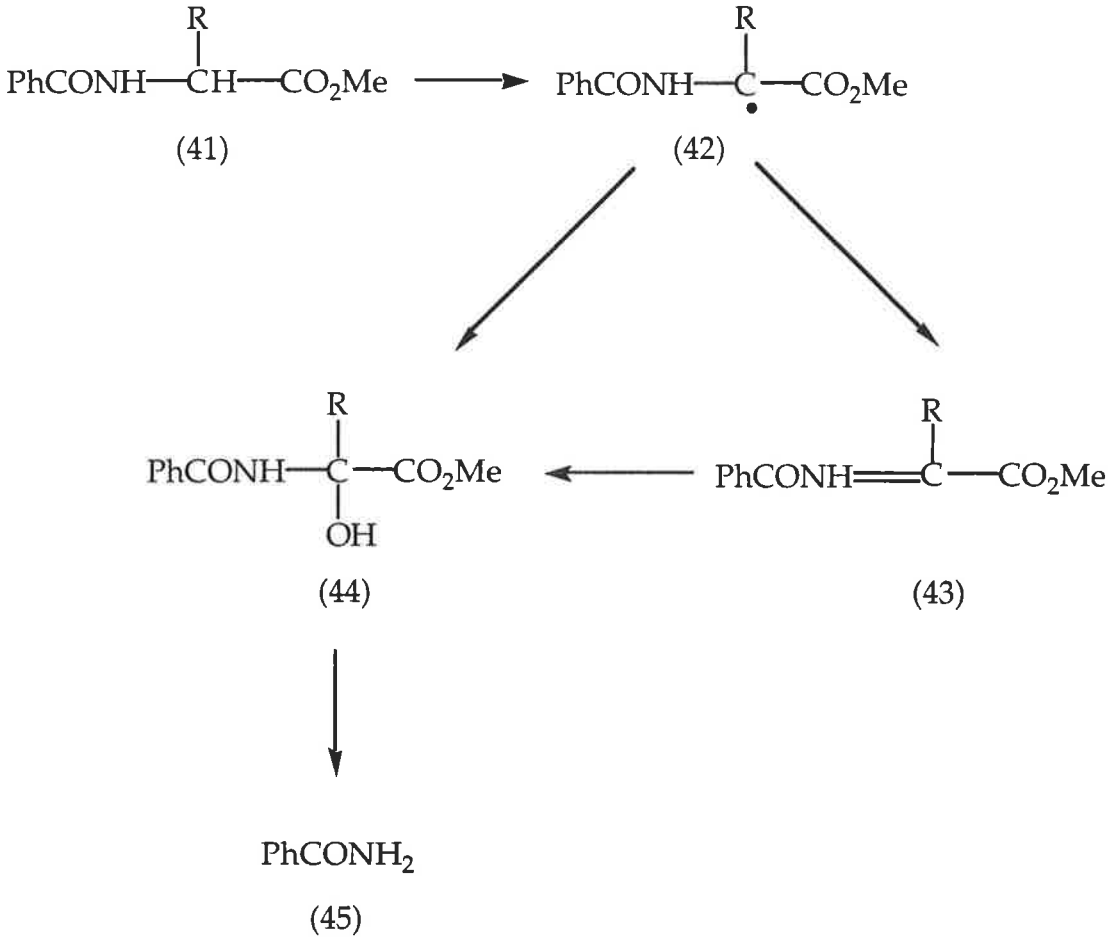
Scheme 9

Ramer and co-workers[57] investigated the stereochemistry of the PAM-catalysed oxidation of *(R)*-tyrosyl-*(S)*-valylglycine (39) to form the dipeptide amide (40). Using stereospecifically α–tritium-labelled glycine residues they showed that the enzyme oxidises the substrate (39) with specific loss of the *pro-S* hydrogen of

the glycyl residue, the *pro-R* hydrogen being retained in the glyoxylate (38). This is consistent with the ability of PAM to convert peptides terminating in *(R)*-alanine but not *(S)*-alanine residues.[58]

Although a number of mechanisms have been proposed for the PAM-catalysed oxidation, one generally accepted mechanism involves direct hydroxylation of the α–carbon of the glycine residue to give an α–hydroxy intermediate. This then undergoes rapid hydrolysis to form the corresponding amide product and glyoxylate (38).[47,59,60]

Another chemical model developed to mimic the reaction catalysed by PAM involves nickel peroxide.[46]



a) R = H
b) R = Me
c) R = $CHMe_2$

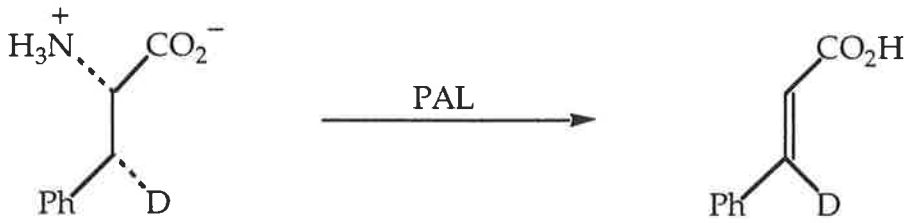
Scheme 10

The amino acid derivatives (41a-c) were shown to undergo oxidative cleavage by nickel peroxide to afford benzamide (45) in a process similar to that catalysed by PAM (Scheme 10).[46] Following their complexation to nickel, hydrogen transfer from the substrates (41a-c) affords the corresponding α–carbon centred radicals (42a-c). Those radicals react to give the corresponding α–hydroxy amino acid derivatives (44a-c) either directly or indirectly *via* the respective *N*-acylimines (43a-c). Subsequent hydrolysis of the α–hydroxy amino acid derivatives (44a-c) affords benzamide (45). Even though a range of chemical models for PAM have been developed,[47,61] the particular feature of the nickel peroxide reaction is that it shows selectivity for reaction of glycine residues akin to that displayed by the enzyme.[62] Thus, in nickel peroxide there is a practical *in vitro* model for the terminal amidation reaction, the characteristics of which closely mimic those of the reaction catalysed by PAM in that it is a free radical process which proceeds *via* an α–hydroxy amino acid intermediate, and is selective for reactions of glycine.[46]

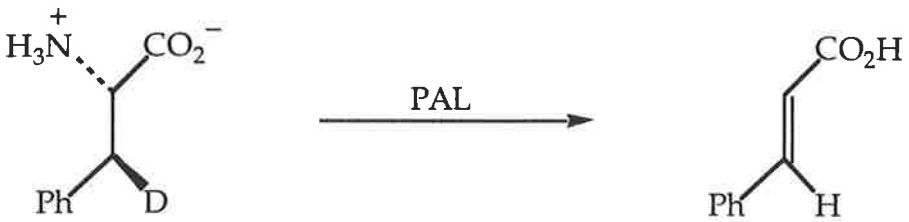
The aim of the work described in Chapter 2 of the Results and Discussion of this thesis was to investigate hydrogen transfer reactions of *N*-protected glycine methyl esters with *tert*-butyl perbenzoate, nickel peroxide and NBS. By using amine protecting groups ranging from strongly electron withdrawing to strongly electron donating, it was thought that the electronic effect of the *N*-substituent on dative radical stabilisation could be established by examining relative rates of reaction of various *N*-protected glycine methyl esters. It was envisaged that the relative reactivity of the glycine derivatives may be reflected in PAM-catalysed reactions, which are thought to proceed through the intermediacy of an α–carbon centred radical, and provide more information about the mechanism of action of the enzyme.

Labelled amino acids have been widely used in the study of enzyme-catalysed reactions. For example, ^{13}C-labelled substrates have been used to determine the stereochemistry of enzyme catalysed decarboxylation reactions,[63] and deuterium labelled tyrosine has been used in the determination of the

stereochemical course of tyramine oxidation by the enzyme semicarbazide sensitive amine oxidase.[64] The *(S)*-phenylalanine derivatives (46a) and (46b), each stereoselectively labelled at the β–centre with deuterium, have been used to study the stereochemical course of the elimination catalysed by the enzyme phenylalanine ammonium lyase (PAL).[65,66] Battersby and co-workers[65] used the deuterium labelled phenylalanines (46a) and (46b) to determine that PAL catalyses the elimination of the *pro-S* hydrogen of *(S)*-phenylalanine (49) (Fig. 3), together with ammonia, to give *trans*-cinnamic acid (48). Reaction of the *(2S,3R)*-isomer (46a, 90% $^2H_1$) with PAL gave deuteriated *trans*-cinnamic acid (47) with approximately 88% deuterium incorporation, indicating a highly stereoselective elimination of the 3-*pro-S* hydrogen from *(S)*-phenylalanine (49) (Scheme 11). Treatment of the *(2S,3S)*-isomer (46b, 90% $^2H_1$) with PAL gave the cinnamic acid (48) with approximately 8% deuterium content, supporting the above conclusion.

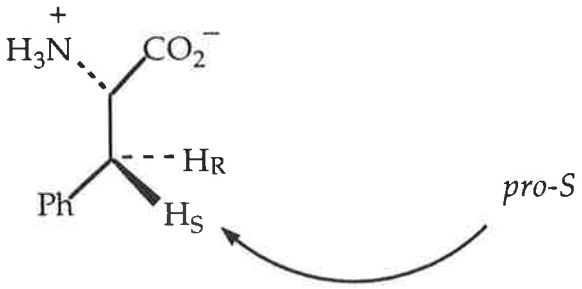


(46a) (47)



(46b) (48)

Scheme 11



(49)

Fig. 3. Designation of the 3-*pro-S* hydrogen of *(S)*-phenylalanine (49) abstracted by PAL.

The active site of PAL contains a prosthetic group for covalent attachment of the amino group. For the substrate to undergo *anti*-elimination, a β–hydrogen must be antiperiplanar to the nitrogen. The phenyl and carboxyl groups must also be *anti* to each other, such that they are *trans* to each other in the product cinnamic acid (48). Therefore, when *(S)*-phenylalanine (49) binds in the active site it must adopt a conformation in which the amino, carboxyl, and phenyl groups and the 3-*pro-S* hydrogen all lie in a plane (Fig. 4). A basic residue is presumably situated in an appropriate position to abstract the 3-*pro-S* hydrogen with *anti*-elimination leading to formation of *trans*-cinnamic acid (48).[68]

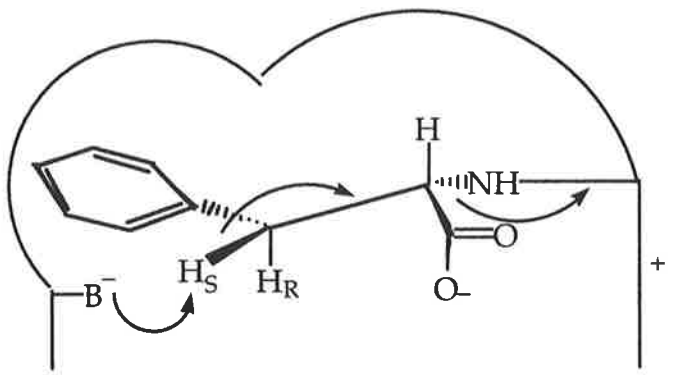
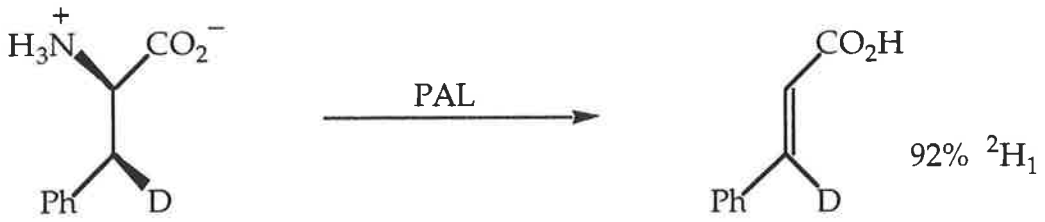


Fig. 4 *(S)*-Phenylalanine (49) in the active site of PAL.

Interestingly, Hanson and Havir[67] showed that the unnatural substrate, *(R)*-phenylalanine (50), is also metabolised by PAL but at a rate one five-thousandth that of *(S)*-phenylalanine (49).

Recently, a stereoselective route was developed to provide each of the stereoisomers of β–deuteriophenylalanine (46c) and (46d) with very high diastereoselectivity and deuterium incorporation.[68] The stereoisomers (46c) and (46d) were used to determine the stereochemical course of the reaction of *(R)*-phenylalanine (50) with the interest being to examine how the enzyme manages to metabolise a substrate with the opposite chirality to its normal substrate. The study showed that treatment of *(2R,3S)*-deuteriophenylalanine (46c) with PAL gave the labelled *trans*-cinnamic acid (47) with 92% deuterium incorporation,

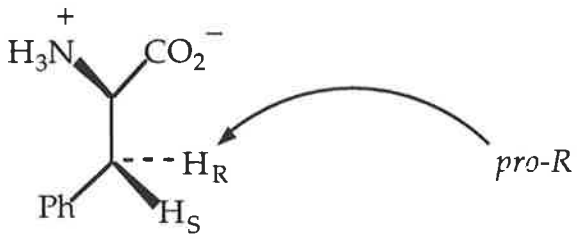


(46c) (47)

(46d) (48)

Scheme 12

whereas treatment of *(2R,3R)*-deuteriophenylalanine (46d) with PAL gave *trans*-cinnamic acid (48) with 27% deuterium incorporation (Scheme 12). These results established that while the loss of hydrogen from *(R)*-phenylalanine (50) in the conversion to *trans*-cinnamic acid (48) is not stereospecific, the enzyme preferentially abstracts the 3-*pro-R* hydrogen from this substrate (Fig. 5).[68]



(50)

Fig. 5 Designation of the 3-*pro-R* hydrogen of *(R)*-phenylalanine (50) abstracted by PAL.

It has been postulated that *(R)*-phenylalanine (50) is situated in the active site of the enzyme with the phenyl, amino, and carboxyl groups bound in the same way as that for the natural substrate (49), even though the chirality at the α–centre is reversed (Fig. 6).[68] In this conformation, the phenyl and carboxyl groups are in the required *anti* orientation, and the amino group and 3-*pro-R* hydrogen are also antiperiplanar.

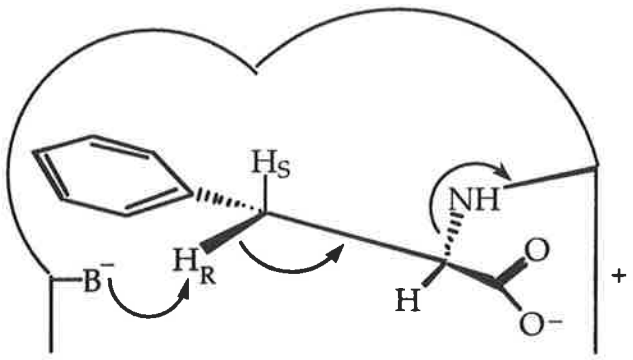


Fig. 6 *(R)*-Phenylalanine (50) in the active site of PAL.

The 3-*pro-R* hydrogen occupies a similar position in the active site to that which the 3-*pro-S* hydrogen of *(S)*-phenylalanine (49) occupies. Thus, abstraction of the 3-*pro-R* hydrogen from *(R)*-phenylalanine (50) occurs preferentially with *anti*-elimination, again giving *trans*-cinnamic acid (48).

Two explanations have been postulated to account for the lack of stereospecificity in the reactions of the deuteriated phenylalanine derivatives (46c) and (46d).[68] In a *syn*-periplanar elimination, abstraction of the 3-*pro-S* hydrogen from *(R)*-phenylalanine (50) may compete with loss of the 3-*pro-R* hydrogen (Fig. 7). If the extent of this reaction is *ca.* 15%, a deuterium isotope effect of *ca.* 1.8 would account for the reaction of the deuteriophenylalanine derivative (46c) to give the labelled *trans*-cinnamic acid (47) with 92% deuterium incorporation and of the deuteriophenylalanine derivative (46d) to give the acid (48) with 27% deuterium incorporation. Alternatively, reversible abstraction of the α–hydrogen from *(R)*-phenylalanine (50), and racemisation, may compete with loss of the 3-*pro-R* hydrogen. The study showed no evidence of racemisation in partially reacted samples of *(R)*-phenylalanine (50),[68] however, it would be unlikely that the concentration of the product *(S)*-phenylalanine (49) would build up to detectable levels under these circumstances. Instead, being a better substrate for the enzyme, *(S)*-phenylalanine (49) would be converted rapidly to *trans*-cinnamic acid (48), with loss of the 3-*pro-S* hydrogen.

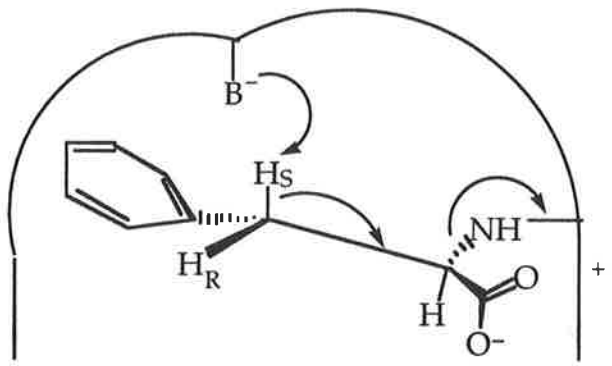
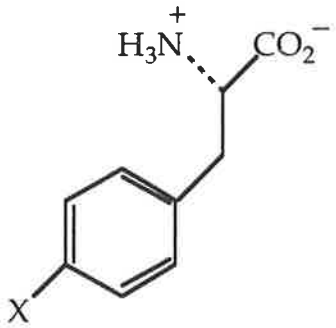


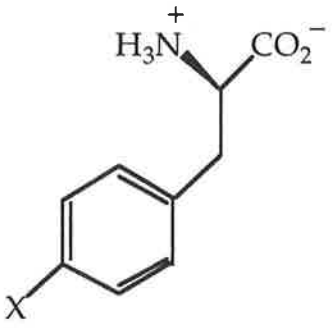
Fig. 7 Abstraction of the 3-*pro-S* hydrogen of *(R)*-phenylalanine (50).

The findings by Easton and Hutton[68] indicated that the primary response of PAL to the change in stereochemistry of the substrate, from *(S)*-phenylalanine (49) to the *(R)*-enaniomer (50), is to reverse the stereoselectivity of β–hydrogen abstraction. Accordingly, the loss of a hydrogen and ammonia from each of the phenylalanine enantiomers (49) and (50) involves mainly antiperiplanar elimination.

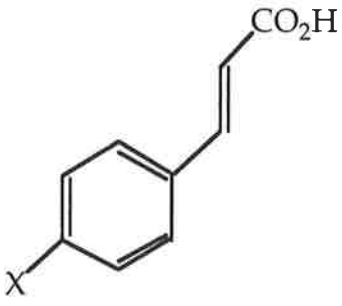
To determine the generality of the minor reaction pathway exhibited in the PAL-catalysed elimination of *(R)*-phenylalanine (50), reactions of analogues of the unnatural substrate (50) were examined in Chapter 3 of the Results and Discussion of this thesis. In an attempt to discriminate between racemisation at the α–centre and *syn*-elimination as the minor reaction pathway, the relative rates of conversion of modified substrates containing β–protons with different acidities were investigated. If removal of the β–proton is the rate determining step in the reaction catalysed by PAL, then this may be reflected in the relative rates of turnover of the modified substrates (51a-c) and (52a-c), to give the corresponding cinnamates (53a-c). It was anticipated that the results obtained would give an indication as to which mechanism is responsible for the minor reaction pathway.



(51)



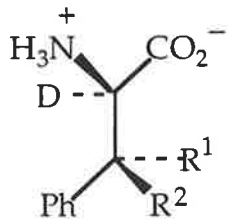
(52)



(53)

a) X = OH
b) X = NO_2
c) X = F

As discussed above, treatment of the deuteriophenylalanine derivative (46c) with PAL gave the labelled cinnamic acid (47) with 92% deuterium incorporation and the deuteriophenylalanine derivative (46d) gave the cinnamic acid (48) with 27% deuterium incorporation.[68] It was thought that by incorporating deuterium at the α–position of the *(2R,3S)*-deuteride (46c) and its diastereomer (46d), to give the dideuterides (54a) and (54b), the rate of competitive racemisation, if it is responsible for the minor reaction pathway, would decrease due to a deuterium isotope effect. Consequently, this would be reflected in the deuterium content at the 3-position in the product cinnamic acid. Reaction of the *(2R,3S)*-2,3-dideuteride (54a) proceeding with >92% deuterium incorporation, and reaction of the *(2R,3R)*-2,3-dideuteride (54b) proceeding with <27% deuterium incorporation, would indicate that the rate of racemisation at the α–centre is being slowed down, indicating that racemisation is responsible for the minor reaction pathway.



(54)

a) R^1 = H, R^2 = D
b) R^1 = D, R^2 = H

Alternatively, reaction of the dideuteride (54a) and its diastereomer (54b) proceeding with the same deuterium incorporation at the 3-position in the products of the reaction as that observed for the non-α–deuteriated analogues (46c) and (46d) would imply that no deuterium isotope effect is associated with the introduction of deuterium at the α–position. This result would indicate that *syn*-elimination of the β–hydrogen is responsible for the minor reaction pathway.

Thus, it was envisaged that the synthesis of the stereoisomers (54a) and (54b) would enable a further investigation into the stereochemical course of reaction of

(R)-phenylalanine (50) with PAL. The results of this investigation are discussed in Chapter 3 of the Results and Discussion of this thesis. The total synthesis of the *(2R,3S)*- and *(2R,3R)*-2,3-dideuterides (54a) and (54b) is also described in this Chapter, with the aim of the work being to ultimately establish if PAL is converting from a lyase to a racemase in an effort to metabolise the unnatural substrate (50), or whether it is altering the mode of elimination from an *anti*- to a *syn*-elimination.

RESULTS AND DISCUSSION : Chapter 1

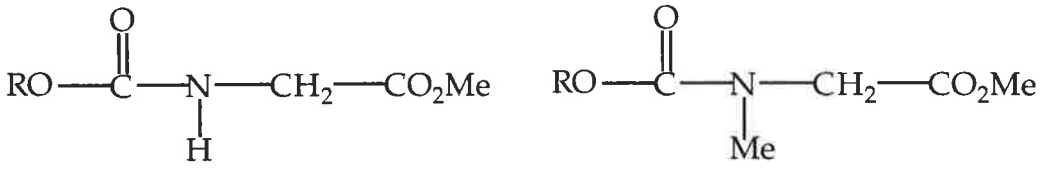
A Study Into the Scope of a New Synthesis of *N*-Methylamino Acid Derivatives and an Investigation into the Novel Mechanism.

As outlined in the Introduction, the aim of the work described in this Chapter was to investigate the mechanism of the novel copper-catalysed reaction, based on the *N*-methylation of the dipeptide (8) to give the *N*-methylated derivative (9). In addition, its potential as a convenient synthetic pathway to the formation of *N*-methylamino acids was examined.

A simple analogue of the dipeptide derivative (8), *N-tert*-butoxycarbonylglycine methyl ester (55a) was chosen as a model for initial investigation. The glycine derivative (55a) was treated with eight equivalents of *tert*-butyl perbenzoate, in the presence of a catalytic amount of cupric octanoate, in benzene at reflux under nitrogen. After work up and chromatography on silica, the corresponding sarcosine derivative (56a) was isolated in 57% yield, and identified by comparison with an authentic sample. A ^{1}H NMR spectrum of the *N*-methyl derivative (56a) showed resonances at δ 2.94 and δ 2.92, attributable to the *N*-methyl group, and showed resonances at δ 3.99 and δ 3.93, due to the α–protons, of the *(E)*-and *(Z)*-isomers, respectively.[69]

Analysis by ^{1}H NMR spectroscopy of a crude mixture from reaction of the glycine derivative (55a) showed the presence of the *N*-methylated product (56a) and residual starting material (55a), in the ratio of approximately 3:1, as well as the by-product of the reaction, methyl benzoate. The extent of reaction increased when a greater molar excess of the perester was used. A decrease in the ratio of the *N*-methylated product (56a) to unreacted starting material (55a) was observed if there was a ten-fold increase in the amount of cupric octanoate used, while no *N*-methylated product (56a) was detected if a one hundred-fold excess of the copper salt was added. By reducing the amount of copper in the reaction mixture,

the reaction time required for *N*-methylation increased significantly. The change in the ratio of the unreacted starting material (55a) to the product (56a) was found to be negligible with respect to the concentration of the glycine derivative (55a) used, within the range of 3.0mM to 30.0mM.



(55) (56)

(a) R = CMe_3
(b) R = CH_2Ph

The reaction of *N*-*tert*-butoxycarbonylglycine methyl ester (55a) is analogous to the reaction of the dipeptide derivative (8) to give the *N*-methyl derivative (9). Each involves the *N*-methylation of a *tert*-butyl carbamate. To determine if *N*-methylation *via* this procedure is in fact general for carbamates, the reaction of *N*-benzyloxycarbonylglycine methyl ester (55b) was also examined. When the carbamate (55b) was treated with eight equivalents of *tert*-butyl perbenzoate in the presence of cupric octanoate, 54% of the *N*-methylated product (56b) was isolated after work up of the reaction mixture, and chromatography on silica. The structure of the *N*-methylated product (56b) was confirmed by comparison of its spectral and physical properties with data reported in the literature.[70] The ^1H NMR spectrum of the product (56b) showed singlets at δ 7.34 and δ 5.13 due to the protons on the aromatic ring and the methylene protons directly adjacent to the ring, respectively. Singlet resonances were observed at δ 4.05 and δ 3.76 due to the α–protons and the methyl ester group, respectively, and a singlet was also observed at δ 3.00 attributable to the *N*-methyl group.

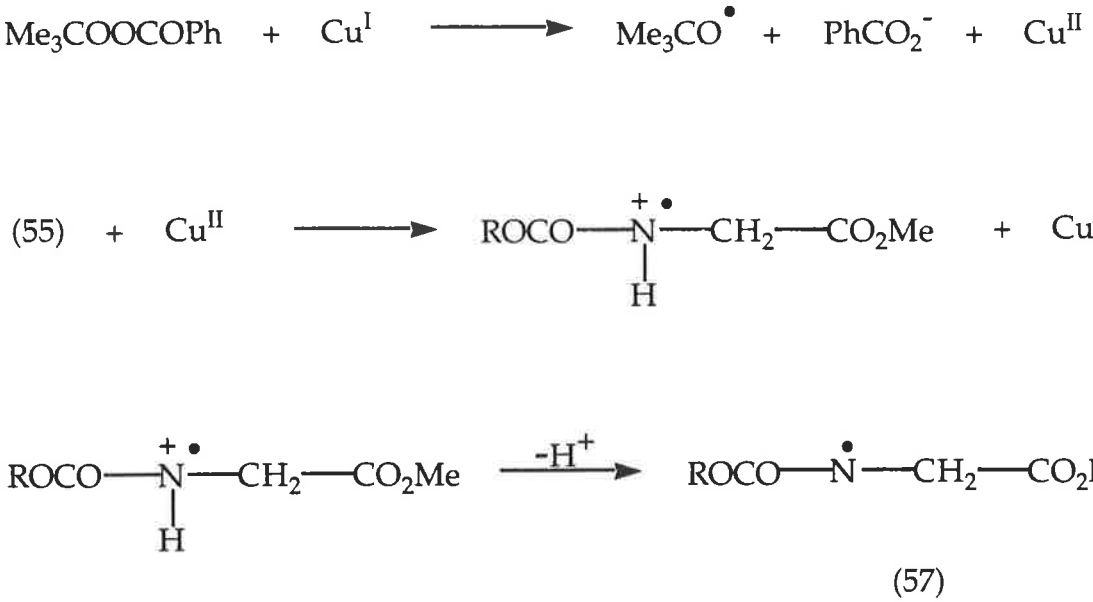
On the basis of the reactions of the glycine derivatives (55a) and (55b), the

N-methylation procedure appears to be general for carbamates.

In order to confirm the product of the contrasting reaction involving *N*-benzoylglycine methyl ester (15) described in the Introduction, this glycine derivative was treated with *tert*-butyl perbenzoate and cupric octanoate under the conditions described for the reactions of the carbamates (55a) and (55b). After work up, analysis of the product by ^{1}H NMR spectroscopy indicated the presence of the α–benzoyloxyglycine derivative (16) and residual starting material (15) in a ratio of approximately 1:3. Doublet resonances were observed at δ 6.84 and δ 4.27 in the ^{1}H NMR spectrum of the product and could be attributed to the α–proton of the benzoate (16), and the methylene protons of the starting material (15), respectively. The resonances at δ 6.84 and δ 4.27 were coupled to the resonances of the amide protons by 9Hz and 5Hz, respectively. The spectral characteristics of the benzoate (16) were in agreement with previous findings.[27]

The greater reactivity of carbamates over amides was shown unambiguously by a competitive reaction between *N*-benzoylglycine methyl ester (15) and *N*-*tert*-butoxycarbonylglycine methyl ester (55a) in the presence of *tert*-butyl perbenzoate and cupric octanoate. Analysis of the crude reaction mixture by ^{1}H NMR spectroscopy and thin layer chromatography (TLC) indicated the presence of *N*-*tert*-butoxycarbonyl-*N*-methylglycine methyl ester (56a), and unreacted *N*-benzoylglycine methyl ester (15). The α–benzoyloxyglycine derivative (16) was not observed, as there was no doublet at δ 6.84 in the ^{1}H NMR spectrum of the crude reaction mixture pertaining to the α–proton, characteristic of this compound.[27]

A possible mechanism to account for the production of the *N*-methyl derivatives (56a) and (56b) is given in Scheme 13. Electron transfer from cuprous ion to *tert*-butyl perbenzoate affords cupric ion, benzoate and *tert*-butoxy radical. In turn, electron transfer from the carbamates (55a) or (55b) to cupric ion, followed by loss of a proton, affords the corresponding carbamate radicals (57a) or (57b) which then react by combination with methyl radical, produced by β–scission of *tert*-butoxy radical, to give the products (56a) and (56b), respectively.

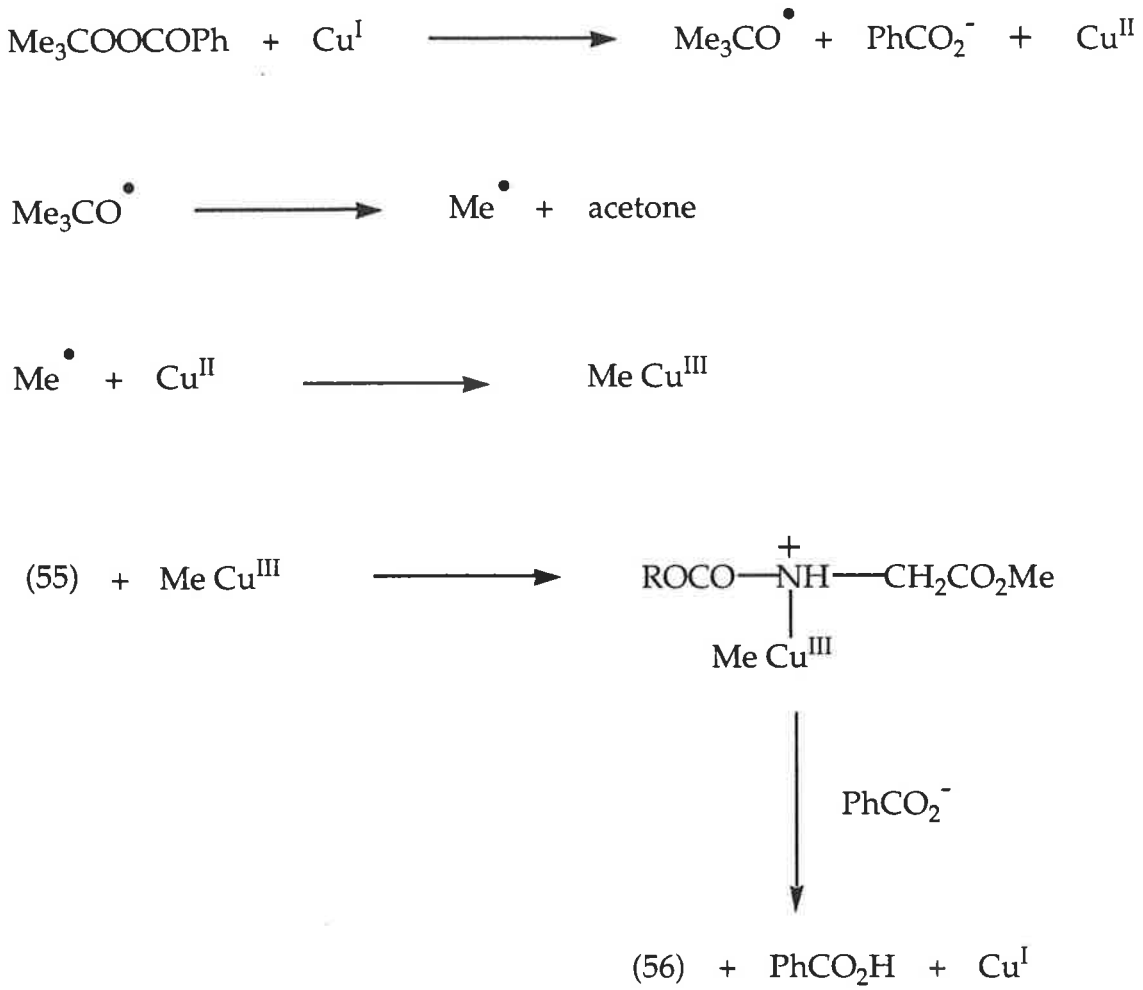


(57)

(a) R = CMe_3
(b) R = CH_2Ph

Scheme 13

An alternative mechanism for the production of the *N*-methyl derivatives (56a) and (56b) is as shown in Scheme 14. Electron transfer from cuprous ion to *tert*-butyl perbenzoate affords cupric ion, benzoate and *tert*-butoxy radical. Methyl radical produced by β–scission of *tert*-butoxy radical combines with cupric ion to give a methyl-copper(III) species, which in turn coordinates to the carbamate nitrogen of the glycine derivatives (55a) and (55b). Subsequent deprotonation yields the corresponding *N*-methylated products (56a) and (56b), and cuprous ion. The different course of reaction of the carbamates (55a) and (55b) to give the *N*-

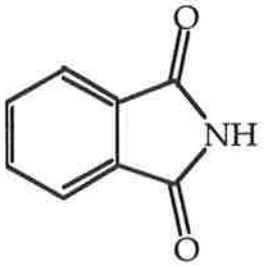


(a) R = CMe_3
(b) R = CH_2Ph

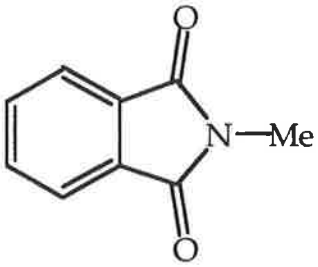
Scheme 14

methylated products (56a) and (56b), respectively, compared with the reaction of the *N*-benzoylglycine derivative (15) to give the benzoate (16) under identical conditions, may be attributed to the relative ease of electron transfer from carbamates, and is consistent with the mechanism described in Scheme 13. Otherwise, it may be due to the different coordinating abilities of the carbamate and amide moieties in the corresponding glycine derivatives (55a,b) and (15). That is, the electrons on the amide nitrogen of the *N*-benzoylglycine derivative (15) may be less readily available for complexation to the copper catalyst, compared with the electrons on the carbamate nitrogen of the glycine derivatives

(55a,b). The different course of reaction of the glycine derivatives (55a,b) and (15) is therefore also consistent with the mechanism described in Scheme 14, since this mechanism implies that complexation between the copper catalyst and the substrate is necessary for *N*-methylation to proceed.



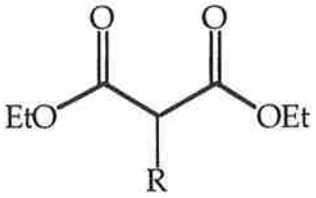
(58)



(59)

To determine the generality of this *N*-methylation procedure, based on the method described above, phthalimide (58) was treated with *tert*-butyl perbenzoate and a catalytic amount of cupric octanoate. After work up and chromatography on the product, *N*-methylphthalimide (59) was isolated in 75% yield. A ^{1}H NMR spectrum of the material showed a singlet resonance at δ 3.19 characteristic of the *N*-methyl group. The spectral characteristics of the *N*-methylated product (59) were consistent with those of an authentic sample. The electrons on the phthalimido nitrogen are delocalised over two adjacent carbonyl groups, and as a result are unlikely to be available for coordination to the copper catalyst prior to *N*-methylation (Scheme 14). However, this implies that the electrons on the nitrogen are also unavailable for electron transfer to cupric ion (Scheme 13). Presumably, *N*-methylation of phthalimide (58) is facilitated through deprotonation of the nitrogen to form the highly resonance stabilised anion, which subsequently coordinates to the copper catalyst to afford the *N*-methylated product (59). This is consistent with the mechanism described in Scheme 14.

The copper-catalysed reaction of diethyl malonate (60a) with *tert*-butyl perbenzoate was subsequently examined to determine whether methylation at



(60)

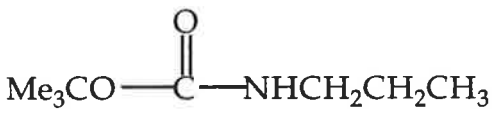
a) R = H
b) R = Me

the α–carbon could be facilitated to give the product (60b), through coordination of the copper catalyst to the resonance stabilised α–carbon centred anion. Analysis of the crude reaction mixture by ^{1}H NMR spectroscopy, however, indicated that diethyl malonate (60a) did not react under the conditions described for the *N*-methylation of phthalimide (58) to give the *N*-methylated product (60b), only starting material could be detected. This tends to suggest that either the nitrogen is required as an initial coordination site for cupric ion, prior to deprotonation and methylation, or the α–carbon centred anion may not form efficiently under these reaction conditions to coordinate to the copper. Both of these explanations would be consistent with the mechanism outlined in Scheme 14. Alternatively, the lack of reaction of diethyl malonate (60a) may give support to the mechanism proposed in Scheme 13, since electron transfer from the substrate (60a) to cupric ion in order to facilitate α–methylation cannot occur.

In summary, the copper-catalysed reactions of the *N*-benzoylglycine derivative (15), the carbamates (55a,b), and phthalimide (58) with *tert*-butyl perbenzoate may be attributed to the coordinating ability of the substrate to the copper catalyst. The *N*-methylation of the carbamates (55a,b) and the lack of *N*-methylation of the glycine derivative (15) may be a result of the carbamate moiety coordinating to copper more efficiently through the readily available electrons on the nitrogen. Interestingly, the electrons on the phthalimido nitrogen are even less available for complexation to the copper catalyst, in comparison to the

electrons on the nitrogen of the *N*-benzoylglycine derivative (15). *N*-Methylation of phthalimide (58) may therefore be due to complexation between the anion on the phthalimido nitrogen and the copper catalyst. Presumably, deprotonation of the nitrogen occurs readily forming the highly resonance stabilised anion. Further, methyl benzoate, the by-product in these reactions, is presumably afforded as a result of complexation between benzoate anion, produced in the initial electron transfer reaction between cuprous ion and *tert*-butyl perbenzoate, and the copper catalyst. These results strongly suggest that the ability of the copper catalyst to coordinate to the substrate, be it through the electrons on the nitrogen or through a formal anion, is a prerequisite for *N*-methylation, which is consistent with the mechanism described in Scheme 14.

To determine whether the methyl ester groups of the glycine derivatives (55a) and (55b) assist *N*-methylation, the copper-catalysed reaction of *N*-*tert*-butoxycarbonylpropylamine (61) with *tert*-butyl perbenzoate was studied. Work up of the reaction mixture, followed by chromatography on the isolated material afforded the *N*-methylated product (62) in 49% yield. A ^{1}H NMR spectrum of the product showed a singlet resonance at δ 2.84 attributable to the *N*-methyl group. The structure was confirmed by comparison of spectral and physical data with that of an authentic sample. The yield of the *N*-methylated product (62) was comparable to the yield of *N*-*tert*-butoxycarbonyl-*N*-methylglycine methyl ester (56a) obtained from reaction of the glycine derivative (55a), under identical conditions. This implies that complexation between the methoxycarbonyl group of the glycine derivatives (55a) and (55b) and the copper catalyst is not required for *N*-methylation to proceed.



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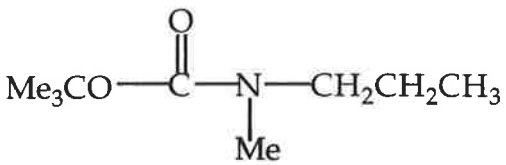
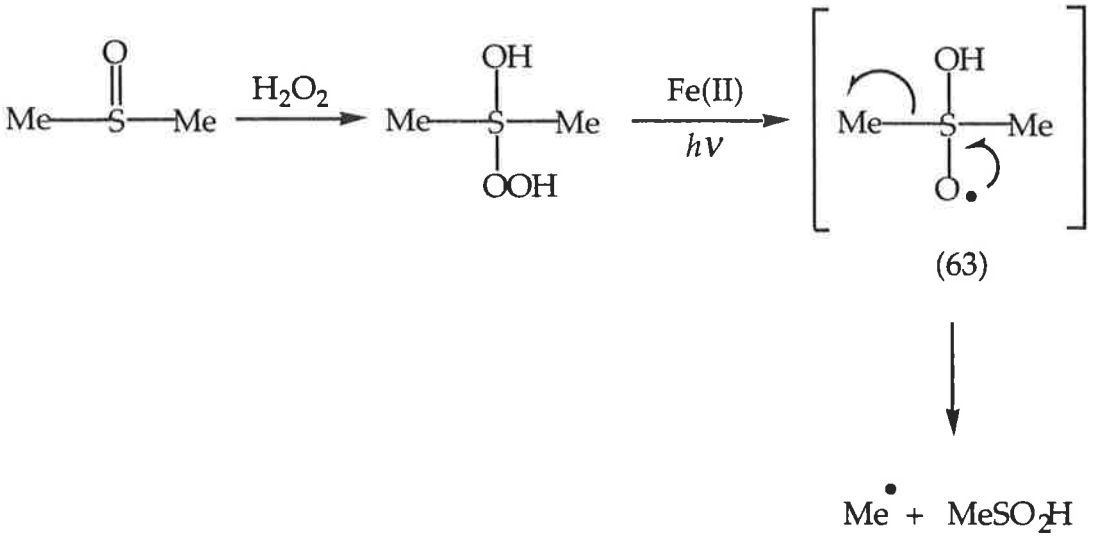


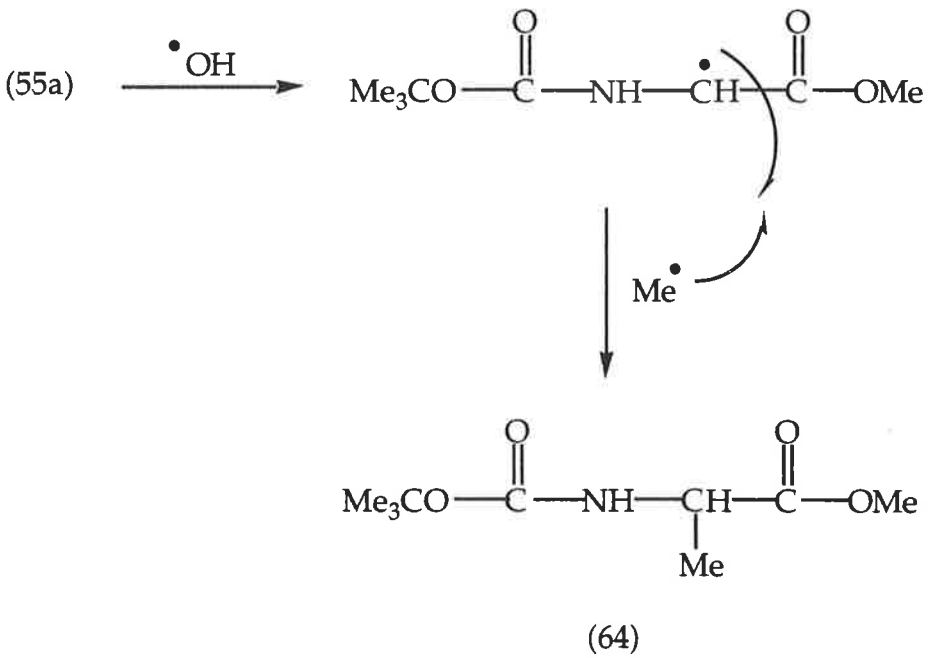
(62)

In an attempt to further probe the mechanism of the *N*-methylation reaction, alternate sources of methyl radical were investigated.

The photoloysis of di-*tert*-butyl peroxide is known to produce *tert*-butoxy radical, which in turns undergoes β–scission to afford methyl radical.[27,71] Accordingly, *N*-*tert*-butoxycarbonylglycine methyl ester (55a) was treated with eight equivalents of di-*tert*-butyl peroxide in the presence of cupric octanoate in benzene, under nitrogen, and the solution was irradiated over 24h. After work up, the isolated material was purified by chromatography on silica to afford the *N*-methylated product (56a) in 42% yield. The structure of the product (56a) was confirmed by comparison of ^{1}H NMR spectral data with that of an authentic sample. When the reaction mixture was irradiated over 48h in an attempt to increase the extent of reaction, the yield of *N*-methylated product (56a) was reduced by a factor of 2, as a result of decomposition of the product. The fact that *N*-methylation of the glycine derivative (55a) occured in the presence of cupric octanoate with a different methyl radical source to *tert*-butyl perbenzoate suggests that only methyl radical and a copper catalyst is required for *N*-methylation to proceed. This is consistent with the mechanisms postulated in both Schemes 13 and 14, except that the methyl radical source does not necessarily have to be *tert*-butyl perbenzoate.

The combination of dimethyl sulfoxide, hydrogen peroxide, and ferrous ions gives rise to methyl radicals and has been used to methylate various substrates.[72] The intermediate (63) is believed to be involved in methylation (Scheme 15). The combination of hydrogen peroxide and ferrous ions, known as Fenton's reagent, gives rise to hydroxy radicals and hydroperoxy radicals *via* electron transfer between ions and the peroxide.[73] Based on a procedure in the literature,[72] the glycine derivative (55a) was treated with Fenton's reagent and dimethyl sulfoxide to examine the efficiency of the reaction as an *N*-methylating procedure. After work up, analysis of the product by TLC and ^{1}H NMR spectroscopy indicated the presence of the starting material (55a) and *N*-*tert*-butoxycarbonylalanine methyl ester (64) in a ratio of approximately 6:1. The



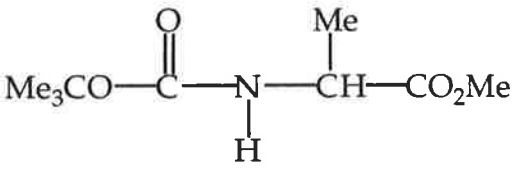


Scheme 15

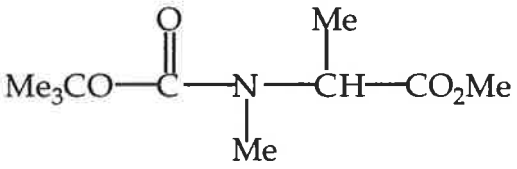
corresponding *N*-methyl derivative (56a) was not produced under these circumstances. This tends to suggest that the ferrous ion does not have the capacity to coordinate to the carbamate moiety in the same way as the cupric ion in order to facilitate *N*-methylation. Instead, a hydrogen transfer reaction

between the carbamate (55a) and hydroxy radical affords a stable α–carbon centred radical, which subsequently combines with methyl radical to afford the α–methylated product (64) (Scheme 15). This result gives further evidence to support the notion that the binding of the copper catalyst to the carbamate moietys of the amino acid derivatives (55a,b) is an important feature of the copper-catalysed *N*-methylation procedure.

To examine the effect of the copper-catalysed reaction with *tert*-butyl perbenzoate on an α–substituted, optically active amino acid derivative, the reaction of *(S)-N-tert*-butoxycarbonylalanine methyl ester (64) was studied. The *N*-methylated product (65) was isolated in 47% yield after work up of the reaction mixture and chromatography on silica. The structure of the product (65) was confirmed by comparison with an authentic sample. The ^{1}H NMR spectrum of the *N*-methylated product (65) showed singlet resonances of approximately equal intensity at δ 2.87 and at δ 2.80 for the *N*-methyl group, and multiplets were observed at δ 4.85 and δ 4.38 for the α–protons of the *(E)*- and *(Z)*-isomers, respectively.[21]



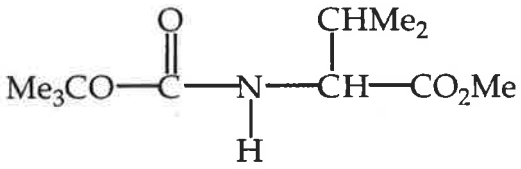
(64)



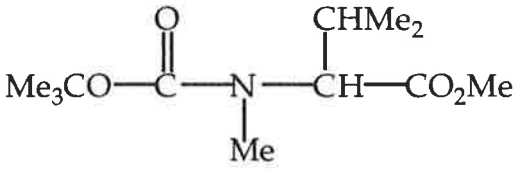
(65)

To ascertain whether racemisation had occurred in the reaction of the optically active starting material (64) to produce the product (65), ^{1}H NMR spectra of the *N*-methylalanine derivative (65) were recorded in the presence of the chiral europium shift reagent, Eu(hfbc)$_3$.[74] When the chiral shift reagent was added to a sample of an authentic racemate of the *N*-methylalanine derivative

(65), the ^1H NMR spectrum indicated the presence of four europium complexes, derived from the *(E)*- and *(Z)*-isomers of each enantiomer. In particular there were four resonances attributable to the *tert*-butoxy groups. By comparison, the sample of the *N*-methyl compound (65) prepared from the optically active material (64) showed only two of these resonances, corresponding to the *(E)*- and *(Z)*-isomers derived from a single enantiomer. Thus, it is evident that reaction of the alanine derivative (64) with *tert*-butyl perbenzoate and cupric octanoate afforded the *N*-methyl compound (65) without racemisation at the α–centre. Reaction of the alanine derivative (64) without racemisation implies that the proton at the α–centre is not removed at any stage of the reaction, which is consistent with the mechanisms postulated in Schemes 13 and 14.



(66)



(67)

Based on the homochirality of the *N*-methyl derivative (65), the *N*-methylation procedure is a complementary method for synthesis of optically active *N*-methylamino acids. To examine the generality of this procedure, *N-tert*-butoxycarbonylvaline methyl ester (66) was treated with *tert*-butyl perbenzoate in the presence of cupric octanoate. However, the valine derivative (66) was found to be inert under the reaction conditions used to afford the products (56a,b) and (65) from the carbamates (55a,b) and (64), respectively. Only the unreacted starting material (66) and methyl benzoate were detected in a ^1H NMR spectrum of the crude reaction mixture. The addition of excess *tert*-butyl perbenzoate and increasing the reaction time still failed to give any *N*-methylated product (67), as the ^1H NMR spectrum did not show a resonance in the vicinity of δ 2.83 which is

characteristic for the *N*-methyl group of the valine derivative (67).[21]

Free radical and electron transfer processes are very susceptible to inhibition by trace impurities. To determine whether impurities were present in the valine derivative (66) which were preventing its reaction, a competitive reaction between the valine derivative (66) and the glycine derivative (55a) was performed. If impurities were present in *N-tert*-butoxycarbonylvaline methyl ester (66), then these would also prevent the glycine derivative (55a) from reacting in the competitive reaction, and no *N*-methylated product (56a) would be produced under these circumstances. With the mixture of the glycine derivative (55a) and the valine derivative (66), the glycine derivative (55a) reacted to give the product (56a), as determined by TLC and ^{1}H NMR spectroscopic analysis of the reaction mixture, and again, none of the *N*-methylvaline derivative (67) was observed. Therefore, the lack of reaction of *N-tert*-butoxycarbonylvaline methyl ester (66) must be due to a decrease in reactivity of the valine derivative (66), compared with the glycine derivative (55a).

To determine if *N*-methylation was similarly selective for the glycine derivative (55a) over the alanine derivative (64), a competitive reaction between the two was performed. Only the glycine derivative (55a) reacted when a mixture of the carbamates (55a) and (64) was treated under the standard reaction conditions. TLC and ^{1}H NMR spectroscopic analysis of the crude reaction mixture, by comparison with TLC and spectral data of authentic samples of the *N*-methyl derivatives (56a) and (65), showed only the formation of the *N*-methylglycine derivative (56a) and a reduction in the ratio of the unreacted starting materials (55a) to (64).

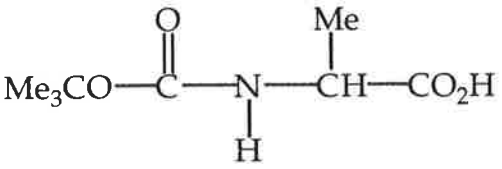
The lack of reaction of the valine derivative (66), and the selective reaction of the glycine derivative (55a) from mixtures of the glycine derivative (55a) and the alanine derivative (64), and the glycine derivative (55a) and the valine derivative (66), may be attributed to the relative ease of complexation of these substrates to the copper catalyst. The glycine derivative (55a) binds selectively to the catalyst and, as a consequence, reacts faster than the alanine derivative (64).

The binding of the valine derivative (66) to copper is even less efficient, to the extent that no reaction occurs. Presumably the relative ease of binding of the amino acid derivatives (55a), (64) and (66) reflects their relative solubilities in the reaction solvent, benzene, and the degree of steric interactions associated with their complexation. The glycine derivative (55a) is least soluble in benzene and will bind to the copper with the least unfavourable steric interactions. These results imply that the binding of the copper catalyst to the carbamate moiety of the amino acid derivatives (55a), (64) and (66) contributes significantly to the efficiency of this *N*-methylation reaction. The selectivity observed in the competitive reactions perfomed between the derivatives of glycine (55a), alanine (64) and valine (66) is therefore consistent with the mechanism outlined in Scheme 14, rather than Scheme 13.

In an earlier study,[34] as discussed in the Introduction, the preferential reaction of glycine residues in radical reactions of amino acid derivatives was attributed to selective hydrogen abstraction from the α–carbon of glycine moieties, as a result of the relative stability and ease of formation of the corresponding α–carbon centred radicals. The thermodynamic preference for the production of tertiary radicals over secondary radicals is outweighed by the destabilising effect of non-bonding interactions. This hypothesis to account for the formation of the radical (20) in preference to the radical intermediates (21) and (22) does not account for the selectivity observed in the reactions of the carbamates (55a), (64) and (66), as an α–centred radical is not produced at any stage in the latter cases. Nor does the rationale pertaining to the selective binding of the copper salt to the glycine derivative (55a) over the alanine derivative (64) and the valine derivative (66), proposed above, provide a satisfactory explanation for the selective reaction to give the glycyl radical (20). Instead, the rationale for the selective reaction of the glycine derivative (55a) to give the *N*-methylated derivative (56a), by preferential binding to the copper catalyst, indicates a different factor which contributes to the selective reaction of glycine derivatives in free radical reactions of amino acid derivatives, and is another factor which may

account for the selectivity observed in biochemical systems.

To determine if the *N*-methylation procedure worked whilst other functional groups were present in the molecule, *N*-*tert*-butoxycarbonylalanine (68) was treated with two equivalents of *tert*-butyl perbenzoate in the presence of cupric octanoate in benzene, at reflux under nitrogen. A five-fold excess of cupric



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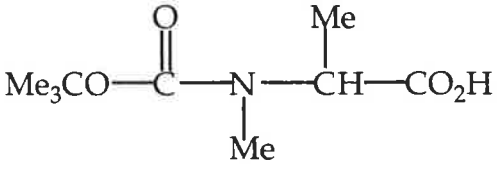
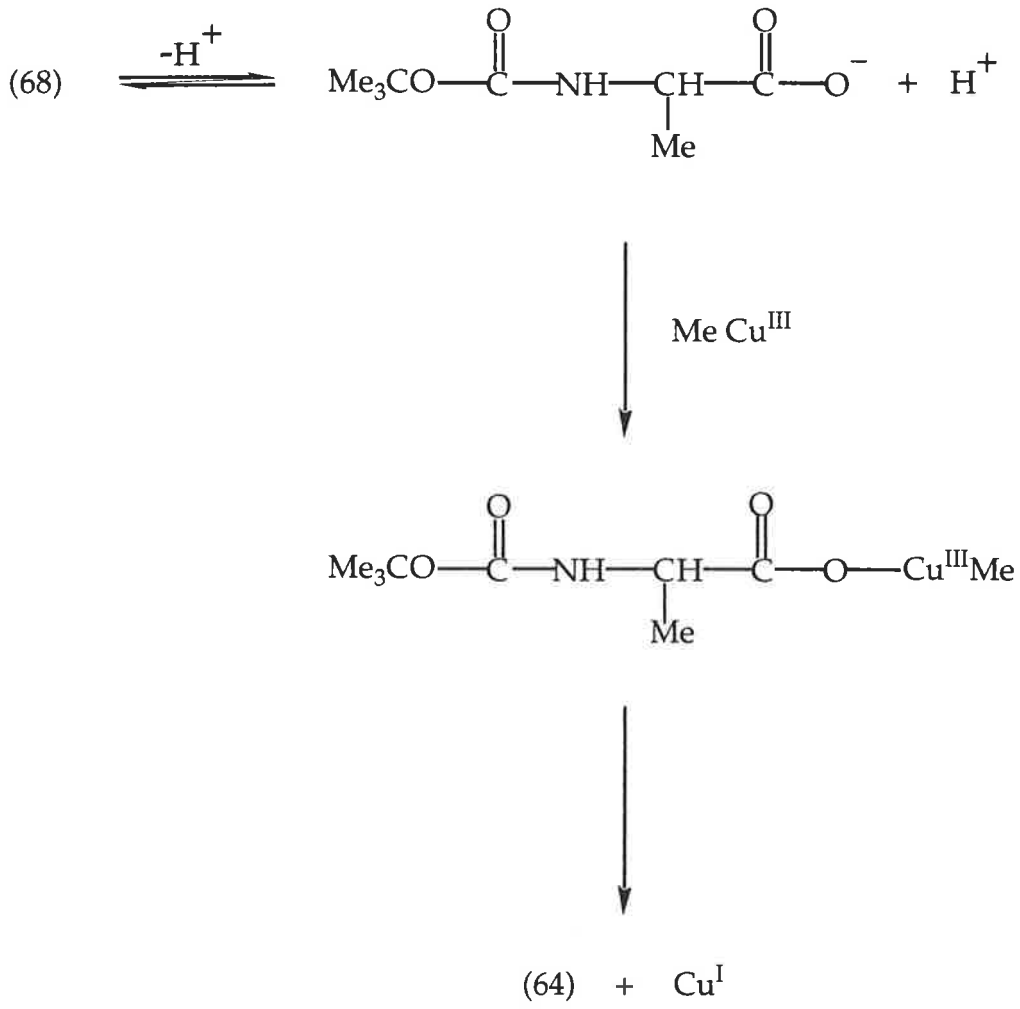


(69)

octanoate, relative to the amount used in the reaction of the glycine derivative (55a) to afford the product (56a), was required to facilitate the reaction. Only starting materials were recovered if a reduced amount of cupric octanoate was used in the reaction involving the *N*-protected free amino acid derivative (68). This is presumably because copper (II) chelates to *N*-*tert*-butoxycarbonylalanine (68) so efficiently that binding to *tert*-butyl perbenzoate necessary for the initial electron transfer, shown in Schemes 13 and 14, does not occur. The reaction mixture was subsequently worked up and the isolated product was purified by flash chromatography on silica. TLC and ^{1}H NMR spectroscopic analysis of the eluted fractions, by comparison with TLC and spectral data of authentic samples of the alanine derivatives (65) and (69), showed that *O*-methylation had occurred as well as *N*-methylation. Both *N*-*tert*-butoxycarbonyl-*N*-methylalanine methyl ester (65) and *N*-*tert*-butoxycarbonyl-*N*-methylalanine (69) were recovered from reaction of the free amino acid derivative (68). Because the reaction of the carbamate (68) with *tert*-butyl perbenzoate and the copper salt yielded a mixture of products, the reaction of the free acid derivative is limited in its synthetic utility. Hence, no attempts were made to optimise the reaction conditions.

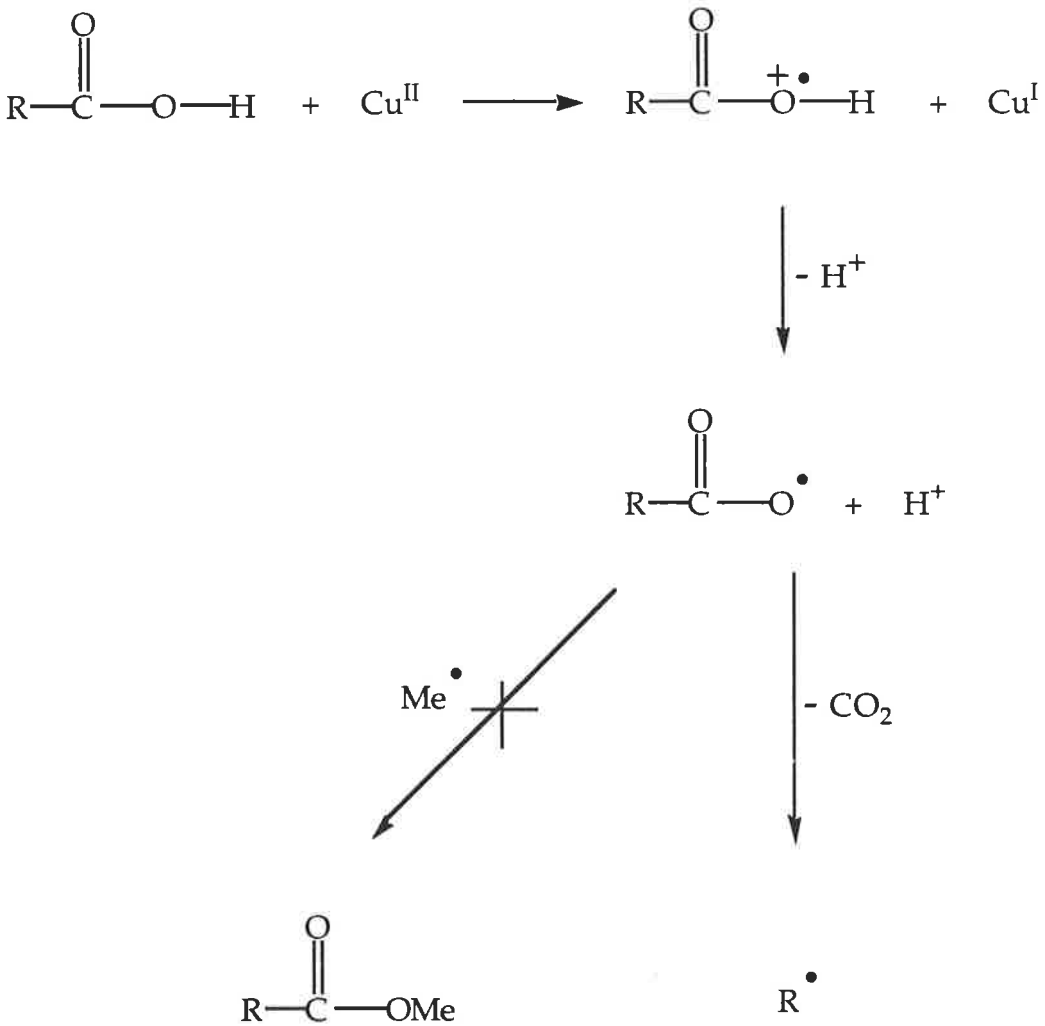
Evidence of *O*-methylation suggests that the reaction of the carbamates (55a), (55b), (64) and (68) with *tert*-butyl perbenzoate in the presence of cupric octanoate is best described by the mechanism shown in Scheme 14 rather than in Scheme 13. Deprotonation of the carboxyl oxygen of the free amino acid derivative (68) to give the resonance stabilised carboxylate anion can occur readily (Scheme 16).



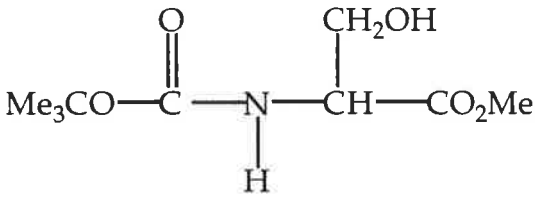
Scheme 16

Presumably, *O*-methylation is facilitated through coordination of the copper catalyst to the carboxylate anion to produce the corresponding methyl ester (64). Furthermore, the mechanism described in Scheme 16 is also consistent with formation of the by-product of the reaction, methyl benzoate. Presumably, methyl benzoate is also afforded as a result of complexation between benzoate

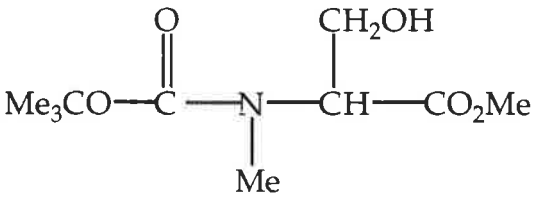
anion, produced in the initial electron transfer reaction between cuprous ion and *tert*-butyl perbenzoate, and the copper catalyst. Therefore, formation of methyl benzoate is also consistent with the mechanism postulated in Scheme 14. In the alternative mechanism, described in Scheme 13, if electron transfer occurs from the carboxyl oxygen of the alanine derivative (68) to cupric ion, followed by proton transfer, β–scission would be expected to occur much faster than combination with methyl radical (Scheme 17). Thus, *O*-methylation is not likely to proceed *via* the mechanism proposed in Scheme 13.



Scheme 17



(70)



(71)

To examine the effect of the copper-catalysed reaction of *tert*-butyl perbenzoate with an amino acid derivative functionalised at the β–position, the reaction of *N*-*tert*-butoxycarbonylserine methyl ester (70) was studied. Previous attempts to *N*-methylate this compound by Olsen and co-workers[21] have proven to be unsuccessful, so the reaction of the serine derivative (70) is of particular interest. After work up, analysis of the crude product by ^{1}H NMR spectroscopy indicated the presence of the *N*-methylated derivative (71) and the unreacted starting material (70) in a ratio of approximately 2:1. The crude product was purified by chromatography on silica to remove methyl benzoate, followed by chromatography on neutral alumina eluting with a gradient of ethyl acetate in hexane to remove the unreacted starting material (70). This afforded the *N*-methylated derivative (71) in 21% yield. The low yield obtained of the *N*-methylated material (71) could be attributed to decomposition of the *N*-methyl derivative (71) *via* lactonization during chromatography. The ^{1}H NMR spectrum of the isolated compound (71) showed resonances at δ 2.96 and δ 2.90 which are characteristic of the *N*-methyl group corresponding to the *(E)*- and *(Z)*-isomers. The spectrum showed no indication of *O*-methylation. An accurate mass measurement of the isolated compound (71) showed a peak at *m/z* 202.108 corresponding to M^+-OMe. The molecular ion peak itself was weak due to the fast lactonization of the product (71), which accounts for the loss of the methoxy group from the ester moiety. The lack of *O*-methylation in the copper-catalysed reaction of the serine derivative (70) with *tert*-butyl perbenzoate is consistent with the mechanisms described in Schemes 13 and 14. The oxygen of the β–

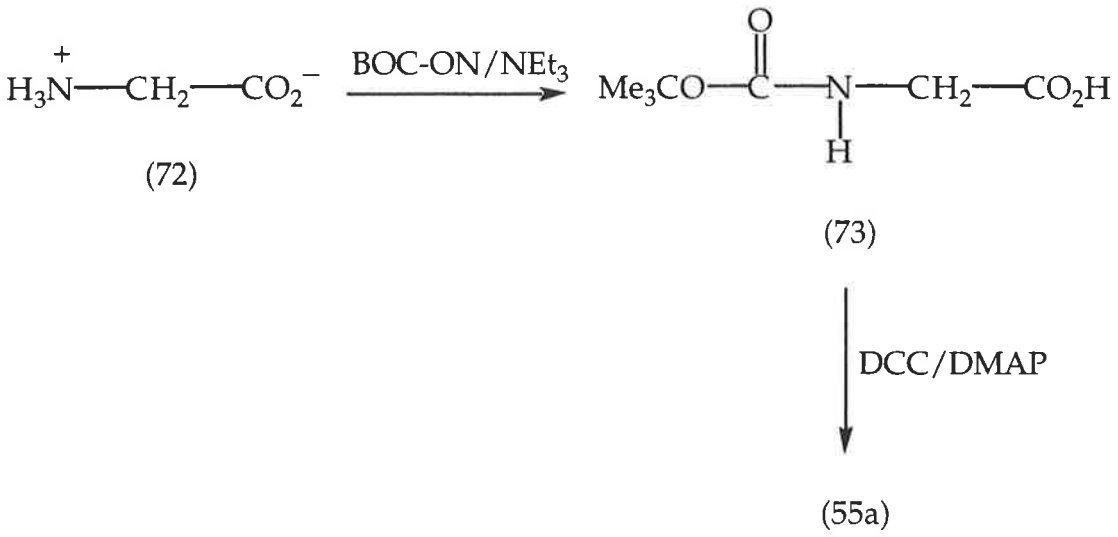
hydroxy group of *N-tert*-butoxycarbonylserine methyl ester (70) is much more electronegative than the carbamate nitrogen, thus, electron transfer from the oxygen to cuprous ion to produce the oxygen-centred radical cation is unlikely (Scheme 13). Electrons on the oxygen are therefore also unavailable for coordination to the copper species, and methoxide formation is not favoured since the anion cannot be stabilised through resonance (Scheme 14). Thus, both mechanisms are consistent with the product (71) observed in the reaction of the serine derivative (70).

In summary, two mechanisms to account for the *N*-methylation reaction have been proposed, both of which involve initial electron transfer from cuprous ion to *tert*-butyl perbenzoate to produce cupric ion, benzoate and *tert*-butoxy radical, which in turn undergoes β–scission to afford methyl radical. Subsequent to this step, it has been postulated that *N*-methylation may proceed *via* electron transfer from the substrate to cupric ion (Scheme 13), or through complexation between the substrate and a methyl-copper(III) species (Scheme 14), the latter suggesting that the coordinating ability of the substrate to the copper catalyst is an important factor in this reaction. The selectivity observed for reaction of the glycine derivative (55a) over the alanine and valine derivatives (64) and (66), respectively, which may account for the selectivity observed in biological systems, is consistent with the mechanism proposed in Scheme 14. In addition, formation of methyl benzoate, a by-product in all of the *N*-methylation reactions described in this Chapter, as well as evidence of *O*-methylation in the reaction of the *N*-protected free amino acid derivative (68), indicates that the *N*-methylation procedure is best described by the mechanism in Scheme 14. As yet, the general mechanism for *N*-methylation *via* the copper-catalysed reaction with *tert*-butyl perbenzoate has not been fully elucidated, as reaction of the serine derivative (70) without methylation at the β–hydroxy position is consistent with both Scheme 13 and 14. However, the study did show that formation of methyl radical in the presence of a copper catalyst was essential for *N*-methylation to proceed, with the actual source of methyl radical being unimportant.

In any case, it has been shown that the copper-catalysed *N*-methylation reaction is general for carbamate derivatives, useful for the formation of optically active *N*-methylamino acids and effective in *N*-methylating *N*-*tert*-butoxycarbonylserine methyl ester (70).

Synthesis of Substrate Amino Acids and Authentic Product Samples

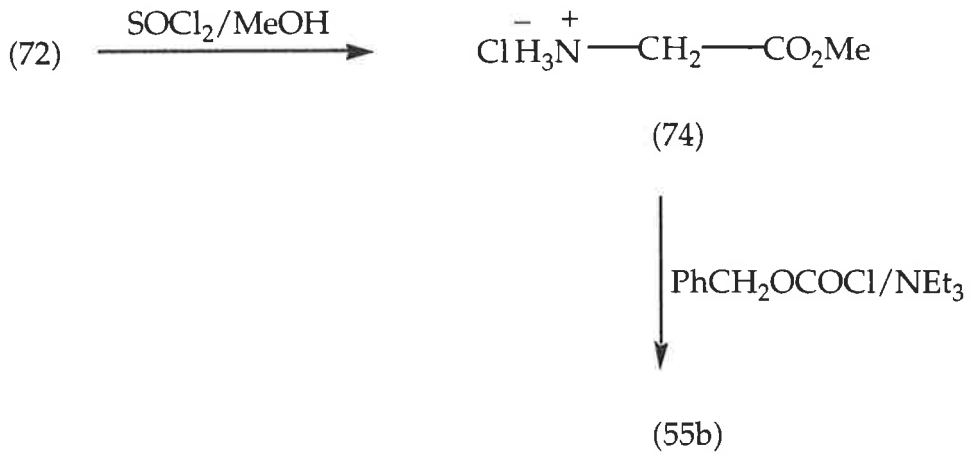
The substrates required for the investigation described in this Chapter were synthesised from the corresponding commercially available amino acids.



Scheme 18

The glycine derivative (55a) was synthesised as shown in Scheme 18. To a solution of glycine (72) in triethylamine and water was added one equivalent of 2-(*tert*-butoxycarbonyloxyimino)-2-phenylacetonitrile, (BOC-ON) in acetone.[75] After work up, recrystallisation of the isolated product from a mixture of ether and hexane gave *N-tert*-butoxycarbonylglycine (73). The ^{1}H NMR spectrum of the product (73) showed a doublet at δ 3.81 due to the α–protons, and a singlet at δ 1.41 attributable to the *tert*-butoxy group. *N-tert*-Butoxycarbonylglycine (73) was esterified using *N,N*-dicyclohexylcarbodiimide (DCC) and dimethylaminopyridine (DMAP) in methanol (Scheme 18).[76] Subsequent work up of the reaction mixture, followed by Kugelrohr distillation yielded *N-tert*-butoxycarbonylglycine methyl ester (55a) as a clear oil, in 76% yield. The product (55a) was identified by comparison of its spectral and physical properties with data reported in the literature.[77] The ^{1}H NMR spectrum of the glycine derivative (55a) showed a doublet resonance at δ 3.92 due to the α–protons, and singlets

were observed at δ 3.75 and δ 1.45 attributable to the methyl ester and *tert*-butoxy group, respectively.



Scheme 19

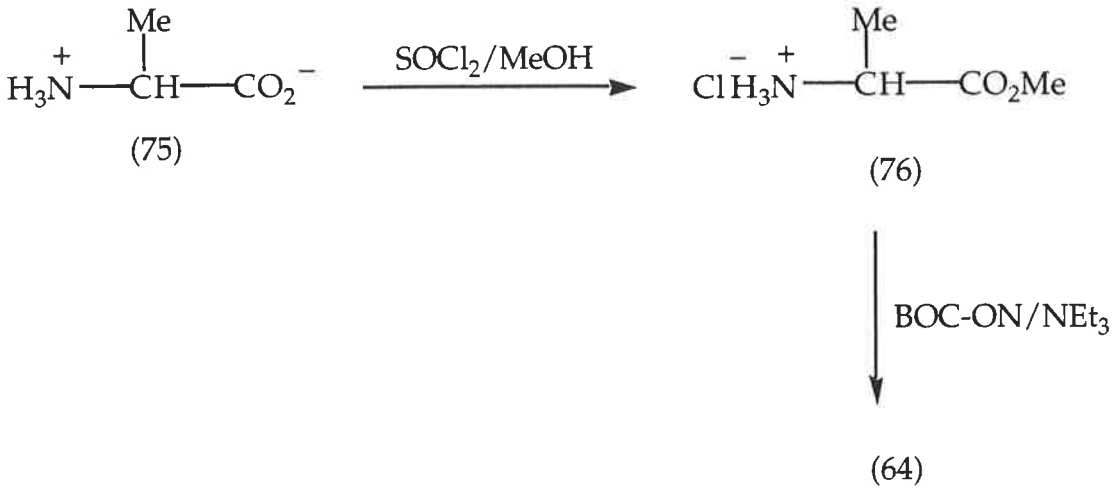
N-Benzyloxycarbonylglycine methyl ester (55b) was synthesised as shown in Scheme 19. To glycine (72) in methanol was added a slight molar excess of thionyl chloride to produce the hydrochloride salt of glycine methyl ester (74).[78] Based on a procedure by Carter and co-workers,[79] a mixture of benzylchloroformate and triethylamine was added to the hydrochloride salt of the glycine methyl ester (74) in toluene. This afforded *N*-benzyloxycarbonylglycine methyl ester (55b), but only in low yield. The ^{1}H NMR spectrum of the product (55b) showed singlets at δ 7.25 and δ 5.10 attributable to the protons on the aromatic ring and the methylene protons directly adjacent to the ring, respectively. A doublet was observed at δ 3.86 and a singlet at δ 3.65 due to the α–protons and the methyl ester group, respectively. The non-nucleophilic base, triethylamine, was used in place of sodium hydroxide, which was used in the above literature procedure, to prevent base hydrolysis of the ester. However, a problem was encountered with this modified procedure, associated with the removal of excess benzylchloroformate which would otherwise have been hydrolysed by sodium hydroxide to the corresponding carboxylate salt, and removed in the work up procedure. Thus, after work up of the crude reaction

mixture, chromatography on the isolated product was required to remove excess benzylchloroformate. Due to the poor yield of the *N*-benzyloxycarbonylglycine derivative (55b) obtained by the above method, the following alternative procedure was implemented. *N*-Benzyloxycarbonylglycine was initially prepared from glycine (72), again, on the basis of a procedure by Carter and co-workers.[79] The previous problems associated with the use of sodium hydroxide as base were not encountered in this instance, as the methyl ester group was put on last using 1-ethyl-3-[3-(dimethylamino)propyl] carbodiimide hydrochloride (EDCI) in methanol.[80] The crude *N*-benzyloxycarbonylglycine methyl ester (55b) thus obtained was purified by Kugelrohr distillation to afford a viscous oil, in 75% yield.

N-tert-Butoxycarbonylpropylamine (61) was synthesised from propylamine using BOC-ON on the basis of a procedure in the literature.[75] The *N*-protected derivative (61) was isolated in 81% yield and purified by Kugelrohr distillation to afford the product as a clear oil. A ^{1}H NMR spectrum of the product (61) showed a singlet at δ 1.44 characteristic of a *tert*-butoxy group. Multiplet resonances were observed at δ 3.07 and δ 1.51 due to the two sets of methylene protons, and a triplet resonance was observed at δ 0.91 attributable to the methyl protons. The structure of the product (61) was confirmed by using ^{1}H NMR spectroscopy and mass spectrometry.

N-tert-Butoxycarbonyl-*(S)*-alanine methyl ester (64) was synthesised from *(S)*-alanine (75), as shown in Scheme 20. To a solution of alanine (75) in methanol was added a slight molar excess of thionyl chloride to produce the hydrochloride salt of alanine methyl ester (76).[78] To the salt (76) was added a mixture of triethylamine and water, with the subsequent addition of BOC-ON in acetone.[75] After work up of the crude reaction mixture, followed by distillation, the protected alanine derivative (64) was produced in 76% yield, as a clear oil. The alanine derivative (64) was identified by comparison of its spectral and physical properties with data reported in the literature.[81] The ^{1}H NMR spectrum of (64) showed a multiplet resonance at δ 4.31 due to the α–proton, singlets were

observed at δ 3.73 and δ 1.43 due to the methyl ester and *tert*-butoxy group, respectively, and a doublet was observed at δ 1.38 attributable to the α–methyl group.

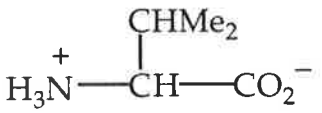


Scheme 20

The valine derivative (66) was synthesised from valine (77) under similar conditions to that described for the formation of the alanine derivative (64) from alanine (75) (Scheme 20). After work up of the crude reaction mixture followed by distillation, the *N*-protected valine derivative (66) was afforded in 71% yield as a clear oil. The structure of the product (66) was confirmed using ^{1}H NMR spectroscopy and elemental analysis. The ^{1}H NMR spectrum showed a multiplet resonance and a singlet resonance at δ 4.18 and δ 3.69 due to the α–proton, and the methyl ester group, respectively. A multiplet resonance was also observed at δ 2.10 due to the β–proton, a singlet was observed at δ 1.40 attributable to the nine protons of the *tert*-butoxy group, and a pair of doublets were observed at δ 0.89 due to the hydrogens of the non-equivalent isopropyl methyl groups.

The serine derivative (70) was prepared from serine (78) as described for the formation of the alanine derivative (64) from alanine (75) (Scheme 20). *N-tert*-Butoxycarbonylserine methyl ester (70) was purified by distillation to afford an oil in 63% yield, and had spectral and physical properties consistent with data found

in the literature.[82] The ^1H NMR spectrum of the product (70) showed multiplets at δ 4.37 and δ 3.95 attributable to the α– and β–protons, respectively, and singlets were observed at δ 3.80 and δ 1.45 due to the corresponding methyl ester and *tert*-butoxy groups.



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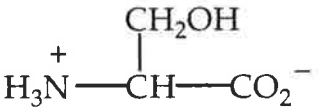


(78)

Authentic samples of *N*-methylated amino acid derivatives were prepared for comparison with the products of the reactions described in this Chapter.

N-tert-Butoxycarbonyl-*N*-methylglycine methyl ester (56a) was prepared from *N*-methylglycine as described in the reaction of alanine (75) to afford the protected alanine derivative (64), shown in Scheme 20. The authentic *N*-methyl derivative (56a) was produced in 80% yield and had spectral and physical properties consistent with data found in the literature.[69]

N-tert-Butoxycarbonyl-*N*-methylalanine methyl ester (65) was prepared from *N*-methylalanine as outlined in Scheme 20. The authentic *N*-methyl derivative (65) was produced in 75% yield, and the structure was confirmed by comparison with spectral and physical data reported in the literature.[21]

Attempts were made to prepare *N-tert*-butoxycarbonyl-*N*-methylvaline (67) from *N*-methylvaline by the methods described in Schemes 18 and 20. Both syntheses failed to give the product (67). The difficulty experienced in protecting *N*-methylvaline using the above methods could be attributed to steric hindrance from the combination of both the methyl group on the carbamate nitrogen and the isopropyl group on the α–carbon.

N-tert-Butoxycarbonyl-*N*-methylalanine (69) was synthesised from *N*-methylalanine by a similar procedure used in the formation of the *N*-protected

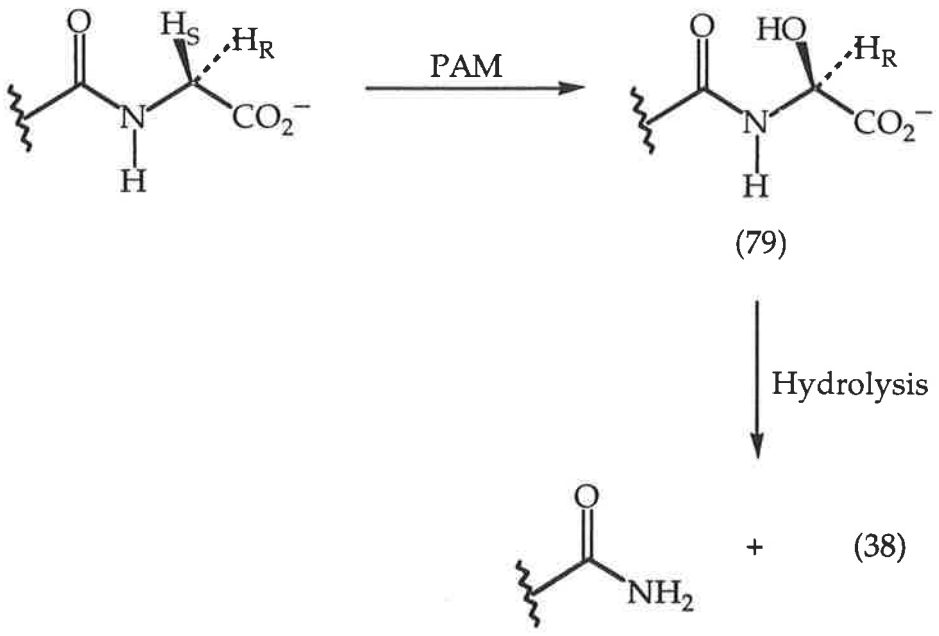
glycine derivative (73) from glycine (72) (Scheme 18). The authentic *N*-methyl derivative (69) was afforded in 71% yield after recrystallisation from a mixture of ether and hexane, and had spectral and physical properties consistent with data found in the literature.[21]

N-*tert*-Butoxycarbonyl-*N*-methylpropylamine (62) was prepared from *N*-methylpropylamine by a similar procedure used in the formation of the *N*-protected glycine derivative (73) from glycine (72) (Scheme 18). The authentic *N*-methyl derivative (62) was purified by distillation to yield a clear oil in 89% yield. The structure of the product (62) was confirmed using ^{1}H NMR spectroscopy and mass spectrometry.

RESULTS AND DISCUSSION : CHAPTER 2

Factors Affecting the Formation of Glycyl Radical Intermediates and their Implication in PAM Inhibition.

The nature of amino acid protecting groups has been shown to markedly control the regioselectivity of many radical reactions, as a result of the electronic and steric constraints these substituents impart during the course of reaction. These factors are of great interest to organic chemists, particularly in the study of biochemical reactions which involve the formation of radical intermediates. Studies have shown that the PAM-catalysed oxidative cleavage of carboxy-terminal glycine extended peptides to produce C-amidated peptides and glyoxylate (38), involves formation of the corresponding α–hydroxy intermediate (79) (Scheme 21).[50,83] In addition, from the results of experiments involving non-enzymatic peptide amidation, discussed in the Introduction, Kizer and co-workers[47] have proposed that the formation of an α–carbon centred peptide



Scheme 21

radical, and its subsequent oxidation by an active oxygen species, must be considered as a possible mechanism for the reaction catalysed by PAM (Scheme 8).

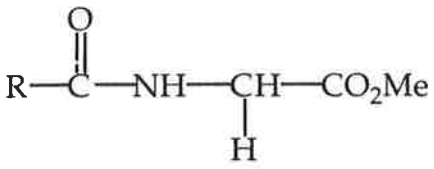
The aim of the work described in this Chapter was to investigate hydrogen transfer reactions of *N*-protected glycine methyl esters with *tert*-butyl perbenzoate, nickel peroxide and NBS. By examining relative rates of reaction of various glycine derivatives, the electronic effect of the *N*-substituent on the formation of an electron deficient α–carbon centre can be established. It was envisaged that the relative reactivity of the glycine derivatives may be reflected in PAM-catalysed reactions, and provide more information as to the role of the amide bond during oxidation by the enzyme. Since PAM catalyses the biosynthesis of numerous hormones, the study of substrates that have an inhibitory affect on the enzyme could lead to the development of pharmaceuticals that control metabolic disorders, associated with hormone activity.

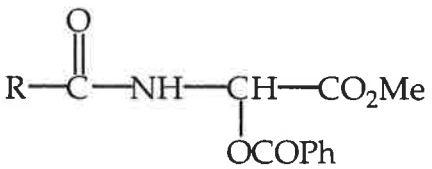
As described in Chapter 1 of this thesis, the reaction of *tert*-butyl perbenzoate with *N*-benzoylglycine methyl ester (15) has been used to introduce the benzoyloxy functionality at the α–carbon through hydrogen abstraction by *tert*-butoxy radical.[27] Reactions of this type were examined, initially to determine to what extent the ease of formation of the transition state, associated with hydrogen transfer from the glycine derivative (15) to *tert*-butoxy radical, is dependent upon the nature of the amide substituent.

To determine the generality of this reaction, *N*-acetylglycine methyl ester (80a) was treated with *tert*-butyl perbenzoate and cupric octanoate under identical conditions to those described for the reaction of *N*-benzoylglycine methyl ester (15) in Chapter 1. After work up, chromatography on the product gave the starting material (80a) and the α–benzoyloxyglycine derivative (81a), in 62% and 18% yield, respectively.

A ^{1}H NMR spectrum of the benzoate (81a) showed a characteristic doublet resonance at δ 6.58 due to the α–proton, coupled to the amide proton resonance by 10Hz, and a singlet was observed at δ 3.78 due to the methyl ester group. The

product (81a) was characterised using high resolution mass spectrometry giving an ion at *m/z* 251.079, corresponding to the molecular ion with a calculated mass of 251.079.





(80) (81)

a) R = Me

b) R = CF_3

Based on the procedure described above, to examine the effect of a fluorinated *N*-acyl substituent on this reaction, *N*-trifluoroacetylglycine methyl ester (80b) was treated with *tert*-butyl perbenzoate and cupric octanoate. After work up, analysis of the crude product by ^{1}H NMR spectroscopy and thin layer chromatography showed no evidence of the corresponding α–benzoyloxyglycine derivative (81b), only starting material (80b) could be detected.

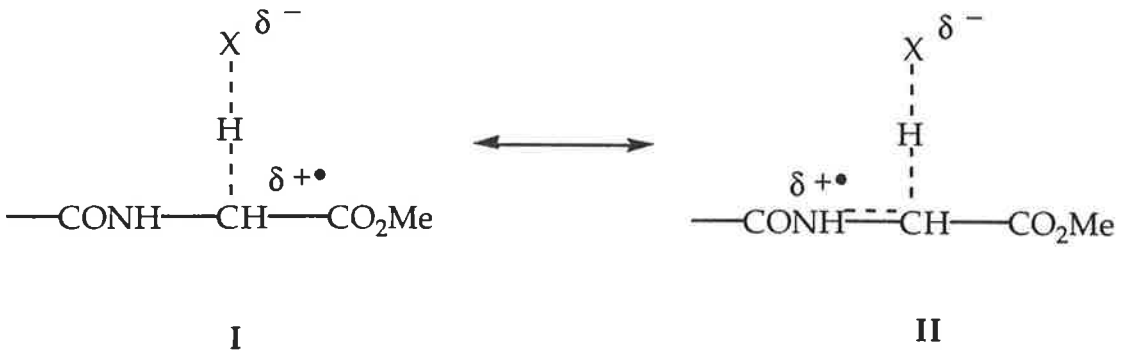
Due to the susceptibility of free radical and electron transfer processes to inhibition by trace impurities, a competitive reaction between the glycine derivatives (80a) and (80b) was performed to determine if there were impurities present in the *N*-trifluoroacetylglycine derivative (80b), which were preventing its reaction. If such impurities were present in the *N*-trifluoroacetylglycine derivative (80b), then these would also prevent the *N*-acetylglycine derivative (80a) from reacting in the competitive reaction, and the benzoate (81a) would not be produced under these circumstances.

Following the procedure described above with an equimolar mixture of the glycine derivatives (80a) and (80b), *N*-acetylglycine methyl ester (80a) reacted to give the corresponding benzoate (81a), as determined by ^{1}H NMR spectroscopic analysis of the reaction mixture. Again, none of the benzoate (81b) was observed. Therefore, the lack of reaction of *N*-trifluoroacetylglycine methyl ester (80b) must

be due to a decrease in reactivity of the glycine derivative (80b) compared with the non-fluorinated analogue (80a).

It has been observed that reaction of *N*-benzoylglycine methyl ester (15) and *N*-acetylglycine methyl ester (80a) with *tert*-butyl perbenzoate and cupric octanoate affords the corresponding α–benzoyloxyglycine derivatives (16) and (81a), however, the *N*-trifluoroacetylglycine derivative (80b) is inert under identical conditions. This contrast in reactivity may be attributed to the profound difference in the electron distribution in the reaction transition states. In the transition states associated with the hydrogen transfer reactions between the glycine derivatives (15) and (80a) and *tert*-butoxy radical, partial positive charge and partial radical character is generated at the site of hydrogen abstraction. It has been proposed that stabilisation of the electron deficient centre results from overlap of the semi-occupied p-orbital, developing at the α–centre, with the π–orbitals of the electron donating amido substituent and the electron withdrawing carboxy substituent.[84] Even though a semi-occupied p-orbital formed adjacent to a methoxycarbonyl substituent is resonance stabilised, formation of such an electron deficient centre by hydrogen transfer to *tert*-butoxy radical is often disfavored by a polar effect, involving the inductive interaction between the electron deficient centre of the substituent and that developing in the transition state at the site of hydrogen abstraction (Fig. 8-I-a). The ability of the acetamido substituent in the glycine derivative (80a) to delocalise the developing electron deficient centre (Fig. 8-II-a) outweighs the polar effect of the methoxycarbonyl substituent, and the rate of formation of the corresponding transition state is enhanced. However, the resonance stabilisation provided by the trifluoroacetamido substituent in the corresponding transition state is much less, due to the electron withdrawing effect of the fluorine substituents. Therefore, the lack of reactivity of the *N*-trifluoroacetylglycine derivative (80b) may be attributed to the inductively electron withdrawing effect of the methoxycarbonyl group in combination with the poor electron donating ability of the corresponding *N*-acyl substituent towards the stabilisation of the electron deficient centre developed in

the transition state. Presumably, in the reaction involving the fluorinated analogue (80b), *tert*-butoxy radical undergoes β–scission to produce methyl radical as an alternative to reacting by hydrogen abstraction.



I II

a) X = $OCMe_3$
b) X = OH
c) X = Br

Fig. 8 **I** Development of an electron deficient centre in the transition state for hydrogen atom abstraction by the species designated by X.
II Delocalisation of the electron deficient centre by an amido substituent.

The low yield of the benzoates (16) and (81a) obtained from reaction of the respective glycine derivatives (15) and (80a) with *tert*-butyl perbenzoate is a reflection of the poor ability of *tert*-butoxy radical to act as a hydrogen abstractor. To examine further the relationship between the nature of the amine protecting group of various glycine derivatives and the rate of formation of the transition state associated with hydrogen transfer reactions, a reaction involving an alternative hydrogen abstracting species was investigated.

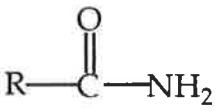
As discussed in the Introduction, a study has shown that the oxidative cleavage of *N*-benzoylamino acid methyl esters by nickel peroxide proceeds *via* a process similar to that catalysed by the PAM enzyme, to give the corresponding benzamides (Scheme 10).[46] The general procedure for the oxidative cleavage involves addition of the *N*-benzoylamino acid methyl ester to a heterogeneous

mixture containing relatively polar nickel peroxide hydrate suspended in the non-polar solvent, benzene. Following complexation of the substrate to the nickel catalyst, hydrogen transfer from the substrate to hydroxy radical proceeds through a transition state in which an electron deficient centre is developed at the α–position, the site of hydrogen abstraction (Fig. 8-I-b). The subsequent formation of an *N*-acylimine and/or α–hydroxyamino acid intermediate, followed by hydrolysis, affords the corresponding benzamide. To confirm the formation of benzamide (45) from oxidative cleavage of the glycine derivative (15) by nickel peroxide in a previous study,[46] a mixture of nickel peroxide and *N*-benzoylglycine methyl ester (15) in benzene was heated at reflux under nitrogen over-night. After work up and chromatography on the product, benzamide (45) was afforded in 36% yield, together with 56% unreacted starting material (15), which was in agreement with previous observations.[46] Both the melting point and ^{13}C NMR spectroscopic data of the isolated product (45) matched those of an authentic sample.

As discussed earlier in this Chapter, treatment of the *N*-acetylglycine derivative (80a) with *tert*-butyl perbenzoate and cupric octanoate afforded the corresponding benzoate (81a), whereas the trifluoroacetylglycine derivative (80b) remained inert under the same conditions. This was attributed to the electron withdrawing effect of the trifluoromethyl group which reduces the electron donating ability of the amide substituent towards the stabilisation of the electron deficient centre developed in the transition state. 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 hydroxy radicals.[109] If the stability of the transition state associated with hydrogen transfer from the glycine derivative (15) to hydroxy radical (Fig. 8-I-b) is similarly affected by the electron donating ability of the amide substituent, then this should be reflected in the reaction of fluorinated analogues of *N*-benzoylglycine methyl ester (15) with nickel peroxide.

To examine this possibility, the reactions of *N*-*p*-fluorobenzoylglycine methyl ester (82a) and *N*-pentafluorobenzoylglycine methyl ester (82b) with

(82)



(83)

a) R = *p*-F-C_6H_4
b) R = C_6F_5

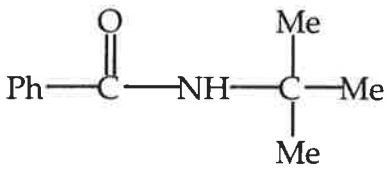
nickel peroxide were investigated.

Based on a procedure in the literature for the oxidative cleavage of the *N*-benzoylglycine derivative (15) with nickel peroxide,[46] the amino acid derivatives (82a) and (82b) were each treated with nickel peroxide to determine the products of the reaction. After work up and chromatography on the products, *p*-fluorobenzamide (83a) was isolated from reaction of the glycine derivative (82a) in 39% yield, and pentafluorobenzamide (83b) was isolated from reaction of the glycine derivative (82b) in 27% yield. In addition, the unreacted starting materials (82a) and (82b) were isolated from each reaction mixture in 52% and 60% yield, respectively. The combined yield of the unreacted starting material (82a) and the product (83a), and the combined yield of the unreacted starting material (82b) and the product (83b), indicates that the benzamides (83a) and (83b) represent major reaction pathways. The products, *p*-fluorobenzamide (83a) and pentafluorobenzamide (83b) were each identified from their mass spectra which showed molecular ions at *m/z* 123 and *m/z* 211, respectively, and by comparison of their melting points and thin layer chromatographs with those of authentic samples.

Under the conditions described above, the glycine derivatives (15), (82a) and (82b) each reacted with nickel peroxide in almost equal efficiency as the yields obtained of the corresponding benzamides (45), (83a) and (83b) were comparable. However, by limiting the concentration of nickel peroxide present in a reaction mixture containing two different glycine derivatives, the hydrogen transfer

reaction proceeding through the most stable transition state would occur in preference, and this should be reflected in the relative rates of formation of the product benzamides. Accordingly, competitive reactions between *N*-benzoylglycine methyl ester (15), *N*-*p*-fluorobenzoylglycine methyl ester (82a) and *N*-pentafluorobenzoylglycine methyl ester (82b) with nickel peroxide were examined in the presence of an internal standard.

Initially, *N*-*tert*-butylbenzamide (84) in benzene was treated with nickel peroxide, and the mixture was heated at reflux under nitrogen for 48h. After work up, the benzamide (84) was recovered in quantitative yield which indicated that it was inert under these reaction conditions. Due to the clear singlet at δ 1.47 in the ^{1}H NMR spectrum, its solubility in benzene, and its unreactivity under the conditions described above, *N*-*tert*-butylbenzamide (84) was chosen as the internal standard for the following competitive experiments with nickel peroxide.



(84)

To determine the rate of reaction of *N*-*p*-fluorobenzoylglycine methyl ester (82a) relative to *N*-benzoylglycine methyl ester (15), an equimolar mixture of the glycine derivatives (82a) and (15) in benzene was treated with nickel peroxide and the mixture was heated at reflux for 16h in the presence of the internal standard (84). ^{1}H NMR spectra of the reaction mixture were taken prior to reaction with nickel peroxide, and after work up. By comparing integrals of resonances characteristic of the starting materials (15) and (82a) with that of the *tert*-butyl singlet of the internal standard (84), it was found that *N*-benzoylglycine methyl ester (15) reacted faster than the fluorinated analogue (82a) by a factor of 1.2, with 39% and 33% of the corresponding starting materials (15) and (82a) consumed.

Similarly, a competitive reaction was performed between *N*-benzoylglycine methyl ester (15) and *N*-pentafluorobenzoylglycine methyl ester (82b). Comparison of the integrals of resonances in the ^{1}H NMR spectra of the crude product, as described above, indicated that the *N*-benzoylglycine derivative (15) reacted faster than the fluorinated analogue (82b) by a factor of 2.0. Further analysis of the reaction mixture by ^{1}H NMR spectroscopy showed that the reaction proceeded until 17% of *N*-pentafluorobenzoylglycine methyl ester (82b) and 31% of *N*-benzoylglycine methyl ester (15) had been consumed.

To increase the extent of reaction of glycine derivatives (15) and (82b) in the reaction with nickel peroxide, it was necessary to repeat the competitive reaction for an extended period of time. Accordingly, an equimolar mixture of the glycine derivatives (15) and (82b) in benzene was treated with nickel peroxide and the mixture was heated at reflux for 48h. After work up, analysis of the product by ^{1}H NMR spectroscopy, as described above, indicated that the reaction proceeded until 18% of the *N*-pentafluorobenzoylglycine derivative (82b), and 45% of the *N*-benzoylglycine derivative (15) had been consumed. Further analysis indicated that the rate of reaction of the *N*-benzoylglycine derivative (15) was greater than that of the fluorinated analogue (82b) by a factor of 2.9. When the competitive reaction was repeated over a period of 3 days, analysis of the crude product by ^{1}H NMR spectroscopy indicated that the *N*-benzoylglycine derivative (15) reacted faster than the fluorinated analogue (82b) by a factor of 1.8.

These results indicate that while there is clearly a selectivity for the *N*-benzoylglycine derivative (15) over the *N*-pentafluorobenzoylglycine derivative (82b) on reaction with nickel peroxide, the variation in the relative rates of reaction observed on changing the reaction time from a period of 16h to 3 days suggests that the magnitudes of these relative rates of reaction are somewhat unreliable. One possible source of error may lie in the extent of reaction of the glycine derivatives (15) and (82b), as this seems to be inherently irreproducible.

A study has shown that changes in temperature can affect the relative rates of reaction of amino acid derivatives with nickel peroxide.[46] To examine the

effect of a drop in temperature on the competitive reaction between the glycine derivatives (15) and (82b) the reaction was repeated as described above, except the reaction mixture was allowed to stir at room temperature for 48h. After work up, analysis of the product by ^{1}H NMR spectroscopy indicated that the reaction proceeded until 26% of the *N*-pentafluorobenzoylglycine derivative (82b) and 72% of the *N*-benzoylglycine derivative (15) had been consumed. This corresponds to a relative rate of 4.3. When the competitive reaction was repeated with a ten-fold increase in the concentration of each substrate (15) and (82b), under the conditions described above, ^{1}H NMR spectroscopic analysis of the crude product indicated that the reaction proceeded until 18% of *N*-pentafluorobenzoylglycine methyl ester (82b) and 46% of *N*-benzoylglycine methyl ester (15) had been consumed, corresponding to a relative rate of 3.1.

The inconsistency observed in the relative rates of reaction of the substrates (15) and (82b) with nickel peroxide on changing the substrate concentration and temperature suggests that factors other than just the ease of formation of the corresponding transition state contribute to the observed selectivity. The relative ease of binding of the glycine derivatives (15) and (82b) to the surface of the nickel peroxide in the heterogeneous reaction mixture may also play a significant role in the relative reactivity of these glycine derivatives.

The substrates (15) and (82b) are presumably bound to the nickel peroxide, at least in part, through electrons on the amide nitrogen. As a consequence of the electron withdrawing effect of the fluorine substituents, the *N*-pentafluorobenzoylglycine derivative (82b) is less likely to bind to the catalyst than the non-fluorinated analogue (15) since the electrons on the amide nitrogen are less available for complexation. In addition, it is likely that the glycine derivatives (15) and (82b) have different solubilities in the solvent, benzene, and therefore in competitive reactions one may preferentially bind to the relatively polar surface of the nickel catalyst. The extent to which these factors affect the rate of reaction with nickel peroxide would depend on the concentration of the substrates and temperature of the reaction mixture.

The nickel peroxide affinity for each substrate is also influenced by the surface area of the nickel catalyst, and its activity, which depends upon the percentage of available oxygen. Both of these factors vary from batch to batch of nickel peroxide produced in the laboratory and could further contribute to the inconsistency observed in the relative rates of reaction between the glycine derivatives (15) and (82b). In addition, even though oxidative cleavage is the major pathway in this reaction, there may be minor competing reaction pathways, such as hydrolysis of the amide bond, which are themselves temperature and concentration dependent.[85]

The results so far have indicated that the relative ease of binding of the substrates (15), (82a) and (82b) to the nickel catalyst, and the comparative ease of formation of the transition states in which semi-occupied p-orbitals are developing at the α–centres (Fig. 8-I-b), both contribute to the overall rate determining step in the reactions of these substrates with nickel peroxide. Therefore, the faster rate of reaction of the glycine derivative (15) with nickel peroxide compared with both fluorinated analogues (82a) and (82b) cannot be attributed only to the greater resonance stabilisation provided by the benzamido substituent in the transition state (Fig. 8-II-b).

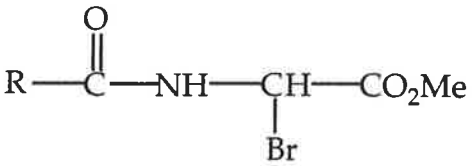
It was anticipated that through the investigation of a series of competitive reactions under homogeneous conditions, more accurate relative rate constants could be obtained, since the problems associated with a heterogeneous reaction mixture, described above, would be non-existent, and so reactions of these substrates with NBS were examined.

Reactions with NBS and *N*-acylglycine derivatives are known to proceed through a transition state in which a semi-occupied p-orbital is developing at the α-centre (Fig. 8-I-c).[41,86] Delocalisation of the electron deficient site is provided by the combined action of the electron donating benzamido substituent and the electron withdrawing methoxycarbonyl substituent (Fig. 8-II-c). Reactions of *N*-benzoylglycine methyl ester (15), *N*-*p*-fluorobenzoylglycine methyl ester (82a) and *N*-pentafluorobenzoylglycine methyl ester (82b) were investigated to examine the

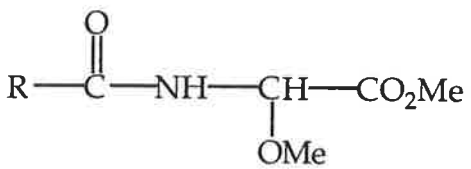
effect of the electron donating ability of the amide substituent on the efficiency of reaction of each substrate with NBS.

A solution of *N*-benzoylglycine methyl ester (15) in carbon tetrachloride was treated with NBS and the mixture was irradiated and heated at reflux under nitrogen for 30min. After work up, the crude product was recrystallised from a mixture of ethyl acetate and hexane to afford the α–bromoglycine derivative (26) in 79% yield. A characteristic doublet resonance was observed in the ^{1}H NMR spectrum at δ 6.70 due to the α–proton of the α–bromoglycine derivative (26), compared with a doublet at δ 4.27 due to the α–protons of the starting material (15). The resonances of the α–proton and amide proton were coupled by 10Hz in the bromide (26) and by 5Hz in the starting material (15). The ^{1}H NMR spectrum of the bromide (26) also showed a signal due to the methyl ester group at δ 3.93, and all observed resonances were consistent with those previously reported.[87]

Following the procedure described above, *N*-*p*-fluorobenzoylglycine methyl ester (82a) and *N*-pentafluorobenzoylglycine methyl ester (82b) were each treated with NBS and the corresponding bromides (85a) and (85b) were afforded in 69% and 61% yield, respectively. A ^{1}H NMR spectrum of the bromide (85a) showed a characteristic doublet resonance at δ 6.66 attributable to the α–proton, compared with a doublet at δ 4.25 due to the α–protons of the starting material (82a). A ^{1}H NMR spectrum of the bromide (85b) showed a doublet resonance at δ 6.57 due to the α–proton, compared with a signal at δ 4.27 attributable to the α–protons of the starting material (82b). In each bromide (85a) and (85b) the resonances of the



(85)



(86)

a) R = *p*-F-C_6H_4
b) R = C_6H_5

α–proton and amide proton were coupled by 10Hz, and these resonances were coupled by 5Hz in the starting materials (82a) and (82b).

Many α–haloglycine derivatives have a propensity to decompose over time through the facile elimination of hydrogen halide to form the *N*-acylimine. Hence, the more stable α–methoxyglycine derivatives (86a) and (86b) were prepared for full characterisation of these compounds. The bromides (85a) and (85b) were each added to methanol and the resultant mixtures were allowed to stir for 20min. After concentration under reduced pressure, chromatography on the crude products obtained from reaction of the bromides (85a) and (85b) gave the corresponding methoxides (86a) and (86b) in 87% and 77% yield, respectively. A ^{1}H NMR spectrum of the methoxide (86a) showed a doublet resonance at δ 5.76 due to the α–proton, and characteristic singlet resonances at δ 3.86 and δ 3.54 for the methyl ester and methoxy group, respectively. A ^{1}H NMR spectrum of the methoxide (86b) showed a doublet at δ 5.71 for the α–proton and signals due to the methyl ester and methoxy groups were observed at δ 3.86 and δ 3.55, respectively. The α–proton and amide proton resonances were coupled by 9Hz in the methoxides (86a) and (86b), the structures of which were confirmed using elemental analysis.

The percentage yields of the bromides (85a) and (85b) obtained from reaction of the corresponding glycine derivatives (82a) and (82b) with NBS was lower than that of the bromide (26) obtained from the reaction of *N*-benzoylglycine methyl ester (15), under the same conditions. This implies that the efficiency of reaction of the fluorinated analogues (82a) and (82b) is lower than that of the non-fluorinated analogue (15). To determine the relative reactivity of the glycine derivatives (15), (82a) and (82b) with NBS, competitive reactions were performed with a limited amount of NBS, in the presence of the internal standard (84).

Accordingly, an equimolar mixture of *N*-benzoylglycine methyl ester (15) and *N*-*p*-fluorobenzoylglycine methyl ester (82a) in carbon tetrachloride was treated with 1 equivalent of NBS. The mixture was irradiated and heated at reflux under nitrogen for 45min. ^{1}H NMR spectra of the reaction mixture were

taken prior to, and after reaction with NBS. By comparing integrals of resonances characteristic of the starting materials (15) and (82a) with that of the *tert*-butyl singlet of the internal standard (84), it was found that the reaction proceeded until 59% of *N*-benzoylglycine methyl ester (15) and 53% of *N*-*p*-fluorobenzoylglycine methyl ester (82a) had been consumed. This indicated that the *N*-benzoylglycine derivative (15) reacted 1.2 times faster than the fluorinated analogue (82a) with NBS. The peaks in the ^{1}H NMR spectrum were confirmed by spiking the crude product with authentic standards.

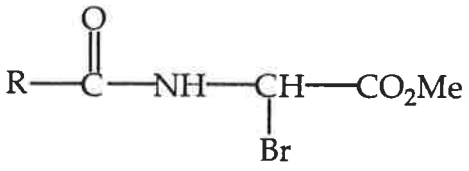
Based on the procedure described above, a competitive reaction between *N*-benzoylglycine methyl ester (15) and *N*-pentafluorobenzoylglycine methyl ester (82b) with NBS was performed. After work up, ^{1}H NMR spectroscopic analysis of the crude product indicated that the reaction proceeded until 71% and 27% of the corresponding starting materials (15) and (82b) had been consumed. This implied that the *N*-benzoylglycine derivative (15) reacted 4.0 times faster than the *N*-pentafluorobenzoylglycine derivative (82b) with NBS. Again, peaks in the ^{1}H NMR spectrum were confirmed by spiking the crude product with authentic standards.

The relative rates of reaction of the glycine derivatives (15), (82a) and (82b) with NBS reflect the comparative ease of formation of the transition states in which a semi-occupied p-orbital is developing at the α–centre. The greater difference in reactivity between the glycine derivatives (15) and (82b) compared to that between the glycine derivatives (15) and (82a), can be attributed to the greater resonance stabilisation provided by the *p*-fluorobenzamido substituent than by the pentafluorobenzamido substituent in the respective transition states. The electron withdrawing effect of five fluorine substituents on the *N*-acyl moiety is greater than the electron withdrawing effect of a single fluorine substituent, such that, in combination with the inductively electron withdrawing methoxycarbonyl substituent, the transition state generated in the hydrogen transfer between the glycine derivative (82b) and bromine atom is markedly less stable than the transition states generated in the reactions of both (15) and (82a).

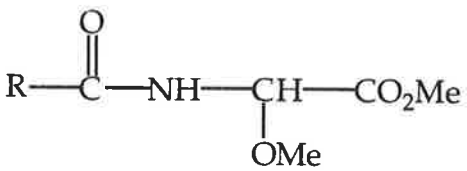
To determine the generality of this phenomenon, reactions of substrates with different amide substituents were investigated. Due to the appreciable difference in reactivity between *N*-acetylglycine methyl ester (80a) and *N*-trifluoroacetylglycine methyl ester (80b) observed with *tert*-butyl perbenzoate and cupric octanoate, reactions of these substrates with NBS were examined.

Based on the procedure described above, treatment of *N*-acetylglycine methyl ester (80a) with NBS gave the corresponding α–bromoglycine derivative (87a) in 60% yield, after chromatography on silica. The ^{1}H NMR spectrum of the bromide (87a) showed a characteristic doublet resonance at δ 6.48 due to the α–proton, compared with a singlet at δ 4.07 due to the α–protons of the starting material (80a). The α–proton and amide proton resonances of the bromide (87a) were coupled by 10Hz, and the spectral characteristics were in accord with those previously reported.[88]

The corresponding stable α–methoxyglycine derivative (88a) was prepared for characterisation. The bromide (87a) was left to stir in methanol for 20min, and after work up gave the methoxide (88a) in 74% yield. The ^{1}H NMR spectrum showed a doublet resonance at δ 5.53 due to the α–proton which was coupled to the amide proton resonance by 9Hz. Characteristic singlet resonances were observed at δ 3.82 and δ 3.46 due to the methyl ester and methoxy group, respectively. The spectral characteristics were consistent with those found in the literature.[89]



(87)



(88)

a) R = Me
b) R = CF_3

Similarly, *N*-trifluoroacetylglycine methyl ester (80b) was treated with NBS to afford the corresponding α–bromoglycine derivative (87b) in 63% yield. A characteristic doublet resonance was observed at δ 6.36 in the ^{1}H NMR spectrum of the product (87b) attributable to the α–proton, compared with a doublet at δ 5.14 for the α–protons of the starting material (80b). The α–proton and amide proton resonances of the bromide (87b) were coupled by 10Hz, and the resonances were coupled by 5Hz in the starting material (80b). The corresponding α–methoxyglycine derivative (88b) was prepared for characterisation, and after work up followed by chromatography on silica, gave the methoxide (88b) in 80% yield. A ^{1}H NMR spectrum of the product (88b) showed a doublet at δ 5.53 attributable to the α–proton and two singlets were observed at δ 3.87 and δ 3.53 characteristic of the methyl ester and methoxy group, respectively. The α–proton and amide proton resonances were coupled by 9Hz, and the structure of the methoxide (88b) was confirmed using elemental analysis.

To determine the rate of reaction of the *N*-acetylglycine derivative (80a) relative to the *N*-benzoylglycine derivative (15) with NBS, a competitive reaction was performed between the two substrates in the presence of the internal standard (84), as described above. Analysis of the crude product by ^{1}H NMR spectroscopy indicated that the reaction proceeded until 75% of the *N*-acetylglycine derivative (80a) and 69% of the *N*-benzoylglycine derivative (15) had been consumed. This indicates that the *N*-acetylglycine derivative (80a) reacts 1.2 times faster than the *N*-benzoylglycine derivative (15) with NBS.

Similarly, a competitive reaction between the *N*-trifluoroacetylglycine derivative (80b) and the *N*-benzoylglycine derivative (15) was performed. Analysis of the mixture by ^{1}H NMR spectroscopy indicated the presence of the α–bromoglycine derivative (26) and the unreacted *N*-trifluoroacetylglycine derivative (80b). The α–bromoglycine derivative (87b) was not produced under these conditions and therefore the rate of reaction of the *N*-trifluoroacetylglycine derivative (80b) relative to that of *N*-benzoylglycine methyl ester (15) could not be measured directly. The relative rate constants measured from the competitive

reactions of *N*-benzoylglycine methyl ester (15) with the glycine derivatives (80a), (82a) and (82b) indicated that the *N*-pentafluorobenzoylglycine derivative (82b) reacted the least efficiently with NBS. It was therefore assumed that the difference in reactivity between the glycine derivatives (80b) and (82b) would be considerably smaller than the difference in reactivity between the glycine derivatives (15) and (80b), such that the rate of reaction of the *N*-trifluoroacetylglycine derivative (80b) relative to the *N*-pentafluorobenzoylglycine derivative (82b) could be quantified. Accordingly, a competitive reaction between the glycine derivatives (80b) and (82b) was performed, as described above. Analysis of the product by ^{1}H NMR spectroscopy indicated that the reaction proceeded until 85% and 36% of the corresponding glycine derivatives (82b) and (80b) had been consumed. This corresponds to a relative rate of 4.2. Therefore, by extrapolation, the *N*-benzoylglycine derivative (15) reacts faster than the *N*-trifluoroacetylglycine derivative (80b) by a factor of approximately 17.

By reducing the electron donating ability of the amide substituent of *N*-benzoylglycine methyl ester (15) and *N*-acetylglycine methyl ester (80a) through the introduction of an electron withdrawing group on the *N*-acyl substituent, the efficiency of reaction of the fluorinated analogues (80b), (82a) and (82b) with NBS was reduced relative to the non-fluorinated analogues (15) and (80a). It seemed likely that replacement of the *N*-acyl substituent with a more efficient electron donating substituent, such as a carbamate, could accelerate reaction with NBS.

Accordingly, *N-tert*-butoxycarbonylglycine methyl ester (55a) was treated with NBS to give the corresponding α–bromoglycine derivative (89) as an oil, in 78% yield, after Kugelrohr distillation. A ^{1}H NMR spectrum of the bromide (89) showed a doublet resonance at δ 5.94 due to the α–proton, coupled to the resonance of the amide proton by 10Hz, and a singlet was observed at δ 3.75 attributable to the methyl ester group. The structure of the bromide (89) was confirmed by comparison of physical and spectral data to that found in the literature.[90]

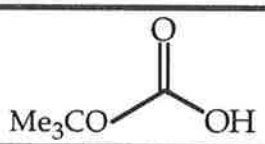
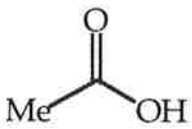
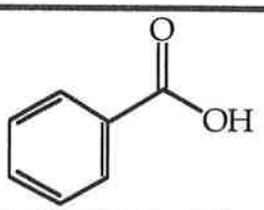
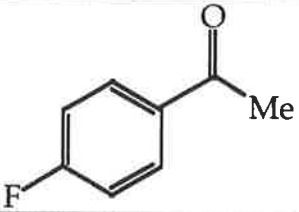
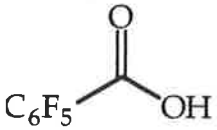
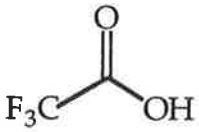
(82b) and the carbamate substituent of the glycine derivative (55a) relate to the electron donating ability of the corresponding *N*-acyl or carbamate substituent, and are presented in Table (1), along with the measured relative rate constants. The rate of reaction of *N*-benzoylglycine methyl ester (15) with NBS has been assigned unity, and k_{rel} refers to the rate of reaction of the substrate with NBS relative to the glycine derivative (15). The pK_a values listed were found in the literature,[91] except for the carboxylic acid corresponding to the carbamate substituent of *N*-*tert*-butoxycarbonylglycine methyl ester (55a). The pK_a of *tert*-butylcarbonic acid was approximated to be in the range 6-7 by analogy with carbonic acid, which has a pK_a of 6.3. It was presumed that the alkyl group on *tert*-butylcarbonic acid would destabilise the anion slightly and may increase the pK_a towards a value of 7.

The results in Table (1) indicate that there is a direct correlation between the relative rate of reaction of each substrate and the electron donating ability of the corresponding *N*-substituent which is reflected in the pK_a values of the acids corresponding to the *N*-acyl substituents of the glycine derivatives (15), (80a), (80b), (82a) and (82b) or the carbamate substituent of the glycine derivative (55a).

The carboxylic acid corresponding to the carbamate substituent of the glycine derivative (55a) has the highest pK_a value, approximated to be in the range 6-7. This implies that the carbamate substituent has the greatest ability to donate electrons towards the stabilisation of the electron deficient site generated in the transition state. This effect is reflected in the relative rate of reaction of the glycine derivative (55a), which is the greatest in this series.

The difference between the pK_a values of the carboxylic acids corresponding to the *N*-acyl substituent of the glycine derivatives (80a), (15) and (82a) is slight. This trend is also reflected in the corresponding relative rates of reaction with NBS, as the k_{rel} values of the glycine derivatives (80a), (15) and (82a) deviate only marginally from unity. It may be deduced from these results that if there is only marginal variation in the electronic nature of the amide substituent, characterised by the variation in the magnitudes of the pK_a values, then the

Table (1) Comparison of the relative rates of reaction of *N*-substituted glycine derivatives with NBS, and their correlation to the *p*Ka values of the carboxylic acids corresponding to the *N*-substituents.

Substrate	k_{rel} **of Substrate**	**Carboxylic Acid**	pK_a **of Carboxylic Acid**
(55a)	2.6		6.0-7.0
(80a)	1.2		4.7
(15)	1.0*		4.2
(82a)	0.86		3.8
(82b)	0.25		1.5
(80b)	0.05		0.3

* Assigned as unity.

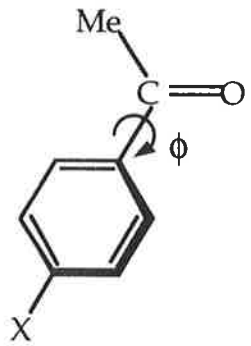
relative rates of reaction with NBS also differ only marginally. Therefore, the energy of the transition states generated in the hydrogen transfer reactions between the glycine derivatives (80a), (15) and (82a) and bromine radical must also be similar, since the extent of delocalisation of the electron deficient centre is controlled, in part, by the electronic nature of the amide substituent.

The difference between the pK_a values of the carboxylic acids corresponding to the *N*-acyl substituents of *N*-benzoylglycine methyl ester (15) and *N*-trifluoroacetylglycine methyl ester (80b) is quite substantial. This may explain the considerable difference in reactivity between the two substrates, and why the product from reaction of the *N*-trifluoroacetylglycine derivative (80b) with NBS could not be detected in competitive reactions between these glycine derivatives.

At first glance, it was thought that the difference in the electron donating ability of the *N*-acyl substituents of *N*-benzoylglycine methyl ester (15) and *N*-pentafluorobenzoylglycine methyl ester (82b) would be greater than the difference in the electron donating ability of the *N*-acyl substituents of *N*-acetylglycine methyl ester (80a) and *N*-trifluoroacetylglycine methyl ester (80b). The reason for this lay in the assumption that the five fluorine substituents on the aromatic ring of the glycine derivative (82b) could operate not only through an inductive effect, but could also interact with the π–system of the benzamido substituent through resonance, unlike the trifluoromethyl group which could only operate through an inductive effect. However, competitive studies with NBS have revealed that the difference in the rate of reaction between the glycine derivatives (80a) and (80b) is greater than the difference in the rate of reaction between the glycine derivatives (15) and (82b). This phenomenon is also exhibited in the relative pK_a values. That is, the difference in the acidity between the carboxylic acids corresponding to the *N*-acyl substituents of the glycine derivatives (80a) and (80b) is greater than the difference between that for the glycine derivatives (15) and (82b). This phenomenon may be explained by comparing these observations with results from a previous study involving *p*-substituted acetophenones.[92]

Earlier studies by Mirarchi and Ritchie[92] using experimental dipole

moments and infinite-dilution molar Kerr constants have shown that acetophenone (90a) favours a uniplanar conformation as a result of conjugation between the acetyl group and the aromatic ring (Fig. 9a). The study also showed that the electron withdrawing inductive and resonance effect of a *p*-fluoro substituent resulted in diminished conjugation between the acetyl group and the ring. From measured Kerr constants, Mirarchi and Ritchie predicted a dihedral angle of 27± 5° between the planes of the carbonyl and aryl groups of *p*-fluoroacetophenone (90b) (Fig. 9b).[92] This is supported by findings by Belsham and co-workers[93] who reported a dihedral angle of 33° for this molecule, measured using ^{19}F NMR spectroscopic data.



(90)

Fig. 9 a) X = H; ϕ ~ 0° dihedral angle
b) X = F; ϕ ~ 27± 5° dihedral angle

As discussed in the Introduction, the radical intermediate (20) formed in the reaction of *N*-benzoylglycine methyl ester (15) with NBS is stabilised through overlap of the semi-occupied p-orbital developing at the α–centre with the electrons on the nitrogen and the π–system of the ring, such that maximum overlap occurs when the ring is coplanar to the carbonyl group. X-Ray crystallographic analysis of the glycine derivative (15) (Appendix 1) indicated a dihedral angle of 14° between the aryl and carbonyl groups. It was thought that the electron withdrawing nature of the five fluorine substituents on the aromatic ring of the *N*-pentafluorobenzoylglycine derivative (82b) was likely to diminish

conjugation relative to the unsubstituted glycine derivative (15), as was observed for *p*-fluoroacetophenone (90b) relative to acetophenone (90a).[92] Indeed, X-ray crystallographic analysis of the *N*-pentafluorobenzoylglycine derivative (82b) (Appendix 2) indicated a dihedral angle of 53° between the planes of the carbonyl and aryl groups. This implies that no interaction is possible between the π–systems of the aromatic ring and the carbonyl group for this molecule.

As a consequence of the non-uniplanarity of the glycine derivative (82b), the degree of stability of the corresponding transition state (Fig. 8-c), generated from reaction of (82b) with NBS, cannot be influenced by resonance as originally assumed, but purely through induction.

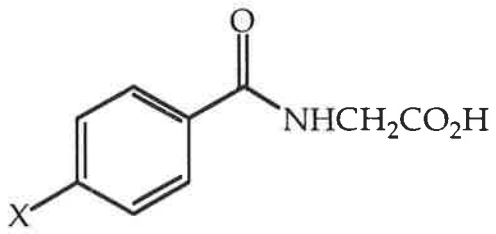
The results discussed so far in this Chapter illustrate the important role of the amine protecting group in determining the reactivity of amino acid derivatives. Competitive experiments involving reactions of the glycine derivatives (80a) and (80b) with *tert*-butyl perbenzoate and cupric octanoate gave a preliminary indication that the electronic nature of the amide substituent can greatly influence the reactivity of the corresponding glycine derivative. The results obtained from competitive reactions of the glycine derivatives (15), (82a) and (82b) with nickel peroxide suggested a selectivity for (15) over the fluorinated analogues (82a) and (82b), however due to complex binding effects associated with the heterogeneity of the nickel peroxide reaction, relative rates of reaction could not be accurately quantified. In any case, a study of the relative rates of reaction of the glycine derivatives (15), (55a), (80a), (80b), (82a) and (82b) with NBS, under homogeneous conditions, indicated a correlation between the relative rates of reaction of these substrates and the pK_a values for the carboxylic acids corresponding to the *N*-acyl substituents of the glycine derivatives (15), (80a), (80b), (82a) and (82b) and the carbamate substituent of the glycine derivative (55a). The results established that the relative rates of reaction of the glycine derivatives (15), (55a), (80a), (80b), (82a) and (82b) with NBS reflect the comparative ease of formation of the corresponding transition state in which an electron deficient centre is developed as a result of hydrogen transfer between the glycine

derivative and bromine atom (Fig. 8-I-c). The stability of this transition state was attributed to the extent of resonance stabilisation provided by the *N*-acyl or carbamate substituent (Fig. 8-II-c).

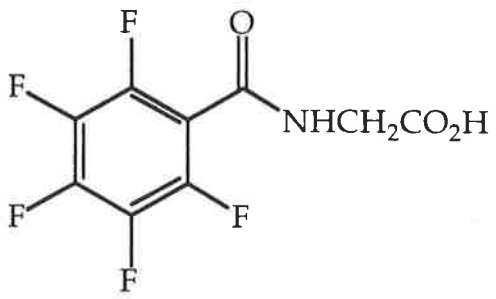
As discussed in the Introduction, the bioactivation of many peptide hormones and neuropeptides involves the PAM-catalysed oxidative cleavage of carboxy-terminal glycine extended precursors. *N*-Benzoylglycine (91a), or hippuric acid, is one of the simplest known peptide substrates of PAM and undergoes oxidative cleavage to produce benzamide (45).[50] It is therefore apparent that only the minimal structure of an acylglycine is required for active site turnover. Although the detailed mechanism of action of PAM is yet to be established, many characteristics of chemical models which proceed through glycyl radical intermediates closely mimic the features displayed in this enzyme-catalysed reaction.[46,47,61] It was anticipated that the difference in reactivity observed between the glycine derivatives (15) and (82b) with NBS may therefore be reflected in reactions of the glycine derivatives (91a) and (92), catalysed by PAM. To ascertain whether the products of the PAM-catalysed reaction of *N*-benzoylglycine (91a) and the fluorinated analogue (92) could be detected by high performance liquid chromatography (HPLC), authentic samples of benzamide (45) and pentafluorobenzamide (83b) were injected onto a C_{18}-reverse phase silica column, using UV detection at 220nm. The amides (45) and (83b) were eluted with a gradient of acetonitrile in water, at a flow rate of 1ml/min and were found to have retention times of 3.9min and 7.8min, respectively. The response ratio of benzamide (45) to pentafluorobenzamide (83b) was measured to be 1.12 : 1. Having established retention times for the authentic standards on a reverse phase HPLC column, it was envisaged that the enzymatically produced benzamides could be detected in the same way.

A competitive experiment was performed between the glycine derivatives (91a) and (92), to examine the effect of the fluorinated analogue (92) on the reaction catalysed by PAM, in relation to the known substrate (91a).

Based on a procedure in the literature,[50] a buffered solution containing



(91)



(92)

a) X = H
b) X = NO_2
c) X = OMe

PAM, sodium ascorbate, copper sulphate and catalase obtained from bovine liver, was incubated at 37° in a constant temperature shaking water bath. Reaction was initiated by the addition of an equimolar amount of *N*-benzoylglycine (91) and *N*-pentafluorobenzoylglycine (92). After a 24h period, the enzymatic assay was quenched with 10% sodium hydroxide to inactivate the PAM enzyme and to convert any of the unreacted starting materials (91a) and (92) to their corresponding sodium carboxylates. The solution was subsequently freeze dried under reduced pressure to yield a residue which was carefully triturated with chloroform, leaving behind the sodium salts of any unreacted starting materials. The combined chloroform extracts were evaporated to dryness and the product obtained was analysed using HPLC under the conditions described above for the analysis of the authentic amides. HPLC analysis indicated the presence of both benzamide (45) and pentafluorobenzamide (83b) which suggested that *N*-pentafluorobenzoylglycine (92) is also a substrate of the enzyme. The identification of the enzymatically produced amides (45) and (83b) was accomplished on the basis of HPLC co-elution with authentic standards. In addition, a FAB mass spectrum of the sample, using glycerol as a matrix, showed a peak at *m/z* 121 corresponding to the molecular ion of benzamide (45), which confirmed its identity. The HPLC trace also showed that the products from the enzyme-catalysed reaction (45) and (83b) were present in a ratio of approximately

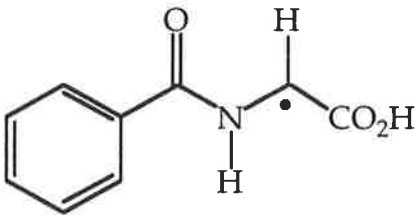
4:1. This indicated that when the substrates were treated with the enzyme, pentafluorobenzamide (83b) was produced at approximately 1/4 of the rate of benzamide (45). This selectivity is comparable to that observed in the competitive reaction involving the glycine derivatives (15) and (82b) with NBS, shown in Table (1).

To confirm the different rates of reaction observed for the glycine derivatives (91a) and (92), the competitive reaction was repeated as described above, except a different supply of enzyme was used. After work up, analysis of the crude product by HPLC indicated the presence of benzamide (45), however chromatography failed to detect any pentafluorobenzamide (83b). The enzymatically produced benzamide (45) co-eluted with an authentic standard, however the peak attributable to authentic pentafluorobenzamide (83b) did not coincide with any peaks in the HPLC trace of the crude product. Taking into consideration the limits of detection of the HPLC, this indicated that the ratio of benzamide (45) to fluorobenzamide (83b) in the HPLC trace was at least 20:1. This implies that *N*-benzoylglycine (91a) reacted faster than the fluorinated analogue (92) by a factor of at least 20.

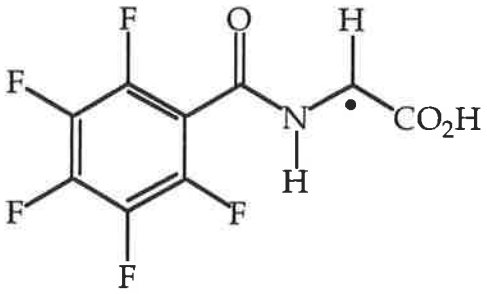
To determine the extent of reaction of the glycine derivatives (91a) and (92) catalysed by PAM, unreacted starting materials were recovered from the reaction mixture and analysed by ^{1}H NMR spectroscopy. The ^{1}H NMR spectrum indicated the presence of both *N*-benzoylglycine (91a) and *N*-pentafluorobenzoyglycine (92) in a ratio of approximately 1:1. This tends to suggest that the enzyme-catalysed reaction was terminated at the initial stages of reaction. Presumably, in the first competitive experiment where *N*-benzoylglycine (91a) was metabolised by the enzyme at 4 times the rate of the fluorinated analogue (92), the extent of reaction was greater as a result of a more active enzyme being used.

In both of the competitive experiments performed between the glycine derivatives (91a) and (92), the fluorinated analogue (92) was metabolised at a slower rate relative to the known substrate (91a). The difference in the reactivity between these substrates may be explained by the following factors. The

fluorinated analogue (92) may bind less readily to the enzymes active site and therefore would have a slower rate of conversion to the amide, or the fluorinated analogue (92) once bound to the enzyme may react less readily to produce the corresponding glycyl radical intermediate (94). It is possible that the difference in reactivity between the substrates (91a) and (92) is due to a combination of these factors. Since fluorine is known to have a small Van der Waals radius, closely resembling that of hydrogen,[94] the binding affinity of the enzyme for *N*-pentafluorobenzoylglycine (92) would presumably be similar to that for the known substrate (91a), since the fluorine substituents on the aromatic ring are unlikely to drastically alter the steric demands of the corresponding *N*-acyl moiety. In the absence of a full kinetic analysis, which is not possible due to the expense of the enzyme, it seems likely that the difference in reactivity between the glycine derivatives (91a) and (92) is due to a difference in reactivity once the substrates are bound in the active site, at least in part, rather than due to binding effects. Consistent with this hypothesis, the faster rate of conversion of *N*-benzoylglycine (91a) compared with that of *N*-pentafluorobenzoylglycine (92) may be attributed to the greater resonance stabilisation provided by the benzamido substituent in the glycyl radical intermediate (93) than by the fluorinated substituent in the intermediate (94).



(93)



(94)

The results obtained from the competitive experiments with PAM therefore give a preliminary indication that the *N*-acyl substituent of *N*-

pentafluorobenzoylglycine (92) has an inhibitory effect on the reaction catalysed by the enzyme.

Table (2) shows the results obtained by Katopodis and May[50] when they incubated each of the substrates *N*-benzoylglycine (91a), *N*-*p*-nitrobenzoylglycine (91b) and *N*-*p*-methoxybenzoylglycine (91c) with PAM. It is evident from their findings that no correlation exists between the pK_a values for the carboxylic acids corresponding to the *N*-acyl substituents of these glycine derivatives, and the relative rates of substrate turnover (V_{max}). Their results are in direct contrast to the trend observed in the competitive reactions involving the glycine derivatives, shown in Table (1) with NBS, even though the catalytic cycle involving PAM presumably proceeds through a similar transition state to that generated in the reactions with NBS. This could be attributed to the *p*-nitro and *p*-methoxy groups which substantially alter the steric demands of the *N*-acyl substituents in the corresponding substrates (91b) and (91c) compared to the *N*-benzoyl substituent in the substrate (91a). As a consequence, the binding effects associated with the substrates (91a), (91b) and (91c) may become a critical factor in the rate determining step for the PAM-catalysed reaction, outweighing the electronic effects of the corresponding amide substituents. These electronic

Table (2) Kinetic parameters of PAM substrates.[50]

Substrate	pK_a*	Binding Constant **K_m (mM)**	Rate of Substrate Turnover **V_{max} (nmol/mg/h)**
(91a)	4.2	1.7	109
(91b)	3.4	1.0	1370
(91c)	4.5	1.6	493

* Refers to the pK_a of the carboxylic acid corresponding to the *N*-acyl substituent of the substrate.

effects, reflected in the pK_a values of the carboxylic acids corresponding to the *N*-acyl substituents of the substrates (91a), (91b) and (91c) differ only marginally from each other. Fluorine, on the other hand, is known to have a Van der Waals radius closely resembling that of hydrogen. Therefore, the steric demands of the *N*-acyl substituents of the glycine derivatives (91a) and (92) would be similar. Since these glycine derivatives have profound differences in the electron density on the nitrogen, the electronic nature of the corresponding *N*-acyl substituents would play a critical role in the rate of conversion of the substrates (91a) and (92) with PAM, which reflects the trend observed in Table (1).

In summary, a change in the catalytic activity of PAM was observed during competitive reactions involving the glycine derivatives (91a) and (92). Assuming that the enzyme-substrate binding constants for these glycine derivatives are similar, the competitive experiments give a preliminary indication that the fluorinated analogue (92) has a significantly lower rate of conversion with the enzyme as a result of the electronic nature of the corresponding amide substituent, rather than due to any binding effects. These preliminary results indicate that the ease of formation of the α–carbon centred radical is a critical factor in the catalytic cycle, and may therefore be the rate determining step.

It is possible that due to the relative ease of formation of glycyl radicals,[39] and the preferential complexation of glycine derivatives by metal ions described in Chapter 1 of this thesis, the naturally occurring substrates of PAM are synthesised with a glycine residue at the *C*-terminus because it is so easily removed by oxidation.

Synthesis of Substrates

The *N*-protected glycine methyl esters (80a), (80b), (82a) and (82b) required for investigation in this Chapter were synthesised as described below.

The hydrochloride salt of glycine methyl ester (74) was obtained by addition of glycine (72) to methanol which had been pre-treated with thionyl chloride.

The *N*-acetylglycine derivative (80a) was prepared by treatment of the hydrochloride salt of glycine methyl ester (74) with acetyl chloride under basic conditions. This gave the methyl ester (80a) as a clear oil in 74% yield. The ^{1}H NMR spectrum of the product (80a) showed singlets at δ 3.77 and δ 2.17 attributable to the methyl ester and acetyl group, respectively. The structure of the product (80a) was confirmed using elemental analysis.

The *N*-trifluoroacetylglycine derivative (80b) was prepared by the addition of trifluoroacetic anhydride to the glycine methyl ester hydrochloride (74), according to a procedure in the literature.[110] After work up, the crude product was purified by Kugelrohr distillation to yield the *N*-trifluoroacetylglycine derivative (80b) in 87% yield. A ^{1}H NMR spectrum of the product (80b) showed a singlet at δ 3.81 and a doublet at δ 4.14, attributable to the methyl ester group and the α–protons, respectively. The spectral characteristics were consistent with those of an authentic sample.

The glycine derivative (82a) was prepared by treatment of the hydrochloride salt of glycine methyl ester (74) with *p*-fluorobenzoyl chloride under basic conditions. After work up, the crude product was recrystallised from a mixture of ethyl acetate and hexane to afford the *N*-protected derivative (82a) in 73% yield. A ^{1}H NMR spectrum of the product (82a) showed a doublet resonance at δ 4.25 and a singlet resonance at δ 3.81, attributable to the α–protons and the methyl ester group, respectively. The structure of the product (82a) was confirmed using elemental analysis.

Similarly, the glycine derivative (82b) was prepared by treatment of the salt (74) with pentafluorobenzoyl chloride under basic conditions. This afforded the

N-protected derivative (82b) in 76% yield. A ^{1}H NMR spectrum of the product (82b) showed a doublet at δ 4.27 and a singlet at δ 3.81 due to the α–protons and the methyl ester group, respectively, and the structure was confirmed using elemental analysis.

N-Pentafluorobenzoylglycine (92) was prepared by hydrolysis of the corresponding methyl ester (82b) under basic conditions. The structure of the product (92) was confirmed using mass spectrometry and infra-red spectroscopy.

RESULTS AND DISCUSSION: CHAPTER 3

An Investigation into the Stereochemical Course of Reaction of *(R)*-Phenylalanine (50) with *(S)*-Phenylalanine Ammonia Lyase

The elimination reaction of *(S)*-phenylalanine (49), catalysed by PAL, proceeds *via* abstraction of the 3-*pro-S* hydrogen with *anti*-elimination of the amino group leading to formation of *trans*-cinnamic acid (48) (Fig. 3).[65] Interestingly, the enzyme is able to metabolise *(R)*-phenylalanine (50) even though it has the opposite chirality to the natural substrate (49).[67] Earlier studies[68] involving the reactions of deuterium labelled *(S)*- and *(R)*-phenylalanine with PAL have shown that even though the major reaction pathway for the unnatural substrate (50) is abstraction of the 3-*pro-R* hydrogen, the reaction is not completely stereospecific, as is the case with *(S)*-phenylalanine (49). A possible mechanism to account for the minor reaction pathway involves the concerted removal of the 3-*pro-S* hydrogen with *syn*-elimination of ammonia (Fig. 7).[68] Alternatively, reversible abstraction of the α–hydrogen from *(R)*-phenylalanine (50), and racemisation, may compete with loss of the 3-*pro-R* hydrogen.

The work described in this Chapter initially examines reactions of modified substrates with PAL to determine whether the mechanism of the β–hydrogen elimination is being altered by the enzyme, in order to accommodate the different structural geometry of the unnatural substrate (50). In addition, the synthesis of the stereoisomers *(2R,3S)*- and *(2R,3R)*-2,3-dideuteriophenylalanine (54a) and (54b) enabled a further investigation into the stereochemical course of the minor reaction pathway.

To determine the generality of the minor reaction exhibited in the PAL-catalysed deamination of *(R)*-phenylalanine (50), analogues of the unnatural substrate (50) were treated with the enzyme, initially to establish if they are

substrates of the enzyme.

The PAL-catalysed reaction of the known substrate, *(S)*-tyrosine (51a), was performed in order to isolate and characterise the major product of the reaction and to determine the relative activity of the enzyme. Based on a procedure in the literature,[68] a solution of *(S)*-tyrosine (51a) and PAL in sodium borate buffer was incubated at 30° over 3 days. After work up, the product from the reaction was obtained in 61% yield and analysed by ^{1}H NMR spectroscopy. This indicated the presence of *trans-p*-hydroxycinnamic acid (53a) and the corresponding *cis*-isomer, in a ratio of approximately 6:1. The *trans*-isomer (53a) was characterised by doublet resonances at δ 6.21 and δ 7.55 attributable to the protons at the 2- and 3-position, respectively, with the resonances mutually coupled by 16Hz. The *cis*-isomer was characterised by doublets at δ 5.72 and δ 7.66 due to the protons at the 2- and 3-position, respectively, with the resonances mutually coupled by 12Hz. The spectral characteristics of *trans-p*-hydroxycinnamic acid (53a) and the corresponding *cis*-isomer were consistent with those of authentic samples.

To determine if PAL catalysed the elimination of ammonia from *(R)*-tyrosine (52a), the unnatural substrate was treated with the enzyme and incubated at 30° over 7 days. After work up, a ^{1}H NMR spectrum of the product indicated the presence of *p*-hydroxycinnamic acid (53a), however only a trace amount had been isolated from the reaction mixture. It was thought that some of the *p*-hydroxycinnamic acid (53a) produced from reaction of *(R)*-tyrosine (52a) with PAL may have decomposed under the work up conditions. To determine if this was the case, the following experiment was performed. An equimolar mixture of commercially available *trans-p*-hydroxycinnamic acid (53a) and *trans*-cinnamic acid (48) was dissolved in sodium borate buffer, and the mixture was left to stir at 30° over several days. A ^{1}H NMR spectrum of the mixture taken prior to incubation confirmed that the cinnamic acids (53a) and (48) were present in a 1:1 ratio by comparison of the integrals of their characteristic resonances. After the incubation period the cinnamic acids (53a) and (48) were recovered under the same conditions used for the work up procedure in the PAL-catalysed

reactions of *(S)*- and *(R)*-tyrosine (51a) and (52a). Analysis of the recovered material by ^{1}H NMR spectroscopy indicated that the doublet resonances attributable to the protons at the 2- and 3-positions of *p*-hydroxycinnamic acid (53a) had both decreased in area by approximately 20%, relative to the resonances due to the protons of cinnamic acid (48). From the ^{1}H NMR spectra, it appeared that *p*-hydroxycinnamic acid (53a) had in fact decomposed to some extent under the conditions used for the work up procedure in the PAL-catalysed reactions of *(S)*- and *(R)*-tyrosine (51a) and (52a). Presumably, decomposition of the product from the reaction of *(S)*-tyrosine (51a) with PAL is not as obvious, since *(S)*-tyrosine (51a) is the natural substrate of the enzyme, and therefore the reaction is relatively more efficient and higher yielding.

To ascertain whether *p*-nitrophenylalanine (51b) is a substrate of PAL, a mixture of the corresponding racemate and the enzyme was incubated over 7 days in a buffered solution. After work up, recrystallisation of the residue gave *trans-p*-nitrocinnamic acid (53b) in 52% yield. A ^{1}H NMR spectrum of the product showed doublet resonances at δ 6.97 and δ 7.32 attributable to the protons at the 2- and 3-position, respectively. These resonances were mutually coupled by 16Hz. The spectral characteristics were consistent with those of an authentic sample.

Having established that *(S)-p*-nitrophenylalanine (51b) is a substrate of the enzyme, attention was turned to *(S)-p*-fluorophenylalanine (51c) as a possible substrate, particularly since the derivative is sterically very similar to *(S)*-phenylalanine (49), and should therefore bind as easily to the enzyme.

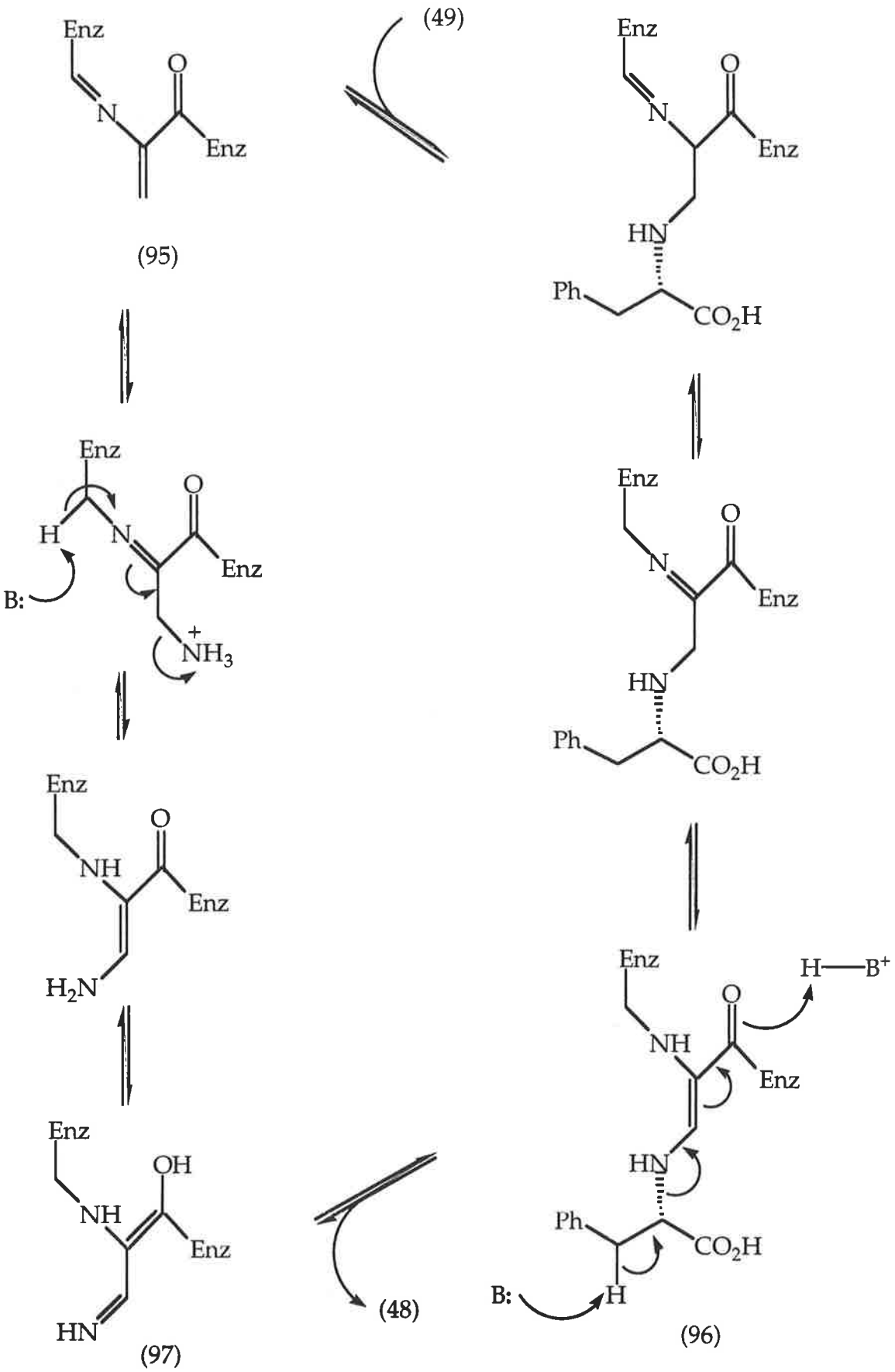
To isolate and characterise the major product from the reaction of *(S)-p*-fluorophenylalanine (51c) with PAL, the substrate was dissolved in sodium borate buffer and treated with the enzyme, as described for the reaction of *(S)*-tyrosine (51a) with PAL. After work up, recrystallisation of the product afforded *trans-p*-fluorocinnamic acid (53c) in 65% yield. Characteristic doublet resonances were observed at δ 6.49 and δ 7.58 in the ^{1}H NMR spectrum, attributable to the protons at the 2- and 3-position, respectively. These resonances were mutually

coupled by 16Hz, and the spectral characteristics of the product (53c) were in accord with those of an authentic sample.

To determine if PAL also catalyses the elimination of *(R)-p*-fluorophenylalanine (52c), the reaction was repeated with the unnatural substrate to give *trans-p*-fluorocinnamic acid (53c), in 29% yield. The resonances of the protons at the 2- and 3-positions in a ^{1}H NMR spectrum of the product (53c) were identical with that reported above.

Thus, it has been established that the analogues of *(R)*-phenylalanine (50) examined, (51a), (52a), (51b), (51c) and (52c) are all substrates of PAL, and react mainly by *anti*-elimination to give the corresponding *trans*-cinnamates (53a), (53b) and (53c). Presumably, upon binding of these modified substrates in an active site of the enzyme, the amino group of the substrate reacts with and becomes covalently bound to an electrophilic centre (95), in a similar process to that described for the reaction of *(S)*-phenylalanine (49) (Scheme 22).[68] Formation of the enamine (96) then forms an electron sink for facile carbon-nitrogen bond cleavage of the substrate, such that removal of a β–proton and subsequent elimination occurs to give the corresponding *trans*-cinnamate (48) and the amino-enzyme complex (97).

The nitro, fluoro and hydroxy substituents in the corresponding substrates (51b), (51c) and (51a), span the range from strongly electron withdrawing to strongly electron donating, and this should be reflected in the relative acidities of the β–protons. It seemed likely that the comparative ease of removal of a β–proton in the substrates (51a), (51b) and (51c) would be determined by the relative acidities of these protons, and if hydrogen abstraction at the β–position is the rate determining step in the catalytic cycle, then this may be reflected in the relative rates of conversion of these modified substrates with PAL. It was assumed, however, that the electronic distribution around the aryl group would be unlikely to influence the acidity of the α–proton due to the proximity of the aryl nucleus. Therefore, if removal of the α–proton, prior to racemisation, is the rate determining step in the catalytic cycle, then the electronic nature of the


Enz
O
N
Enz
(95)
(49)
Enz
O
N
Enz
HN
Ph
CO2H
Enz
H
N
O
Enz
B:
+
NH3
Enz
O
N
Enz
HN
Ph
CO2H
Enz
O
NH
Enz
H2N
Enz
OH
NH
Enz
HN
(97)
(48)
Enz
O
NH
Enz
HN
Ph
CO2H
B:
H
H—B+
(96)

Scheme 22

p-substituent should not affect the overall rate of substrate conversion.

Thus, in an attempt to discriminate between racemisation at the α–centre and *syn*-elimination as the minor reaction pathway, the relative rates of conversion of the substrates (49), (50), (51a)-(51c) and (52a)-(52c) with PAL were investigated.

Initially, a UV assay was developed to quickly and efficiently monitor the production of *trans*-cinnamic acid (48) from the PAL-catalysed reaction of *(S)*-phenylalanine (49). Accordingly, a 1.1mM solution of *(S)*-phenylalanine (49) in sodium borate buffer was transferred to a quartz cell and PAL was added to initiate the reaction. The concentration of *(S)*-phenylalanine (49) used in this reaction was greater than the concentration required for efficient binding to the enzyme but less than the saturation point,[95] and was found to be ideal in order to obtain a satisfactory absorbance of the product cinnamate (48). The solution was allowed to incubate at 30° in a constant temperature water bath and the reaction was monitored by UV absorption at a fixed wavelength of 268nm, the wavelength at which *trans*-cinnamic acid (48) has a maximum absorbance.[96] The rate of change of absorbance of the solution was assigned unity (k_{rel}= 1.0), and referred to as the standard rate of conversion. In a similar fashion, the rate of change of absorbance of a buffered solution containing a mixture of *(R)*-phenylalanine (50) and PAL was measured to be 0.002, relative to the standard rate.

The formation of *trans-p*-hydroxycinnamic acid (53a) from the PAL-catalysed reaction of *(S)*-tyrosine (51a) was also monitored using this method, at a fixed wavelength of 308nm.[96] The rate of change of absorbance of the solution, relative to the standard rate, was measured to be 0.23. Similarly, the rate of change of absorbance of a solution containing the unnatural substrate (52a) and PAL was measured to be 0.0006.

Reactions of *(S)-p*-fluorophenylalanine (51c) and *(R)-p*-fluorophenylalanine (52c), catalysed by PAL, were each monitored by UV absorption at a fixed wavelength of 275nm.[96] The relative rates of change of absorbance of the

solutions containing the modified substrates (51c) and (52c) were measured to be 0.41 and 0.0004, respectively, relative to the standard rate of conversion.

A solution of *(S)*-*p*-nitrophenylalanine (51b) in buffer was treated with PAL, and the production of *trans*-*p*-nitrocinnamic acid (53b) was monitored by UV absorption at 300nm, the wavelength at which *trans*-*p*-nitrocinnamic acid (53b) has a maximum absorbance. However, no change in the absorbance of the solution was observed. As an alternative, the reaction was repeated and monitored at a fixed wavelength of 276nm, the wavelength at which the starting material (51b) has a maximum absorbance. In this way, the rate of depletion of the substrate (51b) could be observed, rather than the rate of production of the product cinnamic acid (53b). However, no change in the absorbance was again observed. It seemed likely that the rate of formation of *trans*-*p*-nitrocinnamic acid (53b) was too slow to be detected by UV spectroscopy. This could be due to inefficient binding between the substrate (51b) and the enzyme as a result of the *p*-nitro substituent being relatively bulky in comparison to the *p*-hydroxy and *p*-fluoro substituents in the corresponding substrates (51a) and (51c).

The concentrations of the modified substrates (51a), (52a), (51c) and (52c) used in these experiments, and the quantity of enzyme added to initiate each reaction was equivalent to that used in the standard assay involving *(S)*-phenylalanine (49). This ensured that a direct comparison could be made between the rate of conversion of *(S)*-phenylalanine (49) and the rates of conversion of each modified substrate (51a), (52a), (51c) and (52c) with PAL. The relative rates of conversion (k_{rel}) are displayed in Table (3).

From the kinetic data summarised in Table (3), it is evident that the relative rates of conversion of these substrates do not reflect the relative acidities of the β–protons and therefore the electronic distribution around the aryl nucleus, governed by the *p*-substituents. In fact, the substrates containing an electron donating hydroxy substituent and an electron withdrawing fluoro substituent are both metabolised by the enzyme at a slower rate compared with the unsubstituted phenylalanine (49). This suggests that β–hydrogen abstraction is

Table (3) Relative rates of conversion of the substrates (49), (50), (51a-c) and (52a-c) with PAL.

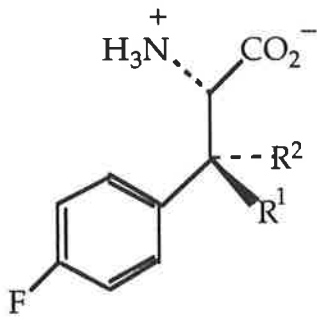
Substrate *(S)*-Enantiomer	k_{rel}	Substrate *(R)*-Enantiomer	k_{rel}	Extinction Coefficient of Corresponding Cinnamate
(49)	1.0*	(50)	0.002	20,417
(51a)	0.23	(52a)	0.0006	20,676
(51b)	-	(52b)	-	no kinetics
(51c)	0.41	(52c)	0.0004	21,167

*Assigned unity

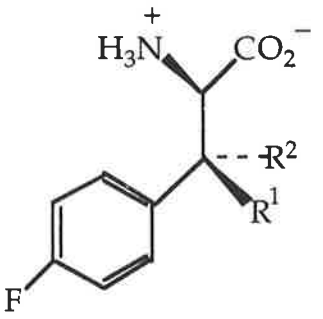
not the rate determining step in the reaction catalysed by PAL.

It was anticipated that the stereoselective synthesis of all four stereoisomers of 3-deuterio-*p*-fluorophenylalanine (98a), (98b), (99a) and (99b) would enable an alternate investigation into the minor reaction pathway by comparison of the deuterium content in the product of the reaction of these stereoisomers with PAL, to that previously reported for the reaction of the non-fluorinated analogues (46a-d).[68] Synthesis of the stereoisomers (98a), (98b), (99a) and (99b) was attempted based on the methodology used for the formation of the stereoisomers of 3-deuteriophenylalanine (46c) and (46d), previously reported.[68]

The free amino acid, *(S)*-*p*-fluorophenylalanine (51c) was initially esterified by treatment with a methanolic hydrogen chloride solution, which was prepared by the slow addition of thionyl chloride to cooled, dry methanol under anhydrous conditions. This afforded the hydrochloride salt of *(S)*-*p*-fluorophenylalanine methyl ester (100) which was *N*-phthaloylated by



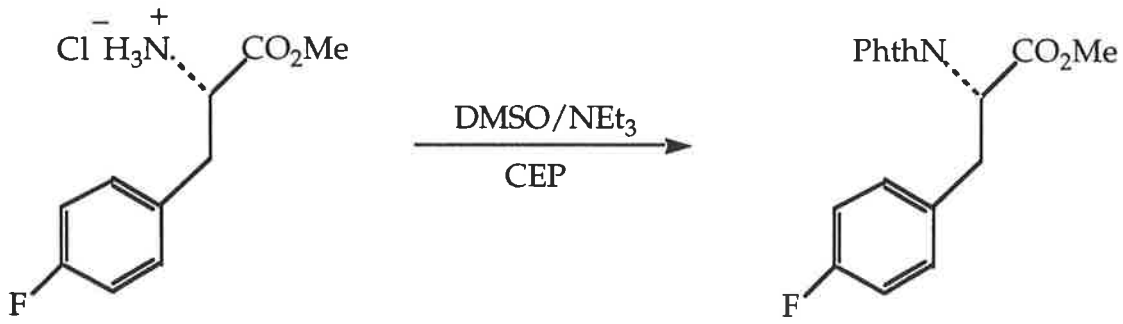
(98)



(99)

a) R^1=H, R^2=D
b) R^1=D, R^2=H

treatment of a solution of the amino acid derivative (100) and triethylamine in anhydrous dimethyl sulfoxide (DMSO) with carboethoxyphthalimide (CEP) (Scheme 23).[97] After work up, the crude oil obtained was adsorbed onto basic alumina and eluted with benzene. Evaporation of the eluent *in vacuo* afforded *(S)-N*-phthaloyl-*p*-fluorophenylalanine methyl ester (101) as a clear viscous oil, in 56% yield. The ^{1}H NMR spectrum of the product (101) showed multiplet resonances within the range δ 7.68 to 7.80, characteristic of an *N*-phthaloyl protecting group, and a singlet was observed at δ 3.78 due to the methyl ester group.

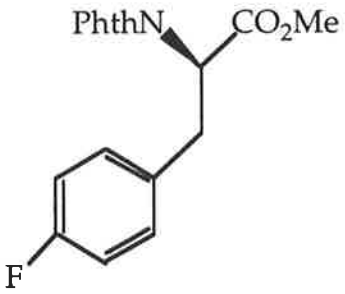


(100) (101)

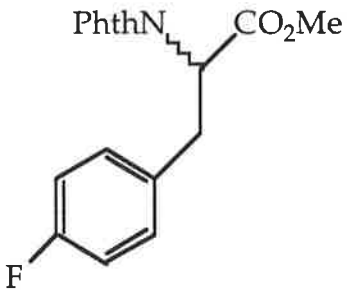
Scheme 23

The synthesis of *(R)*-*N*-phthaloyl-*p*-fluorophenylalanine methyl ester (102) was achieved under the same conditions giving a product with spectral properties identical to those of the other enantiomer (101).

Increasing the concentration of the chiral europium shift reagent, $Eu(hfc)_3$,[98] in an NMR sample of *(S)*-*N*-phthaloyl-*p*-fluorophenylalanine methyl ester (101) resulted in a spectrum which when compared with that of an authentic sample of the racemic phenylalanine derivative (103) indicated that the protection of the amino acid derivative (51c) had proceeded without racemisation at the α–centre.



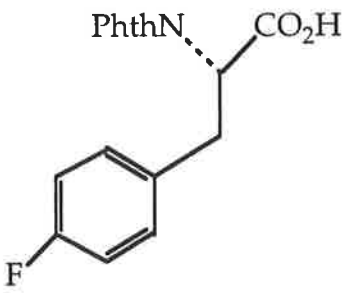
(102)



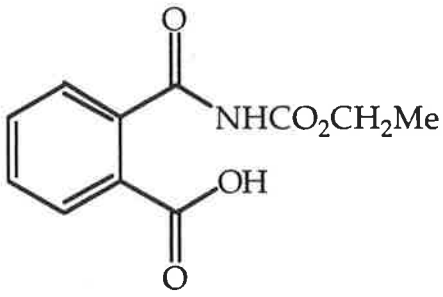
(103)

A more attractive *N*-protection procedure would involve direct *N*-phthaloylation of the free amino acid (51c). However, when *N*-protection of the amino acid (51c) was attempted with CEP and sodium carbonate using a standard procedure under aqueous conditions,[99] analysis of the product by ^1H NMR spectroscopy indicated the presence of *(S)*-*N*-phthaloyl-*p*-fluorophenylalanine (104) and the product (105) from hydrolysis of CEP, in a ratio of 1:4. The phenylalanine derivative (104) was characterised by multiplet resonances in the ^1H NMR spectrum, within the range δ 7.68 to 7.80, attributable to the *N*-phthaloyl protecting group, and a triplet resonance was observed at δ 5.17 due to the α–proton. The resonance of the α–proton was coupled to the two equivalent β–proton resonances by 8.4Hz. The product (105) obtained from hydrolysis of CEP was characterised by a quartet at δ 4.10 due to the methylene protons and a triplet at δ 1.18 due to the methyl protons. The methylene and methyl proton

resonances were coupled by 7.1Hz and were clearly distinguishable from the methylene and methyl signals attributable to CEP itself. The ^{1}H NMR spectral characteristics were found to be consistent with those reported in the literature.[97,100] ^{1}H NMR spectroscopic analysis also indicated that when the reaction was repeated over a prolonged period, the ratio of the by-product (105) to the *N*-phthaloylated derivative (104), increased substantially. When the reaction was repeated in a two-phase system in an attempt to minimise the formation of the unwanted hydrolysis product (105),[100] analysis of the crude reaction mixture by ^{1}H NMR spectroscopy still indicated the presence of the by-product (105) which was difficult to separate from the required *N*-phthaloylated amino acid derivative (104) by chromatography, as the products (104) and (105) had identical Rf values by TLC. Although the preparation of various *N*-phthaloyl-α–amino acids is also possible through the fusion of finely ground phthalic anhydride with an appropriate amino acid,[101] this procedure was avoided as it has been reported to lead to appreciable racemisation.[102]



(104)

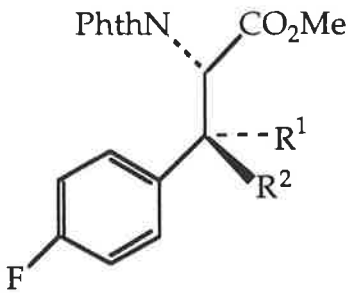


(105)

Thus, the aprotic conditions described above for the *N*-protection of the amino acid derivative (100) were found to be optimal for the production of pure ***(S)-N*-phthaloyl-*p*-fluorophenylalanine methyl ester (101), without racemisation at the α–centre.**

Bromination of the protected amino acid (101) was achieved by irradiating a solution of the ester (101) and NBS in carbon tetrachloride with a 250W mercury

lamp, over 4h.[103] After work up, the bromide (106) was obtained as a 1:1 ratio of diastereomers, in 60% yield.



(106)

a) R^1= H, R^2=Br
b) R^1= Br, R^2=H

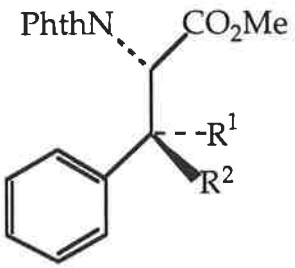
Presumably reaction of the phenylalanine derivative (101) proceeds *via* the benzylic radical to give the bromide (106) as a mixture of diastereomers, with the lack of asymmetric induction attributable to the extremely low activation energy of such halogen transfer reactions.[36] The yield obtained of the bromide (106) from reaction of *(S)-N*-phthaloyl-*p*-fluorophenylalanine methyl ester (101) with NBS was considerably lower than that reported from reaction of the non-fluorinated analogue (31a) with NBS.[103] This may be attributed to the electron withdrawing effect of the fluorinated substituent, such that hydrogen abstraction from the benzylic position by the electrophilic bromine radical, with subsequent development of an electron deficient centre, is disfavoured. The diastereomers (106a) and (106b) were separated by fractional crystallisation from a mixture of hexane and dichloromethane to give the diastereomer (106a) in 30% yield, and the diastereomer (106b) in 28% yield. A ^{1}H NMR spectrum of the bromide (106a) showed doublets at δ 5.55 and δ 5.91 corresponding to the α– and β–protons, respectively, and a singlet was observed at δ 3.82 due to the ester group. The bromide (106b) showed doublets at δ 5.44 and δ 6.00 due to the α– and β–proton, respectively, as well as a singlet at δ 3.57 due to the ester moiety. The mutual coupling constants between the resonances of the α– and β–protons in the

diastereomers (106a) and (106b) were 10.5Hz and 11.2Hz, respectively. The assignment of peaks was based on comparison of the ^{1}H NMR spectral data of the bromides (106a) and (106b) with that of *(2S,3S)*- and *(2S,3R)*-3-bromo-*N*-phthaloylphenylalanine methyl ester (107a) and (107b), which have been characterised in a previous study.[124] The signal attributable to the ester group occurs at higher chemical shift for the diastereomer (106a) than for the diastereomer (106b). Also, the bromide (106a) exhibits a smaller coupling constant between the resonances of the α– and β–protons than the corresponding value for the bromide (106b). The effect has been explained by the most favoured conformations of the bromides (106a) and (106b), which are presumably staggered with the α– and β–protons *anti* to each other to minimise the number of gauche interactions between the bulky groups.[124] When the isomer (106b) adopts the staggered conformation, the phenyl group is situated close to the ester group, and the shielding effect of the phenyl group may explain the lower chemical shift of the ester group of the diastereomer (106b), compared to that of (106a).

Conversion of the bromides (107a) and (107b) to the deuterides (107c) and (107d) with 5% palladium on carbon catalyst, carried out at -20° over 3 days, has been shown to proceed preferentially with retention of configuration.[68] It was anticipated that the bromides (106a) and (106b) could also be converted to their corresponding deuterides (108a) and (108b) with similar retention of configuration.

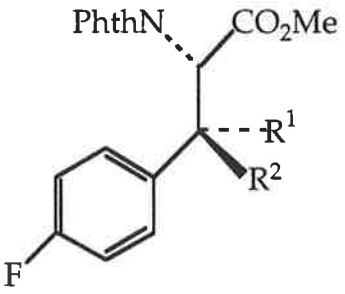
Initially, hydrogenolysis of the bromide (106a) was performed using a 5% palladium on carbon catalyst, with methanol as the solvent, under an atmosphere of hydrogen at -20°, to examine whether reduction of the bromide (106a) was effected under these conditions. This reaction proceeded to give the reduced product (101) in 85% yield. Having established benzylic reduction, deuteriolysis of the bromide (106a) was investigated, conducting the reaction under an atmosphere of deuterium, as described above. After work up of the reaction mixture, ^{1}H NMR spectroscopic analysis indicated that reduction of the bromide (106a) proceeded with low diastereoselectivity to give the corresponding

deuteride (108) as a 3:2 ratio of diastereomers. The spectrum showed doublets at δ 3.58 and δ 3.52, attributable to the β–protons of the respective diastereomers (108a) and (108b), and integration of these signals was used to determine the diastereomeric ratio. The assignment of peaks was based on comparison of the ^{1}H NMR spectral data of the deuterides (107c) and (107d) which have been characterised in a previous study.[68]



(107)

a) R^1=H, R^2=Br
b) R^1=Br, R^2=H
c) R^1=H, R^2=D
d) R^1=D, R^2=H



(108)

a) R^1=H, R^2=D
b) R^1=D, R^2=H

Although little has been reported on the stereochemical course of the catalytic reduction of benzylic halides, presumably, a transition state for the reduction of the bromide (106a) involves an electron deficient centre at the benzylic position due to abstraction of a bromine atom. Formation of this electron deficient site may not be favourable if it is in close proximity to an electron withdrawing fluorinated substituent. The lack of high diastereoselectivity observed in the reduction of the bromide (106a) as opposed to the >98% diastereoselectivity observed in the reduction of the bromide (107a), under identical conditions,[68] may be attributed to a relatively high activation energy associated with the formation of an electron deficient site adjacent to an electron withdrawing substituent, such that one diastereomer is not preferentially produced.

In addition to the low diastereoselectivity observed in the catalytic reduction of the bromide (106a), the presence of complex aromatic peaks in the region δ 7.69 to δ 7.81 in the ^{1}H NMR spectrum indicated that ring opening of the phthaloyl group had taken place during the course of reaction. ^{1}H NMR spectroscopic analysis also indicated the presence of products due to decomposition.

Since reduction of the bromide (106a) did not proceed with high retention of configuration, as originally anticipated, the deuterides (108) could not be used for study into the mechanism of the PAL-catalysed reaction of *(R)*-phenylalanine (50). Previous attempts[111] to stereoselectively reduce the bromides (107a) and (107b) with tri-*n*-butyltin deuteride and triphenylgermanium deuteride resulted in either poor diastereoselectivity or no reaction at all, and preliminary reactions with sodium borohydride lead only to reduction of the *N*-phthaloyl substituent. Thus, reduction of the structurally similar bromides (106a) and (106b) was not attempted with these reagents. As an alternative, the synthesis of the 2,3-dideuterides (54a) and (54b) was developed in order to examine the minor reaction pathway.

In a previous study,[68] treatment of the *(2R,3S)*-deuteride (46c) with PAL gave *trans*-3-deuteriocinnamic acid (47) with 92% deuterium incorporation, whereas treatment of the *(2R,3R)*-deuteride (46d) gave *trans*-cinnamic acid (48) with only 27% deuterium contained at the 3-position. It was thought that by incorporating deuterium at the α–positions of the deuterides (46c) and (46d), to give the corresponding dideuterides (54a) and (54b), the rate of racemisation, if it is responsible for the minor reaction pathway, would decrease due to a deuterium isotope effect. Consequently, this would be reflected in the percentage of deuterium contained at the 3-position, in the product cinnamic acids. If reaction of the dideuteride (54a) is shown to proceed with >92% deuterium incorporation at the 3-position, and reaction of the dideuteride (54b) is shown to proceed with <27% deuterium incorporation at the 3-position, then this would imply that the rate of racemisation at the α–centre is being slowed down, indicating that racemisation is responsible for the minor reaction pathway. Alternatively,

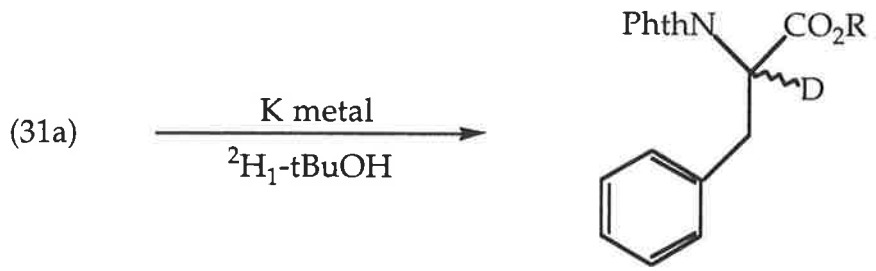
reactions of the dideuterides (54a) and (54b) proceeding with the same deuterium incorporation at the 3-position in the products of the reaction as that observed for the non-α–deuteriated analogues (46c) and (46d) would imply no deuterium isotope effect associated with the introduction of deuterium at the α–position. This result would indicate that *syn*-elimination of the β–hydrogen is responsible for the minor reaction pathway.

Thus, attention was turned to the synthesis of *(2R,3S)*- and *(2R,3R)*-2,3-dideuteriophenylalanine (54a) and (54b) to establish if PAL is converting from a lyase to a racemase in an effort to metabolise the unnatural substrate (50), or whether it is altering the mode of elimination from an *anti*- to a *syn*-elimination.

Protection of commercially available racemic phenylalanine (49) was accomplished by heating the amino acid (49) and finely ground phthalic anhydride at 150° for 30min, according to a procedure in the literature.[101] The *N*-protected substrate was subsequently esterified by treatment with a methanolic hydrogen chloride solution. After work up, the crude product was recrystallised from a mixture of ethyl acetate and hexane to afford the ester (31a) in 85% yield. A ^{1}H NMR spectrum of the product (31a) showed multiple resonances in the region δ 7.79 to δ 7.70, characteristic of the *N*-phthaloyl group, and a singlet was observed at δ 3.79 attributable to the methyl ester moiety. The spectral characteristics were consistent with those previously reported.[108] The single enantiomers of phenylalanine (49) and (50) were not individually protected since the subsequent introduction of deuterium at the α–carbon centre would destroy chirality anyway. Instead, it was anticipated that the mixture of enantiomers would be resolved by the use of an enzyme at a later stage.

Incorporation of deuterium at the α–position of *N*-phthaloylphenylalanine methyl ester (31a) was achieved by addition of the ester (31a) to a solution of potassium *tert*-butoxide, which was prepared by the careful addition of a catalytic amount of potassium metal to deuteriated *tert*-butanol, under anhydrous conditions (Scheme 24). After work up, the product (109b) was afforded as a pale yellow oil, in 73% yield. Analysis of the crude product by ^{1}H NMR spectroscopy

indicated the presence of 2-deuterio-*N*-phthaloylphenylalanine *tert*-butyl ester (109b) with 75% deuterium incorporation. The deuterium content was determined by comparing the integration of the signal at δ 5.10, attributable to the residual α–proton, to the integration of the signal at δ 3.51, due to the β–protons, in the ^{1}H NMR spectrum. The disappearance of the singlet at δ 3.79 attributable to the methyl ester group, and the appearance of a signal at δ 1.48, characteristic of a *tert*-butyl ester group, suggested that *trans*-esterification had also taken place under these reaction conditions.



(109)

a) R = Me
b) R = CMe_3

Scheme 24

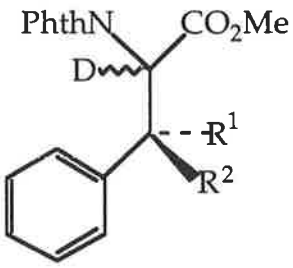
To increase the percentage of deuterium incorporated at the α–position, the reaction was repeated on the partially deuteriated substrate (109b), as described above. After work up, analysis of the product by ^{1}H NMR spectroscopy indicated the presence of the *tert*-butyl ester (109b) with 96% deuterium incorporation.

2-Deuterio-*N*-phthaloylphenylalanine *tert*-butyl ester (109b) was re-esterified to the corresponding methyl ester (109a) by leaving the substrate to reflux in a methanolic hydrogen chloride solution for 4h. After work up, the crude product was recrystallised from a mixture of ethyl acetate and hexane to afford the corresponding methyl ester (109a) in 94% yield, without loss of deuterium content.

Treatment of the amino acid derivative (109a) with NBS gave the

corresponding bromides (110) in 72% yield.[103] Analysis of the product by ^1H NMR spectroscopy indicated the presence of the bromides (110) as a 1:1 mixture of diastereomers, each as a racemate, with ^1H NMR spectral data consistent with that previously reported.[124] The diastereomer (110b) was separated by crystallisation from a mixture of hexane and dichloromethane, and isolated in 25% yield. The mother liquor from the crystallisation was evaporated *in vacuo* and the residue was purified by HPLC to afford the other diastereomer (110a) in 48% yield. A ^1H NMR spectrum of the diastereomer (110a) showed doublets at δ 5.59 and δ 5.92, due to the residual α–proton and β–proton, respectively, with a mutual coupling constant of 10.3Hz between these resonances. Integration of these signals indicated that the diastereomer (110a) had 95% deuterium incorporation at the α–position. A ^1H NMR spectrum of the other diastereomer (110b) showed characteristic doublet resonances at δ 5.52 and δ 6.02 due to the residual α–proton and β–proton, respectively, with a coupling constant between these resonances of 11.2Hz. Integration of these signals indicated that the diastereomer (110b) had 94% deuterium content at the α–position. Signals attributable to the methyl ester groups were observed at δ 3.82 and δ 3.55 for the diastereomers (110a) and (110b), respectively.

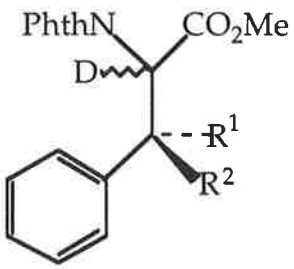
Conversion of the bromides (110a) and (110b) to their corresponding dideuterides (111a) and (111b) with 5% palladium on carbon catalyst, was performed at -15° under an atmosphere of deuterium.[68]



(110)

a) R^1=H, R^2=Br
b) R^1=Br, R^2=H

The ^1H NMR spectra of the products obtained from reduction of the bromoesters (110a) and (110b) showed broad doublet resonances at δ 3.59 and δ 3.53 for the respective β–protons, with integration of these peaks used to determine the diastereomeric ratio of the deuterides (111a) and (111b). Spectral analysis of the product dideuterides indicated that in each case the reduction proceeded with retention of configuration at the β–position, and with >95% diastereoselectivity. Integration of the signals in the ^1H NMR spectra indicated that the dideuterides (111a) and (111b) had 94% and 95% deuterium content at the α–position, respectively, and 94% and 90% deuterium content at the β–position, respectively, which was confirmed by mass spectrometric analysis. The assignment of peaks was based on comparison of the ^1H NMR spectral data of the deuterides (107c) and (107d) which have been characterised in a previous study.[68]

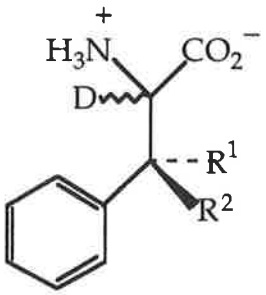


(111)

a) R^1=H, R^2=D
b) R^1=D, R^2=H

Removal of the phthaloyl and methyl ester protecting groups from the dideuterides (111a) and (111b) by heating the substrates at reflux in a 2:1 mixture of 6N HCL and acetic acid, has been shown to proceed without loss of stereochemical integrity.[68] Accordingly, to minimise any loss of deuterium incorporated at the α–position through racemisation, the dideuterides (111a) and (111b) were each hydrolysed in a 2:1 mixture of 6N HCL and acetic acid, with subsequent treatment with aniline in ethanol to give the corresponding free amino acids (112a) and (112b). A ^1H NMR spectrum of the free amino acid (112a)

showed a broad doublet resonance at δ 3.92 attributable to the residual α–proton, and a broad singlet at δ 3.17 due to the β–proton. The corresponding resonances were coupled by 7.9Hz. Integration of these signals indicated that the free amino acid (112a) had 92% deuterium incorporation at the α–position. The ^{1}H NMR spectrum of the free amino acid (112b) showed a broad doublet resonance at δ 3.90 attributable to the residual α–proton, and a broad singlet due to the β–proton was observed at δ 3.00. These resonances were coupled by 4.9Hz. Integration of these signals indicated that the free amino acid (112b) had 93% deuterium incorporation at the α–position. Based on mass spectrometric analysis of the free amino acids (112a) and (112b) removal of the protecting groups did not change the deuterium content at the β–position. Assignments of the stereochemistry of the free amino acids (112a) and (112b) were based on the coupling constants between the resonances of the residual α–protons and β–protons. The coupling constants between the α– and β–proton resonances of the deuterides (46c) and (46d) were reported to be 8.0Hz and 5.0Hz, respectively.[68] These are consistent with the coupling constants measured for the dideuteriated amino acid derivatives (112a) and (112b), which were measured to be 7.9Hz and 4.9Hz, respectively.



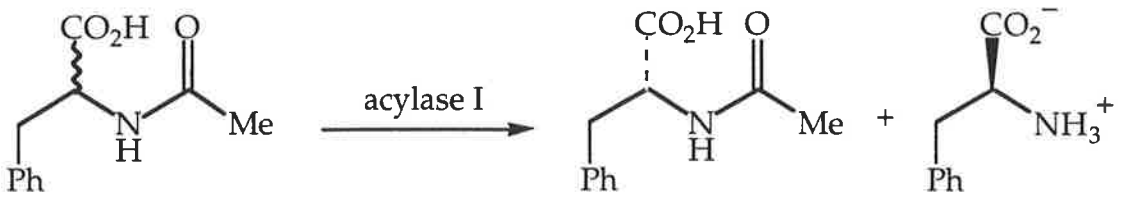
(112)

a) R^1=H, R^2=D
b) R^1=D, R^2=H

Having synthesised *(2R,3S)(2S,3S)*-2,3-dideuteriophenylalanine (112a) and *(2R,3R)(2S,3R)*-2,3-dideuteriophenylalanine (112b), it was necessary to resolve the

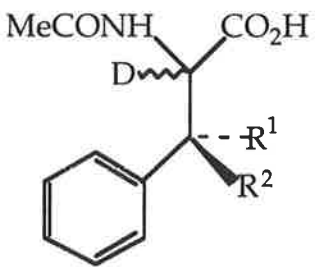
enantiomers of each diastereomer so that the PAL-catalysed reactions of the dideuteriated amino acids (54a) and (54b) could be investigated without the presence of the natural substrate, which would react in preference.

The resolution of racemic phenylalanine has been shown to be readily achieved through the asymmetric hydrolytic action of hog renal acylase I (acylase I) on *N*-acyl derivatives (Scheme 25).[104]



Scheme 25

Previous studies have indicated that acylase I has almost absolute enantioselectivity for the hydrolysis of *(S)*-*N*-acyl-α–amino acids, and that it accepts substrates having a wide range of structure and functionality.[105] Thus, the use of acylase I seemed a practical method for the preparation of the enantiomerically pure amino acid derivatives (54a) and (54b) from their corresponding racemic *N*-acetyl derivatives (113a) and (113b).



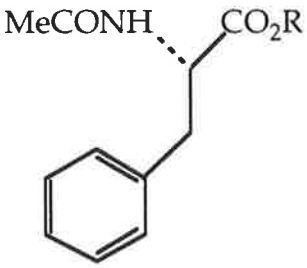
(113)

a) R^1=H, R^2=D
b) R^1=D, R^2=H

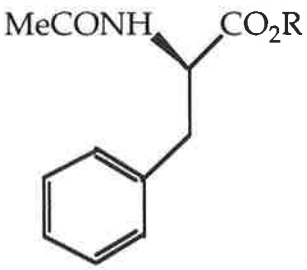
One of the standard methods for *N*-acetylating amino acids involves addition of glacial acetic acid to the amino acid followed by treatment with acetic anhydride.[104,105] However, in view of the rapid racemisation which optically active amino acids undergo in the presence of these reagents, it was necessary to find a non-racemising *N*-acetylation procedure such that *N*-acetylation of the deuteriated amino acid derivatives (112a) and (112b) would occur without loss of deuterium content at the α–centre.

Initially, the *N*-acetylation of authentic *(S)*-phenylalanine (49) was examined. Treatment of *(S)*-phenylalanine (49) with glacial acetic acid and acetic anhydride affords the corresponding mixed anhydride, and as a consequence the α–proton becomes more labile and easily exchangeable. It was thought that since treatment of *(S)*-phenylalanine (49) with acetylchloride could not afford the mixed anhydride, the possibility of racemisation at the α–centre would be low. Accordingly, a solution of *(S)*-phenylalanine (49) in a mixture of ice and water was treated with excess acetylchloride, based on a procedure in the literature.[105] After work up, *(S)*-*N*-acetylphenylalanine (114a) was isolated in only 23% yield. The structure of the product (114a) was verified by comparison of ^{1}H NMR spectral data with that found in the literature,[106] and by infra-red (IR) spectroscopy which showed a strong amide stretch at 3356 cm^{-1}. *(S)*-*N*-Acetylphenylalanine (114a) possessed $[a]_D$ = +40.0° in methanol which is consistent with that previously reported.[104] This implied that the reaction proceeded without racemisation at the α–centre. However, as a result of the poor yield obtained of the *N*-acetylated product (114a), presumably due to competing hydrolysis of acetylchloride, this procedure was abandoned.

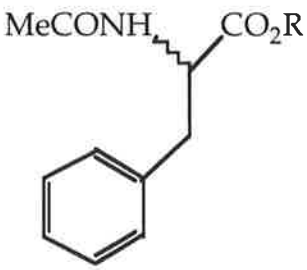
As an alternative, the *N*-acetylation of *(S)*-phenylalanine (49) was attempted using acetic anhydride under basic conditions. A solution of *(S)*-phenylalanine (49) and triethylamine in water was treated with acetic anhydride to give *(S)*-*N*-acetylphenylalanine (114a) in 98% yield. The spectral characteristics of the product (114a) were consistent with those reported in the literature.[106] *(S)*-*N*-Acetylphenylalanine (114a) possessed $[a]_D$ = +39.0° in methanol which implied



(114)



(115)



(116)

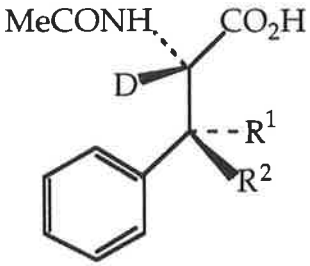
a) R = H
b) R = Me

that a small amount of racemisation may have occurred under these reaction conditions. In view of the deuterium isotope effect associated with proton exchange at the α–centre of the deuteriated amino acid derivatives (112a) and (112b), it was assumed that the small amount of racemisation observed under these reaction conditions would be almost non-existent, when applied to the labelled amino acids (112a) and (112b).

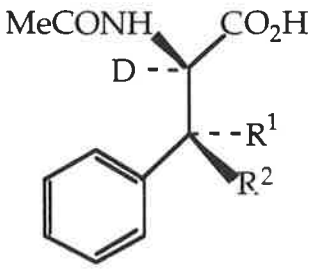
Accordingly, the labelled amino acids (112a) and (112b) were each *N*-acetylated by treatment with a mixture of acetic anhydride and triethylamine, as described above. After work up, the *N*-acetylated products (113a) and (113b) from reaction of the corresponding amino acids (112a) and (112b) were isolated in 64% and 54% yield, respectively, both with 92% deuterium contained at the α–centres. The structures were confirmed by ^{1}H NMR and IR spectroscopy, and the deuterium content at the α–centre was determined by comparison of the integration of the signals attributable to the residual α–protons and β–protons in the ^{1}H NMR spectra.

In order to develop a method to accurately determine the enantiomeric purity of the labelled amino acids (118a) and (118b) once resolved by acylase I enzyme, authentic unlabelled samples of each enantiomer of *N*-acetylphenylalanine (114a) and (115a) and the racemate (116a) were prepared as

described above. It was hoped that a direct separation of the racemic compound (116a) could be developed without prior derivatisation or conversion to diastereomers, as it was envisaged that only small quantities of the labelled amino acids (118a) and (118b) would be obtained after the enzyme resolution.



(117)



(118)

a) R^1=H, R^2=D
b) R^1=D, R^2=H

Initially, the use of ^{1}H NMR spectroscopy as an analytical tool in determining enantiomeric purity was examined. However, the addition of various chiral europium shift reagents to samples of the authentic racemate (116a) proved unsuccessful in separating the ^{1}H NMR signals corresponding to the protons of the *(R)*- and *(S)*-enantiomers. As an alternative to ^{1}H NMR spectroscopy, various HPLC methods were investigated.

β–Cyclodextrin can form inclusion complexes with derivatised and underivatised amino acids, however, chiral recognition has been shown to be significant mainly for larger species such as dansyl or naphthyl derivatised *(R)*- and *(S)*-amino acids.[107] In any case, the separation of racemic *N*-acetylphenylalanine (116a) was attempted using liquid chromatography with a chiral stationary phase composed of chemically bonded β–cyclodextrin (Cyclobond I). Various combinations of methanol, water, acetonitrile, acetic acid, and triethylammonium acetate as the mobile phase, as well as adjustments in the flow rate, however, failed to separate the enantiomers.

α–Cyclodextrin is significantly smaller than β–cyclodextrin and tends to complex many smaller molecules. Therefore, separation of the racemate (116a)

was attempted using HPLC with an α–cyclodextrin column (Cyclobond III).[107] Again, various mixtures of solvents, listed above, failed to effectively separate the enantiomers of racemic *N*-acetylphenylalanine (116a).

Armstrong and co-workers[107] were able to separate racemic phenylalanine using a 25cm α–cyclodextrin column. However, this separation procedure was not employed since the peaks in the HPLC trace corresponding to the *(R)*- and *(S)*-enantiomers were not resolved to the base-line. In fact, the error in the percentage enantiomeric excess was as high as ±10%. The resolution of racemic phenylalanine, accomplished by Armstrong and co-workers[107] was therefore inappropriate for the purposes of obtaining very accurate percentages of enantiomeric purity. It was anticipated that the investigation into the stereochemical course of reaction of the unnatural substrates (54a) and (54b) with PAL would only be significant if <5% of the natural substrate were present. The resolution of phenylalanine using an α–cyclodextrin column was therefore inadequate and not attempted.

Attention was subsequently turned to liquid chromatography with amylase *tris* (*(S)*-α–methyl benzylcarbonate) bonded to silica gel as a chiral stationary phase, (Chiral Pak) using mixtures of isopropanol and hexane as the mobile phase. However, this column also failed to separate the enantiomers of the authentic racemate (116a), even when the separation was attempted on the corresponding methyl ester derivative (116b).

The efficient, direct separation of racemic compounds by HPLC, without prior derivatisation or conversion to diastereomers has not been well documented in the literature. Since HPLC appeared to be ineffective in separating the enantiomers of the racemate (116a) and the corresponding methyl ester (116b), attention was turned to chiral gas chromatography (GC).

Authentic samples of *(S)*- and *(R)*-*N*-acetylphenylalanine (114a) and (115a) and the corresponding racemate (116a) were converted to their methyl esters (114b), (115b) and (116b), respectively, by treatment with a methanolic hydrogen chloride solution in anticipation of the esters being more easily separated by GC

than the corresponding free acids. The methyl esters (114b), (115b) and (116b) were characterised by comparison of their ^{1}H NMR spectra and melting points to those found in the literature.[106] Racemic *N*-acetylphenylalanine methyl ester (116b) was injected onto a Chirasil-Val capillary column in conjunction with a temperature gradient of 190-200° over 10min. Under these conditions, two sharp peaks, present in a 1:1 ratio were recorded having retention times of 4.26 and 4.40min. Under identical conditions *(R)*-*N*-acetylphenylalanine methyl ester (115b) and *(S)*-*N*-acetylphenylalanine methyl ester (114b) were each injected onto the GC column. A GC trace of the *(R)*-enantiomer (115b) showed a single peak with a retention time of 4.25min, and a trace of the *(S)*-enantiomer (114b) showed a single peak with a retention time of 4.39min. The retention time of each enantiomer (114b) and (115b) was confirmed by spiking the racemate (116b) with each enantiomer and analysing the mixture by chiral GC.

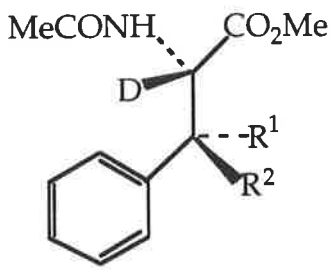
Thus, having established a method of separating the enantiomers of racemic *N*-acetylphenylalanine methyl ester (116b) using chiral GC, it was envisaged that the enantiomeric purity of the product from the enzymatic resolution of the *N*-acetyl protected amino acid derivative (113a) and its diastereomer (113b) could be determined in the same way, and with high accuracy.

Initially, resolution of the authentic unlabelled racemate (116a) by acylase I was attempted to determine the optimum conditions required for 100% hydrolysis of the unwanted *(S)*-enantiomer (114a). Based on a procedure in the literature,[105] to a buffered solution was added racemic *N*-acetylphenylalanine (116a) and cobalt (II) chloride, followed by a large excess of acylase I. The mixture was allowed to stir at 38° over 48h after which time the pH was adjusted to pH 1 and unreacted *(R)*-*N*-acetylphenylalanine (115a) was extracted from the aqueous solution with ethyl acetate. After concentration under reduced pressure, a small portion of the product, which was isolated in 30% yield, was treated with a methanolic hydrogen chloride solution to convert the acid (115a) to the corresponding methyl ester to determine the enantiomeric purity of the product by GC. The sample was injected onto a chiral GC column, under the same

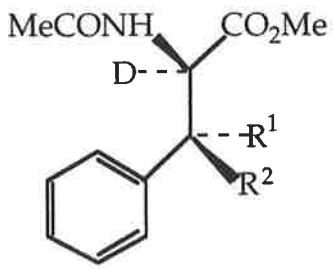
conditions used for the analysis of the authentic standards. GC analysis of the methyl ester showed a single peak with a retention time of 4.25min, corresponding to that of the *(R)*-enantiomer (115b). The peak pertaining to the *(S)*-enantiomer (114b) was not present indicating that >95% of the *N*-acetyl derivative (114a) had been hydrolysed by the enzyme, within the limits of detection. This result was confirmed by spiking the methyl ester (115b) derived from the resolved product (115a) with authentic standards and examining the mixtures by GC.

It should be noted that the enzyme-catalysed hydrolysis of *(S)*-*N*-acetylphenylalanine (114a) did not go to completion if the incubation period was less than 30h, if cobalt (II) chloride was not added to the reaction mixture, if the reaction was not performed in buffer, or if less than 4000 units of enzyme was added, per mg of substrate.

Having established the optimum conditions for complete hydrolysis of the *(S)*-enantiomer (114a), enantiomeric resolution of the labelled *N*-acetyl derivatives (113a) and (113b) was repeated under the same conditions. This afforded the corresponding *N*-acetylated products (118a) and (118b), in 27% and 26% yield, respectively. Small portions of the *N*-acetylated products (118a) and (118b) were subsequently converted to their corresponding methyl esters (120a) and (120b), and examined by chiral GC.



(119)



(120)

a) R^1=H, R^2=D
b) R^1=D, R^2=H

GC analysis of the methyl esters indicated the presence of the deuteriated amino acid derivatives (120a) and (120b) in >95% purity, within the limits of detection. In both cases, the corresponding enantiomers (119a) and (119b) could not be detected. These results were confirmed by spiking samples of each methyl ester (120a) and (120b) derived from the enzymatically produced acids (118a) and (118b) with authentic standards, and examining the mixtures by GC.

The products (118a) and (118b) obtained from resolution of the corresponding racemates (113a) and (113b) were converted to their respective free amino acids (54a) and (54b) by heating the substrates at reflux in a 2:1 mixture of 6N HCL and acetic acid. The ^{1}H NMR spectral characteristics of the products (54a) and (54b) were consistent with those reported in the literature.[68] The melting points of the dideuterides (54a) and (54b) were analogous to those reported for the deuterides (46c) and (46d),[68] although somewhat broadened due to the extra deuterium contained at the α–position.

Having synthesised the amino acid derivatives (54a) and (54b) it was anticipated that the mechanism of the minor reaction pathway, exhibited in the PAL-catalysed reaction of *(R)*-phenylalanine (50), could be elucidated by comparison of the deuterium content in the products of the reactions of the dideuterides (54a) and (54b) with PAL, to the deuterium content in the products of the reactions of the deuterides (46c) and (46d), previously reported.[68]

Initially, to confirm the deuterium isotope effect observed in a previous study in the reaction of *(2R,3R)*-3-deuteriophenylalanine (46d) with PAL,[68] a solution of the deuteride (46d) in sodium borate buffer was incubated with PAL at 30° over 8 days. After work up, ^{1}H NMR spectroscopic analysis of the product indicated the presence of *trans*-cinnamic acid (48) with approximately 25% deuterium incorporated at the 3-position, which was in agreement with previous findings.[68]

Similarly, the dideuterides (54a) and (54b) were each dissolved in sodium borate buffer and treated with PAL, except the enzyme to substrate ratio was greater by a factor of 15, thus ensuring a fast rate of conversion. After work up,

analysis of the products of the reactions of the dideuterides (54a) and (54b) by ^1H NMR spectroscopy indicated that both (54a) and (54b) afforded *trans*-cinnamic acid (48) with approximately 25% deuterium contained at the 3-position.

It was originally anticipated that reaction of the dideuteride (54a) with PAL would proceed with >92% deuterium incorporation at the 3-position if the minor reaction pathway could be accounted for by racemisation at the α–centre, or 92% deuterium incorporation if *syn*-elimination were responsible. In addition, it was also anticipated that reaction of the dideuteride (54b) would yield the product cinnamate with either 27% deuterium incorporation at the 3-position if *syn*-elimination were occurring, or <27% deuterium incorporation if the mechanism could be accounted for by racemisation. It was, however, not envisaged that deuterium contained at the α–position would be removed at any stage in the reaction. Since the PAL-catalysed reactions of the dideuterides (54a) and (54b) both afforded *trans*-cinnamic acid (48) with approximately 25% deuterium contained at the 3-position, this tends to suggest that the mechanism responsible for the minor reaction pathway cannot be explained solely by either racemisation at the α–centre or *syn*-elimination of the 3-*pro*-*S* hydrogen, as previously suggested.[68] It seemed likely that an exchange process was responsible for the removal of deuterium contained at the α–position and this process may also be partly responsible for removal of deuterium contained at the β–position.

To determine if this were the case, *(R)*-phenylalanine (50) was dissolved in sodium borate buffer which was prepared in 99% deuteriated water, and the solution was treated with PAL under identical conditions to that described for the reactions of the dideuterides (54a) and (54b). After work up, analysis of the product by ^1H NMR spectroscopy indicated the presence of *trans*-cinnamic acid (48) with approximately 25% deuterium incorporation at the 2-position, and 50% deuterium incorporation at the 3-position. This implies that protons contained at the α– and β–positions in the substrate (50) have at some point during the catalytic cycle undergone reversible abstraction to incorporate deuterium. When the reaction was repeated in deuteriated buffer with a much lower enzyme to

substrate ratio, analysis of the product by ^{1}H NMR spectroscopy indicated the presence of *trans*-cinnamic acid (48) with no deuterium incorporation. These results indicate that the enzyme to substrate ratio may be an important factor in the enzyme-catalysed reaction. Evidently, when the reaction is performed with a high enzyme to substrate ratio, proton exchange at the α– and β–positions becomes a competing process for the elimination reaction.

To determine if the enzyme is directly involved in the mechanism of the exchange process, an equimolar mixture of *(R)*-phenylalanine (50) and *trans*-cinnamic acid (48) in deuteriated buffer was left to stir at 30° over several days, without the presence of PAL. Analysis of the mixture by ^{1}H NMR spectroscopy indicated no deuterium incorporation in either *(R)*-phenylalanine (50) or *trans*-cinnamic acid (48). This implies that the exchange process must initially occur between the enzyme and deuteriated buffer, followed by exchange between the enzyme and the substrate and/or product of the reaction.

While reaction of *(R)*-phenylalanine (50) with the enzyme involves mainly loss of the 3-*pro*-*R* hydrogen and ammonia in an antiperiplanar elimination process, as previously reported,[68] it appears that the minor reaction pathway may be due, at least in part, to proton exchange. Consequently, the study could not differentiate between isomerisation to *(S)*-phenylalanine (49), before elimination, or synperiplanar elimination as the minor reaction.

Nevertheless, the investigation in this Chapter does indicate that proton exchange at the β–centre, and more importantly at the α–centre occurs only in the presence of the enzyme, which tends to suggest that the enzyme may well be converting from a lyase to a racemase in an effort to metabolise the unnatural substrate (50).

CONCLUSION

The work described in Chapter 1 of this thesis shows that the reactions of the carbamates (55a), (55b), (64) and (70) with *tert*-butyl perbenzoate in the presence of cupric octanoate, represent a novel mode of reaction of organic substrates on treatment with peresters in the presence of copper salts. Two mechanisms to account for the *N*-methylation reaction have been proposed, both of which involve initial electron transfer from cuprous ion to *tert*-butyl perbenzoate to produce cupric ion, benzoate and *tert*-butoxy radical, which in turn undergoes β–scission to afford methyl radical. Subsequent to this step, it is postulated that *N*-methylation may proceed *via* electron transfer from the substrate to cupric ion, or through complexation between the substrate and a methyl-copper(III) species, the latter suggesting that the coordinating ability of the substrate to the copper catalyst is an important factor. The selective reaction of the glycine derivative (55a), in preference to the corresponding alanine and valine derivatives (64) and (66), indicates that the relative reactivity of these substrates is determined by the comparative ease of their complexation to the copper. This provides another aspect to account for the selective reaction of glycine residues in free-radical reactions of α–amino acid derivatives. The reactions of the glycine derivatives (55a), (55b), (64) and (70) illustrate a complementary[17,21,23,24] new procedure for the *N*-methylation of α–amino acid derivatives, without racemisation.

In Chapter 2 of this thesis, the relative reactivity of the glycine derivatives (15), (55a), (80a), (80b), (82a) and (82b) with NBS is shown to be markedly affected by the electronic nature of the amine protecting group. Indeed, there is a direct correlation between the relative rates of reaction of these glycine derivatives and the pK_a values for the carboxylic acids corresponding to the *N*-substituents. The relative reactivity of these glycine derivatives with NBS is attributed to the ability of the *N*-substituent to donate electrons towards the stabilisation of an electron deficient site, developed in the transition state, at the site of hydrogen abstraction. This effect was exploited in reactions of *N*-benzoylglycine (91a) and *N*-

pentafluorobenzoylglycine (92) catalysed by PAM, since PAM-catalysed oxidative cleavage proceeds through a similar transition state. Competitive reactions involving the glycine derivatives (91a) and (92) with PAM give a preliminary indication that the *N*-acyl substituent of the fluorinated analogue (92) has an inhibitory effect on the reaction catalysed by the enzyme. This suggests that the ease of formation of the α–carbon centred radical is a critical factor in the catalytic cycle, and may therefore be the rate determining step. It is not yet clear to what extent the electronic nature of the *N*-acyl substituent reflects the rate of oxidative cleavage by PAM, a more detailed kinetic study of various *N*-protected glycine derivatives would help define the role of the amide moiety. Since PAM catalyses the biosynthesis of numerous hormones, further investigation could lead to the development of new pharmaceuticals that control metabolic disorders, associated with hormone activity.

It is possible that due to the relative ease of formation of glycyl radicals,[39] and the preferential complexation of glycine derivatives by metal ions described in Chapter 1 of this thesis, the naturally occurring substrates of PAM are synthesised with a glycine residue at the *C*-terminus because it is so easily removed by oxidation.

Synthesis of the dideuterides (54a) and (54b), described in Chapter 3 of this thesis, enabled a further investigation into the stereochemical course of the minor reaction pathway exhibited in the PAL-catalysed deamination of *(R)*-phenylalanine (50).[68] Treatment of each of the dideuterides (54a) and (54b) with PAL gives *trans*-cinnamic acid (48) with no deuterium content at the 2-position and approximately 25% deuterium content at the 3-position. Treatment of unlabelled *(R)*-phenylalanine (50) with PAL in deuteriated buffer gives the cinnamic acid (48) with approximately 25% deuterium content at the 2-position and 50% deuterium content at the 3-position. While reaction of *(R)*-phenylalanine (50) with the enzyme involves mainly loss of the 3-*pro*-*R* hydrogen and ammonia in an antiperiplanar elimination process,[68] the results in Chapter 3 indicate that a minor pathway for reaction of *(R)*-phenylalanine (50)

with the enzyme may be attributed, at least in part, to proton exchange. Consequently, the study could not differentiate between isomerisation to *(S)*-phenylalanine (49), before elimination, or synperiplanar elimination as the minor reaction.[68] Nevertheless, the investigation shows that proton exchange at the β–centre, and more importantly at the α–centre occurs only in the presence of the enzyme, which indicates another mechanism to account for the minor reaction. This suggests that PAL may well be converting from a lyase to a racemase in an effort to metabolise the unnatural substrate (50).

Experimental

General

Melting points were determined on a Reichert Kofler hot-stage micro-melting apparatus and are uncorrected. All temperatures were measured in degrees Celsius (°).

Infrared spectra were recorded on a Hitachi 270-30 spectrophotometer as nujol mulls between sodium chloride plates or as solutions in chloroform.

The micro analysis was performed by the Canadian Microanalytical Service Ltd., British Columbia.

Analytical thin layer chromatography was performed using Merck Kieselgel 60 F_{254} silica on aluminium backing plates. Preparative chromatography was performed using positive pressure flash chromatography[112] or dry column flash chromatography[113] using Merck Kieselgel 60 (230-400 mesh ASTM) or grade (1) neutral or basic alumina. The eluting solvents are stated in the procedures. Solvents were purified and dried using standard procedures.[114]

Nuclear magnetic resonance spectra were recorded on either a Bruker CXP-300 or a Varian VXR-500S spectrometer. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded at 300 MHz, unless otherwise specified, and carbon nuclear magnetic resonance (^{13}C NMR) spectra were also recorded at 300 MHz. Spectra were either recorded in deuteriochloroform ($CDCl_3$) using tetramethylsilane (TMS) as an internal standard, in deuterium oxide (D_2O), deuteriated dimethylsulfoxide (d^6-DMSO) or deuteriated methanol (d^4-MeOH). Chemical shifts are quoted as δ in parts per million. Multiplicities are abbreviated to; s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad.

Electron impact mass spectra were recorded with an AEI MS-30 double focussing mass spectrometer operating at 70eV. Fast atom bombardment (FAB) mass spectra were recorded on a Vacuum Generators ZAB 2HF mass spectrometer.

Ultraviolet (UV) spectra were recorded on an SP8-100 spectrophotometer, unless otherwise specified.

Organic solutions were dried by the addition of anhydrous magnesium sulphate, unless otherwise specified.

Experimental

Chapter 1

N-tert-Butoxycarbonyl-*N*-methylglycine Methyl Ester (56a)

To a solution of *N-tert*-butoxycarbonylglycine methyl ester (55a) (100mg, 0.53mmol) in benzene (40ml), was added cupric octanoate (2mg) and the mixture was treated with *tert*-butyl perbenzoate (0.82g, 4.2mmol). The reaction mixture was heated at reflux under nitrogen for 24h, and subsequently washed with saturated sodium metabisulphite (5 x 25ml) to remove the copper salt. The organic layer was dried with anhydrous sodium sulphate, and the solvent removed *in vacuo* to afford *N-tert*-butoxycarbonyl-*N*-methylglycine methyl ester (56a). Methyl benzoate and unreacted starting material (55a) were separated from the *N*-methyl derivative (56a) in the crude reaction mixture by flash chromatography on silica gel, eluting with a mixture of 20% ethyl acetate in hexane. The R_f values of (55a) and (56a) in this solvent system were (0.27) and (0.35), respectively, and the spots were visualised using a molybdic acid solution made up in a mixture of ethanol and water. Subsequent Kugelrohr distillation of (56a) isolated from the flash column afforded *N-tert*-butoxycarbonyl-*N*-methylglycine methyl ester (56a) as a clear oil (61mg, 57%), oven temp. (100°/0.05mm). ^{1}H NMR ($CDCl_3$) δ 3.99 (s, 2H, α–H -*E*-isomer), 3.93 (s, 2H, α–H -*Z*-isomer), 3.79 (s, 3H, CO_2Me -*E*-isomer), 3.77 (s, 3H, CO_2Me -*Z*-isomer), 2.94 (s, 3H, N-Me -*E*-isomer), 2.92 (s, 3H, N-Me -*Z*-isomer), 1.48 (s, 9H, $C(Me)_3$ -*E*-isomer), 1.43 (s, 9H, $C(Me)_3$ -*Z*-isomer). Spectral characteristics were consistent with those reported in the literature.[69]

The reaction conditions described above were found to be optimal after a series of reactions which involved varying each condition with respect to a standard reaction. The standard reaction was carried out as follows: To a

solution of *N-tert*-butoxycarbonylglycine methyl ester (55a) (100mg, 0.53mmol) in benzene (40ml), was added cupric octanoate (0.02g) and the mixture was treated with *tert*-butyl perbenzoate (0.4g, 2.1mmol). The reaction mixture was heated at reflux under nitrogen for 24h, and subsequently washed with saturated sodium metabisulphite (5 x 25ml) to remove the copper salt. The organic layer was dried with anhydrous sodium sulphate, and the solvent removed *in vacuo* to afford the crude mixture containing (56a) and the unreacted starting material (55a). Analysis of the crude reaction mixture by ^{1}H NMR spectroscopy determined the approximate yield of the *N*-methylglycine derivative (56a) to be 20-30%.

To a solution of (55a) (100mg, 0.53mmol) in benzene (150ml), was added cupric octanoate (0.02g) and the mixture was treated with *tert*-butyl perbenzoate (0.4g, 2.1mmol). The reaction mixture was heated at reflux under nitrogen for 24h, and subsequently worked up as described above. Analysis of the crude reaction mixture of (56a) by ^{1}H NMR spectroscopy showed a negligible change in the yield of the *N*-methylglycine derivative (56a) with respect to that obtained in the standard reaction, described above.

To a solution of (55a) (100mg, 0.53mmol) in benzene (15ml), was added cupric octanoate (0.02g) and the mixture was treated with *tert*-butyl perbenzoate (0.4g, 2.1mmol). The reaction mixture was heated at reflux under nitrogen for 24h, and subsequently worked up as described above. Analysis of the crude reaction mixture of (56a) by ^{1}H NMR spectroscopy showed a negligible change in the yield of the *N*-methylglycine derivative (56a) with respect to that obtained in the standard reaction.

To a solution of (55a) (100mg, 0.53mmol) in benzene (40ml), was added cupric octanoate (0.02g), and the mixture was treated with *tert*-butyl perbenzoate (4.1g, 21.1mmol). The reaction mixture was heated at reflux under nitrogen for 24h, and subsequently worked up as described above. Analysis of the crude reaction mixture of (56a) by ^{1}H NMR spectroscopy determined the yield of the *N*-methylglycine derivative (56a) to be 60-70 %.

To a solution of (55a) (100mg, 0.53mmol) in benzene (40ml) was added

cupric octanoate (0.27g) and the mixture was treated with *tert*-butyl perbenzoate (0.4g, 2.1mmol). The reaction mixture was heated at reflux under nitrogen for 24h, and subsequently worked up as described above. Analysis of the crude reaction mixture of (56a) by ^{1}H NMR spectroscopy showed no presence of the *N*-methylglycine derivative (56a).

N-Benzyloxycarbonyl-N-methylglycine Methyl Ester (56b)

N-Benzyloxycarbonyl-*N*-methylglycine methyl ester (56b) was prepared from *N*-benzyloxycarbonylglycine methyl ester (55b) (58mg, 0.26mmol) by the method described above for the formation of (56a) from (55a). Methyl benzoate and the unreacted starting material (55a) were separated from the *N*-methylglycine derivative (56b) in the crude reaction mixture by flash chromatography on silica gel, eluting with a mixture of 50% ether in hexane. The R_f values of (55b) and (56b) in this solvent system were (0.23) and (0.29), respectively, and the spots were visualised using a solution of molybdic acid made up in a mixture of ethanol and water. Subsequent Kugelrohr distillation of (56b) isolated from the flash column afforded *N*-benzyloxycarbonyl-*N*-methylglycine methyl ester (56b) as a clear oil (33mg, 54%), oven temp. (170°/0.2 mm). ^{1}H NMR ($CDCl_3$) δ 7.34 (s, 5H, Ph), 5.13 (s, 2H, $PhCH_2$), 4.05 (s, 2H, α–H), 3.76 (s, 3H, CO_2Me), 3.00 (s, 3H, N-Me). Spectral characteristics were in accord with those reported.[70]

N-Benzoylglycine Methyl Ester (15)

Thionyl chloride (1.9g, 15.4mmol) was added to a cooled and stirred solution of hippuric acid (2.5g, 14.0mmol) in methanol (150ml) and the mixture was allowed to stir at room temperature, under anhydrous conditions over-night. The reaction mixture was concentrated under reduced pressure, diluted with methanol and concentrated to dryness. Crystallisation from a mixture of ethyl acetate and hexane gave the *title compound* (15) as colourless crystals (2.4g, 89%),

m.p. 82-83° (Lit.[115] 82-83°). ^{1}H NMR ($CDCl_3$) δ 7.2-7.7 (m, 5H, Ph), 6.70 (br-d, J 5Hz, 1H, NH), 4.25 (d, J 5Hz, 2H, α-H), 3.80 (s, 3H, CO_2Me). Spectral characteristics were consistent with those previously reported.[27]

Treatment of *N*-Benzoylglycine Methyl Ester (15) with *tert*-Butyl Perbenzoate and Cupric Octanoate

To a solution of *N*-benzoylglycine methyl ester (15) (0.40g, 2.0mmol) in benzene (40ml) was added a catalytic amount of cupric octanoate (8mg), and the mixture was treated with *tert*-butyl perbenzoate (0.80ml, 4.0mmol) according to a procedure in the literature.[27] The mixture was heated at reflux under nitrogen over-night then subsequently worked up as described for the formation of (56a) from (55a). Analysis of the isolated material by ^{1}H NMR spectroscopy indicated the presence of the starting material (15) and *N*-benzoyl-2-benzoyloxyglycine methyl ester (16). Chromatography of the product mixture gave *N*-benzoylglycine methyl ester (15) (0.26mg, 64% recovery), with spectral characteristics consistent with those reported above, and the benzoate (16) (0.13g, 20%), m.p. 118-121° (Lit.[27] 122-123°) ^{1}H NMR ($CDCl_3$) δ 7.2-7.9 (m, 10H, Ph), 6.82 (d, J 9Hz, 1H, α-H), 3.85 (s, 3H, CO_2Me) The spectral characteristics of the benzoate (16) were found to be consistent with those previously reported.[27]

Treatment of a Mixture of *N*-Benzoylglycine Methyl Ester (15) and *N-tert*-Butoxycarbonylglycine Methyl Ester (55a) with *tert*-Butyl Perbenzoate and Cupric Octanoate

To a solution of *N*-benzoylglycine methyl ester (15) (20mg, 0.10mmol) and *N-tert*-butoxycarbonylglycine methyl ester (55a) (20mg, 0.10mmol) in benzene (40ml) was added a catalytic amount of cupric octanoate (2mg), and the mixture was treated with *tert*-butyl perbenzoate (0.16g, 0.8mmol). The mixture was heated at reflux over-night and worked up as described for the preparation of (56a) from (55a). Analysis of the product by ^{1}H NMR spectroscopy indicated the presence of

N-tert-butoxycarbonyl-*N*-methylglycine methyl ester (56a) and unreacted *N*-benzoylglycine methyl ester (15). The spectral characteristics for the *N*-methyl derivative (56a) and the *N*-benzoylglycine derivative (15) were in accord with those previously reported in the Experimental section. The α–benzoyloxyglycine derivative (16) was not produced, as the characteristic doublet at δ 6.82 attributable to the α–proton was not observed in the ^{1}H NMR spectrum.[27]

Treatment of Phthalimide (58) with *tert*-Butyl Perbenzoate and Cupric Octanoate

To a solution of phthalimide (58) (0.04g, 0.27mmol) in benzene (40ml) was added a catalytic amount of cupric octanoate (2mg), and the mixture was treated with *tert*-butyl perbenzoate (0.42g, 2.2mmol) as described for the formation of the *N*-methylamino acid derivative (56a) from (55a). After work up, the product mixture was purified by flash chromatography on silica gel eluting with 10% hexane in dichloromethane to afford *N*-methylphthalimide (59) as a white solid (0.03g, 75%), m.p. 132-134° (Lit.[116] 135°) ^{1}H NMR ($CDCl_3$) δ 7.84 (m, 2H, Ph), 7.72 (m, 2H, Ph), 3.19 (s, 3H, N-Me). The spectral and physical characteristics were consistent with those of an authentic sample.

Treatment of Diethyl Malonate (60a) with *tert*-Butyl Perbenzoate and Cupric Octanoate

To a solution of diethyl malonate (60a) (0.48g, 3.0mmol) in benzene (40ml) was added a catalytic amount of cupric octanoate (2mg) and the mixture was treated with *tert*-butyl perbenzoate (4.8g, 24.7mmol) as described for the formation of the *N*-methylamino acid derivative (56a) from (55a). Analysis of the crude reaction mixture by ^{1}H NMR spectroscopy indicated the presence of the unreacted starting material (60a). The spectral characteristics were consistent with those of an authentic sample.

Treatment of *N-tert*-Butoxycarbonylpropylamine (61) with *tert*-Butyl Perbenzoate and Cupric Octanoate

To a solution of *N-tert*-butoxycarbonylpropylamine (61) (0.11g, 0.69mmol) in benzene (40ml) was added a catalytic amount of cupric octanoate (2mg), and the mixture was treated with *tert*-butyl perbenzoate (1.1g, 5,7mmol), as described for the formation of the *N*-methylamino acid derivative (56a) from (55a). Methyl benzoate and the unreacted starting material (61) were separated from the *N*-methyl derivative (62) in the crude reaction mixture by flash chromatography on silica gel eluting with a mixture of ethyl acetate and hexane. Subsequent Kugelrohr distillation of the product afforded *N-tert*-butoxycarbonyl-*N*-methylpropylamine (62) as a clear oil (0.059g, 49%), oven temp. (65°/0.08mm). ^{1}H NMR ($CDCl_3$) δ 3.16 (m, 2H, α–H), 2.84 (s, 3H, N-Me), 1.54 (m, 2H, β–H), 1.46 (s, 9H, $C(Me)_3$, 0.89 (t, J 7.4 Hz, 3H, Me). The ^{1}H NMR spectrum of the product (62) was consistent with that of an authentic sample.

Treatment of *N-tert*-Butoxycarbonylglycine Methyl Ester (55a) with Di-*tert*-Butyl Peroxide and Cupric Octanoate

To a solution of *N-tert*-butoxycarbonylglycine methyl ester (55a) (0.03g, 0.16mmol) in benzene (15ml) was added di-*tert*-butyl peroxide (0.2g, 1.4mmol) and a catalytic amount of cupric octanoate (2mg). The mixture was irradiated continuously for 24h with ultraviolet light (350nm) in a Rayonet photochemical reactor. The reaction mixture was subsequently washed with sodium metabisulphite (3 x 25ml) to remove the copper salt, dried and concentrated *in vacuo*. The crude product was purified by flash chromatography on silica gel to afford *N-tert*-butoxycarbonyl-*N*-methyglycine methyl ester (56a) as a clear oil (0.014g, 42%), oven temp. (100°/0.05mm), with spectral characteristics consistent with those previously reported in the Experimental section.

Treatment of *N-tert*-Butoxycarbonylglycine Methyl Ester (55a) with Fenton's Reagent and DMSO

A stirred solution of *N-tert*-butoxycarbonylglycine methyl ester (55a) (100mg, 0.53mmol) and ferrous sulphate (220mg, 0.8mmol) in DMSO (1ml) was treated dropwise with a mixture of hydrogen peroxide (2ml) in DMSO, according to a procedure in the literature.[72] After work up, analysis of the crude product by TLC and ^{1}H NMR spectroscopy indicated the presence of the starting material (55a), with spectral characteristics consistent with those reported earlier in the Experimental section, and *N-tert*-butoxycarbonylalanine methyl ester (64). ^{1}H NMR ($CDCl_3$) δ 4.31 (m, 1H, α–H), 3.73 (s, 3H, CO_2Me), 1.43 (s, 9H, $C(Me)_3$, 1.38 (d, J 7.3 Hz, 3H, α–Me). Integration of the resonances characteristic of the starting material (55a) and the product (64) indicated that they were present in a 6:1 ratio.

***(S)-N-tert*-Butoxycarbonyl-*N*-methylalanine Methyl Ester (65)**

The *title compound* (65) was prepared from *(S)-N-tert*-butoxycarbonylalanine methyl ester (64) (100mg, 0.49mmol) by the method described above for the formation of the *N*-methyl derivative (56a) from (55a). Methyl benzoate and unreacted starting material (64) were separated from the *N*-methylalanine derivative (65) in the crude reaction mixture by flash chromatography on silica gel, eluting with 20% ethyl acetate in hexane. The R_f values of (64) and (65) in this solvent system were (0.30) and (0.39), respectively, and the spots were visualised using a molybdic acid solution made up in a mixture of ethanol and water. Subsequent Kugelrohr distillation of (65) isolated from the flash column afforded *(S)-N-tert*-butoxycarbonyl-*N*-methylalanine methyl ester (65) as a clear oil (51mg, 47%), oven temp. (100°/0.1 mm). ^{1}H NMR ($CDCl_3$) δ 4.85 (m, 1H, α–H), 4.38 (m, 1H, α–H) (mixture of *E*- and *Z*- isomers), 3.72 (s, 3H, CO_2Me), 2.87 (s, 3H, N-Me), 2.80 (s, 3H, N-Me) (mixture of *E*- and *Z*-isomers), 1.47 (s, 9H, $C(Me)_3$), 1.43 (s, 9H, $C(Me)_3$) (mixture of *E*- and *Z*-isomers),

1.38 (d, J 7.3 Hz, 3H, α–Me). Spectral characteristics were consistent with those reported in the literature.[21]

Increasing concentrations of the chiral europium shift reagent, $Eu(hfbc)_3$, in an NMR sample of the product (65) resulted in a spectrum which when compared with that of an authentic sample of racemic (65) indicated that the reaction proceeded without racemisation.

Treatment of *N*-tertButoxycarbonylvaline Methyl Ester (66) with *tert*-Butyl Perbenzoate and Cupric Octanoate

To a solution of *N-tert*-butoxycarbonylvaline methyl ester (66) (50mg, 0.22mmol) in benzene (40ml), was added cupric octanoate (2mg) and the mixture was treated with *tert*-butyl perbenzoate (0.34g, 1.8mmol) according to the method described in the formation of the *N*-methyl derivative (56a) from (55a), except the reaction mixture was heated at reflux under nitrogen, over 5 days. Analysis of the crude reaction mixture by ^1H NMR spectroscopy indicated the presence of only the unreacted starting material (66). ^1H NMR ($CDCl_3$) δ 5.04 (br, 1H, NH), 4.18 (m, 1H, α–H), 3.69 (s, 3H, CO_2Me), 2.10 (m, 1H, β–H), 1.40 (s, 9H, $C(Me)_3$), 0.89 (pair d, J 6.9 Hz, 6H, non-equivalent isopropyl methyl groups)

The reaction was repeated using *N-tert*-butoxycarbonylvaline methyl ester (66) (100mg, 0.43mmol) with addition of a greater excess of *tert*-butyl perbenzoate (2.5g, 13.2mmol). Analysis of the crude reaction mixture by ^1H NMR spectroscopy, again showed only the presence of the unreacted starting material (66).

Treatment of a Mixture of *N-tert*-Butoxycarbonylglycine Methyl Ester (55a) and *N-tert*-Butoxycarbonylalanine Methyl Ester (64) with *tert*-Butyl Perbenzoate and Cupric Octanoate

To a mixture of *N-tert*-butoxycarbonylglycine methyl ester (55a) (59mg, 0.31mmol) and *N-tert*-butoxycarbonylalanine methyl ester (64) (64mg, 0.31mmol) in benzene (40ml), was added cupric octanoate (2mg) and the mixture was treated

with *tert*-butyl perbenzoate (0.4g, 2.1mmol), as described for the formation of the *N*-methylamino acid derivative (56a) from (55a). After work up, analysis of the product by ^{1}H NMR spectroscopy indicated the presence of the *N*-methyl derivative (56a) and the unreacted alanine derivative (64), which implied that only the glycine derivative (55a) reacted with *tert*-butyl perbenzoate in the presence of cupric octanoate to afford the corresponding *N*-methylglycine derivative (56a). This was confirmed by analysis of ^{1}H NMR spectra of the product spiked with authentic standards.

Treatment of a Mixture of *N-tert*-Butoxycarbonylglycine Methyl Ester (55a) and *N-tert*-Butoxycarbonylvaline Methyl Ester (66) with *tert*-Butyl Perbenzoate and Cupric Ocatanoate

To a mixture of *N-tert*-butoxycarbonylglycine methyl ester (55a) (50mg, 26.4mmol) and *N-tert*-butoxycarbonylvaline methyl ester (66) (60mg, 26.1mmol), was added cupric octanoate (2mg) and the mixture was treated with *tert*-butyl perbenzoate (0.4g, 2.1mmol). The reaction mixture was heated at reflux under nitrogen for 24h, and worked up as described for the formation of the *N*-methylamino acid derivative (56a) from (55a). Analysis of the product by ^{1}H NMR spectroscopy indicated the presence of the *N*-methyl derivative (56a) and the unreacted valine derivative (66), which indicated that only the glycine derivative (55a) reacted with *tert*-butyl perbenzoate in the presence of cupric octanoate to afford the corresponding *N*-methylglycine derivative (56a). This was confirmed by analysis of ^{1}H NMR spectra of the product spiked with authentic standards.

Treatment of *N-tert*-Butoxycarbonylalanine (68) with *tert*-Butyl Perbenzoate and Cupric Ocatanoate

To a solution of *N-tert*-butoxycarbonylalanine (68) (0.19g, 1.0mmol) in benzene (50ml), was added cupric octanoate (10mg) and the mixture was treated with *tert*-butyl perbenzoate (0.39g, 2.0mmol). The reaction mixture was heated at

reflux under nitrogen for 48h, and subsequently washed with a minimum amount of saturated sodium metabisulphite (3 x 5ml) to remove the copper salt. The organic layer was dried with anhydrous sodium sulphate, and the solvent removed *in vacuo* to afford the crude product. The components were separated using flash chromatography on silica gel, eluting with 100% hexane initially, followed by a (1-5)% gradient of acetic acid in chloroform. On the basis of TLC and ^{1}H NMR spectra taken of the eluted fractions, it was determined that the product contained *N-tert*-butoxycarbonyl-*N*-methylalanine methyl ester (65), (spectral data reported above) (0.09g, 41%) and *N-tert*-butoxycarbonyl-*N*-methylalanine (69) (0.04g, 20%), m.p. 83-86° (Lit.[21] 89°) ^{1}H NMR ($CDCl_3$) δ 4.31 (m, 1H, α–H), 2.83 (s, 3H, N-Me), 2.88 (s, 3H, N-Me) (mixture of *E*- and *Z*-isomers), 1.45 (s, 9H, $C(Me)_3$), 1.40 (d, J 7.3 Hz, 3H, α–CH_3). The spectral characteristics were in accord with those reported in the literature.[21]

When the reaction was repeated using a reduced amount of the copper salt (2mg), a ^{1}H NMR spectrum of the crude reaction mixture showed only the presence of the starting material (68).

When the reaction was repeated using a four-fold molar excess or greater of *tert*-butyl perbenzoate, the main products observed by ^{1}H NMR spectroscopy were those due to decomposition.

N-tert-Butoxycarbonyl-_N_-methylserine Methyl Ester (71)

To a solution of *N-tert*-butoxycarbonylserine methyl ester (70) (0.37g, 1.7mmol) in benzene (50ml) was added cupric octanoate (2mg) and the mixture was treated with *tert*-butyl perbenzoate (2.6g, 13.4mmol). The reaction mixture was heated at reflux under nitrogen for 48h, and washed with sodium metabisulphite (5 x 15ml) to remove the copper salt. The organic layer was dried with anhydrous sodium sulphate, and the solvent was removed *in vacuo*. A ^{1}H NMR spectrum of the crude product showed the presence of *N-tert*-butoxycarbonyl-*N*-methylserine methyl ester (71) and the unreacted starting

material (70) in a ratio of approximately 3:1. Methyl benzoate was removed from the crude product using chromatography on silica, eluting with a (0-20)% gradient of ethyl acetate in hexane. The unreacted starting material (70) was separated from the *N*-methyl derivative (71) using chromatography on neutral alumina, eluting with a (0-80)% gradient of ethyl acetate in hexane. This afforded *N-tert*-butoxycarbonyl-*N*-methylserine methyl ester (71) in low yield (80mg, 21%) (Found *m/z* 202.108; $C_9H_{16}NO_4$ requires *m/z* 202.108) ^{1}H NMR ($CDCl_3$) δ 4.45 (m, 1H, α–H), 4.12 (m, 2H, CH_2OH), 3.77 (s, 3H, CO_2Me), 2.96 (s, 3H, N-Me), 2.90 (s, 3H, N-Me) (mixture of *E*-and *Z*- isomers), 1.47 (s, 9H, $C(Me)_3$), 1.43 (s, 9H, $C(Me)_3$) (mixture of *E*- and *Z*-isomers).

Synthesis of Substrate Amino Acids and Authentic Product Samples

N-tert-Butoxycarbonylglycine Methyl Ester (55a)

N-tert-Butoxycarbonylglycine methyl ester (55a) was prepared from glycine (72) (2.0g, 26.6mmol) *via N-tert*-butoxycarbonylglycine (73) using methods by Nagasawa and co-workers,[75] and Hassner and Alexanian.[76] The *title compound* (55a) was afforded as a clear oil after Kugelrohr distillation (3.8g, 76%), oven temp. (60°/0.03mm) ^{1}H NMR ($CDCl_3$) δ 5.03 (br, 1H, NH), 3.92 (d, J 5.7 Hz, 2H, α–H), 3.75 (s, 3H, Me), 1.45 (s, 9H, $C(Me)_3$). Spectral characteristics were consistent with those reported in the literature.[77]

N-Benzyloxycarbonylglycine Methyl Ester (55b)

To a stirring solution of glycine (72) (2.0g, 26.6mmol) in methanol (100ml) was slowly added thionyl chloride (4.7g, 39.9mmol) to afford the hydrochloride salt of glycine methyl ester (74).[78] Based on a modified literature procedure,[79] a solution of the crude salt (74) in triethylamine (10ml) and toluene (20ml) was placed in a round bottom flask fitted with two dropping funnels. The flask was cooled in an ice bath, and a mixture of benzylchloroformate (4.1g, 23.9mmol) in toluene (20ml), and triethylamine (10ml) were added simultaneously to the stirred solution over a period of 20min. The mixture was left to stir over-night. To remove unreacted salt in the crude reaction mixture, water (100ml) was added and the toluene layer was separated. The aqueous layer was subsequently extracted with toluene (2 x 50ml). The toluene layers were combined and stirred with 10% sodium hydroxide (20ml) to remove the excess benzylchloroformate. The organic solution was dried, and the solvent removed *in vacuo*. The crude *N*-benzyloxycarbonylglycine methyl ester (55b) thus obtained was purified using chromatography on silica to remove the residual benzylchloroformate. *N*-

benzyloxycarbonylglycine methyl ester (55b) was finally isolated as a clear oil (2.5 g, 39%), oven temp. (170°/0.3mm). ^{1}H NMR ($CDCl_3$) δ 7.25 (s, 5H, Ar), 5.42 broad, 1H, NH), 5.10 (s, 2H, $PhCH_2$), 3.86 (d, J 5.7 Hz, 2H, α–H), 3.65 (s, 3H, Me). Spectral characteristics were in accord with those reported in the literature.[70]

N-tert-Butoxycarbonylpropylamine (61)

_N_-Protection of propylamine (3.0g, 0.05mol) with BOC-ON was performed on the basis of a procedure in the literature.[75] The crude product was purified by Kugelrohr distillation to afford _N-tert_-butoxycarbonylpropylamine (61) as a clear oil (6.5g, 81%), oven temp. (50°/0.2mm). ^{1}H NMR ($CDCl_3$) δ 4.64 (b, 1H, NH), 3.07 (m, 2H, α–H), 1.51 (m, 2H, β–H), 1.44 (s, 9H, $C(Me)_3$), 0.91 (t, J 7.5 Hz, 3H, Me). (Found _m/z_ 159.126; $C_8H_{17}NO_2$ requires _m/z_ 159.126)

N-tert-Butoxycarbonylalanine Methyl Ester (64)

To a stirring solution of alanine (75) (5.0g, 56.1mmol) in methanol (150ml) was slowly added thionyl chloride (8.0g, 67.2mmol) to afford the hydrochloride salt of alanine methyl ester (76).[78] _N_-Protection of the salt (76) with BOC-ON was performed on the basis of a procedure in the literature.[75] The crude product was purified by Kugelrohr distillation to afford _N-tert_-butoxycarbonylalanine methyl ester (64) as a clear oil (8.7g, 76%), oven temp. (140°/0.5 mm). ^{1}H NMR ($CDCl_3$) δ 5.10 (br, 1H, NH), 4.31 (m, 1H, α–H), 3.73 (s, 3H, CO_2Me), 1.43 (s, 9H, $C(Me)_3$), 1.38 (d, J 7.3 Hz, 3H, α–Me). Spectral characteristics were in accord with those reported in the literature.[21]

N-tert-Butoxycarbonylalanine (68)

_N_-Protection of alanine (75) (2.0g, 22.4mmol) with BOC-ON was performed on the basis of a procedure in the literature.[75] The crude product was purified by repeated recrystallisations from mixtures of ether in hexane to give _N-tert_-butoxycarbonylalanine (68) (3.3g, 77%), m.p. 80-81° (Lit[117] 83-84°). ^{1}H NMR ($CDCl_3$) δ 5.15 (br, 1H, NH), 4.25 (m, 1H, α–H), 1.47 (s, 9H, $C(Me)_3$), 1.44 (d, J 7.4 Hz, 3H, α–Me). Spectral characteristics were in accord with those reported in the literature.[118]

N-tert-Butoxycarbonylvaline Methyl Ester (66)

To a stirring solution of valine (77) (5.0g, 42.7mmol) in methanol (150ml), was added thionyl chloride (6.1g, 51.3mmol) to afford the hydrochloride salt of valine methyl ester. _N_-Protection of the salt with BOC-ON was performed on the basis of a procedure in the literature.[75] The valine derivative (66) was afforded as a clear oil after Kugelrohr distillation (7.0g, 71%), oven temp. (100°/0.08mm). (Found: C, 56.7; H, 8.7; N, 6.1; O, 27.7. $C_{11}H_{21}NO_4$ requires C, 57.1; H, 9.1; N, 6.1; O, 27.7%). ^{1}H NMR ($CDCl_3$) δ 5.04 (br, 1H, NH), 4.18 (m, 1H, α–H), 3.69 (s, 3H, CO_2Me), 2.10 (m, 1H, β–H), 1.40 (s, 9H, $C(Me)_3$), 0.89 (pair d, J 6.9 Hz, 6H, non-equivalent isopropyl methyl groups)

N-tert-Butoxycarbonylserine Methyl Ester (70)

To a stirring solution of serine (78) (5.0g, 47.5mmol) in methanol (200ml) was slowly added thionyl chloride (6.7g, 56.3mmol) to afford the hydrochloride salt of serine methyl ester.[78] _N_-protection of the hydrochloride salt with BOC-ON was performed on the basis of a procedure in the literature.[75] The crude product was purified by flash chromatography on silica gel, eluting with a (0-30)% gradient of ethyl acetate in hexane. Kugelrohr distillation of the product isolated

from the column yielded *N-tert*-butoxycarbonylserine methyl ester (70) as a clear oil (7.0g, 64%), oven temp. (110°/0.03mm). ^{1}H NMR ($CDCl_3$) δ 5.62 (br d, J 7.8 Hz, 1H, NH), 4.37 (m, 1H, α–H), 3.95 (m, 2H, CH_2OH), 3.80 (s, 3H, CO_2Me), 3.16 (broad, 1H, OH), 1.45 (s, 9H, $C(Me)_3$). The spectral characteristics of the product (70) were consistent with those found in the literature.[119]

N-tert-Butoxycarbonyl-_N_-methylglycine Methyl Ester (56a)

To a stirring solution of *N*-methylglycine (1.0g, 11.2mmol) in methanol (40ml) was slowly added thionyl chloride (1.6g, 13.4mmol) to afford the hydrochloride salt of *N*-methylglycine methyl ester.[78] *N*-Protection of the salt with BOC-ON was performed on the basis of a procedure in the literature.[75] The crude product was purified by Kugelrohr distillation to yield the *title compound* (56a) as a clear oil, (1.8g, 80%), oven temp. (100°/0.05mm). The ^{1}H NMR spectral data for the product (56a) was reported earlier in the Experimental section, and is consistent with spectral data reported in the literature.[69]

N-tert-Butoxycarbonyl-_N_-methylalanine Methyl Ester (65)

To stirring solution of *N*-methylalanine (0.25g, 2.4mmol) in methanol (20ml) was slowly added thionyl chloride (0.35g, 2.9mmol) to afford the hydrochloride salt of *N*-methylalanine methyl ester.[78] *N*-Protection of the hydrochloride salt with BOC-ON was performed on the basis of a procedure in the literature.[75] After work up, the crude product was purified by Kugelrohr distillation to yield the *title compound* (65) as a clear oil (0.39g, 75%), oven temp. (100°/0.1mm). The ^{1}H NMR spectral data for the product (65) was reported earlier in the Experimental section and is in accord with spectral data reported in the literature.[21]

N-tert-Butoxycarbonyl-_N_-methylpropylamine (62)

_N_-Protection of _N_-methylpropylamine (0.13g, 1.8mmol) with BOC-ON was performed on the basis of a procedure in the literature.[75] After work up, the crude product was purified by Kugelrohr distillation to afford the *title compound* (62) as a clear oil (0.25g, 79%), oven temp. (65°/0.08mm). ^{1}H NMR ($CDCl_3$) δ 3.16 (m, 2H, α–H), 2.84 (s, 3H, N-Me), 1.54 (m, 2H, β–H), 1.46 (s, 9H, $C(Me)_3$, 0.89 (t, J 7.4 Hz, 3H, Me). (Found *m/z* 173.141; $C_9H_{19}NO_2$ requires 173.141)

Attempts to Prepare _N-tert_-Butoxycarbonyl-_N_-methylvaline Methyl Ester (67) From _N_-methylvaline

To a stirring solution of _N_-methylvaline (0.30g, 2.3mmol) in methanol (20ml) was slowly added thionyl chloride (0.34g, 2.9mmol) to afford the hydrochloride salt of _N_-methylvaline methyl ester, in quantitative yield.[78] Subsequent _N_-protection using BOC-ON on the basis of a procedure in the literature,[75] failed to give the desired product (67).

Addition of BOC-ON to _N_-methylvaline prior to esterification also failed to give the desired product (67).[75]

N-tert-Butoxycarbonyl-_N_-methylalanine (69)

_N_-Protection of _N_-methylalanine (0.5g, 4.8mmol) with BOC-ON was performed on the basis of a procedure in the literature.[75] After work up, the crude product was recrystallised twice from a mixture of ether in hexane to give _N-tert_-butoxycarbonyl-_N_-methylalanine (69) (0.69g, 71%), m.p. 86-87° (Lit[21] 89°). The spectral data of (69) was reported earlier in the Experimental section and is in accord with data reported in the literature.[21]

Chapter 2

N-Acetylglycine Methyl Ester (80a)

Potassium carbonate (14g, 0.1mol) was added to a stirred mixture of ice and crude glycine methyl ester hydrochloride (74) (8.5g, 0.07mol), and the resultant solution was cooled in an ice bath. Acetylchloride (5.0g, 0.06mol) was subsequently dissolved in chloroform and added to the cooled solution. The reaction mixture was allowed to stir over-night. The product was extracted into chloroform (3 x 50ml) and the combined organic extracts were washed with a saturated sodium bicarbonate solution (2 x 25ml), dried and concentrated *in vacuo*. The residue was purified by Kugelrohr distillation to yield *N*-acetylglycine methyl ester (80a) as a clear, viscous oil (5.8g, 74%), oven temp. (125-130°/0.05mm). (Found: C, 45.2; H, 6.9; N, 10.8. $C_5H_9NO_3$ requires C, 45.8; H, 6.9; N,10.7%) ^{1}H NMR ($CDCl_3$) δ 5.40 (br, 1H, NH), 4.07 (s, 2H, α–H), 3.77 (s, 3H, CO_2Me), 2.17 (s, 3H, Me).

Treatment of *N*-Acetylglycine Methyl Ester (80a) with *tert*-Butyl Perbenzoate and Cupric Octanoate

To a solution of *N*-acetylglycine methyl ester (80a) (0.1g, 0.8mmol) in benzene (50ml) was added a catalytic amount of cupric octanoate (2mg), and the mixture was treated with *tert*-butyl perbenzoate (0.6g, 3.0mmol) as described for the formation of the *N*-methyl derivative (56a) from (55a). After work up, chromatography on the product afforded the unreacted starting material (80a) (59.9mg, 62%) (spectral data reported above) and *N*-acetyl-2-benzoyloxyglycine methyl ester (81a), as a viscous oil (36.1mg, 18%). (Found *m/z* 251.079, $C_{12}H_{13}NO_5$ requires 251.079) ^{1}H NMR ($CDCl_3$) δ 8.11-7.82 (m, 5H, Ph), 6.58 (d, J 10Hz, 1H, α–H), 3.78 (s, 3H, CO_2Me), 2.10 (s, 3H, Me).

N-Trifluoroacetylglycine Methyl Ester (80b)

To a stirring solution of glycine (72) (5.0g, 66.6mmol) in methanol (150ml) was added thionyl chloride to afford the hydrochloric salt of glycine methyl ester (74) (8.3g, 66.0mmol).[78] Trifluoroacetic anhydride (9.4g, 66.0mmol) in dichloromethane (50ml) was subsequently added to a mixture of the glycine methyl ester hydrochloride (74) (8.3g, 66.0mmol) and triethylamine (16.8g, 0.16mol), and the mixture was left to stir over-night, under nitrogen.[110] The reaction mixture was washed with sodium bicarbonate (2 x 40ml), dried, and the solvent was removed under reduced pressure. The product was purified by Kugelrohr distillation to yield *N*-trifluoroacetylglycine methyl ester (80b) as an oil (10.7g, 87%), oven temp. (75°/0.3mm). ^{1}H NMR ($CDCl_3$) δ 6.9 (br, 1H, NH), 4.14 (d, J 5 Hz, 2H, α–H), 3.81 (s, 3H, CO_2Me). The spectral characteristics were consistent with those of an authentic sample.

Treatment of *N*-Trifluoroacetylglycine Methyl Ester (80b) with *tert*-Butyl Perbenzoate and Cupric Octanoate

To a solution of *N*-trifluoroacetylglycine methyl ester (80b) (0.05g, 0.27mmol) in benzene (40ml) was added a catalytic amount of cupric octanoate (2mg), and the mixture was treated with *tert*-butyl perbenzoate (0.42g, 2.2mmol), as described for the formation of the *N*-methyl derivative (56a) from (55a). Analysis of the crude reaction mixture by TLC and ^{1}H NMR spectroscopy indicated the presence of the unreacted starting material (80b), with spectral characteristics consistent with those reported above.

Treatment of a Mixture of *N*-Acetylglycine Methyl Ester (80a) and *N*-Trifluoroacetylglycine Methyl Ester (80b) with *tert*-Butyl Perbenzoate and Cupric Octanoate

To a mixture of *N*-acetylglycine methyl ester (80a) (100mg, 0.76mmol) and *N*-trifluoroacetylglycine methyl ester (80b) (141mg, 0.76mmol) in benzene (40ml) was added a catalytic amount of cupric octanoate (2mg), and the mixture was treated with *tert*-butyl perbenzoate (1.2g, 6.1mmol) by the method described above. Analysis of the crude reaction mixture by ^{1}H NMR spectroscopy indicated the presence of the α–benzoyloxyglycine derivative (81a), and the unreacted *N*-trifluoroacetylglycine derivative (80b), both with spectral characteristics consistent with those previously reported in the Experimental section.

***N*-*p*-Fluorobenzoylglycine Methyl Ester (82a)**

Potassium carbonate (1.5g, 0.01mol) was added to a stirred mixture of ice and glycine methyl ester hydrochloride (74) (280mg, 2.2mmol). The resulting solution was cooled in an ice bath while *p*-fluorobenzoyl chloride (350mg, 2.2mmol) was added, and the mixture was left to stir for 3h at room temperature. The product was extracted into chloroform (3 x 40ml) and the combined organic extracts were washed with a saturated sodium bicarbonate solution (2 x 30ml), dried, and evaporated *in vacuo*. The crude product was recrystallised from a mixture of ethyl acetate and hexane to afford *N*-*p*-fluorobenzoylglycine methyl ester (82a) as a white solid (0.34g, 73%), m.p. 115-117° (Found: C, 56.95; H, 4.81; N, 6.97. $C_{10}H_{10}FNO_3$ requires C, 56.85; H, 4.77; N, 6.63%). ^{1} NMR ($CDCl_3$) δ 7.14 (d, J 9 Hz, 2H, Ph), 7.10 (d, J 9Hz, 2H, Ph), 4.25 (d, J 5 Hz, 2H, α–H), 3.81 (s, 3H, CO_2Me).

N-Pentafluorobenzoylglycine Methyl Ester (82b)

Potassium carbonate (7.0g, 0.05mol) was added to a stirred mixture of ice and glycine methyl ester hydrochloride (74) (2.2g, 0.017mol), followed by the addition of pentafluorobenzoyl chloride (2.7g, 0.012mol), as described above. After work up, the crude product was recrystallised from a mixture of ethyl acetate and hexane to afford *N*-pentafluorobenzoylglycine methyl ester (82b) as a white solid (2.5g, 76%), m.p. 121-122° (Found: C, 42.40; H, 2.22; N, 4.97. $C_{10}H_6F_5NO_3$ requires C, 42.39; H, 2.14; N, 4.95%). ^{1}H NMR ($CDCl_3$) δ 6.70 (br, 1H, NH), 4.27 (d, J 5 Hz, 2H, α–H), 3.81 (s, 3H, CO_2Me).

Nickel Peroxide

A mixture of 6% sodium hypochlorite solution (20ml) and sodium hydroxide (3.4g) was added dropwise to nickel sulphate hexahydrate (3.4g) in water (20ml), and stirred for 1h at room temperature, according to a procedure in the literature.[120] The resulting black nickel peroxide was collected by filtration and washed with water to remove active chlorine. The resulting cake was dissolved in benzene and subsequently evaporated *in vacuo* to dryness. The black solid obtained was dried over anhydrous calcium chloride under reduced pressure for several days.

Treatment of *N*-Benzoylglycine Methyl Ester (15) with Nickel Peroxide

N-Benzoylglycine methyl ester (15) (50.0mg, 0.26mmol) in benzene (10ml) was treated with nickel peroxide (0.5g) at reflux under nitrogen for 2h, according to a procedure in the literature.[46] The reaction mixture was filtered while hot, the filtrate was concentrated under reduced pressure, and the residue was purified by flash chromatography on silica gel eluting with a (50-100)% gradient of ethyl acetate in hexane. Benzamide (45) was isolated as a white solid (11.3mg,

36%), m.p. 127-129° (Lit.[121] 128°). ^{13}C NMR ($CDCl_3$) δ 127.25, 128.51, 131.82, 169.55. The ^{13}C NMR spectral characteristics were consistent with those of an authentic sample.

Treatment of *N*-*p*-fluorobenzoylglycine Methyl Ester (82a) with Nickel Peroxide

N-*p*-Fluorobenzoylglycine methyl ester (82a) (50mg, 0.24mmol) in benzene (10ml) was treated with nickel peroxide (0.5g) at reflux, under nitrogen over-night, as described above. After work up the residue was purified by flash chromatography on silica gel eluting with a (50-100)% gradient of ethyl acetate in hexane. *p*-Fluorobenzamide (83a) was isolated as a white solid (13.0mg, 39%), m.p. 153-155° (Lit.[121] 154-157°). Thin layer chromatographs of the isolated product were found to be identical to those of an authentic sample. Mass spectrum; *m/z* 139 ($M^{+\bullet}$, 28%), 123 (41), 95 (31).

Treatment of *N*-Pentafluorobenzoylglycine Methyl Ester (82b) with Nickel Peroxide

N-Pentafluorobenzoylglycine methyl ester (82b) (0.11g, 0.39mmol) in benzene (10ml) was treated with nickel peroxide (1.0g) at reflux under nitrogen over-night, as described above. After work up the residue was purified by flash chromatography on silica gel eluting with 55% ethyl acetate in hexane. Pentafluorobenzamide (83b) was isolated as a white solid (20.0mg, 27%), m.p. 144-146° (Lit.[121] 146-149°). Thin layer chromatographs of the isolated product were found to be identical to those of an authentic sample. Mass spectrum; *m/z* 211 ($M^{+\bullet}$, 34%), 195 (44), 157 (22).

Treatment of *N-tert*-Butylbenzamide (84) with Nickel Peroxide

A solution of *N-tert*-butylbenzamide (84) (0.05g) in benzene (15ml) was treated with nickel peroxide (0.5g) and the mixture was heated at reflux under nitrogen over-night, as described above. After work up, the benzamide (84) was recovered in quantitative yield (0.05g, 100% recovery).

Treatment of a Mixture of *N*-Benzoylglycine Methyl Ester (15) and *N-p*-fluorobenzoylglycine Methyl Ester (82a) with Nickel Peroxide

A mixture of *N*-benzoylglycine methyl ester (15) (19.5mg, 0.10mmol) and *N-p*-fluorobenzoylglycine methyl ester (82a) (21.3mg, 0.10mmol) in benzene (20ml) was treated with nickel peroxide (0.16g), in the presence of the internal standard (84) (4mg). The reaction mixture was heated at reflux for 16h, and worked up as described above. The relative reactivity of the glycine derivatives (15) and (82a) was determined using ^{1}H NMR spectroscopy by examining the extent of depletion of each substrate relative to the internal standard (84). Analysis of the product by ^{1}H NMR spectroscopy indicated that 39% and 33% of the starting materials (15) and (82a) respectively, had been consumed. The ratio of the logarithmic values of the percentages of the unreacted starting materials (15) and (82a), 61% and 67%, respectively, is 1.2. This corresponds to the relative rate of reaction. The signals in the ^{1}H NMR spectrum were confirmed by spiking the product with authentic standards.

Treatment of a Mixture of *N*-Benzoylglycine Methyl Ester (15) and *N*-Pentafluorobenzoylglycine Methyl Ester (82b) with Nickel Peroxide

A mixture of *N*-benzoylglycine methyl ester (15) (38.0mg, 0.2mmol) and *N*-pentafluorobenzoylglycine methyl ester (82b) (55.7mg, 0.2mmol) in benzene (25ml) was treated with nickel peroxide (0.15g) in the presence of the internal standard (84) (7mg), as described above. After work up, analysis of the product by ^{1}H NMR spectroscopy, as described above, indicated that 17% and 31% of the starting materials (82b) and (15) respectively, had been consumed. This corresponds to a relative rate of 2.0.

A mixture of *N*-benzoylglycine methyl ester (15) (20.2mg, 0.1mmol) and *N*-pentafluorobenzoylglycine methyl ester (82b) (29.6mg, 0.1mmol) in benzene (25ml) was treated with nickel peroxide (0.15g) in the presence of the internal standard (84) (7mg), as described above, except the mixture was heated at reflux over 48h. After work up, analysis of the product by ^{1}H NMR spectroscopy indicated that 18% and 45% of the starting materials (82b) and (15), respectively, had been consumed, corresponding to a relative rate of 2.9.

A mixture of *N*-benzoylglycine methyl ester (15) (47.1mg, 0.24mmol) and *N*-pentafluorobenzoylglycine methyl ester (82b) (69.0mg, 0.24mmol) in benzene (25ml) was treated with nickel peroxide (0.15g) in the presence of the internal standard (84) (7mg), as described above, except the mixture was heated at reflux over 3 days. After work up, ^{1}H NMR spectroscopic analysis of the product indicated a relative rate between these substrates of 1.8.

A mixture of *N*-benzoylglycine methyl ester (15) (22.9mg, 0.12mmol) and *N*-pentafluorobenzoylglycine methyl ester (82b) (33.6mg, 0.12mmol) in benzene (25ml) was treated with nickel peroxide (0.15g) in the presence of the internal standard (84) (7mg), as described above, except the mixture was left to stir at room temperature over-night. After work up, analysis of the product by ^{1}H NMR spectroscopy indicated that 26% and 72% of the glycine derivatives (82b) and (15), respectively, had been consumed, corresponding to a relative rate of 4.3.

A mixture of *N*-benzoylglycine methyl ester (15) (0.25g, 1.3mmol) and *N*-pentafluorobenzoylglycine methyl ester (82b) (0.37mg, 1.3mmol) in benzene (30ml) was treated with nickel peroxide (0.15g) in the presence of the internal standard (84) (7mg). The mixture was left to stir at room temperature over-night. After work up, ^{1}H NMR spectroscopic analysis of the product indicated that the reaction proceeded until 18% and 46% of the glycine derivatives (82b) and (15), respectively, had been consumed corresponding to a relative rate of 3.1.

In each experiment, the signals in the ^{1}H NMR spectra were confirmed by spiking the products with authentic standards.

N-Benzoyl-α–bromoglycine Methyl Ester (26)

To a solution of *N*-benzoylglycine methyl ester (15) (50.0mg, 0.26mmol) in carbon tetrachloride (40ml) was added NBS (46mg, 25.9mmol). The mixture was irradiated with a 250W mercury lamp, and heated at reflux under nitrogen for 30min.[34] The reaction mixture was allowed to cool, then was filtered and the filtrate was washed with water (3 x 25ml). The organic phase was dried, then was concentrated under reduced pressure to give the *title compound* (26) which was purified by recrystallisation from a mixture of ethyl acetate and hexane (45.0mg, 79%), m.p. (85-88°) (Lit.[87] 87-90°). ^{1}H NMR ($CDCl_3$) δ 7.83 (m, 2H, Ph), 7.5 (m, 4H, Ph), 6.70 (d, J 10 Hz, 1H, α–H), 3.93 (s, 3H, CO_2Me). The spectral and physical characteristics were consistent with those found in the literature.[87]

N-*p*-Fluorobenzoyl-α–bromoglycine Methyl Ester (85a)

To a solution of *N*-*p*-fluorobenzoylglycine methyl ester (82a) (0.16g, 0.76mmol) in carbon tetrachloride (40ml) was added NBS (0.13g, 0.76mmol), as described above. This afforded the corresponding α–bromoglycine derivative (85a) (0.11g, 69%). ^{1}H NMR ($CDCl_3$) δ 7.19 (d, J 9Hz, 2H, Ph), 7.14 (d, J 9 Hz, 2H, Ph), 6.66 (d, J 10 Hz, 1H, α–H), 3.93 (s, 3H, CO_2Me).

The corresponding α–methoxyglycine derivative (86a) was prepared for characterisation, by allowing the bromide (85a) to stir in methanol for 30min. After concentration under reduced pressure, the crude product was purified by flash chromatography on silica gel to afford *N*-*p*-fluorobenzoyl-α–methoxyglycine methyl ester (86a) as a white solid (0.16g, 87%), m.p. 98-100° (Found: C, 54.85; H, 5.09; N, 6.01. $C_{11}H_{12}FNO_4$ requires C, 54.75; H, 5.02; N, 5.81%) ^{1}NMR ($CDCl_3$) δ 7.15 (d, J 9 Hz, 2H, Ph), 7.14 (d, J 9 Hz, 2H, Ph), 5.76 (d, J 9 Hz, 1H, α–H), 3.86 (s, 3H, CO_2Me), 3.54 (s, 3H, OMe).

N-Pentafluorobenzoyl-α–bromoglycine Methyl Ester (85b)

To a solution of *N*-pentafluorobenzoylglycine methyl ester (82b) (0.11g, 0.37mmol) in carbon tetrachloride (40ml) was added NBS (0.07g, 0.37mmol), as described above. This afforded the corresponding α–bromoglycine derivative (85b) (0.06g, 61%). ^{1}H NMR ($CDCl_3$) δ 6.73 (br, 1H, NH), 6.57 (d, J 5 Hz, 1H, α–H), 3.82 (s, 3H, CO_2Me).

The corresponding α–methoxyglycine derivative (86b) was prepared for characterisation, as described above. The crude product was purified by flash chromatography on silica gel to afford *N*-pentafluorobenzoyl-α–methoxyglycine methyl ester (86b) as a white solid, in 77% yield, m.p. 104-105° (Found: C, 42.28; H, 2.63; N, 4.57. $C_{11}H_8F_5NO_4$ requires C, 42.16; H, 2.57; N, 4.47%). ^{1}NMR ($CDCl_3$) δ 7.40 (br, 1H, NH), 5.71 (d, J 9 Hz, 1H, α–H), 3.86 (s, 3H, CO_2Me), 3.55 (s, 3H, OMe).

Treatment of a Mixture of *N*-Benzoylglycine Methyl Ester (15) and *N*-*p*-Fluorobenzoylglycine Methyl Ester (82a) with NBS

To a mixture of *N*-benzoylglycine methyl ester (15) (17.5mg, 0.09mmol) and *N*-*p*-fluorobenzoylglycine methyl ester (82a) (19.0mg, 0.09mmol) in carbon tetrachloride (40ml), was added NBS (16.0mg, 0.09mmol) and the internal

standard (84) (3.0mg, 0.02mmol), as described above. The relative reactivity of the glycine derivatives (15) and (82a) was determined using ^{1}H NMR spectroscopy in d^4-methanol, by examining the extent of depletion of each substrate relative to the internal standard (84). Analysis of the product by ^{1}H NMR spectroscopy indicated that 59% and 53% of the *N*-benzoylglycine derivative (15) and the *N*-*p*-fluorobenzoylglycine derivative (82a), respectively, had been consumed during the course of reaction. The ratio of the logarithmic values of the percentages of the unreacted starting materials (15) and (82a), 41% and 47%, respectively, is 1.2. This corresponds to the relative rate of reaction. The signals in the ^{1}H NMR spectrum were confirmed by spiking the product with authentic standards.

Treatment of a Mixture of *N*-Benzoylglycine Methyl Ester (15) and *N*-Pentafluorobenzoylglycine Methyl Ester (82b) with NBS

To a mixture of *N*-benzoylglycine methyl ester (15) (12.5mg, 0.06mmol) and *N*-pentafluorobenzoylglycine methyl ester (82b) (18.8mg, 0.06mmol) in carbon tetrachloride (40ml) was added NBS (11.0mg, 0.06mmol) and the internal standard (84) (2.0mg, 0.01mmol), as described above. Analysis of the product by ^{1}H NMR spectroscopy indicated that 71% and 27% of the respective starting material (15) and (82b) had been consumed, which corresponds to a relative rate of 4.0. The signals in the ^{1}H NMR spectrum were confirmed by spiking the product with authentic standards.

***N*-Acetyl-α–bromoglycine Methyl Ester (87a)**

To a solution of *N*-acetylglycine methyl ester (80a) (20.0mg, 0.15mmol) in carbon tetrachloride (40ml) was added NBS (27.1mg, 0.15mmol), as described above. After work up, the *title compound* (87a) was isolated (19mg, 60%), which tended to decompose over time. ^{1}H NMR ($CDCl_3$) δ 7.10 (br, 1H, NH), 6.48 (d, J 10 Hz, 1H, α–H), 3.87 (s, 3H, CO_2Me), 2.10 (s, 3H, Me). Spectral characteristics were in accord with those reported.[88]

The corresponding α–methoxyglycine derivative (88a) was prepared for characterisation, as described above. After concentration under reduced pressure, the product was purified by Kugelrohr distillation, oven temp. (100°/0.6mm), followed by recrystallisation from petroleum ether to yield *N*-acetyl-α–methoxyglycine methyl ester (88a), (17.8mg, 74%) m.p. 44-45° (Lit.[89] 44-46°). ^{1}H NMR ($CDCl_3$) δ 6.8 (br, 1H, NH), 5.53 (d, J 9 Hz, 1H, α–H), 3.82 (s, 3H, CO_2Me), 3.46 (s, 3H, OMe), 2.1 (s, 3H, Me). The spectral and physical characteristics were consistent with those found in the literature.[89]

_N_-Trifluoroacetyl-α–bromoglycine Methyl Ester (87b)

To a solution of *N*-trifluoroacetylglycine methyl ester (80b) (0.11g, 0.59mmol) in carbon tetrachloride (40ml) was added NBS (0.10g, 0.59mmol), as described above. This afforded the corresponding α–bromoglycine derivative (87b) (98mg, 63%) after work up. ^{1}H NMR ($CDCl_3$) δ 7.10 (br, 1H, NH), 6.36 (d, J 10 Hz, 1H, α–H), 3.94 (s, 3H, CO_2Me).

The corresponding α–methoxyglycine derivative (88b) was prepared for characterisation, as described above. The crude product was purified by flash chromatography on silica gel to afford *N*-trifluoroacetyl-α–methoxyglycine methyl ester (88b) as a white solid, in 80% yield, m.p. 96-99° (Found: C, 33.58; H, 3.88; N, 6.63. $C_6H_8F_3NO_4$ requires C, 33.48; H, 3.75; N, 6.51%) ^{1}H NMR ($CDCl_3$) δ 7.40 (br, 1H, NH), 5.53 (d, J 9 Hz, 1H, α–H), 3.87 (s, 3H, CO_2Me), 3.53 (s, 3H, OMe).

Treatment of a Mixture of _N_-Acetylglycine Methyl Ester (80a) and _N_-Benzoylglycine Methyl Ester (15) with NBS

To a mixture of *N*-acetylglycine methyl ester (80a) (20.2mg, 0.15mmol) and *N*-benzoylglycine methyl ester (15) (29.8mg, 0.15mmol) in carbon tetrachloride (40ml) was added NBS (27.0mg, 0.15mmol) and the internal standard (84) (4.0mg, 0.02mmol). The mixture was irradiated and heated at reflux under nitrogen over

30min, and worked up as described above. Analysis of the product by ^{1}H NMR spectroscopy indicated that 75% and 69% of the *N*-acetylglycine derivative (80a) and the *N*-benzoylglycine derivative (15), respectively, had been consumed during the course of reaction. This corresponds to a relative rate of 1.2. The signals in the ^{1}H NMR spectrum were confirmed by spiking the product with authentic standards.

Treatment of a Mixture of *N*-Benzoylglycine Methyl Ester (15) and *N*-Trifluoroacetylglycine Methyl Ester (80b) with NBS

To a mixture of *N*-benzoylglycine methyl ester (15) (23.2mg, 0.12mmol) and *N*-trifluoroacetylglycine methyl ester (80b) (22.2mg, 0.12mmol) in carbon tetrachloride (40ml) was added NBS (21.4mg, 0.12mmol) and the internal standard (84) (4.0mg, 0.02mmol), as described above. Analysis of the product by ^{1}H NMR spectroscopy indicated the presence of the α–bromoglycine derivative (26) and the unreacted starting material (80b).

Treatment of a Mixture of *N*-Trifluoroacetylglycine Methyl Ester (80b) and *N*-Pentafluorobenzoylglycine Methyl Ester (82b) with NBS

To a mixture of *N*-trifluoroacetylglycine methyl ester (80b) (20.3mg, 0.11mmol) and *N*-pentafluorobenzoylglycine methyl ester (82b) (31.0mg, 0.11mmol) in carbon tetrachloride (40ml), was added NBS (20.0mg, 0.11mmol), and the internal standard (84) (4.0mg) as described above. Analysis of the product by ^{1}H NMR spectroscopy indicated that 85% and 36% of the respective glycine derivative (82b) and (80b) had been consumed during the course of reaction, which corresponds to a relative rate of 4.2. The signals in the ^{1}H NMR spectrum were confirmed by spiking the product with authentic standards.

_N_-*tert*-Butoxycarbonyl-α–bromoglycine Methyl Ester (89)

To a solution of *N*-*tert*-butoxycarbonylglycine methyl ester (55a) (30mg, 0.16mmol) in carbon tetrachloride (40ml) was added NBS (28.5mg, 0.16mmol), as described above. After work up, the corresponding α–bromoglycine derivative (89) was isolated as an oil (27.3mg, 78%). ^{1}H NMR ($CDCl_3$) δ 6.90 (br, 1H, NH), 5.94 (d, J 10 Hz, 1H, α–H), 3.75 (s, 3H, CO_2Me), 1.52 (s, 9H, $C(Me)_3$. The spectral characteristics were consistent with those found in the literature.[90]

Treatment of a Mixture of *N*-Benzoylglycine Methyl Ester (15) and *N*-*tert*-Butoxycarbonylglycine Methyl Ester (55a) with NBS

To a mixture of *N*-benzoylglycine methyl ester (15) (21.0mg, 0.11mmol) and *N*-*tert*-butoxycarbonylglycine methyl ester (55a) (20.6mg, 0.11mmol) in carbon tetrachloride (40ml), was added NBS (15.0mg, 0.08mmol) and the internal standard (84) (4.0mg, 0.02mmol), as described above. Analysis of the product by ^{1}H NMR spectroscopy indicated that 78% and 46% of the respective starting material (55a) and (15) had been consumed during the course of reaction. This corresponds to a relative rate of 2.6. The signals in the ^{1}H NMR spectrum were confirmed by spiking the product with authentic standards.

_N_-Pentafluorobenzoylglycine (92)

To a solution of *N*-pentafluorobenzoylglycine methyl ester (82b) (177mg, 0.63mmol) in water (40ml) was added potassium carbonate (130mg, 0.94mmol) and the mixture was heated at reflux for 2h. The reaction mixture was acidified with dilute HCl and the aqueous layer was extracted with ether (5 x 30ml). The ether layer was dried and evaporated *in vacuo* and the residue was recrystallised in chloroform to afford *N*-pentafluorobenzoylglycine (92) as a white solid (0.14g,

83%), m.p. 156-159° Mass spectrum; *m/z* 270 (M^++ 1, 56%), 195 (39), 185 (45). ν_{max} 3000-2500, 1725-1700 cm^{-1}.

Treatment of a Mixture of *N*-benzoylglycine (91a) and *N*-Pentafluorobenzoylglycine (92) with PAM

Stock Solutions:

Sodium MES Buffer (100mM, pH 6.6)

Sodium 2-[*N*-morpholino]ethanesulfonate (MES) (2.17g, 0.01mol) was dissolved in water (100ml). The pH was reduced to 6.6 by the addition of conc. HCl.

Sodium Ascorbate (0.01M)

Sodium Ascorbate (10mg, 0.05mmol) was dissolved in sodium MES buffer (5ml, pH 6.6)

Copper Sulphate Hexahydrate (0.04M)

Copper sulphate hexahydrate (10mg, 0.04mmol) was dissolved in water (1000ml).

Initially, the response ratio of an equimolar mixture containing authentic samples of benzamide (45) and pentafluorobenzamide (83b) was measured on a reverse phase HPLC column. Chromatography was carried out using a Waters 6000A solvent pump, a Waters U6K injector and a Waters model 441 absorbance detector operating at 220nm, in conjunction with an ICI DP-700 data station. Analysis was performed using a Waters Nova Pak C_{18}-reverse phase 10 micron silica column, (100mm x 8mm), eluting with a gradient of (30-40)% acetonitrile in water with a flow rate of 1ml/min. HPLC analysis of the mixture showed the authentic standards (45) and (83b) to have retention times of 4.1 and 8.0 min, respectively. Integration of the peaks indicated that the amides (45) and (83b) were present in a ratio of 1.12 : 1.

Sodium ascorbate (0.4ml, 0.01M) and copper sulphate (0.05ml, 0.04M) taken from the stock solutions prepared above, were added to a 1ml volumetric flask containing bovine liver catalase (0.008g, 12,800 units), and the solution was made up to a total volume of 1ml with sodium-MES buffer (100mM, pH 6.6).[50] To this 1ml buffered solution was added PAM enzyme (Wako, recombinant from *Xenopus laevis*, solution in 100mM Tris-HCL buffer solution, pH 9.3, containing 0.3M NaCl; 1μL, 3000units). The incubation was performed at 37° in a constant temperature shaking water bath, and was initiated by the addition of a mixture of *N*-benzoylglycine (91a) (1.5mg, 0.008mmol) and *N*-pentafluorobenzoylglycine (92) (2.2mg, 0.008mmol). The mixture was left to incubate over-night and was subsequently dissolved in 10% sodium hydroxide to inactivate the enzyme and to convert any unreacted starting materials to their corresponding sodium carboxylates. The mixture was freeze dried under reduced pressure to afford a residue which was triturated with chloroform (10 x 15ml). The chloroform extracts were combined and evaporated *in vacuo* to yield the product which was dissolved in HPLC grade methanol (30μL) and analysed by HPLC, as described for the authentic standards. HPLC analysis indicated the presence of benzamide (45) and pentafluorobenzamide (83b) in a ratio of approximately 4:1, at retention times of 3.9 and 7.8 min, respectively. The peaks in the HPLC trace were confirmed by spiking the enzymatically produced material with authentic standards.

Using a different supply of PAM, the competitive experiment was repeated, as described above. The reaction was initiated by the addition of equimolar amounts of *N*-benzoylglycine (91a) (1.5mg) and *N*-pentafluorobenzoylglycine (92) (2.2mg). After work up, HPLC analysis of the product indicated the presence of benzamide (45) with a retention time of 4.2 min, however, chromatography failed to detect any pentafluorobenzamide (83b). Taking into account the limits of detection of the HPLC, this indicated that benzamide (45) and pentafluorobenzamide (83b) were present in a ratio of at least 20:1.

Unreacted starting materials were recovered from the reaction mixture by re-acidifying the corresponding sodium carboxylates with conc. HCL and

extracting with chloroform (5 x 25ml). The organic extracts were dried and concentrated under reduced pressure. Analysis of the recovered material by ^{1}H NMR spectroscopy indicated the presence of *N*-benzoylglycine (91a) and *N*-pentafluorobenzoylglycine (92) in a ratio of approximately 1:1. The signals were confirmed by spiking the recovered material with authentic standards.

Chapter 3

Treatment of *(S)*-Tyrosine (51a) with PAL

Sodium borate buffer (0.04M, pH 8.7) was prepared by dissolving sodium tetraborate decahydrate (0.38g, 1mmol) in water (25ml). The pH was reduced to 8.7 by the addition of conc. HCl. To a solution of *(S)*-tyrosine (51a) (33.5mg, 0.18mmol) in sodium borate buffer (25ml) was added PAL (Sigma, from *Rhodotorula glutinis*, grade 1, solution in 60% glycerol, 3mM, Tris-HCl, pH 7.5; 0.2ml, 0.5 units). The solution was stirred at 30° in a constant temperature water bath for 3 days. The mixture was acidified with conc. HCl to pH 1 and freeze dried under reduced pressure. The residue was triturated several times with chloroform and the organic extracts were combined, dried and evaporated *in vacuo*. Recrystallisation of the crude product gave a mixture of *cis-* and *trans-p*-hydroxycinnamic acid (53a) in a ratio of 1:6, (18.0mg, 61%), m.p. 211-213° (Lit.[121] 214°). ^{1}H NMR (d^6 DMSO) *trans*-isomer: δ 7.55 (d, 1H, J 16Hz, 3-position), 7.37 (d, 2H, J 8.6Hz, Ph), 6.8 (d, 8.6Hz, Ph), 6.21 (d, 1H, J 16Hz, 2-position); *cis*-isomer: δ 7.66 (d, 1H, J 12.8Hz, 3-position), 7.15 (d, 2H, J 8.4Hz, Ph), 6.76 (d, 2H, J 8.4Hz, Ph), 5.72 (d, 1H, J 12.8Hz, 2-position). The spectral characteristics were consistent with those of authentic samples.

Treatment of *(R)*-Tyrosine (52a) with PAL

(R)-Tyrosine (52a) (50mg, 0.28mmol) in sodium borate buffer (25ml) was treated with PAL (0.2ml, 0.5 units), as described above, except the mixture was incubated for 7 days. After work up, only a trace amount of *trans-p*-hydroxycinnamic acid (53a) was isolated from the reaction mixture. The spectral properties of the product (53a) were consistent with those reported above. To determine whether *trans-p*-hydroxycinnamic acid (53a) was decomposing during

the work up procedure, the following experiment was performed: Equimolar amounts of commercially available *transp*-hydroxycinnamic acid (53a) (23mg) and *trans*-cinnamic acid (48) (20mg) were incubated in sodium borate buffer (25ml) over 5 days at 30°. A ^{1}H NMR spectrum of the mixture taken prior to incubation showed the cinnamic acids (53a) and (48) to be present in a 1:1 ratio. After the incubation period the cinnamic acids (53a) and (48) were recovered as described above in the work up procedure for the reaction of *(S)*-tyrosine (51a) with PAL. A ^{1}H NMR spectrum of the mixture showed that the doublet resonances at δ 6.21 and δ 7.55 attributable to the protons at the 2- and 3-positions of *transp*-hydroxycinnamic acid (53a) had decreased in area by approximately 20%, relative to the doublets at δ 7.81 and δ 6.47 due to the protons at the 2- and 3-positions of *trans*-cinnamic acid (48). The signals were confirmed by spiking the mixture with authentic standards.

Treatment of *p*-Nitrophenylalanine (51b) with PAL

p-Nitrophenylalanine (51b) (33.0mg, 0.16mmol) in sodium borate buffer (25ml) was treated with PAL (0.2ml, 0.5 units) and the mixture was left to incubate over 7 days, as described above. After work up, the organic phase was dried and the solvent was removed under reduced pressure to give *trans-p*-nitrocinnamic acid (53b) (16.1mg, 52%). ^{1}H NMR (d^6 DMSO) δ 7.78-7.65 (m, 4H, Ph), 7.32 (d, J 16Hz, 1H, 3-position), 6.97 (d, J 16Hz, 1H, 2-position). The spectral characteristics were consistent with those of an authentic sample.

Treatment of *(S)-p*-Fluorophenylalanine (51c) with PAL

To a solution of *(S)-p*-fluorophenylalanine (51c) (23.0mg, 0.12mmol) in sodium borate buffer (20ml) was added PAL (0.2ml, 0.5units), and the mixture was incubated at 30° for 3 days. The reaction mixture was acidified to pH 1 with conc. HCL and extracted with dichloromethane (5 x 25ml). The organic phase was

dried and the solvent was removed under reduced pressure. Recrystallisation of the residue gave *trans-p*-fluorocinnamic acid (53c) (13.0mg, 65%), m.p. 206-209° (Lit.[121] 209-210°) ^{1}H NMR (d^6 DMSO) δ 7.75 (m, 2H, Ph), 7.58 (d, J 16Hz, 1H, 3-position), 7.24 (m, 2H, Ar), 6.49 (d, J 16Hz, 1H, 2-position). The spectral characteristics were consistent with those of an authentic sample.

Treatment of *(R)-p*-Fluorophenylalanine (52c) with PAL

(R)-p-Fluorophenylalanine (52c) (22.0mg, 0.11mmol) in sodium borate buffer (20ml) was treated with PAL (0.2ml, 0.5 units) as described above, except the mixture was allowed to incubate over 7 days. After work up, recrystallisation of the residue gave *trans-p*-fluorocinnamic acid (53c) (5.6mg, 29%). The spectral characteristics were in accord with those reported above.

Treatment of *(S)*-Phenylalanine (49) with PAL,
Reaction Monitored by UV Spectroscopy

To a solution of *(S)*-phenylalanine (49) (3ml, 1.1mM) in sodium borate buffer (pH 8.7) was added PAL (0.2ml, 0.5units). The solution was incubated at 30° in a constant temperature water bath and the reaction was monitored by UV spectroscopy for 30min, using a DMR-10 Zeiss Recording Spectrophotometer. The rate of change of absorbance of the solution was monitored at the fixed wavelength of 268nm,[96] and was assigned unity and referred to as the standard rate of conversion.

Treatment of *(R)*-Phenylalanine (50) with PAL,
Reaction Monitored by UV Spectroscopy

To a solution of *(R)*-phenylalanine (50) (3ml, 1.1mM) in sodium borate buffer was added PAL (0.2ml, 0.5 units), and the reaction was monitored by UV spectroscopy for 90min, as described above. The rate of change of absorbance of

the solution was monitored at the fixed wavelength of 268nm,[96] and was measured to be 0.002, relative to the standard rate of conversion.

Treatment of *(S)*-Tyrosine (51a) with PAL,
Reaction Monitored by UV Spectroscopy

To a solution of *(S)*-tyrosine (51a) (3ml, 1.1mM) in sodium borate buffer was added PAL (0.2ml, 0.5 units), and the reaction was monitored by UV spectroscopy for 45min, as described above. The rate of change of absorbance of the solution was monitored at the fixed wavelength of 308nm,[96] and was measured to be 0.23, relative to the standard.

Treatment of *(R)*-Tyrosine (52a) with PAL,
Reaction monitored by UV Spectroscopy

To a solution of *(R)*-tyrosine (52a) (3ml, 1.1mM) in sodium borate buffer was added PAL (0.2ml, 0.5 units) and the reaction was monitored by UV spectroscopy for 90min, as described above. The rate of change of absorbance of the solution was monitored at the fixed wavelength of 308nm,[96] and was measured to be 0.0006, relative to the standard rate.

Treatment of *(S)*-*p*-Nitrophenylalanine (51b) with PAL,
Reaction monitored by UV Spectroscopy

To a solution of *(S)*-*p*-nitrophenylalanine (51b) (3ml, 1.1mM) in sodium borate buffer was added PAL (0.2ml, 0.5 units) and the reaction was monitored by UV spectroscopy for 45min, as described above. The rate of change of absorbance of the solution was monitored at the fixed wavelength of 300nm.[96] No change in absorbance was observed.

The reaction was repeated, as described above, except the reaction was monitored by UV spectroscopy at a fixed wavelength of 276nm[96] over 12h, in

order to measure the rate of depletion of the starting material (51b). Again, no change in absorbance was observed.

Treatment of *(S)*-*p*-Fluorophenylalanine (51c) with PAL, Reaction monitored by UV Spectroscopy

To a solution of *(S)*-*p*-fluorophenylalanine (51c) (3ml, 1.1mM) in sodium borate buffer was added PAL (0.2ml, 0.5 units) and the reaction was monitored by UV spectroscopy for 35min, as described above. The rate of change of absorbance of the solution was monitored at the fixed wavelength of 275nm,[96] and was measured to be 0.41, relative to the standard rate.

Treatment of *(R)*-*p*-Fluorophenylalanine (52c) with PAL, Reaction monitored by UV Spectroscopy

To a solution of *(R)*-*p*-fluorophenylalanine (52c) (3ml, 1.1mM) in sodium borate buffer was added PAL (0.2ml, 0.5 units) and the reaction was monitored by UV spectroscopy for 120min, as described above. The rate of change of absorbance of the solution was monitored at the fixed wavelength of 275nm,[96] and was measured to be 0.0004, relative to the standard.

***(S)*-*N*-Phthaloyl-*p*-fluorophenylalanine Methyl Ester (101)**

METHOD I

(S)-*p*-Fluorophenylalanine (51c) (27.0mg, 0.15mmol) was suspended in water (10ml) with sodium carbonate (16.0mg, 0.15mmol) and the mixture was left to stir for 15min at room temperature. Carboethoxyphthalimide (CEP) (32mg, 0.15mmol) was subsequently added and the reaction mixture was left to stir over-night.[99] The solution was filtered, and the filtrate was extracted with ether (2 x 25ml) to remove the urethane by-product. The aqueous layer was acidified with

dilute HCL to pH 3, extracted with ether (5 x 25ml) and the resulting organic extracts were dried and concentrated under reduced pressure. Analysis of the product by ^{1}H NMR spectroscopy indicated the presence of a mixture of *(S)-N*-phthaloyl-*p*-fluorophenylalanine (104) ^{1}H NMR ($CDCl_3$) δ 7.80-7.68 (m, 4H, Phth), 7.13-7.11 (m, 2H, Ph), 6.90-6.87 (m, 2H, Ph), 5.17(t, J 8.4Hz, 1H, α–H), 3.56 (d, J 8.4Hz, 2H, β–H); and the product (105) from hydrolysis of CEP ^{1}H NMR ($CDCl_3$) δ 7.50 (m, 4H, Ph), 4.10 (q, J 7.1Hz, 2H, CH_2), 1.18 (t, J 7.1Hz, 3H, Me), in a ratio of 1:4. The spectral characteristics for the hydrolysis product (105) were found to be consistent with those reported in the literature.[100] When the reaction was performed over a prolonged period, a ^{1}H NMR spectrum of the crude reaction mixture showed a substantial decrease in the ratio of *(S)-N*-phthaloyl-*p*-fluorophenylalanine (104) to the hydrolysis product (105).

METHOD II

According to a procedure in the literature,[100] *(S)-p*-fluorophenylalanine (51c) (40mg, 0.2mmol) and sodium carbonate (19mg, 0.2mmol) were dissolved in water (8ml) and left to stir for 10min. CEP (38mg, 0.2mmol) in ethyl acetate (15ml) was subsequently added and the reaction mixture was vigorously stirred for 30min at room temperature. Analysis of the mixture by TLC indicated that all of the CEP had been consumed after this time. The organic layer was separated from the aqueous phase and the aqueous layer was washed with ether (2 x 25ml). The aqueous phase was subsequently acidified to pH 2 using dilute HCL, extracted with ether (5 x 25ml), and the combined organic extracts were dried and evaporated under reduced pressure. Analysis of the crude product by ^{1}H NMR spectroscopy indicated the presence of both *(S)-N*-phthaloyl-*p*-fluorophenylalanine (104) and the product from hydrolysis of CEP (105). The spectral characteristics of the products (104) and (105) were consistent with those reported above.

METHOD III

The hydrochloride salt of *(S)-p*-fluorophenylalanine methyl ester (100) (640g, 2.7mmol) and triethylamine (0.5ml) were dissolved in anhydrous DMSO (8ml), and the solution was treated with CEP (660mg, 3.0mmol).[97] The mixture was heated at 60-70° under nitrogen for 6h. After cooling, water was added to the reaction mixture (20ml) and the aqueous layer was extracted with dichloromethane (5 x 20ml). The extract was washed with water (2 x 20ml), dried, and the solvent was removed under reduced pressure to afford crude *(S)-N*-phthaloyl-*p*-fluorophenylalanine methyl ester (101) as an oil. The crude oil was adsorbed onto basic alumina and eluted with benzene (200ml). Evaporation of the eluent *in vacuo* afforded the *title compound* (101) as a clear, viscous oil. (0.50g, 56%) ^{1}H NMR ($CDCl_3$) δ 7.80-7.68 (m, 4H, Phth), 7.11 (m, 2H,Ph), 6.87 (m, 2H, Ph), 5.10 (dd, J 5.8Hz, J 10.7Hz, α–H), 3.78 (s, 3H, CO_2Me), 3.55 (dd, J 5.8Hz, 14.5Hz, 1H, β–H), 3.51 (dd, J 10.7Hz, 14.5Hz, 1H, β–H). ^{13}C NMR ($CDCl_3$) δ 33.8, 52.9, 53.1, 115.3, 115.6, 123.5, 130.3, 130.4, 131.4, 132.3, 134.2, 160.1, 163.3, 167.4, 169.1. Mass spectrum; *m/z* 327 ($M^{+}\bullet$, 17%), 307 (2), 268 (40), 250 (26), 218 (18), 190 (32), 180 (100), 163 (9), 149 (52), 130 (38), 121 (24), 109 (34), 76 (37). (Found *m/z* 327.091; $C_{18}H_{14}NO_4F$ requires *m/z* 327.091)

Increasing concentrations of the chiral europium shift reagent, $Eu(hfc)_3$, in an NMR sample of the product (101) resulted in a spectrum which when compared with that of the racemate (103) indicated that the reaction proceeded without racemisation.

_(R)-N_-Phthaloyl-_p_-fluorophenylalanine Methyl Ester (102)

Treatment of a solution of *(R)-p*-fluorophenylalanine (52c) (0.13g, 0.7mmol) in methanol (50ml) with thionyl chloride (1ml) to afford the corresponding hydrochloride salt (0.17g, 100%), followed by *N*-protection with CEP (0.16g, 0.7mmol), as described above, in METHOD III, gave the *title compound* in 61%

yield. The spectral characteristics were consistent with those of the *(S)*-enantiomer (101), reported above.

N-Phthaloyl-*p*-fluorophenylalanine Methyl Ester (103)

Treatment of a solution of racemic *p*-fluorophenylalanine (51c) (0.52g, 2.8mmol) in methanol (100ml) with thionyl chloride (1.5ml) to afford the corresponding hydrochloride salt (0.66g, 100%), followed by *N*-protection with CEP (0.68g, 3.1mmol), as described above in METHOD III, gave the *title compound* as a white solid, in 67% yield, m.p. 96-99°. The spectral characteristics were found to be consistent with those reported for the corresponding enantiomers (101) and (102).

(2S,3S)-3-Bromo-*N*-phthaloyl-*p*-fluorophenylalanine Methyl Ester (106a) and (2S,3R)-3-Bromo-*N*-phthaloyl-*p*-fluorophenylalanine Methyl Ester (106b)

To a solution of *(S)*-*N*-phthaloyl-*p*-fluorophenylalanine methyl ester (101) (250mg, 0.76mmol) in carbon tetrachloride (25ml) was added NBS (150mg, 0.84mmol), and the mixture was heated at reflux for 4h whilst irradiated with a 250W mercury lamp. The mixture was allowed to cool, then was filtered and the filtrate was washed with water (20ml). The organic phase was dried, then was concentrated under reduced pressure to give the *title compounds* (106a) and (106b) (181mg, 60%) as a 1:1 mixture. Fractional recrystallisation from a mixture of ethyl acetate and hexane gave the isomer (106b) (54.3mg, 30%) m.p. 149-151°. ^{1}H NMR ($CDCl_3$) δ 7.97-7.79 (m, 4H, Phth), 7.58-7.55 (m, 2H, Ph), 7.11-7.08 (m, 2H, Ph), 6.00 (d, J 11.2Hz, 1H, β–H), 5.44 (d, J 11.2Hz, 1H, α–H), 3.57 (s, 3H, CO_2Me). Mass spectrum; *m/z* 407 and 405 ($M^{+}\bullet$, 10%), 346 (4), 326 (50), 294 (75), 267 (100), 234 (7), 218 (53), 180 (67), 149 (32), 135 (9), 120 (52), 104 (61), 91 (37), 76 (62). (Found *m/z* 404.999 and 406.998; $C_{18}H_{13}NO_4FBr$ requires *m/z* 405.000 and $C_{18}H_{13}NO_4F^{81}Br$ requires *m/z* 406.998) Further recrystallisation gave the second diastereomer (106a) (50.0mg, 28%), m.p. 130-133°. ^{1}H NMR ($CDCl_3$) δ 7.73-7.67 (m,

4H, Phth), 7.37-7.33 (m, 2H, Ph), 6.90-6.85 (m, 2H, Ph), 5.91 (d, J 10.5Hz, 1H, β–H), 5.55 (d, J 10.3Hz, 1H, α–H), 3.82 (s, 3H, CO_2Me). ^{13}C NMR ($CDCl_3$) δ 49.9, 53.0, 56.8, 115.7, 116.0, 124.0, 130.0, 130.1, 131.5, 134.5, 135.1, 161.0, 164.3, 166.2, 167.0.

Hydrogenolysis of *(2S,3S)*-3-Bromo-*N*-phthaloyl-*p*-fluorophenylalanine Methyl Ester (106a)

A mixture of the bromide (106a) (10.2mg, 0.025mmol) and 5% Pd/C (2mg) in methanol (8ml) was stirred at -15° under an atmosphere of hydrogen over 3 days, in a refrigerated room.[68] The palladium catalyst was removed by filtration, and the solvent was removed under reduced pressure. The residue was taken up in dichloromethane (20ml) and washed with 10% sodium carbonate (2 x 10ml), then dried, and the solvent was removed *in vacuo.* This afforded the reduced product (101) (6.9mg, 85%), with spectral characteristics consistent with those previously reported in the Experimental section.

Treatment of *(2S,3S)*-3-Bromo-*N*-phthaloyl-*p*-fluorophenylalanine Methyl Ester (106a) with 5% Pd/C Under an Atmosphere of Deuterium.

A mixture of the bromide (106a) (20.0mg, 0.05mmol) and 5% Pd/C (2mg) in $^2H_1$-methanol (99.5% $^2H_1$, 2.5ml) was stirred at -15° under an atmosphere of deuterium over 4 days, in a refrigerated room, and worked up as described above. This gave the deuteride (108) as a 3:2 ratio of diastereomers. ^1H NMR ($CDCl_3$) δ 7.81-7.69 (m, 4H, Phth), 7.10-7.15 (m, 2H, Ph), 6.89-6.84 (m, 2H, Ph), 5.10 (br-d, 1H, α–H), 3.78 (s, 3H, CO_2Me), 3.58 (d, J 5.1Hz, 0.38H, β–H), 3.52 (d, J 12.0Hz, 0.62H, β–H) Complex multiplets in the region δ 7.69-7.81 indicated that ring opening of the phthaloyl protecting group had occurred during the course of reaction. The ^1H NMR spectrum also indicated the presence of products due to decomposition.

N-Phthaloylphenylalanine Methyl Ester (31a)

A mixture of commercially available racemic phenylalanine (10.0g, 60.5mmol) and finely ground phthalic anhydride (9.0g, 60.8mmol) was heated in an oil bath at 145-150° for 30min. The mixture was allowed to cool, then the solid was dissolved in hot methanol (60ml). The solution was filtered and then water (60ml) was added. *N*-Phthaloylphenylalanine crystallised on cooling and was isolated as fine colourless crystals (17g, 95%) m.p. 178-182° (lit.[122] 162-164°). ^{1}H NMR ($CDCl_3$) δ 10.69 (br-s, 1H, CO_2H), 7.77-7.61 (m, 4H, Phth), 7.18 (s, 5H, Ph), 5.23 (t, J 8.5Hz, α–H), 3.59 (d, J 8.5Hz, 2H, β–H). Spectral data was in agreement with previous reports.[111]

Racemic *N*-phthaloylphenylalanine (5.0g, 16.9mmol) was dissolved in dry methanol (100ml) which had been pre-treated with thionyl chloride (1ml). The solution was stirred under anhydrous conditions for 16h, then the solvent was removed under reduced pressure. The residue was taken up in dichloromethane (40ml), and the organic solution was washed with 10% sodium carbonate and water, then was dried. Removal of the solvent under reduced pressure and recrystallisation of the residue from a mixture of ethyl acetate in hexane gave the *title compound* (31a) as colourless crystals (4.40g, 85%), m.p. 89-92° (Lit.[123] 73-75°) ^{1}H NMR ($CDCl_3$) δ 7.79-7.70 (m, 4H, Phth), 7.18 (s, 5H, Ph), 5.21 (dd, J 5.6Hz, 10.9 Hz, 1H, α–H), 3.79 (s, 3H, CO_2Me), 3.62 (dd, J 5.6Hz, 14.3 Hz, 1H, β–H), 3.57 (dd, J 10.9 Hz, 14.3 Hz, 1H, β–H). The spectral characteristics were consistent with those previously reported.[108]

2-Deuterio-*N*-phthaloylphenylalanine Methyl Ester (109a)

To a flame dried round bottom flask was added $^2H_1$-*tert*-butanol (98% $^2H_1$, 5ml) and potassium metal (50mg) and the solution was stirred under anhydrous conditions over-night. To the resultant potassium *tert*-butoxide solution was added *N*-phthaloylphenylalanine methyl ester (31a) (1.8g, 5.8mmol), and the

mixture was stirred vigorously for 2h. Saturated ammonium chloride solution was added (2ml) to neutralise the *tert*-butoxide. Excess *tert*-butanol was removed *in vacuo* and the residue was partitioned between ether and brine. The organic layer was separated, dried and was evaporated under reduced pressure to afford 2-deuterio-*N*-phthaloylphenylalanine *tert*-butyl ester (109b) as a viscous oil, which solidified on standing (1.5g, 73%) ^{1}H NMR ($CDCl_3$) δ 7.79-7.67 (m, 4H, Phth), 7.17 (s, 5H, Ph), 5.10 (dd, J 5.6Hz, 10.9 Hz, 0.22H, α–H), 3.55 (dd, J 5.6Hz, 14.3 Hz, 0.78H, β–H), 3.51 (dd, J 10.9 Hz, 14.3 Hz, 0.78H, β–H), 1.48 (s, 9H, $CO_2C(Me)_3$). The percentage of deuterium contained at the α–position was determined by comparison of the integration of signals in the ^{1}H NMR spectrum at δ 5.10 and δ 3.53, attributable to the α– and β–proton, respectively. This gave 78% deuterium incorporation. The spectral characteristics were consistent with previous findings.[111]

To increase the percentage of deuterium contained at the α–position, the partially deuteriated *tert*-butyl ester (109b) (1.5g, 4.3mmol) was re-dissolved in $^2H_1$-*tert*-butanol (5ml) which had been pre-treated with potassium metal (50mg), as described above. This afforded the *tert*-butyl ester (109b) (1.1g, 70%) with spectral characteristics consistent with those described above, and 96% deuterium incorporation at the α–position.

2-Deuterio-*N*-phthaloylphenylalanine *tert*-butyl ester (109b) (1.1g, 3.1mmol) was subsequently dissolved in dry methanol (100ml) which had been pre-treated with thionyl chloride (1ml), and the reaction mixture was heated at reflux for 4h. The solvent was removed under reduced pressure and the residue was taken up in dichloromethane (40ml). The organic solution was washed with 10% sodium carbonate (3 x 20ml) and water (2 x 25ml), then was dried. Removal of the solvent under reduced pressure and recrystallisation of the residue from a mixture of ethyl acetate and hexane gave the *title compound* (109a) as white crystals (0.90g, 94%), m.p. 88-94°, with 96% deuterium contained at the α–position. ^{1}H NMR ($CDCl_3$) δ 7.79-7.70 (m, 4H, Phth), 7.18 (s, 5H, Ph), 5.21 (dd, J 5.6Hz, 10.9 Hz, 0.04H, α–H), 3.79 (s, 3H, CO_2Me), 3.62 (dd, J 5.6Hz, 14.3 Hz, 0.96H,

β–H), 3.57 (dd, J 10.9 Hz, 14.3 Hz, 0.96H, β–H). Mass spectrum; *m/z* 310 ($M^{+}\bullet$, 12%, 96% $^{2}H_1$), 251 ($C_{16}H_{11}DNO_2^{+}$, 24), 233 ($C_{12}H_9DNO_4^{+}$, 13), 219 (7), 205 (3), 191 (14), 163 (100), 132 (30), 104 (36), 91 (17), 76 (27). The ^{1}H NMR spectral characteristics were consistent with previous findings.[108]

(2*R*,2*S*)-Deuterio-(3*S*)-bromo-*N*-phthaloylphenylalanine Methyl Ester (110a) and (2*R*,2*S*)-Deuterio-(3*R*)-bromo-*N*-phthaloylphenylalanine Methyl Ester (110b).

To a solution of 2-deuterio-*N*-phthaloylphenylalanine methyl ester (109a) (0.3g, 0.96mmol) in carbon tetrachloride (15ml) was added NBS (0.19g, 1.0mmol), and the mixture was heated at reflux for 4h whilst irradiated with a 250W mercury lamp. The mixture was allowed to cool, then was filtered and the filtrate was washed with water (20ml). The organic phase was dried, then was concentrated under reduced pressure to give the *title compounds* (110a) and (110b) (0.27g, 72%) as a 1:1 mixture. Crystallisation from a mixture of hexane and dichloromethane gave the diastereomer (110b) as a mixture of enantiomers (68mg, 25%), m.p. 144-146°. ^{1}H NMR ($CDCl_3$) δ 7.96-7.79 (m, 4H, Phth), 7.60-7.38 (m, 5H, Ph), 6.02 (s, 0.94H, β–H), 5.52 (d, J 11.2Hz, 0.06H, α–H), 3.55 (s, 3H, CO_2Me). Mass spectrum; *m/z* 390 and 388 ($M^{+}\bullet$, 17%, 94% $^{2}H_1$), 331 (7), 329 ($C_{16}H_{10}BrDNO_2^{+}$, 8), 309 ($C_{18}H_{13}DNO_4^{+}$, 50), 276 (33), 250 (100), 219 (49), 191 (54), 162 (33), 130 (28), 104 (74), 90 (16), 76 (64).

The mother liquor was concentrated under reduced pressure and purified using HPLC. An SGE polymer coated C_{18}-silica column (5 microns, 250mm x 9.7mm) was used in conjunction with a Waters 717 auto sampler, two Waters 510 pumps, and a Waters 486 UV detector, operating at 256nm, with a semi-preparative flow cell. The system was controlled by a 486 computer with millennium software. Elution of the mother liquor through the HPLC column, with the solvent gradient described below, at a flow rate of 1ml/min gave the diastereomer (110a), as a mixture of enantiomers, (0.13g, 48%), m.p. 126-130°. ^{1}H NMR ($CDCl_3$) δ 7.72-7.63 (m, 4H, Phth), 7.37-7.11 (m, 5H, Ph), 5.92 (s, 0.95H, β–H), 5.59 (d, J 10.5Hz, 0.05H, α–H), 3.82 (s, 3H, CO_2Me). Mass spectrum; *m/z* 390 and

388 ($M^{+}\bullet$, 13%, 95% $^{2}H_1$), 331 (4), 329 ($C_{16}H_{10}BrDNO_2^{+}$, 5), 309 ($C_{18}H_{13}DNO_4^{+}$, 42), 276 (39), 250 (89), 219 (52), 191 (54), 162 (26), 130 (33), 104 (81), 90 (12), 76 (100). The spectral characteristics were consistent with previous findings.[124]

HPLC Solvent Gradient:

Time (min)	0	35	37	38
A (%)	95	40	95	95
B (%)	5	60	5	5

A = methanol : water, 40 : 60, and 0.4ml acetic acid/L
B = acetonitrile

(2*R*,3*S*)(2*S*,3*S*)-Dideuterio-*N*-phthaloylphenylalanine Methyl Ester (111a).

Treatment of a solution of the bromide (110a) (0.11g, 0.28mmol) in $^{2}H_1$-methanol (99.5% $^{2}H_1$, 5ml) with 5% Pd/C (50mg) under an atmosphere of deuterium for 4 days in a refridgerated bath, as described for the hydrogenolysis of the bromide (106a), gave the *title compound* (111a) as a white solid (0.07g, 79%) (98% d.e.), m.p. 92-100°. ^{1}H NMR ($CDCl_3$) δ 7.80-7.68 (m, 4H, Phth), 7.20-7.13 (m, 5H, Ph), 3.79 (s, 3H, CO_2Me), 5.10 (br-d, 0.05H, α–H), 3.53 (br-s, 0.95H, β–H). Mass spectrum; *m/z* 311 ($M^{+}\bullet$, 97% $^{2}H_1$) Assignment of the stereochemistry of the product (111a) was based on comparison of the signals in the ^{1}H NMR spectrum with spectral data obtained in a previous study.[68]

(2*R*,3*R*)(2*S*,3*R*)-Dideuterio-*N*-phthaloylphenylalanine Methyl Ester (111b)

A mixture of the bromide (110b) (0.12g, 0.3mmol) and 5% Pd/C (50mg) in $^{2}H_1$-methanol (99.5% $^{2}H_1$, 5ml) was stirred at -15° under an atmosphere of deuterium, as described above. This afforded the *title compound* (111b) (0.07g,

75%) (98% d.e.) m.p. 94-104°. ^{1}H NMR ($CDCl_3$) δ 7.80-7.68 (m, 4H, Phth), 7.19-7.15 (m, 5H, Ph), 5.10 (br-d, 0.06H, α–H), 3.79 (s, 3H, CO_2Me), 3.59 (br-s, 0.94H, β–H) Mass spectrum; *m/z* 311 ($M^{+}\bullet$, 96% $^2H_1$) Assignment of the stereochemistry of the product (111b) was based on comparison of the signals in the ^{1}H NMR spectrum with that obtained in a previous study.[68]

***(2R,3S)(2S,3S)*-Dideuteriophenylalanine (112a)**

A solution of the 2,3-dideuteriophenylalanine derivative (111a) (0.11mg, 0.35mmol) in 6N HCL/acetic acid (2:1, 15ml) was heated at reflux for 6h. The mixture was allowed to cool, then was evaporated to dryness under reduced pressure. The residue was taken up in water (20ml) and the phthalic acid was removed by filtration. The filtrate was evaporated to dryness, then the residue was dissolved in dry ethanol (2ml). A few drops of aniline was added and the solution was left to crystallise over a few days. The precipitate was isolated by filtration and was washed with acetone to give the *title compound* (112a) (42mg, 70%), as a white powder. m.p. 259-266° (Lit.[121] 266-267°, for the unlabelled derivative) ^{1}H NMR (D_2O) δ 7.33-7.21 (m, 5H, Ph), 3.92 (br-d, J 7.9Hz, 0.08H, α–H), 3.17 (br-s, 0.92H, β–H). Mass Spectrum *m/z* 167 ($M^{+}\bullet$, 95%, $^2H_1$, 33%); 122(82), 92($PhCHD^+$, 74); 75(100) The spectral characteristics were consistent with previous findings.[68]

***(2R,3R)(2S,3R)*-Dideuteriophenylalanine (112b).**

Treatment of the 2,3-dideuteriophenylalanine derivative (111b) (69mg, 0.22mmol) with 6N HCL/acetic acid (12ml), as described above, gave the *title compound* (112b) (27mg, 73%) as a white powder, m.p. 262-267° (Lit.[91] 266-267°, for the unlabelled derivative) ^{1}H NMR (D_2O) δ 7.32-7.20 (m, 5H, Ph), 3.90 (br-d, J 4.9Hz, 0.07H, α–H), 3.00 (br-s, 0.93H, β–H). Mass spectrum *m/z* 167($M^{+}\bullet$, 95% $^2H_1$, 25%); 122(77), 92($PhCHD^+$, 79); 75(100). The spectral characteristics were

consistent with those previously reported.[68]

(*S*)-*N*-Acetylphenylalanine (114a)

METHOD I

(*S*)-Phenylalanine (49) (0.10g, 0.6mmol) was dissolved in a mixture of water (10ml) and excess sodium carbonate (0.4g) with a small amount of crushed ice. Acetylchloride (0.1g, 0.12mmol) was subsequently added with vigorous stirring to the chilled solution, and the reaction mixture was left to stir for 50min, whilst the temperature was maintained below 0°.[105] The reaction mixture was acidified with conc. HCl to pH 1, extracted with ethyl acetate (5 x 25ml), dried and evaporated *in vacuo* to afford the *title compound* (114a) in low yield (29mg, 23%) m.p. 170-171° (Lit.[104] 171°) ν_{max} 3356 cm^{-1}, amide stretch; $[a]_D$ +40° (c, 1 in methanol) (Lit.[104] $[a]_D$ +41° (c, 1 in methanol)). ^1H NMR ($CDCl_3$ + d^6 DMSO) δ 9.73 (br-s, 1H, OH), 7.32-7.16 (m, 5H, Ph), 5.88 (br-d, 1H, NH), 4.87 (m, 1H, α–H), 3.19 (m, 2H, β–H), 2.00 (s, 3H, Me). The spectral characteristics were consistent with those reported in the literature.[106]

METHOD II

To a solution of (*S*)-phenylalanine (49) (0.11g, 0.67mmol) in water (10ml) was added triethylamine (0.15ml) and the solution was treated with acetic anhydride (0.1ml, 0.97mmol). The mixture was stirred vigorously for 2h, acidified to pH 1 with conc. HCL, and the aqueous layer was extracted with ethyl acetate (5 x 20ml). The organic layers were combined, dried, and evaporated under reduced pressure to afford the *title compound* (114a) as a white powder (0.13g, 98%), m.p. 170-171° (Lit.[104] 171°) $[a]_D$ +39° (c, 1 in methanol) (Lit.[104] $[a]_D$ +41° (c, 1 in methanol)) The spectral data was identical to that reported above.

(*S*)-*N*-Acetylphenylalanine Methyl Ester (114b)

A solution of *(S)-N*-acetylphenylalanine (114a) (0.1g, 0.48mmol) in methanol (60ml) was treated with thionyl chloride (0.5ml) and the mixture was left to stir at room temperature over-night. The mixture was concentrated under reduced pressure to give a residue which was recrystallised from a mixture of ether and petroleum ether. This afforded the *title compound* (114b) (0.09g, 88%), m.p. 85-87° (Lit.[106] m.p. 84-85°). ^{1}H NMR ($CDCl_3$ + d^6 DMSO) δ 7.33-7.08 (m, 5H, Ph), 5.90 (br-s, 1H, NH), 4.90 (dt, J 9Hz, 6Hz, 1H, α–H), 3.73 (s, 3H, CO_2Me), 3.10 (d, J 6Hz, 2H, β–H), 1.99 (s, 3H, Me) The spectral characteristics were consistent with those previously reported.[106]

The enantiomeric purity of the methyl ester (114b) was determined using gas chromatography. The product (114b) was injected onto a Chirasil-Val Capillary Column, 25m x 0.30mm with a temperature gradient of 190-200° over a period of 10min. A GC trace of the material showed a sharp, single peak, at a retention time of 4.40min, which when compared to that of the authentic racemate (116b) under identical conditions, indicated that the reaction proceeded without racemisation at the α-centre.

(*R*)-*N*-Acetylphenylalanine Methyl Ester (115b)

To a solution of *(R)*-phenylalanine (50) (0.11g, 0.67mmol) in water (15ml) was added triethylamine (0.17ml) and the solution was treated with acetic anhydride (0.1ml, 0.97mmol), as described for the preparation of *(S)-N*-acetylphenylalanine (114a). After work up, *(R)-N*-acetylphenylalanine (115a) was afforded as a white powder (0.12g, 96%) $[a]_D$ -39° (c, 1 in methanol) (Lit.[104] $[a]_D$ -41° (c, 1 in methanol)). The physical and spectral characteristics were consistent with those obtained for the other enantiomer (114a). The corresponding methyl ester (115b) was produced as described above, and had spectral characteristics consistent with those of the other enantiomer (114b).

The enantiomeric purity of the methyl ester (115b) was determined using chiral gas chromatography, as described above. A GC trace of the methyl ester (115b) showed a sharp, single peak at a retention time of 4.26min, which when compared with that of the authentic racemate (116b), indicated that the reaction proceeded without racemisation at the α-centre.

(*R,S*)-*N*-Acetylphenylalanine Methyl Ester (116b)

To a solution of racemic phenylalanine (0.11g, 0.67mmol) in water (10ml) was added triethylamine (0.15ml) and the solution was treated with acetic anhydride (0.1ml, 0.97mmol) as described above. After work up, the *N*-acetyl derivative (116a) was afforded as a white powder (0.13g, 98%) m.p. 145-146° (Lit.[104] 146°) and had spectral characteristics consistent with those of *(S)*-*N*-acetylphenylalanine (114a). The *N*-acetyl derivative (116a) was subsequently converted to the corresponding methyl ester (116b), m.p. 62-63° (Lit.[106] 60-61°), as described for the formation of (114b). The product (116b) had spectral properties identical to that of the *(S)*-enantiomer (114b), reported above.

GC analysis of the racemate (116b), under identical conditions to that described above, showed two sharp peaks in a 1:1 ratio, with retention times of 4.40min and 4.26min, corresponding to the *(S)*- and *(R)*-enantiomers.

(*2R,3S*)(*2S,3S*)-Dideuterio-*N*-acetylphenylalanine (113a)

To the deuteriophenylalanine derivative (112a) (39mg, 0.23mmol) in water (3.5ml) was added triethylamine (0.03ml) and the solution was treated with acetic anhydride (0.03ml) as described in METHOD II for the preparation of *(S)*-*N*-acetylphenylalanine (114a). After work up, the *title compound* (113a) was afforded as a white powder (26mg, 54%), m.p. 142-149° (Lit.[104] 146°, for the unlabelled derivative). ^{1}H NMR ($CDCl_3$) δ 7.31-7.13 (m, 5H, Ph), 6.07 (br-s, 1H, NH), 4.90 (br-d, J 5.9Hz, 0.08H, α–H), 3.10 (br-s, 0.92H, β–H), 1.99 (s, 3H, Me).

ν_{max} 3356 cm^{-1}, amide stretch. The spectral characteristics were consistent with those reported for the unlabelled derivative (114a).

(2R,3R)(2S,3R)-Dideuterio-*N*-acetylphenylalanine (113b)

To the deuteriophenylalanine derivative (112b) (40mg, 0.24mmol) in water (4ml) was added triethylamine (0.025ml) and the solution was treated with acetic anhydride (0.3ml), as described above. After work up, the *title compound* (113b) was afforded as a white powder (32mg, 64%), m.p.138-144° (Lit.[104] 146°, for the unlabelled derivative) ^1H NMR ($CDCl_3$) δ 7.32-7.15 (m, 5H, Ph), 5.95 (br-s, 1H, NH), 4.90 (br-d, J 5.9Hz, 0.08H, α–H), 3.21 (br-s, 0.92H, β–H), 2.00 (s, 3H, Me). ν_{max} 3356 cm^{-1}, amide stretch. The spectral characteristics were consistent with those of the corresponding unlabelled derivative (114a).

Enantiomeric Resolution of *N*-Acetylphenylalanine (116a) with Acylase I

Potassium phosphate buffer (pH 7.5, 0.1M) was prepared by dissolving potassium dihydrogen orthophosphate (0.68g, 5.0mmol) in water (50ml). The pH was adjusted to 7.5 with addition of lithium hydroxide (0.5M). Cobalt (II) chloride hexahydrate was added such that the total concentration of the cobalt salt would be approximately 1mM.

To a solution of *N*-acetylphenylalanine (116a) (30mg, 0.14mM) in phosphate buffer (1ml) was added acylase I (*N*-acylamino-acid amidohydrolase, grade 1 from porcine kidney lyophilised powder; 50mg, 200,000units) and the mixture was allowed to stir at 38° for 48h.[105] The pH was adjusted to pH 1 with conc. HCL and unreacted *(R)-N*-acetylphenylalanine (115a) was extracted from the aqueous solution with ethyl acetate (5 x 25ml). The organic layers were combined, dried and evaporated under reduced pressure to afford the product (115a) (9.0mg, 37%), m.p. 169-171° (Lit.[104] 171°). The ^1H NMR spectral characteristics were consistent with those previously reported in the Experimental section.

To determine the enantiomeric purity of the *N*-acetyl derivative (115a), a small portion of the isolated material was treated with a methanolic hydrogen chloride solution to afford the corresponding methyl ester (115b). The sample was injected onto a chiral GC column, under the same conditions as that described above, for the analysis of the authentic methyl ester (114b). A GC trace showed a single peak with a retention time of 4.25min, corresponding to the *(R)*-enantiomer (115b), indicating that >95% of the *(S)*-enantiomer (114a) had been metabolised by the enzyme, within the limits of detection. This was confirmed by spiking the methyl ester (115b) derived from the enzymatically produced *N*-acetyl derivative (115a) with authentic standards.

It should be noted that the enzyme-catalysed hydrolysis of *(S)*-*N*-acetylphenylalanine (114a) did not go to completion under the following conditions:

- if the incubation period was less than 30h
- if cobalt (II) chloride was not added to the buffered solution
- if the reaction was not performed in buffer
- if less than 4000 units of enzyme was added per mg of substrate

Enantionmeric Resolution of *(2R,3S)(2S,3S)*-Dideuterio-*N*-acetylphenylalanine (113a) with Acylase I

To a solution of the deuteriated phenylalanine derivative (113a) (26mg, 0.13mM) in phosphate buffer (1ml), was added acylase I (50mg, 200,000units) and the mixture was allowed to stir at 38° for 48h, as described above. After work up, the *N*-acetyl derivative (118a) (7mg, 26%), m.p. 164-169° (Lit.[104] 171°) was isolated, and had spectral characteristics consistent with those reported for (113a). A small portion was converted to the corresponding methyl ester (120a) and injected onto a chiral GC column, as described above, to determine the enantiomeric purity. A GC trace of the product showed a single peak with a retention time of 4.26min, corresponding to the *(R)*-enantiomer (120a), indicating

that >95% of the *(S)*-enantiomer (117a) had been metabolised by the enzyme. This was confirmed by spiking the methyl ester (120a), derived from the resolved product with authentic standards.

Enantiomeric Resolution of *(2R,3R)(2S,3R)*-Dideuterio-*N*-acetylphenylalanine (113b) with Acylase I

As described above, to a solution of the deuteriated phenylalanine derivative (113b) (30mg, 0.14mM) in phosphate buffer (1ml) was added acylase I (50mg, 200,000units) and the mixture was allowed to stir at 38° for 48h. This afforded the product (118b) (8.2mg, 27%), after work up. The ^{1}H NMR spectral characteristics were identical to those reported for (113b).

To determine the enantiomeric purity of the *N*-acetyl derivative (118b), a small portion of the isolated material was treated with a methanolic hydrogen chloride solution to afford the corresponding methyl ester (120b). The sample was injected onto a chiral GC column, as described above. A GC trace of the material showed a single peak with a retention time of 4.25min, corresponding to the *(R)*- enantiomer (120b), indicating that >95% of the *(S)*-enantiomer (117b) had been metabolised by the enzyme, within the limits of detection. This was confirmed by spiking the methyl ester (120b), derived from the resolved product with authentic standards.

***(2R,3S)*-2,3-Dideuteriophenylalanine (54a)**

Treatment of the *N*-acetyl derivative (118a) (6mg, 0.03mmol) with a mixture of 6N HCL/acetic acid (2:1, 9ml), as described for the preparation of (112a) from (111a), gave the *title compound* (54a) as a white powder (42mg, 80%) m.p. 264-269° (Lit.[68] 268-272°), with 91% deuterium contained at the α–position. The ^{1}H NMR spectral characteristics were identical to those reported for (112a). Mass spectrum; *m/z* 167 ($M^{+\bullet}$, 91%, $^2H_1$, 6%); 122(76), 92($PhCHD^+$, 81), 75(100).

(2*R*,3*R*)-2,3-Dideuteriophenylalanine (54b)

Treatment of the *N*-acetyl derivative (118b) (8mg, 0.04mmol) with a mixture of 6N HCL/acetic acid (2:1, 12ml), as described for the preparation of the derivative (112a) from (111a), gave the *title compound* (54b) as a white powder (5.2mg, 78%) m.p. 268-273° (Lit.[68] 270-273°), with 90% deuterium contained at the α–position. The ^{1}H NMR spectral characteristics were consistent with those reported for (112b). Mass Spectrum *m/z* 167 ($M^{+\bullet}$, 91%, $^2H_1$, 4%); 122(78), 92($PhCHD^+$, 79), 75(100).

Treatment of (2*R*,3*R*)-3-Deuteriophenylalanine (46d) with PAL

To a solution of (2*R*,3*R*)-3-deuteriophenylalanine (46d) (10mg, 0.06mmol) in sodium borate buffer (8ml) was added PAL (0.2ml, 0.5units). The solution was stirred at 30° in a constant temperature air incubator for 8 days. The solution was acidified to pH 1 by the addition of conc. HCL, then was extracted with dichloromethane (3 x 50ml). The organic phase was dried and the solvent was removed under reduced pressure. Recrystallisation of the residue gave *trans*-cinnamic acid (48) (5.4mg, 60%), m.p. 134-137° (Lit.[68] 135-137°). ^{1}H NMR (500MHz, $CDCl_3$) δ 7.81 (d, J 16Hz, 0.73H, 3-position), 7.41-7.58 (m, 5H, Ph), 6.47 (d, J 16Hz, 0.73H, 2-position), 6.46 (br-s, 0.27H, 2-position). ^{1}H NMR spectroscopic analysis indicated that the product (48) contained approximately 27% deuterium at the 3-position, which is consistent with previous findings.[68]

Treatment of (2*R*,3*S*)-2,3-Dideuteriophenylalanine (54a) with PAL

Treatment of a solution of (2*R*,3*S*)-2,3-dideuteriophenylalanine (54a) (2.2mg, 0.01mmol) in sodium borate buffer (2ml) with PAL (0.2ml, 0.5 units), as described above, gave *trans*-cinnamic acid (48) (0.85mg, 57%). ^{1}H NMR spectroscopic analysis indicated that the product (48) contained approximately 25% deuterium

at the 3-position, and no deuterium could be detected at the 2-position. The spectral characteristics were consistent with those reported above.

Treatment of *(2R,3R)*-2,3-Dideuteriophenylalanine (54b) with PAL

Treatment of a solution of *(2R,3R)*-2,3-dideuteriophenylalanine (54b) (2.0g, 0.01mmol) in sodium borate buffer (2ml) with PAL (0.2ml, 0.5 units), as described above, gave *trans*-cinnamic acid (48) (0.9mg, 61%). ^{1}H NMR spectroscopic analysis indicated that the product (48) contained approximately 25% deuterium at the 3-position, and no deuterium could be detected at the 2-position. The spectral characteristics were consistent with those reported above.

Treatment of *(R)*-phenylalanine (50) with PAL in Deuteriated Buffer

Deuteriated sodium borate buffer (0.04M, pH 8.7) was prepared by dissolving sodium tetraborate decahydrate (0.38g, 1.0mmol) in D_2O (25ml). The pH was reduced to 8.7 by the addition of conc. HCL. Treatment of *(R)*-phenylalanine (50) (1.1mg, 0.006mmol) in deuteriated sodium borate buffer (2ml) with PAL (0.2ml, 0.5units), as described above, gave *trans*-cinnamic acid (48). ^{1}H NMR spectroscopic analysis indicated that the product (48) contained approximately 50% deuterium at the 3-position and 25% deuterium at the 2-position.

The reaction was repeated using a lower enzyme to substrate ratio as follows: To a solution of *(R)*-phenylalanine (50) (44.0mg, 0.27mmol) in deuteriated sodium borate buffer (25ml) was added PAL (0.2ml, 0.5 units). The solution was stirred at 30° in a constant temperature water bath for 7 days. After work up, analysis of the crude product by ^{1}H NMR spectroscopy indicated the presence of *trans*-cinnamic acid (48), with no deuterium contained at either the 2- or 3-positions. ^{1}H NMR (500MHz, $CDCl_3$) δ 7.79 (d, J 16Hz, 1H, 3-position), 7.56 (m, 2H, Ph), 7.42 (m, 2H, Ph), 6.45 (d, J 16Hz, 1H, 2-position). The spectral characteristics were consistent with those of an authentic sample.

Incubation of a Mixture of *(R)*-Phenylalanine (50) and *trans*-Cinnamic acid (48) at 30° in Deuteriated Buffer

Deuteriated sodium borate buffer (0.04M, pH 8.7) was prepared by dissolving sodium tetraborate decahydrate (0.38g, 1.0mmol) in D_2O (25ml), as described above. An equimolar mixture of *(R)*-phenylalanine (50) (3.3mg) and *trans*-cinnamic acid (48) (3.0mg) was suspended in deuteriated buffer (5ml), and left to incubate at 30° over several days. A ^1H NMR spectrum of the mixture indicated the presence of *(R)*-phenylalanine (50) and *trans*-cinnamic acid (48) with no deuterium incorporation in either substrate.

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Appendix 1

EXPERIMENTAL DETAILS

A. Crystal Data

Empirical Formula	$C_{10}H_{11}NO_3$
Formula Weight	193.20
Crystal Color, Habit	colorless, needle
Crystal Dimensions	0.10 X 0.15 X 0.20 mm
Crystal System	orthorhombic
Lattice Type	Primitive
No. of Reflections Used for Unit	
Cell Determination (2θ range)	25 (46.8 - 63.8°)
Omega Scan Peak Width	
at Half-height	0.32°
Lattice Parameters	a = 17.707(2)Å b = 12.830(1) Å c = 8.742(1) Å
	V = 1986.0(9) $Å^3$
Space Group	Pccn (#56)
Z value	8
D_{calc}	1.292 g/cm^3
F_{000}	816.00
μ(CuKα)	7.63 cm^{-1}

B. Intensity Measurements

Diffractometer	Rigaku AFC6R
Radiation	CuKα (λ = 1.54178 $\AA$) graphite monochromated
Take-off Angle	6.0°
Detector Aperture	7.0 mm horizontal 7.0 mm vertical
Crystal to Detector Distance	400 mm
Temperature	23.0°C
Scan Type	ω-2θ
Scan Rate	8.0°/min (in ω) (up to 4 scans)
Scan Width	(0.80 + 0.30 tan θ)°
$2\theta_{max}$	120.2°
No. of Reflections Measured	Total: 1739
Corrections	Lorentz-polarization Absorption (trans. factors: 0.9607 - 1.0000) Secondary Extinction (coefficient: 1.6(2)e-06)

C. Structure Solution and Refinement

Structure Solution	Direct Methods (SIR92)
Refinement	Full-matrix least-squares
Function Minimized	$\Sigma w(\|Fo\| - \|Fc\|)^2$
Least Squares Weights	$\frac{1}{\sigma^2(Fo)} = \frac{4Fo^2}{\sigma^2(Fo^2)}$
p-factor	0.0040
Anomalous Dispersion	All non-hydrogen atoms
No. Observations (I>3.00σ(I))	673
No. Variables	128
Reflection/Parameter Ratio	5.26
Residuals: R; Rw	0.036 ; 0.025
Goodness of Fit Indicator	1.58
Max Shift/Error in Final Cycle	0.01
Maximum peak in Final Diff. Map	0.12 $e^-/\AA^3$
Minimum peak in Final Diff. Map	-0.12 $e^-/\AA^3$

Table 1. Atomic Coordinates and Isotropic Displacement Parameters for $C_{10}H_{11}NO_3$

atom	x	y	z	B_{eq}
O(1)	-0.0537(1)	0.1574(2)	0.5086(3)	4.89(7)
O(2)	-0.2116(1)	0.3846(2)	0.5495(4)	7.66(10)
O(3)	-0.0898(1)	0.4175(2)	0.5108(3)	5.53(8)
N(1)	-0.0727(2)	0.2625(2)	0.3070(3)	4.27(8)
C(1)	0.0474(2)	0.1711(3)	0.3289(5)	3.84(10)
C(2)	0.0825(2)	0.2277(3)	0.2141(5)	4.8(1)
C(3)	0.1537(2)	0.2001(4)	0.1637(5)	6.1(1)
C(4)	0.1893(2)	0.1161(4)	0.2296(6)	6.7(2)
C(5)	0.1556(2)	0.0583(3)	0.3431(6)	6.1(1)
C(6)	0.0842(2)	0.0867(3)	0.3924(5)	5.1(1)
C(7)	-0.0303(2)	0.1967(3)	0.3877(5)	3.9(1)
C(8)	-0.1484(2)	0.2882(3)	0.3572(5)	5.0(1)
C(9)	-0.1543(2)	0.3676(3)	0.4833(5)	4.9(1)
C(10)	-0.0925(2)	0.4975(4)	0.6276(5)	7.3(1)
H(1)	-0.0519	0.2947	0.2103	5.1085
H(2)	0.0574	0.2857	0.1697	5.7467
H(3)	0.1777	0.2388	0.0848	7.3692
H(4)	0.2385	0.0974	0.1958	7.9892
H(5)	0.1807	0.0000	0.3866	7.2826
H(6)	0.0602	0.0477	0.4710	6.1436
H(8a)	-0.1754	0.3143	0.2713	5.9984
H(8b)	-0.1717	0.2258	0.3920	5.9984
H(10a)	-0.0434	0.5259	0.6420	8.7587
H(10b)	-0.1261	0.5513	0.5969	8.7587

Table 1. Atomic Coordinates and Isotropic Displacement Parameters for $C_{10}H_{11}NO_3$ (cont...)

atom	x	y	z	B_{eq}
H(10c)	-0.1096	0.4680	0.7212	8.7587

$$B_{eq} = \frac{8}{3}\pi^2(U_{11}(aa^*)^2 + U_{22}(bb^*)^2 + U_{33}(cc^*)^2 + 2U_{12}aa^*bb^*\cos\gamma + 2U_{13}aa^*cc^*\cos\beta + 2U_{23}bb^*cc^*\cos\alpha)$$

Table 2. Anisotropic Displacement Parameters for $C_{10}H_{11}NO_3$

atom	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
O(1)	0.066(2)	0.065(2)	0.055(2)	-0.002(2)	0.006(2)	0.002(2)
O(2)	0.068(2)	0.098(2)	0.126(3)	0.014(2)	0.035(2)	-0.022(2)
O(3)	0.066(2)	0.067(2)	0.077(2)	0.005(2)	0.004(2)	-0.012(2)
N(1)	0.047(2)	0.059(2)	0.057(2)	0.009(2)	0.007(2)	0.007(2)
C(1)	0.042(2)	0.044(2)	0.061(3)	0.004(2)	-0.001(2)	-0.010(2)
C(2)	0.044(2)	0.070(3)	0.069(3)	0.000(2)	0.000(2)	0.001(3)
C(3)	0.048(3)	0.096(4)	0.089(4)	0.001(3)	0.009(3)	0.010(3)
C(4)	0.050(3)	0.089(4)	0.115(5)	0.007(3)	0.000(3)	-0.018(4)
C(5)	0.063(3)	0.058(3)	0.111(4)	0.009(3)	-0.012(3)	-0.004(3)
C(6)	0.058(3)	0.052(3)	0.085(4)	0.004(2)	0.001(3)	0.000(3)
C(7)	0.053(3)	0.046(3)	0.050(3)	-0.004(2)	0.002(2)	-0.010(2)
C(8)	0.055(3)	0.062(3)	0.073(3)	0.007(2)	0.001(3)	-0.002(3)
C(9)	0.055(3)	0.057(3)	0.073(4)	0.010(2)	0.007(3)	0.006(3)
C(10)	0.111(4)	0.069(3)	0.097(4)	0.015(3)	-0.010(3)	-0.023(3)

The general temperature factor expression:

$$\exp(-2\pi^2(a^{*2}U_{11}h^2 + b^{*2}U_{22}k^2 + c^{*2}U_{33}l^2 + 2a^*b^*U_{12}hk + 2a^*c^*U_{13}hl + 2b^*c^*U_{23}kl))$$

Table 3. Interatomic Distances (Å) Involving Non-Hydrogen Atoms for $C_{10}H_{11}NO_3$

atom	atom	distance	atom	atom	distance
O(1)	C(7)	1.242(4)	O(2)	C(9)	1.189(4)
O(3)	C(9)	1.331(4)	O(3)	C(10)	1.449(4)
N(1)	C(7)	1.332(4)	N(1)	C(8)	1.449(4)
C(1)	C(2)	1.385(5)	C(1)	C(6)	1.379(4)
C(1)	C(7)	1.506(4)	C(2)	C(3)	1.381(5)
C(3)	C(4)	1.376(5)	C(4)	C(5)	1.375(6)
C(5)	C(6)	1.385(5)	C(8)	C(9)	1.504(5)

Table 4. Interatomic Distances ($\AA$) Involving Hydrogen Atoms for $C_{10}H_{11}NO_3$

atom	atom	distance	atom	atom	distance
N(1)	H(1)	1.01	C(2)	H(2)	0.95
C(3)	H(3)	0.95	C(4)	H(4)	0.95
C(5)	H(5)	0.95	C(6)	H(6)	0.95
C(8)	H(8a)	0.95	C(8)	H(8b)	0.95
C(10)	H(10a)	0.95	C(10)	H(10b)	0.95
C(10)	H(10c)	0.95			

Table 5. Interatomic Angles(°) Involving Non-Hydrogen Atoms for $C_{10}H_{11}NO_3$

atom	atom	atom	angle	atom	atom	atom	angle
C(9)	O(3)	C(10)	116.1(3)	C(7)	N(1)	C(8)	120.4(3)
C(2)	C(1)	C(6)	119.4(4)	C(2)	C(1)	C(7)	122.9(4)
C(6)	C(1)	C(7)	117.7(4)	C(1)	C(2)	C(3)	120.4(4)
C(2)	C(3)	C(4)	119.0(4)	C(3)	C(4)	C(5)	121.7(4)
C(4)	C(5)	C(6)	118.6(4)	C(1)	C(6)	C(5)	120.8(4)
O(1)	C(7)	N(1)	121.3(4)	O(1)	C(7)	C(1)	120.5(4)
N(1)	C(7)	C(1)	118.2(4)	N(1)	C(8)	C(9)	116.1(3)
O(2)	C(9)	O(3)	123.7(4)	O(2)	C(9)	C(8)	122.7(4)
O(3)	C(9)	C(8)	113.6(4)				

Table 6. Interatomic Angles(°) Involving Hydrogen Atoms for $C_{10}H_{11}NO_3$

atom	atom	atom	angle	atom	atom	atom	angle
C(7)	N(1)	H(1)	119.8	C(8)	N(1)	H(1)	119.8
C(1)	C(2)	H(2)	119.8	C(3)	C(2)	H(2)	119.8
C(2)	C(3)	H(3)	120.5	C(4)	C(3)	H(3)	120.5
C(3)	C(4)	H(4)	119.2	C(5)	C(4)	H(4)	119.1
C(4)	C(5)	H(5)	120.7	C(6)	C(5)	H(5)	120.7
C(1)	C(6)	H(6)	119.6	C(5)	C(6)	H(6)	119.6
N(1)	C(8)	H(8a)	107.8	N(1)	C(8)	H(8b)	107.8
C(9)	C(8)	H(8a)	107.8	C(9)	C(8)	H(8b)	107.8
H(8a)	C(8)	H(8b)	109.4	O(3)	C(10)	H(10a)	109.6
O(3)	C(10)	H(10b)	109.6	O(3)	C(10)	H(10c)	109.5
H(10a)	C(10)	H(10b)	109.4	H(10a)	C(10)	H(10c)	109.3
H(10b)	C(10)	H(10c)	109.4				

Table 7. Torsion Angles(°) Involving Non-Hydrogen Atoms for $C_{10}H_{11}NO_3$

atom	atom	atom	atom	angle	atom	atom	atom	atom	angle
O(1)	C(7)	N(1)	C(8)	-1.4(6)	O(1)	C(7)	C(1)	C(2)	-166.3(4)
O(1)	C(7)	C(1)	C(6)	14.4(5)	O(2)	C(9)	O(3)	C(10)	-0.8(6)
O(2)	C(9)	C(8)	N(1)	-169.0(4)	O(3)	C(9)	C(8)	N(1)	12.1(5)
N(1)	C(7)	C(1)	C(2)	13.5(5)	N(1)	C(7)	C(1)	C(6)	-165.9(4)
C(1)	C(2)	C(3)	C(4)	-0.2(7)	C(1)	C(6)	C(5)	C(4)	0.2(7)
C(1)	C(7)	N(1)	C(8)	178.8(3)	C(2)	C(1)	C(6)	C(5)	0.2(6)
C(2)	C(3)	C(4)	C(5)	0.6(8)	C(3)	C(2)	C(1)	C(6)	-0.2(6)
C(3)	C(2)	C(1)	C(7)	-179.5(4)	C(3)	C(4)	C(5)	C(6)	-0.6(8)
C(5)	C(6)	C(1)	C(7)	179.6(4)	C(7)	N(1)	C(8)	C(9)	79.4(5)
C(8)	C(9)	O(3)	C(10)	178.0(3)					

Table 8. Non-bonded Contacts out to 3.60 $\AA$ for $C_{10}H_{11}NO_3$

atom	atom	distance	ADC	atom	atom	distance	ADC
O(1)	N(1)	2.824(4)	8	O(1)	C(6)	3.295(4)	55605
O(1)	C(2)	3.349(4)	8	O(1)	C(8)	3.548(5)	8
O(1)	C(5)	3.549(5)	55605	O(2)	C(5)	3.373(5)	45606
O(3)	N(1)	3.483(4)	8	N(1)	C(9)	3.589(5)	55408
C(7)	C(10)	3.549(6)	55408				

Hydrogen Bonds

A	H	B	A-H	H...B	A...B	A-H...B
N(1)	H(1)	O(1)	1.01	1.87	2.824(4)	157

The ADC (atom designator code) specifies the position of an atom in a crystal. The 5-digit number shown in the table is a composite of three one-digit numbers and one two-digit number: TA (first digit) + TB (second digit) + TC (third digit) + SN (last two digits). TA, TB and TC are the crystal lattice translation digits along cell edges a, b and c. A translation digit of 5 indicates the origin unit cell. If TA = 4, this indicates a translation of one unit cell length along the a-axis in the negative direction. Each translation digit can range in value from 1 to 9 and thus ± 4 lattice translations from the origin (TA=5, TB=5, TC=5) can be represented.

The SN, or symmetry operator number, refers to the number of the symmetry operator used to generate the coordinates of the target atom. A list of symmetry operators relevant to this structure are given below.

For a given intermolecular contact, the first atom (origin atom) is located in the origin unit cell and its position can be generated using the identity operator (SN=1). Thus, the ADC for an origin atom is always 55501. The position of the second atom (target atom) can be generated using the ADC and the coordinates of the atom in the parameter table. For example, an ADC of 47502 refers to the target atom moved through symmetry operator two, then translated -1 cell translations along the a axis, +2 cell translations along the b axis, and 0 cell translations along the c axis.

An ADC of 1 indicates an intermolecular contact between two fragments (eg. cation and anion) that reside in the same asymmetric unit.

Symmetry Operators:

(1)	X,	Y,	Z	(2)	1/2-X,	1/2-Y,	Z
(3)	1/2+X,	-Y,	1/2-Z	(4)	-X,	1/2+Y,	1/2-Z
(5)	-X,	-Y,	-Z	(6)	1/2+X,	1/2+Y,	-Z
(7)	1/2-X,	Y,	1/2+Z	(8)	X,	1/2-Y,	1/2+Z

Table 9. Selected Least Squares Planes for $C_{10}H_{11}NO_3$

Plane number 1

Atoms defining plane	Distance
C(1)	0.002(4)
C(2)	-0.001(4)
C(3)	-0.002(4)
C(4)	0.004(5)
C(5)	-0.002(4)
C(6)	-0.001(4)

Plane number 2

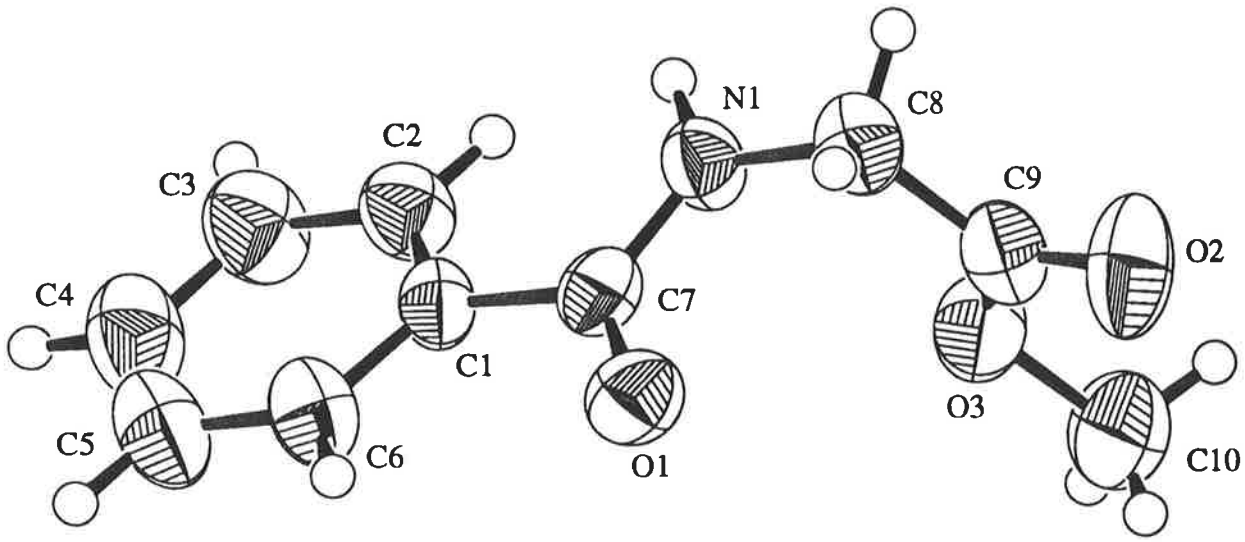
Atoms defining plane	Distance
O(1)	0.0
N(1)	0.0
C(7)	0.0

Summary

plane	mean deviation	χ^2
1	0.0018	1.0
2	0.0000	0.0

Dihedral angles between planes (°)

plane	1
2	13.94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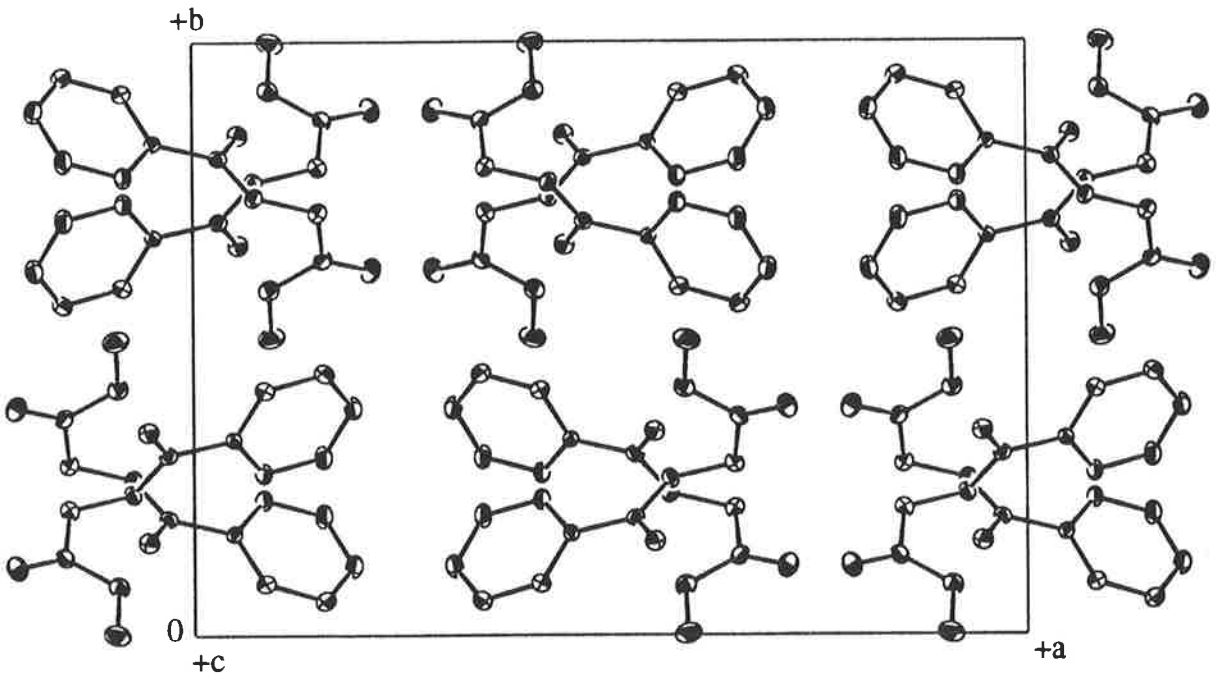
C8
C2
C3
C9
O2
C4
C7
C1
O3
C5
C6
O1
C10


+b
0
+c
+a

Appendix 2

EXPERIMENTAL DETAILS

A. Crystal Data

Empirical Formula	$C_{10}H_6F_5NO_3$
Formula Weight	283.15
Crystal Color, Habit	colourless, needle
Crystal Dimensions	0.28 X 0.12 X 0.08 mm
Crystal System	monoclinic
Lattice Type	Primitive
No. of Reflections Used for Unit	
Cell Determination (2θ range)	25 (95.6 - 99.6°)
Omega Scan Peak Width	
at Half-height	0.47°
Lattice Parameters	a = 4.819(2)Å b = 17.770(2) Å c = 12.640(2) Å β = 98.91(2)°
	V = 1069.3(5) $Å^3$
Space Group	$P2_1/n$ (#14)
Z value	4
D_{calc}	1.759 g/cm^3
F_{000}	568.00
μ(CuKα)	16.78 cm^{-1}

B. Intensity Measurements

Diffractometer	Rigaku AFC6R
Radiation	CuKα (λ = 1.54178 $\AA$) graphite monochromated
Take-off Angle	6.0°
Detector Aperture	7.0 mm horizontal 7.0 mm vertical
Crystal to Detector Distance	400 mm
Temperature	23.0°C
Scan Type	ω-2θ
Scan Rate	32.0°/min (in ω) (up to 4 scans)
Scan Width	(1.20 + 0.30 tan θ)°
$2\theta_{max}$	120.1°
No. of Reflections Measured	Total: 1887 Unique: 1669 (R_{int} = 0.018)
Corrections	Lorentz-polarization Absorption (trans. factors: 0.8718 - 1.0000) Secondary Extinction (coefficient: 3.0(4)e-06)

C. Structure Solution and Refinement

Structure Solution	Direct Methods (SHELXS86)
Refinement	Full-matrix least-squares
Function Minimized	$\Sigma w(\|Fo\| - \|Fc\|)^2$
Least Squares Weights	$\frac{1}{\sigma^2(Fo)} = \frac{4Fo^2}{\sigma^2(Fo^2)}$
p-factor	0.0120
Anomalous Dispersion	All non-hydrogen atoms
No. Observations (I>3.00σ(I))	1339
No. Variables	197
Reflection/Parameter Ratio	6.80
Residuals: R; Rw	0.029 ; 0.033
Goodness of Fit Indicator	1.91
Max Shift/Error in Final Cycle	<0.01
Maximum peak in Final Diff. Map	0.19 $e^-/\AA^3$
Minimum peak in Final Diff. Map	-0.19 $e^-/\AA^3$

Table 1. Atomic Coordinates and Isotropic Displacement Parameters for $C_{10}H_6F_5NO_3$

atom	x	y	z	B_{eq}
F(2)	-0.4609(3)	0.93885(7)	0.74990(10)	3.17(3)
F(3)	-0.2537(3)	0.88158(9)	0.5825(1)	4.20(4)
F(4)	0.1250(3)	0.76692(9)	0.6114(1)	4.78(4)
F(5)	0.2843(3)	0.70880(8)	0.8099(1)	4.29(4)
F(6)	0.0725(3)	0.76352(7)	0.9772(1)	3.38(3)
O(1)	-0.5533(3)	0.89853(9)	0.9663(1)	2.67(3)
O(2)	0.2600(3)	0.87548(9)	1.2221(1)	2.89(4)
O(3)	0.0304(3)	0.94828(9)	1.3248(1)	2.89(4)
N(1)	-0.0986(4)	0.9103(1)	1.0424(1)	2.30(4)
C(1)	-0.1992(4)	0.8536(1)	0.8697(2)	1.97(4)
C(2)	-0.2808(4)	0.8808(1)	0.7670(2)	2.31(5)
C(3)	-0.1753(5)	0.8522(1)	0.6803(2)	2.75(5)
C(4)	0.0159(5)	0.7940(1)	0.6947(2)	3.07(5)
C(5)	0.0970(4)	0.7648(1)	0.7950(2)	2.80(5)
C(6)	-0.0114(4)	0.7939(1)	0.8807(2)	2.33(5)
C(7)	-0.3020(4)	0.8895(1)	0.9650(2)	1.92(4)
C(8)	-0.1558(5)	0.9408(1)	1.1433(2)	2.34(5)
C(9)	0.0686(4)	0.9169(1)	1.2322(2)	2.12(5)
C(10)	0.2372(6)	0.9273(2)	1.4160(2)	3.68(7)
H(1)	0.072(5)	0.903(1)	1.035(2)	3.4(6)
H(8a)	-0.329(5)	0.923(1)	1.160(2)	2.3(5)
H(8b)	-0.162(5)	0.995(1)	1.143(2)	3.0(5)
H(10a)	0.440(7)	0.938(2)	1.402(2)	6.4(8)
H(10b)	0.226(6)	0.873(2)	1.428(2)	5.1(7)

Table 1. Atomic Coordinates and Isotropic Displacement Parameters for $C_{10}H_6F_5NO_3$ (cont...)

atom	x	y	z	B_{eq}
H(10c)	0.198(6)	0.958(2)	1.475(2)	6.3(8)

$$B_{eq} = \frac{8}{3}\pi^2(U_{11}(aa^*)^2 + U_{22}(bb^*)^2 + U_{33}(cc^*)^2 + 2U_{12}aa^*bb^*\cos\gamma + 2U_{13}aa^*cc^*\cos\beta + 2U_{23}bb^*cc^*\cos\alpha)$$

Table 2. Anisotropic Displacement Parameters for $C_{10}H_6F_5NO_3$

atom	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
F(2)	0.0438(8)	0.0389(8)	0.0368(7)	0.0150(6)	0.0036(6)	0.0038(6)
F(3)	0.067(1)	0.0639(10)	0.0278(7)	0.0042(8)	0.0043(7)	-0.0031(7)
F(4)	0.0579(10)	0.075(1)	0.0518(9)	0.0067(9)	0.0189(7)	-0.0314(8)
F(5)	0.0407(8)	0.0439(9)	0.076(1)	0.0179(7)	0.0016(7)	-0.0203(8)
F(6)	0.0449(8)	0.0366(8)	0.0450(8)	0.0102(6)	0.0015(6)	0.0082(6)
O(1)	0.0185(8)	0.0473(10)	0.0365(9)	0.0012(7)	0.0067(6)	-0.0018(7)
O(2)	0.0301(9)	0.0430(9)	0.0372(9)	0.0082(8)	0.0071(7)	0.0050(7)
O(3)	0.0390(9)	0.0418(9)	0.0279(8)	0.0035(7)	0.0018(7)	-0.0033(7)
N(1)	0.0189(10)	0.039(1)	0.030(1)	0.0019(8)	0.0068(8)	-0.0026(8)
C(1)	0.019(1)	0.025(1)	0.031(1)	-0.0008(9)	0.0031(9)	-0.0024(9)
C(2)	0.024(1)	0.027(1)	0.036(1)	0.0017(10)	0.0022(10)	-0.0028(10)
C(3)	0.037(1)	0.039(1)	0.027(1)	-0.003(1)	0.002(1)	-0.004(1)
C(4)	0.033(1)	0.043(1)	0.042(1)	-0.005(1)	0.011(1)	-0.022(1)
C(5)	0.024(1)	0.030(1)	0.051(1)	0.004(1)	0.003(1)	-0.013(1)
C(6)	0.024(1)	0.027(1)	0.035(1)	-0.0016(9)	-0.0008(9)	-0.0007(10)
C(7)	0.023(1)	0.023(1)	0.028(1)	-0.0005(9)	0.0057(9)	0.0020(9)
C(8)	0.026(1)	0.035(1)	0.030(1)	0.002(1)	0.0077(10)	-0.003(1)
C(9)	0.024(1)	0.027(1)	0.031(1)	-0.0037(10)	0.0084(9)	0.0016(9)
C(10)	0.054(2)	0.053(2)	0.029(1)	0.002(1)	-0.005(1)	0.000(1)

The general temperature factor expression:

$$\exp(-2\pi^2(a^{*2}U_{11}h^2 + b^{*2}U_{22}k^2 + c^{*2}U_{33}l^2 + 2a^*b^*U_{12}hk + 2a^*c^*U_{13}hl + 2b^*c^*U_{23}kl))$$

Table 3. Interatomic Distances ($\AA$) Involving Non-Hydrogen Atoms for $C_{10}H_6F_5NO_3$

atom	atom	distance	atom	atom	distance
F(2)	C(2)	1.344(2)	F(3)	C(3)	1.342(2)
F(4)	C(4)	1.338(2)	F(5)	C(5)	1.337(2)
F(6)	C(6)	1.339(2)	O(1)	C(7)	1.224(2)
O(2)	C(9)	1.202(2)	O(3)	C(9)	1.334(3)
O(3)	C(10)	1.451(3)	N(1)	C(7)	1.326(3)
N(1)	C(8)	1.451(3)	C(1)	C(2)	1.383(3)
C(1)	C(6)	1.387(3)	C(1)	C(7)	1.513(3)
C(2)	C(3)	1.375(3)	C(3)	C(4)	1.378(3)
C(4)	C(5)	1.370(3)	C(5)	C(6)	1.374(3)
C(8)	C(9)	1.495(3)			

Table 4. Interatomic Distances ($\AA$) Involving Hydrogen Atoms for $C_{10}H_6F_5NO_3$

atom	atom	distance	atom	atom	distance
N(1)	H(1)	0.85(2)	C(8)	H(8a)	0.95(2)
C(8)	H(8b)	0.97(2)	C(10)	H(10a)	1.04(3)
C(10)	H(10b)	0.98(3)	C(10)	H(10c)	0.96(3)

Table 5. Interatomic Angles(°) Involving Non-Hydrogen Atoms for $C_{10}H_6F_5NO_3$

atom	atom	atom	angle	atom	atom	atom	angle
C(9)	O(3)	C(10)	114.7(2)	C(7)	N(1)	C(8)	122.3(2)
C(2)	C(1)	C(6)	116.6(2)	C(2)	C(1)	C(7)	121.3(2)
C(6)	C(1)	C(7)	122.1(2)	F(2)	C(2)	C(1)	120.1(2)
F(2)	C(2)	C(3)	117.7(2)	C(1)	C(2)	C(3)	122.1(2)
F(3)	C(3)	C(2)	120.3(2)	F(3)	C(3)	C(4)	120.0(2)
C(2)	C(3)	C(4)	119.6(2)	F(4)	C(4)	C(3)	120.2(2)
F(4)	C(4)	C(5)	120.1(2)	C(3)	C(4)	C(5)	119.7(2)
F(5)	C(5)	C(4)	120.1(2)	F(5)	C(5)	C(6)	120.0(2)
C(4)	C(5)	C(6)	119.9(2)	F(6)	C(6)	C(1)	120.0(2)
F(6)	C(6)	C(5)	117.9(2)	C(1)	C(6)	C(5)	122.1(2)
O(1)	C(7)	N(1)	125.0(2)	O(1)	C(7)	C(1)	120.8(2)
N(1)	C(7)	C(1)	114.2(2)	N(1)	C(8)	C(9)	109.9(2)
O(2)	C(9)	O(3)	124.2(2)	O(2)	C(9)	C(8)	124.9(2)
O(3)	C(9)	C(8)	111.0(2)				

Table 6. Interatomic Angles(°) Involving Hydrogen Atoms for $C_{10}H_6F_5NO_3$

atom	atom	atom	angle	atom	atom	atom	angle
C(7)	N(1)	H(1)	119(1)	C(8)	N(1)	H(1)	117(1)
N(1)	C(8)	H(8a)	111(1)	N(1)	C(8)	H(8b)	112(1)
C(9)	C(8)	H(8a)	107(1)	C(9)	C(8)	H(8b)	107(1)
H(8a)	C(8)	H(8b)	108(1)	O(3)	C(10)	H(10a)	111(1)
O(3)	C(10)	H(10b)	109(1)	O(3)	C(10)	H(10c)	105(1)
H(10a)	C(10)	H(10b)	106(2)	H(10a)	C(10)	H(10c)	109(2)
H(10b)	C(10)	H(10c)	114(2)				

Table 7. Torsion Angles(°) Involving Non-Hydrogen Atoms for $C_{10}H_6F_5NO_3$

atom	atom	atom	atom	angle	atom	atom	atom	atom	angle
F(2)	C(2)	C(1)	C(6)	-179.6(2)	F(2)	C(2)	C(1)	C(7)	-1.9(3)
F(2)	C(2)	C(3)	F(3)	-0.5(3)	F(2)	C(2)	C(3)	C(4)	178.2(2)
F(3)	C(3)	C(2)	C(1)	-178.1(2)	F(3)	C(3)	C(4)	F(4)	0.4(3)
F(3)	C(3)	C(4)	C(5)	179.3(2)	F(4)	C(4)	C(3)	C(2)	-178.4(2)
F(4)	C(4)	C(5)	F(5)	-0.4(3)	F(4)	C(4)	C(5)	C(6)	178.6(2)
F(5)	C(5)	C(4)	C(3)	-179.4(2)	F(5)	C(5)	C(6)	F(6)	-1.3(3)
F(5)	C(5)	C(6)	C(1)	177.9(2)	F(6)	C(6)	C(1)	C(2)	-178.5(2)
F(6)	C(6)	C(1)	C(7)	3.8(3)	F(6)	C(6)	C(5)	C(4)	179.7(2)
O(1)	C(7)	N(1)	C(8)	5.6(3)	O(1)	C(7)	C(1)	C(2)	53.4(3)
O(1)	C(7)	C(1)	C(6)	-129.1(2)	O(2)	C(9)	O(3)	C(10)	-1.3(3)
O(2)	C(9)	C(8)	N(1)	-3.4(3)	O(3)	C(9)	C(8)	N(1)	176.0(2)
N(1)	C(7)	C(1)	C(2)	-125.5(2)	N(1)	C(7)	C(1)	C(6)	52.0(3)
C(1)	C(2)	C(3)	C(4)	0.7(3)	C(1)	C(6)	C(5)	C(4)	-1.1(3)
C(1)	C(7)	N(1)	C(8)	-175.5(2)	C(2)	C(1)	C(6)	C(5)	2.3(3)
C(2)	C(3)	C(4)	C(5)	0.6(3)	C(3)	C(2)	C(1)	C(6)	-2.1(3)
C(3)	C(2)	C(1)	C(7)	175.6(2)	C(3)	C(4)	C(5)	C(6)	-0.3(3)
C(5)	C(6)	C(1)	C(7)	-175.3(2)	C(7)	N(1)	C(8)	C(9)	146.1(2)
C(8)	C(9)	O(3)	C(10)	179.2(2)					

Table 8. Non-bonded Contacts out to 3.60 $\AA$ for $C_{10}H_6F_5NO_3$

atom	atom	distance	ADC	atom	atom	distance	ADC
F(2)	O(3)	3.135(2)	57703	F(2)	C(9)	3.172(2)	57703
F(2)	C(8)	3.253(3)	47703	F(2)	O(3)	3.412(2)	47703
F(2)	O(2)	3.441(2)	57703	F(2)	C(10)	3.452(3)	57703
F(2)	C(4)	3.595(3)	45501	F(3)	F(6)	2.962(2)	46404
F(3)	C(10)	3.084(3)	45401	F(3)	O(3)	3.359(2)	57703
F(3)	C(10)	3.397(3)	57703	F(3)	C(10)	3.497(4)	55401
F(4)	F(6)	2.976(2)	46404	F(4)	F(6)	2.991(2)	56404
F(4)	C(7)	3.387(2)	56404	F(4)	O(2)	3.496(2)	46404
F(4)	O(1)	3.501(2)	56404	F(4)	N(1)	3.579(2)	56404
F(5)	C(9)	2.872(2)	56404	F(5)	O(2)	2.999(2)	46404
F(5)	O(3)	3.027(2)	56404	F(5)	O(2)	3.085(2)	56404
F(5)	C(10)	3.392(3)	56404	F(5)	C(8)	3.431(3)	56404
F(5)	C(1)	3.578(3)	65501	F(6)	O(1)	3.017(2)	65501
F(6)	C(3)	3.366(2)	56504	F(6)	C(4)	3.368(3)	56504
O(1)	N(1)	2.942(3)	45501	O(1)	C(6)	2.959(3)	45501
O(1)	C(1)	3.262(3)	45501	O(1)	C(8)	3.382(3)	47703
O(1)	C(5)	3.470(3)	45501	O(1)	O(2)	3.512(2)	45501
O(2)	C(5)	3.039(3)	56504	O(2)	C(4)	3.293(3)	56504
O(2)	C(8)	3.336(3)	65501	O(3)	C(2)	3.530(3)	57703
N(1)	N(1)	3.541(4)	57703				

Hydrogen Bonds

A	H	B	A-H	H...B	A...B	A-H...B
N(1)	H(1)	O(1)	0.85(2)	2.13(2)	2.942(2)	161(2)

The ADC (atom designator code) specifies the position of an atom in a crystal. The 5-digit number shown in the table is a composite of three one-digit numbers and one two-digit number: TA (first digit) + TB (second digit) + TC (third digit) + SN (last two digits). TA, TB and TC are the crystal lattice translation digits along cell edges a, b and c. A translation digit of 5 indicates the origin unit cell. If TA = 4, this indicates a translation of one unit cell length along the a-axis in the negative direction. Each translation digit can range in value from 1 to 9 and thus ± 4 lattice translations from the origin (TA=5, TB=5, TC=5) can be represented.

The SN, or symmetry operator number, refers to the number of the symmetry operator used to generate the coordinates of the target atom. A list of symmetry operators relevant to this structure are given below.

For a given intermolecular contact, the first atom (origin atom) is located in the origin unit cell and its position can be generated using the identity operator (SN=1). Thus, the ADC for an origin atom is always 55501. The position of the second atom (target atom) can be generated using the ADC and the coordinates of the atom in the parameter table. For example, an ADC of 47502 refers to the target atom moved through symmetry operator two, then translated -1 cell translations along the a axis, +2 cell translations along the b axis, and 0 cell translations along the c axis.

An ADC of 1 indicates an intermolecular contact between two fragments (eg. cation and anion) that reside in the same asymmetric unit.

Symmetry Operators:

(1)	X,	Y,	Z	(2)	1/2-X,	1/2+Y,	1/2-Z
(3)	-X,	-Y,	-Z	(4)	1/2+X,	1/2-Y,	1/2+Z

Table 9. Selected Least Squares Planes for $C_{10}H_6F_5NO_3$

Plane number 1

Atoms defining plane	Distance
C(1)	0.012(2)
C(2)	-0.008(2)
C(3)	-0.002(2)
C(4)	0.007(2)
C(5)	0.000(2)
C(6)	-0.009(2)

Plane number 2

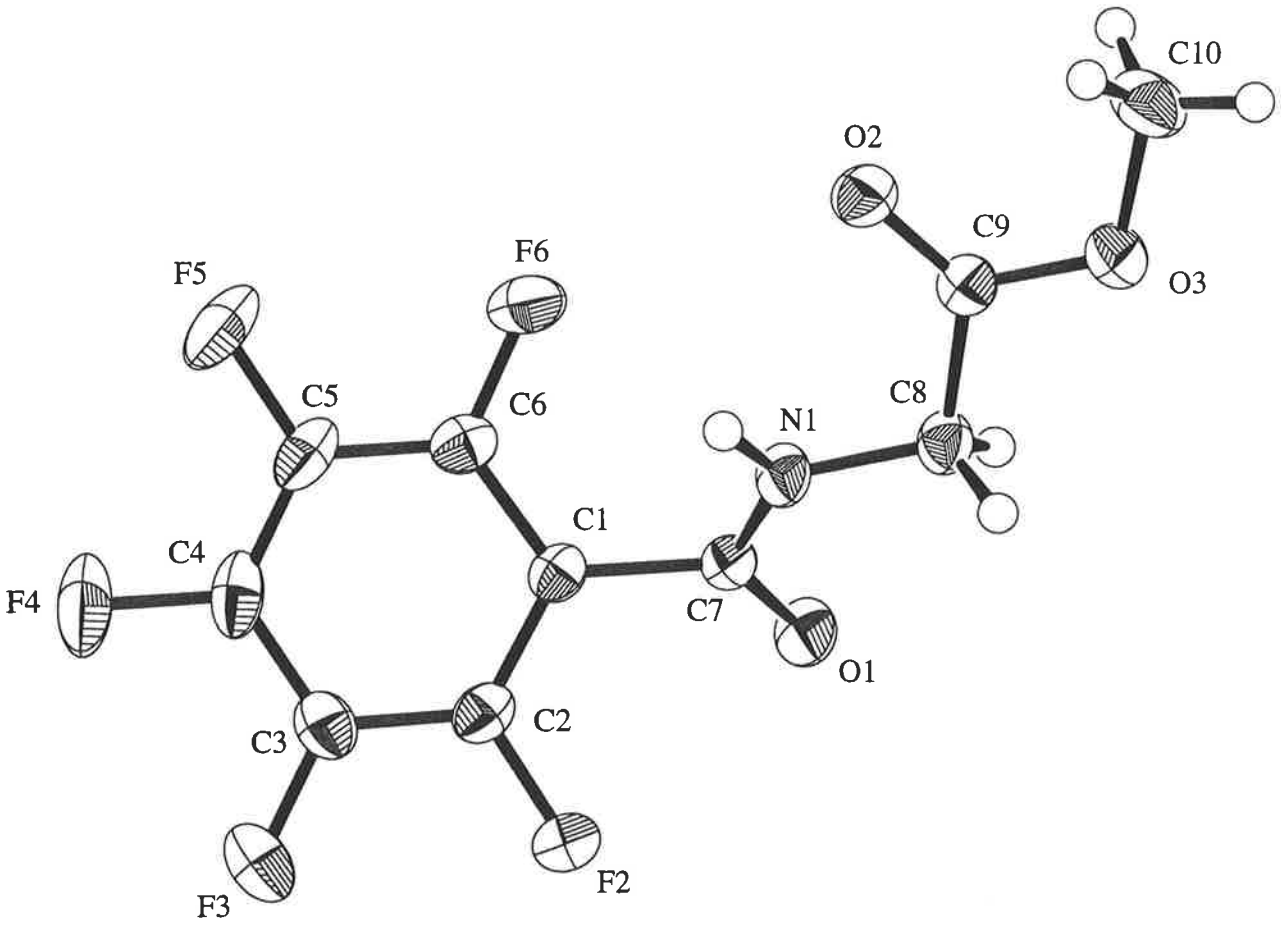
Atoms defining plane	Distance
O(1)	0.0
N(1)	0.0
C(7)	0.0

Summary

plane	mean deviation	χ^2
1	0.0064	78.1
2	0.0000	0.0

Dihedral angles between planes (°)

plane	1
2	127.03


C10
O2
C9
O3
F5
F6
C5
C6
N1
C8
C1
C4
F4
C7
O1
C3
C2
F3
F2

".....that's not what Chris said."

ERRATUM

Page 4, Line1:	'amino' should read 'amido'
Page 9:	(21) is not a secondary radical but a tertiary radical
Page 11:	'PH representing a phenyl group on structure (23) should be 'Ph'
Page 20:	Structure (43) should not have an H on the N
Page 21, Line 7:	The reaction of the α-hydroxy derivative is not a hydrolysis but a reverse condensation
Page 40, Line 5:	'moietys' should be spelled 'moieties'
Page 43, Line 10:	'perfomed' should read 'performed'
Page 44, Line 10:	Copper (II) can be chelated by a ligand but cannot itself 'chelate'
Page 52, Line 29:	Reference 81 should be 21
Page 75:	4th structure should be a carboxylic acid
Page 92-94, Clarification:	The k_{rel} values calculated from changes in absorbance took into account the relative extinction coefficients of the product cinnamates in addition to the difference in extinction coefficients of the substrate and product between derivatives.
Page 105, Lines 15 & 18:	'HCL' should read 'HCl'
Page 108, Clarification:	The discussion on the cause of racemisation due to the formation of the mixed anhydride is not entirely correct. The mixed anhydride of the acetylated amino acid reacts to form an azlactone, through a cyclisation, which makes the α-proton much more acidic. Subsequent ring opening produces the *N*-acetylated amino acid.
Page 120:	Carbon Nuclear Magnetic Resonance spectra were recorded at 75 MHz not 300 MHz