THE SYNTHESIS OF ANTIBIOTICS

AND AMINO ACIDS

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in the University of Oxford



by

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Michaelmas Term 1989

ABSTRACT

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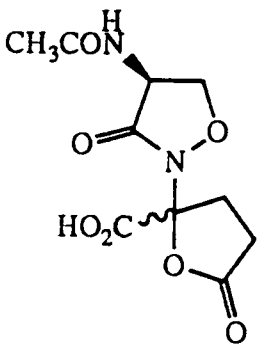
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The Synthesis of Antibiotics and Amino Acids

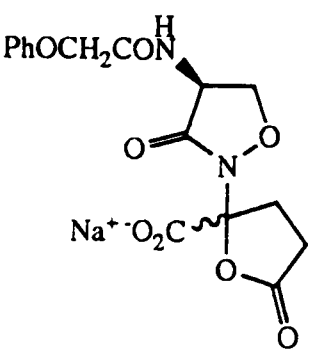
This thesis is divided into two parts:

Part One describes synthetic approaches to the naturally occurring antibiotic Lactivicin (1) and its acylamino derivatives, involving the condensation of protected *L*-cycloserine with 2-oxoglutaric acid. The phenoxyacetyl-amino derivative (2) exhibited antibacterial activity.

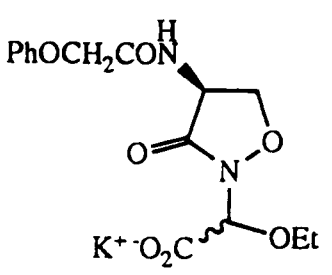
A method was developed for the selective deprotonation of the endocyclic amide of the protected cycloserine; the resulting anion was then quenched with various electrophiles. In this manner, the Lactivicin analogues (3) and (4) were prepared. Whilst (3) was biologically inactive, (4) showed appreciable antibacterial activity.



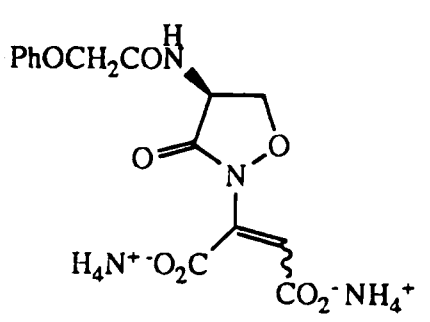
(1)



(2)



(3)



(4)

Part Two details the use of *N*-benzoylamino-2-bromoglycine methyl ester, a glycidyl radical precursor, as a novel α-amino acid synthon. Its synthetic utility in free radical reactions was demonstrated with 2-functionalised allylstannanes as allyl transfer reagents, to produce a range of substituted α-allylglycines. Using this procedure, the naturally occuring amino acid, 4-methyleneglutamic acid, was prepared in its racemic form. Studies on the extension of this methodology to the asymmetric synthesis of α-amino acids are also described.

A synthesis of α-allenylglycine, a previously undocumented amino acid, was developed. The synthetic route involves the Lewis acid mediated reaction of propargyltriphenylstannane with *N*-benzoylamino-2-bromoglycine methyl ester, followed by aqueous acidic deprotection.

To Mum, Dad
and Helen

"We often discover what will do,
by finding out what will not do;
and probably he who never made a mistake,
never made a discovery."

Samuel Smiles
1812 - 1904

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GLOSSARY

Ac	Acetyl
Bn	Benzyl
BOC	*tert*-Butyloxycarbonyl
^{n}Bu, Bu	*n*-Butyl
^{t}Bu	*tert*-Butyl
CBZ	Benzyloxycarbonyl
Et	Ethyl
Me	Methyl
Ph	Phenyl
PNB	4-Nitrobenzyl
^{i}Pr	*iso*-Propyl
Tf	Trifluoromethanesulphonyl
TMS	Trimethylsilyl
p-TolSO$_2$	4-Toluenesulphonyl
Tr	Trityl
V	Phenoxyacetyl
AIBN	Azobisisobutyronitrile
9-BBN	9-Borabicyclo[3.3.1]nonane
DCC	1,3-Dicyclohexylcarbodiimide
DEAD	Diethyl azodicarboxylate
DMAD	Dimethyl azodicarboxylate
DMAP	4-Dimethylaminopyridine
DMF	*N*,*N*-Dimethylformamide
DMSO	Dimethylsulphoxide
DOPA	3-(3,4-Dihydroxyphenyl)-alanine
GABA	4-Aminobutyric acid
HMPA	Hexamethylphosphoric triamide
LDA	Lithium diisopropylamide
LHMDS	Lithium hexamethyldisilazide
NBS	*N*-Bromosuccinimide
PLP	Pyridoxal 5-phosphate
TFA	Trifluoroacetic acid
B	General base
Enz	General enzyme
Nu	General nucleophile
HPLC	High performance liquid chromatography

PART ONE

ANTIBIOTIC SYNTHESIS

1. INTRODUCTION

1.1. The Recognition of Micro-organisms[1]

In 1546, Girolamo Fracastro of Verona proposed the then remarkable theory that diseases were transmitted by minute particles of living matter capable of multiplication and airborne dissemination. These particles were later characterised as 'animalcules' by van Leeuwenhoek in 1863, and visualised with the aid of a primitive microscope. The theory of spontaneous generation of these lower forms of life persisted until 1861, when Pasteur's experiments on fermentation demonstrated how to sterilise liquids and how to maintain sterility by the exclusion of airborne micro-organisms.

With a knowledge of Pasteur's work, the surgeon Joseph Lister in 1865 was able to develop a technique for controlling surgical wound infection using a phenol spray. His work did much to stimulate interest in applying the germ theory to all infectious diseases.

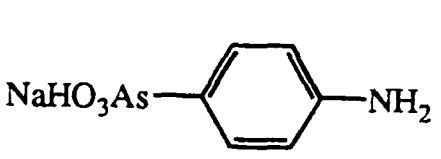
At about the same time, Robert Koch in Germany identified the causative organisms of diseases such as anthrax, tuberculosis and cholera, and developed methods for staining organisms for examination under the microscope. Consequently, numerous other organisms were soon identified as the causative agents of particular diseases.

1.2. The Development of Antibiotics

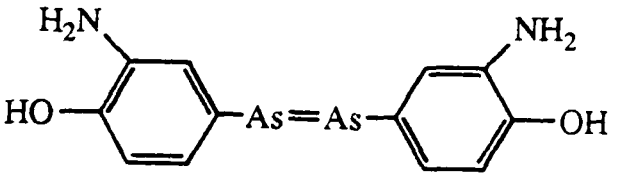
Armed with a basic knowledge of the properties of micro-organisms, organic chemists turned their attention to chemical methods of curing

infectious diseases and embarked upon the syntheses of new compounds, the structure and properties of which could be subjected to endless modification in the search for such remedies.

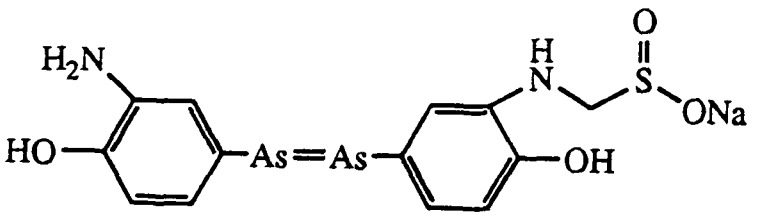
At the end of the nineteenth century, an infection of African cattle carried by tsetse flies (trypanosomiosis) was shown to cause the fatal sleeping sickness disease in man. In 1905, Thomas, in Liverpool, discovered that this could be cured by an organo-arsenical compound - atoxyl (1). Ehrlich, working on similar compounds, followed up this observation by developing salvarsan (2)[2] which was found to be particularly effective not only in curing trypanosomiosis in mice, but also in curing syphilis in man. The slightly less toxic neosalvarsan (3) remained the standard treatment for syphilis until it was superseded by penicillin in 1945.



(1)



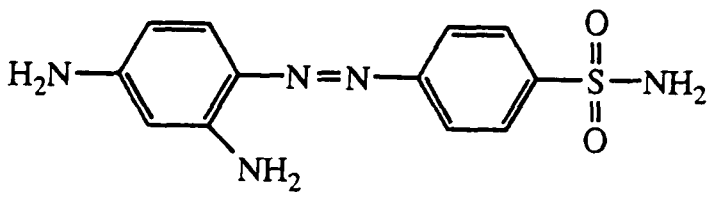
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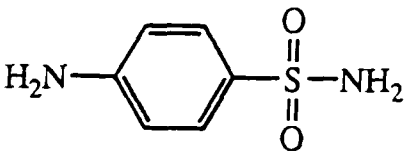
(3)

In the 1930's, a red dye named Prontosil (4) was found to cure streptococcal infections in patients, despite its apparent inactivity *in vitro*. It was soon discovered that the active part of the dye liberated in the body was a substance called sulphanilamide (5). Countless modifications of the molecular structure of sulphanilamide yielded the sulphonamide class of antibacterials, exemplified by sulphafurazole (6) and sulphamerazine (7). These substances were able to cure infections due to streptococci,

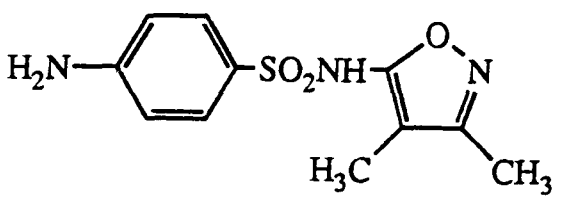
meningococci, pneumococci, gonococci and dysentery bacilli, and were later used to treat leprosy and malaria.



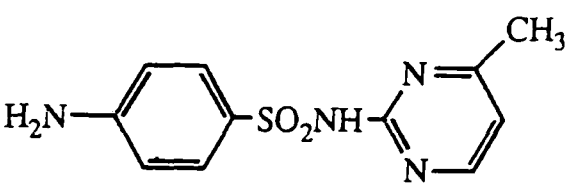
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(5)



(6)



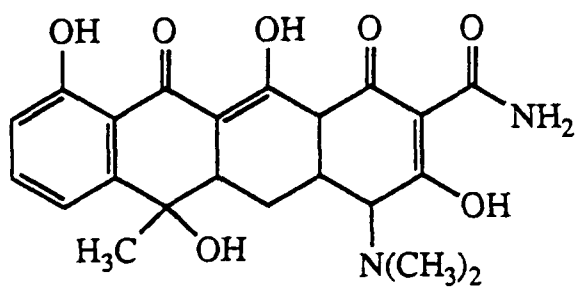
(7)

Although in 1928, Fleming[3] had noted the antibacterial properties of a mould of the fungus *Penicillium notatum*, the extract of this mould, named penicillin, was not developed further at the time due to difficulties in its preparation, its instability and the advent of the sulphonamides which diverted interest away from Fleming's observations.

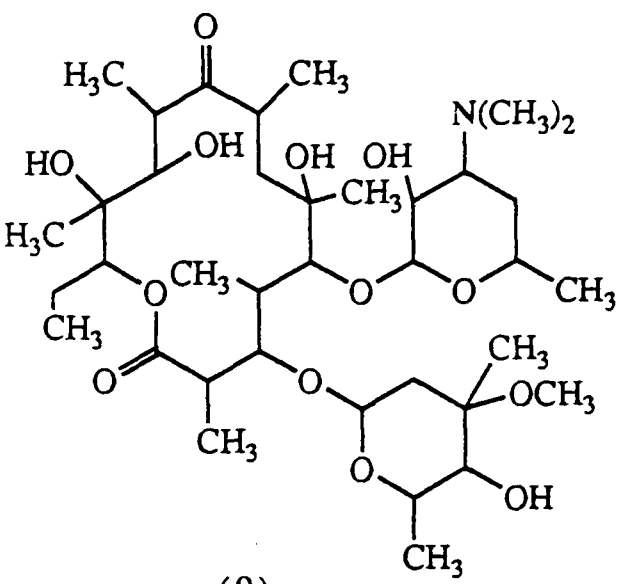
It was not until 1938 that Florey and Chain[4] isolated a crude sample of penicillin and demonstrated its antibacterial spectrum, many of its chemical properties and its lack of toxicity in mice. Although supplies of penicillin were very limited, the first seriously ill patients were successfully treated for severe staphylococcal and streptococcal infections in 1941. However, *Penicillium chrysogenum* was then found to be a richer source than Fleming's original *P. notatum*, enabling the treatment of large numbers of World War II casualties.

The β-lactam antibiotics represent the most important class of antibacterial agents so far discovered. However other antibacterial metabolites have been discovered in various micro-organisms. These include the tetracyclines, e.g. oxytetracycline (8), the aminoglycoside antibiotics, e.g.

streptomycin and kanamycin, and the macrolide antibiotics, e.g. erythromycin (9). These classes of antibiotic, whilst not as important as the β-lactams, are essential in chemotherapy due to the problems of the development of bacterial resistance to the latter.



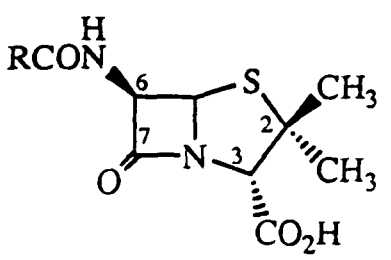
(8)



(9)

1.3. The β-Lactam Antibiotics

The isolation and characterisation of the active compounds within penicillin cultures revealed species containing a fused β-lactam-thiazolidine nucleus, proven by X-ray crystallographic studies.[5] The penicillins, the discovery and development of which have been extensively reviewed,[6-8] possess varying side chains at the 6-position, with concomitant variations in antibacterial activity. Some examples are illustrated below (10-12), together with their biosynthetic precursor, isopenicillin N (13).



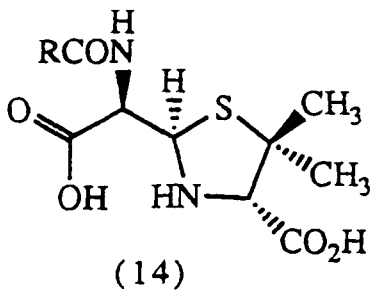
(10) R= $PhCH_2$ Penicillin G

(11) R= $PhOCH_2$ Penicillin V

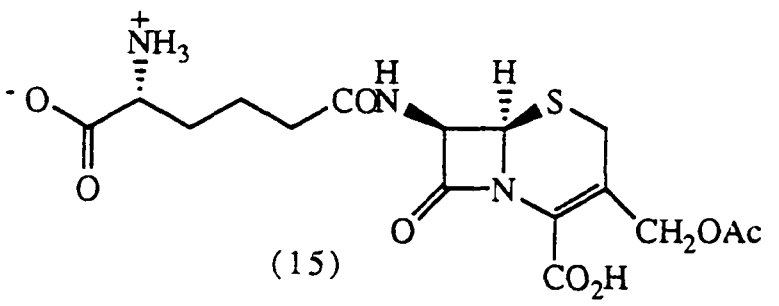
(12) R= (D)-$O_2CCH(NH_3^+)(CH_2)_3$ Penicillin N

(13) R= (L)-$O_2CCH(NH_3^+)(CH_2)_3$ Isopenicillin N

Soon after their introduction to clinical medicine, it was discovered that many bacterial strains produce β-lactamase enzymes,[9] able to catalytically hydrolyse penicillins to penicilloic acids (14), thereby inactivating them. The discovery[10] and structural determination[11] of Cephalosporin C (15), first isolated from the mould *Cephalosporium acremonium*, were significant events as the cephalosporins were the first type of β-lactam antibiotic to exhibit substantial resistance to the β-lactamases.

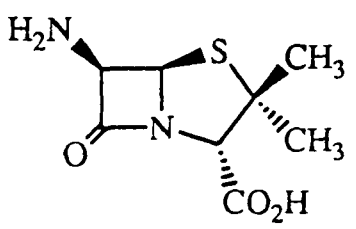


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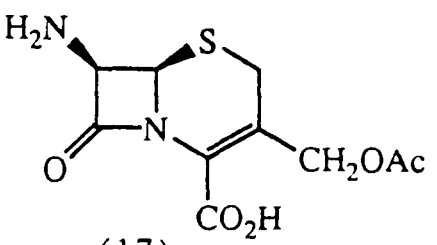


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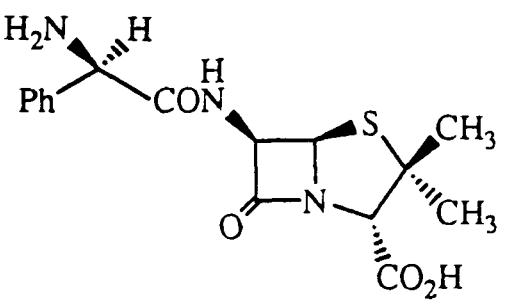
The first total synthesis of a β-lactam antibiotic, penicillin V (11), was achieved by Sheehan[12] in 1959. However, as a method of entering this class of antibiotic, total synthesis is fraught with difficulty and is often inefficient owing to the multifunctionalised strained bicyclic ring system of penicillins. Consequently, modified fermentation processes utilising high producing mutant strains have been used to produce the nuclei of penicillins and cephalosporins, 6-aminopenicillanic acid (16)[13] and 7-aminocephalosporanic acid (17)[14] respectively, leading to many semi-synthetic antibiotics, the first of which to be used clinically was ampicillin (18).[15]



(16)



(17)



(18)

The main objectives of the medicinal chemists have been to widen the spectrum of antibacterial activity, to produce compounds that are resistant to penicillinase, and to produce compounds that are stable in gastric acid and hence are effective after oral administration.

1.4. The Mode of Action of β-Lactam Antibiotics

The bacteriocidal mode of action of the β-lactam antibiotics has been indicated, by extensive biological studies, to involve inhibition of cell wall biosynthesis.[16,17] To understand this fully, it is therefore necessary to appreciate the constitution and biosynthesis of the cell wall.

Bacteria are categorised according to their staining characteristics with Gram's stain[18] into Gram positive and Gram negative groups. In the cell walls of Gram positive bacteria, the main structural component is peptidoglycan, a biopolymer of sugars and amino acids. However, Gram negative bacteria have an inner membrane composed mainly of peptidoglycan, and in addition, a complex outer membrane constructed of phospholipids, lipopolysaccharides and lipoprotein, the latter of which is covalently linked to the inner layer. In general, Gram negative bacteria are less sensitive to penicillin action than the Gram positive genotype. This may be explained by the more complex cell wall of the latter hindering the penetration of the penicillin into the cell.

The peptidoglycan constituent, present in all bacteria and essential for cell survival, consists of linear polysaccharide chains cross-linked to one another by short peptides, thus imparting structural integrity to the bacterial cell wall.

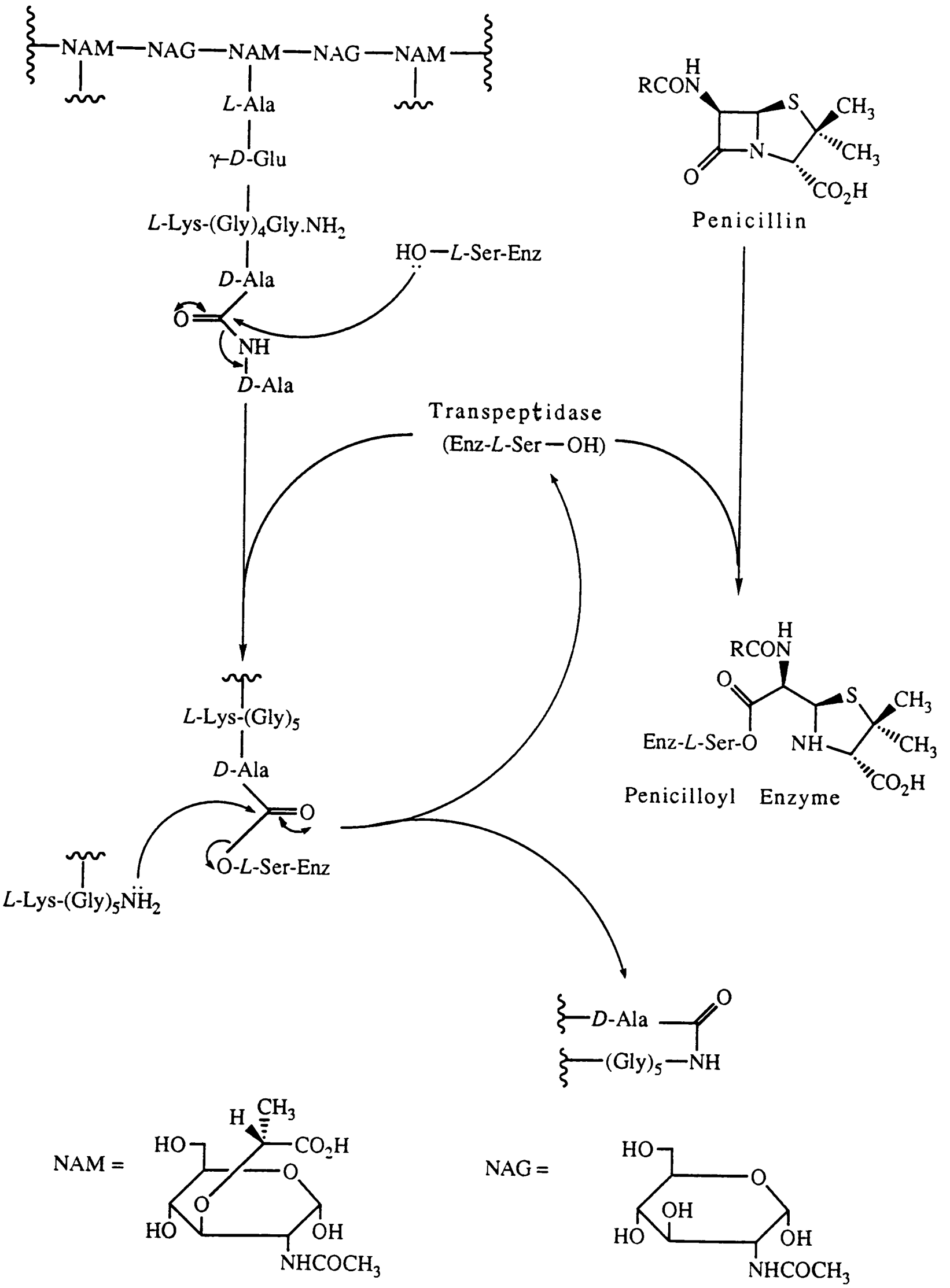
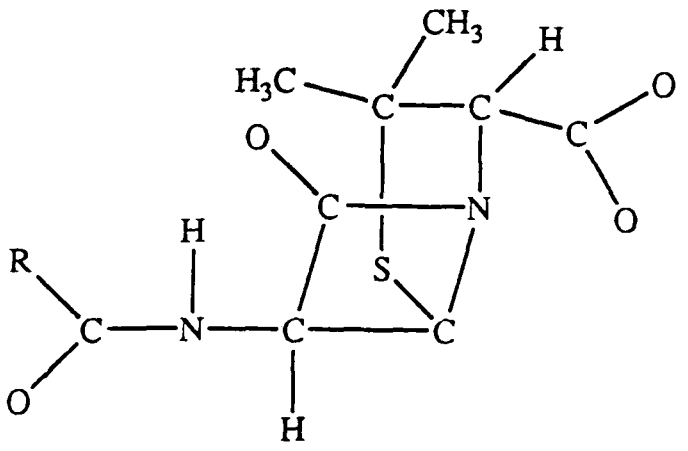

NAM—NAG—NAM—NAG—NAM
L-Ala
γ-D-Glu
L-Lys-(Gly)4Gly.NH2
D-Ala
HO—L-Ser-Enz
O
NH
D-Ala
Transpeptidase
(Enz-L-Ser—OH)
L-Lys-(Gly)5
D-Ala
O
O-L-Ser-Enz
L-Lys-(Gly)5NH2
RCON
H
S
CH3
CH3
N
O
CO2H
Penicillin
RCON
H
O
S
CH3
Enz-L-Ser-O
NH
CH3
CO2H
Penicilloyl Enzyme
D-Ala
O
(Gly)5—NH
NAM =
H
CH3
HO
CO2H
O
O
HO
OH
NHCOCH3
NAG =
HO
O
OH
HO
OH
NHCOCH3

Figure 1.1

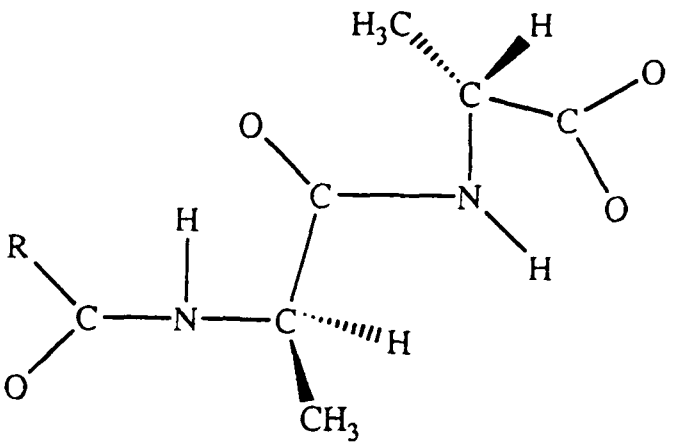
The initial stages of peptidoglycan biosynthesis involve the construction of a linear peptidoglycan[19] consisting of alternating *N*-acetyl-*D*-glucosamine (NAG) and *N*-acetylmuramic acid (NAM) units linked to a decapeptide (Figure 1.1). In the latter stages, the linear peptidoglycan strands are cross-linked by the formation of peptide bonds. A specific transpeptidase enzyme cleaves the terminal *D*-alanyl-*D*-alanine bond of the decapeptide and forms a peptide linkage between the residual alanine and the terminal residue of a pentaglycine unit in a neighbouring chain, involving the loss of the terminal *D*-alanine residue.[20]

A mechanism for the mode of action of penicillin was proposed by Tipper and Strominger[16] in 1965. Based upon molecular models, they suggested that penicillins bear a structural resemblance to the terminal *D*-alanyl-*D*-alanine moiety of the linear peptidoglycan, as illustrated in Figure 1.2, and inhibit transpeptidation by reacting with the enzyme binding site for this substrate. The highly reactive amide bond of the β-lactam ring is then the equivalent of the peptide bond in *D*-alanyl-*D*-alanine in the conformation which is bound to the enzyme. A facile acylation of the transpeptidase active site occurs with opening of the β-lactam ring, irreversibly forming a penicilloyl enzyme, thereby inactivating the transpeptidase (Figure 1.1).

The cell wall so generated is defective and cannot support the fragile cytoplasmic membrane against the internal osmotic pressure. The cell fluid bursts through the membrane and the organism is killed.[21]



Penicillin

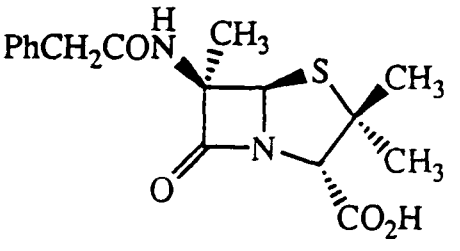


D-Alanyl-*D*-Alanine

Figure 1.2

Support for this hypothesis has come from the observation that penicillins inhibit transpeptidation in *Escherichia coli*[22,23] and a DD-carboxypeptidase in *Bacillus subtilis*[24,25]. In the latter case, reversal of inhibition could be achieved by treatment of the enzyme penicilloate complex with nucleophiles such as hydroxylamine or thiols. Further support comes from X-ray crystallographic data[26,27], which has shown that the catalysis of the peptidoglycan cross-linkage and binding of penicillins and cephalosporins involve the same serine hydroxyl residue on the transpeptidase enzyme.

However, the structural analogy between the penicillins and the natural substrate is imperfect.[28] The N-CO (lactam) bond length in penicillin V (11) is 0.12Å longer than in *D*-alanyl-*D*-alanine, and the inherent ring strain in penicillins causes the β-lactam to assume a pyramidal rather than planar geometry. Based on the Strominger hypothesis, it was predicted that the 6α-methyl derivatives of penicillins should act as better inhibitors of transpeptidase enzymes because of the closer structural analogy to the natural substrate.[29] However, synthetic 6α-methyl penicillin G (19)[30] was found to be devoid of antibacterial activity.



(19)

Lee[28] has postulated that the β-lactam antibiotics are analogues of a transitory intermediate formed during cleavage of the *D*-alanyl-*D*-alanine dipeptide moiety, since the penicillin molecule bears a greater resemblance to the natural substrate when the *D*-alanyl-*D*-alanine peptide bond is weakened, allowing the amide nitrogen to attain tetrahedral character

more closely. The distortion is energetically unfavourable and the only driving force would be binding to the enzyme. Binding will however be preferential, since penicillins are already distorted. This viewpoint is in good accord with the observation that enzymes catalyse the formation of the transition state for such a process; in this case, the 'tetrahedral intermediate' of the acylation reaction.

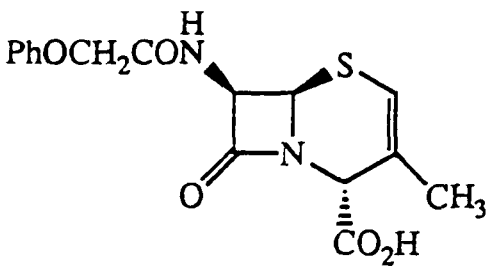
Further criticism of the hypothesis comes from the isolation of several different penicillin binding proteins from *E. coli* and *P. aeruginosa*[31], thus suggesting a more complex mechanism of action.

Thus penicillins may be considered as both suicide inhibitors and 'transition state analogue' inhibitors.

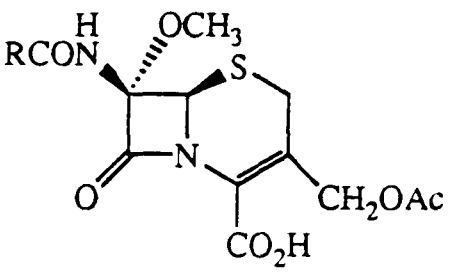
1.5. Structure-Activity Relationships

A naïve interpretation of the Strominger hypothesis may suggest that certain structural features, namely the acylamino side chain, the pendant carboxylic acid and the β-lactam ring itself, should be essential for the best fit of the antibiotic into the active site of the enzyme. However, even a cursory examination of the structural/antibiotic activity relationships for the β-lactam family of antibiotics indicates that this is not so. For instance, the Δ-3 cephalosporins (15)[10] are potent antibiotics, yet the Δ-2 cephalosporins (20)[8], produced by olefin migration display no antibacterial activity. Cephamycins (21)[32], bearing a 7α-methoxy group possess high antibacterial activity and are also resistant to β-lactamase enzymes, yet substitution of a 7α-methyl group renders the species inactive. Thienamycin (22)[33] is active against a wide range of bacteria and possesses

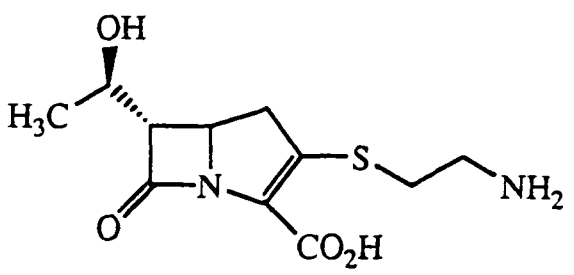
high β-lactamase resistance, but the acylamino side chain is here replaced with a 6α-hydroxyethyl side chain. It is of interest to note that penem carboxylic acid (23)[34] possesses no side chain, yet exhibits significant antibacterial activity.



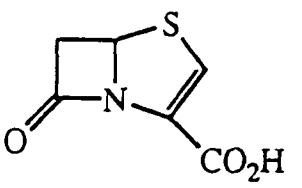
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(21)

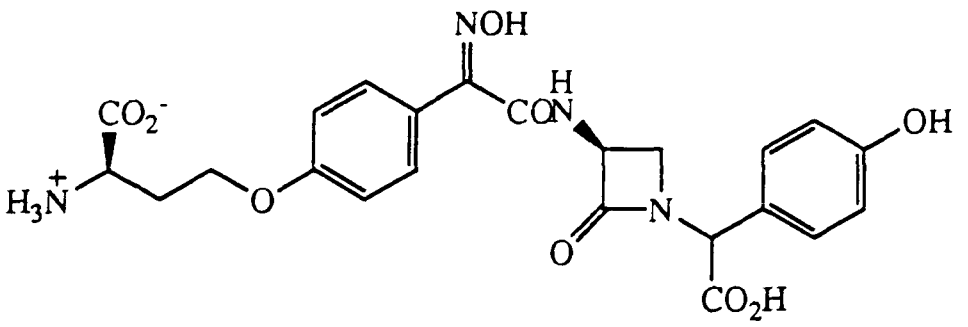


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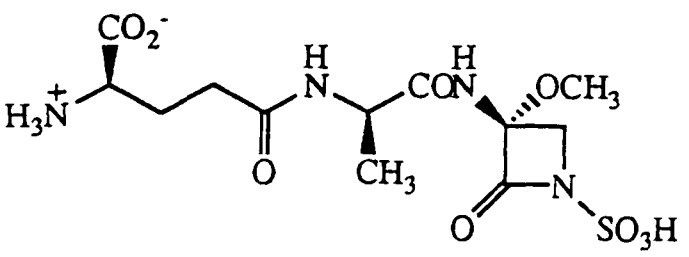


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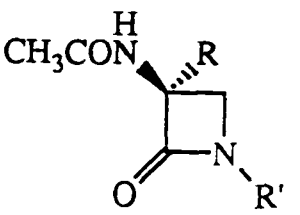
Nocardicin A (24)[35] was the first example of a monocyclic β-lactam with high activity to be discovered, and has been subsequently followed by the discovery of sulfazecin C (25)[36] and the monobactams (26-29)[37] possessing a relatively basic structure, yet remarkably high biological activity.



(24)



(25)



(26) R=H R'=H

(27) R=OCH_3 R'=H

(28) R=H R'=SO_3H

(29) R=OCH_3 R'=SO_3H

These observations suggest the minimum structural requirement for antibacterial activity to be a suitably activated β-lactam and an appropriately disposed acid residue.

Attempts have been made to relate structural features of antibiotics to both their reactivity and antibacterial activity. Such parameters that have been considered are the departure of the bonds about nitrogen from planarity[38,39], infra-red stretching frequencies of the β-lactam carbonyl group[40], C-N and C=O bond length differences[41], ^{13}C and ^{15}N chemical shifts[42] and extensive theoretical calculations[43].

The departure of the nitrogen atom from planarity may be judged by determining the sum of the three angles formed by the bonds between nitrogen and its substituents,[38] or more conveniently by the distance 'h' of the nitrogen atom above the plane defined by the three atoms attached to it[39], as illustrated in Figure 1.3.

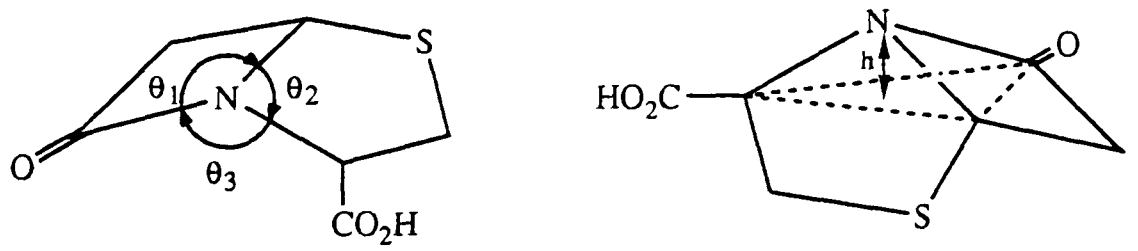
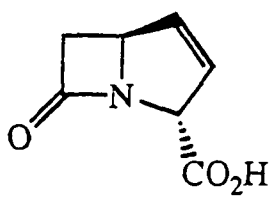


Figure 1.3

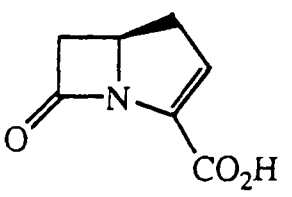
In penicillins 'h' is *ca* 0.4Å, whereas in Δ-3 cephalosporins it is 0.2-0.3Å. It has been proposed that bicyclic β-lactams with high 'h' values should be active, and those with low 'h' values should be too stable to hydrolysis and hence inactive.

However, ease of hydrolysis is not necessarily a measure of biological activity. For instance, Δ-1-carbapenemcarboxylic acid (30), which,

although it has a high 'h' value (0.54Å) and is susceptible to hydrolysis[44], is inactive as an antibiotic. Conversely, Δ-2-carbapenemcarboxylic acid (31), which has a lower 'h' value (0.50Å), is less easily hydrolysed as expected, but possesses antibacterial activity.[38]



(30)



(31)

This outcome can be explained by the inability of (30) to delocalise the unshared nitrogen electron pair into a π-orbital system. These electrons are delocalised into the carbonyl group and serve to strengthen the lactam bond.

The reduced amide resonance in a β-lactam compound with an acyclic amide may be expected to increase the carbonyl stretching frequency, increase the C-N bond length, decrease the C=O bond length and in theory, increase the activity. However, application of this theory to known compounds uncovers several discrepancies. For instance, the carbonyl bond length is the same for the active penicillin V and Δ-3 cephalosporins as well as the inactive Δ-2 cephalosporins (1.2 ± 0.01Å). Page[45] has pointed out that while the carbonyl stretching frequency increases with pyramidal distortion, the interpretation of this is not simple, and concludes that there is no significant reduction of amide resonance in the penicillins; that the pyramidal geometry of the β-lactam nitrogen is not necessary for either high chemical reactivity or antibiotic activity, and that the most important contribution to antibacterial activity emanates from the transport and binding of the antibiotic to its receptor site, and inhibition of the relevant enzyme.

Despite the lack of correlation between chemical reactivity and biological activity, it would seem that a minimum of reactivity is required in addition to conformational prerequisites.

An alternative rationalisation of the observed relationships has been made by Frere[27], who has proposed that the structures of the side chains of bicyclic β-lactams determine the position of the β-lactam carbonyl on the active site of the enzyme. In contrast, Cohen[46] has suggested that the conformationally significant parameter is the interatomic distance 'l' between the β-lactam oxygen atom and the carboxylate carbon (Figure 1.4).

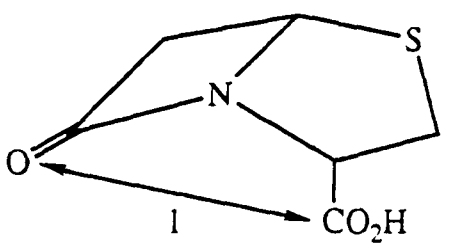


Figure 1.4

A value of 3.0 - 3.9Å is predicted for active compounds; any value exceeding this renders the geometry inactive. In the case of monobactams bearing a sulphonate group, the distance between the lactam oxygen and the sulphur is taken [e.g. (29), 'l' = 3.355Å, 'h' = 0.13Å]. Inevitably, there are exceptions to this rule; for instance in penicillin G (10), where 'l' = 4.263Å. It must of course be remembered that these distances are derived from crystal structures which are not necessarily representative of the solvated compounds.

Recently, Wolfe[47] used sophisticated molecular mechanics to perform conformational analyses on several biologically active and inactive penicillins. It was found that antibacterial activity is associated with a global minimum having a compact structure, whose convex face is

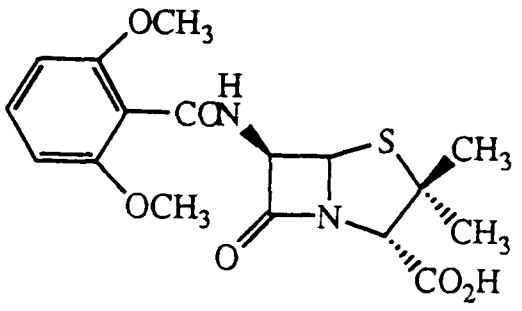
accessible to a penicillin binding protein, with the C-3 carboxyl group and the side chain N-H exposed on this face. In conjunction with a model of a penicillin binding protein, it was proposed that all active penicillins bind through strong hydrogen bonds to the aforementioned two sites, with a four-centred relationship between the O-H of serine and the (O)C-N of the β-lactam ring.

The majority of hypotheses, as outlined above, attempting to account for β-lactam antibacterial activity assume the target of all β-lactam antibiotics to be the same. However, it has been discovered that there are multiple receptor sites for β-lactams within the plasma membrane of bacteria.[31] The exact role of all of these penicillin binding proteins is unclear and hence the precise mode of action of penicillins cannot be stated with any certainty.

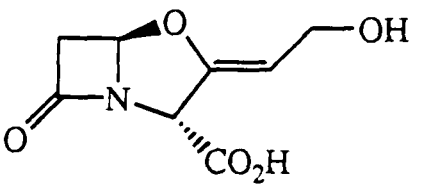
It should also be pointed out that other factors such as toxicity and the effectiveness of targeting the antibiotic to the desired site of attack are important considerations for the design of a useful antibiotic.

1.6. β-Lactamase Inhibitors

The early types of β-lactamase inhibitors tended to be semi-synthetic penicillins or cephalosporins, such as methicillin (32)[48]. These were of little use in clinical medicine since they are competitive inhibitors of the β-lactamases and therefore must be present in relatively high concentrations to protect β-lactamase susceptible antibiotics.

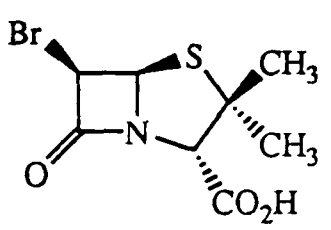


(32)

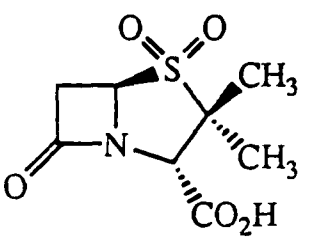


(33)

Clavulanic acid (33)[49] was the first compound to be discovered that acts as an irreversible inhibitor of β-lactamases, and in contrast to methicillin (32), is able to penetrate cell walls efficiently. 6β-Bromopenicillanic acid (34)[50,51] and penicillanic acid sulphone (35)[52] are also known to be irreversible inhibitors of β-lactamases.



(34)



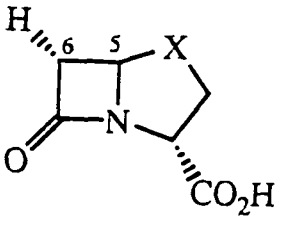
(35)

Knowles[53] has suggested that the primary chemical features required for effectiveness of a β-lactamase inhibitor are:-

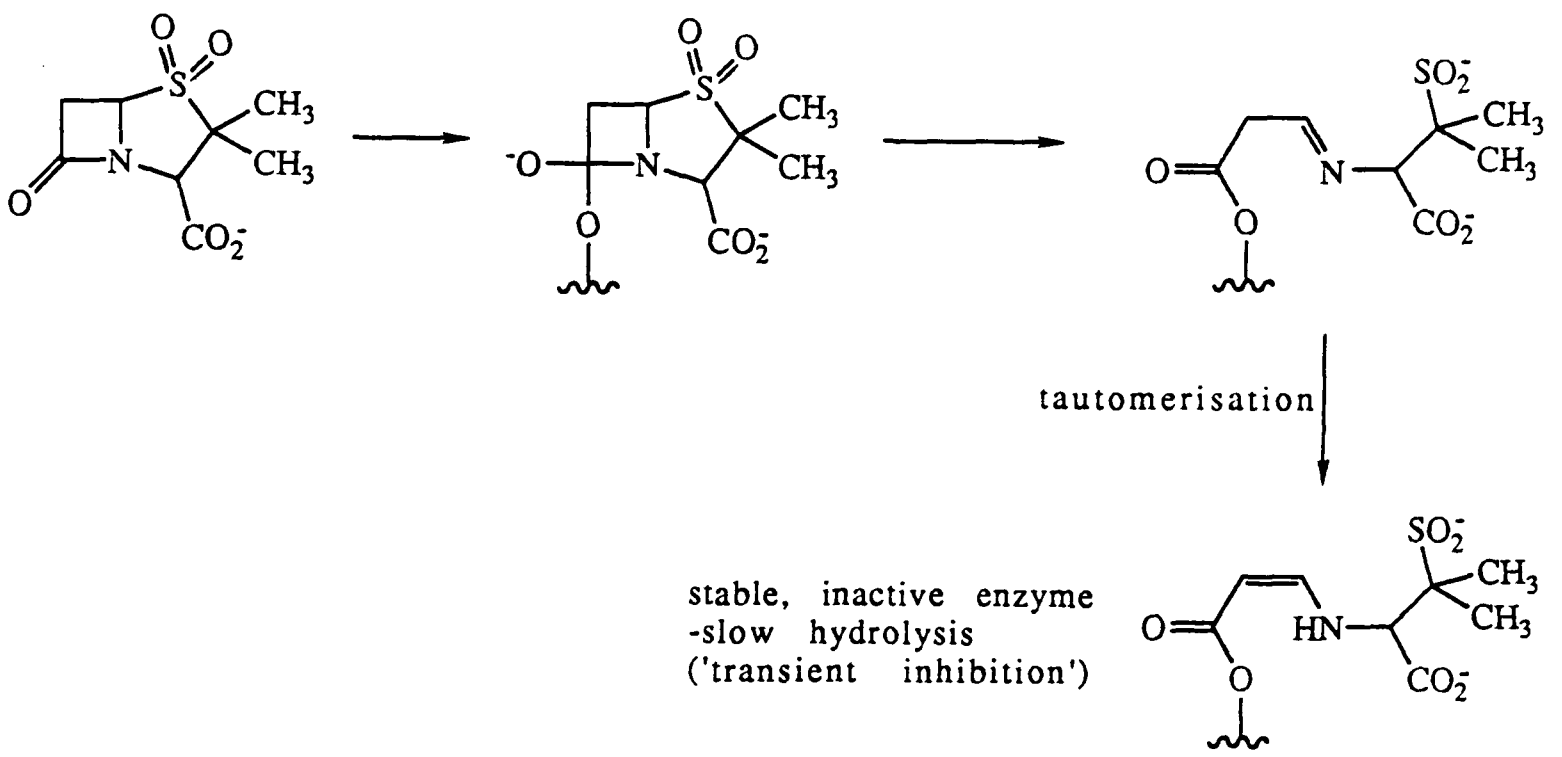
(a) a bicyclic β-lactam nucleus which can be recognised as a (poor) substrate,

(b) an α-hydrogen at C-6 of the β-lactam ring, and

(c) a good leaving group (X) attached at C-5.



A mechanism was proposed for the mode of action of the irreversible β-lactamase inhibitors, which involves the initial formation of an acyl-enzyme, subsequently forming a stable, inactive enzyme (Scheme 1.1).[53]



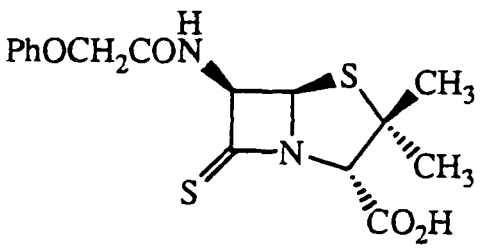
Scheme 1.1

A few crystal structures of β-lactamase enzymes are now known; for example, that of the β-lactamase from *Staphylococcus aureus* PCI.[54] It has been suggested[55] that extensive conformational changes in the enzyme accompanying its interaction with the inhibitor may be responsible for the irreversible loss in activity. Recently, this hypothesis has been further substantiated by Fink *et al*[56], who suggested that the conformational change results in displacement of the catalytic groups with respect to the acyl-enzyme bond such that catalysis of deacylation is prevented, thus causing irreversible inhibition.

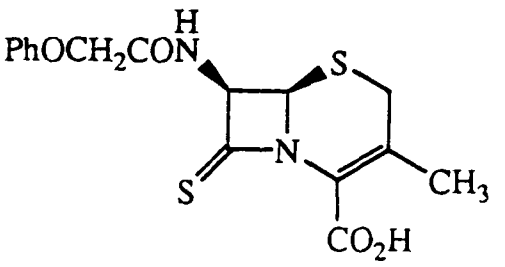
1.7. Analogues of the β-Lactam Antibiotics

In recent years, several studies have been directed towards the synthesis of biologically active analogues of the β-lactam antibiotics without a β-lactam ring.

The thiono-analogues (36) and (37) have been synthesised[57], though their biological activities are less than the corresponding penicillin or cephalosporin, as might be predicted on the basis of stereoelectronic theory.

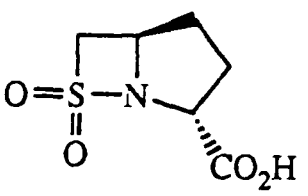


(36)

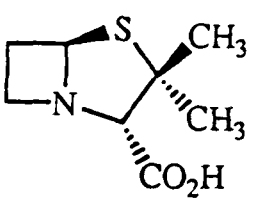


(37)

The β-sultam analogue (38)[58] has been synthesised and was reported to be inactive, as was the deoxopenicillanic acid analogue (39)[59].

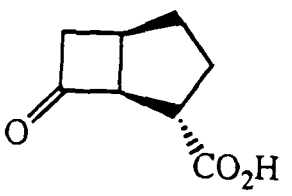


(38)

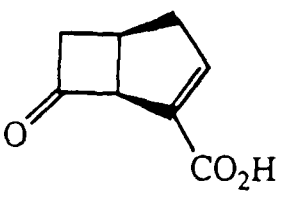


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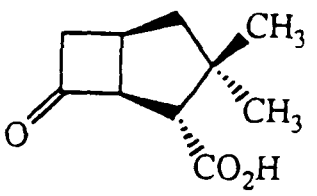
The syntheses of several cyclobutanone analogues (40-42)[60,61] have been completed, but none has been shown to exhibit any antibacterial activity. However (43)[62], synthesised as an analogue of the active *in vivo* antibiotic 1-oxabisnorpenicillin G (44)[63], exhibited weak antibacterial activity against some *Streptomyces* strains.



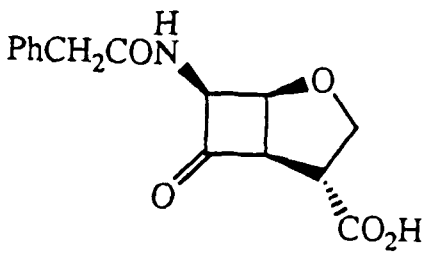
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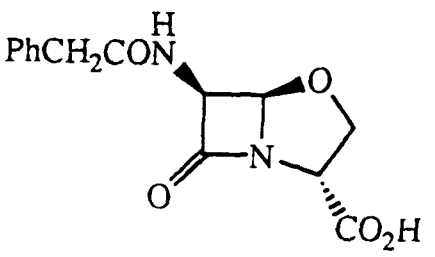
(41)



(42)



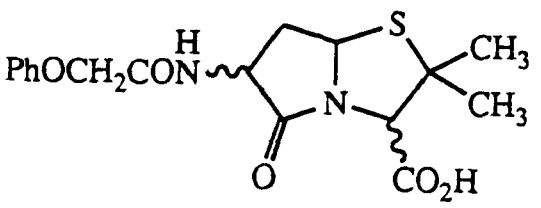
(43)



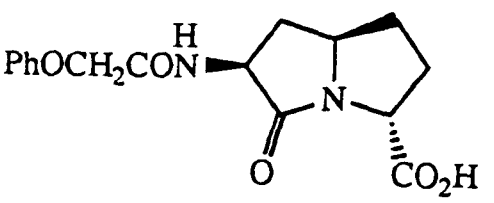
(44)

1.8. γ-Lactams as Antibiotics

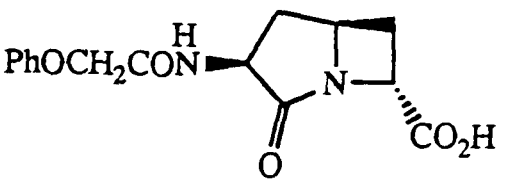
In the hope of discovering a new class of antibiotics, several γ-lactams have been prepared and tested for antibacterial activity, for example (45)[64], (46)[65] and (47)[66]. These were all found to be inactive, presumably due to the lack of reactivity of the γ-lactam in comparison with a β-lactam.



(45)

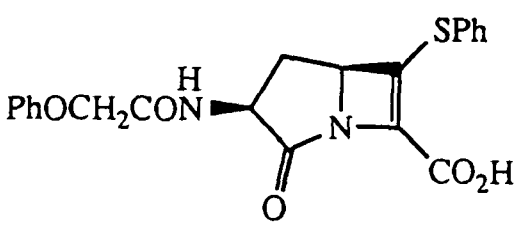


(46)

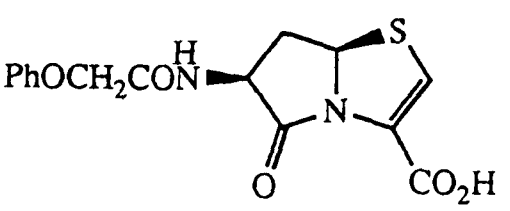


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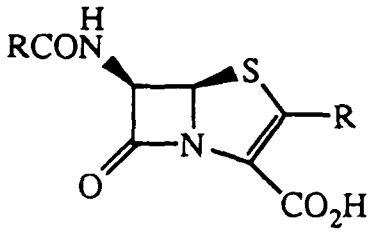
However, the recently synthesised azete (48)[67], and the γ-lactam analogue (49)[68] were the first such species found to display antibacterial activity.



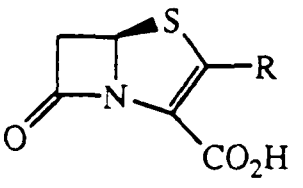
(48)



(49)



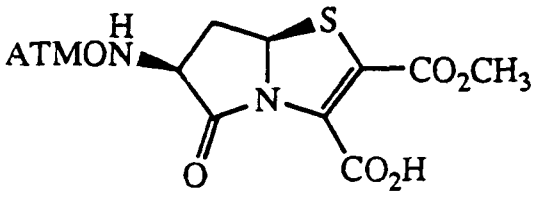
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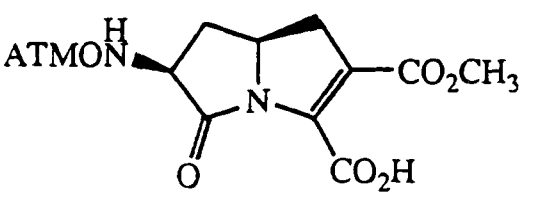
(51)

It is of interest to note that both the 6β-acylaminopenem (50)[69], of which (49) is an analogue, and the counterpart (51)[70], devoid of the 6β-acylamino side chain, exhibit powerful antibacterial activity; however they have been considered too reactive for employment as practical antibiotics.[71]

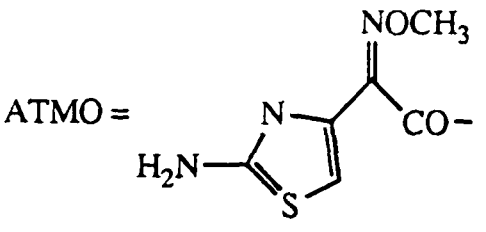
These results stimulated enormous interest in the field of γ-lactam antibiotics, resulting in the syntheses of the related compounds (52)[72] and the carbapenem analogue (53)[73], both of which displayed weak antibacterial activity.



(52)



(53)



The activity in this type of compound was attributed[68] to both the increase in strain inherent in the molecule by incorporation of the unsaturation into the thiazolidine ring, and the increased γ-lactam reactivity due to delocalisation of the lactam-N lone pair through the double bond with stabilisation of the resulting electron density by an electron withdrawing group and/or the α-sulphur atom (Figure 1.5).

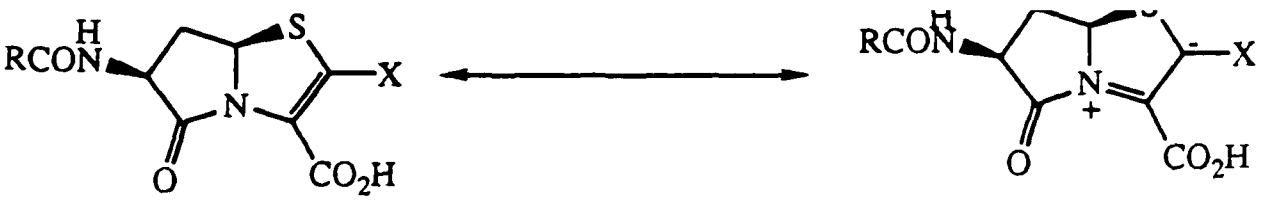
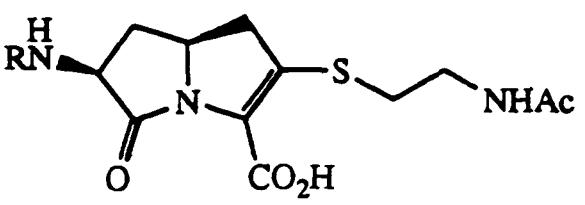
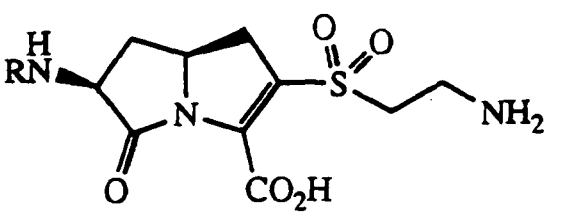


Figure 1.5

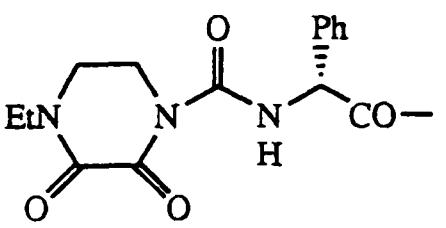
Other new synthetic γ-lactams exhibiting antibacterial activity are exemplified by (54-57)[74], and the bicyclic pyrazolidinones (58-60)[75].

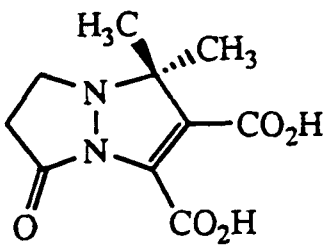


(54) R=ATMO
(55) R=EPPA

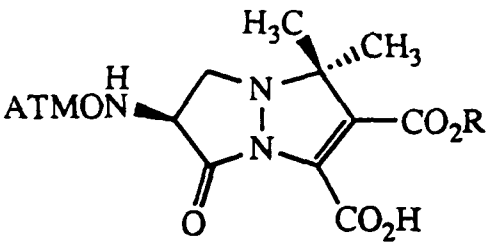


(56) R=ATMO
(57) R=EPPA

EPPA = 



(58)

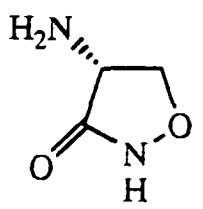


(59) R=H
(60) R=Me

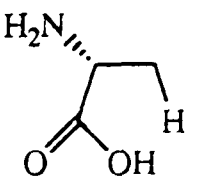
Computational chemistry and molecular superpositioning have demonstrated that these novel γ-lactam structures with a 7β-acylamino side chain present the pharmacophoric groups in close spatial similarity to the groups in biologically active cephalosporin and penicillin antibiotics, suggesting that they can be accommodated in the same active sites of essential penicillin binding proteins where cephalosporins and penicillins are recognised.[76]

1.9. γ-Lactam Antibiotics Incorporating the Cycloserine Nucleus

D-Cycloserine (61)[77], marketed as Oxamycin by Merck, Sharpe and Dohme, is an antibiotic in its own right. It is produced by certain strains of *Streptomyces orchidaceus* or *S. garyphalus*, or it may be synthesised[78]. It inhibits the growth of virulent strains of *Mycobacterium tuberculosis* and other related bacteria, and has been used in the treatment of severe pulmonary tuberculosis. As a structural analogue of *D*-alanine (62), it acts as a competitive inhibitor of alanine racemase and *D*-alanyl-*D*-alanine synthetase. Cycloserine therefore inhibits cell wall biosynthesis by preventing the formation of the pentapeptide, as described in Section 1.4.

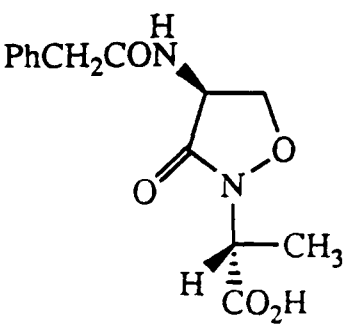


(61)

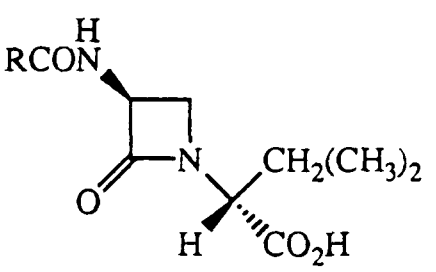


(62)

Inevitably, the *L*-cycloserine nucleus has been used in an attempt to mimic the action of β-lactam antibiotics, since the endocyclic oxygen atom increases the reactivity of the ring. However, the synthetic derivative (63)[79] was found to be devoid of biological activity. This result was hardly surprising since the analogous dethiopenicillins (64)[80] arising from the desulphurisation of penicillins were also shown to be inactive.

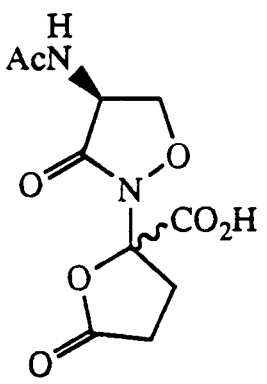


(63)

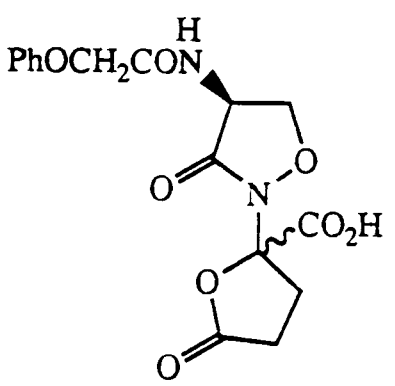


(64)

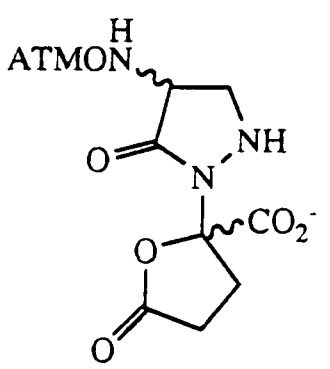
Recently the novel antibiotic Lactivicin (LTV, 65)[81], containing the *L*-cycloserine moiety, was isolated from culture filtrates of *Empedobacter lactagenus* YK-258 and *Lysobacter albus* YK-422 (first obtained from Japanese soil samples), and its structure elucidated[82]. LTV was active against Gram positive and negative bacteria and was shown to possess biological activities similar to those of β-lactam antibiotics in its affinity for penicillin binding proteins and susceptibility to β-lactamases.



(65)



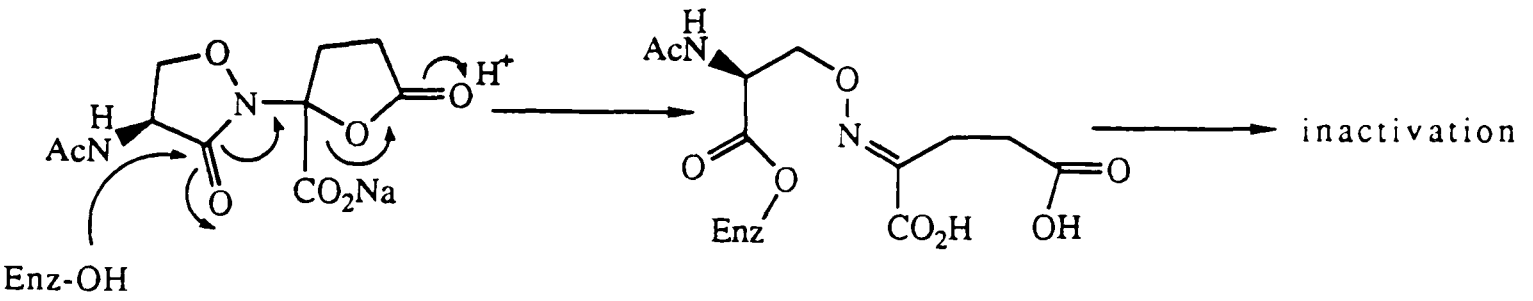
(66)



(67)

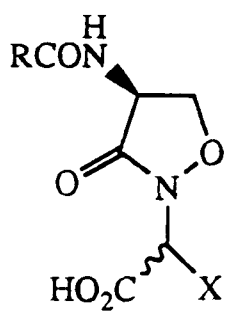
In the current study, a total synthesis of LTV (65) was undertaken, and extended to include alternative acylamino side chains, e.g. (66). However, towards completion of this work, the synthesis of (65) and its derivatives appeared in the literature[83], followed by a description of its chemistry[84], its activity and its mode of action[85]. The synthesis of the aza-analogue of LTV (67)[86] has also been reported and exhibits *in vitro* antibacterial activity.

The mode of action of LTV is believed to involve irreversible acylation of the penicillin binding protein to yield an acylated enzyme, with concomitant opening of the γ-lactone ring and formation of an oxime, which may subsequently lead to further degradation (Scheme 1.2).

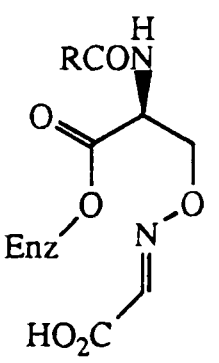


Scheme 1.2

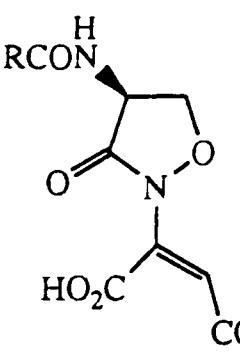
Consequently, in the hope of discovering related antibiotics, the synthesis of a derivative of the type (68) was undertaken, where X is a suitable leaving group. This would be expected to yield the acylated enzyme (69) *via* a similar mechanism, involving the loss of X and thus providing the driving force for the process.



(68)

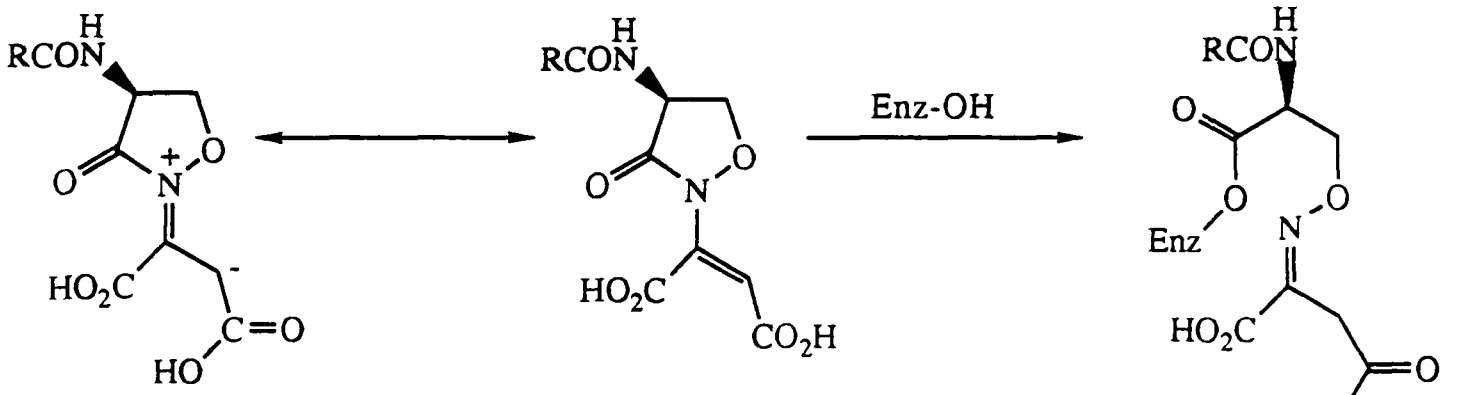


(69)



(70)

In addition, the synthesis of an analogue of the type (70) was carried out. Here, delocalisation of the nitrogen lone pair through the double bond and stabilisation by the carboxylate functionality would be expected to activate the γ-lactam ring, as well as providing a pathway for the movement of electrons upon acylation of the appropriate penicillin binding protein (Scheme 1.3).

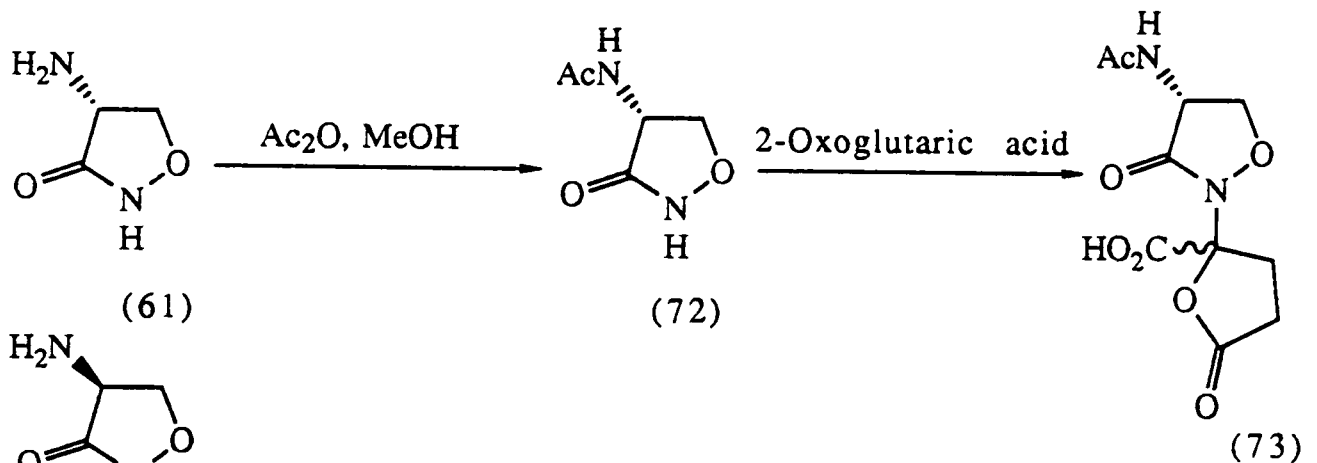


Scheme 1.3

2. RESULTS AND DISCUSSION

2.1. The Synthesis of Acylamino Derivatives of Lactivicin

In order to obtain LTV (65) in the correct stereochemical configuration, the *L*-isomer of cycloserine (71) has to be used as the starting material. However, owing to the high cost of this material (£27.90/100mg, Aldrich Chemical Co. Ltd.), its enantiomer, *D*-cycloserine (61) was used to investigate the envisaged reaction sequence outlined in Scheme 2.1.

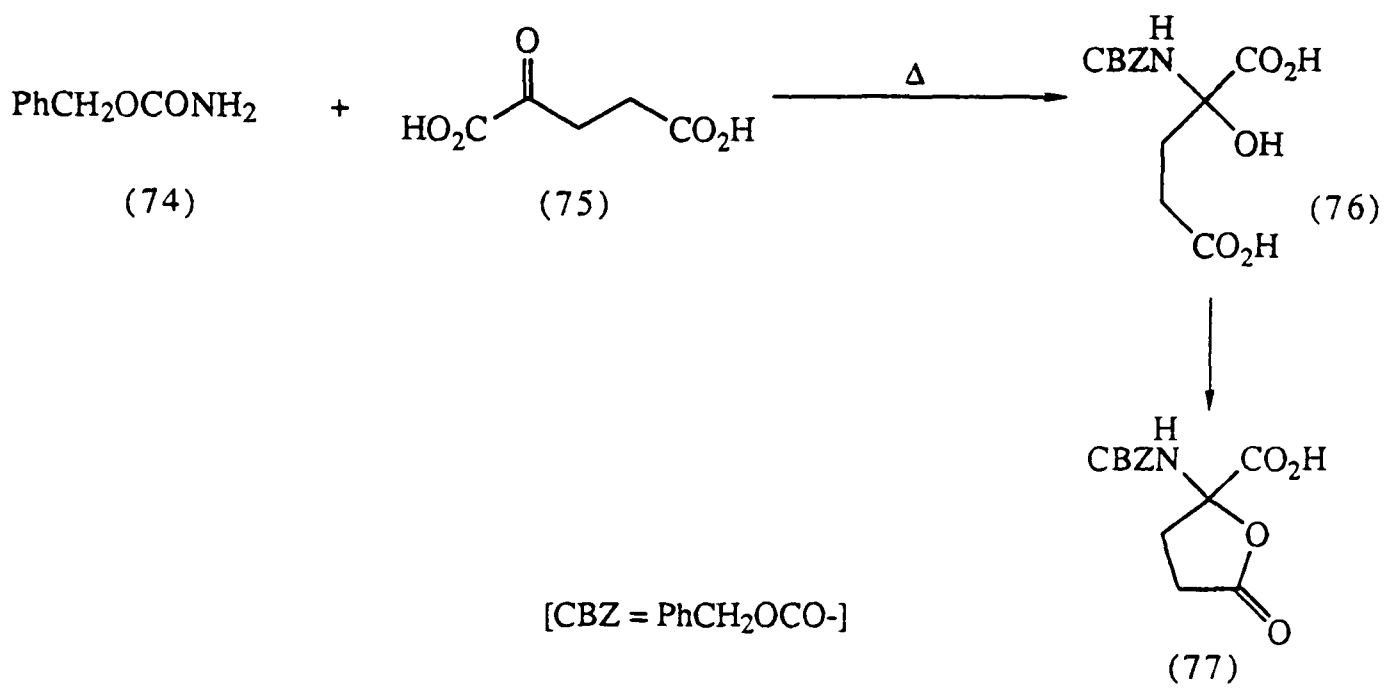


Scheme 2.1

Attachment of the acetyl sidechain was accomplished by the dropwise addition of acetic anhydride to a solution of *D*-cycloserine (61) in dry methanol at room temperature.[87] After recrystallisation from hot methanol, *D*-4-acetamido-3-isoxazolidinone (72) was collected as a white crystalline solid {m.p. 176-177°C; $[\alpha]_D$ +74.5° (c=0.86, H_2O)} in 77% yield. Other standard procedures involving the addition of acetyl chloride or acetic anhydride to a solution of (61) in aqueous sodium hydroxide failed to give reasonable yields of the acylated product (72).

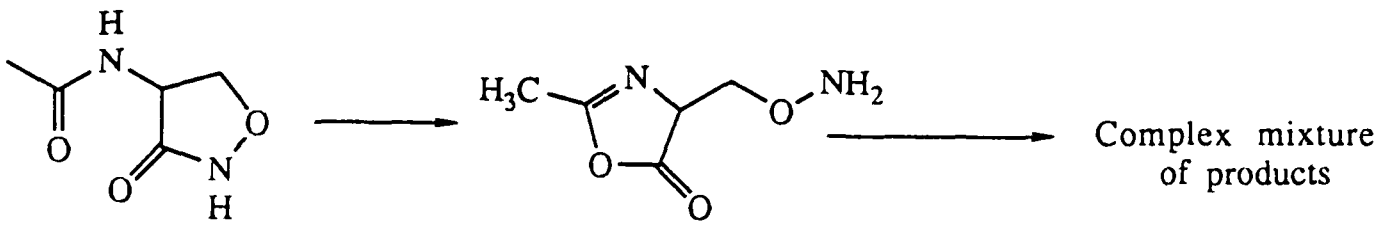
Literature precedent[88] exists for the condensation of amides with α-keto acids. Indeed, the lactone acid (77) has been prepared in 90% yield by

fusing benzyl carbamate (74) and 2-oxoglutaric acid (75) under reduced pressure. The reaction proceeds *via* the intermediate (76) which is subsequently stabilised by lactone formation (Scheme 2.2).



Scheme 2.2

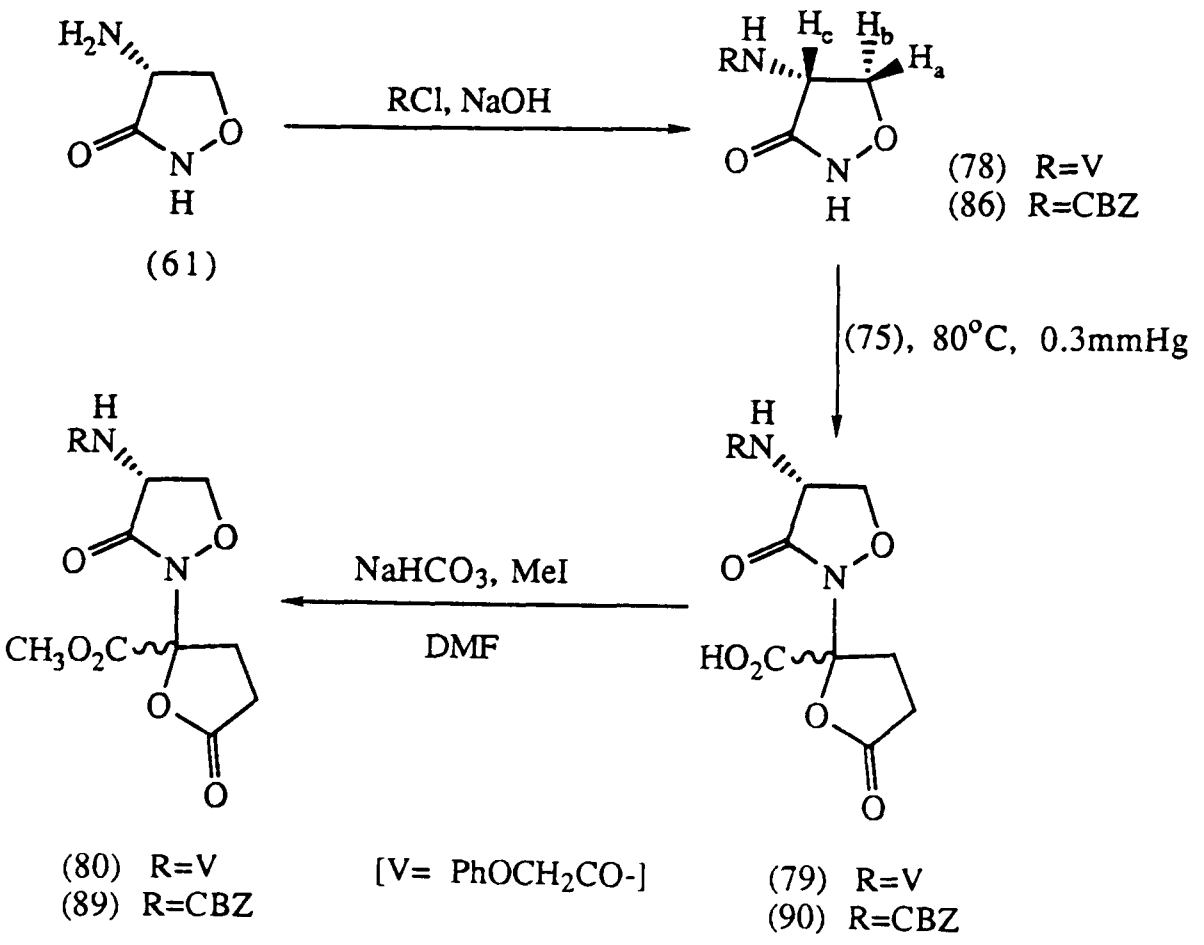
However, ^1H nmr analysis of the crude product obtained from the fusion of (72) with 2-oxoglutaric acid (75) at 80°C for 4 hours at a pressure of *c a* 10mmHg was inconclusive. Esterifications of the reaction mixture in DMF with both methyl iodide and benzyl bromide produced complex mixtures of products. These could neither be successfully separated by chromatography nor identified by mass spectrometry and ^1H nmr. Complex mixtures were also obtained by reacting (72) with (75) in the presence of activated 3Å molecular sieves in either DMSO at 80-160°C or refluxing DMF. The failure of this process may be attributed to either reaction of the side chain amide functionality with (75), or azlactone formation (Scheme 2.3).



Scheme 2.3

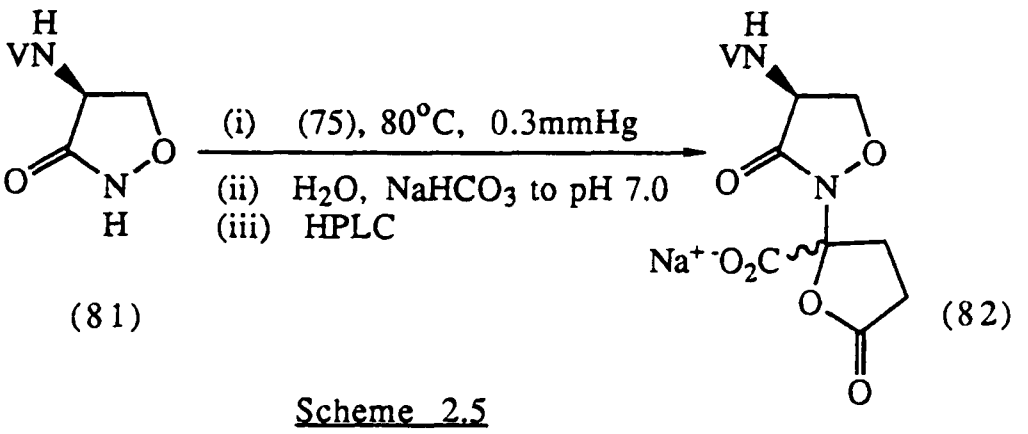
Consequently, it was decided to introduce bulkier, less reactive acylamino side chains in an attempt to direct the reaction towards the endocyclic nitrogen atom. The phenoxyacetyl (penicillin V side chain) protecting group was introduced by the dropwise addition of phenoxyacetyl chloride to a cooled solution of (61) in aqueous sodium hydroxide. Acidic work up and recrystallisation of the product from ethyl acetate furnished *D*-4-phenoxyacetylamino-3-isoxazolidinone (78) in 48% yield {m.p. 121-122°C; $[\alpha]_D$ +32.5° (c=0.9, CH_3OH)}. The ^1H nmr spectrum of (78) in DMSO-d_6 showed ring hydrogen signals at δ 4.05 (1H, dd, H_a), δ 4.52 (1H, t, H_b) and δ 4.88 (1H, m, H_c). The signal due to H_b is situated significantly further down field than H_a as a result of the field effect of the *cis* C(4)-N bond. The endocyclic proton appears as a broad singlet at δ 11.47, whilst the side chain amide -NH resonance is a doublet at δ 8.66 (*J* 8 Hz).

The lactone (79), successfully prepared by the fusion of (78) with 2-oxoglutaric acid (75) under high vacuum, was characterised as its methyl ester (80) after chromatographic purification (Scheme 2.4).



Scheme 2.4

L-Cycloserine (71) was subsequently protected with the phenoxyacetylamino side chain, in an identical manner to that previously described, to produce (81) in 42% yield {m.p. 120-121°C; $[\alpha]_D$ -30.3° (c=1.01, CH_3OH)}. Condensation of (81) with (75) under high vacuum, basification of the reaction mixture in aqueous solution with sodium hydrogen carbonate to pH 7.0, and purification by reverse phase HPLC yielded the sodium salt (82) (Scheme 2.5). The complex ^1H nmr spectrum (300 MHz, D_2O) was almost identical in the lactone and cycloserine ring regions to that of the natural product LTV (65)[83]. On bioassay plates at a concentration of ca 1mg ml^{-1}, (82) exhibited strong inhibition of both *Escherichia coli* and *Staphylococcus aureus*.



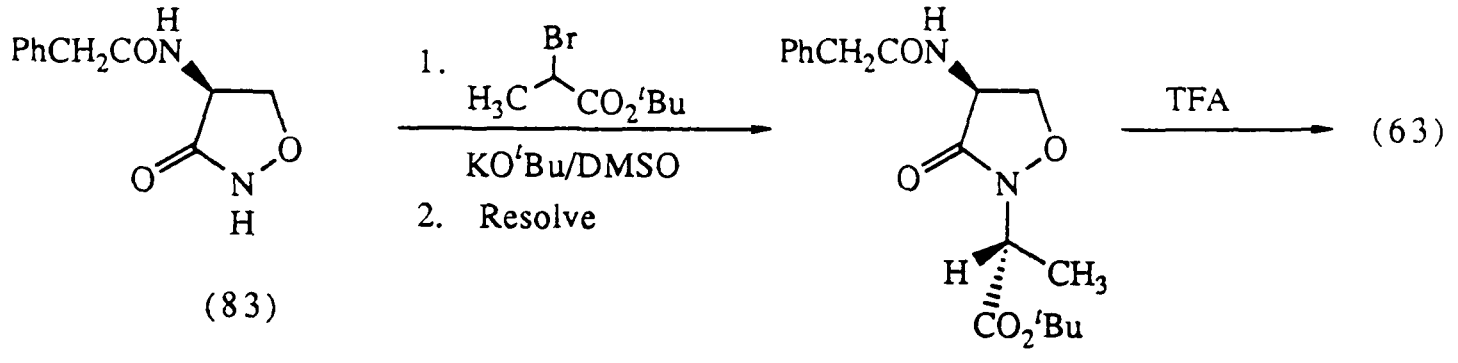
(81) (82)

Scheme 2.5

Two approaches were then considered to both (a); alkylate the cycloserine ring nitrogen efficiently, and (b); introduce an acylamino side chain that could be readily removed, after condensation with 2-oxoglutaric acid, and replaced with the acetyl side chain.

(a) In their synthesis of (63)[79], Tsuji and Yamana reported that treatment of (83) with potassium *t*-butoxide in DMSO followed by *t*-butyl-2-bromopropionate resulted in the formation of (84) (Scheme 2.6). This occurs by selective deprotonation of the more acidic ring amide proton (pKa *ca* 8-10 due to the inductive effect of the adjacent oxygen atom) as

opposed to the exocyclic amide proton (pKa 16)[89], followed by nucleophilic displacement of bromide ion from the ester.

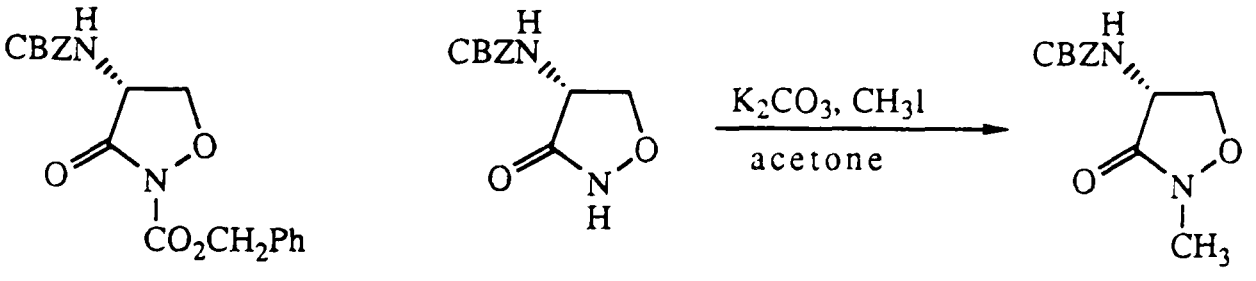


Scheme 2.6

Likewise, in order to ascertain whether (72) could be selectively deprotonated, it was treated with 1.15 equivalents of potassium *t*-butoxide in DMSO and quenched with excess methyl iodide. However, only very low yields of what appeared to be a dimethylated product were obtained.

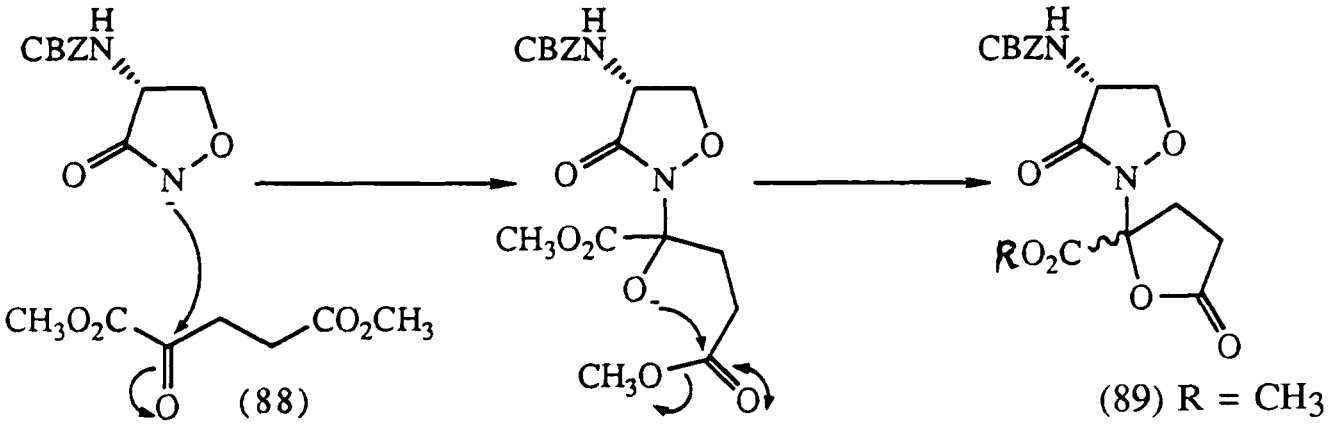
However, Stammer[90] has reported that even methylation of *N*-acetyl-*D*-cycloserine with methyl iodide/potassium carbonate gives an oily product having the expected spectral properties, but devoid of optical activity. He also states that *N*-benzyloxycarbonyl-*D*-cycloserine can be alkylated under the same conditions to form optically active and apparently pure products; the CBZ group appears to influence greatly the susceptibility of the ring system to racemisation.

Hence benzyloxycarbonylamino-*D*-cycloserine (86)[90] was prepared in an analogous manner to (78), maintaining a high pH throughout to prevent the formation of *N*,2-dibenzyloxycarbonyl-*D*-cycloserine (85)[91]. Alkylation of (86) was then achieved using potassium carbonate/methyl iodide in acetone to give the mono-methylated product (87) in 93% yield (Scheme 2.7).



Scheme 2.7

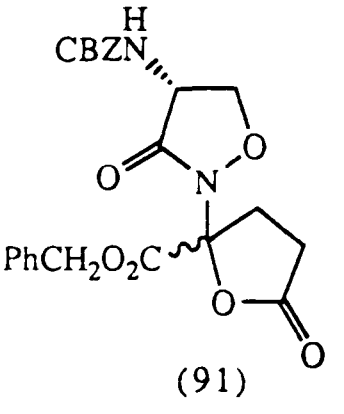
It was then hoped that this process could be exploited by the reaction of (86) with 2-oxoglutaric acid, dimethyl ester (88), prepared trivially, to yield the *N*-CBZ lactivicin analogue (89) (Scheme 2.8).

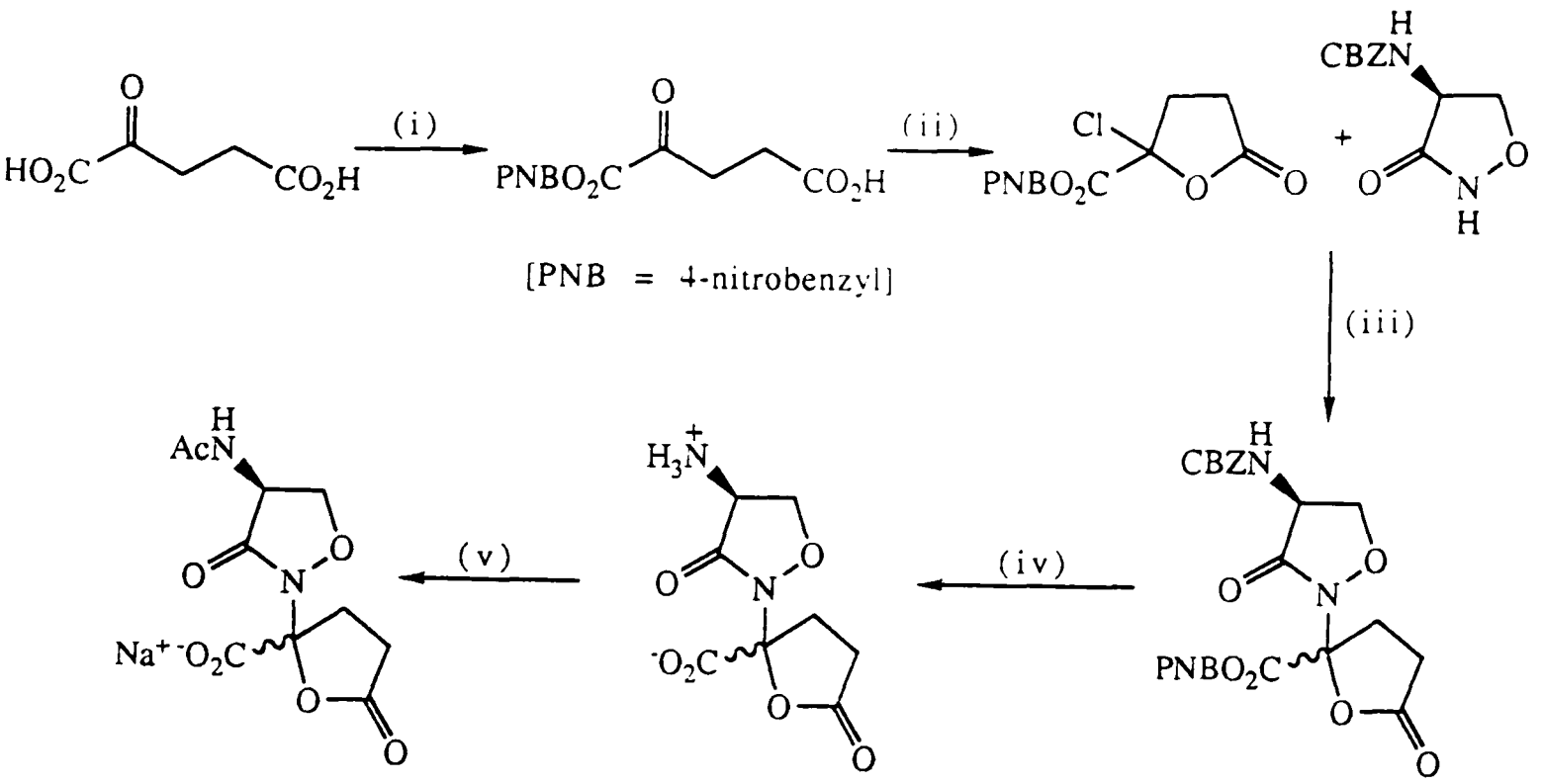


Scheme 2.8

(b) The fusion reaction was performed with (86) and 2-oxoglutaric acid to give the lactone (90), subsequently characterised both as its methyl ester (89), obtained in 25% yield from (86), and as its benzyl ester (91), obtained in 31% yield from (86).

During attempts to deprotect (91) by hydrogenation using 10% palladium on charcoal catalyst, a total synthesis of LTV (65) and its acylamino derivatives appeared in the literature.[82] This synthesis is illustrated below in Scheme 2.9, and adopts similar protocol to that described above.

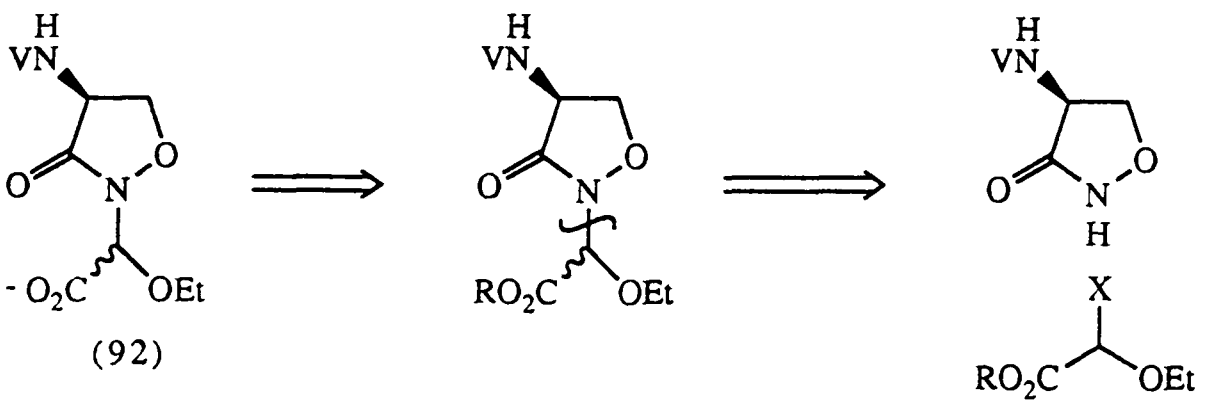




Scheme 2.9. Reagents: (i) PNBBr, DCHA, DMF; (ii) $SOCl_2$ (5.5 equiv.), $ClCH_2CH_2Cl$; (iii) Et_3N, CH_2Cl_2; (iv) H_2, Pd-C, EtOAc - pH 7.0 buffer; (v) Ac_2O, $NaHCO_3$, $THF-H_2O$.

2.2. The Synthesis of Lactivicin Analogues

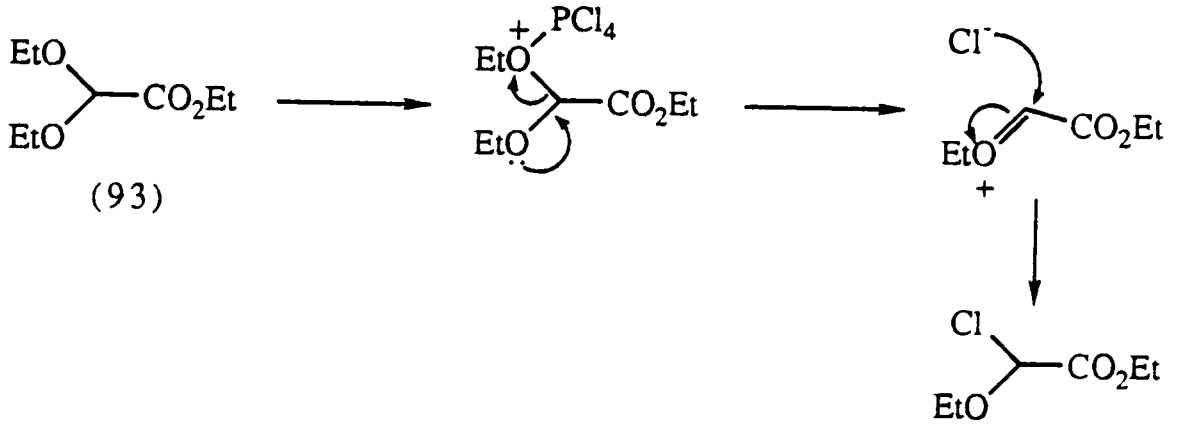
Attention was then turned to the synthesis of analogues of LTV that might inhibit bacterial growth in a similar manner to LTV itself. The ring opening of the γ-lactone appears to be a crucial step in the mechanism of action, since 2-(4-phenylacetamido-3-oxo-2-isoxazolidinyl)propionic acids (63)[77] display no antibacterial activity; so it was hoped that simplification of the molecule and replacement of the lactone with a suitable leaving group would lead to a compound with demonstrable biological activity, e.g. (92). An appropriate retrosynthesis for (92) is outlined in Scheme 2.10.



X = Good leaving group, e.g. halogen

Scheme 2.10

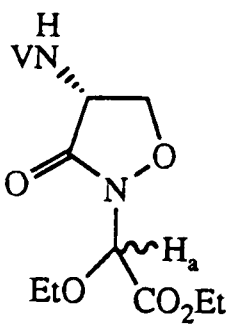
To evaluate the methodology for such a process, ethyl 2-chloro-2-ethoxyacetate (94)[92] was prepared from ethyl diethoxyacetate (93) by heating with phosphorus pentachloride (Scheme 2.11).



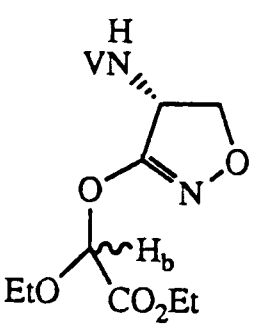
(93) (94)

Scheme 2.11

Reaction of (94) with phenoxyacetylamino-*D*-cycloserine (78) in THF at room temperature yielded no identifiable products. At reflux, however, small quantities of an impure compound assumed to be the *N*-alkylated product (95) were isolated. The two sharp singlets at δ 5.63 and δ 5.69 in the ^{1}H nmr spectrum were assigned to the proton H_a, corresponding to the two diastereoisomers formed in a ratio of *ca* 1:1.



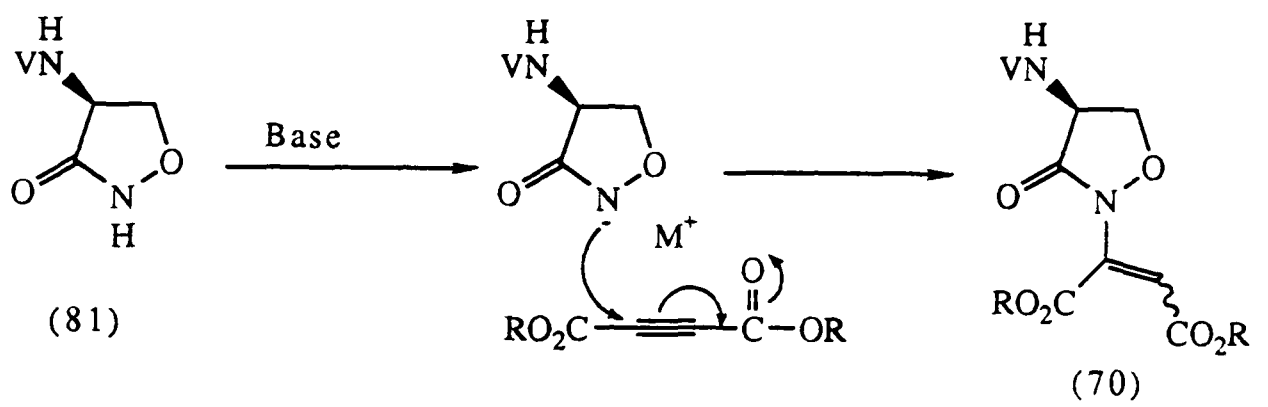
(95)



(96)

This assumption was substantiated by stirring (78) with potassium carbonate in acetone, followed by the addition of (94) and refluxing the suspension for *ca* 4.5 hours. The only identifiable product showed two singlets in the ^{1}H nmr spectrum at δ 5.73 and δ 5.83, i.e. further downfield than for (95), and an additional peak in the infra-red spectrum at 1628cm^{-1}. Thus this latter product was assigned the structure (96), the product of *O*-alkylation.

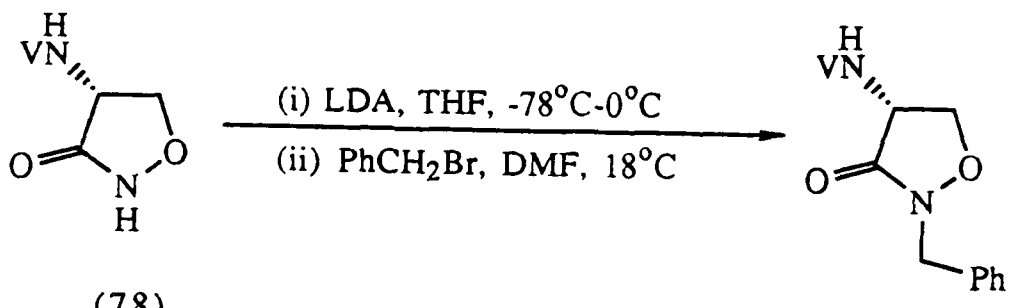
Concurrent with this study, attempts were made to prepare derivatives of the type (70) for the reasons discussed in Section 1.9. The procedure envisaged required initial deprotonation of the endocyclic N-H, followed by a Michael type 1,4 nucleophilic addition to acetylene dicarboxylates, as illustrated in Scheme 2.12. It was during these efforts that different bases and reaction conditions were evaluated for their ability to deprotonate (78) cleanly, and hence produce good yields of *N*-alkylated products, as described later in this chapter. The eventual procedure chosen was the deprotonation of (78) in THF from -78°C to +5°C with lithium diisopropylamide (LDA), prepared from *n*-butyllithium standardised by the Gilman Double Method[93]. After removal of the THF *in vacuo*, the resulting lithium salt was treated with the alkylating agent in DMF solution.



(81) (70)

Scheme 2.12

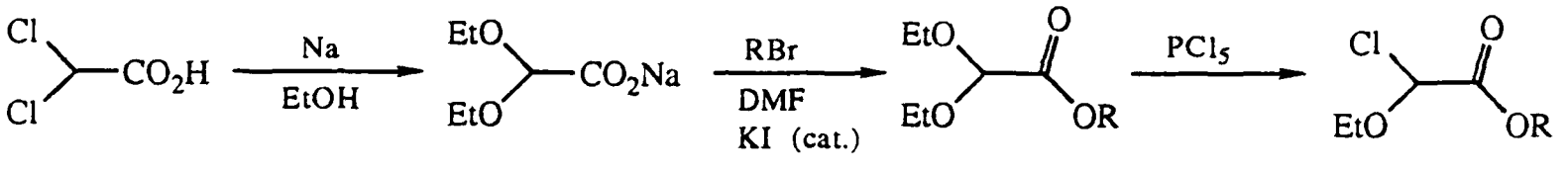
Using this procedure for a model reaction between (78) and alkyl halides, *D*-4-phenoxyacetylamino-2-benzyl-3-isoxazolidinone (97) was prepared in 57% yield (Scheme 2.13).



(78) (97)

Scheme 2.13

A more suitable electrophile than the ethyl ester (94) had to be chosen, since acid or base catalysed saponification of (95) could possibly result in either hydrolysis of the activated lactam or displacement of the strategic leaving group as ethanol. Initially, the 4-nitrobenzyl ester was chosen, since it may be trivially removed by hydrogenation. Hence 4-nitrobenzyl diethoxyacetate (96') was prepared by the treatment of dichloroacetic acid with sodium ethoxide, followed by esterification with 4-nitrobenzyl bromide in DMF (Scheme 2.14). The reaction of (96') with phosphorus pentachloride yielded (97') which could neither be easily purified by bulb to bulb distillation, nor by chromatography due to hydrolysis.

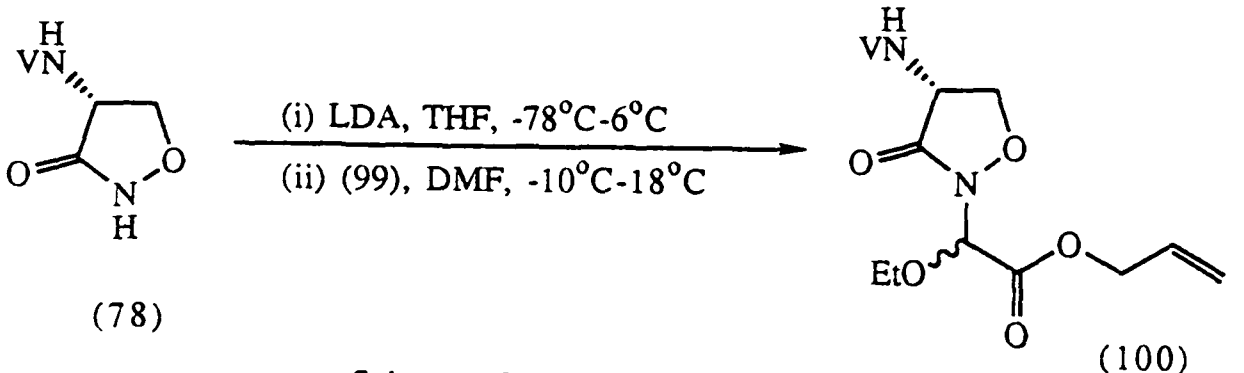


(96') R= PNB
(98) R= CH_2=CH-CH_2-

(97') R= PNB
(99) R= CH_2=CH-CH_2-

Scheme 2.14

However, allyl 2-chloro-2-ethoxyacetate (99) was successfully prepared in a similar manner from (98) (Scheme 2.14). Unfortunately, the phosphorus pentachloride caused the occurrence of ester exchange to a small extent, and so (99) was obtained (after distillation) with (94) as a 25% contaminant; redistillation also failed to separate the two components. The desired *N*-alkylated cycloserine derivative (100) was isolated as its allyl ester in 54% yield from the reaction of (78) with LDA and subsequent quenching of the anion with allyl 2-chloro-2-ethoxyacetate (99) (Scheme 2.15).

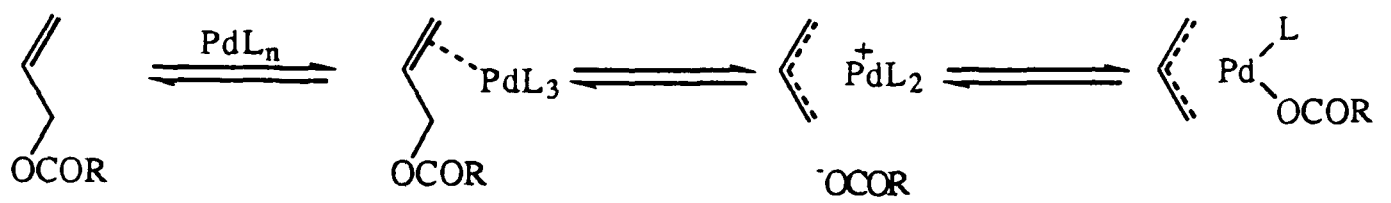


(78)

(100)

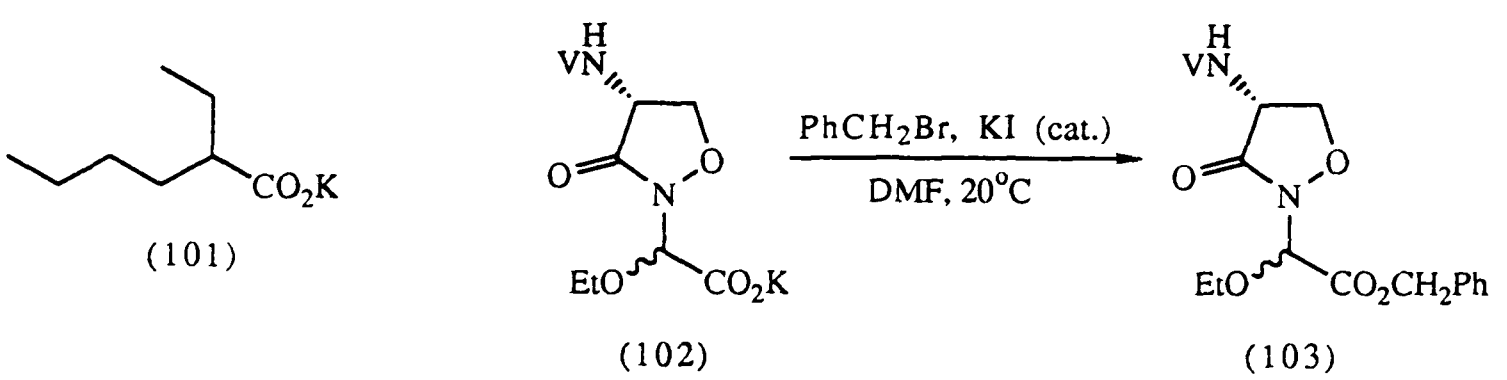
Scheme 2.15

Removal of the allyl ester was achieved by homogeneous palladium(0) catalysed exchange deprotection with tetrakis(triphenylphosphine)-palladium(0).[94] The process uses catalytic π-allyl activation involving a series of equilibria, illustrated in Scheme 2.16. Displacement of the



Scheme 2.16

exchange equilibrium over to the desired product was assured by employing the alkali metal salts of 2-ethylhexanoic acid, which were soluble in most common solvents. Thus potassium 2-ethylhexanoate (101), a white waxy solid, was prepared trivially from 2-ethylhexanoic acid and aqueous potassium hydroxide.

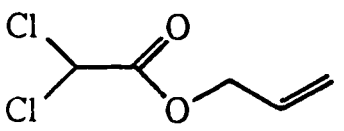


Scheme 2.17

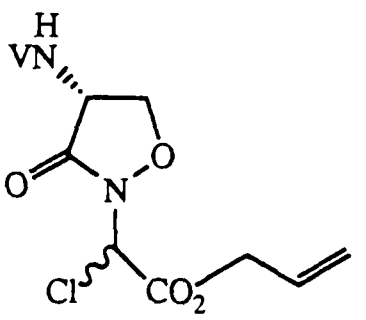
Treatment of the allyl ester (100) with potassium 2-ethylhexanoate (101) (*c a* 1.7 equivalents in dichloromethane-ethyl acetate) in the presence of tetrakis(triphenylphosphine)palladium(0) (5mol%) and additional triphenylphosphine (10 mol%) (as aerial oxidation to the phosphine oxide is catalysed by the palladium complex) yielded the salt (102) which was characterised as its benzyl ester (103) (Scheme 2.17).

To parallel this sequence, allyl dichloroacetate (104) was prepared from dichloroacetic acid, sodium hydrogen carbonate and allyl bromide in DMF

solution. Reaction of (104) with phenoxyacetylamino-*D*-cycloserine (78) using the procedure described above, gave crude (105) which proved to be unstable and decomposed readily upon silica gel chromatography. Therefore, this approach was pursued no further.

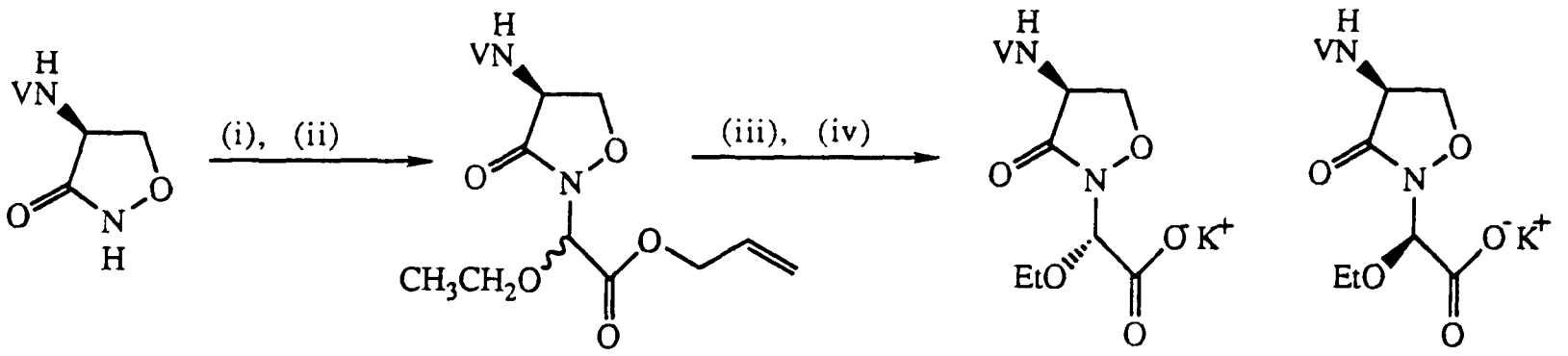


(104)



(105)

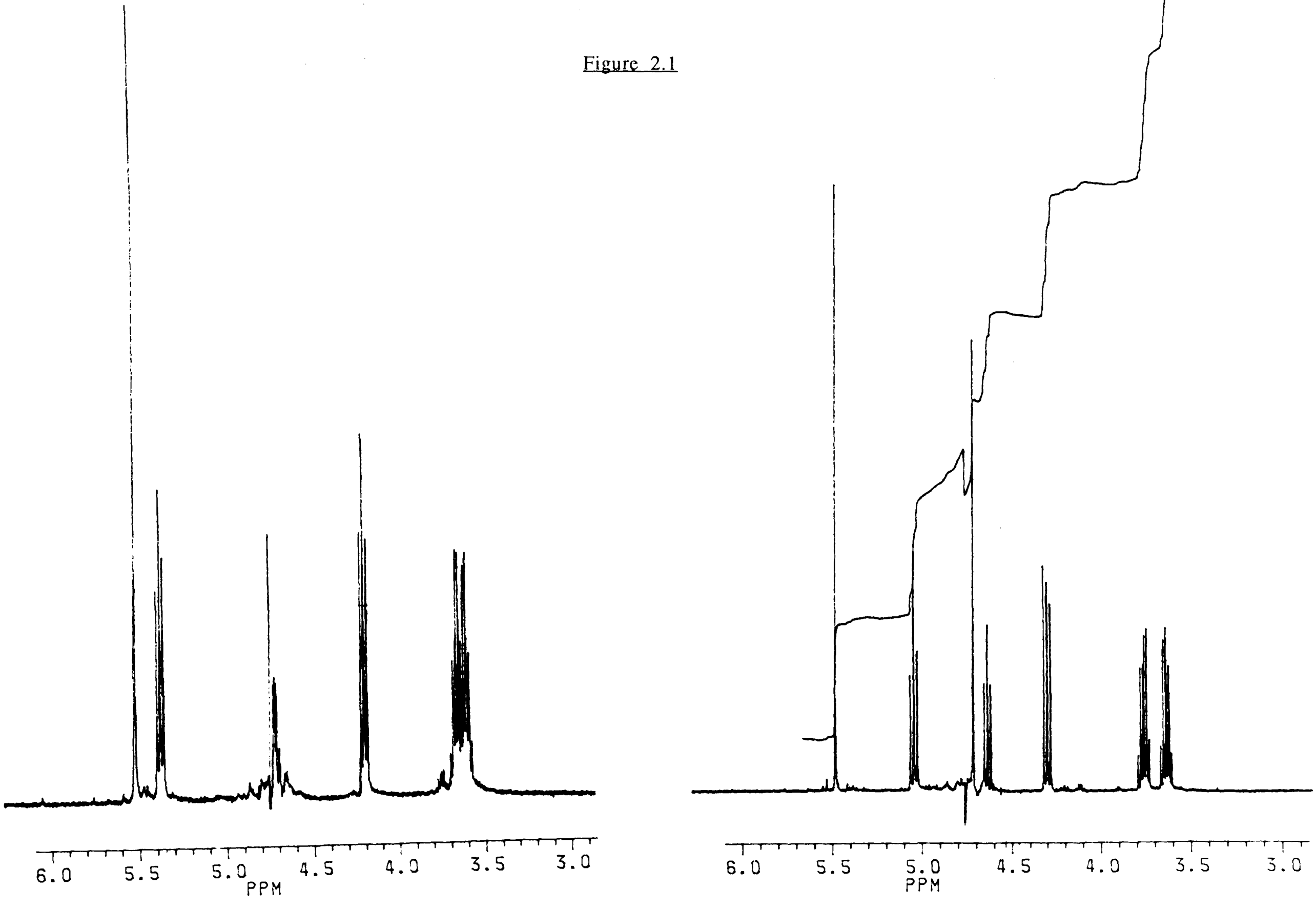
The next step was to repeat the alkylation procedure previously described using phenoxyacetylamino-*L*-cycloserine (81) in order to obtain the desired stereochemistry at C-4. Thus, treatment of (81) with LDA in THF at -78°C and reaction of the intermediate lithium salt with (99) in DMF furnished (106) in 32% yield (Scheme 2.18). Palladium(0) catalysed deprotection of the allyl ester as before furnished crude (107) as a 1:1 mixture of diastereoisomers (107a,b), which could be separated by reverse phase HPLC; the ^1H nmr spectra (500 MHz, D_2O) are shown in Figure 2.1.



(81) (106) (107a) (107b)

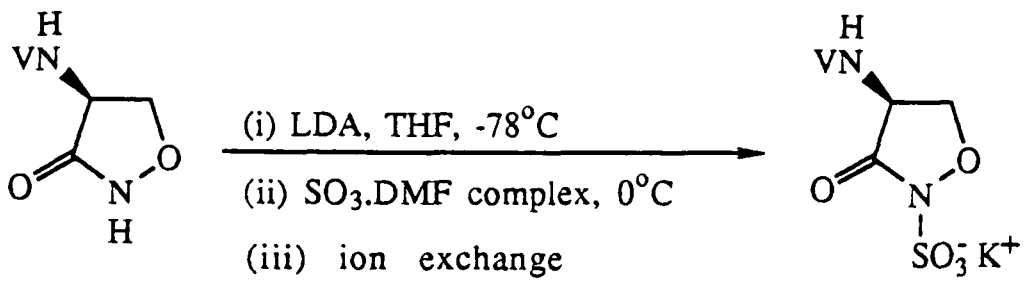
Scheme 2.18. Reagents: (i) LDA, THF, -78°C-6°C, 1.5 h; (ii) (99), DMF, -10°C-18°C, 16 h; (iii) $Pd(PPh_3)_4$, PPh_3, potassium 2-ethylhexanoate (101), CH_2Cl_2-EtOAc, 1 h; (iv) HPLC.

Figure 2.1



Both (107a) and (107b) were tested for antibacterial activity against *E. coli* and *S.aureus*, but were inactive at concentrations of up to 1mg ml^{-1}.

A similar alkylation procedure was then used, in this laboratory, to prepare the *N*-sulphonated *L*-cycloserine derivative (108)[95] as an analogue of the monobactam (27) (Scheme 2.19). However on bioassay, (108) did not display any antibacterial activity against a wide range of organisms.



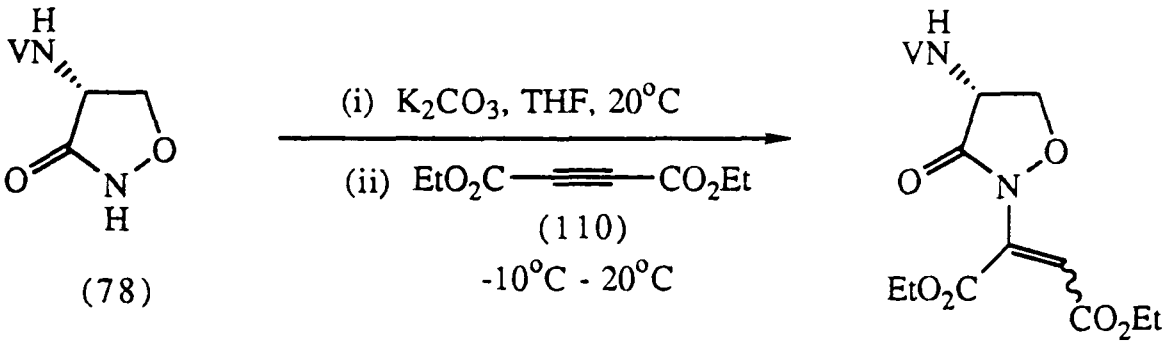
(81) (108)

Scheme 2.19

As mentioned earlier in this chapter, the eventual elucidation of the optimum alkylation conditions occurred after experimentation with several bases under various reaction conditions whilst attempting the synthesis of (109), *via* 1,4-additions of deprotonated cycloserine derivatives to acetylene dicarboxylates, as depicted in Scheme 2.12.

Initially, reflux of phenoxyacetylamino-*D*-cycloserine (78) with diethyl acetylenedicarboxylate (110) in chloroform furnished only starting materials after 3 days. Deprotonation was first attempted using potassium carbonate in THF, followed by quenching with diethyl acetylene-dicarboxylate at -10°C and warming to room temperature. The adduct (109) was obtained in only 9% yield after chromatography (Scheme 2.20). The low optical rotation {$[\alpha]_D$ +11.6° (c=0.85, $CHCl_3$)}, compared to that obtained for the same compound prepared using alternative procedures (see later), suggested that a degree of racemisation had occurred at C-4 of the

cycloserine ring, in accordance with Stammer's observations on the alkylation of *N*-acyl cycloserine derivatives.[90]



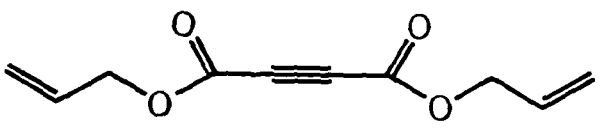
(78) (109)

Scheme 2.20

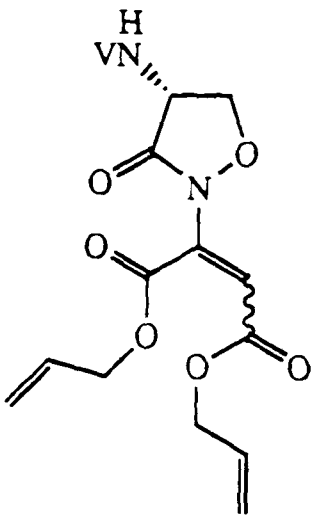
Attempts to optimise the yield of (109) are summarised in Table 2.1.

Thus the deprotonation of (78) with LDA occurred efficiently as the reaction mixture was warmed from -78°C to +5°C, a yellow suspension forming as the anion was generated. It was then necessary to change the solvent to DMF (for solubility reasons) before quenching the lithium salt with (110) at -60°C for *ca* 30 minutes. Longer reaction times or higher temperatures for the latter step resulted in complex reaction mixtures, attributed to polymerisation of the initial adduct.

The requirement for an acetylenic dicarboxylic ester bearing protecting groups removable with no risk of hydrolysis of the γ-lactam was satisfied by diallyl acetylenedicarboxylate (111)[96], synthesised by the action of allyl alcohol on acetylene dicarboxylic acid, catalysed by *p*-toluenesulphonic acid, with hydroquinone present to prevent polymerisation. Reaction of (111) with (78) using the optimised alkylation procedure furnished the diallyl ester (112) in 22% yield.



(111)



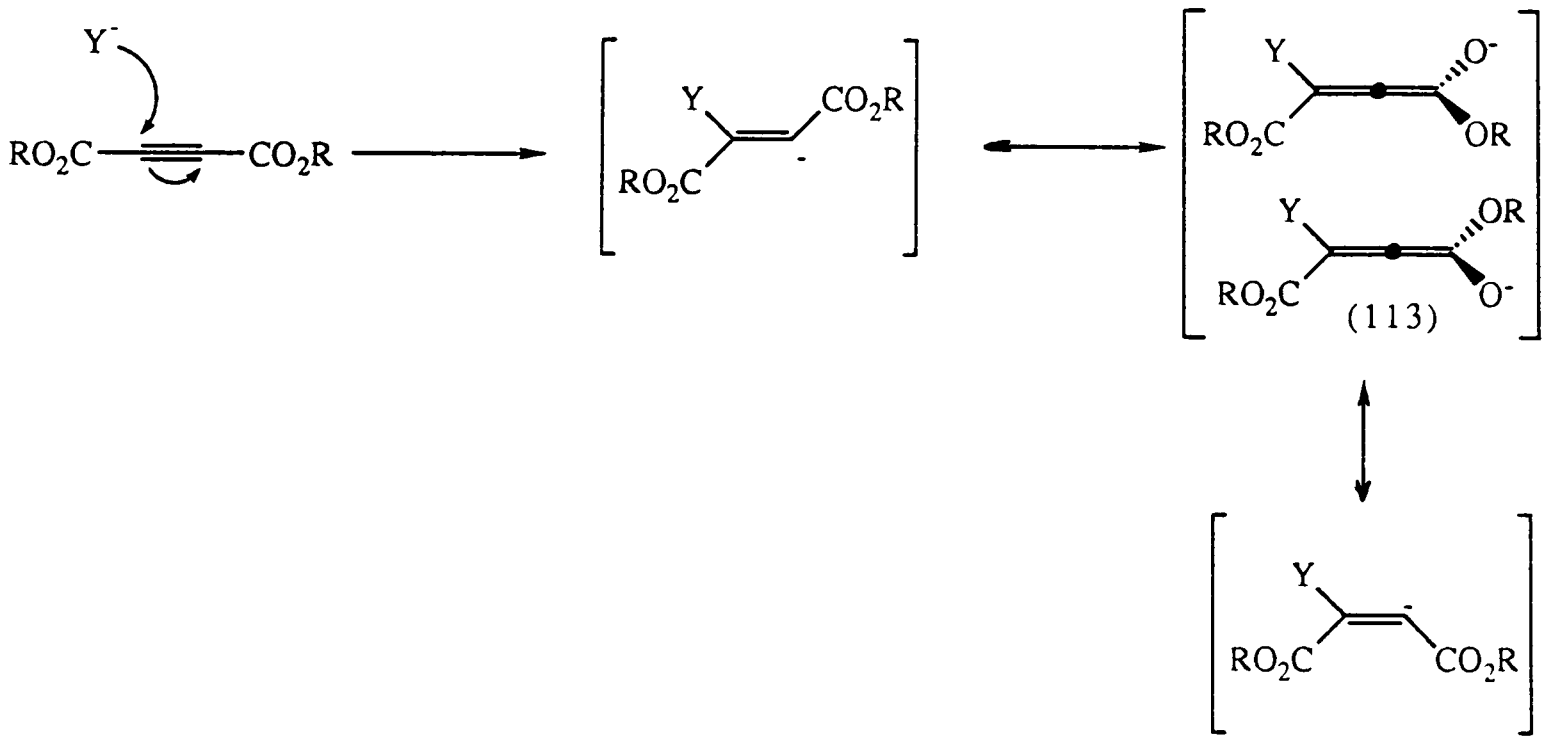
(112)

	CONDITIONS	COMMENT
1.	(i) KO^{t}Bu (1 equiv.)/THF/15°C, (ii) (110) (1 equiv.)/0°C - 15°C. (iii) H_3O^+ work-up.	Starting materials by ^{1}H nmr; complex tlc.
2.	(i) KO^{t}Bu (2 equiv.)/THF (ii), (iii) as 1.	Complex ^{1}H nmr.
3.	(i) K_2CO_3 (0.5 equiv.)/THF, (ii) (110) (2 equiv.)/-10°C - 15°C.	(109) present after 2 h by tlc; absent after 4.5 h.
4.	(i) K_2CO_3 (1 equiv.)/THF, (ii) (110) (2 equiv.)/-10°C - 15°C.	8% yield of (109).
5.	(i) NaH/DMF/0°C, (ii) (110) (2 equiv.)/0°C - 15°C.	Complex tlc. and ^{1}H nmr; no trace of (109).
6.	(i) ^{n}BuLi/THF/-78°C - 0°C, (ii) (110) (2 equiv.)/-70°C - 20°C, (iii) Aqueous NH_4Cl quench.	Trace of (109) observed by tlc and ^{1}H nmr.
7.	(i) ^{n}BuLi (2 equiv.)/THF/-78°C - -50°C, (ii) (110) (2 equiv.)/-70°C - 5°C, (iii) Glacial AcOH/-20°C - 20°C.	Starting materials and unidentifiable products.
8.	(i) LDA/THF/-78°C - 0°C, (ii) (110) (2 equiv.)/-30°C - 15°C, (iii) Aqueous NH_4Cl quench.	Complex mixture of products.
9.	(i) LDA/THF/-78°C - 0°C, (ii) (110) (2 equiv.)/-50°C, (iii) Aqueous NH_4Cl quench.	(78) recovered.
10.	(i) LDA/THF/-78°C - 5°C, (ii) (110) (1.1 equiv.)/DMF/-60°C, (iii) Glacial AcOH/-60°C - 0°C.	30% yield of (109).

Table 2.1

At this stage, the geometry of the trisubstituted double bond could not be specified with any certainty. Unless the protons of the *cis* and *trans* adducts are exactly coincident, it must be assumed that only one isomer was produced by this reaction as the only olefinic proton resonance in the ^1H nmr spectrum of (112) appeared at δ 5.85. Spectroscopic analysis was of no value, since nuclear Overhauser experiments would reveal nothing about the isolated proton and ultra-violet spectroscopy would provide no clues since the absorption maxima of fumaric and maleic acids differ by only 2nm.

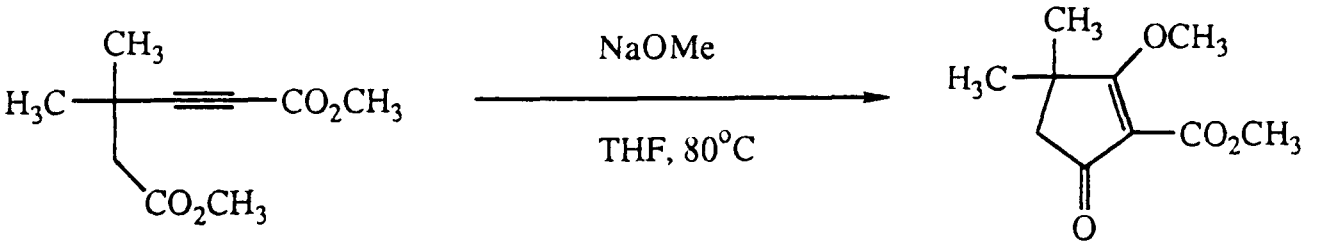
A tentative attempt to predict the geometry was envisaged by considering both experimental evidence and stereoelectronic effects. The principle of microscopic reversibility predicts that nucleophilic addition to simple acetylenes will occur in a specifically *trans* fashion. However, as shown in Scheme 2.21, for the addition to conjugated acetylenic diesters, there is the possibility of isomerisation of the initially formed vinylic anion *via* the structure (113)[97], where Y^- is the incoming nucleophile.



Scheme 2.21

Quenching of (113) by, in this case, the electrophile H^+ would be expected to occur initially on oxygen, followed by collapse to the α,β-unsaturated dicarboxylic ester. If under kinetic control, the more sterically hindered trisubstituted olefin should be favoured, or conversely, thermodynamic control should lead to the less sterically hindered olefin. However, the relative steric requirements of the substituents are not clear cut, and even experimental evidence for similar additions provides no general rule.

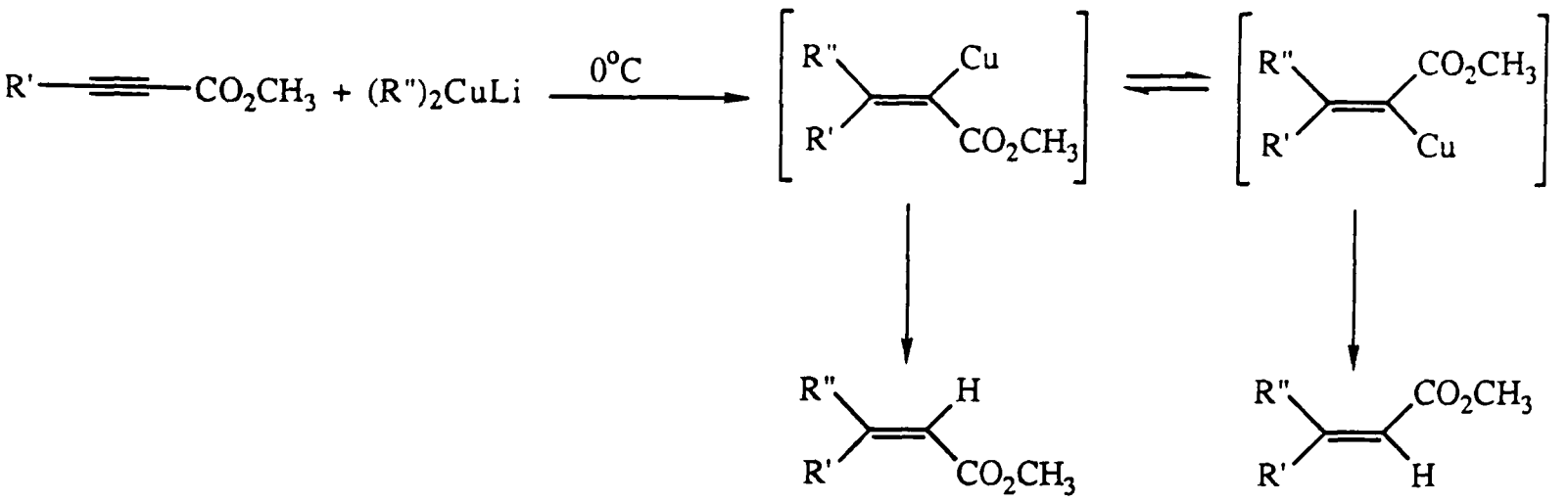
Landesberg and Kellner[98], when investigating the addition of nucleophiles to α,β-unsaturated hexynedioic esters (114), found that sodium methoxide in THF produced the cyclised product (115) exclusively, resulting from internal trapping of the initial vinylic anion from net *trans* addition to the double bond (Scheme 2.22). However, sodium methoxide in methanol, i.e. an external proton source, resulted in a mixture of products emanating from both *cis* and *trans* addition. The former product is due to a steric-approach controlled protonation step of the intermediate allenic enolate rather than a product-development controlled process.



(114) (115)

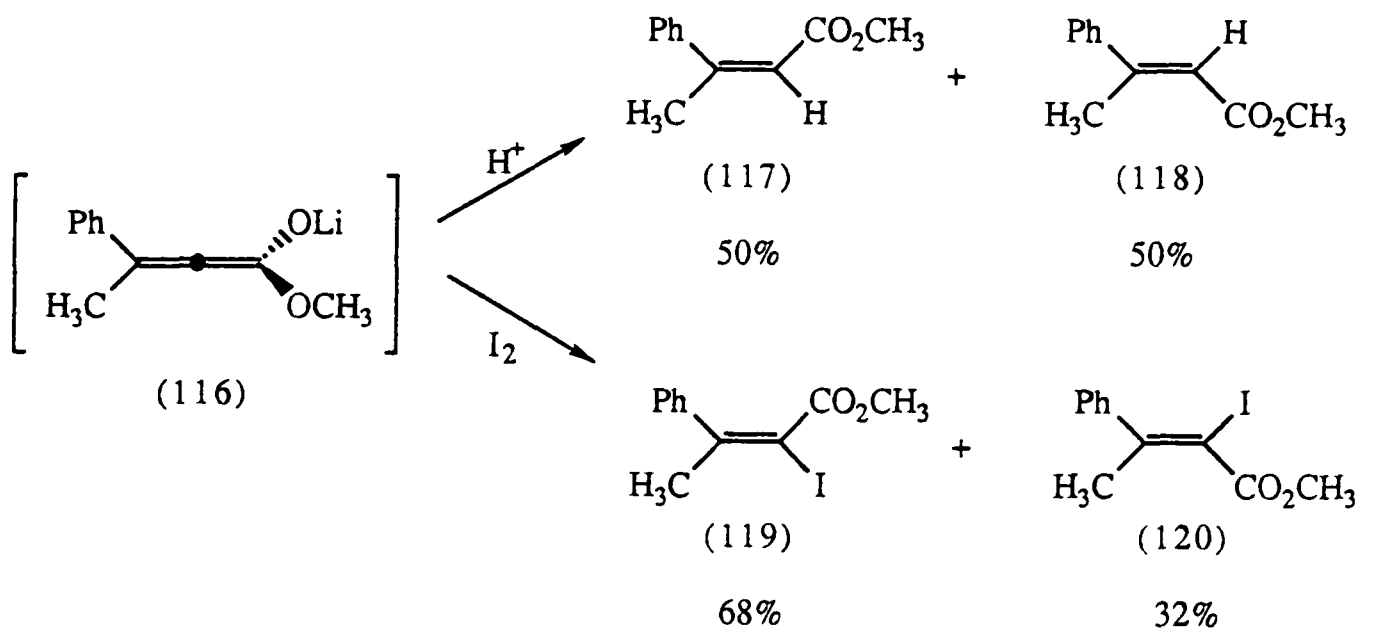
Scheme 2.22

Corey[99] has found that the addition of lithium cuprates to acetylenic diesters in THF produces mixtures of *cis* and *trans* adducts at 0°C, yet exclusively *cis* adducts at -78°C. This has been attributed to the equilibration of the intermediate 'enolates' at the higher temperature (Scheme 2.23). Not too much importance should be attached to this however, since cuprate additions often display radical-type mechanisms.



Scheme 2.23

Klein[100] has shown that an anion (116) can be generated by treatment of methyl 3-phenylprop-2-ynoate with dimethylcopperlithium and methyllithium in sequence. The anion showed allenic character (ν_{max} 1900cm^{-1}) and reacted with a proton (small electrophile) to yield a 50:50 mixture of the *E*-(118) and *Z*-(117) α,β-unsaturated esters. However, treatment with iodine (bulky electrophile) gave primarily the *E*-product (119) (Scheme 2.24). The product is thus determined in this case by the approach of the quenching agent.

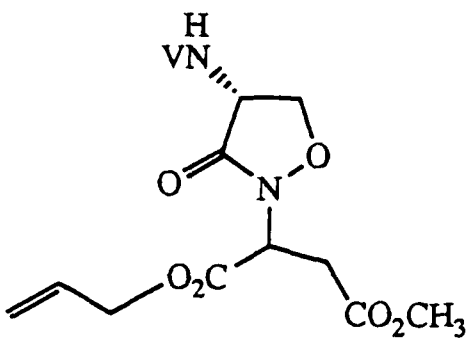


Scheme 2.24

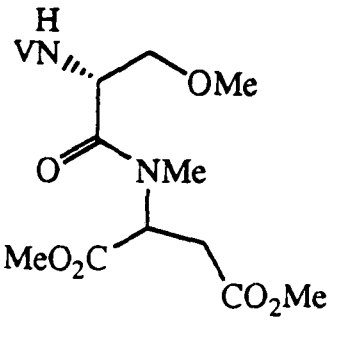
Returning to the case of (113), there also exists the possibility that the amide N-H of the sidechain may be acting as an internal proton source, causing instantaneous quenching of the initially formed vinylic anion.

Bearing all of these variables in mind, it seems likely that (113) might possess a *trans*-diester configuration. Attempts to crystallise the adduct in a form suitable for X-ray crystallography have so far proved unsuccessful; thus, the uncertain geometry is represented on the diagrams herein.

Standard palladium(0) catalysed deprotection conditions were then applied to (112). However, after work-up no olefinic proton could be observed by ^{1}H nmr (500 MHz), and re-esterification with methyl iodide in DMF only produced trace amounts of products tentatively identified as (121) and (122) by ^{1}H nmr analysis and mass spectrometry.



(121)



(122)

An alternative palladium(0) catalysed deprotection procedure[101] using highly nucleophilic amines such as pyrrolidine to displace the activated allyl ester was also employed. A complex mixture of products resulted, ^{1}H nmr analysis (500 MHz) indicating one allyl ester to be still intact. However, extended reaction times and higher temperatures tended to destroy the olefin.

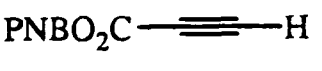
Attempts to deprotect the diethyl ester (109) using lithium hydroxide[102] as a mild base appeared both to hydrolyse the lactam and to cause the disappearance of the olefinic proton resonance in the ^{1}H nmr spectrum.

It is interesting to note that the N-O bond in *N*-2 substituted cycloserine derivatives remains unaffected by hydrogenation conditions.[83]

Consequently, the 4-nitrobenzyl ester was selected as a potential protecting group. The only documented procedures[96] for esterifying acetylene dicarboxylic acid involve treatment with a catalytic amount of *p*-toluenesulphonic acid in a solution of the alcohol; however, treatment of acetylene dicarboxylic acid with a concentrated solution of 4-nitrobenzyl alcohol in chloroform in the presence of *p*-toluenesulphonic acid failed to produce the diester (123). Also, treatment of acetylene dicarboxylic acid with 2 equivalents of sodium hydrogen carbonate and subsequent esterification with 4-nitrobenzyl bromide in the presence of dicyclohexano-18-crown-6[103] only produced high yields of 4-nitrobenzyl propiolate (124), presumably due to the facile decarboxylation of the dicarboxylate salt.



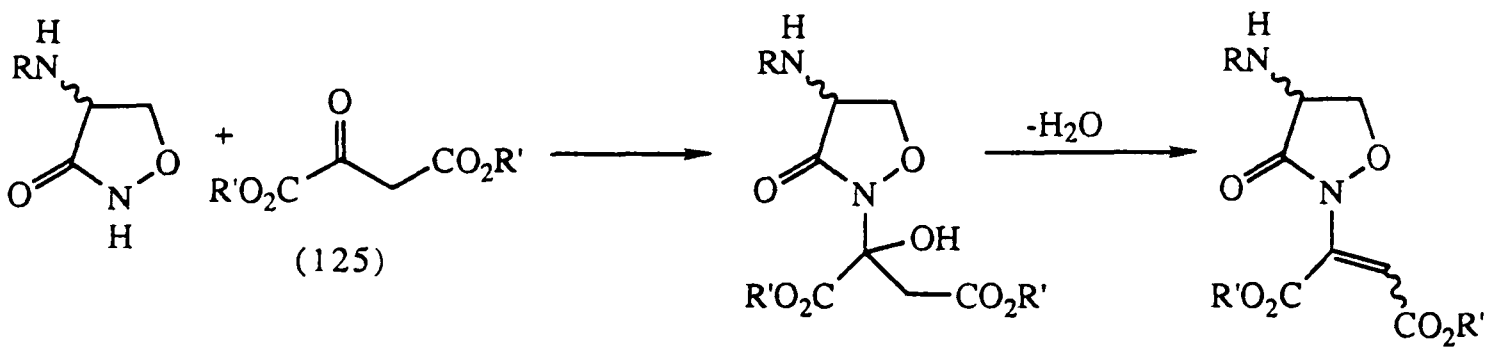
(123)



(124)

Preparation of the di-trimethylsilylethyl ester[104], easily removed by fluoride ion, was attempted by treatment of 2-(trimethylsilyl)ethanol, 1,3-dicyclohexylcarbodiimide and 4-dimethylaminopyridine with acetylene dicarboxylic acid at -15°C. This procedure gave rise to many products as a result of polymerisation.

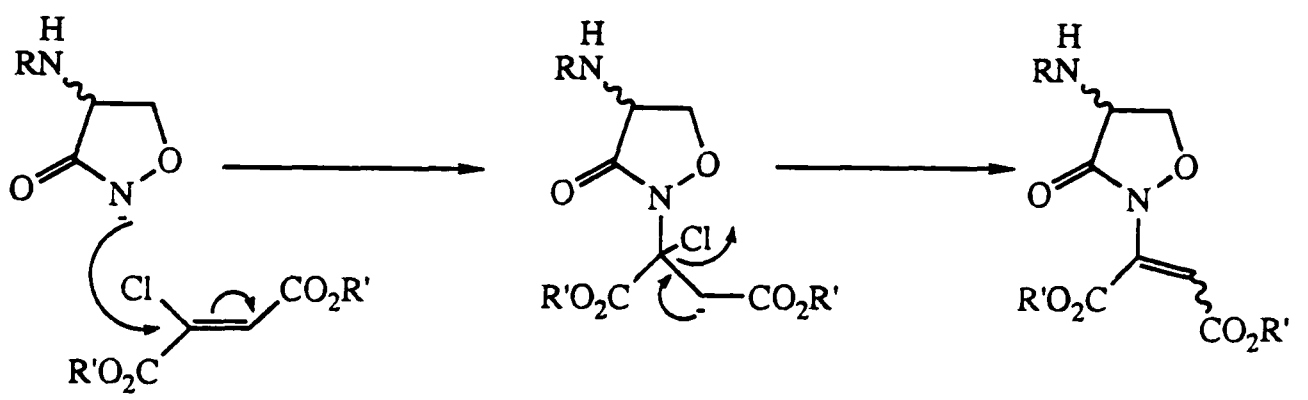
An alternative means of enamine synthesis was envisaged in the condensation of cycloserine derivatives with α-keto esters as shown in Scheme 2.25.



(125)

Scheme 2.25

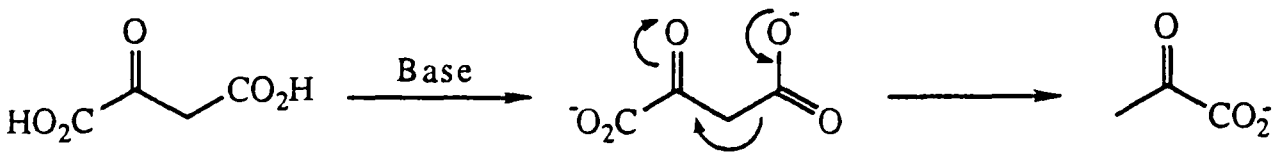
The general method employed for enamine synthesis usually involves condensation of an *amine* (primary or secondary) with a carbonyl compound with water separation by azeotropic distillation with benzene or toluene.[105] However, as cycloserine is essentially an amide, it may be necessary to deprotonate the endocyclic N-H to enable condensation to occur, followed by dehydration of the initial adduct. Alternatively, by treatment of diprotected oxalacetic acid (125) with phosphorus pentachloride[106], the reactive vinyl halide (126) could be prepared from which the 'enamine' could be formed *via* a vinylic substitution mechanism (Scheme 2.26).



(126)

Scheme 2.26

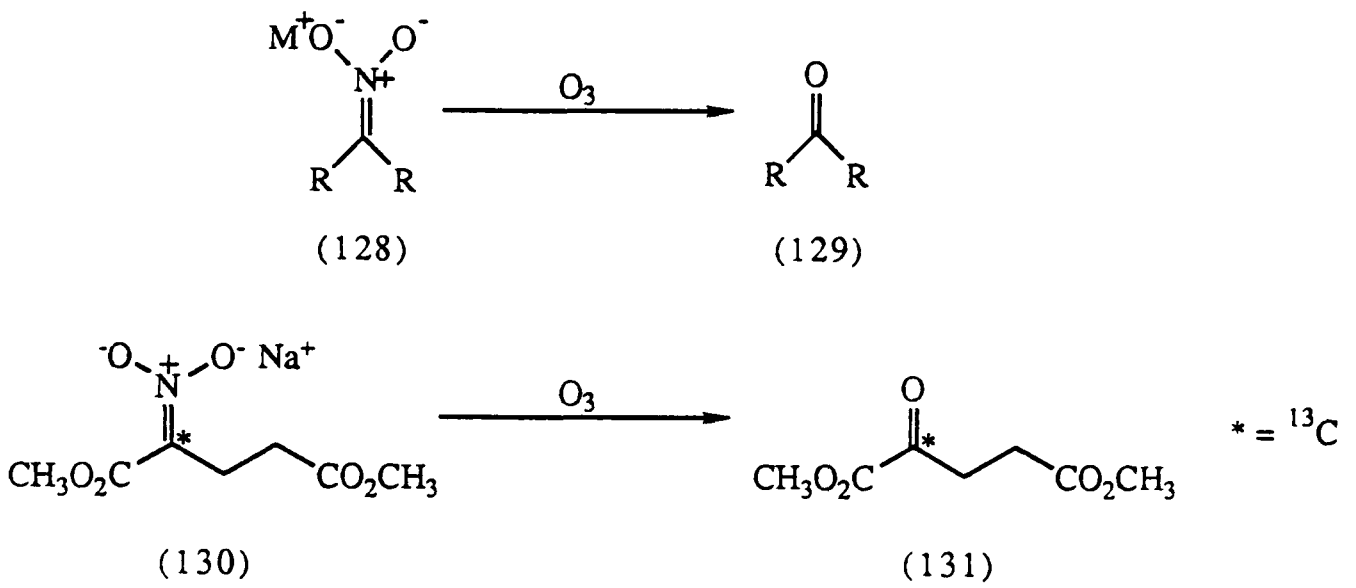
However, esterification of oxalacetic acid (127) to yield the diester (125, R' = PNB) could not be achieved under basic conditions due to the spontaneous decarboxylation of the intermediate carboxylate salt that ensued (Scheme 2.27). Attempts to couple (127) and 4-nitrobenzyl alcohol, mediated by 1,3-dicyclohexylcarbodiimide both with and without 4-dimethylaminopyridine, yielded unidentifiable products.



(127)

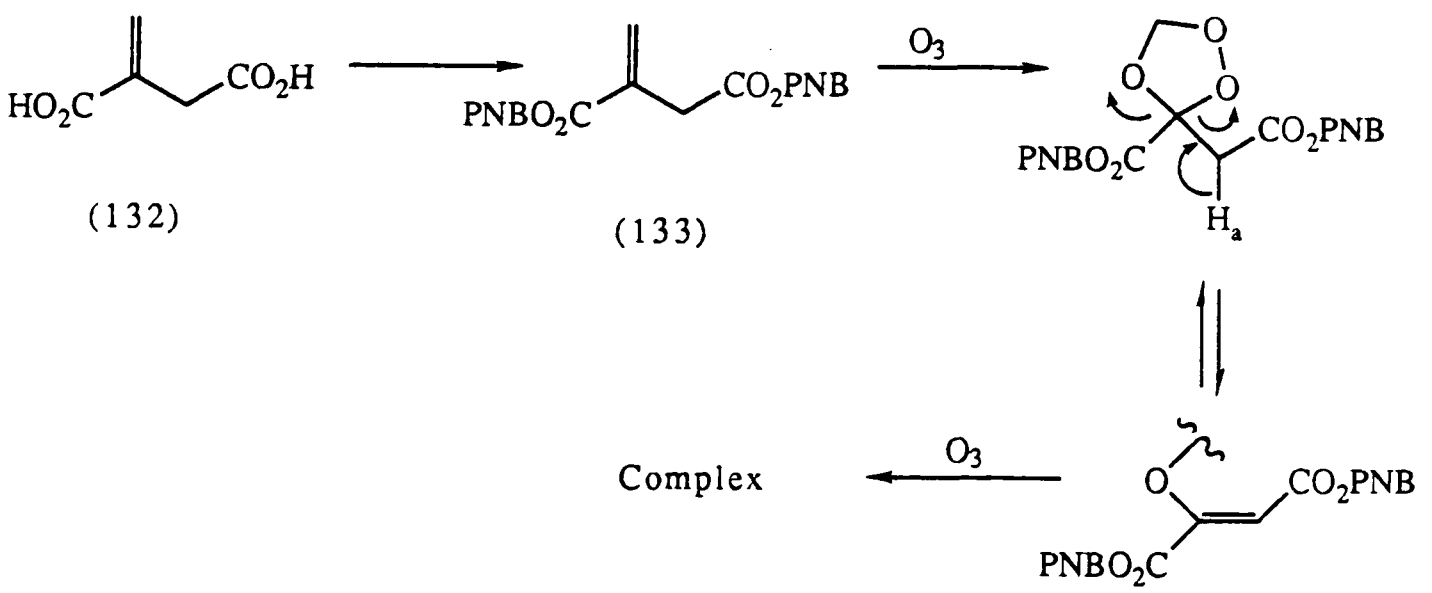
Scheme 2.27

It is known that the ozonolyses of nitronate salts (128) furnish the corresponding ketones (129) in good yield.[107] Indeed, the α-keto ester (131)[108] has been successfully prepared by the ozonolysis of the sodium nitronate salt (130) (Scheme 2.28). Thus the di-(4-nitrobenzyl) ester (133)



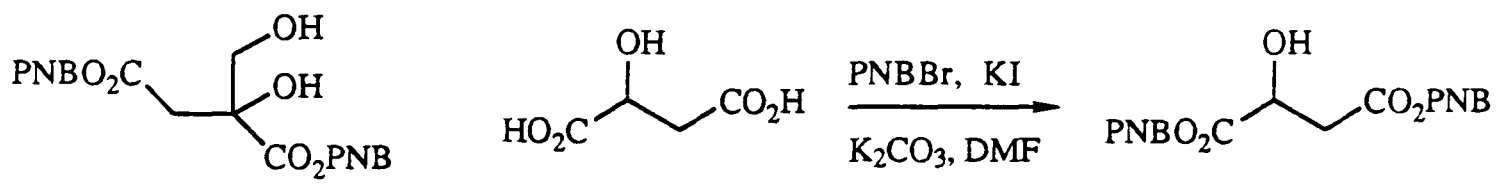
Scheme 2.28

was prepared trivially by treatment of 2-methylenebutan-1,4-dioic acid (itaconic acid) (132) with potassium carbonate and 4-nitrobenzyl bromide in DMF. However, ozonolysis[109] of (132) at -78°C followed by quenching of the ozonide with dimethylsulphide revealed the presence of many highly polar materials, none corresponding to the desired product, possibly as a result of the relatively high acidity of the 'enolic' proton (H_a) leading to over-reaction (Scheme 2.29).



Scheme 2.29

Oxidation of (133) with a catalytic amount of osmium tetroxide[110] in aqueous THF, using 1.5 equivalents of sodium metaperiodate produced the diol (134) in good yield. However further oxidation of the latter with sodium metaperiodate failed to furnish the desired α-keto ester; ^{1}H nmr indicated the presence of several products which t.l.c. revealed to be polar, again possibly due to the oxidation of (125) on its formation.



(134) (135) (136)

Scheme 2.30

Various methods exist for the mild oxidation of alcohols to ketones. Hence the diester (136) was prepared simply by esterification of malic acid (2-hydroxybutan-1,4-dioic acid) with potassium carbonate and 4-nitrobenzyl bromide in DMF (Scheme 2.30). The attempts made to oxidise (136) proved unsuccessful and are summarised below.

(i) The Swern[111] oxidation by dimethylsulphoxide 'activated' with oxalyl chloride at -50°C in dichloromethane produced an unidentifiable product mixture; no starting material could be isolated.

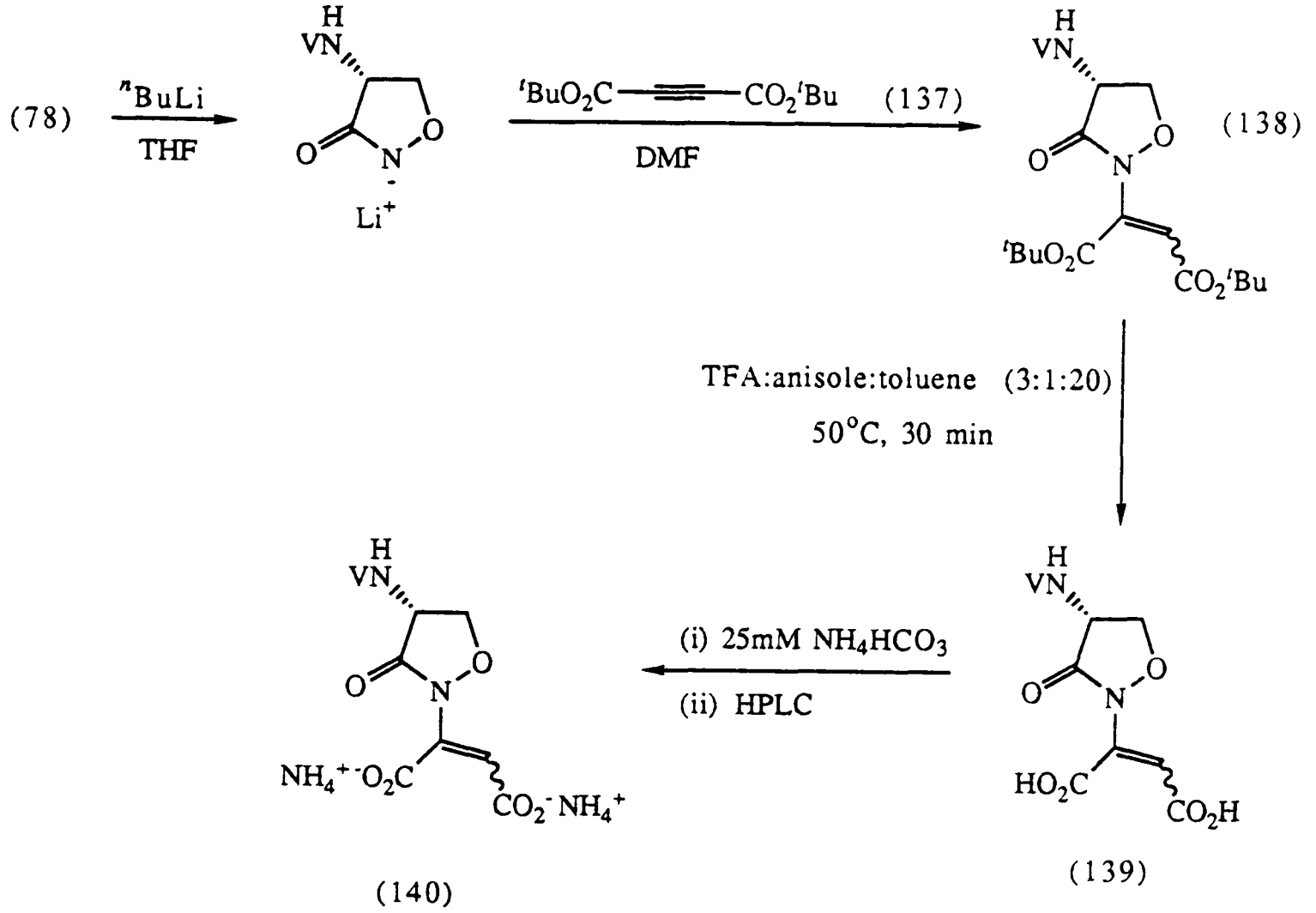
(ii) The Moffatt[112] oxidation, employing a mixture of dimethyl-sulphoxide, 1,3-dicyclohexylcarbodiimide and trifluoroacetic acid also failed to produce the desired ketone; only highly polar products could be observed on t.l.c..

(iii) Silver carbonate supported on celite is a highly selective and relatively mild oxidant previously used for the oxidation of primary and secondary alcohols to aldehydes and ketones.[113] The reagent was prepared by adding purified celite to an aqueous solution of silver nitrate, subsequent addition of aqueous sodium carbonate decahydrate, and filtering

off the resulting yellow/green precipitate. However, no oxidation of (136) by this reagent was observed by ^1H nmr or t.l.c. even after reflux in dichloromethane for 2 days.

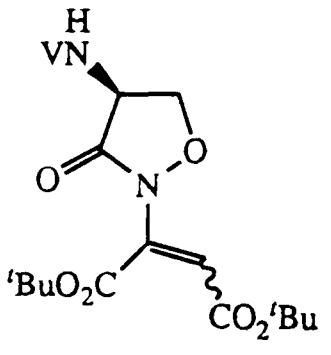
(iv) The recently developed tetra-*n*-propylammonium perruthenate (ruthenium VII)[114] functions as a mild catalytic oxidant, in conjunction with *N*-methylmorpholine *N*-oxide, for the high yield conversion of alcohols to aldehydes and ketones. However, in our hands, its reaction with (136) proved to be very slow, only producing several minor products.

These latter approaches were then abandoned in favour of the 1,4 nucleophilic addition of the cycloserine anion with di-*t*-butyl acetylenedicarboxylate (137), previously avoided because of the sensitivity of the cycloserine nucleus to the acidic conditions required for the removal of *t*-butyl esters. The adduct (138) was formed in 30% yield by treatment of phenoxyacetylamino-*D*-cycloserine (78) with LDA as before, isolation of the lithium salt, and reaction with (137) at -50°C in DMF for 3 hours, before quenching with glacial acetic acid (Scheme 2.31). The longer reaction time was found to be necessary, presumably due to both the nucleophilic attack at the acetylenic carbon atom being hindered by the bulky esters and the steric crowding in the resulting trisubstituted olefin. Deprotection of the carboxylic esters could be achieved by prolonged stirring of (138) at room temperature in a solution of trifluoroacetic acid, anisole and toluene in a ratio of 3:1:20 respectively. However, the reaction proceeded more cleanly if the solution was heated at 50°C for 30 minutes. As the diacid (139) proved to be only sparingly water soluble, the crude product was dissolved in 25m*M* ammonium hydrogen carbonate buffer for purification by HPLC. On bioassay plates, the diammonium salt showed no activity against either *S. aureus* or *E. coli* X580, as expected.

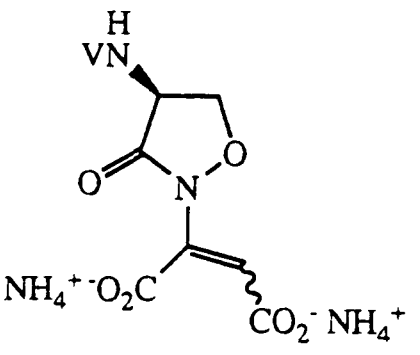


Scheme 2.31

The procedure outlined in Scheme 2.31 was repeated with the corresponding *L*-isomer of (78), to give the di-*t*-butyl ester (141) {$[\alpha]_D$ -39.4° (c=1.0, CH_3OH)} in comparable yield; the ^1H nmr spectrum (500 MHz, $CDCl_3$) is shown in Figure 2.2. Deprotection in a similar manner yielded the diammonium salt (142) after purification by HPLC. To speed up the passage of (142) through the reverse phase column, 10m*M* aqueous ammonium hydrogen carbonate buffer was employed, containing 1% v/v acetonitrile to sharpen the peaks on the HPLC trace.



(141)



(142)

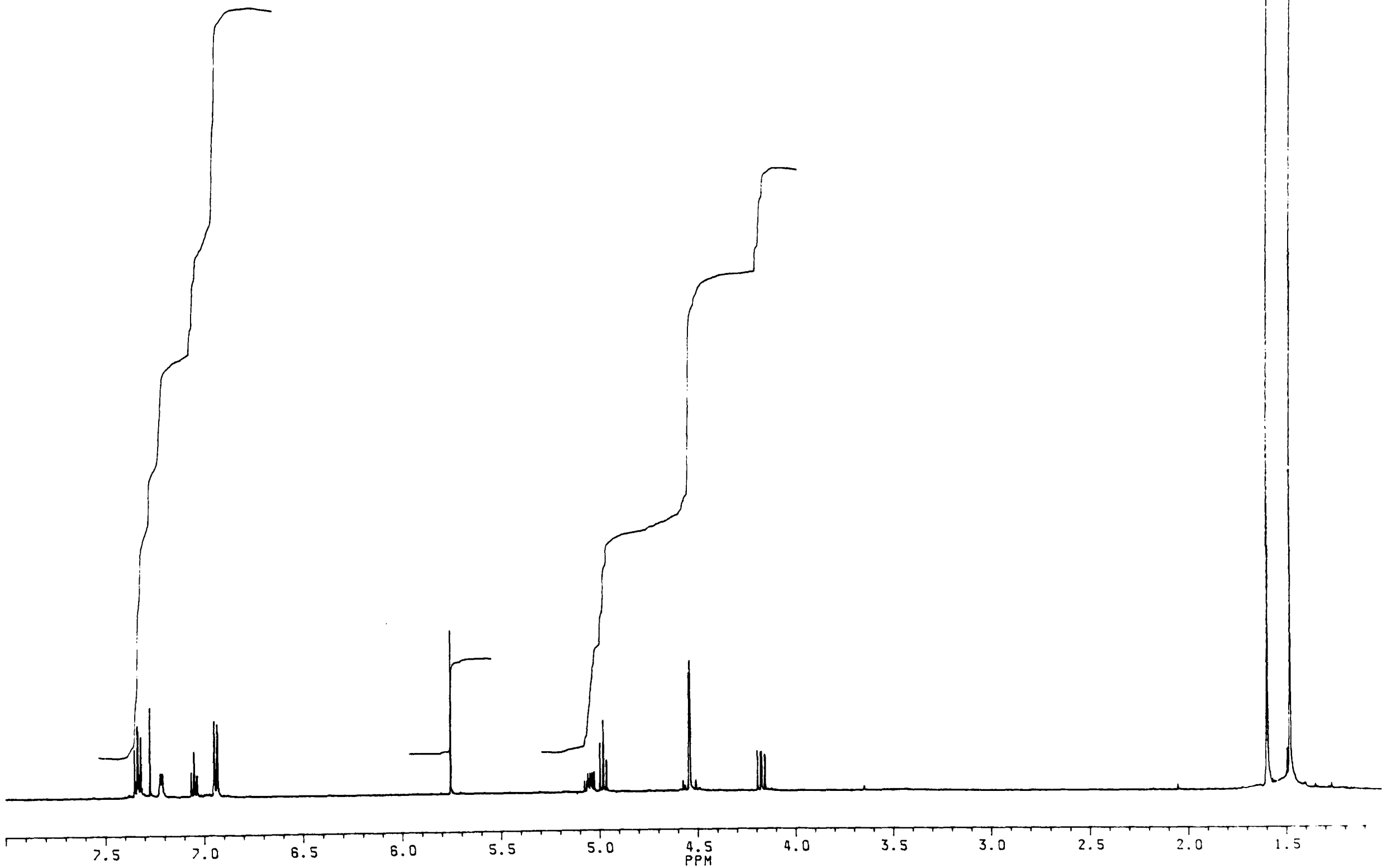


Figure 2.2

On bioassay plates, (142) exhibited good activity against both *S. aureus* and *E. coli* X580 at a concentration of *ca* 1mg ml^{-1}. [The unfunctionalised cycloserine derivatives (78) and (81) were also tested on bioassay plates in 10m*M* ammonium hydrogen carbonate buffer, but no antibacterial activity was apparent]. On *E. coli* bioassay plates containing a penicillinase ('Penase concentrate', ex. Difco Ltd.), an identical inhibition zone of 20mm was formed, indicative of its resistance to basic penicillinase enzymes. However, approximately 1mg of a cephalosporinase enzyme (Penicillinase Type IV from *Enterobacter cloacae* was introduced into a ^1H nmr sample of (142) in D_2O, and left to stand at room temperature for 3 days. Over this period, the intensity of all protons visible in the ^1H nmr spectrum (500 MHz) diminished considerably, suggesting that (142) is sensitive to cephalosporinases, as is LTV (65) itself. A control experiment revealed that (142) alone remains unchanged in water at room temperature for 4 days. More extensive quantitative biological evaluation of (142) is currently being undertaken by Eli Lilly & Co., Indianapolis, U.S.A..

3. EXPERIMENTAL

General Experimental

Melting points (m.p.) were determined on a Büchi 510 capillary apparatus and are uncorrected.

Infra-red spectra were recorded as nujol mulls, thin films, KBr discs or in chloroform solution on Perkin-Elmer 681 and Perkin-Elmer 1750 Fourier Transform spectrometers. Absorption maxima were recorded in wavenumbers. The following abbreviations were used: vs, very strong; s, strong; m, medium; w, weak and b, broad.

Low resolution mass spectra (m/e) in the chemical ionisation (CI), desorption chemical ionisation (DCI) or alternating chemical ionisation and electron impact (ACE) modes were recorded on VG Micromass 30F, VG 20-250 or ZAB 1F spectrometers. Samples requiring electron impact (EI) were run on a VG 20-250 spectrometer. Fast atom bombardment (FAB) and field ion (FI) mass spectra were performed on a ZAB 1F spectrometer. Gas chromatography mass spectra (GCMS) were recorded on a VG Micromass Trio 1 spectrometer. The m/e values are quoted with the relative abundance (base ion = 100%) and assignment in parentheses. Only molecular ions (M^+), fragments of molecular ions and major peaks were reported.

Proton magnetic resonance spectra (^1H nmr) were recorded at 200MHz, 250MHz, 300MHz and 500MHz on Varian Gemini, Bruker AM250, Bruker WH300 and Bruker AM500 spectrometers respectively. For ^1H nmr spectra recorded in $CDCl_3$, DMSO-d_6 and acetone-d_6, chemical shifts (δH) were reported in parts per million (ppm) downfield of tetramethylsilane at

0.00ppm. For ^{1}H nmr spectra recorded in D_2O, chemical shifts were reported in ppm downfield of sodium (3-trimethylsilyl)propionate-2,2,3,3-d_4 (TSP) at 0.00ppm. The following abbreviations were used: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet and b, broad. Coupling constants were recorded in Hertz to the nearest 0.5Hz.

Carbon magnetic resonance spectra (^{13}C nmr) were recorded at 50.31MHz, 62.38MHz and 125.77MHz on Varian Gemini, Bruker AM250 and Bruker AM500 spectrometers respectively. Chemical shifts (δC) are quoted in ppm and are referenced to $CDCl_3$ (δ_{ref} = 77.0ppm) unless otherwise stated. Spectra recorded in D_2O are referenced to 1,4-dioxan (δ_{ref} = 67.6).

Thin layer chromatography (tlc) was performed using Merck aluminium foil backed plates, pre-coated with Kieselgel 60 GF254 (No. 5554). Plates were developed with either 5% w/v *dodeca*-phosphomolybdic acid in ethanol or 10% w/v ammonium molybdate in 2*N* sulphuric acid. Preparative layer chromatography (PLC) was performed using 200 x 200 x 1mm layers of Merck Kieselgel 60 GF254 (No. 7730). Flash column chromatography was performed with Merck Kieselgel 60 GF254, 230-400 mesh.

Optical rotations, determined on a Perkin-Elmer 241 polarimeter at 20°C, and microanalyses were performed by the Dyson Perrins Laboratory staff.

All starting materials were purified and dried unless otherwise stated. Solvents were distilled prior to use. THF was distilled from sodium in the presence of benzophenone under nitrogen.

Reaction times were recorded in minutes (min) or hours (h). Reactions were studied by tlc or ^1H nmr prior to work-up. Solvents were evaporated at 30°C or below on a Büchi R110 Rotavapor; high-boiling solvents were evaporated on a Büchi R110 Rotavapor fitted with a dry-ice condenser at <2mmHg.

Reverse phase high performance liquid chromatography (HPLC) was performed using two Gilson 303 pumps, a rheodyne 7125 injector, a Gilson HM holochrome detector set at 254nm and a column packed with Zorbax ODS (250mm x 9.4mm internal diameter).

Bioassays were carried out using the 'holed-plate' method[115] with the following organisms:

Staphylococcus aureus NCTC 6571,

Escherichia coli ESS and X580.

The samples for bioassay were applied in aqueous solution (0.10ml) on to agar plates containing the desired organism and incubated at 37°C for 15 h.

D-4-Acetylamino-3-isoxazolidinone (72)

D-Cycloserine (2.03g, 19.9mmol) was suspended in methanol (400ml). Acetic anhydride (2.0ml, 27.2mmol) was added dropwise to the reaction mixture at room temperature over a period of *ca* 20 min. Stirring was continued at 20°C for 2.5 h. The methanol was removed *in vacuo* and the residue recrystallised from hot methanol to yield (72) (2.20g, 77%) as white crystals; m.p. 176-177°C, (Lit.[87], 175-177°C); $[\alpha]_D$ +74.5° (c = 0.86, H_2O), (Lit.[87], +73°); ν_{max} (nujol) 3302 m, 1705 s, 1640 s, 1563 s; δH (300MHz, DMSO-d_6) 1.86 (3H, s, CH_3CO-), 3.88 (1H, dd, *J* 9.5, 8.5 Hz, 5-H), 4.49 (1H, t, *J* 8.5 Hz, 5-H), 4.68-4.77 (1H, m, 4-H), 8.43 (1H, d, *J* 7.5 Hz, CH_3CONH-); δC (62.5 MHz, DMSO-d_6) 22.5 (q, -CH_3), 51.3 (d, 4-C), 72.7 (t, 5-C), 169.8 (s, CO), 170.7 (s, CO); m/e (EI) 144 (M^+, 27%), 84 (40), 43 (100); [Found C, 41.48%; H, 5.77%; N, 19.74%. $C_5H_8N_2O_3$ requires C, 41.67%; H, 5.59%; N, 19.43%].

D-4-Phenoxyacetylamino-3-isoxazolidinone (78)

To a solution of *D*-cycloserine (2.97g, 29.0mmol) in aqueous sodium hydroxide solution (1.25*M*, 60ml) cooled to *ca* 3°C in an ice-water bath was added phenoxyacetyl chloride (6.48 g, 38.0 mmol) dropwise over *ca* 15 min and the mixture allowed to warm to room temperature with stirring. The reaction mixture was stirred for a further 2h at 20°C and then washed with diethyl ether (50ml). Aqueous hydrochloric acid (1*N*, 45ml) was added and the resulting white precipitate was filtered off, washed with water and dried in a vacuum desiccator. The crude product was recrystallised from ethyl acetate to yield (78) (3.30g, 48%) as a white crystalline solid; m.p. 121-122°C; $[\alpha]_D$ +32.5° (c = 0.9, CH_3OH); ν_{max} ($CHCl_3$) 3408 m, 1715 s, 1685 s, 1601 m, 1525 s, 1496 s, 1442 m, 1240 s; δH (300 MHz, DMSO-d_6) 4.05 (1H, dd, *J* 10.5, 8.5 Hz, 5-H), 4.52 (1H, t, *J* 8.5 Hz, 5-H), 4.54 (2H, s, $PhOCH_2$-), 4.88 (1H, m, 4-H), 6.95-7.00, 7.27-7.33 (5H, 2 x m, Ar), 8.66 (1H, d, *J* 8 Hz, $PhOCH_2CONH$-), 11.47 (1H, bs, -O-NH-CO); δC (62.5 MHz, DMSO-d_6) 50.9 (d, 4-C), 66.9 (t, $PhOCH_2$-),

71.9 (t, 5-C), 114.9, 121.1, 129.5 (3 x d, Ar), 157.8 [s, Ar(*ipso*-C)], 168.3, 170.3 (2 x s, 2 x CO); m/e (NH_3 CI) 254 (MNH_4^+, 25%), 237 (MH^+, 100), 194 (40), 152 (10).

[4*R*]-2*R*,*S*-(4-Phenoxyacetylamino-3-oxo-2-isoxazolidinyl)-5-oxo-tetrahydrofuran-2-carboxylic acid, methyl ester (80)

A finely powdered mixture of *D*-4-phenoxyacetylamino-3-isoxazolidinone (49mg, 0.21mmol) and 2-oxoglutaric acid (39mg, 0.27mmol) was heated at 80°C for 4 h under vacuum (0.3mmHg). The initial clear melt eventually solidified and this residue (85mg) was dissolved in dry, distilled DMF (1.0ml). Sodium hydrogen carbonate (20mg, 0.24mmol) was then added and the resulting mixture stirred at 20°C for 1 h. Methyl iodide (237µl, 3.81mmol) was added and stirring was continued for a further 24 h at 20°C. The reaction mixture was poured into water (10ml) and extracted with ethyl acetate (3 x 10ml). The organic layer was washed with water (2 x 10ml), dried (sodium sulphate), filtered and evaporated *in vacuo* to yield an oil. The crude product was purified by PLC (ethyl acetate) R_f 0.7 to yield (80) (25mg, 31%) as an oil; ν_{max} ($CDCl_3$) 3405 bm, 1809 s, 1757 s, 1710 s, 1690 s, 1601 m, 1521 m, 1496 s, 1442 m; δH (300 MHz, $CDCl_3$) 2.49, 2.63, 2.85, 3.22 (4H, 4 x m, lactone 3-H, 4-H), 3.92, 3.93 (3H, 2 x s, $-CO_2CH_3$), 4.14, 4.88, 5.08 (3H, 3 x m, cycloserine 4-H, 5-H), 4.56 (2H, s, $-C\underline{H}_2CONH-$), 6.91-7.35 (5H, m, Ar); m/e (CI) 378 (M^+, 2%), 268 (35), 145 (38), 115 (95), 90 (100), 88 (78).

L-4-Phenoxyacetylamino-3-isoxazolidinone (81)

Prepared in a manner analogous to (78) using *L*-cycloserine (296mg, 2.90mmol), aqueous sodium hydroxide solution (1.25*M*, 6.0ml) and phenoxyacetyl chloride (679mg, 3.98mmol). The crude product was recrystallised from ethyl acetate to yield (81) (285mg, 42%); m.p. 120-121°C; $[\alpha]_D$ -30.3° (c = 1.01, CH_3OH); ^1H nmr, ν_{max}, m/e identical to data for (78).

[4*S*]-2*R*,*S*-(4-Phenoxyacetylamino-3-oxo-2-isoxazolidinyl)-5-oxo-tetrahydrofuran-2-carboxylic acid, sodium salt (82)

A finely powdered mixture of *L*-4-Phenoxyacetylamino-3-isoxazolidinone (235mg, 1.0mmol) and 2-oxoglutaric acid (190mg, 1.3mmol) was heated at 80°C for 5 h under vacuum (0.3mmHg) to yield crude (82). Sodium hydrogen carbonate (200mg) was added to a solution of the residue in water (1.5ml) to pH 7. A sample of this solution after filtration was purified by HPLC [Zorbax ODS, water : acetonitrile (9:1), flow rate 4ml min^{-1}, retention time 6 min] to yield (82) as a white powder; δH (300 MHz, D_2O) 2.27-2.40, 2.49-2.63, 2.67-2.80, 2.89-3.01 (4H, 4 x m, lactone 3-H, 4-H), 3.98-4.18, 4.39-4.55, 4.90-5.11 (3H, 3 x m, cycloserine 4-H, 5-H), 6.80-6.94, 7.14-7.23 (5H, 2 x m, Ar). The sample was tested on bioassay plates as described in Chapter 2.

Attempted 2-methylation of *D*-4-acetamido-3-isoxazolidinone (72)

A solution of (72) (144mg, 1.00mmol) in dry, distilled DMSO (1.0ml) was added dropwise over 15 min to a stirred solution of freshly sublimed potassium *t*-butoxide (131mg, 1.15mmol) in DMSO (2.0ml). The solution was stirred for 0.5 h under nitrogen at 20°C, then quenched with excess methyl iodide (1.0ml). The reaction mixture was then stirred at 20°C under nitrogen for 20 h, and quenched by pouring into distilled water (30ml). The mixture was extracted with ethyl acetate (2 x 40ml) and washed with water (25ml). The organic layer was dried (sodium sulphate), filtered and evaporated *in vacuo* to yield a crude product (13mg). ^1H nmr analysis did not indicate the desired product, however mass spectrometry (EI) indicated possible dimethylation [172 (M^+ + 14), 158 (M^+)]. Attempted purification by PLC revealed no identifiable products.

D-4-Benzyloxycarbonylamino-3-isoxazolidinone (86)

Prepared in an analogous manner to (78) using *D*-cycloserine (4.0g, 39mmol), aqueous sodium hydroxide solution (1.25*M*, 80ml) and benzylchloroformate (95%w/v, 8.7g, 5mmol). Recrystallisation of the crude product from ethanol : water (1:1) gave (86) (2.96 g, 32%) as a white powder; m.p. 131-132°C, (Lit.[90], 140-142°C); $[\alpha]_D$ +38° (c = 0.98, CH_3OH), [Lit.[90], +43° (c = 1.0, CH_3OH)]; ν_{max} (nujol) 3327 m, 1696 s, 1676 s; δH (300 MHz, DMSO-d_6) 3.94 (1H, dd, *J* 10, 8.5 Hz, 5-H), 4.50 (1H, t, *J* 8.5 Hz, 5-H), 4.57 (1H, m, 4-H), 5.05 (2H, s, PhC<u>H</u>$_2$-), 7.36 (5H, m, Ar), 7.86 (1H, d, *J* 8.5 Hz, $PhCH_2OCONH$-), 11.50 (1H, bs, 2-H); δC (62.5 MHz, DMSO-d_6) 53.0 (d, 4-C), 66.0 (t, PhC<u>H</u>$_2$-), 72.0 (t, 5-C), 128.0, 128.1, 128.6 (3 x s, Ar), 137.0 [s, Ar(*ipso*-C)], 156.2 (s, CO), 170.6 (s, CO); m/e (NH_3 CI) 254 (MNH_4^+, 25%), 237 (MH^+, 100), 193 (15), 108 (23), 91 (84), 49 (16). [Found C, 55.87%; H, 5.01%; N, 11.62%. $C_{11}H_{12}N_2O_4$ requires C, 55.93%; H, 5.12%; N, 11.86%].

D-4-Benzyloxycarbonylamino-2-methyl-3-isoxazolidinone (87)

To a solution of (86) (246mg, 1.04mmol) in acetone (4.5ml) was added potassium carbonate (170mg, 1.23mmol) with stirring at room temperature. Methyl iodide (798 mg, 5.62 mmol) was then added and the mixture refluxed under nitrogen for 24 h. The reaction mixture was filtered and the filtrate evaporated *in vacuo* to yield a crude product (378mg) which was recrystallised from methanol to yield (87) (242mg, 93%) as an off-white powder; R_f 0.5 (diethyl ether); ν_{max} ($CHCl_3$) 1721 m, 1691 m, 1550 m, 1462 s, 1378 m; δH (300 MHz, acetone-d_6) 3.08 (3H, s, -NCH_3), 4.06 (1H, dd, *J* 10, 8.5 Hz, 5-H), 4.57 (1H, t, *J* 8.5 Hz, 5-H), 4.68 (1H, *m*, 4-H), 5.10 (2H, s, PhOC<u>H</u>$_2$-), 7.31-7.37 (5H, m, Ar) ; m/e (NH_3 DCI) 268 (MNH_4^+, 20%), 251 (MH^+, 100), 250 (M^+, 22), 91 (40).

2-Oxoglutaric acid, dimethyl ester (88)

Hydrogen chloride gas was bubbled through a solution of 2-oxoglutaric acid (2.20g, 15.1mmol) in methanol (100ml) for 5 h, and the solution stirred at room temperature for a further 15 h. The solvent was removed *in vacuo*, the residue dissolved in ethyl acetate (30ml) and washed with sodium hydrogen carbonate solution (5%w/v, 2 x 25ml). The organic layer was dried (sodium sulphate), filtered and evaporated *in vacuo* to yield the crude product which was purified by bulb to bulb distillation (90-95°C, 0.4mmHg) to yield (88) (1.42g, 54%) as a colourless liquid; ν_{max} (thin film) 2958 m, 1735 bs, 1439 s; δH (300 MHz, $CDCl_3$) 3.53 (2H, *ca* t, *J* 6.5 Hz, 4-H), 4.03 (2H, *ca* t, *J* 6.5 Hz, 3-H), 4.54 (3H, s, $-OCH_3$), 4.74 (3H, s, $-COCO_2CH_3$); m/e (NH_3 CI) 192 (MNH_4^+, 100%), 175 (MH^+, 25), 115 (45), 100 (33); [Found C, 48.06%; H, 6.09%. $C_7H_{10}O_5$ requires C, 48.28%; H, 5.79%].

[4*R*]-2*R*,*S*-(4-Benzyloxycarbonylamino-3-oxo-2-isoxazolidinyl)-5-oxo-tetrahydrofuran-2-carboxylic acid, methyl ester (89)

Prepared in an analogous manner to (80) using (86) (102mg, 0.43mmol), 2-oxoglutaric acid (90mg, 0.62mmol), DMF (2.0ml), sodium hydrogen carbonate (90mg, 1.07mmol) and methyl iodide (605µl, 9.73mmol). The crude product was purified by PLC [diethyl ether : ethyl acetate (4:1)] R_f 0.75 to yield (89) (40mg, 25%) as an oil; ν_{max} ($CHCl_3$) 3422 m, 1808 s, 1760 bs, 1727 bs, 1508 s; δH (300MHz, $CDCl_3$) 2.42-2.54, 2.55-2.67, 2.77-2.89, 3.11-3.23 (4H, 4 x m, lactone 3-H, 4-H), 3.87, 3.88 (3H, 2 x s, $-CO_2CH_3$), 4.08-4.17, 4.65-4.80, 4.83-4.91 (3H, 3 x m, cycloserine 4-H, 5-H), 5.13 (2H, s, $PhC\underline{H}_2$-), 5.47, 5.57 (1H, 2 x bd, *J* 5Hz, -CONH-), 7.32-7.37 (5H, m, Ar); m/e (NH_3 DCI) 396 (MNH_4^+, 30%), 378 (M^+, 10), 251 (35), 237 (43), 160 (31), 108 (38), 91 (100).

[4*R*]-2*R*,*S*-(4-Benzyloxycarbonylamino-3-oxo-2-isoxazolidinyl)-5-oxo-tetrahydrofuran-2-carboxylic acid, benzyl ester (91)

Prepared in an analogous manner to (89) using (86) (315mg, 1.33mmol), 2-oxoglutaric acid (250mg, 1.71mmol), DMF (3ml), sodium hydrogen carbonate (151mg, 1.80mmol), benzyl bromide (0.37ml, 3.10mmol) and a catalytic quantity of sodium iodide. The crude product was purified by chromatography [(flash silica, 70g), ethyl acetate] R_f 0.6 to yield (91) (185mg, 31%) as an oil; ν_{max} ($CHCl_3$) 3425 w, 1808 s, 1763 bs, 1730 bs, 1508 m, 1270 m, 1186 s, 1056 m; δH (300 MHz, $CDCl_3$) 2.37-2.49, 2.53-2.64, 2.76-2.88, 3.11-3.22 (4H, 4 x m, lactone 3-H, 4-H), 4.05-4.18, 4.60-4.77, 4.82-4.91 (3H, 3 x m, cycloserine 4-H, 5-H), 5.13 (2H, s, PhC$\underline{H}_2$OCONH-), 5.28, 5.30 (2H, 2 x s, -$CO_2C\underline{H}_2$Ph), 5.44, 5.50 (1H, 2 x bd, *J* 5 Hz, -CONH-), 7.34-7.39 (10H, m, Ar); m/e (NH_3 DCI) 472 (MNH_4^+, 40%), 455 (MH^+, 2), 327 (20), 108 (20), 91 (100).

Ethyl-2-chloro-2-ethoxyacetate (94)

Phosphorus pentachloride (21.0g, 0.101mol) and ethyl diethoxyacetate (18.0g, 0.102mol) were warmed together at *ca* 35°C under argon in dry conditions until dissolution occurred. The solution was allowed to stand for 2 h before heating to 140°C for 3 h, then distilled under aspirator pressure. The title compound (94) (17.02g, 99%) was obtained as the fraction boiling at 82-83°C (15mmHg); ν_{max} ($CHCl_3$) 2941 m, 1757 s, 1300 m, 1195 m, 1111 m, 1018 m, 910 s; δH (300 MHz, $CDCl_3$) 1.19, 1.21 (2 x 3H, 2 x t, *J* 7 Hz, 2 x CH_3-), 3.57, 3.90 [2 x 1H, 2 x dq, *J* 10, 7 Hz, -CH(Cl)OC$\underline{H}_2$C H$_3$], 4.17 (2H, q, *J* 7Hz, -$CO_2C\underline{H}_2CH_3$), 5.71 (1H, s, 2-H); m/e (EI) 131 (M^+-Cl, 10%), 103 (100), 93 (30), 75 (65), 59 (30), 45 (95), 36 (100).

Attempted preparations of [4*R*]-2*R*,*S*-(4-Phenoxyacetylamino-3-oxo-2-isoxazolidinyl)-2-ethoxyethanoic acid, ethyl ester (95)

Method 1

A solution of ethyl-2-chloro-2-ethoxyacetate (94) (117mg, 0.70mmol) and *D*-4-phenoxyacetylamino-3-isoxazolidinone (78) (151mg, 0.64mmol) in THF (2.5ml) was gently refluxed until the starting materials had disappeared (by tlc). After evaporation of the solvent *in vacuo*, the residue was partitioned between ethyl acetate (5ml) and water (5ml). The organic layer was dried (sodium sulphate), filtered and evaporated *in vacuo* to yield a brown oil which was purified by chromatography [(flash silica, 20g), gradient elution, diethyl ether : ethyl acetate (1:1 - 1:3)] to yield an oil (25mg). Though the ^{1}H nmr spectrum was very complex, the mass spectrum was consistent with (95); m/e (NH_3 DCI) 367 (MH^+, 50%), 366 (M^+, 40%).

Method 2

D-4-Phenoxyacetylamino-3-isoxazolidinone (78) (125mg, 0.53mmol) and anhydrous potassium carbonate (80mg, 0.58mmol) were suspended in acetone (2.0ml) and stirred for 20 min at 15°C. Ethyl-2-chloro-2-ethoxyacetate (94) (92mg, 0.55mmol) was then added and the white suspension refluxed for *ca* 4.5 h. The solvent was removed *in vacuo* and the residue partitioned between ethyl acetate (10ml) and aqueous hydrochloric acid (0.2*N*, 10ml). The organic layer was dried (sodium sulphate), filtered and evaporated *in vacuo* to yield an oil. This was purified by chromatography [(flash silica, 20g), ethyl acetate]. The major product (R_f 0.6; yield 31.5mg) again had a mass spectrum consistent with (95) (M^+ = 366), yet ^{1}H nmr and ν_{max} differed from those obtained in Method 1 above, suggesting that *O*-alkylation may have occurred.

Comparative data:

Method 1

δH (250 MHz, $CDCl_3$) 5.63, 5.69 (1H, 2 x s, -C<u>H</u>CO_2Et), 7.17 [1H, d, -NH-(sidechain)]; ν_{max} ($CHCl_3$) 1746 s, 1685 s.

Method 2

δH (300 MHz, $CDCl_3$) 5.73, 5.83 (1H, 2 x s, -C<u>H</u>CO_2Et), 7.18, 7.75 [1H, 2 x d, -NH-(sidechain)]; ν_{max} ($CHCl_3$) 1752 s, 1688 s, 1628 m (imine).

Standardisation of *n*-butyllithium[90]

Two 950μl aliquots of a solution of *n*-butyllithium in hexanes were titrated against 0.1*N* hydrochloric acid, using phenolphthalein as indicator, to ascertain the total base content. Further 950μl aliquots were quenched with 1,2-dibromoethane, then titrated as above to establish the total inorganic base content. The latter titre value was subtracted from the former titre value to reveal the total organic base content, from which the molarity was calculated.

D-4-Phenoxyacetylamino-2-benzyl-3-isoxazolidinone (97)

A solution of lithium diisopropylamide was prepared by dropwise addition of a standardised solution of *n*-butyllithium in hexanes (1.70*M*, 392μl, 0.67mmol) to a solution of diisopropylamine (110μl, 0.79mmol) in THF (1.5ml), over 10 min at -78°C. The solution was allowed to warm to 0°C over *ca* 30 min, before being recooled to -78°C. *D*-4-Phenoxyacetylamino-3-isoxazolidinone (78) (150mg, 0.64mmol) was dissolved in THF (1.5ml) and cooled to -78°C under argon. The solution of lithium diisopropylamide was added to the latter *via* cannula under positive pressure of argon, and the resulting solution allowed to warm to 0°C over 1.5 h. Stirring was continued at 0°C for 10 min before removal of the solvent *in vacuo*. To a solution of the residue in DMF (1.5ml) was added benzyl bromide (122mg, 0.72mmol) and

the resulting mixture stirred for 16 h at room temperature. The solvent was removed *in vacuo*; the residue was dissolved in ethyl acetate (20ml) and washed with water (3 x 20ml). The organic layer was dried (sodium sulphate), filtered and evaporated *in vacuo*. The crude product was purified by chromatography [(flash silica, 50g), gradient elution, diethyl ether : 30-40 petroleum ether (3:1 - 1:0)] to yield (97) (117mg, 57%) as a white powder; R_f 0.25 (diethyl ether); ν_{max} ($CHCl_3$) 3405 w, 1701 s, 1685 bs, 1601 w, 1521 m, 1496 m, 1442 m, 1240 m; δH (500 MHz, $CDCl_3$) 3.98 (1H, dd, *J* 10, 8 Hz, 5-H), 4.72, 4.81 (2H, ABq, *J* 15 Hz, PhC<u>H</u>$_2$-), 4.54 (2H, s, PhOC<u>H</u>$_2$-), 4.80-4.89 (2H, m, 4-H, 5-H), 6.95 [2H, d, *J* 8 Hz, ArO(*o*-H)], 7.05 [1H, t, *J* 7 Hz, ArO(*p*-H)], 7.21 (1H, d, *J* 4.5 Hz, -NH-), 7.34-7.39 [7H, m, ArO(*m*-H), <u>Ph</u>CH$_2$-]; m/e (NH_3 DCI) 327 (MH^+, 100%), 326 (M^+, 60), 91 ($PhCH_2^+$, 70). [Found C, 66.49%; H, 5.80%; N, 8.44%. $C_{18}H_{18}N_2O_4$ requires C, 66.24%; H, 5.56%; N, 8.59%.]

<u>4-Nitrobenzyl diethoxyacetate (96)</u>

Ethanol (35ml) was added to sodium (2.40g, 0.11mol) over *ca* 25 min with gentle refluxing under an inert atmosphere. The sodium had completely reacted after a further 0.5 h, and the mixture was then refluxed for a further 1 h. Dichloroacetic acid (2.5ml, 0.03mol) was added over 5-10 min, ensuring the mixture refluxed smoothly. The reaction mixture was refluxed for a further 5 h and the ethanol removed *in vacuo*. To a solution of the residue in DMF (50ml) was added 4-nitrobenzyl bromide (9.73g, 0.05mol) and potassium iodide (1 crystal). The mixture was stirred under argon at 30-35°C for 24 h. The DMF was removed *in vacuo* and the residue partitioned between ethyl acetate (200ml) and water (200ml). The organic layer was washed with water (200ml), dried (sodium sulphate), filtered and evaporated *in vacuo* to yield a brown oil (7.6g). This was purified by chromatography [(flash silica, 450g), diethyl ether : 30-40 petroleum ether (1:1)] R_f 0.35 to yield (96) (3.90g, 46%) as a pale yellow liquid; ν_{max} ($CHCl_3$) 3025 m, 2983 m,

2888 m, 1757 s, 1608 m, 1526 s, 1349 s; δH (250 MHz, $CDCl_3$) 1.24 (6H, t, *J* 7 Hz, 2 x $C\underline{H}_3CH_2O$-), 3.66, 3.70 (4H, 2 x q, *J* 9 Hz, 2 x $CH_3C\underline{H}_2O$-), 4.98 (1H, s, 2-H), 5.30 (2H, s, $ArC\underline{H}_2O$-), 7.54, 8.21 (2 x 2H, 2 x d, Ar); m/e (NH_3 DCI) 301 (MNH_4^+, 100%), 103 (65).

(4-Nitrobenzyl) 2-chloro-2-ethoxyacetate (97')

Phosphorus pentachloride (2.20g, 10.6mmol) and 4-nitrobenzyl diethoxyacetate (3.0g, 10.6mmol) were stirred together under argon at 40°C for 1.5 h until dissolution occurred. The solution was allowed to stand at room temperature for 2 h and then heated to 140°C for 3 h. The crude product could not be successfully purified by bulb to bulb distillation, but ^1H nmr analysis showed (97') to be present, though very impure; δH (300 MHz, $CDCl_3$) 1.25 (3H, t, *J* 7 Hz, $C\underline{H}_3CH_2O$-), 3.60 [2H, q (obscured), *J* 7 Hz, $CH_3C\underline{H}_2O$-], 5.31 (2H, s, $ArC\underline{H}_2O$-), 5.86 (1H, s, 2-H), 7.53, 8.20 (2 x 2H, 2 x d, Ar).

Allyl diethoxyacetate (98)

Ethanol (35ml) was added to clean sodium (2.41g, 0.11mol) over *ca* 25 min with stirring and gentle refluxing under argon. After a further 0.5 h the sodium had completely reacted, and the mixture was refluxed for a further 1 h. Dichloroacetic acid (2.5ml, 0.03mol) was added dropwise over *ca* 10 min so that the mixture refluxed smoothly. The brown/orange mixture was refluxed for a further 4 h and the ethanol was removed *in vacuo*. To a solution of the residue in DMF (50ml) was added allyl bromide (3.9ml, 0.05mol) and potassium iodide (1 crystal). The suspension was then stirred under argon for 36 h, the DMF was removed *in vacuo* and the residue partitioned between ethyl acetate (200ml) and water (200ml). The organic layer was washed with water (2 x 100ml), dried (sodium sulphate), filtered and evaporated *in vacuo* to yield a brown oil (4.5g). The crude product was purified by chromatography [(flash silica, 400g), diethyl ether : 30-40

petroleum ether (1:1)] R_f 0.55 to yield (98) (2.93g, 52%) as a colourless liquid; ν_{max} ($CHCl_3$) 2983 s, 2934 m, 2888 m, 1751 vs, 1446 m, 1281 m, 1211 s, 1120 s, 1067 s, 986 s, 939 s; δH (300 MHz, $CDCl_3$) 1.22 (6H, t, *J* 7 Hz, 2 x C<u>H</u>$_3$CH$_2$O-), 3.63, 3.65 (4H, 2 x q, *J* 9 Hz, 2 x CH$_3$C<u>H</u>$_2$O-), 4.65 (2H, dt, *J* 6 Hz, $J_{allylic}$ 1 Hz, CH$_2$=CH-C<u>H</u>$_2$O-), 4.90 [1H, s, (EtO)$_2$C<u>H</u>-], 5.23 (1H, dd, J_{cis} 10 Hz, $J_{allylic}$ 1 Hz, C<u>H</u>$_2$=CH-), 5.32 (1H, dd, J_{trans} 17 Hz, $J_{allylic}$ 1 Hz, C<u>H</u>$_2$=CH-), 5.84-5.97 (1H, m, CH$_2$=C<u>H</u>-); m/e (NH_3 CI) 206 (MNH_4^+, 100%), 189 (MH^+, 15), 114 (30), 103 (70), 48 (16).

<u>Allyl 2-chloro-2-ethoxyacetate (99)</u>

Phosphorus pentachloride (1.55g, 7.44mmol) and allyl diethoxyacetate (98) (1.40g, 7.44mmol) were warmed together at 35°C for 2 h, then heated to 135°C for 2.5 h. The brown solution was distilled to yield (99) (900mg, 68%) as a colourless liquid; b.p. 52-58°C (0.5mmHg). The data obtained indicated a 3:1 ratio of the allyl and ethyl esters respectively; redistillation again failed to separate the two compounds which codistilled in the same ratio. Data for (99); ν_{max} ($CHCl_3$) 2987 m, 2942 m, 1759 vs, 1477 m, 1446 m, 1415 m, 1331 m, 1299 s, 1284 s, 1191 vs, 1144 s, 1112 s, 1016 m, 987 m, 942 m; δH (250 MHz, $CDCl_3$) 1.32 (3H, t, *J* 7 Hz, C<u>H</u>$_3$CH$_2$O-), 3.68, 4.05 [2 x 1H, 2 x dq, *J* 9.5, 7 Hz, -CH(Cl)OC<u>H</u>$_2$CH$_3$], 4.73 (2H, dt, *J* 6 Hz, $J_{allylic}$ 1Hz, CH$_2$=CH-C<u>H</u>$_2$O-), 5.30 (1H, dd, J_{cis} 10 Hz, $J_{allylic}$ 1Hz, C<u>H</u>$_2$=CH-), 5.39 (1H, dd, J_{trans} 17 Hz, $J_{allylic}$ 1 Hz, C<u>H</u>$_2$=CH-), 5.85 [1H, s, EtOC<u>H</u>(Cl)-], 5.86-6.02 (1H, m, CH$_2$=C<u>H</u>-); m/e (NH_3 CI) 196 (MNH_4^+, 100%), 114 (33), 102 (18), 93 (20), 59 (10).

<u>[4*R*]-2*R*,*S*-(4-Phenoxyacetylamino-3-oxo-2-isoxazolidinyl)-2-ethoxyacetate, allyl ester (100)</u>

An identical quantity of lithium diisopropylamide was prepared in the same manner as for the preparation of (95). *D*-4-Phenoxyacetylamino-3-isoxazolidinone (78) (150mg, 0.64mmol) was dissolved in THF (1.5ml) and

cooled to -78°C under argon. The solution of lithium diisopropylamide was added to the latter solution *via* cannula under positive pressure of argon, with stirring at -78°C. The reaction mixture was allowed to warm to 0°C over 1.5 h, then stirred at 6°C for 10 min. The solvent was removed *in vacuo* and the fine yellow powder was dissolved in DMF (1.5ml). To this solution was added a solution of (99) (135mg, 0.76mmol) in DMF (1.5ml) at -10°C under argon and the reaction mixture allowed to warm to room temperature over 16 h. After removal of the DMF *in vacuo*, the residue was dissolved in ethyl acetate (20ml) and washed with water (4 x 20ml). The organic layer was dried (sodium sulphate), filtered and evaporated *in vacuo*. The crude product was purified by chromatography [(flash silica, 40g), diethyl ether] R_f 0.35 to yield (100) (130mg, 54%) as a mixture of diastereoisomers in a ratio of *ca* 1:1; $[\alpha]_D$ -7° (c = 1.2, $CHCl_3$); ν_{max} ($CHCl_3$) 3407 m, 2986 m, 2940 m, 2888 m, 1748 s, 1735 s, 1688 s, 1601 m, 1591 m, 1521 s, 1496 s, 1442 m, 1240 s, 1210 m, 1176 m, 1107 m, 984 m, 941 m; δH (500 MHz, $CDCl_3$) 1.30, 1.31 (3H, 2 x t, *J* 7 Hz, $C\underline{H}_3CH_2O$-), 3.62-3.84 (2H, m, $CH_3C\underline{H}_2O$-), 4.09 (1H, dd, *J* 11, 9 Hz, 5-H), 4.53 (2H, s, $PhOC\underline{H}_2$-), 4.67-4.75 (2H, m, CH_2=CH-$C\underline{H}_2O$-), 4.81-4.83 (1H, *ca* dd, 5-H), 4.79-4.86, 5.03-5.08 (1H, 2 x m, 4-H), 5.26, 5.28 (1H, 2 x dd, J_{cis} 10 Hz, $J_{allylic}$ 1 Hz, $C\underline{H}_2$=CH-), 5.35 (1H, 2 x dd, J_{trans} 17 Hz, $J_{allylic}$ 1 Hz, $C\underline{H}_2$=CH-), 5.64, 5.71 (1H, 2 x s, CH_3CH_2O-$C\underline{H}$-), 5.87-5.95 (1H, m, $C\underline{H}_2$=CH-), 6.91-6.94 [2H, m, Ar(*o*-H)], 7.01-7.05 [1H, m, Ar(*p*-H)], 7.17 (1H, d, *J* 5 Hz, -NH-), 7.28-7.33 [2H, m, Ar(*m*-H)]; δC (125 MHz, $CDCl_3$) 14.5 (2 x q, $\underline{C}H_3CH_2O$-), 51.1, 51.9 (2 x d, 4-C), 65.2, 65.3 (2 x t, $CH_3\underline{C}H_2O$-), 67.1 (t, $PhO\underline{C}H_2$-), 71.6 (t, 5-C), 73.7 (t, CH_2=CH-$\underline{C}H_2$-), 81.9 (2 x d, CH_3CH_2O-$\underline{C}H$-), 117.4 [d, Ar(*o*-C)], 119.5, 119.6 (2 x t, $\underline{C}H_2$=CH-), 122.4 [2 x d, Ar(*p*-C)], 129.8 [2 x d, Ar(*m*-C)], 130.9 (2 x d, CH_2=$\underline{C}$H-), 156.9, 157.0 [2 x s, Ar(*ipso*-C)], 164.2, 164.8, 169.0, 169.1, 169.2, 169.7 (6 x s, 3 x CO); m/e (NH_3 DCI) 396 (MNH_4^+, 1%), 379 (MH^+, 5), 378 (M^+, 18), 333 (100), 293 (80), 107 (30), 94 (40), 35 (40); [Found C, 57.43%; H, 6.22%; N, 7.01%. $C_{18}H_{22}N_2O_7$ requires C, 57.2%; H, 5.9%; N, 7.4%].

Allyl dichloroacetate (104)

Aqueous sodium bicarbonate (0.8*M*, 40ml) was added to a solution of dichloroacetic acid (4.0g, 30mmol) in water (40ml) and stirred for 20 min. The water was removed *in vacuo* and the residue dissolved in DMF (30ml). To this solution was added allyl bromide (3.95g, 33mmol) dropwise over *ca* 5 min, and the reaction mixture was stirred at 20°C for 16 h. The DMF was removed *in vacuo* and the residue partitioned between ethyl acetate (60ml) and water (60ml). The organic layer was washed with water (60ml), dried (sodium sulphate), filtered and evaporated *in vacuo* to yield (104) (3.8g, 73%) as a pale oil; δH (500 MHz, $CDCl_3$) 4.75 (2H, dt, *J* 7,1 Hz, CH_2=CH-C<u>H</u>$_2$-), 5.33 (1H, d, J_{cis} 10 Hz, $J_{allylic}$ 1 Hz, C<u>H</u>$_2$=CH-CH_2-), 5.42 (1H, d, J_{trans} 17 Hz, $J_{allylic}$ 1 Hz, C<u>H</u>$_2$=CH-CH_2-), 5.91-5.96 (1H, m, CH_2=C<u>H</u>-CH_2-), 5.97 (1H, s, -$CHCl_2$); m/e (NH_3 CI) 74 (100%), 58 (93).

Potassium 2-ethylhexanoate (101)

To a solution of 2-ethylhexanoic acid (14.6g, 0.10mol) in diethyl ether (30ml) was added a solution of potassium hydroxide (5.5g, 0.10mol) in water (50ml). The mixture was vigorously stirred for 1 h. The aqueous phase was washed with diethyl ether (2 x 50ml). The water was removed *in vacuo* to yield (101) (17.9g, 100%) as an oil which crystallised at -15°C to a white waxy solid.

[4*R*]-2*R*,*S*-(4-Phenoxyacetylamino-3-oxo-2-isoxazolidinyl)-2-ethoxyacetate, benzyl ester (103)

To a solution of the ester (100) (22mg, 0.06mmol) in dichloromethane (210µl) was added a solution of potassium 2-ethylhexanoate (101) (18mg, 0.10mmol) in ethyl acetate (200µl). Triphenylphosphine (2mg, 0.008mmol) and tetrakis(triphenylphosphine)palladium(0) (3mg, 0.003mmol) were then added and the resulting solution stirred at 25°C for 1 h. After removal of the

solvent *in vacuo* and shaking the residue with diethyl ether (2ml), a cream coloured precipitate was formed which was washed with further diethyl ether (3 x 2ml), the ethereal layers being decanted each time. Drying in a vacuum desiccator yielded the potassium salt of the free acid (26mg) as a cream coloured powder. ^1H nmr analysis at this stage showed the absence of the allyl protecting group and so it was reprotected as the benzyl ester for identification purposes. To a solution of the potassium salt in DMF (300µl) was added benzyl bromide (10µl, 0.08mmol) and potassium iodide (1 crystal). The reaction mixture was stirred under argon at 20°C for 5 h, then partitioned between ethyl acetate (10ml) and water (10ml). The organic layer was washed with water (2 x 10ml), dried (sodium sulphate), filtered and evaporated *in vacuo*. The residue was purified by chromatography [(flash silica, 10g), diethyl ether] R_f 0.4 to yield (103) (5mg, 20%) as a mixture of diastereoisomers. Data for the major diastereoisomer: δH (500 MHz, $CDCl_3$) 1.25 (3H, t, *J* 7 Hz, $C\underline{H}_3CH_2$-), 3.53-3.78 (2H, m, $CH_3C\underline{H}_2$-), 3.93-3.97 (1H, *ca* dd, 5-H), 4.47 (2H, s, $PhOC\underline{H}_2$-), 4.56-4.63, 4.92-4.98 (2H, 2 x m, 4-H, 5-H), 5.10, 5.29 (2H, ABq, *J* 9.5 Hz, $PhC\underline{H}_2O$-), 5.59 (1H, s, EtO-C<u>H</u>-), 7.02 (1H, d, *J* 5 Hz, -NH-), 6.73-6.88, 6.94-7.00, 7.24-7.32 (10H, 3 x m, Ar); m/e (NH_3 DCI) 428 (M^+, 10%), 383 (M^+ - OCH_2CH_3, 30), 293 (30), 91 (100).

<u>[4*S*]-2*R*,*S*-(4-Phenoxyacetylamino-3-oxo-2-isoxazolidinyl)-2-ethoxyacetate, allyl ester (106)</u>

Prepared in an analogous manner to (100) using (81) (208mg, 0.88mmol), *n*-butyllithium (1.70*M*, 536µl, 0.91mmol), diisopropylamine (150µl, 1.07mmol) and (99) (187mg, 1.05mmol). The title compound (106) (107mg, 32%) was isolated as a mixture of diastereomers in a ratio of *ca* 1:1; $[\alpha]_D$ +2.5° (c = 1.1, $CHCl_3$); δH (500 MHz, $CDCl_3$) data identical to 100; m/e (NH_3 DCI) 396 (MNH_4^+, 15%), 379 (MH^+, 10), 378 (M^+, 16), 333 (100), 293 (40).

[4*S*]-2*R* and 2*S*-(4-Phenoxyacetylamino-3-oxo-2-isoxazolidinyl)-2-ethoxy-acetic acid, potassium salt (107a,107b)

To a solution of the ester (106) (60mg, 0.16mmol) in dichloromethane (0.6ml) was added a solution of potassium 2-ethylhexanoate (101) (50mg, 0.27mmol) in ethyl acetate (0.6ml). Triphenylphosphine (6mg, 0.023mmol) and tetrakis(triphenylphosphine)palladium(0) (6mg, 0.005mmol) were then added, and the resulting solution stirred at 25°C for 1 h. After removal of the solvent *in vacuo* and shaking with diethyl ether (10ml), the resulting precipitate was further washed with diethyl ether (2 x 10ml), decanting the ethereal layer each time, and dried in a vacuum desiccator. The crude product (63mg) was redissolved in water (2ml), filtered, and a sample purified by HPLC [ODS Hypersil reverse phase column, 5% acetonitrile/water, flow rate 4ml min^{-1}] to yield the title compounds as separate diastereoisomers in a ratio of *ca* 1:1;

one diastereoisomer; δH (500 MHz, D_2O) 1.23 (3H, t, *J* 7 Hz, $C\underline{H}_3CH_2O$-), 3.59-3.69 (2H, m, $CH_3C\underline{H}_2O$-), 4.20 (1H, dd, *J* 10, 9 Hz, 5-H), 5.39 (1H, dd, *J* 10, 9 Hz, 4-H), 5.53 (1H, s, EtO-C<u>H</u>-), 7.05 [2H, d, *J* 8.5 Hz, Ar(*o*-H)], 7.11 [1H, t, *J* 7.5 Hz, Ar(*p*-H)], 7.41 [2H, dd, *J* 8.5, 7.5 Hz, Ar(*m*-H)]; δC (125 MHz, D_2O) 13.8 (q, $\underline{C}H_3CH_2O$-), 51.2 (d, 4-C), 64.9 (t, PhO$\underline{C}H_2$-), 66.7, 71.3 (2 x t, $CH_3\underline{C}H_2O$-, 5-C), 81.8 (d, EtO-<u>C</u>H-), 114.8 [d, Ar(*o*-C)], 122.2 [d, Ar(*p*-C)], 129.9 [d, Ar(*m*-C)], 157.1 [s, Ar(*ipso*-C)], 168.2, 170.1, 171.9 (3 x s, 3 x CO); m/e (FAB+) 415 (MK^+, 5%), 399 (MNa^+, 18), 377 (MH^+, 8), 131 (60), 115 (100), 93 (60), 39 (100).

other diastereoisomer; δH (500 MHz, D_2O) 1.24 (3H, t, *J* 7 Hz, $C\underline{H}_3CH_2O$-), 3.63, 3.76 (2H, 2 x dq, *J* 8.5, 7 Hz, $CH_3C\underline{H}_2O$-), 4.30 (1H, dd, *J* 10, 9 Hz, 5-H), 4.64 (1H, dd, *J* 10, 9 Hz, 5-H), 4.72 (2H, s, PhOC$\underline{H}_2$-), 5.04 (1H, dd, *J* 10, 9 Hz, 4-H), 5.48 (1H, s, EtO-C<u>H</u>-), 7.05 [2H, d, *J* 8.5 Hz, Ar(*o*-H)], 7.11 [1H, t, *J* 7.5 Hz, Ar(*p*-H)], 7.41 [2H, dd, *J* 8.5, 7.5 Hz, Ar(*m*-H)]; δC (125 MHz, D_2O) 13.8 (q, $\underline{C}H_3CH_2O$-), 52.3 (d, 4-C) , 64.9 (t, PhO$\underline{C}H_2$-), 66.7, 69.6 (2 x t, $CH_3\underline{C}H_2O$-, 5-C), 82.0 (d, EtO-<u>C</u>H-), 114.8 [d, Ar(*o*-C)], 122.2 [d, Ar(*p*-C)], 129.9 [d, Ar(*m*-C)], 157.1 [s, Ar(*ipso*-C)], 168.2,

170.0, 171.9 (3 x s, 3 x CO); m/e (FAB+) 415 (MK^+, 5%), 399 (MNa^+, 40), 377 (MH^+, 9), 131 (46), 115 (70), 107 (70), 77 (100), 39 (100).

[4*R*]-1-(4-Phenoxyacetylamino-3-oxo-2-isoxazolidinyl)-1,2-dicarboxylic acid, diethyl ester (109)

Method 1

To a solution of *D*-4-phenoxyacetylamino-3-isoxazolidinone (78) (150mg, 0.64mmol) in THF (2.5ml) under argon was added potassium carbonate (93mg, 0.67mmol). The mixture was stirred at 20°C for 10 min, then cooled to -10°C before dropwise addition of (110) (102µl, 0.64mmol) over *ca* 10 min. The mixture was then stirred at room temperature for a further 2 h. Upon disappearance of the starting materials, observed by tlc, the reaction mixture was filtered and the filtrate evaporated *in vacuo*. The residue was partitioned between ethyl acetate (10ml) and water (10ml), and the organic layer dried (sodium sulphate), filtered and evaporated *in vacuo*. The crude product was purified by chromatography [(flash silica, 25g), diethyl ether : ethyl acetate (1:1)] R_f 0.5 to yield (109) (22mg, 9%); $[\alpha]_D$ +11.6° (c=0.85, $CHCl_3$).

Method 2

A solution of lithium diisopropylamide was prepared by dropwise addition, over 10 min, of a standardised solution of *n*-butyllithium (1.70*M* in hexanes, 400µl, 0.67mmol) to a solution of diisopropylamine (110µl, 0.79mmol) in THF (1.5ml), cooled to -78°C under argon. This solution was then allowed to warm to 0°C over 0.5 h. A solution of *D*-4-phenoxyacetylamino-3-isoxazolidinone (78) (150mg, 0.64mmol) in THF (1.5ml) was cooled to -78°C under argon. The lithium diisopropylamide solution was added to the latter *via* cannula under positive pressure of argon at -78°C and the solution allowed to warm to 4°C over 2 h with stirring at this temperature for a

further 10 min. The THF was removed *in vacuo* and the resulting powder dissolved in DMF (2.5ml) and cooled to -60°C under argon with stirring. Acetylene dicarboxylic acid diethyl ester (110) (120µl, 0.75mmol) was added dropwise over 15 min. The orange/brown solution was stirred at -60°C for 30 min, quenched with glacial acetic acid (58µl, 1.01mmol) and allowed to warm to 0°C over ca 1 h. The DMF was removed *in vacuo* (<1mmHg) and the residue partitioned between ethyl acetate (20ml) and water (20ml). The organic layer was washed with water (20ml), dried (sodium sulphate), filtered and evaporated *in vacuo*. The crude product was purified by chromatography [(flash silica, 35g), diethyl ether] R_f 0.35, to yield (109) (76mg, 30%) as an oil; $[\alpha]_D$ +33.2° (c = 0.9, $CHCl_3$); ν_{max} ($CHCl_3$) 1748 bs, 1710 bs, 1695 bs, 1623 s, 1520 m, 1497 m, 1370 s, 1352 m, 1244 s, 1177 s, 1099 w, 1024 w, 992 w; δH (500 MHz, $CDCl_3$) 1.27, 1.37 (2 x 3H, 2 x t, *J* 7 Hz, 2 x $C\underline{H}_3CH_2O$-), 4.18 (2H, q, *J* 7 Hz, $CH_3C\underline{H}_2O$-), 4.23 (1H, dd, *J* 11, 8.5 Hz, 5-H), 4.38-4.43 (2H, m, $CH_3C\underline{H}_2O$-), 4.52 (2H, s, $PhOC\underline{H}_2$-), 4.97 (1H, *ca* t, *J* 8.5 Hz, 5-H), 5.06-5.11 (1H, m, 4-H), 5.78 (1H, s, -C=C<u>H</u>-), 6.91 [2H, d, *J* 8 Hz, Ar(*o*-H)], 7.04 [1H, t, *J* 7.5 Hz, Ar(*p*-H)], 7.32 [3H, m, -NH-, Ar(*m*-H)]; δC (125 MHz, $CDCl_3$) 13.7, 14.1 (2 x q, 2 x $\underline{C}H_3CH_2O$-), 51.4 (d, 4-C), 60.8, 63.0, 67.1 (3 x t, $CH_3\underline{C}H_2O$-, 5-C), 73.0 (*ca* t, $PhO\underline{C}H_2$-), 101.2 (d, -C=<u>C</u>H-), 114.7 [d, Ar(*o*-C)], 122.4 [d, Ar(*p*-C)], 129.8 [d, Ar(*m*-C)], 137.8 (s, -<u>C</u>=CH-), 156.9 [s, Ar(*ipso*-C)], 160.5, 163.6, 164.9, 169.2 (4 x s, 4 x CO); m/e (NH_3 DCI) 424 (MNH_4^+, 100%), 407 (MH^+, 83), 361 (25), 221 (30), 204 (93), 188 (30), 107 (10), 94 (15).

<u>Acetylene dicarboxylic acid, diallyl ester (111)</u>

A mixture of acetylene dicarboxylic acid (5.0g, 43.9mmol), dry allyl alcohol (20.0g, 344.8mmol), 4-toluenesulphonic acid (0.3g, 1.74mmol) and hydroquinone (0.02g, 0.18mmol) in a flask fitted with a 10cm vigreux column and a condenser set for distillation was heated on an oil-bath, the temperature of which was raised from 125-150°C until *ca* 14.5g allyl alcohol

had distilled at 95-98°C over *ca* 2 h. The solution was cooled, poured into water (55ml), extracted with diethyl ether (3 x 20ml), washed with sodium hydrogen carbonate solution (3 x 20ml), dried (sodium sulphate), filtered and evaporated *in vacuo*. Hydroquinone (0.02g, 0.18mmol) was added and the product distilled at reduced pressure to yield (111) (4.05g, 48%); bp 103-104°C (1.5mmHg); ν_{max} (thin film) 1727 vs, 1363 m, 1251 vs, 1036 m, 993 m, 937 m; δH (500 MHz, $CDCl_3$) 4.71 (4H, dt, *J* 6, 1.4 Hz, -CH_2O-), [5.31 (2H, *ca* dd, J_{cis} 10.5 Hz, $J_{allylic}$ 1.5 Hz), 5.37 (2H, *ca* dd, J_{trans} 17 Hz, $J_{allylic}$ 1.5 Hz), C$\underline{H}_2$=CH-], 5.86-5.94 (2H, m, CH_2=C$\underline{H}$-); m/e (NH_3 CI) 212 (MNH_4^+, 100%), 195 (MH^+, 5), 58 (54).

[4*R*]-1-(4-Phenoxyacetylamino-3-oxo-2-isoxazolidinyl)-1,2-dicarboxylic acid, diallyl ester (112)

Prepared in an analogous manner to (109) using identical quantities of lithium diisopropylamide and *D*-4-phenoxyacetylamino-3-isoxazolidinone (78). Acetylene dicarboxylic acid diallyl ester (111) (142mg, 0.73mmol) was added dropwise at -60°C over 20 min, and the reaction mixture stirred for a further 20 min at -60°C. The reaction was quenched with a solution of glacial acetic acid (50µl, 0.87mmol) in DMF (1ml), and allowed to warm to 0°C over 1 h. The reaction mixture was diluted with ethyl acetate (30ml) and washed with water (5 x 30ml). The organic layer was dried (sodium sulphate), filtered and evaporated *in vacuo*. The crude product was purified by chromatography [(flash silica, 30g), diethyl ether] R_f 0.5 to yield (112) (60mg, 22%] as an oil; δH (500 MHz, $CDCl_3$) 4.24 (1H, dd, *J* 10, 8 Hz, 5-H), 4.54, 4.55 (2H, ABq, *J* 7 Hz, PhOC$\underline{H}_2$-), 4.64 (2H, dt, *J* 6, 1 Hz, CH_2=CH-C$\underline{H}_2$-), 4.84-4.86 (2H, m, CH_2=CH-C$\underline{H}_2$-), 5.01-5.10 (2H, m, 4-H, 5-H), 5.24-5.45 (2 x 2H, m, 2 x C$\underline{H}_2$=CH-), 5.85 (1H, s, 2-H), 5.88-5.96, 5.99-6.07 (2 x 1H, 2 x m, 2 x CH_2=C$\underline{H}$-), 6.93 [2H, *ca* d, *J* 9 Hz, Ar(*o*-H)], 7.05 [1H, t, *J* 7.5 Hz, Ar(*p*-H)], 7.20 (1H, d, *J* 5 Hz, -NH-), 7.34 [2H, dd, *J* 9, 7.5 Hz, Ar(*p*-H)]; δC (50 MHz, $CDCl_3$) 51.2 (d, 4-C), 65.5,

67.0, 67.6 (3 x t, CH_2=CH-$\underline{C}H_2$-, 5-C), 72.3 (t, PhO$\underline{C}H_2$-), 100.8 (d, 2-C), 114.8 [d, Ar(*o*-C)], 118.7, 119.8 (2 x t, 2 x $\underline{C}H_2$=CH-), 125.5 [d, Ar(*p*-C)], 130.0 [d, Ar(*m*-C)], 131.3, 131.9 (2 x d, 2 x CH_2=$\underline{C}$H-), 137.9 (s, 1-C), 157.1 [s, Ar(*ipso*-C)], 160.6, 164.0, 164.8, 169.5 (4 x s, 4 x CO); m/e (NH_3 DCI) 448 (MNH_4^+, 100%), 431 (MH^+, 2), 245 (50), 169 (40).

Attempted deprotection of (112)

To a solution of (112) (50mg, 0.12mmol) in dichloromethane (0.5ml) was added triphenylphosphine (3mg, 0.012mmol) and tetrakis(triphenyl-phosphine)palladium(0) (7mg, 0.006mmol), followed by dropwise addition at 0°C of a solution of pyrrolidine (21µl, 0.24mmol) in dichloromethane (50µl). Stirring was continued for 20 min at 20°C. The reaction mixture was diluted with ethyl acetate (25ml) and extracted with sodium bicarbonate solution (3 x 25ml). The combined aqueous layers were acidified to pH 2 with 5% aqueous hydrochloric acid and immediately re-extracted with dichloromethane (3 x 50ml). The combined organic layers were dried (sodium sulphate), filtered and evaporated *in vacuo*. ^{1}H nmr analysis of the residue revealed a complex product mixture and the possible removal of only one allyl ester.

Attempted preparation of 2-oxobutan-1,4-dioic acid, di-(4-nitrobenzyl) ester (125)

To a solution of 4-nitrobenzyl alcohol (232mg, 1.52mmol), dicyclohexylcarbodiimide (313mg, 1.52mmol), 4-dimethylaminopyridine (10mg, 0.08mmol) and anhydrous sodium sulphate (*ca* 20mg) in DMF (1.0ml) cooled to -15°C under argon, was added a solution of 2-oxobutan-1,4-dioic acid (100mg, 0.76mmol) in DMF (1.0mmol) over 25 min. After stirring at -15°C for a further 15 min, the solution was allowed to warm to room temperature and stirred for 17 h. The reaction mixture was diluted with

ethyl acetate (20ml), washed with water (4 x 25ml), dried (sodium sulphate), filtered and evaporated *in vacuo*. Analysis of the residue by tlc and ^1H nmr indicated a complex mixture of 4-nitrobenzyl esters.

2-Methylenebutan-1,4-dioic acid, di-(4-nitrobenzyl) ester (133)

To a solution of 2-methylenebutan-1,4-dioic acid (900mg, 6.92mmol) in DMF (18ml) was added potassium carbonate (960mg, 6.95mmol) with stirring at 18°C under argon. To this mixture was added 4-nitrobenzyl bromide (3.13g, 14.5mmol) and potassium iodide (2 crystals). The reaction mixture was stirred at room temperature for 18 h. The DMF was removed *in vacuo* and the residue partitioned between ethyl acetate (100ml) and water (100ml). The organic layer was washed with water (3 x 100ml) and brine (100ml), dried (sodium sulphate), filtered and evaporated *in vacuo*. The oily residue was purified by chromatography [(flash silica, 300g), diethyl ether] to yield (133) (1.85g, 67%) as a pale yellow solid; ν_{max} ($CHCl_3$) 1729 m, 1609 m, 1526 s, 1350 s, 1317 m, 1210 m, 1180 m, 1142 m; δH (200 MHz, $CDCl_3$) 3.48 (2H, s, 3-H), 5.22, 5.29 (2 x 2H, 2 x s, 2 x ArCH_2-), 5.85, 6.46 (2 x 1H, 2 x s, CH_2=C-), 7.48, 7.50 [2 x 2H, 2 x d, *J* 9 Hz, Ar(*o*-H)], 8.20, 8.21 [2 x 2H, 2 x d, *J* 9 Hz, Ar(*m*-H)]; m/e (NH_3 CI) 418 (MNH_4^+, 100%), 401 (MH^+, 5), 122 (17), 106 (15); [Found C, 56.87%; H, 4.00%; N, 6.87%. $C_{19}H_{16}N_2O_8$ requires C, 57.00%; H, 4.03%; N, 7.00%].

Ozonolysis of (133)

A solution of 2-methylenebutan-1,4-dioic acid, di-(4-nitrobenzyl) ester (133) (260mg, 0.65mmol) in dichloromethane (5ml) in a three-necked 100ml flask equipped with a stirrer bar, calcium chloride drying tube and bubbler was cooled to -70°C. Ozone, prepared by a silent electric discharge (200 V) through a stream of oxygen gas, was bubbled through the solution for 0.5 h, followed by argon for 0.5 h to remove excess ozone. To the solution at 0°C

was added dimethylsulphide (1ml); the solution was allowed to warm to room temperature and stirred for 6 h. The solvent was removed *in vacuo* and the residue partitioned between ethyl acetate (40ml) and water (40ml). The organic layer was washed with water (2 x 40ml), dried (sodium sulphate) and evaporated *in vacuo* to yield a pale yellow crystalline solid. Analysis by tlc revealed many polar products, and ^{1}H nmr analysis suggested cleavage/over-oxidation of the molecule.

Oxidation of (133) with osmium tetroxide/sodium periodate

To a solution of (133) (100mg, 0.25mmol) in THF : water (3:1, 1.3ml) at room temperature was added a catalytic amount of osmium tetroxide. The reaction mixture rapidly became black, and was stirred for a further 15 min. Sodium metaperiodate (80mg, 0.38mmol) was added in small portions over *ca* 25 min, and the reaction mixture stirred at room temperature for 2 h. The solvent was removed *in vacuo* and the residue partitioned between ethyl acetate (20ml) and water (20ml). The organic phase was washed with water (3 x 20ml), dried (sodium sulphate), filtered and evaporated *in vacuo*. The resulting pale yellow oil was purified by chromatography [(flash silica, 20g), diethyl ether] R_f 0.2 to yield 2-hydroxy-2-hydroxymethyl-butan-1,4-dioic acid, di-(4-nitrobenzyl) ester (134) (70mg, 65%) as an oil; δH (500 MHz, $CDCl_3$) 2.42 (1H, bs, -OH), 2.87, 3.01 (2H, ABq, *J* 16 Hz, 3-H), 3.71, 3.81 (2H, ABq, *J* 11 Hz, -C$\underline{H}_2$OH), 3.96 (1H, bs, -OH), 5.18, 5.22 (2H, ABq, *J* 13 Hz, ArC$\underline{H}_2$O-), 5.31, 5.35 (2H, ABq, *J* 13 Hz, ArC$\underline{H}_2$O-), 7.48, 7.51 [2 x 2H, 2 x d, *J* 8.5 Hz, Ar(*m*-H)], 8.20, 8.21 [2 x 2H, 2 x d, *J* 8.5 Hz, Ar(*o*-H)]; δC (50 MHz, $CDCl_3$) 39.5 (t, 3-C), 65.3, 65.4, 67.6 (3 x t, 2 x Ar$\underline{C}H_2$-, -$\underline{C}H_2$OH), 123.9 [d, Ar(*m*-C)], 128.4 [d, Ar(*o*-C)], 142.4, 142.6, 147.9 [3 x s, Ar(*p*-C), Ar(*ipso*-C), 2-C], 170.1, 173.4 (2 x s, 2 x CO).

Oxidation of (134) with sodium periodate

To a stirred solution of the diol (134) (176mg, 0.4mmol) in THF : water (3:1, 2.0ml) was added sodium metaperiodate (260mg, 1.22mmol) at room temperature. After 1 h, a white slurry was obtained. The solvent was removed *in vacuo*, and the residue partitioned between ethyl acetate (30ml) and water (30ml). The organic layer was washed with water (30ml), dried (sodium sulphate), filtered and evaporated *in vacuo* to yield a white solid. Analysis by tlc and ^{1}H nmr revealed a complex mixture of products.

2-Hydroxybutan-1,4-dioic acid, di-(4-nitrobenzyl) ester (136)

Prepared in an analogous manner to (133) using 2-hydroxybutan-1,4-dioic acid (900mg, 6.72mmol) in DMF (18ml), potassium carbonate (954mg, 6.90mmol), 4-nitrobenzyl bromide (3.05g, 14.1mmol) and potassium iodide (catalytic amount). After purification by chromatography [(flash silica, 300g), gradient elution, diethyl ether - ethyl acetate], the title compound (136) (1.55g, 57%) was obtained as a cream coloured powder; R_f 0.4 [diethyl ether : ethyl acetate (1:1)]; δH (500 MHz, $CDCl_3$) 2.93-2.97, 2.99-3.03 [2H, 2 x 4 line multiplets, J_{AB} 16.5 Hz, -C$\underline{H}_2$CH(OH)-], 3.19 (1H, d, *J* 5 Hz, -OH), 4.60-4.63 [1H, m, -CH$_2$C$\underline{H}$(OH)-], 5.23, 5.24 (2H, ABq, *J* 13 Hz, ArC$\underline{H}_2$-), 5.32 (2H, s, ArC$\underline{H}_2$-), 7.49, 7.52 (4H, 2 x d, *J* 8.5 Hz, Ar), 8.21, 8.22 (4H, 2 x d, *J* 8.5 Hz, Ar); m/e (NH_3 DCI) 422 (MNH_4^+, 100%), 153 (15), 122 (22); [Found C, 53.27%; H, 4.08%; N, 6.75%. $C_{18}H_{16}N_2O_9$ requires C, 53.47%; H, 3.99%; N, 6.93%].

Swern oxidation of (136)

To a solution of oxalyl chloride (26µl, 0.27mmol) in dichloromethane (0.6 ml) at -60°C was added DMSO (38µl, 0.54mmol). The reaction mixture was stirred for 2 min and a solution of the alcohol (136) (100mg, 0.25mmol) in dichloromethane (0.3ml) and DMSO (0.1ml) was added within 5 min. Stirring was continued for 15 min at -50°C; triethylamine (171µl, 1.23mmol)

was added, and stirring was continued for 5 min before allowing the reaction mixture to warm to room temperature. Water (2ml) was added and the aqueous layer re-extracted with dichloromethane (2ml). The combined organic layers were dried (magnesium sulphate), filtered and evaporated *in vacuo*. The residue was purified by chromatography [(flash silica, 20g), diethyl ether] to give low yields of many unidentifiable products.

Moffatt oxidation of (136)

The alcohol (136) (200mg, 0.50mmol) was dissolved in DMSO (0.75ml, 10.7mmol) and benzene (0.75ml) containing pyridine (0.04ml, 0.50mmol) and trifluoroacetic acid (0.02ml, 0.25mmol) at room temperature. After addition of dicyclohexylcarbodiimide (310mg, 1.50mmol), the sealed reaction mixture was left for 16 h at room temperature. Ethyl acetate (20ml) was added and the insoluble dicyclohexylurea filtered off. The organic phase was washed with water (2 x 20ml), dried (sodium sulphate), filtered and evaporated *in vacuo* to yield a yellow solid (400mg). Analysis by tlc and ^{1}H nmr suggested the presence of only very polar, possibly over-oxidised products.

Preparation of silver carbonate supported on celite

Celite was purified by washing it successively with methanol containing 10% concentrated hydrochloric acid, and then with distilled water until the washings were neutral and was then dried at 100°C. The purified celite (7.0g) was added to a stirred solution of silver nitrate (7.93g, 46.7mmol) in water (47ml). A solution of sodium carbonate decahydrate (7.0g, 24.5mmol) in water (70ml) was slowly added to the homogeneous suspension. When addition was complete, stirring was continued at room temperature for a further 10 min. The resulting yellow/green precipitate was filtered off and

dried on a rotary evaporator for 4 h. [The reagent system contains *c a* 1mmol of silver carbonate *per* 0.57g of material.][113]

Oxidation of (136) with silver carbonate on celite

Traces of moisture were removed from a suspension of silver carbonate on celite in benzene by azeotropic distillation. After removal of the benzene *in vacuo*, the reagent was stored under argon. To a stirred suspension of silver carbonate on celite (290mg, 0.5mmol) in dichloromethane (2ml) under argon was added a solution of the alcohol (136) (100mg, 0.25mmol) in dichloromethane (2ml). The resulting green mixture was stirred at 20°C for 18 h, then refluxed for 48 h. The reaction mixture was filtered and the filtrate was evaporated *in vacuo*. Analysis of the residue by tlc and ^{1}H nmr indicated that no reaction had occurred.

Oxidation of (136) with tetra-*n*-propylammonium perruthenate[114]

To a solution of (136) (100mg, 0.25mmol) in dichloromethane (3ml) was added 4-methylmorpholine-*N*-oxide (50mg, 0.375mmol) and activated 4Å molecular sieves. After stirring the mixture for 10 min at room temperature, tetra-*n*-propylammonium perruthenate (6mg, 0.015mmol) was added and the reaction stirred for a further 20 h. The reaction mixture was diluted with dichloromethane (20ml), washed with sodium sulphite solution (20ml), brine (20ml) and copper sulphate solution (20ml), dried (sodium sulphate), filtered and evaporated *in vacuo* to yield a dark brown oil. Analysis by tlc and ^{1}H nmr revealed starting material and several minor products.

Attempted preparation of acetylene dicarboxylic acid, di-(4-nitrobenzyl) ester (123)

To a solution of acetylene dicarboxylic acid (1.00g, 8.8mmol) in water (10ml) was slowly added potassium hydrogen carbonate (1.76g, 17.5mmol); the resulting solution was freeze-dried. A solution of this potassium salt (100mg, 0.53mmol), 4-nitrobenzyl bromide (260mg, 1.20mmol), dicyclohexano-18-crown-6 (370mg, 1.00mmol), hydroquinone (ca 10mg) and potassium iodide (catalytic amount) in acetonitrile (6.0ml) was refluxed under argon for 18 h. The solvent was removed *in vacuo* and the residue purified by chromatography to yield propiolic acid, (4-nitrobenzyl) ester (124) (100mg) as a pale yellow solid; δH (500 MHz, $CDCl_3$) 2.99 (1H, s, H-C≡C-), 5.32 (2H, s, Ar$C\underline{H}_2$-), 7.55, 8.24 (2 x 2H, 2 x d, *J* 9 Hz, Ar); δC (50 MHz, $CDCl_3$) 66.1 (t, Ar$\underline{C}H_2$-), 73.9 (s, H-C≡$\underline{C}$-), 76.0 (d, H-$\underline{C}$≡C-), 124.0, 128.8 [2 x d, Ar], 128.7, 141.8 [2 x s, Ar], 152.3 (s, CO); m/e (NH_3 CI) 223 (MNH_4^+, 100%), 205 (M^+, 18), 153 (38), 136 (27), 122 (24), 53 (18).

[4*R*]-1-(4-Phenoxyacetylamino-3-oxo-2-isoxazolidinyl)-1,2-dicarboxylic acid, di-*t*-butyl ester (138)

Prepared by a procedure analogous to that of (112) using (78) (375mg, 1.59mmol) in THF (3.75ml), *n*-butyllithium (1.37*M* in hexanes, 1.25ml, 1.71mmol) and diisopropylamine (0.28ml, 1.98mmol) in THF (3.75ml). After addition of di-*t*-butylacetylene dicarboxylate (431mg, 1.91mmol) in DMF (6.25ml) at -60°C, the reaction mixture was stirred at -50°C for 3 h before quenching with glacial acetic acid (90μl) in DMF (0.5ml) and warming to room temperature over 1.5 h. After standard work-up procedure, the residue was purified by chromatography [(flash silica, 80g), diethyl ether] R_f 0.5 to yield (138) (220mg, 30%) as a white powder; $[\alpha]_D$ +45.5° (c = 1, CH_3OH); ν_{max} ($CHCl_3$) 3401 m, 2984 m, 1734 s, 1706 s, 1690 s, 1622 m, 1521 m, 1496 m, 1394 m, 1370 s, 1255 s, 1162 s, 1140 s; δH (500 MHz, $CDCl_3$) 1.47, 1.58 [2

x 9H, 2 x s, 2 x ($C\underline{H}_3)_3C$-], 4.18 (1H, dd, *J* 11, 8.5 Hz, 5-H), 4.52, 4.54 (2H, ABq, *J* 14 Hz, $PhOC\underline{H}_2$-), 4.96 (1H, *ca* t, *J* 8.5 Hz, 5-H), 5.03-5.08 (1H, m, 4-H), 5.74 (1H, s, -C=C-H), 6.92 [2H, d, *J* 8 Hz, Ar(*o*-H)], 7.04 [1H, t, *J* 7.5 Hz, Ar(*p*-H)], 7.26 (1H, d, *J* 6 Hz, -NH-), 7.32 [2H, dd, *J* 8, 6.5 Hz, Ar(*m*-H)]; δC (50 MHz, $CDCl_3$) 27.6, 28.0 [2 x q, 2 x ($\underline{C}H_3)_3C$-], 51.3 (d, 4-C), 66.9 (t, 5-C), 72.9 (t, $PhO\underline{C}H_2$-), 81.0, 84.7 [2 x s, 2 x $(CH_3)_3\underline{C}$-], 103.6 (d, -C=$\underline{C}$-H), 114.8 [d, Ar(*o*-C)], 122.5 [d, Ar(*p*-C)], 130.0 [d, Ar(*m*-C)], 138.0 (s, -$\underline{C}$=C-H), 157.1 [s, Ar(*ipso*-C)], 160.9, 163.7, 164.3, 169.4 (4 x s, 4 x CO); m/e (NH_3 DCI) 480 (MNH_4^+, 50%), 463 (MH^+, 10), 424 (80), 368 (100), 169 (50); [Found C, 59.63%; H, 6.75%; N, 5.83%. $C_{23}H_{30}N_2O_8$ requires C, 59.73%; H, 6.54%; N, 6.06%].

[4*R*]-1-(4-Phenoxyacetylamino-3-oxo-2-isoxazolidinyl)-1,2-dicarboxylic acid, diammonium salt (140)

A solution of (138) (51.6mg, 0.112mmol) in trifluoroacetic acid : anisole : toluene (3:1:20, 12ml) was heated at 50°C for 30 min. The solvent was removed *in vacuo* and the residue re-evaporated from toluene (2 x 10ml). The crude product was dissolved in aqueous ammonium hydrogen carbonate buffer (25m*M*, 20ml) and extracted with diethyl ether (2 x 20ml). The aqueous phase was freeze-dried, and a small sample purified by HPLC (Zorbax ODS reverse phase silica, eluted with 25m*M* aqueous ammonium hydrogen carbonate buffer, flow rate 4ml min^{-1}, retention time 44 min) to yield the title compound (140) as a white fluffy solid; ν_{max} (KBr disc) 3238 m, 1723 m, 1672 s, 1619 s, 1546 m, 1495 m, 1403 m, 1330 m, 1083 s; δH (500 MHz, D_2O) 4.29 (1H, dd, *J* 10, 9 Hz, cycloserine 4-H), 4.75 (2H, s, obscured, $PhOC\underline{H}_2$-), 5.29 (1H, dd, *J* 10, 9 Hz, cycloserine 5-H), 5.85 (1H, s, 2-H), 7.05 [2H, d, *J* 8.5 Hz, Ar(*o*-H)], 7.12 [1H, t, *J* 7.5 Hz, Ar(*p*-H)], 7.41 [2H, dd, *J* 8.5, 7.5 Hz, Ar(*m*-H)]; m/e (FAB, acidified with oxalic acid) 351 (MH^+, 100%).

[4*S*]-1-(4-Phenoxyacetylamino-3-oxo-2-isoxazolidinyl)-1,2-dicarboxylic acid, di-*t*-butyl ester (141)

Prepared in an identical manner to (138) using *L*-4-phenoxyacetylamino-3-isoxazolidinone (81) (375mg, 1.59mmol). Purification by chromatography [(flash silica, 75g), diethyl ether] R_f 0.5 and recrystallisation from dichloromethane/30-40 petroleum ether furnished the title compound (141) (202mg, 28%) as white needles; m.p. 159-160°C; $[\alpha]_D$ -39.4° (c = 1, CH_3OH); remaining data identical to (138).

[4*S*]-1-(4-Phenoxyacetylamino-3-oxo-2-isoxazolidinyl)-1,2-dicarboxylic acid, diammonium salt (142)

A solution of (141) (30.9mg, 0.067mmol) in trifluoroacetic acid : anisole : toluene (3:1:20, 6ml) was heated at 50°C for 30 min. The solvent was removed *in vacuo* and the residue re-evaporated from toluene (2 x 10ml). The crude product was dissolved in aqueous ammonium hydrogen carbonate buffer (25m*M*, 20ml) and extracted with diethyl ether (2 x 25ml). The aqueous phase was freeze-dried and purified by HPLC (Zorbax ODS reverse phase silica, eluted with 10m*M* aqueous ammonium hydrogen carbonate buffer containing 1% v/v acetonitrile, flow rate 4ml min^{-1}, retention time 16 min) to yield the title compound (142) (16.0mg, 62%) as a white, fluffy solid; all data identical to that for (140).

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PART TWO

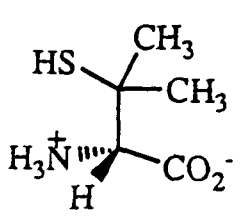
AMINO ACID SYNTHESIS

1. APPROACHES TO α-AMINO ACID SYNTHESIS

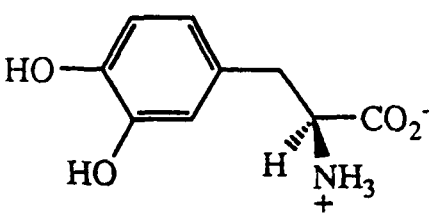
1.1. Naturally Occurring Amino Acids

Major advances in the understanding of enzyme mechanisms, protein conformations and properties related to molecular recognition and other important regulatory interactions, involving the basic twenty or so proteinogenic amino acids, have placed the study of amino acid chemistry at the forefront of modern chemical science. The number of naturally occurring non-proteinogenic amino acids is rapidly approaching 1000, most of which have been discovered in the last 35 years.[1] The majority are the products of secondary metabolism in plants and micro-organisms; some, such as homoserine, are intermediates in the biosynthesis of proteinogenic amino acids;[2] others perform vital natural functions, such as neurotransmitter action.[3]

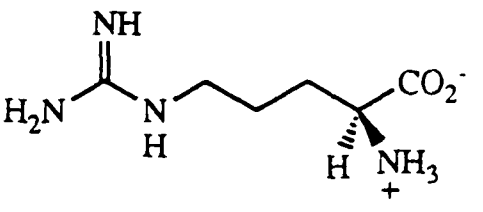
In addition to their biological functions, both natural and unnatural amino acids have found a wide variety of applications. Some possess medicinal properties, e.g. *D*-penicillamine (1), used to remove heavy metals from the body,[4] and *L*-dopa (2), used in the treatment of Parkinson's disease.[5] *L*-Arginine (3) is related to the biosynthesis of urea, and is used for the treatment of hepatic disorders.



(1)



(2)



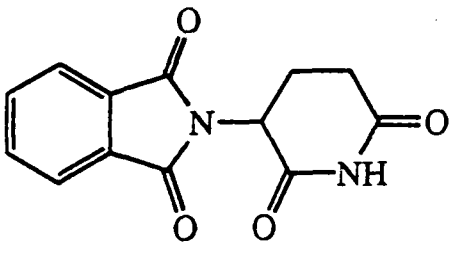
(3)

Many amino acids have been found to be inhibitors of certain enzymes, enabling the mode of action of that enzyme to be investigated.[6,7]

Since the proteinogenic amino acids are readily available and inexpensive, they have been used as chiral starting materials in many organic syntheses,[8] including the synthesis of the non-proteinogenic and unnatural members of this class of compounds.

1.2. General Amino Acid Synthesis

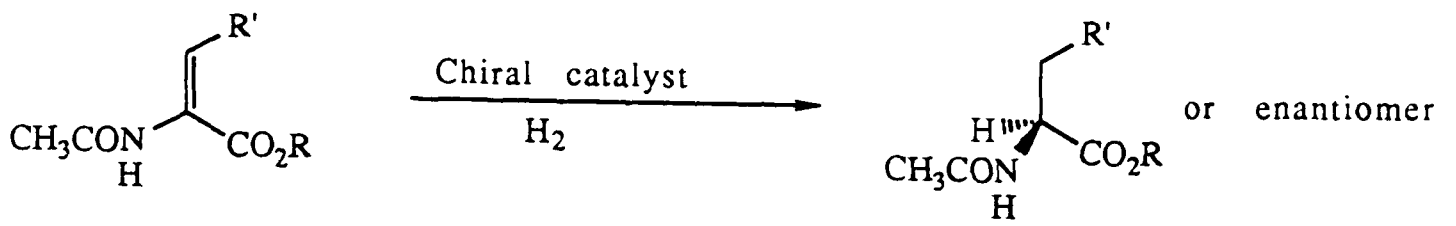
All amino acids exist in living systems in predominantly one enantiomeric form, and thus an enantiospecific synthetic strategy will normally be preferred by a chemist attempting to prepare such compounds. This requirement was ignored in all early amino acid syntheses, such as those developed by Strecker[9], Gabriel[10] and even more modern syntheses[11]. However the two enantiomers of amino acids often have different biological properties; for example, the *L*-isomers of leucine, phenylalanine, tyrosine and tryptophan all taste bitter, whereas the corresponding *D*-isomers taste sweet.[12] A renowned example demonstrating the need for stereospecific syntheses is thalidomide (4). In the 1960's, it was used as a sedative and hypnotic, but was administered in its racemic form. It was subsequently shown that both enantiomers had the required medicinal properties, but that only the *L*-isomer was responsible for the teratogenic effects observed after its administration to pregnant women, leading to the withdrawal of the drug.[13]



(4)

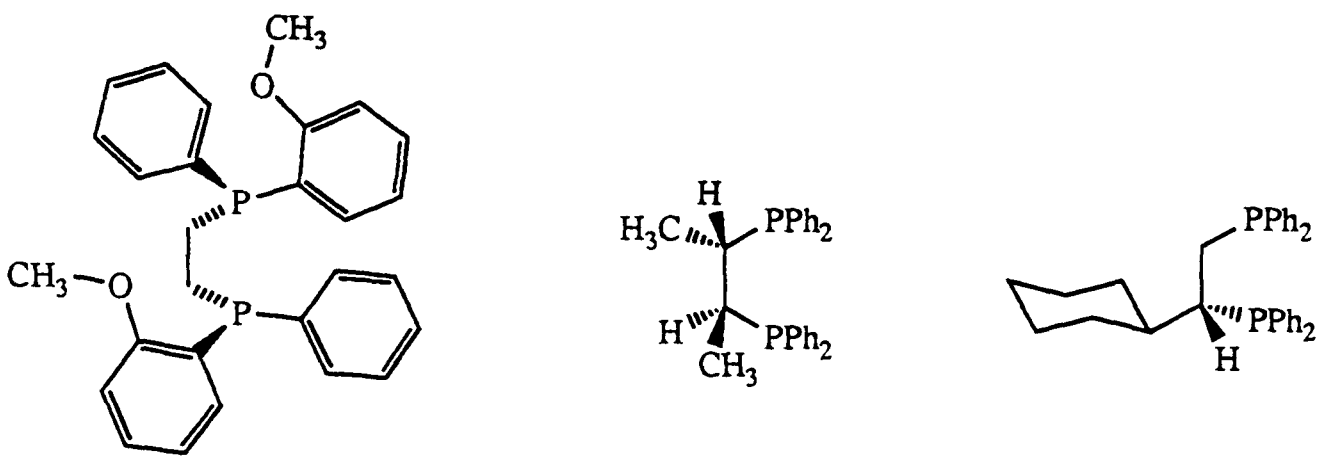
The first successful stereospecific syntheses of α-amino acids were realised by the asymmetric hydrogenations of prochiral dehydroalanine derivatives

using rhodium complexes containing chiral biphosphine ligands (Scheme 1.1).[14]



Scheme 1.1

Typical chiral ligands employed are shown in Figure 1.1.



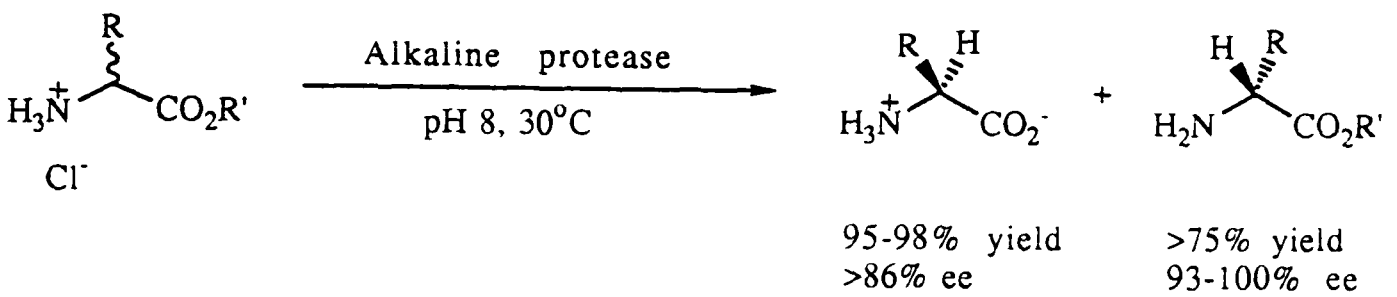
(R,R)-DIPAMP (S,S)-CHIRAPHOS CYCPHOS

Figure 1.1

However, the limitations are that the highest enantiomeric excesses are generally restricted to the Z-isomers of the dehydroalanines, and it is not possible to predict which chiral ligands will give the best enantiomeric excess for a particular substrate. There also exist the problems of the initial syntheses of the chiral ligands (often involving many steps and subsequent resolution), and the functional group incompatibility of the process.

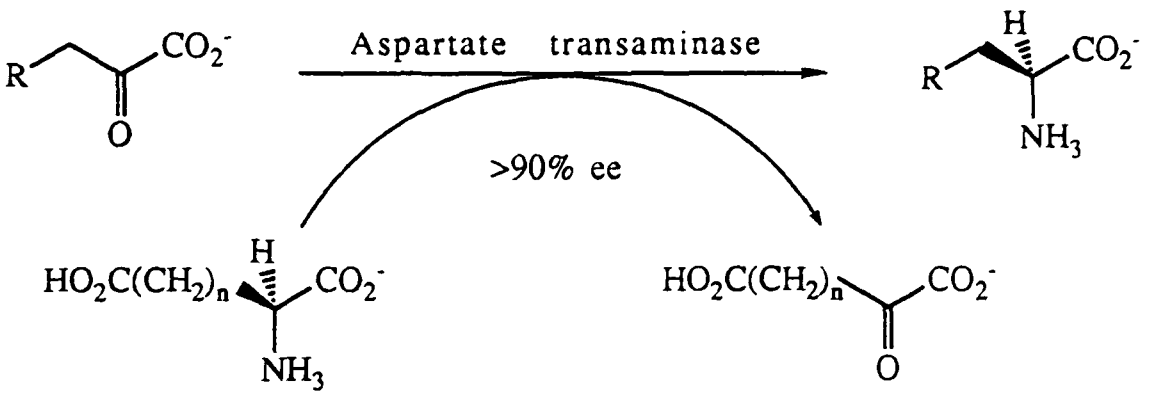
Syntheses of amino acids with purified enzymes have become an important commercial method to prepare amino acids on both large and intermediate scales.[15] An early application of enzymes involved the hog-kidney acylase hydrolyses of *N*-acyl amino acids for enantiomer separations.[16] Acylases are specific for *L*-enantiomer hydrolysis, and the unreactive *N*-acyl-*D*-

amino acids may either be cleaved chemically to give the corresponding *D*-amino acids, or racemised and recycled. A more recent disclosure[17] employs alkaline protease from *Bacillus licheniformis* to resolve racemic amino acid esters. Methyl and benzyl *L*-esters are enantioselectively hydrolysed, leaving the unreactive *D*-esters in high optical purity (Scheme 1.2).



Scheme 1.2

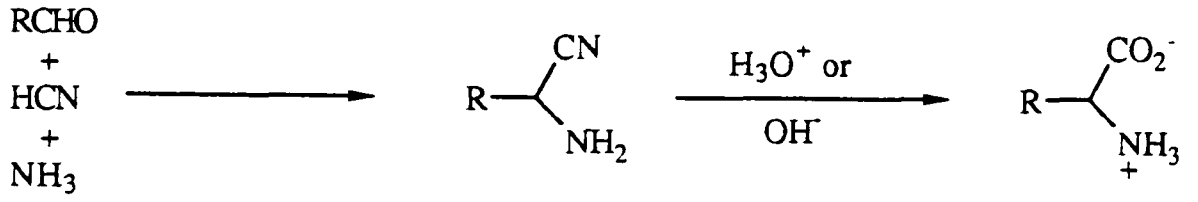
The biosyntheses of α-amino acids often occur via α-keto acids which are enzymatically reductively aminated. Work in this laboratory has demonstrated that this process can be used to synthesise α-amino acids from α-keto acids using cloned aspartate transaminase from *E. coli* (Scheme 1.3).[18] The only drawbacks of this approach are that the unnatural isomer of an amino acid is unobtainable and that the syntheses of the precursor α-keto acids are not always straightforward.



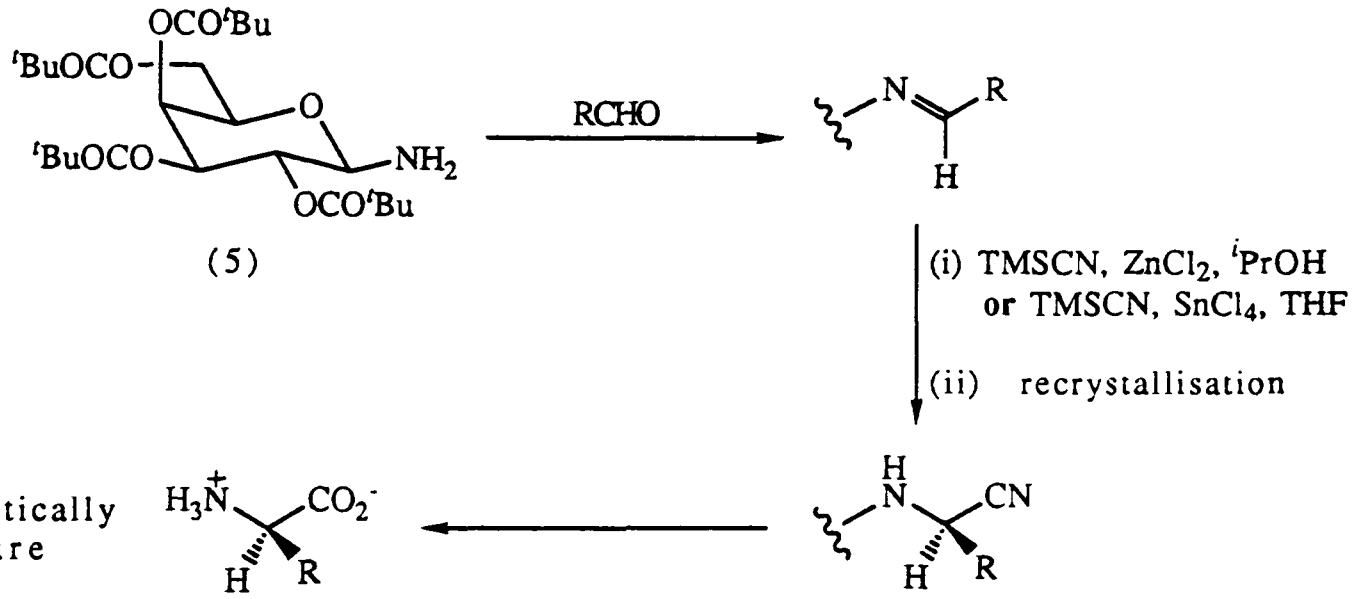
Scheme 1.3

The Strecker synthesis is a trusted method for the synthesis of racemic amino acids (Scheme 1.4); consequently, many attempts have been made to

modify the process to introduce some degree of asymmetric induction into the reaction. One specific example outlines a simple procedure to produce *R*-amino acids in optically pure form from tetra-*O*-pivaloyl-β-*D*-galactopyranosylamine (5) (Scheme 1.5).[19] However, with only a few exceptions chiral induction in these cases is generally low and irreproducible.

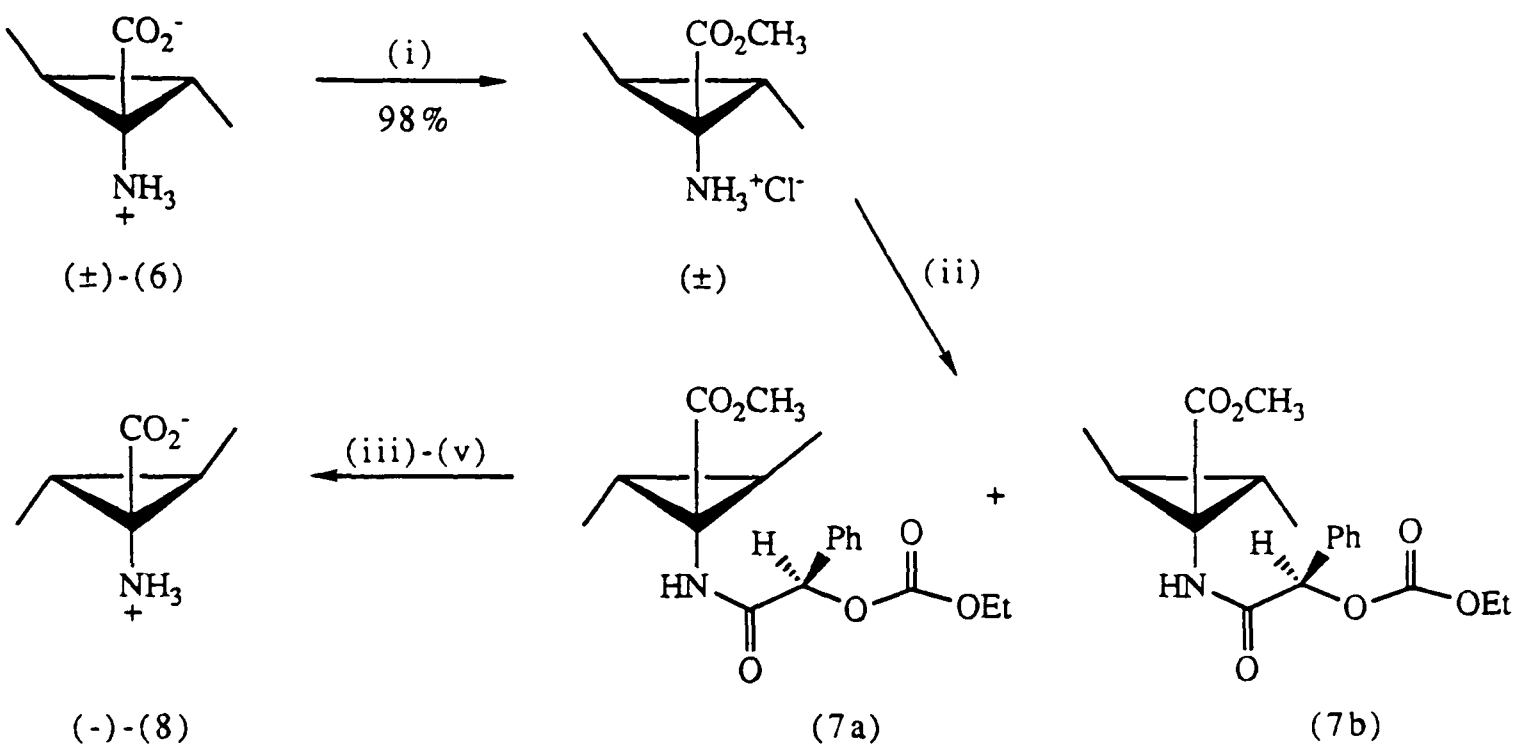


Scheme 1.4



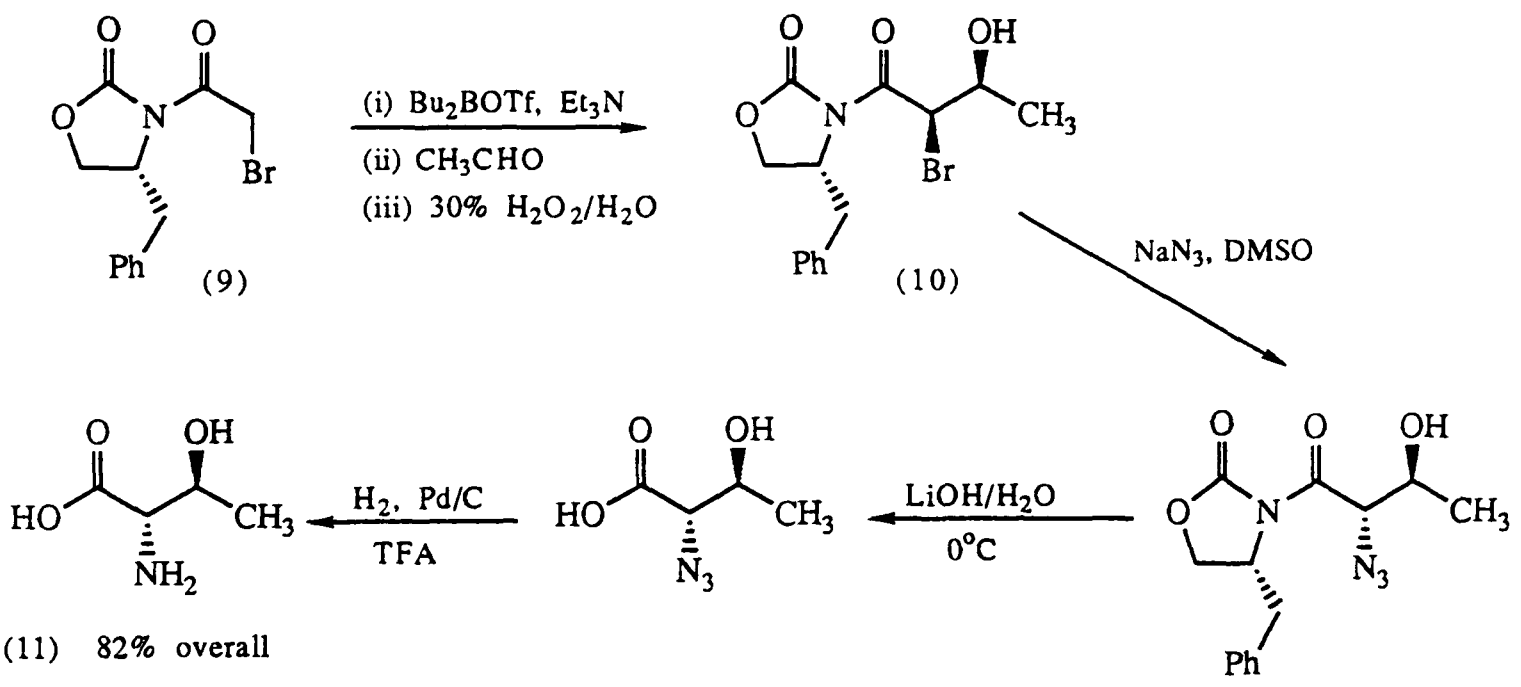
Scheme 1.5

A common procedure to obtain both isomers of an amino acid in optically pure form involves derivatisation of the racemate with a chiral auxiliary, allowing the resulting diastereomers to be separated. For example, derivatisation of the racemic *trans*-2,3-dimethylated 1-aminocyclopropane-carboxylate (6) as the *R*-mandelate derivative, allows (7a) to be separated by fractional crystallisation, releasing (1*R*,2*R*,3*R*)-(8) in an optically pure form (Scheme 1.6).[20]



Scheme 1.6. Reagents: (i) MeOH, HCl; (ii) ethyl 1,2-dihydro-2-ethoxy-1-quinoline carboxylate (EEDQ) (2 equiv.), (R)-(-)-2-hydroxy-2-phenylacetic acid, Et_3N (1 equiv.), CH_2Cl_2, 18 h, 65%; (iii) recrystallisation; (iv) 6*N* HCl, 100°C, 8 h; (v) Dowex 50W-X8 (H) ion exchange.

A useful application of a chiral auxiliary has been demonstrated by Evans.[22] An efficient route to β-hydroxy-α-amino acids was achieved by carrying out highly diastereoselective aldol condensations on the chiral α-haloacetate (9) (Scheme 1.7).[21]



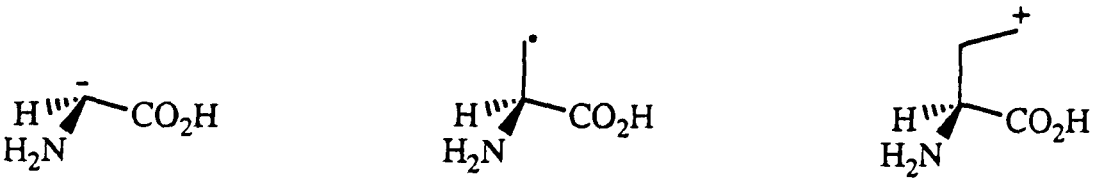
Scheme 1.7

Formation of the dibutylboron enolates followed by condensation with an aldehyde and oxidative removal of the boron provides the α-halo aldol

adduct (10). The utility of such adducts was demonstrated by the subsequent generation of optically pure *L*-allo-threonine (11) without loss of stereochemical integrity. Use of the same chiral auxiliary has led to the syntheses of many other α-amino acid derivatives.[22] Other chiral auxiliaries have been employed by Oppolzer[23], Gennari[24], Vederas[25] and Steglich[26] with varying degrees of enantiomeric excesses.

The limitations of many of the above syntheses has encouraged studies of the asymmetric conversion of inexpensive proteinogenic amino acids into non-proteinogenic amino acids, with which the remainder of this chapter shall be concerned.

Carbon atoms in amino acids are labelled with Greek letters in the order of increasing distance from the carboxyl group. It is thus most convenient to adopt the synthon terminology, the various synthons being described by their distance from the amino acid functionality and the nature of the charge they bear. Examples are shown in Figure 1.2. The various methodologies adopted for the syntheses of these species are reviewed below.

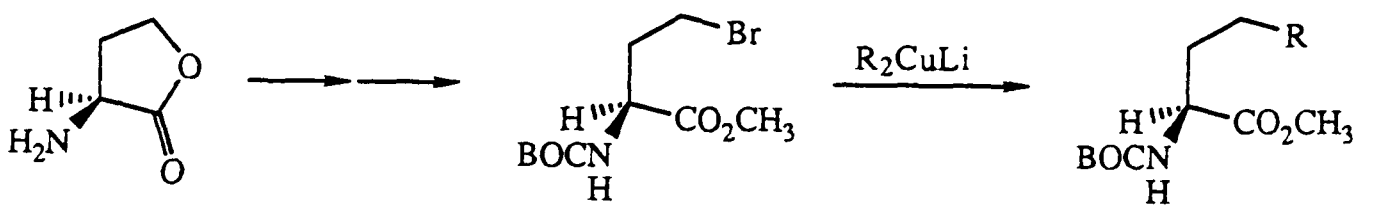


α-anion β-radical γ-cation

Figure 1.2

1.3. The γ-Cation

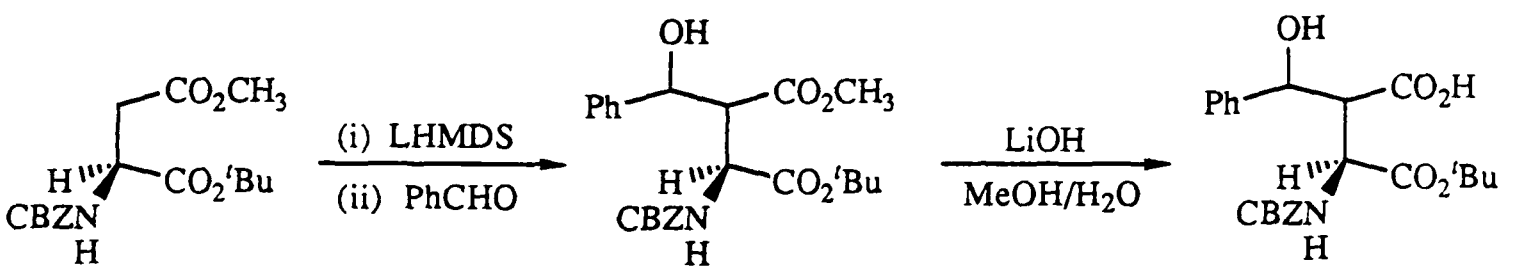
The only work done on this synthon has been reported by Viallefont[27], and involves the ring opening of homoserine lactones with nucleophiles leading to a series of γ-substituted amino acids. Thus, treatment of *L*-homoserine lactone (12) with hydrogen bromide in acetic acid followed by protection, gave methyl 2-*t*-butyloxycarbonylamino-4-bromo-*L*-butyrate (13). Reaction of (13) with organocuprates gave amino acid derivatives with no detectable racemisation (Scheme 1.8).



Scheme 1.8

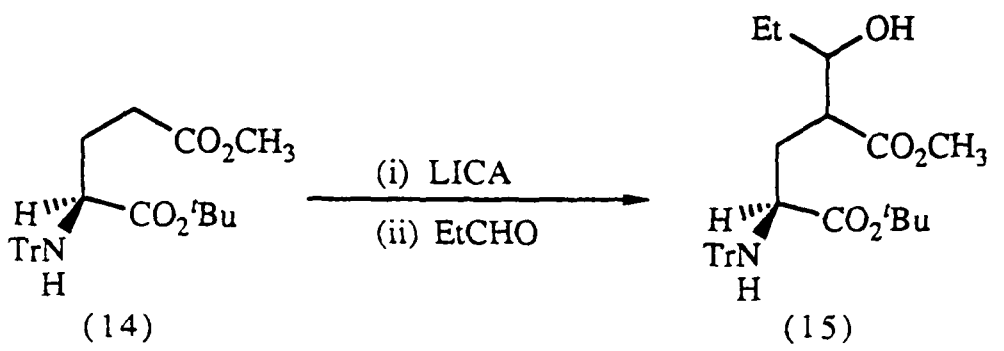
1.4. The β- and γ-Anions

Work carried out in this laboratory has demonstrated the synthetic utility of the β-anion[28] and the γ-anion[29] synthons. The β-anion was generated by the deprotonation of aspartic acid using sterically demanding protecting groups and a sterically hindered base to prevent α-deprotonation. The potential problem of β-elimination was alleviated by using a urethane protecting group. Subsequent treatment with electrophiles gave rise to a series of β-substituted aspartates (Scheme 1.9).



Scheme 1.9

Similar methodology was employed for the generation of the γ-anion. Thus, treatment of α-*t*-butyl γ-methyl *N*-trityl-*L*-glutamate (14) with lithium isopropylcyclohexamide (LICA) and quenching of the anion with electrophiles such as propanal yielded the the γ-hydroxyalkylated product (15) (Scheme 1.10).

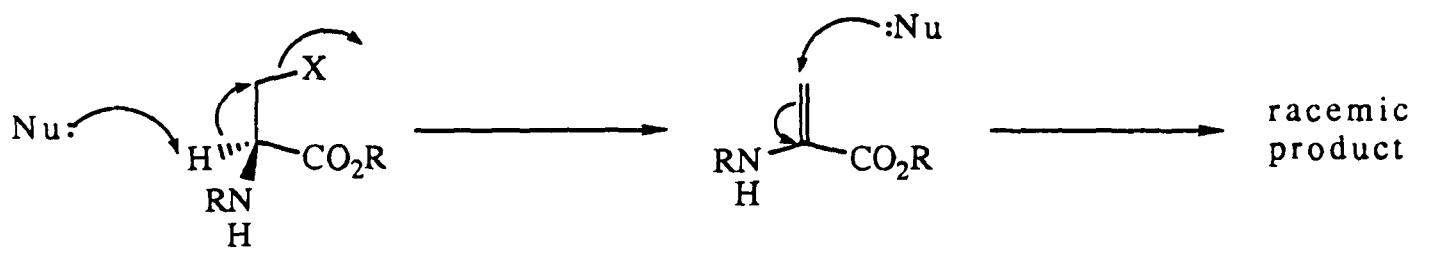


(14) (15)

Scheme 1.10

1.5. The β-Cation

This synthon has been studied by several groups[30,31], but until a few years ago synthetic approaches had met with only a limited degree of success. This was primarily due to the tendency of β-heterosubstituted alanines (16), when treated with nucleophiles (Nu), to undergo elimination of HX giving the corresponding dehydroalanines (17) (Scheme 1.11).



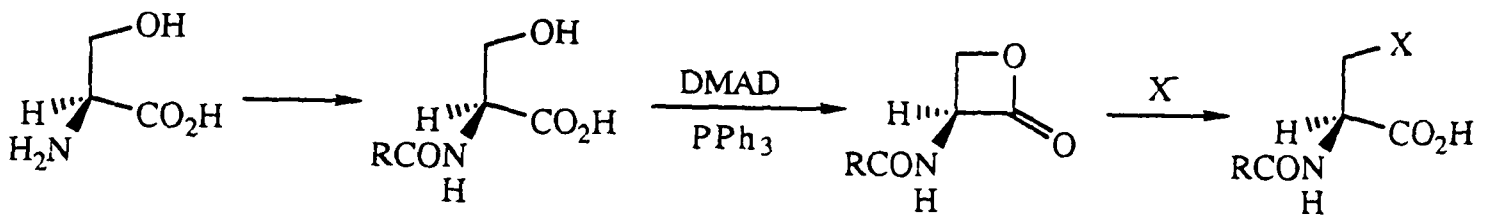
(16) (17)

Scheme 1.11

Jacquier[31], however, exploited the high nucleophilicity and relatively poor basicity of lithium diorganocuprate reagents in an attempt to circumvent the racemisation problems mentioned above. Moderate yields of β-alkylated

alanine derivatives were obtained by treatment of diprotected iodoalanine with 4 equivalents of di-*n*-butyl copper lithium at -60°C.

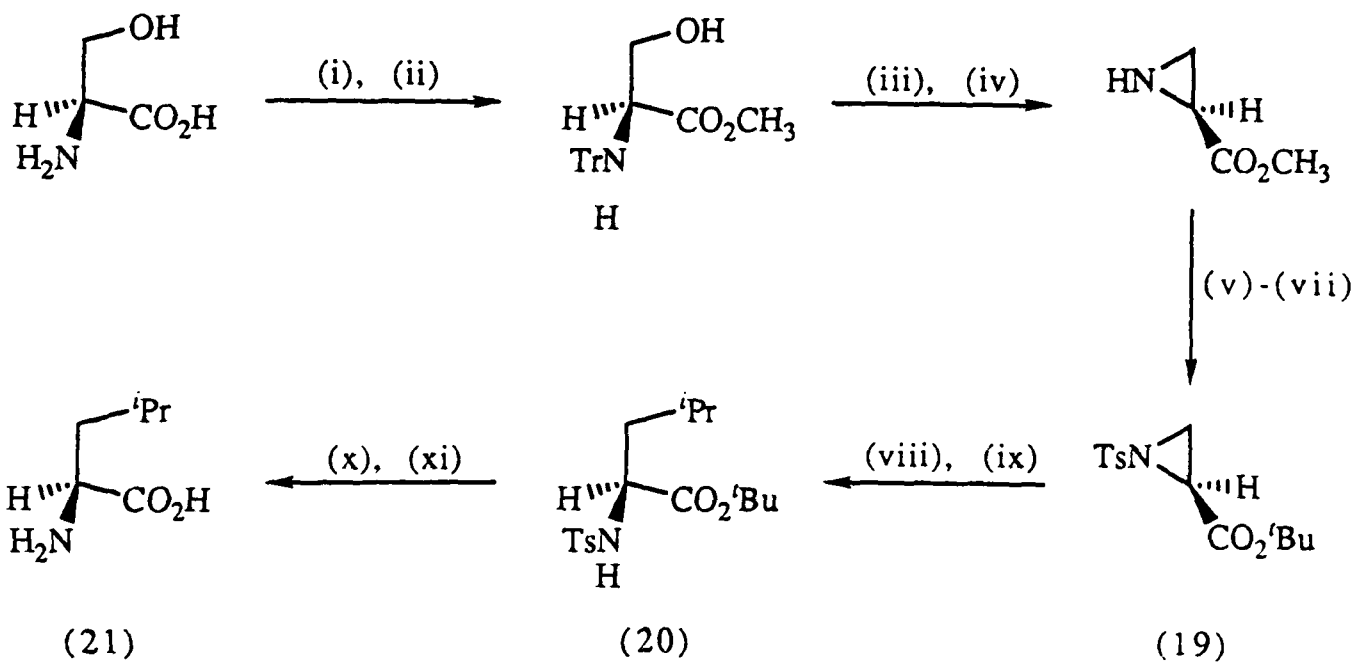
The most successful procedure is probably that developed by Vederas[32]. Either *L*- or *D*-serine was converted in two steps to a β-lactone (18). Reaction of (18) with a range of nucleophiles, including organocuprates, gave β-substituted alanine derivatives in good yield and generally with very good enantiomeric excesses (Scheme 1.12).



(18)

Scheme 1.12

In related procedures developed by workers in this laboratory[33,34], *L*-serine was converted into the aziridine (19). Ring opening with the 'higher order' isopropyl cuprate gave (20), before hydrolysis to the parent amino acid (21), obtained with high optical purity (>95% ee) (Scheme 1.13).



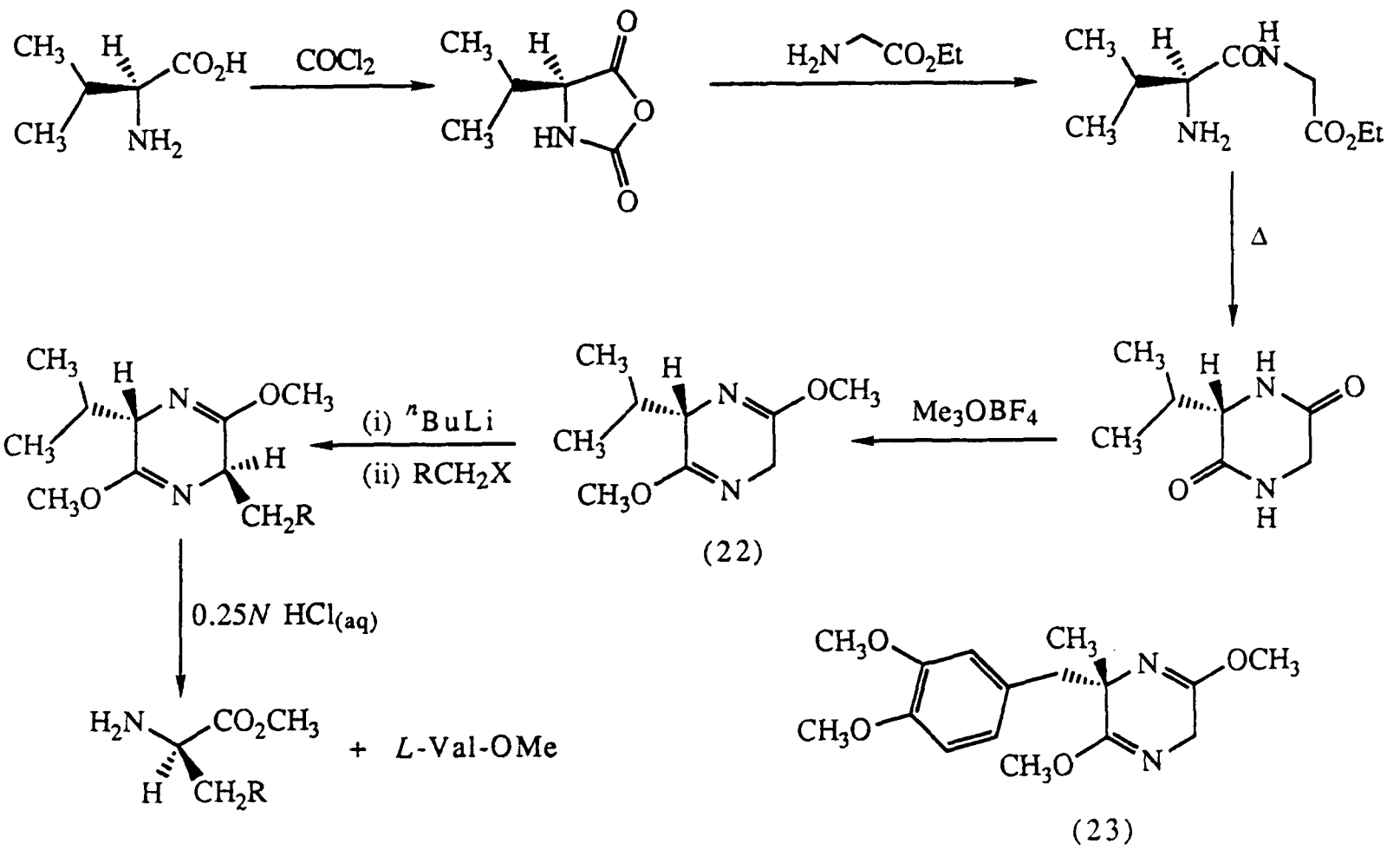
(21) (20) (19)

Scheme 1.13. Reagents: (i) MeOH, HCl; (ii) TrCl, Et_3N; (iii) TsCl, base; (iv) CF_3CO_2H; (v) *p*-$CH_3C_6H_4SO_2Cl$, K_2CO_3, CH_2Cl_2; (vi) LiOH, dioxan/water; (vii) $CCl_3C(NH)O^tBu$ (2 equiv.), $BF_3.Et_2O$, CH_2Cl_2, cyclohexane, 0°C; (viii) ^{i}PrMgCl (5 equiv.), CuBr.SMe_2 (0.2 equiv.), THF/HMPA; (ix) $NH_4Cl_{(aq)}$, 0°C; (x) 33% HBr/AcOH, phenol, 25°C; (xi) ion exchange.

1.6. The α-Anion

This has been the most widely studied synthon[35-46], and in most cases the methodology has involved the synthesis of a chiral, cyclic molecule containing an acidic proton[35-42]. Deprotonation with a strong non-nucleophilic base gives a cyclic anion, with which electrophiles react from the less hindered face, resulting in asymmetric induction.

Schöllkopf[35] has devised probably the most versatile and useful procedure, based upon the formation of bis-lactim ethers. The general protocol involves peptide coupling of two amino acids, usually *L*-valine and glycine, piperazinedione formation, and bis-lactim ether (22) formation with trimethyloxonium tetrafluoroborate (Meerwein's salt) (Scheme 1.14).



Scheme 1.14

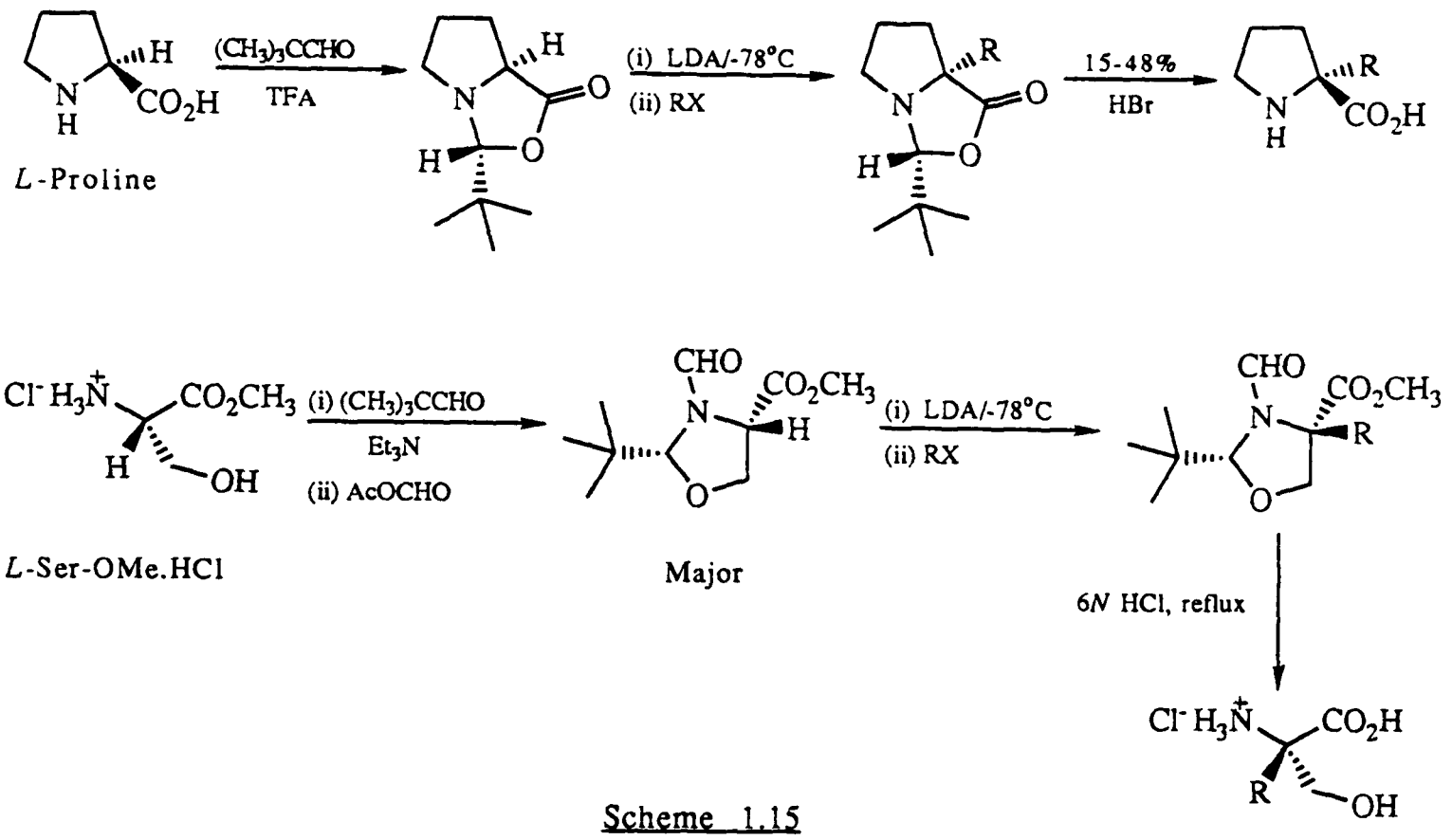
Metallation of the bis-lactim ether (22) with *n*-butyllithium in THF at low temperature (usually -78°C) followed by electrophilic quench furnishes the homologated bis-lactim ethers, the electrophile adding *anti*- to the isopropyl group in a highly stereoselective fashion, with typical diastereomeric excesses of greater than 90%. Recrystallisation followed by mild acidic deprotection gives amino acid esters with high enantiomeric purity. Generally, the free zwitterionic amino acids may be obtained by hydrolysing the methyl esters in 6*N* aqueous hydrochloric acid at reflux for 1 hour.

β-Hydroxyamino acids can be prepared in a similar manner, by quenching the metallated bis-lactim ether with either aldehydes or ketones. High asymmetric induction is again obtained at the alanine α-carbon, but only modest diastereoselection is found at the β-carbon. Other electrophiles successfully employed include nitro-olefins, dihalides, thioketones, acrylates and epoxides.

Diastereomeric excesses in some alkylations can be improved by using the bis-lactim ether (23) derived from *L*-*O*,*O*-dimethyl-α-methyl dopa and glycine. Indeed, several hindered electrophiles give good to excellent alkylation yields from (23).

The major weaknesses of the bis-lactim ether method involve some of the difficulties associated with the hydrolysis of the derivatised bis-lactim ethers to the amino acid methyl esters, and finally (under harsher conditions), to the free amino acids. The separation of the chiral auxiliary from the new amino acid can also be problematical and time-consuming.

Seebach *et al*[40] have made a useful contribution to the practical asymmetric synthesis of amino acids *via* the formation of various cyclic aminals of numerous amino acids and the subsequent enolate alkylation of such systems with a high degree of diastereoselectivity. Two examples are illustrated in Scheme 1.15 below.



Scheme 1.15

The latter approach culminated in the development of a general and practical method for preparing a wide variety of novel amino acids employing optically active oxazolidinone and imidazolidinone derivatives, shown in Figure 1.3. These are discussed further in Chapter 3.

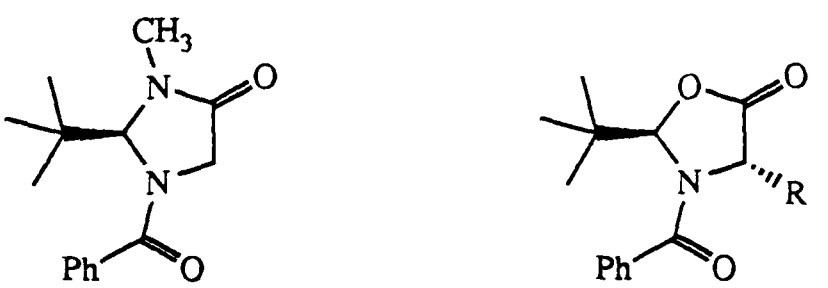
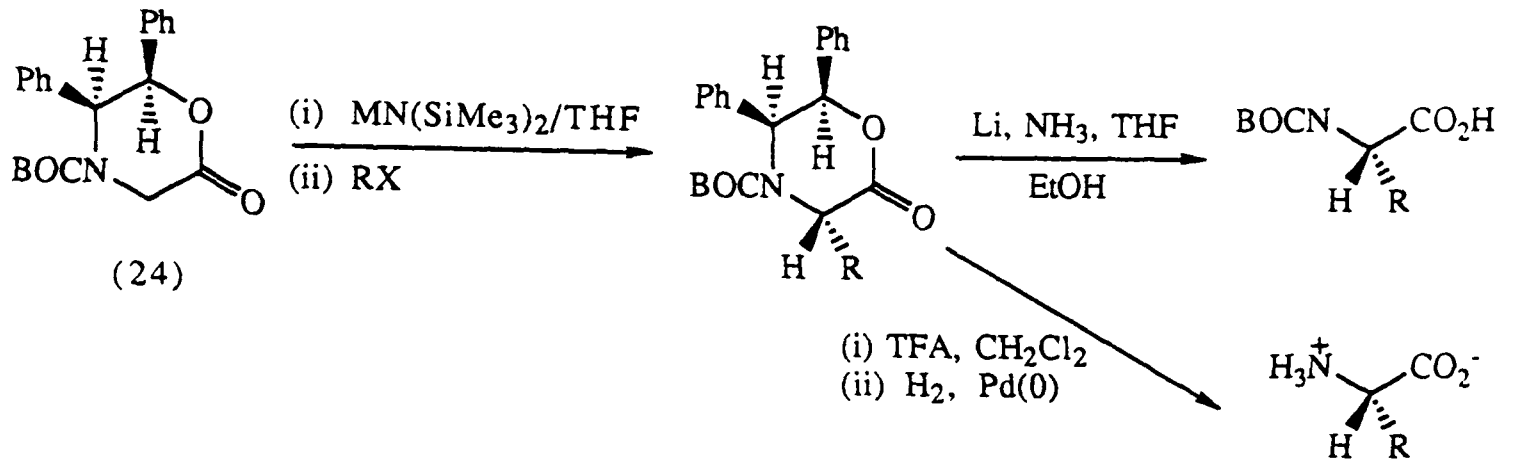


Figure 1.3

Williams and Im[41c] examined the enolate alkylation of the 5,6-diphenyl-2,3,5,6-tetrahydro-4H-oxazin-2-ones (24), as shown in Scheme 1.16.

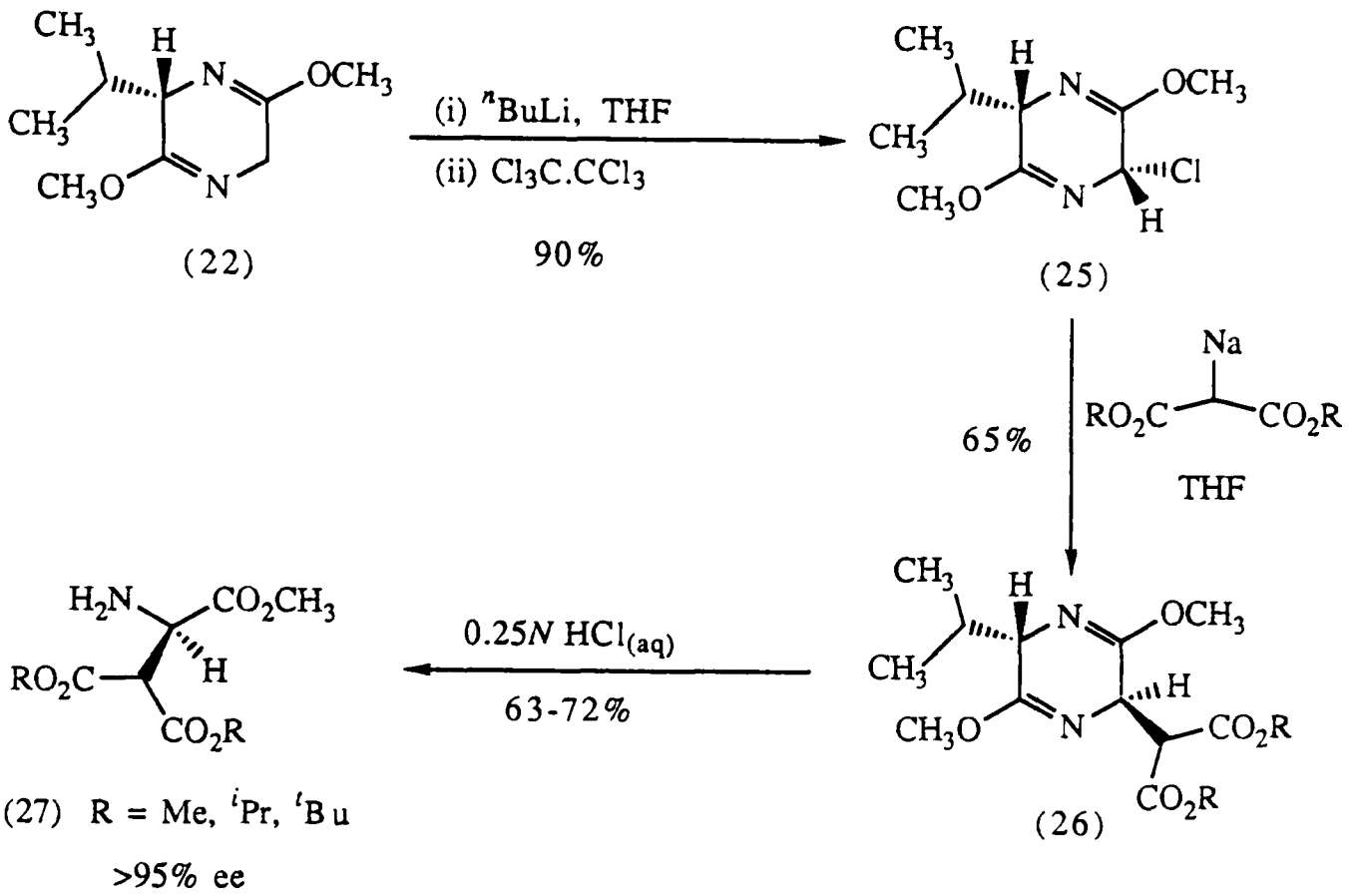
However, these systems have been much more extensively examined as electrophilic glycinates, as discussed in section 1.7 below.



Scheme 1.16

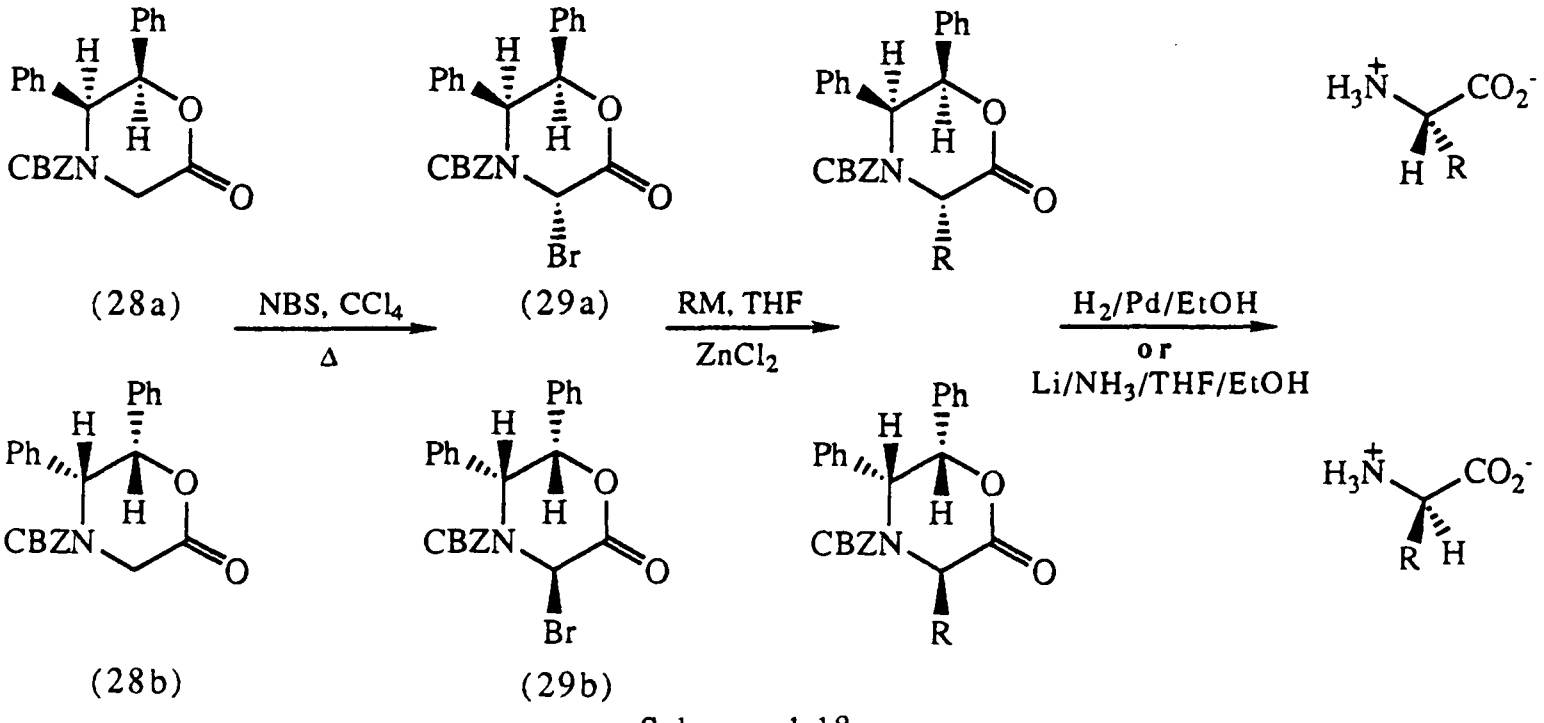
1.7. The α-Cation

The α-cation has been less intensively studied than the α-anion, although this area is currently the focus of much interest. Schöllkopf *et al*[47] found that chlorination of the enolate derived from the Val-Gly bis-lactim ether (22) with hexachloroethane provided the labile chloride (25) as a 94:6 *cis*:*trans* mixture (Scheme 1.17). Elimination of HCl from (25) to form the corresponding aromatic 2,5-dimethoxy-3-isopropylpiperazine was found to occur very readily; thus immediate condensation of (25) with esters of sodium malonate provided the malonates (26) in *ca* 65% yield. The malonate anions attacked the electrophilic glycine cation of (25) with inversion to give the *trans*-isomer as the major product. Hydrolysis with 0.25*N* aqueous hydrochloric acid provided the corresponding methyl, isopropyl and *t*-butyl esters (27) of β-carboxyaspartic acid methyl ester in good yields and with >95% ee.



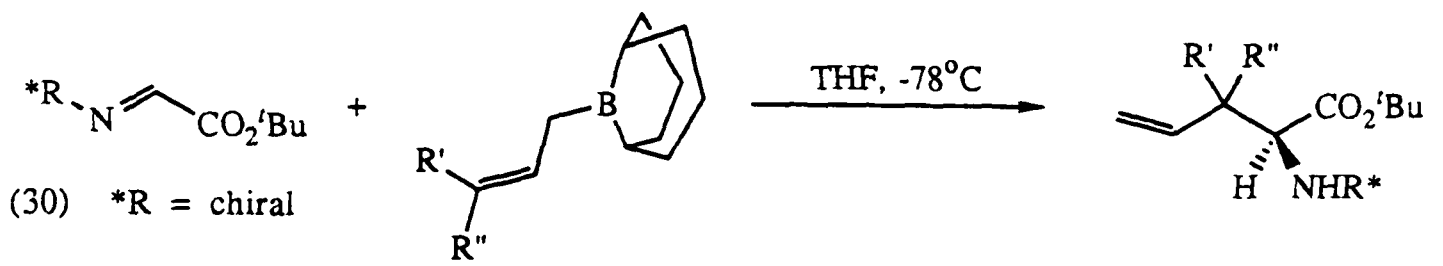
Scheme 1.17

However, the most extensively studied optically active electrophilic glycine template has been developed by Williams *et al*[48]. Thus bromination of the oxazinones (28a, 28b) with *N*-bromosuccinimide yielded the corresponding bromides (29a, 29b). Subsequent reaction with various organometallic reagents in the presence of zinc chloride gives, after reduction, *L*- and *D*-amino acids (Scheme 1.18).



Scheme 1.18

Yamamoto *et al*[49] have studied the additions of various allylic organometallic reagents to the chiral imines (30) with the chiral auxiliary attached to the glycine nitrogen atom (Scheme 1.19). It was found that allyl-9-BBN reagents gave the best yields and highest diastereoselectivities, whereas the corresponding zinc, magnesium and titanium reagents gave poor results.



Scheme 1.19

1.8. The α-Radical

No general procedure for the synthesis of α-amino acids *via* an α-radical system has yet been developed; work in this area has been the subject of Part Two of this thesis and is discussed in Chapters 3 and 4. However, assuming that an α-radical may be generated, a suitable series of radicalphiles is needed to gain access to a range of homologated glycines. This requirement is satisfied by the organotin reagents, whose potential to provide synthetically viable and selective transformations under neutral conditions has only been exploited during the last two decades.[50] A review of the use of organotin reagents, particularly allylic stannanes, is presented in the following chapter.

2. FREE RADICAL APPLICATIONS OF ORGANOSTANNANES

2.1. Conditions for Radical Reactions

One of the most useful synthetic applications of organotin compounds involves the generation of carbon-centred radicals to produce carbon-carbon[51,52] and carbon-heteroatom bonds[53]. These procedures generally involve the reactions of radicals with non-radicals, since this method has obvious advantages over radical-radical processes:

(i) radical character is not destroyed during the bond-forming reaction; thus initiators need only be present in catalytic quantities;

(ii) the concentration of non-radicals is relatively simple to control; and

(iii) as the reactions are not diffusion controlled, the selectivities can be influenced by variation of substituents.

Thus, synthetically viable radical-non-radical reactions must involve chain processes. For these free radical chain processes to be successful, a number of the reaction processes must be controlled. Keck[52] has delineated several important constraints:

(i) specific generation of initiator radicals;

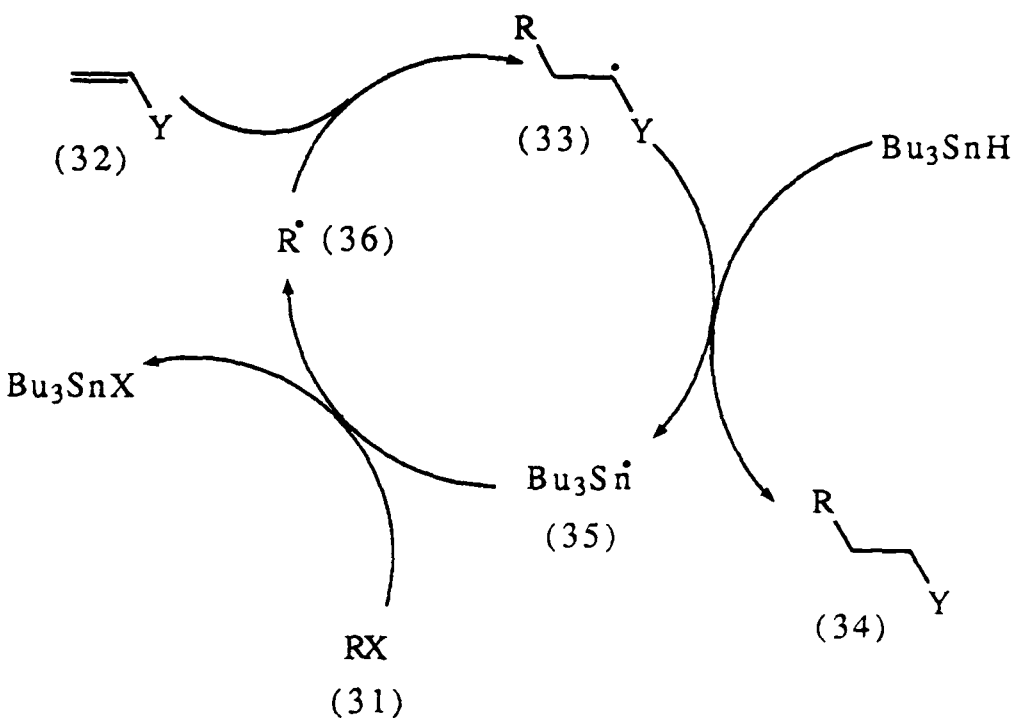
(ii) selective, low energy pathways for the production of substrate radicals;

(iii) chain carrying steps employing reagents which preclude the formation of highly reactive, indiscriminate radicals; and

(iv) reasonable termination steps to produce innocuous by-products which do not disturb the chain.

These rules can be illustrated by a chain reaction that has gained increasing synthetic importance in the last few years.[51] Alkyl halides (31)

and alkenes (32) react in the presence of tri-*n*-butyltin hydride to give the products (34) (Scheme 2.1). For a successful application of the tin method, alkyl radicals (36) must attack alkenes (32) to form adduct radicals (33). Trapping of (33) yields product (34) and the tributyl tin radical (35), which react with alkyl halides (31) to give back educt radicals (36).



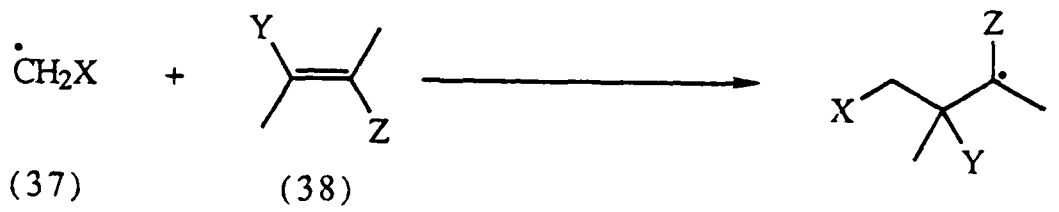
Scheme 2.1

The tin method can be synthetically useful only if these processes are faster than all other possible reactions of radicals (36), (33) and (35). Therefore the radicals in the chain must meet certain selectivity and reactivity prerequisites. The influence of substituents on radical reactivity, regioselectivity and stereoselectivity has been exhaustively studied by Giese *et al* [54], and is briefly summarised below.

2.2. Influence of Substituents

The addition of alkyl radicals to alkenes involves forming a strong carbon-carbon σ-bond from a carbon-carbon π-bond, thus resulting in an exothermic reaction; according to Hammond's postulate[55], the transition

states should lie very early on the reaction coordinate and should resemble the reagents. Thus the rates of addition of a radical to an alkene depend largely on (i) the substituent X on the attacking radical (37) and (ii) the substituents Y(α) and Z(β) on the alkene (38) (Scheme 2.2).



(37) (38)

Scheme 2.2

These substituent effects can be described by Frontier Molecular Orbital (FMO) Theory[54]. The singly occupied orbital (SOMO) of the radical interacts with the lowest occupied orbital (LUMO) and/or the highest occupied orbital (HOMO) of the C-C multiple bond. Radicals with a high lying SOMO interact preferentially with the LUMO of the alkene (Figure 2.1). Electron withdrawing substituents at the alkene, which lower the LUMO energy, increase the addition rate by reducing the SOMO-LUMO difference.

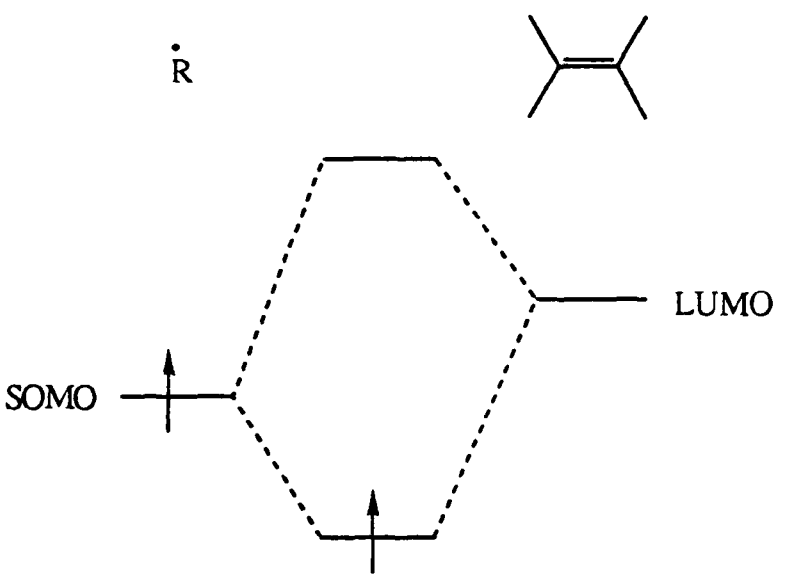


Figure 2.1

This is illustrated by the fact that cyclohexyl radicals react approximately 9000 times faster with acrolein than 1-hexene (Table 2.1).[51] Thus β-substituents exert on the whole, only polar effects on the rate of addition of

free radicals. Radical stabilising and space filling β-substituents have little effect on the rate of addition.

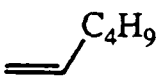
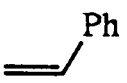
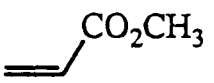
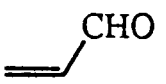
Olefin	k_{rel}
	1
	84
	3000
	8500

Table 2.1

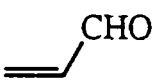
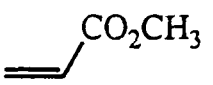
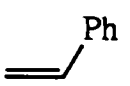
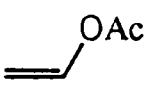
Olefin	k_{rel}
	34.0
	6.7
	1.0
	0.016

Table 2.2

If, instead of electron withdrawing groups, the alkene contains electron donating groups, the LUMO energy is raised with a concomitant increase in the SOMO-LUMO energy difference, causing a decrease in the rate of addition of n-hexyl radicals to the alkene (Table 2.2).

The effect of α-substituents is markedly different from that of the β-effect. This is due to the steric hindrance by the substituent Y offered to the approach of free radicals. In contrast to the small steric β-effect, the steric α-effect is very large. When the latter is taken into consideration, it has been found that polar α-effects are much smaller than polar β-effects.

Substituents on the attacking radical exert both polar and steric effects on the rate of addition to alkenes. It has been found[56,57] that alkyl groups X increase the reactivity of radicals (37) in addition reactions with electron deficient alkenes whilst increasing the stability of those radicals. For relatively small alkyl substituents, polar effects, which increase the nucleophilicity of radicals with increasing number of alkyl substituents at

the radical centre, are apparently more important than the effects of stability of the radical; thus more substituted radicals tend to react faster. More strongly electron donating alkoxy substituents further increase the reactivity of radicals in addition reactions to electron deficient alkenes whilst electron withdrawing substituents decrease the rate of addition.[57]

These observations may also be explained in terms of FMO theory. Electron donating substituents incorporated into the radical decrease the SOMO-LUMO energy difference;[58] hence the *t*-butyl radical reacts more rapidly than a secondary or primary radical. Strongly electron withdrawing substituents decrease the SOMO energy of the radical to such an extent that it becomes electrophilic and the SOMO-HOMO interaction becomes dominant (Figure 2.2).[59] Electron donating substituents raise the HOMO-energy of the alkene, so that the SOMO-HOMO energy decreases, thus explaining the greater reactivity of electrophilic radicals towards electron rich alkenes.

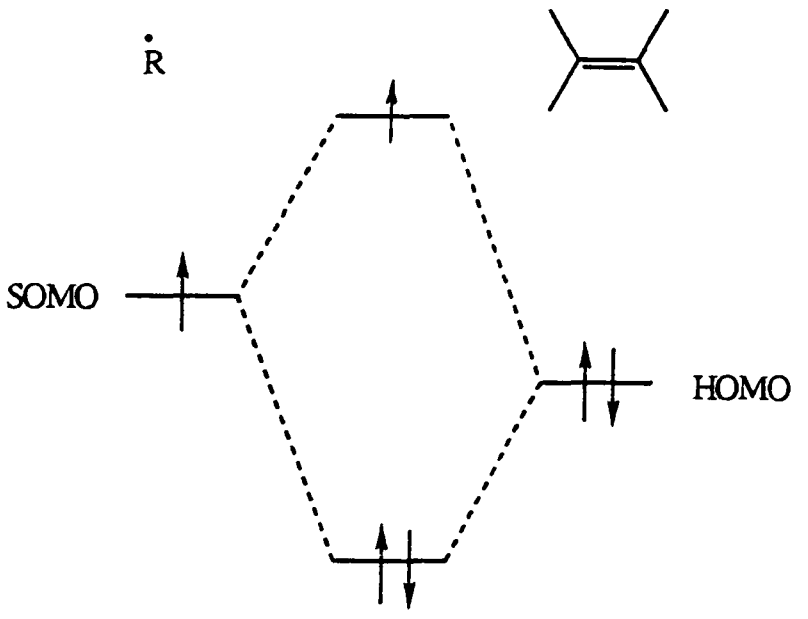
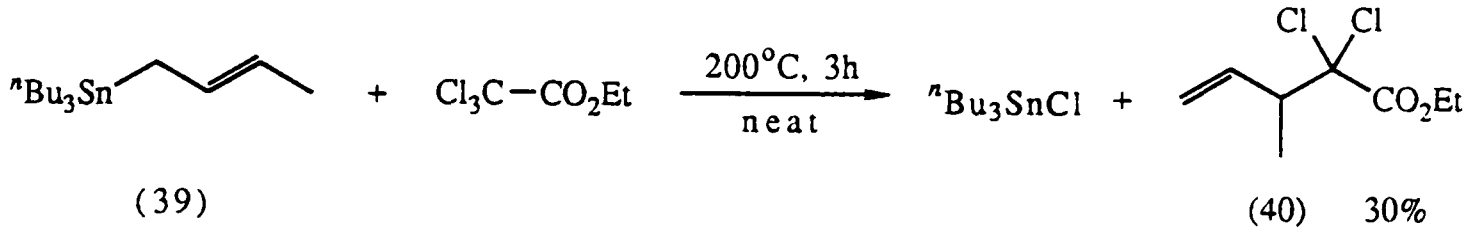


Figure 2.2

2.3. The Allyl Transfer Reaction

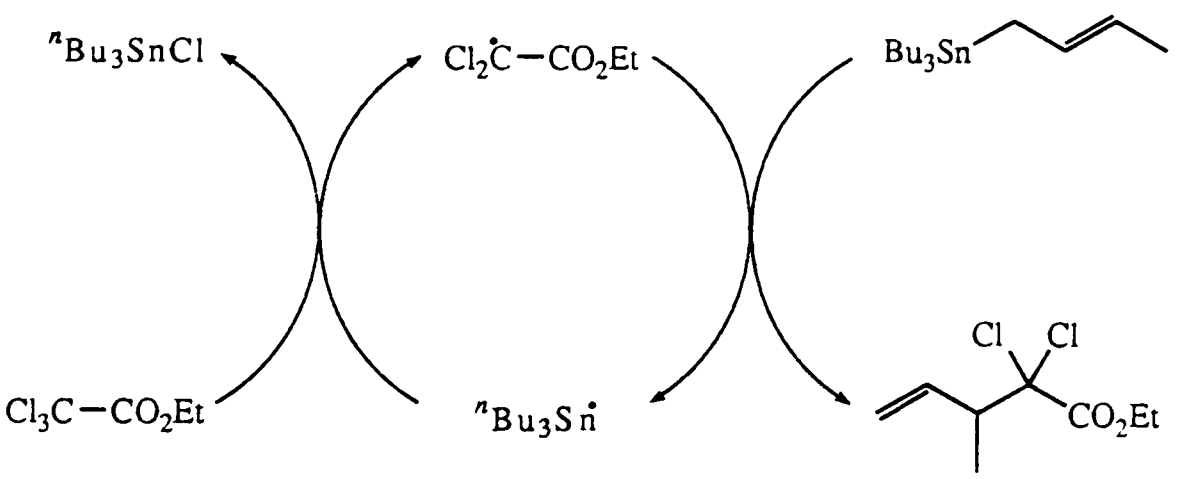
The utility of organotin compounds was first demonstrated by the mild organotin hydride reductions of alkyl halides and multiple bonds to the corresponding alkanes,[60,61] and more recently in the more interesting carbon-carbon bond forming reaction of trialkylallylstannanes with alkyl halides and other suitable substrates, to give allylated products. The reaction was initially described by Pereyre[62] and Migita[63], and was subsequently exploited by Keck[64,66-70].

Pereyre *et al*[62] utilised the crotyl stannane (39) to give the product (40) derived from allylic transposition *via* an intermolecular process (Scheme 2.3).



(39) (40) 30%

Scheme 2.3



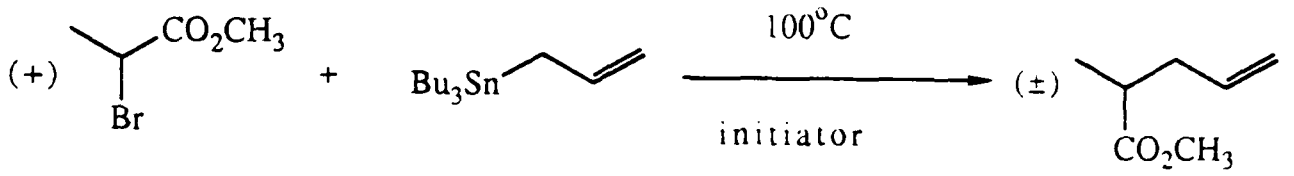
Scheme 2.4

Evidence[63,65] for the proposed S_H2' mechanism (bimolecular homolytic substitution with allylic transposition) (Scheme 2.4) was based on the following experimental observations:

(i) the reaction was promoted by initiators such as AIBN and inhibited by radical scavengers such as *p*-quinone;

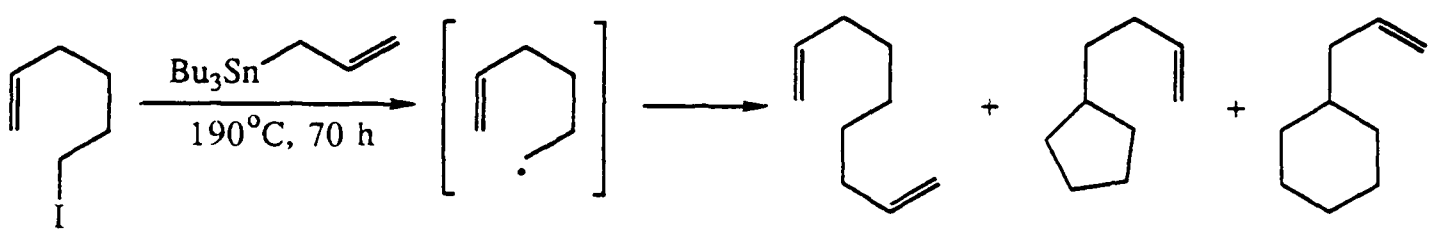
(ii) the order of reactivity of alkyl halides was RI > RBr > RCl;

(iii) a chiral substrate with a bromine atom at the chiral centre gave a racemic product (Scheme 2.5);



Scheme 2.5

(iv) 5-hexenyl radicals generated during an allyl transfer reaction gave cyclised products along with the allyl transfer products (Scheme 2.6);



Scheme 2.6

(v) in an experiment involving Me_3CBr, Me_2CHBr and $Me(CH_2)_2Br$, the relative rate constants for the allyl transfer from allyltri-*n*-butylstannane were 8.6, 2.1 and 1.0 respectively.

(vi) transfer of the crotyl group occurred with transposition; similar transposition was assumed for an unsubstituted allyltin reagent.

The original highly chlorinated radical precursors gave poor yields. Reactions typically involved the use of neat reagents (dibenzoyl peroxide

initiation) within a reaction tube which was degassed, evacuated, and heated to 80°C for 15 hours.

Recently Keck *et al*[52] developed a general set of conditions which facilitate efficient allyl transfer reactions of the type outlined above. Typically, two equivalents of allyltri-*n*-butylstannane, one equivalent of substrate and 0.15 equivalents of AIBN in dry degassed toluene or benzene are either heated at 80°C for 8 hours, or subjected to photolysis (in the absence of AIBN) for 5 hours at room temperature. Higher yields have also been obtained by the use of alkyl bromides[66], selenides[66], thiophenyls[64] and thiocarbonates[68] as radical precursors. Examples of the utility of this method are shown in Table 2.3; the reactions are also successful if 2-methylallyltri-*n*-butylstannane is used as the allyl transfer reagent.

Thus, free radical alkylations using allyl or methallyl tri-*n*-butylstannanes are often stereospecific and are extraordinarily tolerant of complex functionality in the substrate, which in many cases would be expected to be incompatible with 'two electron' approaches to achieve the same net bond construction.

This method is a powerful complement to various oxidative addition reactions, such as halolactonisation and selenolactonisation, enabling a high degree of stereocontrol. This has been illustrated in the total syntheses of (±)-perhydrohistrionicotoxin[67], (+)-compactin[69] and (+)-pseudomonic acid C (43)[70]. The latter is synthesised from *L*-lyxose (41), the vital C-C bond forming reaction utilising allyl transfer from allyltri-*n*-butylstannane to (42) (Scheme 2.7).

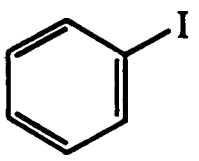
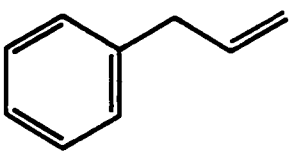
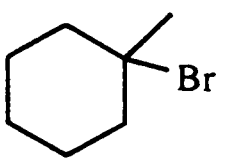
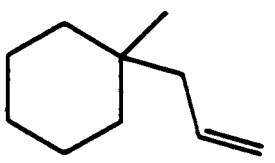
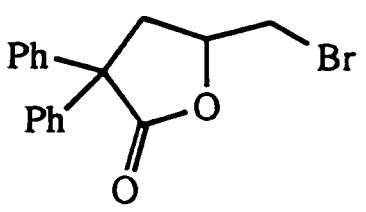
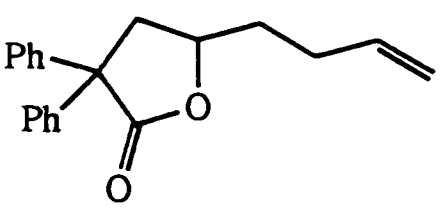
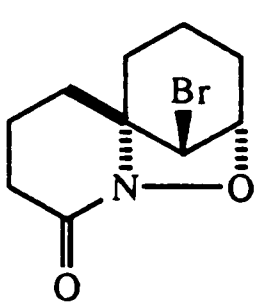
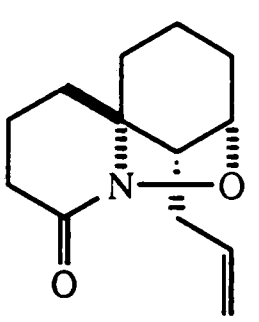
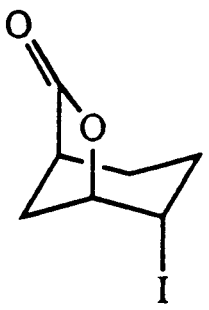
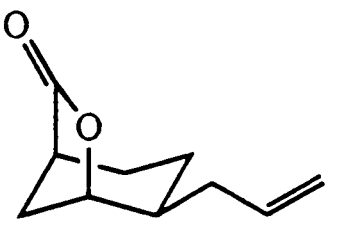
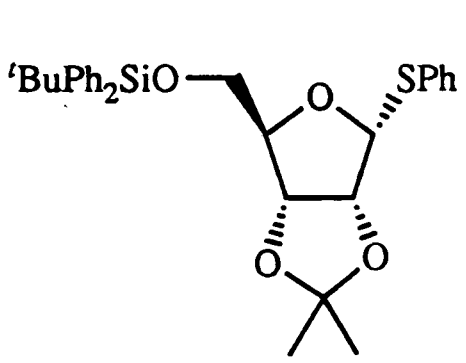
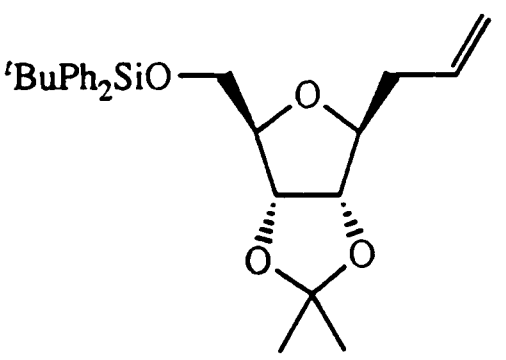
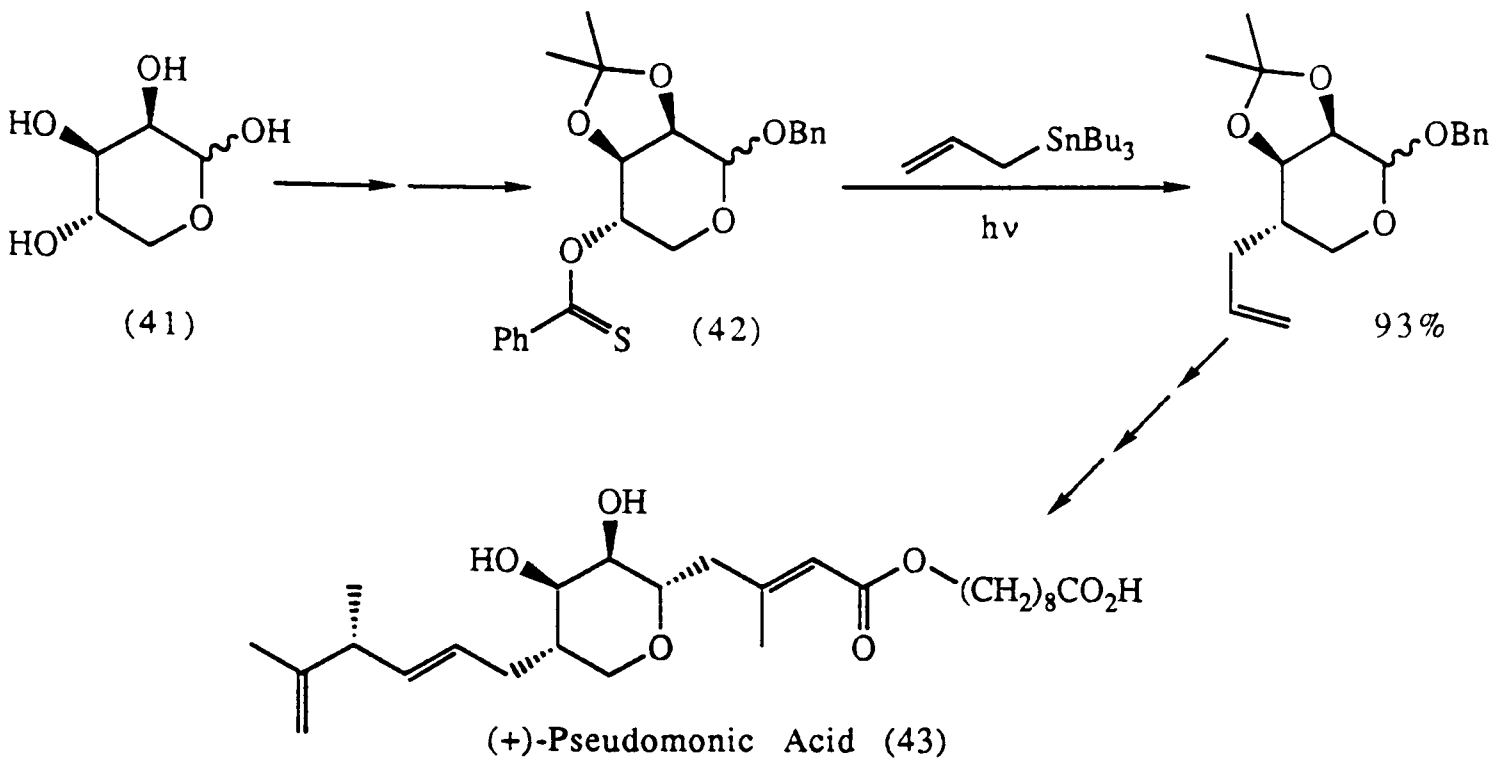
Substrate	Product	Yield %
		Δ 57
		Δ 90
		Δ 88 hν 82
		Δ 93 hν 88
	 major isomer (*ca* 2:1)	Δ 65 hν 81
	 major isomer (*ca* 5:1)	hν 86

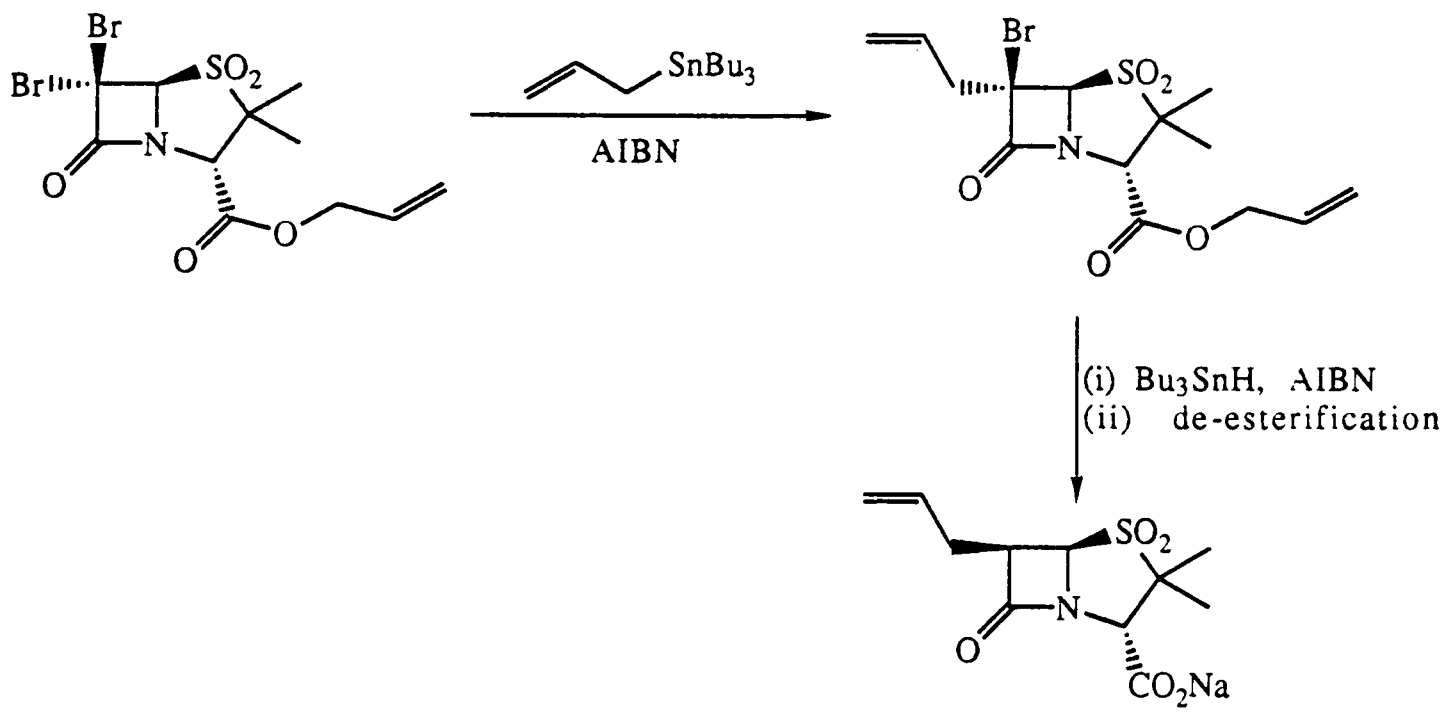
Table 2.3



(+)-Pseudomonic Acid (43)

Scheme 2.7

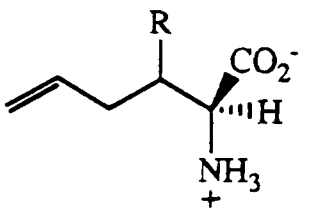
Hanessian[71] has observed that highly efficient radical induced allyl transfer reactions are possible with 6-bromo and 6,6-dibromo penams, giving excellent chemo- and stereoselectivity; 6β-Allylpenicillin-1,1-dioxide sodium salt (44) was synthesised as a novel β-lactamase inhibitor (Scheme 2.8).



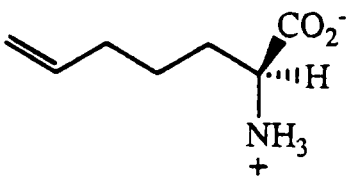
Scheme 2.8

(44)

The amino acids (45-47)[72] which were incorporated into tripeptides and employed in studies on penicillin biosynthesis have also been synthesised *via* allyl transfer.

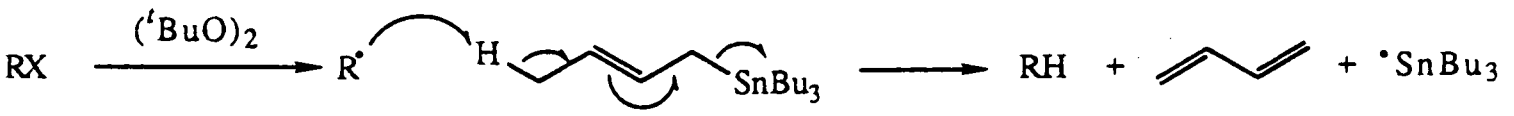


(45) R=H
(46) R=CH_3



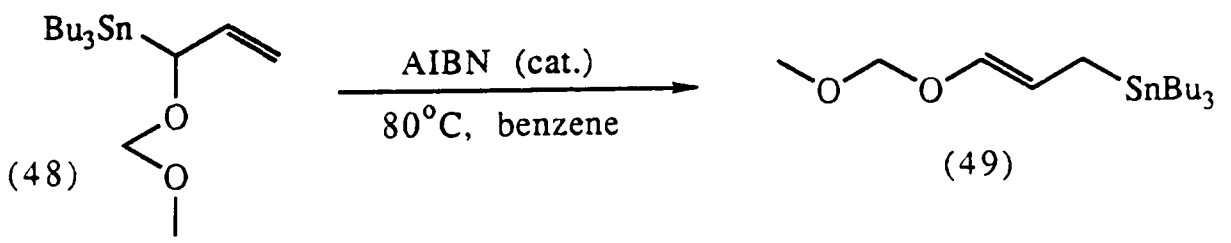
(47)

It has been found[73] that crotyl tri-*n*-butylstannane (3-methylallyl tri-*n*-butylstannane) is not a synthetically useful allylic transfer reagent using the general reaction protocol described above; under the forcing conditions[52] required for it to react, namely reflux in xylene or chlorobenzene with di-*t*-butyl peroxide as initiator, the only products observed correspond to simple reduction (Scheme 2.9).



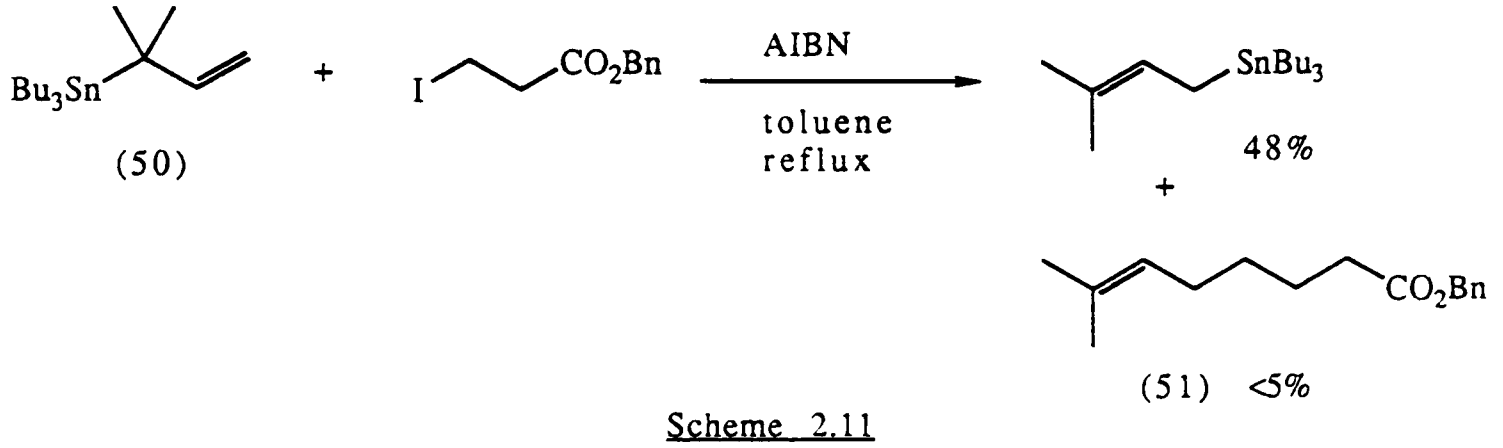
Scheme 2.9

In an attempt to incorporate heteroatom functionality into allyl transfer reagents, (1-methoxymethoxy)prop-2-enyltri-*n*-butylstannane (48)[74] was synthesised, but gave only the rearranged product (49) when subjected to the standard allyl transfer conditions (Scheme 2.10).[75]



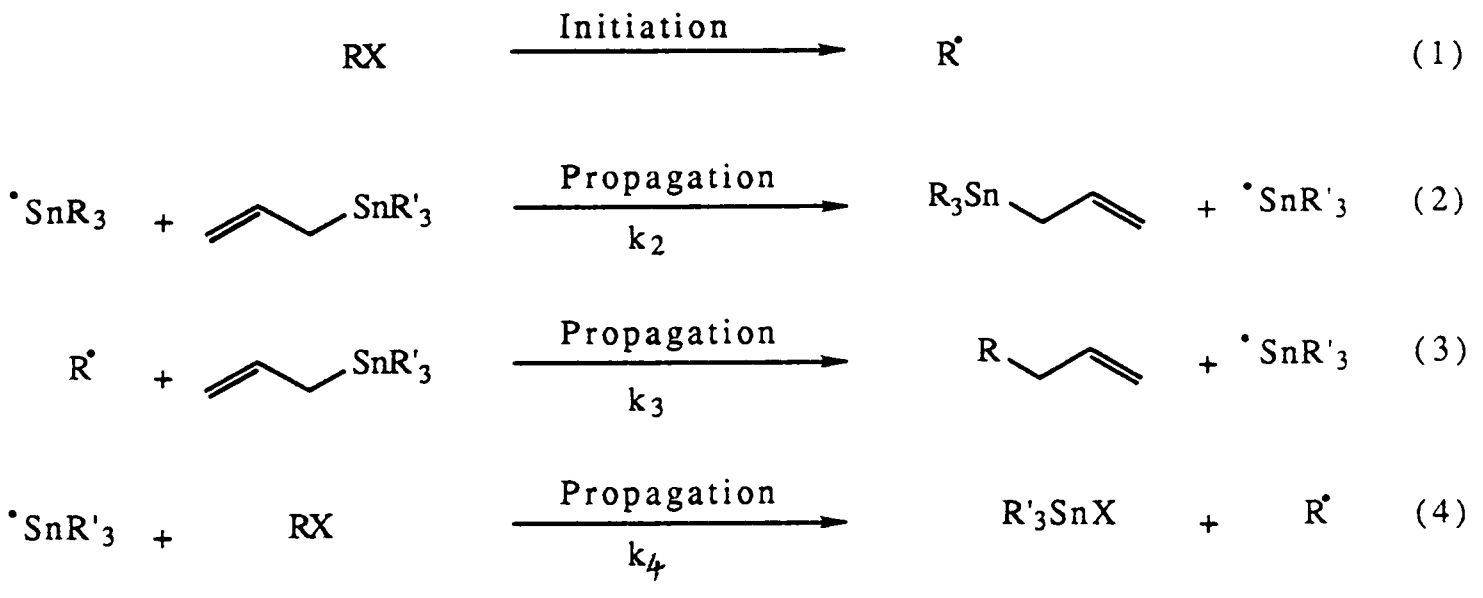
Scheme 2.10

Similar results were obtained when 1,1-dimethylallyltri-*n*-butylstannane (50)[76] was used as a potential free radical prenylating agent. Reaction with benzyl-3-iodopropionate in refluxing toluene yielded mainly the rearranged stannane and only a small amount of the desired product (51) (Scheme 2.11).[75]



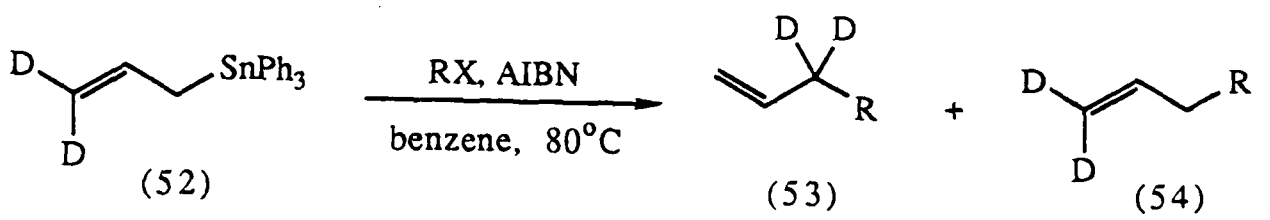
Scheme 2.11

A competing step must therefore be included in the generally accepted mechanism for the allyl transfer reactions of allylstannanes, namely addition of the stannyl radical to the allyl stannane, thus accounting for the observed rearrangements (Scheme 2.12).



Scheme 2.12

Thus, the relative rates of steps (2) and (3) determine whether or not rearrangement of the allylstannane is important with respect to allyl transfer. This mechanism was investigated using the specifically deuterated allylstannane, 3,3-dideuterioallyltriphenylstannane (52)[75] with various alkyl halides to give alkylated products (53) and (54) (Scheme 2.13).



Scheme 2.13

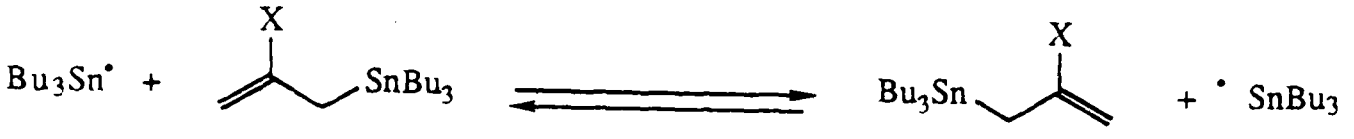
If the rearrangement, step 2 above, was fast compared to radical allylation, step 3, a 1:1 ratio of (53):(54) should be observed. If this step was relatively slow, then the S_H2' mechanism should lead to the predominance of (53). Some results are shown in Table 2.4.

R	X	Yield (53 + 54) %	Ratio (53):(54)
1-adamantyl	Br	88	> 10:1
n-decyl	I	45	> 10:1
n-decyl	Br	16	3:1
n-decyl	Cl	4	1:1

Table 2.4

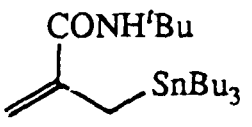
Thus, for a given alkyl substituent, the strength of the C-X bond determines the degree of rearrangement of the allylstannane; the comparatively weak C-I bond makes k_4 relatively fast for *n*-decyl iodide, whereas the comparatively strong C-Cl bond makes k_4 relatively slow. The stability of the radical produced from cleavage of the carbon-halogen bond also has an obvious importance.

The scope of allyl transfer reactions with respect to the structure of the stannane is therefore restricted. Only the parent allyltri-*n*-butylstannane or 2-substituted derivatives possessing a latent symmetry (e.g. 2-methyl-allyltri-*n*-butylstannane) have been used without complication since the competing radical rearrangement is degenerate (Scheme 2.14).

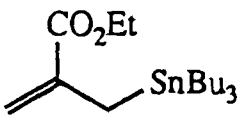


Scheme 2.14

Recent investigations into the electronic nature of the 2-substituent in trialkyl and triarylallylstannanes have been conducted in this laboratory.[75] The double bond in allylstannanes is electron rich due to C-Sn σ-bond electron donation and thus the rate of addition of nucleophilic alkyl radicals to allylstannanes should increase with the electron-withdrawing nature of the 2-substituent. The allylstannanes, α-(tri-*n*-butylstannylmethyl)acrylamide (54)[77,78,79] and ethyl α-(tri-*n*-butyl-stannylmethyl)acrylate (56)[75] were synthesised and shown to be efficient allyl transfer reagents with several alkyl halides under thermal conditions (Table 2.5).[75,79]



(55)



(56)

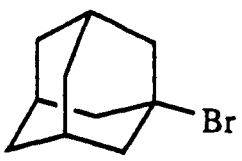
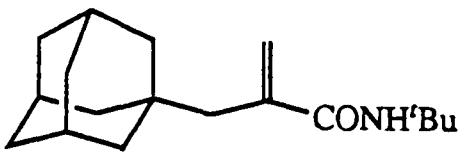
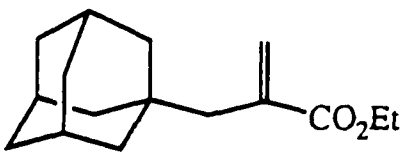
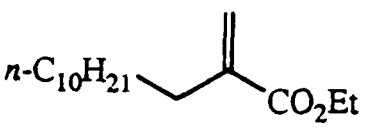
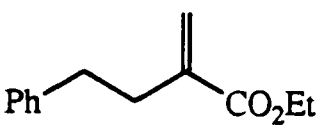
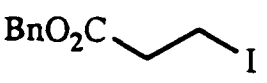
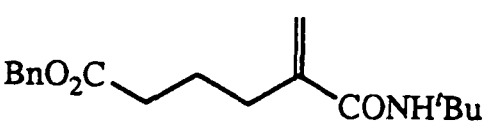
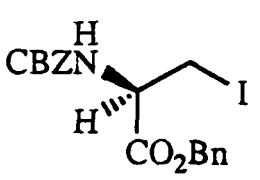
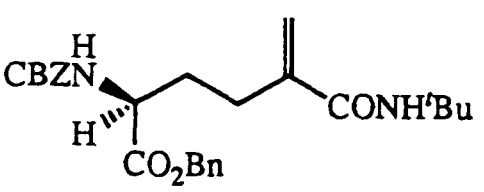
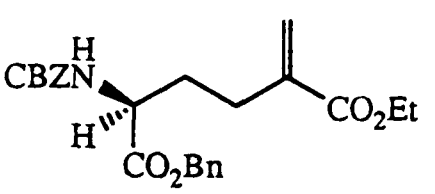
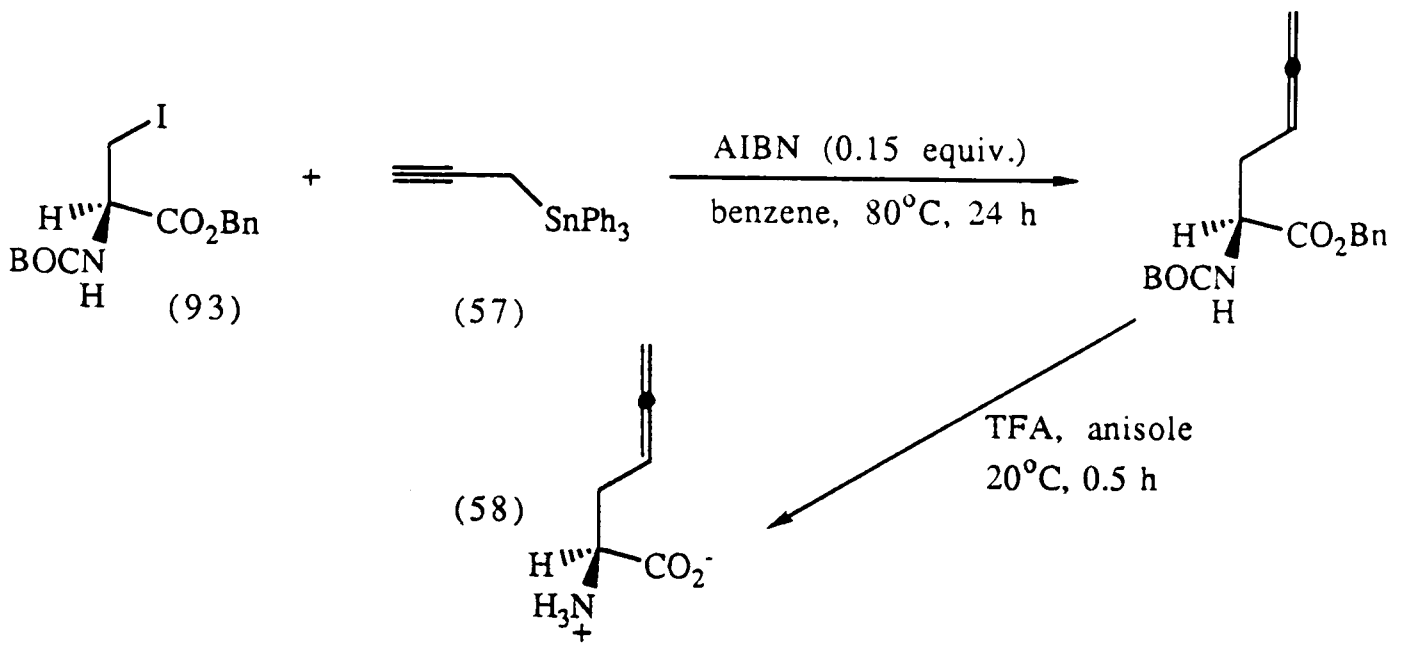
Alkyl halide RX	Alkylation product from (55) (%)	Alkylation product from (56) (%)
	 76%	 70%
n-$C_{10}H_{21}I$	——	 70%
$PhCH_2Br$	——	 76%
	 62%	——
	 62%	 70%

Table 2.5

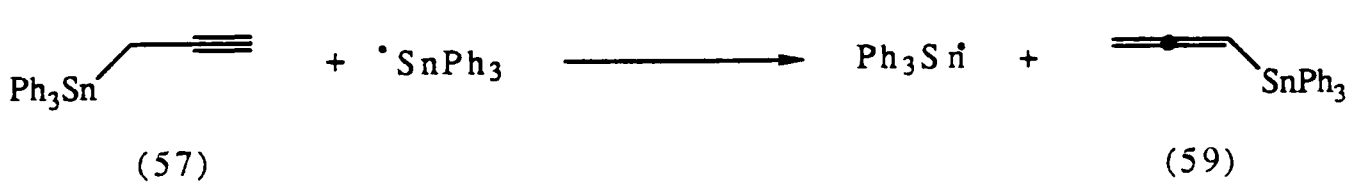
2.4. The Allene Transfer Reaction

'Allene transfer', a S_H2' reaction analogous to allyl transfer has been used in this laboratory in the synthesis of natural products. For example, a relatively simple and stereodefined synthesis of the naturally occurring *S*-2-aminohexa-4,5-dienoic acid (58)[80] was achieved, the crucial reaction being allene transfer from the propargylic stannane (prop-2-ynyltriphenylstannane) (57) to the optically active β-iodoalanine derivative (93) (Scheme 2.15).



Scheme 2.15

For good preparative yields, however, it was necessary to use four equivalents of the propargyl stannane, since a competing S_H2' reaction of the stannyl radical with (57) occurred to give the relatively unreactive 1,2-dienylstannane (59) (Scheme 2.16).

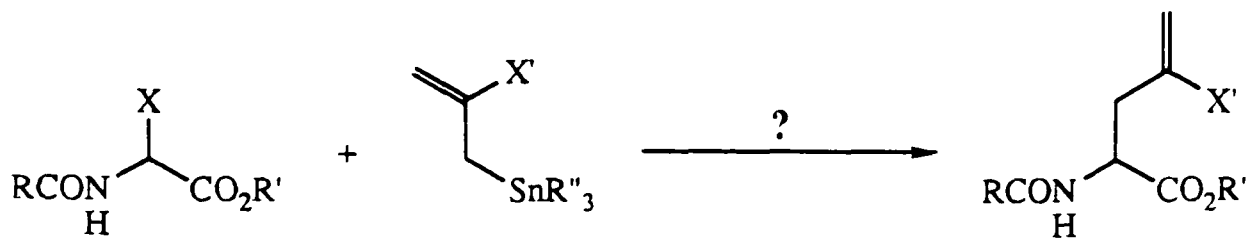


(57) (59)

Scheme 2.16

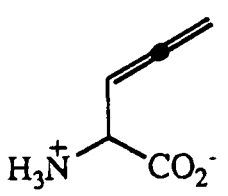
2.5. Research Objectives

The aim of the research described in Part Two of this thesis was to synthesise 2-substituted allylstannanes, and investigate the synthetic utility of these reagents in allyl transfer reactions with an α-radical generated on a suitable glycine derivative, as shown in the generalised Scheme 2.17 below, and to attempt to extend the methodology to asymmetric syntheses of α-amino acid derivatives.[81]



Scheme 2.17

Additionally, it was decided to assess the ability of the propargyl stannane (57) to undergo an allene transfer reaction with the same glycidyl radical, in an attempt to synthesise the previously undocumented α-amino acid, allenylglycine (60).



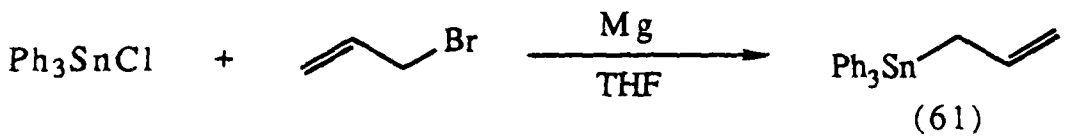
(60)

3. GENERATION AND REACTIONS OF A GLYCIDYL RADICAL

3.1. Synthetic Approaches to Allylstannanes

Most of the possibilities for the synthesis of allylstannanes fall into one of the methods outlined below.

(i) Preparation of an allylic organometallic compound which is subsequently reacted with an organotin halide.[66] For example, allyltriphenylstannane (61)[82] was prepared by the addition of a solution of triphenyltin chloride and allyl bromide, in dry THF, to a vigorously stirred mixture of an excess of magnesium turnings in THF (Scheme 3.1). After refluxing overnight, quenching with aqueous ammonium chloride and standard work-up procedure, the product was obtained as a white crystalline solid in 86% yield.

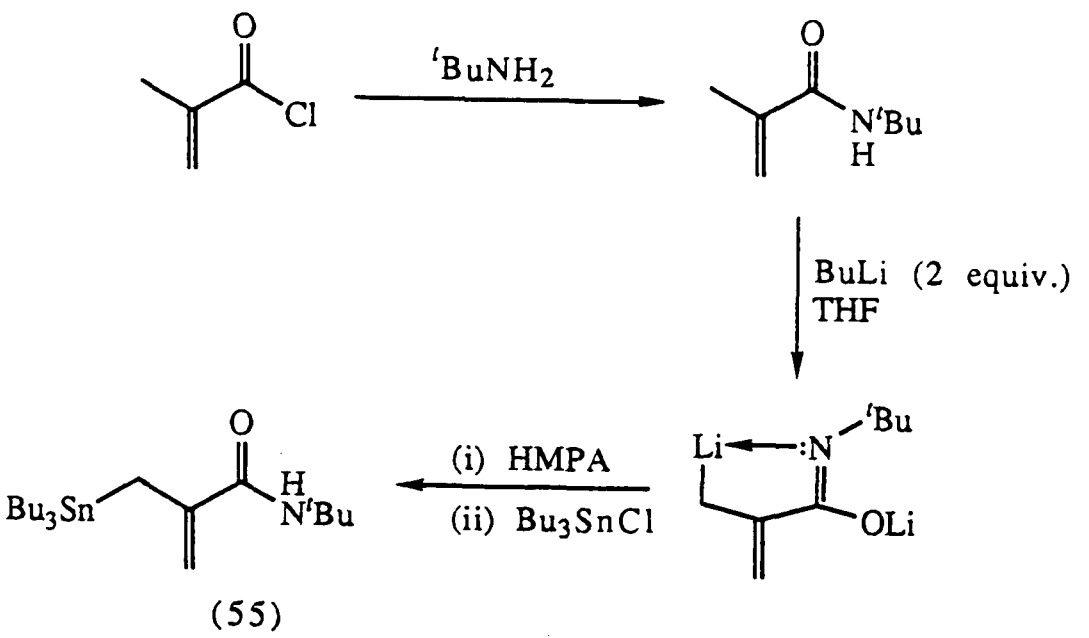


(61)

Scheme 3.1

(ii) Dimetalation of *N*-*t*-butylmethacrylamide with *n*-butyllithium to give a reagent equivalent to the dianion of methacrylic acid.[77,78] Subsequent quenching with triphenyltin chloride as an electrophile produced the acrylamide (55) (Scheme 3.2).[79]

(iii) Grignard reagents are most generally used in the preparation of allylstannanes. However, in the preparation of 2-substituted allylstannanes, the reactivity of Grignard reagents would impose certain limitations on the nature of the 2-substituent. Ueno's methodology for the



(55)

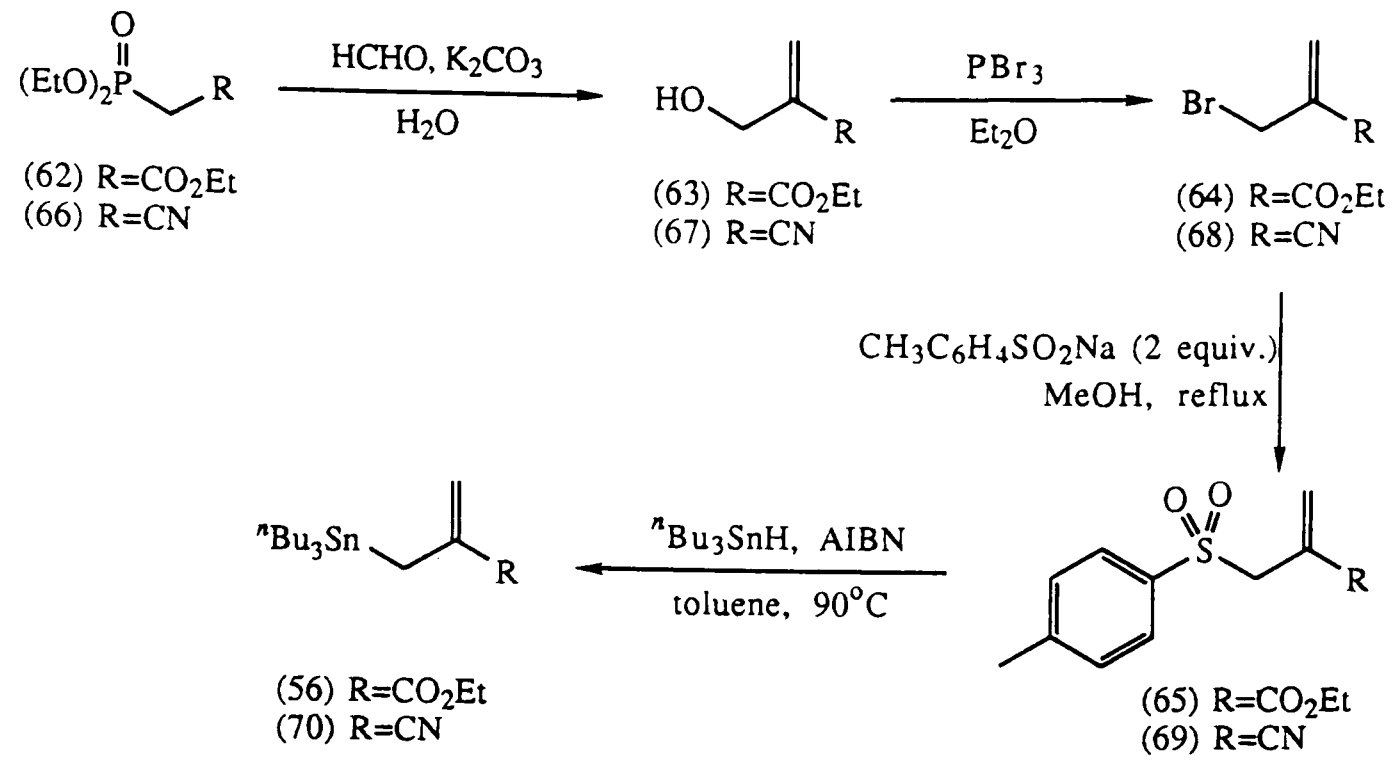
Scheme 3.2

synthesis of allylstannanes, the regioselective desulphonation of allylic sulphones with organotin hydrides, provides a route which is general and tolerant of a variety of functionality (Scheme 3.3).[83]



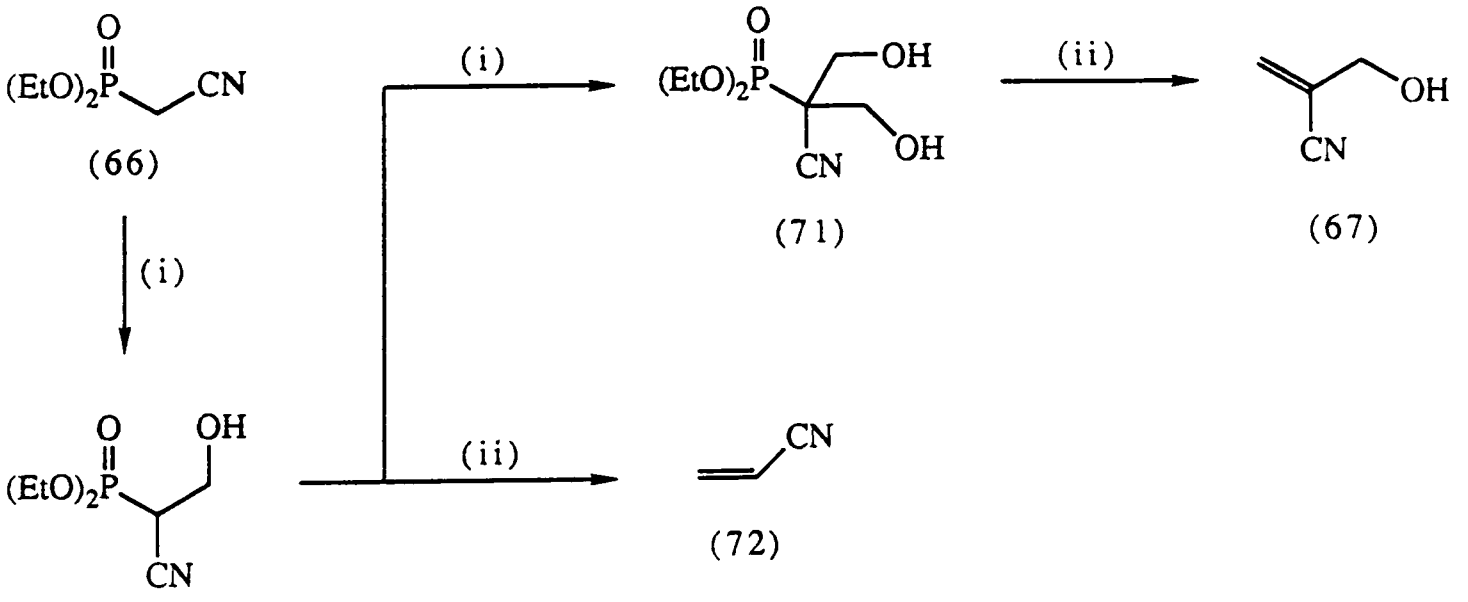
Scheme 3.3

Ethyl α-(tri-*n*-butylstannylmethyl)acrylate (56)[75] was first prepared from the analogous sulphone using this method (Scheme 3.4). This reagent was considered valuable, since not only should it react faster with nucleophilic radicals, due to the presence of an electron withdrawing group, but it should also enable a relatively simple entry into modified reagents *via* subsequent modification of the ester moiety. As a complement to this reagent, α-(tri-*n*-butylstannylmethyl)acrylonitrile (70) was prepared using a similar process (Scheme 3.4).



Scheme 3.4

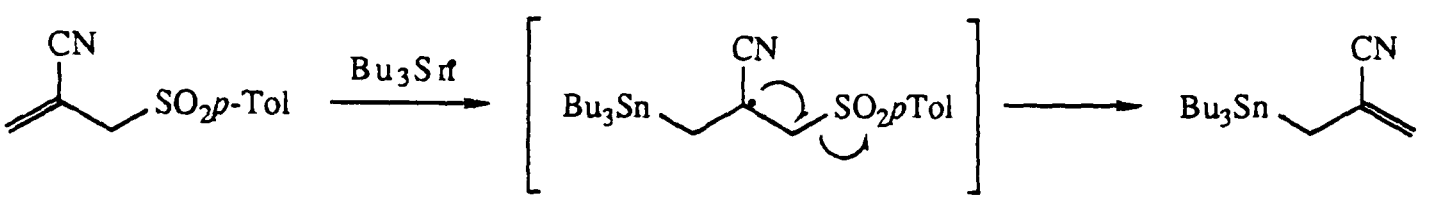
Thus, reaction of diethylcyanomethylphosphonate (66) with a large excess of formaldehyde in saturated aqueous potassium carbonate solution at room temperature, produced the bis(hydroxymethyl) derivative (71) (Scheme 3.5).[84] The latter underwent the Wittig-Horner elimination *in situ*, with the production of α-(hydroxymethyl)acrylonitrile (67) in only 30% yield from (66), compared to a 66% yield of (63) from triethylphosphonoacetate (62). The excess potassium carbonate suppressed a competing reaction - the initially formed potassium diethylphosphonate acting as a base to form cyanoethene (72).



Scheme 3.5. (i) HCHO, K_2CO_3, H_2O; (ii) $(EtO)_2P(O)OK$.

α-(Bromomethyl)acrylonitrile (68) was prepared by the addition of phosphorus tribromide to (67) at -10°C. After stirring at room temperature and quenching with water at -10°C, extraction and distillation under reduced pressure gave (69) in 27% yield [c.f. (64) produced in 82% yield from (63)].

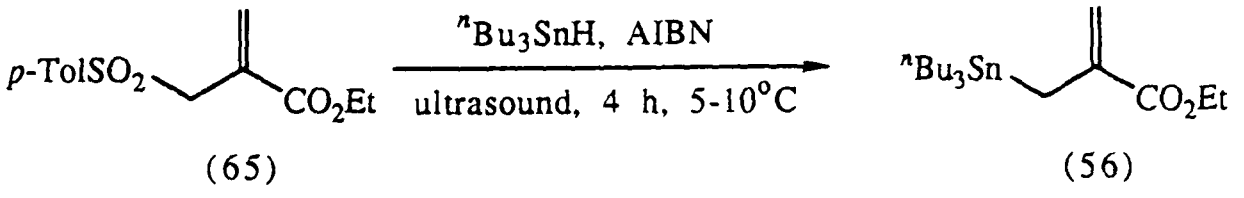
Reflux of (68) in methanol with sodium 4-tolylsulphinate furnished the sulphone (69) in 77% yield after chromatography. α-(Tri-*n*-butylstannyl-methyl)acrylonitrile (70) was then prepared by free radical stannylation of (69) using Ueno's method,[83] to give (70) in 76% yield after chromatography. The reaction mechanism can be described as S_H2' and is shown in Scheme 3.6.[85]



Scheme 3.6

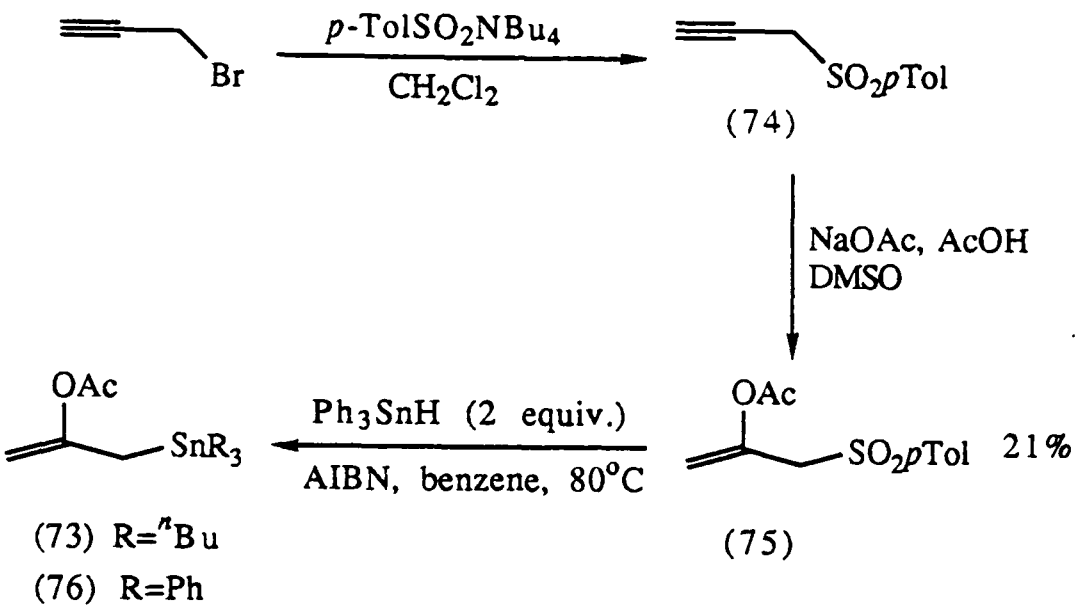
Low intensity ultrasonication[86] can dramatically increase the rates of free radical reactions, and often improves yields. This enhancement is due to cavitation, producing huge increases in local pressures and temperatures.

To assess the effectiveness of ultrasound, the sulphone (65) was converted to the stannane (56) by sonication in toluene at 5-10°C for 4 hours with tri-*n*-butyltin hydride (Scheme 3.7). The reaction proceeded cleanly, furnishing (56) in 86% yield, compared to 76% by thermal means.



Scheme 3.7

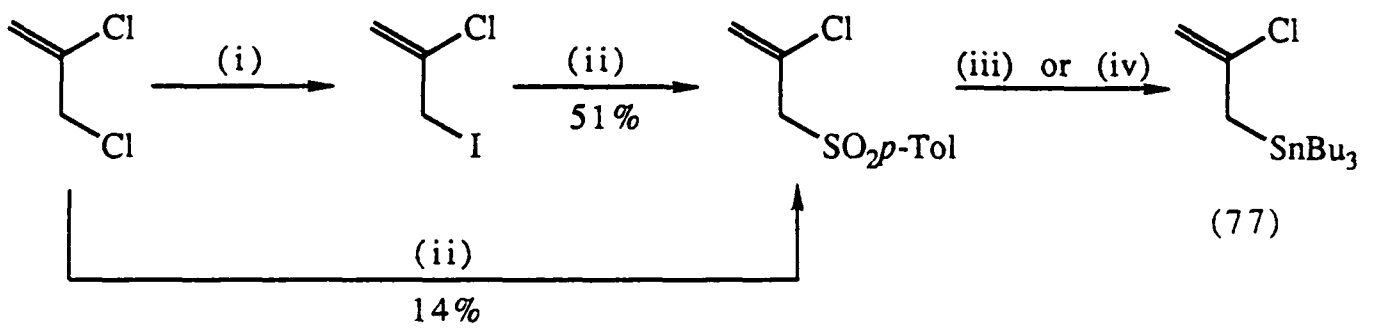
The enol acetate reagent (73)[83] is a reported compound. The corresponding allyltriphenylstannane (76) was prepared in a similar fashion (Scheme 3.8).



Scheme 3.8

The enol acetate sulphone (75) was obtained in low yield from the reaction of the propargyl sulphone (74) with sodium acetate and acetic acid in dimethylsulphide at room temperature.[87]

The 2-chlorinated allylstannane (77) was also prepared in this laboratory by the method shown in Scheme 3.9.[81]

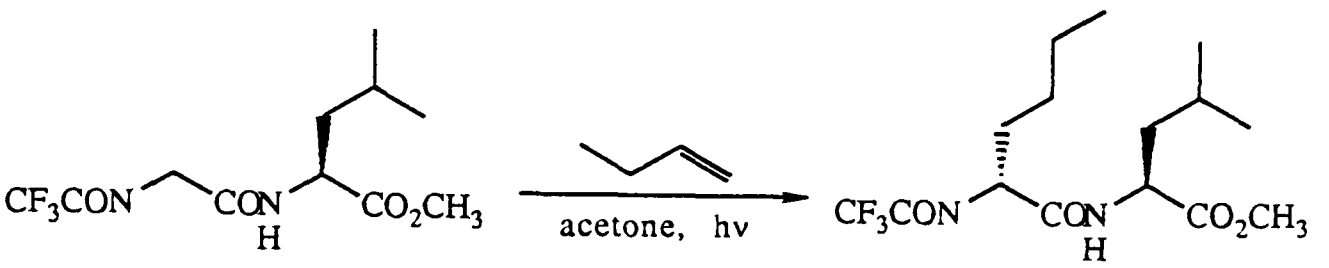


Scheme 3.9. Reagents: (i) NaI, acetone; (ii) p-TolSO$_2$Na (2 equiv.), MeOH, reflux; (iii) Bu$_3$SnH, AIBN (cat.), toluene, 80°C; (iv) Bu$_3$SnH, AIBN (cat.), toluene, ultrasound.

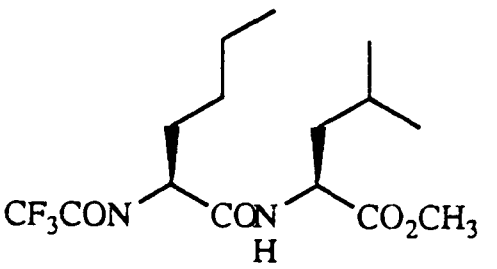
We now had two electrophilic 2-substituted allylstannanes in hand, (56) and (70), and two nucleophilic allylstannanes (76) and (77), with which to investigate allyl transfer reactions with glycidyl radicals.

3.2. Generation of a Glycidyl Radical

Free radical photochemical alkylations of glycine dipeptide derivatives have been reported.[88] They involve the glycine residues in glycyl-alanine dipeptides selectively, leading to conversion of the glycine residue into a branched chain α-amino acid residue. Furthermore, a 1,4-asymmetric induction effect was noted in the acetone initiated photoalkylation reaction of glycyl-leucine dipeptide derivatives with but-1-ene (Scheme. 3.10).[89] However, the diastereoselectivity of the process was poor, indicated by the ratio of products (78):(79) being only 3:2.



Tfa-Gly-*L*-Leu-OMe (78)

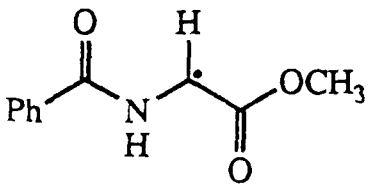


(79)

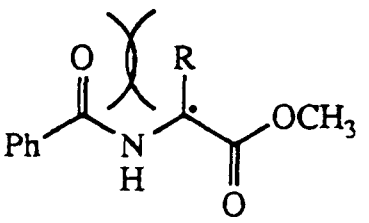
Scheme 3.10

The preferred conversion of the glycine residues of peptides into branched chain α-amino acids *via* free radical pathways is due to the stability of the radicals produced by their atom transfer reactions,[90] although this is contrary to the expectation that tertiary radicals should be formed in preference to secondary ones on the grounds of radical stability. Glycine residues afford secondary radicals by α-C-H bond homolysis, whereas analogous reactions of derivatives of other amino acids produce tertiary radicals. Easton *et al*[90,91] attributed the stability of the captodative[92] glycine radical equivalent (80) to a particularly favourable planar

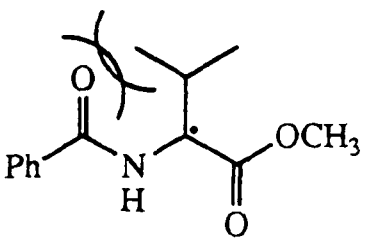
geometry, allowing maximum overlap of the semi-occupied p-orbitals with the π-orbitals of the amido and methoxycarbonyl substituents to occur. For the radicals (81) and (82) however, a planar geometry would result in destabilising steric interactions, especially in the latter, which outweigh the normal thermodynamic preference for the production of tertiary radicals. These predictions were borne out by experiment, in that the rate of reaction of *N*-bromosuccinimide with the glycine derivatives specifically deuterated at the α-position decreased in the order (80) > (81) > (82).



(80)

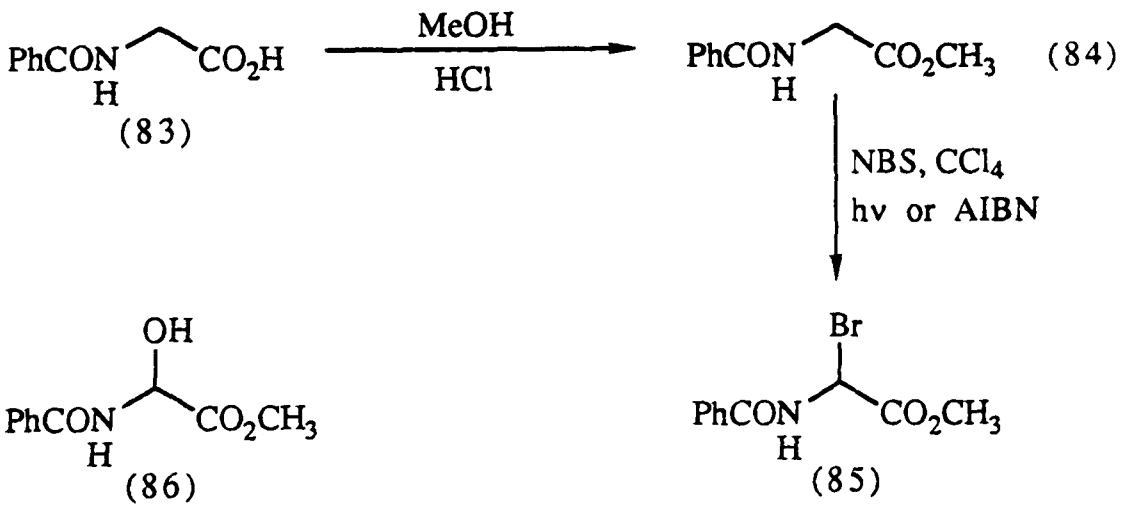


(81)



(82)

In the current study, the α-brominated glycine derivative, *N*-benzoyl-2-bromoglycine methyl ester (85)[90] was selected as a suitable radical precursor to explore the feasibility of allyl transfer reactions (Scheme 3.11).



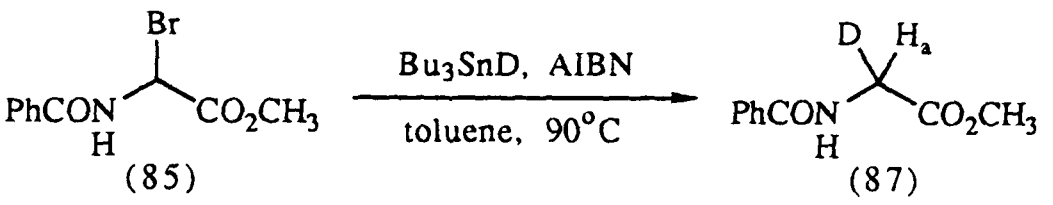
Scheme 3.11

Esterification of hippuric acid (83) in methanol saturated with hydrogen chloride gas produced the protected glycine (84)[93] in 93% yield. Reaction of (84) with *N*-bromosuccinimide in refluxing carbon tetrachloride, initiated either photolytically or by a catalytic quantity of AIBN, yielded the

desired bromide (85) in near quantitative yield. However, the latter method generally produced (85) in a cleaner fashion (by ^{1}H nmr). As suspected, (85) was unstable to silica gel chromatography, leading only to the isolation of *N*-benzoyl-2-hydroxyglycine methyl ester (86).

3.3. Reactions of the Glycidyl Radical

The radical precursor (85) was first treated with tri-*n*-butyltin deuteride in degassed toluene at 90°C in the presence of AIBN. (Toluene was generally preferred to benzene as a solvent, primarily for toxicity considerations.) As expected, the monodeuterated compound (87) was obtained in 67% yield (Scheme 3.12). In the ^{1}H nmr spectrum (500 MHz), the proton resonance corresponding to H_a could be resolved to a multiplet due to additional coupling with the deuterium atom. In addition, mass spectrometry indicated a >95% deuterium incorporation.

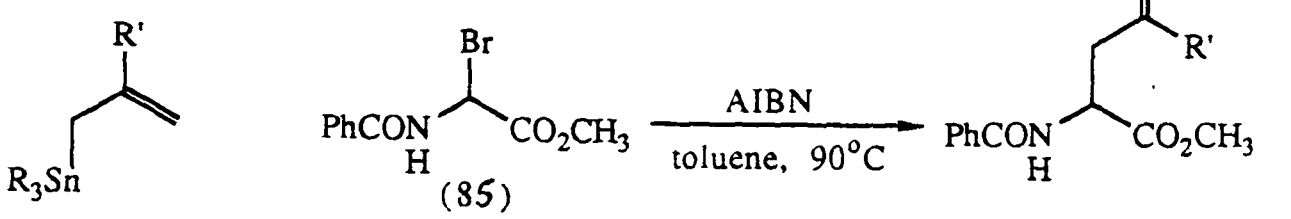


Scheme 3.12

Two methods were generally employed for the work-up of such reactions. Hexane/acetonitrile partitioning provided a means of removing hexane soluble tin residues. Alternatively, a solution of the crude product in ethyl acetate was shaken with 5% aqueous potassium fluoride. This converted the tin residues to insoluble stannyl fluorides which could be removed by filtration. In practice, this proved to be more effective than the former procedure. In all cases, further purification by silica gel chromatography was used; chromatography without $KF_{(aq)}$ work-up proved impractical due

to the tendency of organotin bromides [by-products of all reactions involving (85)] to 'streak'.

The reactions of the allyl transfer reagents with (85), using standard free radical conditions with AIBN initiation and standard work-up procedures, are summarised in Table 3.1 below.



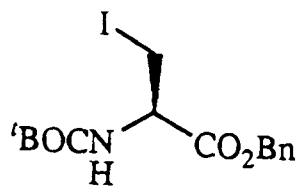
		Yield (%)
(61) R=Ph, R'=H	(88) R'=H	67
(56) R=^{n}Bu, R'=CO_2Et	(89) R'=CO_2Et	74
(70) R=^{n}Bu, R'=CN	(90) R'=CN	50
(76) R=Ph, R'=OAc	(91) R'=OAc	<5
(77) R=^{n}Bu, R'=Cl	(92) R'=Cl	62

Table 3.1

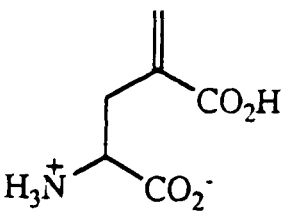
These results demonstrate the ability of the electrophilic radicalphiles (61), (56) and (70) to undergo efficient allyl transfer reactions with the glycidyl radical. The production of (92)[81] in a moderate yield suggests that the glycidyl radical, by nature of the electron withdrawing groups attached to the radical centre, may also function as a source of electrophilic radicals. However, the reaction of the stannane (76) proved less successful, with only trace amounts of the allyl transfer product (91) being observed in the ^1H nmr spectrum of the crude product [δH (500 MHz) 2.85-2.91, 2.93-2.98 (2H, 2 x 4 line multiplets, AB part of ABX system), 4.89, 4.92 (2 x 1H, 2 x s, CH_2=C-), 4.95-4.99 (1H, m, X part of ABX system)]. Attempted purification by chromatography failed to isolate (91), possibly due to hydrolysis.

As predicted, the nucleophilic allylic stannanes (76) and (77) failed to undergo allyl transfer reactions under standard conditions with sources of

nucleophilic radicals, such as 1-bromoadamantane and the protected 3-iodo-*L*-alanine derivative (93).[94]



(93)

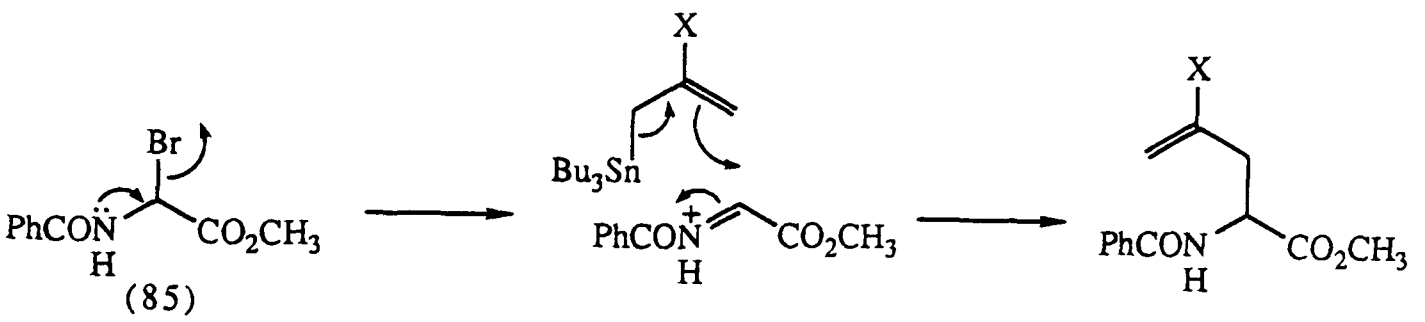


(94)

The utility of this synthetic methodology was demonstrated by the hydrolysis of the allyl transfer product (89) [3*N* HCl : dioxane (1:1), 24 h, reflux], which provided racemic 4-methyleneglutamate (94)[95,96] in 52% yield, identical by ^{1}H nmr analysis to the naturally occurring material.

3.4. Inhibition of the Radical Process

To verify that the reaction process described above was solely radical in nature, and that it was not proceeding *via* a thermal 2-electron pathway (Scheme 3.13), it was necessary to introduce radical inhibitors into the reaction mixture.



Scheme 3.13

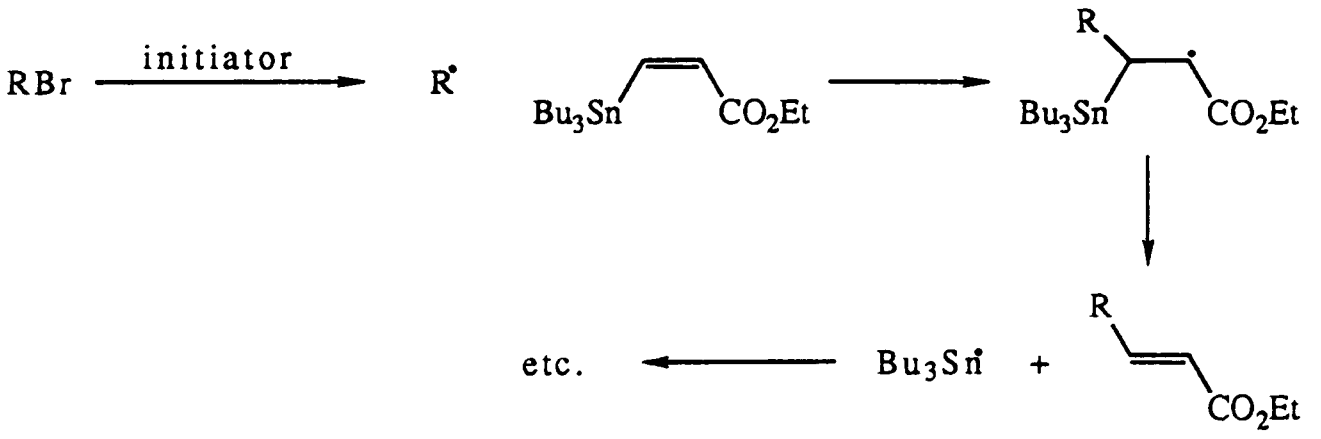
Reaction of (85) with the allylic stannane (56) in toluene at 90°C in the absence of AIBN still produced the allyl transfer product (89) in good yield. However, repetition of the process in the dark with a small quantity of

hydroquinone present as a radical inhibitor, prevented the formation of (89) - confirmation of the free radical nature of the reaction.

It is interesting to note that attempted inhibition of the reaction of (85) with tri-*n*-butyltin hydride using an excess of hydroquinone still produced the monodeuterated compound (87) in good yield (as evidenced by ^1H nmr). This may be due to the tin hydride acting as a '2-electron' hydride donor when the radical processes are inhibited.

3.5. Reactions of Vinylstannanes with the Glycidyl Radical

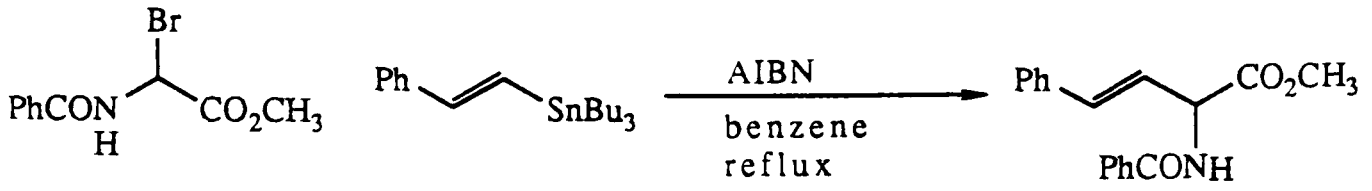
In these laboratories, a method of carbon-carbon bond formation based on a radical addition-elimination process has been developed, and was found to be general for a range of primary, secondary and tertiary bromides (Scheme 3.14).[97] The process involves initial addition of an alkyl radical to a vinylstannane, followed by proximal elimination of a trialkylstannyl radical to propagate the chain mechanism.



Scheme 3.14

As expected, the reaction proceeds more readily if the vinylic substituent is electron withdrawing, although reaction of β-tri-*n*-butylstannylstyrene (95) was demonstrated to give products resulting from addition-

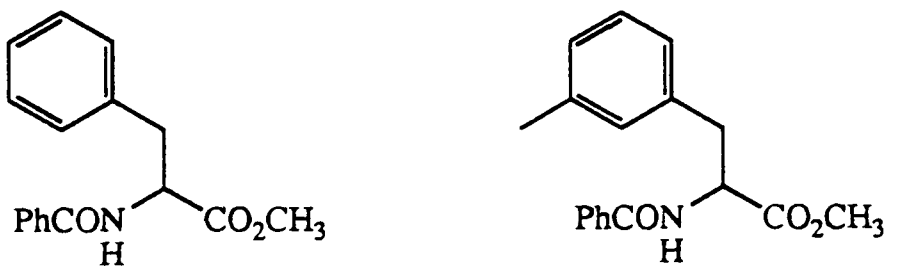
elimination.[98] Indeed, reaction of (95) with the glycidyl radical precursor (85) in refluxing benzene with AIBN initiation, furnished (96) in 25% yield, a product of addition-elimination (Scheme 3.15).[99]



(85) (95) (96)

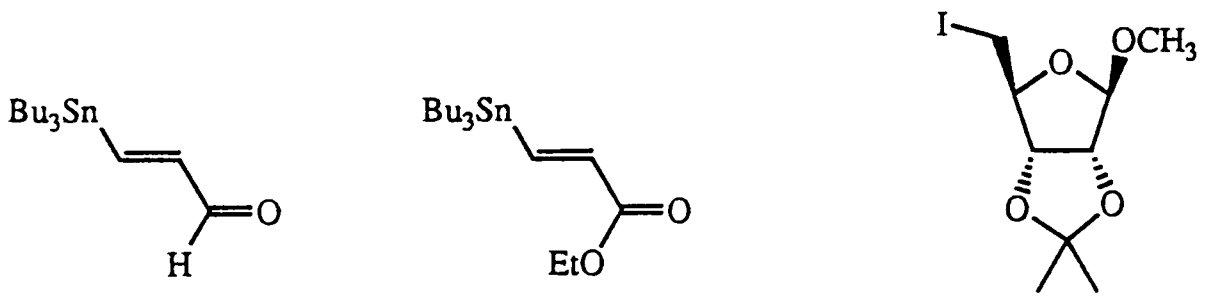
Scheme 3.15

When the same reaction was carried out in both toluene and *m*-xylene, the only products observed were (97) and (98) respectively, in *ca* 30% yield. These presumably result from a chain termination step involving the glycidyl radical and solvent radicals.



(97) (98)

However, the reaction of (85) with tri-*n*-butylstannylacrolein (99)[100] using either photolytic or AIBN initiation failed to furnish any products resulting from addition-elimination.

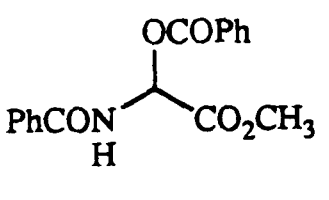


(99) (100) (101)

It has been reported[100] that the *trans*-β-stannylacrylate (100) does undergo a very slow addition-elimination reaction with the methyl riboside iodide (101), which is even more sluggish with (99). The problem was overcome

by the use of benzoyl peroxide[101] as a radical initiator, which has the advantages of a longer half-life than AIBN, and of being a generator of more reactive radicals than AIBN. The optimum conditions were found to be heating a solution of 1 equivalent of the iodide (101), 1.5 equivalents of the stannyl aldehyde (99) and 2 equivalents of benzoyl peroxide in degassed solvent to 65-70^{0}C.

However, under these conditions, the only product observed from the reaction of (85) with (99) was the benzoylated product (102). This resulted from a preferential recombination of the glycidyl radical with the initiator radicals, possibly due to steric effects. Optimisation of this reaction, by heating (85) together with benzoyl peroxide (2 equiv.) in toluene at 80^{o}C furnished (102) in 64% yield.



(102)

3.6. The Use of Ultrasound

As mentioned in Section 3.1, ultrasound could be successfully employed to facilitate generation of the stannane (56) from the corresponding sulphone, using tri-*n*-butyltin hydride. This method was then applied to the reaction of (85) with allyltriphenylstannane (61). However, after sonication for 4 hours, only a trace of the desired allyl transfer product was observed (by ^{1}H nmr), the bulk of the reaction mixture being residual starting materials. Similarly, the methacrylstannane (56) gave disappointing results, the only observed product being *N*-benzoylglycine methyl ester (84), i.e. reduced (85).

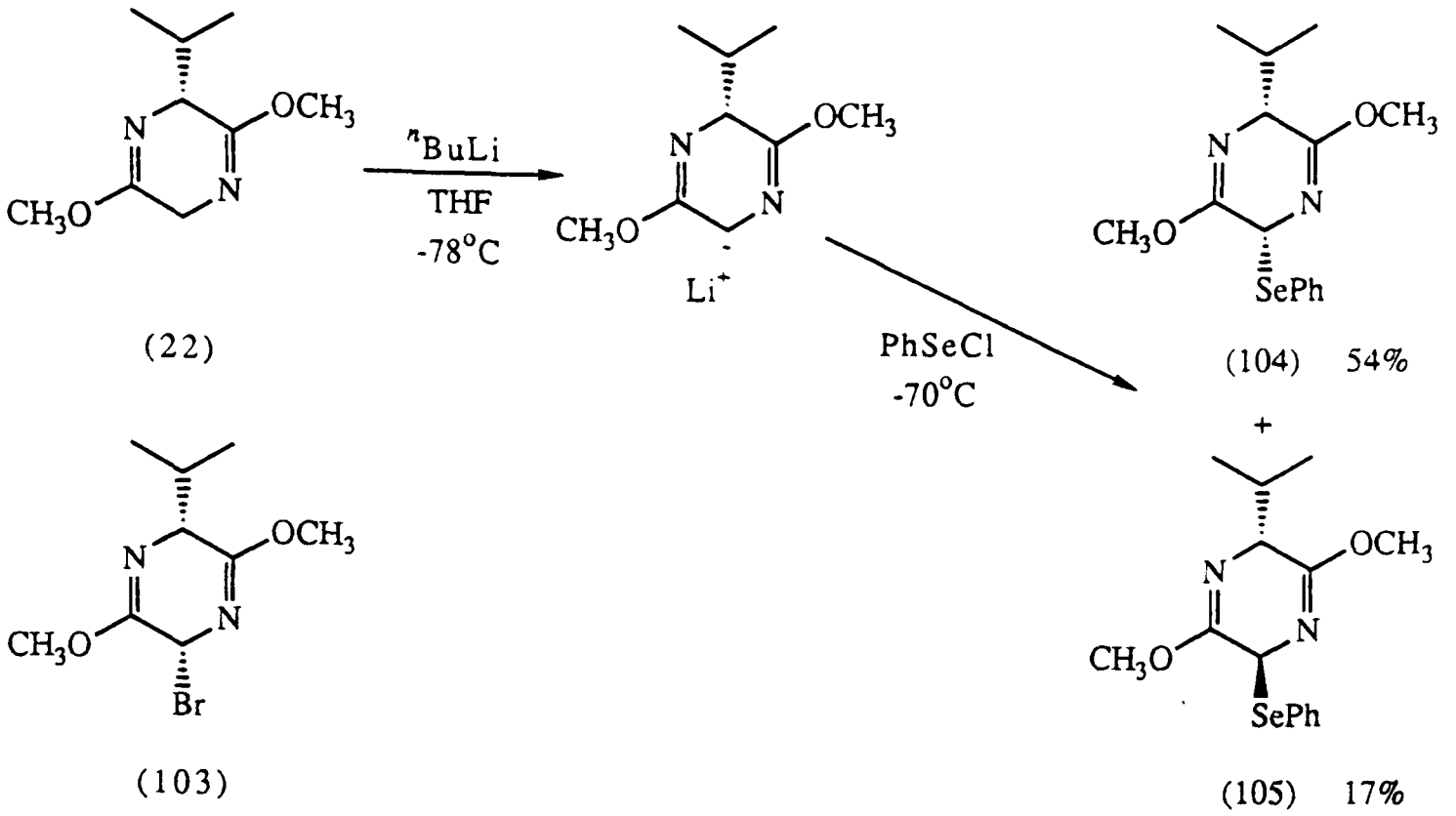
Sonication of (85) with tri-*n*-butyltin deuteride in toluene at 5-10°C gave the monodeuterated product (87) in 42% yield. Attempts to inhibit this process using large excesses of hydroquinone or benzoquinone in the absence of AIBN still produced (87) in moderate quantities. This suggests that a radical pathway is not being followed in this case, rather the tin deuteride may be behaving as a '2-electron' hydride donor.

3.7. Chiral Glycidyl Radical Equivalents

The logical progression of this chemistry was to attempt to extend the above methodology to achieve a general asymmetric synthesis of novel allylic α-amino acids. Several of the 'glycine templates' discussed in Chapter 1 were available; the results obtained from these are documented below.

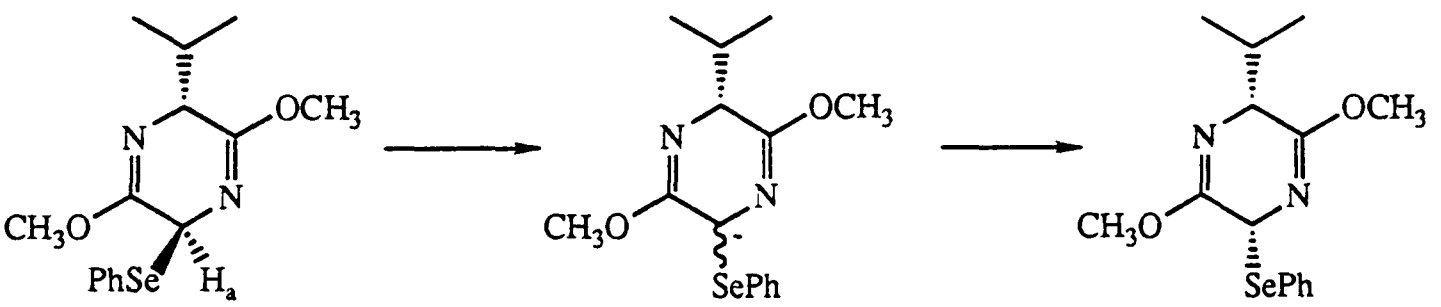
3.8. The Schöllkopf Method

Schöllkopf has documented the formation of the chlorinated bis-lactim ether (25).[47] However, due to its propensity to eliminate HCl quite readily, subsequent nucleophilic displacements of chloride ion had to be executed at -70°C, thus precluding its use as a radical precursor. This factor also rules out the possibility of the corresponding brominated bis-lactim ether (103) as a suitable radical precursor. As an alternative, the selenide was prepared as shown in Scheme 3.16.



Scheme 3.16

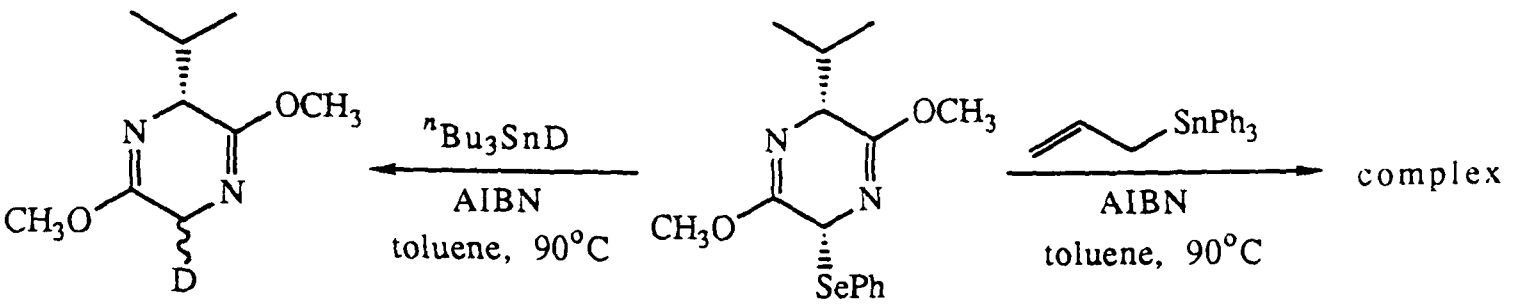
Metalation of (22) with *n*-butyllithium (1.1 equiv.) in THF at -78°C, followed by the addition of a solution of phenylselenyl chloride in THF at -70°C furnished, after work-up and chromatography, the two separate diastereomers (104) and (105) in 54% and 17% yields respectively. The major isomer, bore the isopropyl and phenylseleno functionalities in a *cis* relationship. This was attributed to the lability of the proton H_a once selenated, causing further deprotonation to form (106) which, in the work-up, was reprotonated from the least hindered face of the molecule (Scheme 3.17).



Scheme 3.17

Treatment of (104) with tri-*n*-butyltin deuteride in toluene at 90°C with AIBN initiation, produced the monodeuterated product (107) in 37% yield. However, under identical conditions, the reaction of (104) with

allyltriphenylstannane gave rise to a complex mixture of products: only the reduced product (22) could be identified with any certainty (Scheme 3.18).



(107) (104)

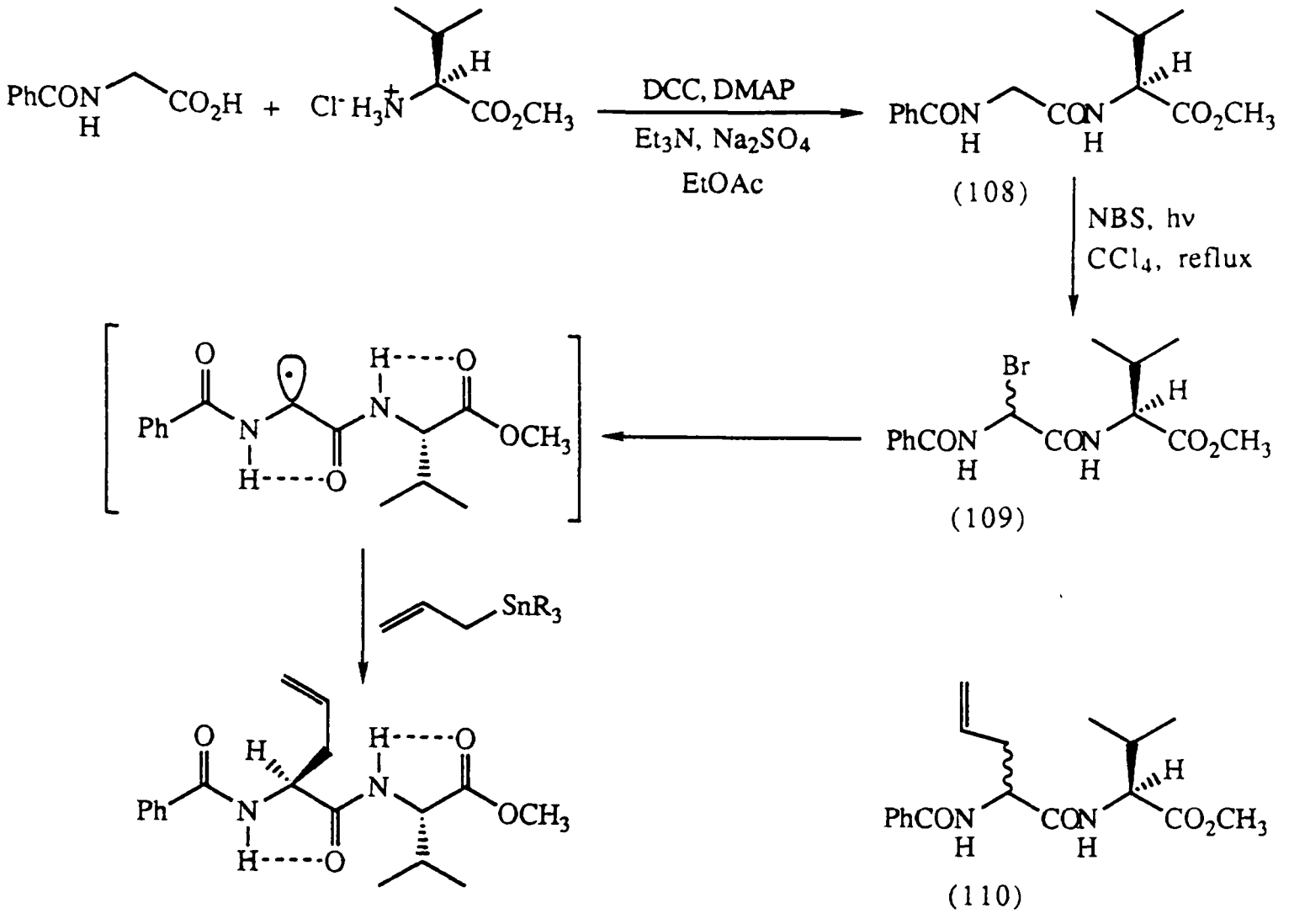
Scheme 3.18

The use of benzoyl peroxide as initiator, and lower temperatures (65°C) also failed to produce the desired product. It was subsequently noted that the starting material (104) decomposed even at room temperature over a period of 1-2 days, and thus is not suitable for high temperature radical processes.

3.9. The Dipeptide Method

Based on Elad's observation[88] of asymmetric induction during the acetone initiated photoalkylation reactions of glycyl-dipeptides, it was decided to attempt a thermal allyl transfer onto a selectively brominated glycine residue in such a dipeptide.

Hippuric acid was coupled with *L*-valine methyl ester hydrochloride using dicyclohexylcarbodiimide with DMAP[102] as a catalyst, to furnish the protected dipeptide (108) in 37% yield (Scheme 3.19).

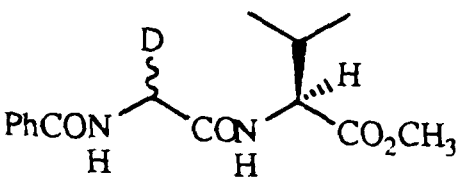


Scheme 3.19

Free radical bromination of (108) using *N*-bromosuccinimide with photoinitiation produced the bromodipeptide (109) in essentially quantitative yield. ^{1}H nmr analysis (500 MHz) and integration suggested a 60:40 ratio of diastereomers at this stage. A degree of asymmetric induction would be expected in subsequent reactions of (109) due to the H-bonded possibilities indicated in Scheme 3.19.

However, allyl transfer reactions with (109) and allyltriphenylstannane produced repeatedly low yields (<20%) of a mixture of diastereomeric products (110). These were inseparable by silica gel chromatography but ^{1}H nmr analysis (500 MHz) indicated that the ratio of diastereomers was *ca* 55:45. Subsequent to obtaining these results, Easton *et al*[103] published a report demonstrating an allyl transfer reaction to the bromodipeptide derived from racemic Gly-Val (±)-(108), proceeding in 35% yield and with no diastereoselectivity.

Deuteration of (109) using tri-*n*-butyltin deuteride under standard conditions produced the monodeuterated dipeptide (111) in 54% yield; ^{1}H nmr (500 MHz) and mass spectrometric analyses suggested *ca* 93% deuterium incorporation, though nothing could be stated about stereoselectivity.



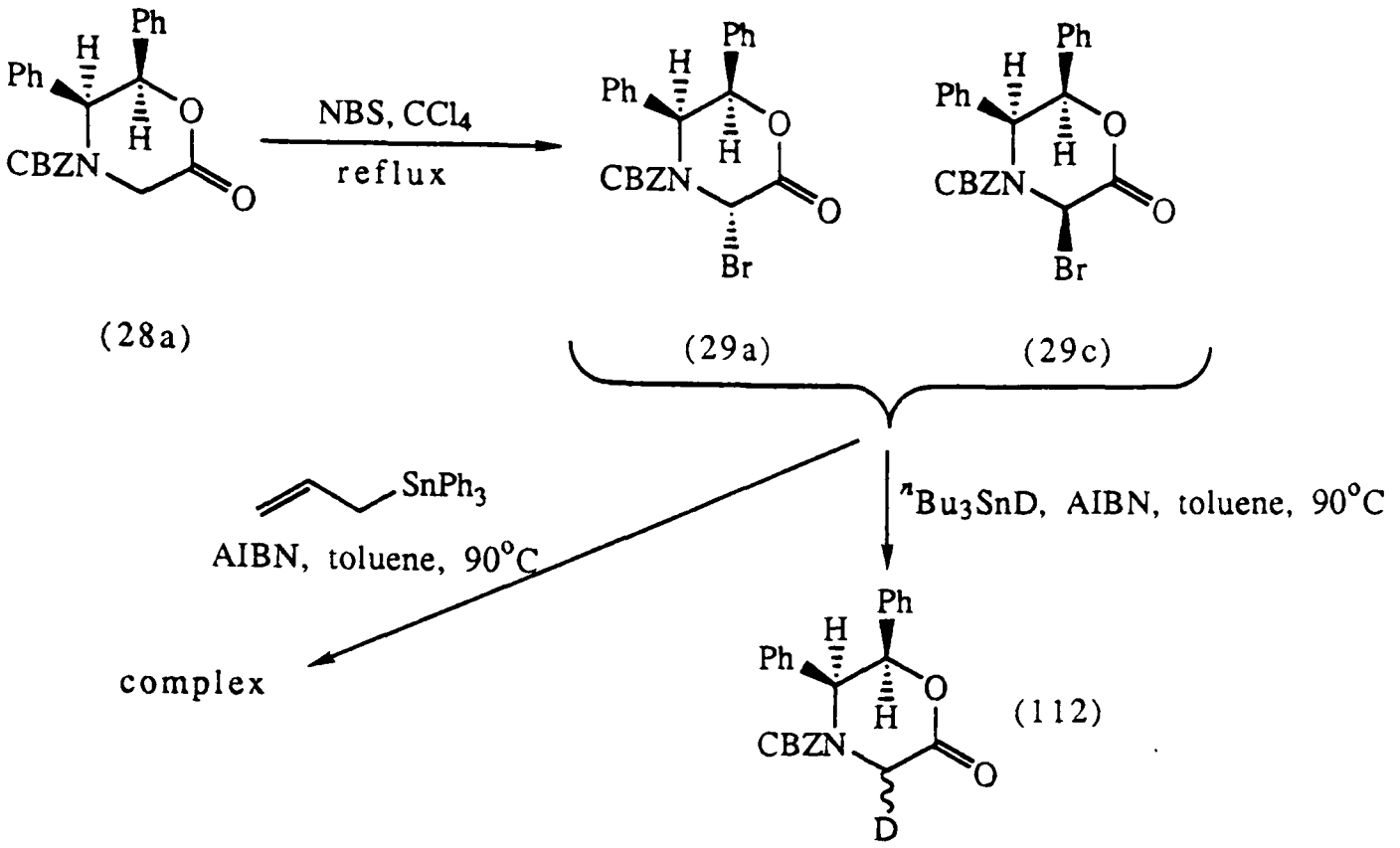
(111)

In these acyclic systems, it is expected that good stereocontrol will not be easy to obtain since the stereogenic centre is three atoms away from the reacting centre, and is subject to conformational motion at higher temperatures.

3.10. The Williams Method

The brominated oxazinone (29a) first prepared by Williams[41a] was then selected as a potential glycidyl radical equivalent. Thus, bromination of (28a) with *N*-bromosuccinimide under standard conditions proceeded quantitatively, but ^{1}H nmr analysis (300 MHz, 373K) indicated an approximate 3:1 ratio of diastereomers (29a):(29c) (Scheme 3.20). [It was necessary to record all ^{1}H nmr spectra at high temperatures (*ca* 100°C), since hindered rotation gave rise to complex spectra at normal operating temperatures.

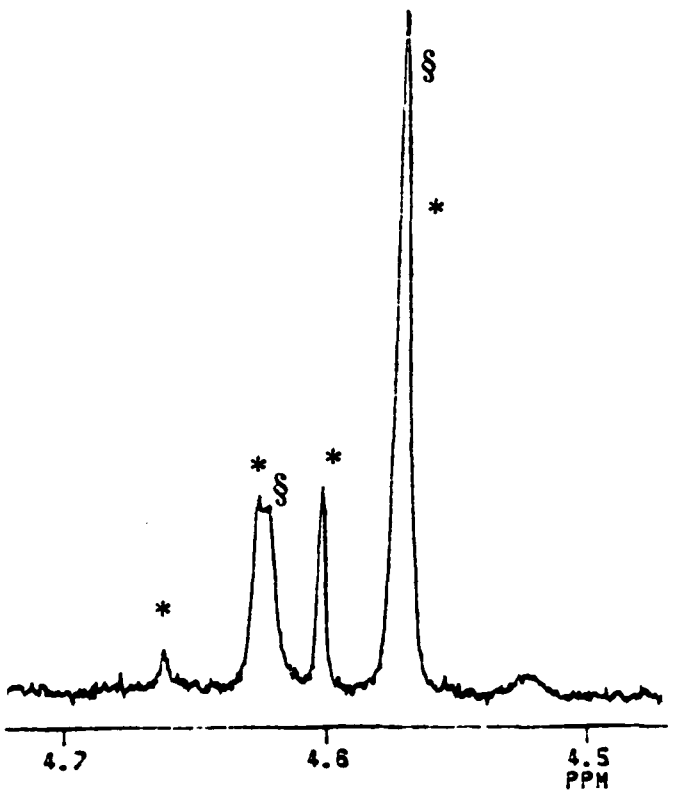
Deuteriation of the crude bromide (29a,c) under standard conditions furnished (112), which was purified by silica gel chromatography. Mass



Scheme 3.20

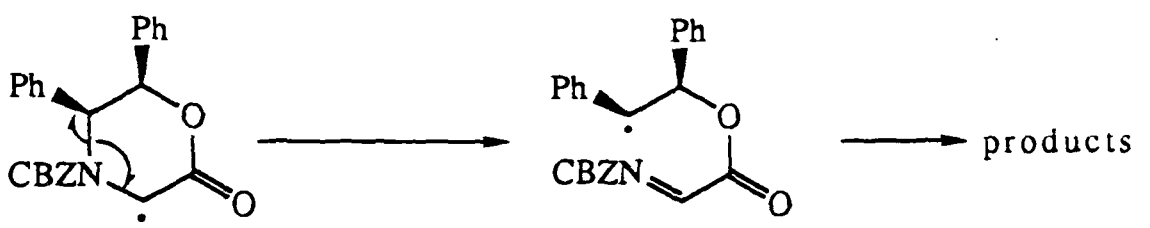
spectrometric analysis suggested that there had been *ca* 75% deuterium incorporation. A ^{1}H-(^{2}H decoupled) nmr spectrum (500 MHz) revealed the residual AB quartet of (28a) at C-3 (*) plus two additional singlets (§) corresponding to single 3-H proton resonances in the deuterated compound (112). Comparison of the integrals revealed a 4:1 diastereoselectivity ratio (Figure 3.1).

Figure 3.1



However, allyl transfer reactions using allyltriphenylstannane failed to furnish the desired allyl transfer product; only a complex mixture of unidentifiable products was observed. The only apparent conclusion to be

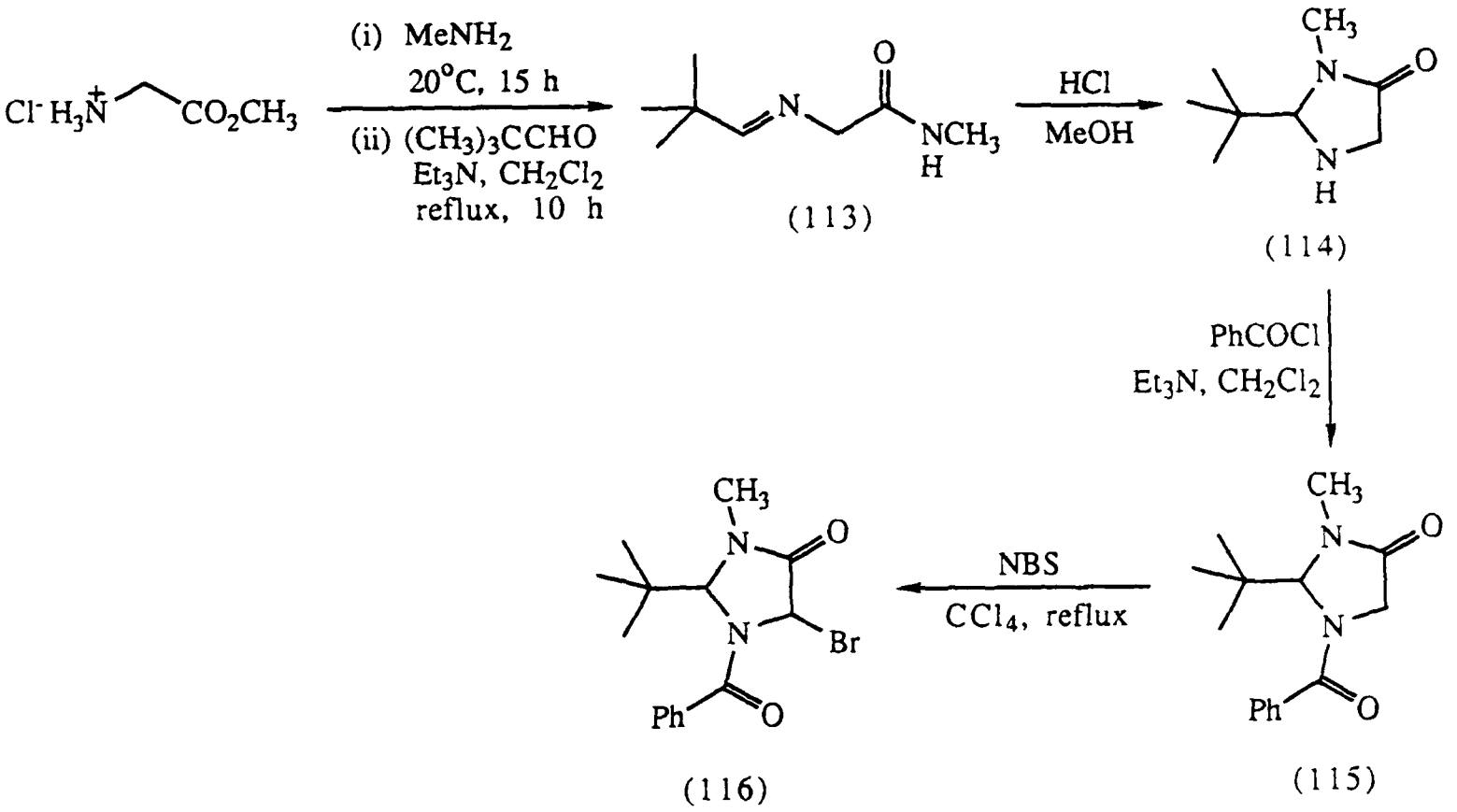
drawn here is that the initially formed radical was unstable, and unless immediately trapped by the reactive tri-*n*-butyltin hydride, decomposed, possibly as postulated in Scheme 3.21. This may arise because the radical possesses no stabilising influences due to the non-planar nature of the cyclic species, thus preventing stabilisation by overlap of the semi-occupied p-orbital with the adjacent π-orbitals on the carbonyl and urethane functionalities. This approach was thus abandoned.



Scheme 3.21

3.11. The Seebach Method

The cyclic imidazolidinone (115), first produced by Seebach[40b] for use as a chiral anionic glycine equivalent, was prepared according to Scheme 3.22.[40e]

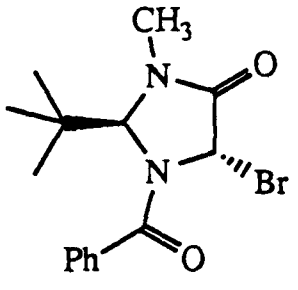


Scheme 3.22

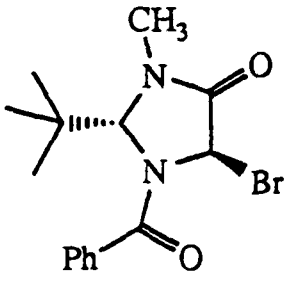
Thus, formation of the imine (113) was achieved by stirring glycine methyl ester hydrochloride with an 8*M* solution of methylamine in methylated spirits, followed by removal of the solvent and condensation of the resulting amine with pivalaldehyde. Acid catalysed cyclisation of (113) furnished the imidazolidinone (114), which, when treated with benzoyl chloride, yielded (115) - a cyclic template suitable for functionalisation of the inbuilt glycine moiety. The convenience of this synthesis is obvious: the first four steps can be carried out essentially as a 'one-pot' process, producing (115) in an overall 42% yield after chromatography.

Seebach then resolved the two isomers of (115) to continue the asymmetric synthesis; however in this case, it was decided to proceed with the racemic mixture to assess the practicality of allyl transfer reactions.

Selective bromination[104] at the 5-position was achieved quantitatively by reflux of (115) in carbon tetrachloride in the presence of AIBN to yield (116). As (116) was unstable to chromatography, ^{1}H nmr analysis showed the crude product to be free of any significant impurity, and the presence of only one peak corresponding to the 5-H suggested that the free radical bromination had proceeded with extremely high diastereoselectivity; thus (116) consisted only of (116a) and (116b).



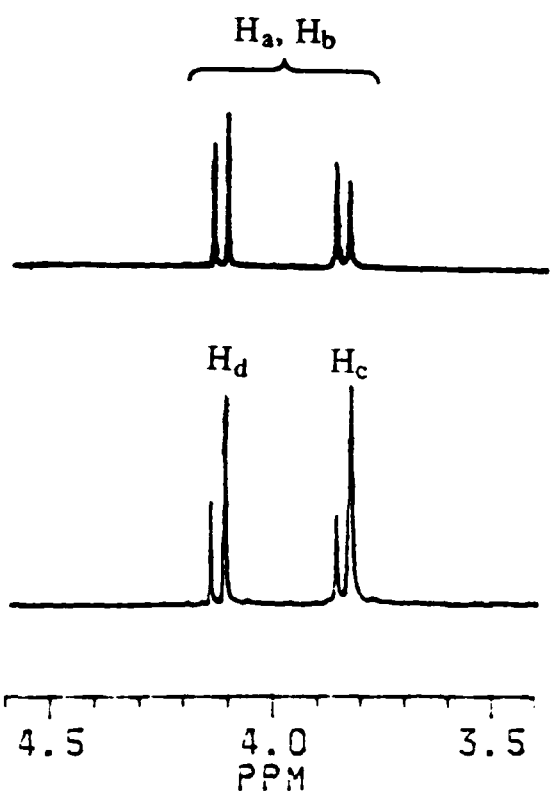
(116a)

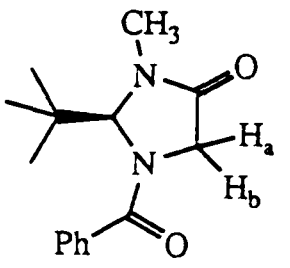


(116b)

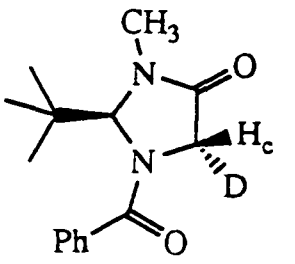
Unfortunately, treatment of (116) with tri-*n*-butyltin deuteride (not documented by Seebach) produced a mixture of four identifiable products

which could only be partially separated by chromatography. Apart from the reduced compound (115) (12%), the deuterated compounds (117) (16%) and (118) (8%) were observed by virtue of two broad singlets in a ratio of *ca* 2:1 in the 500 MHz ^1H nmr spectrum. These peaks, corresponding to H_c and H_d were superimposed upon the AB quartet (H_a,H_b) of (117), but shifted slightly upfield (Figure 3.2 - only one pair of diastereoisomers is shown for simplicity). A ^2H nmr experiment (38 MHz) also indicated the ratio of (117):(118) to be 2:1.

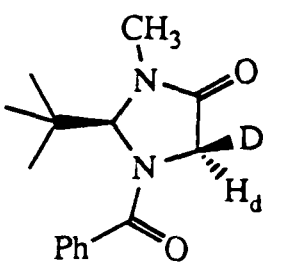




(115)



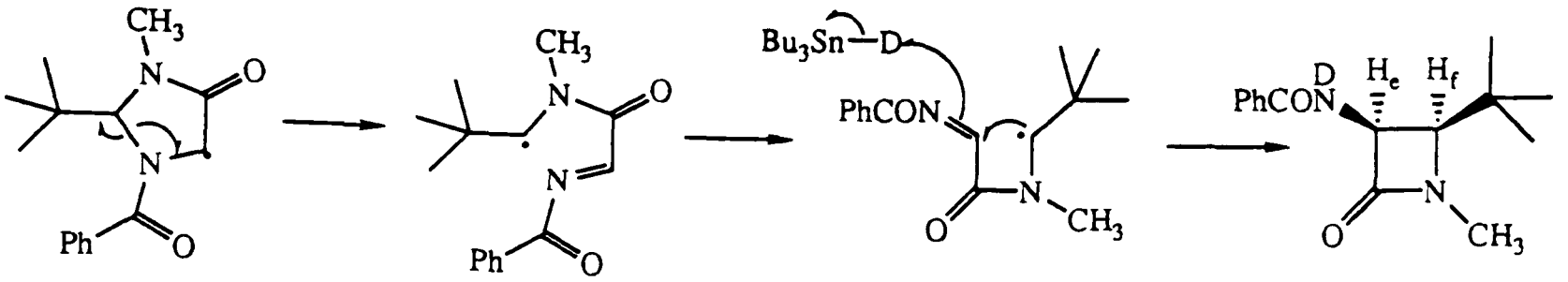
(117)



(118)

Figure 3.2

Also observed in the ^1H nmr spectrum was an AB quartet centred around δ 4.83 and δ 5.06 corresponding to the protons H_e and H_f in the β-lactam compound (120) (ν_{max} 1735cm^{-1}). Formation of this unexpected compound may be rationalised by Scheme 3.23. According to Baldwin's rules[105], based on the stereochemical requirements of the transition states for ring closure, the latter ring closure may be defined as '4-*exo*-trig', and is thus a favoured process.



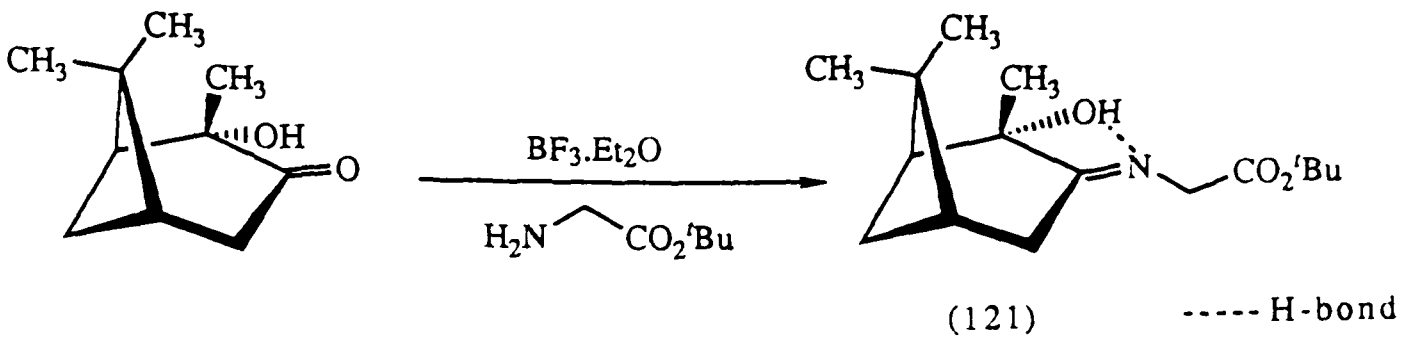
Scheme 3.23

Attempted allyl transfer reactions using allyltriphenylstannane (61) and ethyl α-(tri-*n*-butylstannylmethyl)acrylate (56) produced only unidentifiable products under standard conditions. These results again suggest that the radical (119) is unstable (as discussed in Section 3.10), its lack of selectivity leading to a variety of products from uncontrolled radical processes.

3.12. Conclusions

Whilst the general allyl transfer process to a glycidyl radical equivalent in good yield provides a useful addition to the synthetic methodologies currently available for the synthesis of α-amino acids, its extension to asymmetric synthesis has so far proved disappointing. This may be attributed to the instability of the radicals produced from cyclic glycine templates, and in acyclic systems, to the stereogenic centre being inherently too distant from the reacting centre to encourage stereocontrolled reactions.

These problems may be overcome in the future by employing chiral Schiff bases formed from glycine esters, such as those used by Yamada[46a] (Scheme 3.24).



(121) ----- H-bond

Scheme 3.24

However, the use of (121) as an anionic glycine equivalent only produces acceptable diastereomeric excesses (i.e. >80%) with very bulky electrophiles; careful modification of the chiral precursor may therefore be required.

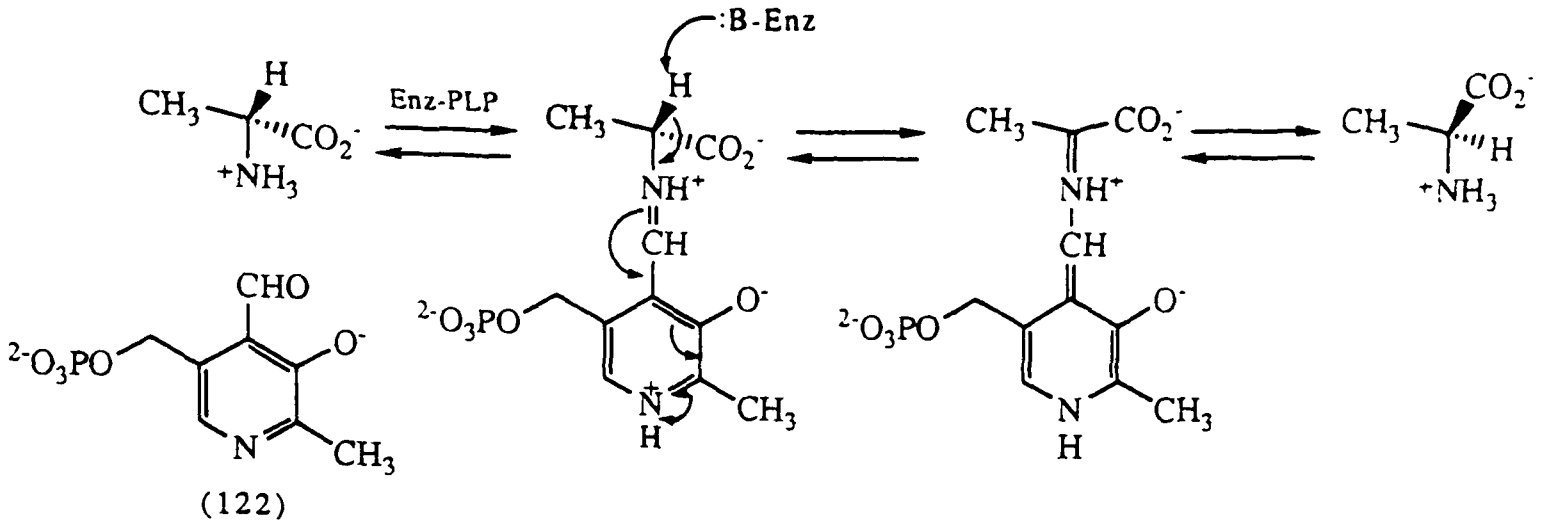
4. SYNTHESIS OF α-ALLENYLGLYCINE

4.1. Unsaturated α-Amino Acids as Enzyme Inhibitors[106]

A large variety of molecules, both naturally occurring as well as synthetic, have been shown, over the past 15 years or so, to cause specific inactivation of some target enzyme. Many of these species have been shown to act as irreversible mechanism-based enzyme inhibitors, or 'suicide substrates' by covalent binding to the active site of the target enzyme. If covalent capture is efficient, then the enzyme molecule is modified and its catalytic activity destroyed.

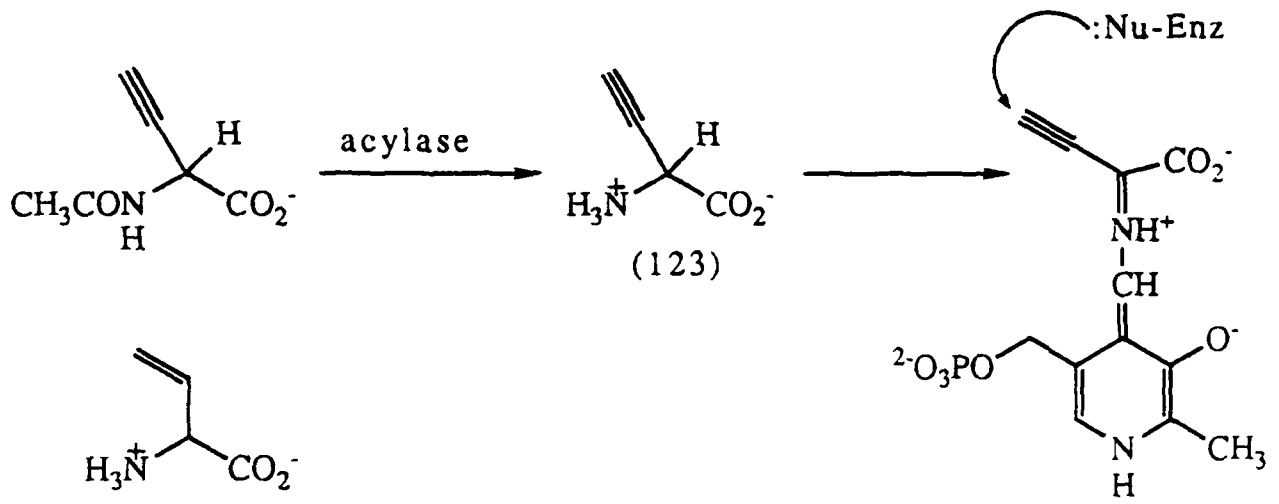
There are many naturally occurring toxins which function by specifically and irreversibly inhibiting various vitamin B_6 containing enzymes. Since most B_6-cofactored enzymes function in amino acid metabolism, the known inhibitors of these enzymes are almost exclusively amino acid derivatives. For instance, the bacterial enzyme alanine racemase, involved in provision of *D*-alanine as the first step in bacterial cell wall glycopeptide synthesis, uses vitamin B_6, pyridoxal phosphate (PLP) (122), as a tightly bound cofactor to stabilise a substrate-carbanion equivalent (Scheme 4.1).[107] The planar *p*-quinoid contributor to the carbanion structure, if achirally reprotonated, yields the racemic alanine mixture back from either starting enantiomer.

In 1980, *L*-ethynylglycine (123) was isolated as its *N*-acetyl derivative from a fermentation broth as an antimicrobial agent.[108] Although rather



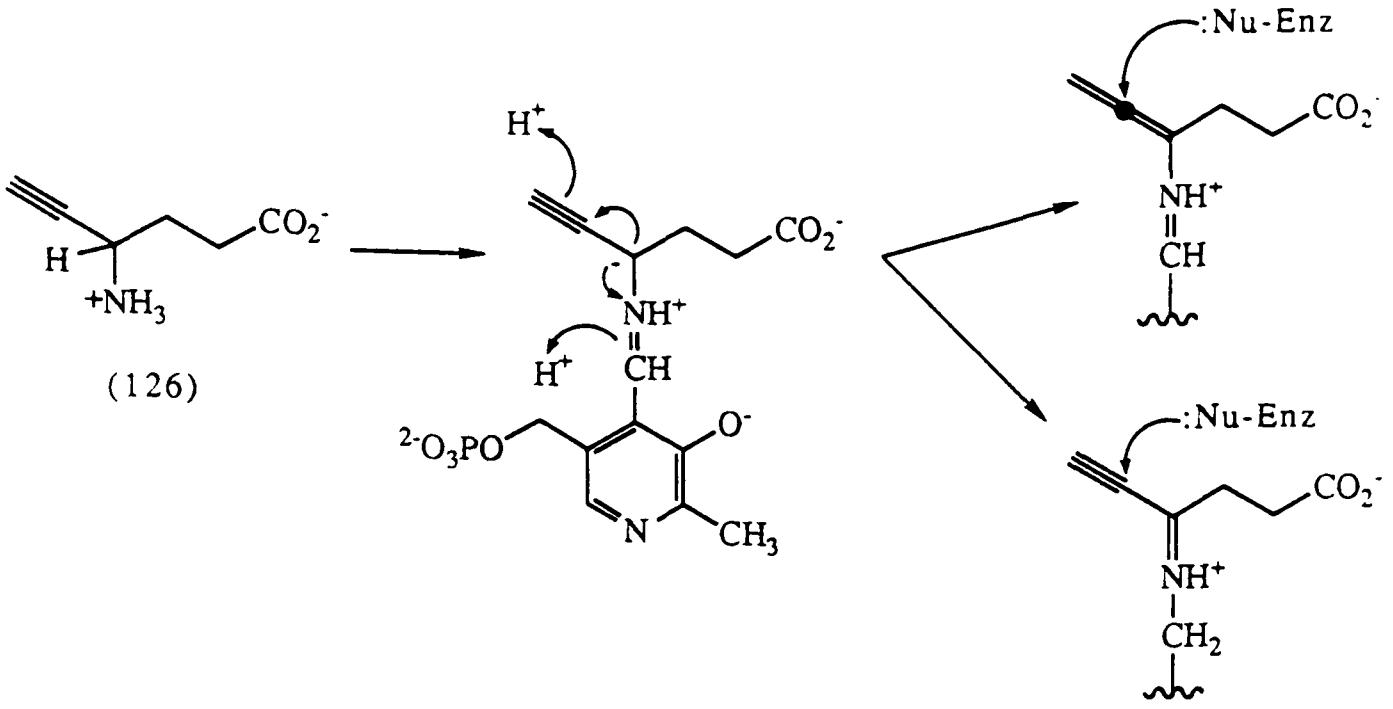
Scheme 4.1

unstable as the free amino acid, it was reported to inactivate alanine racemase, possibly *via* Michael addition to the carbanion equivalent (124) (Scheme 4.2). Vinylglycines e.g. (125) also function in much the same way as PLP-enzyme inhibitors.



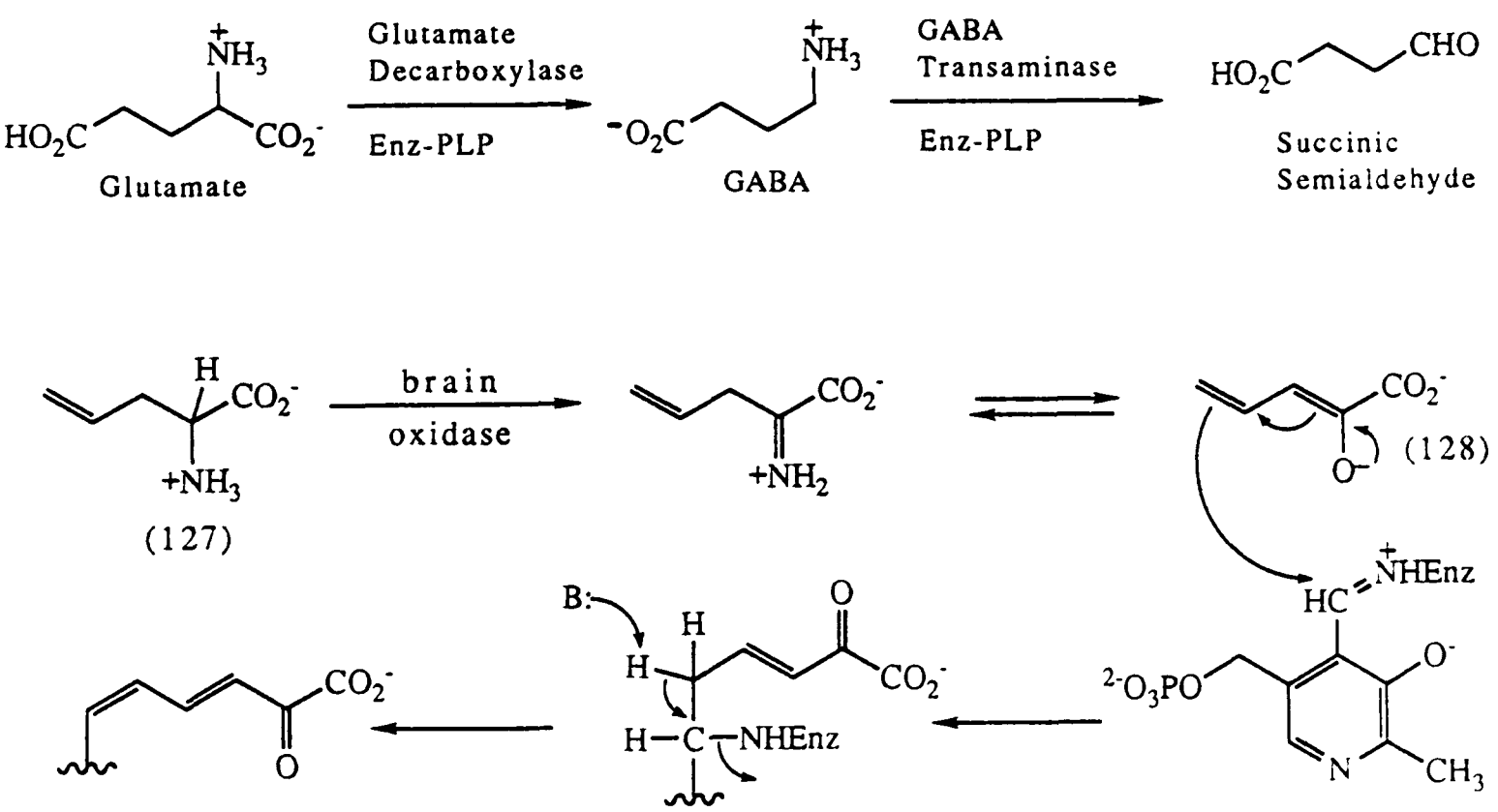
Scheme 4.2

In several other cases, acetylenic substrate analogues have been fashioned to inactivate target enzymes. When transaminases such as γ-aminobutyrate transaminase (GABA-T), a brain enzyme target for anti-epileptic drugs, is inactivated by γ-acetylene GABA (126) it is unclear whether rearrangement of a carbanion to a conjugate allene or oxidation to the conjugated acetylenic imino acid-PLP complex is the step leading to the alkylating species (Scheme 4.3).[109]



Scheme 4.3

While olefinic inactivators of GABA-T, the enzyme responsible for removal of the inhibitory neurotransmitter in the brain, GABA, are anti-epileptic,[110] inhibitors of the PLP-enzyme producing GABA in the brain, glutamate decarboxylase, are neuroconvulsive. Thus, the natural allylglycine (127) on entry into brain tissue is first oxidised by brain amino acid oxidases to an imino acid and then to 2-keto-4-pentenoate (128) (Scheme 4.4).[111] This product is thus neuroconvulsant by virtue of glutamate decarboxylase blockage.

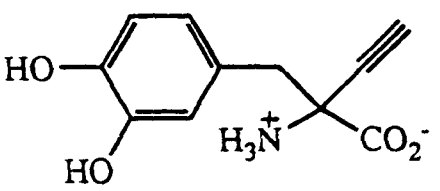


Scheme 4.4

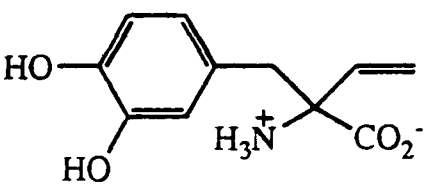
It has also been suggested[112] that α-allenic α-amino acids are attractive candidates for the specific inhibition of vitamin B_6-linked decarboxylases. Consequently, much interest in recent years has centred on the syntheses of allenic, as well as other β,γ-unsaturated α-amino acids.

4.2. Synthetic Approaches to β,γ-Unsaturated α-Amino Acids

The first examples of α-substituted α-amino acids bearing unsaturation attached to the quaternary α-carbon to be prepared, were α-ethynyl-3,4-dihydroxyphenylalanine (129) and α-vinyl-3,4-dihydroxyphenylalanine (130).[113] These were both found to be potent DOPA decarboxylase inhibitors.

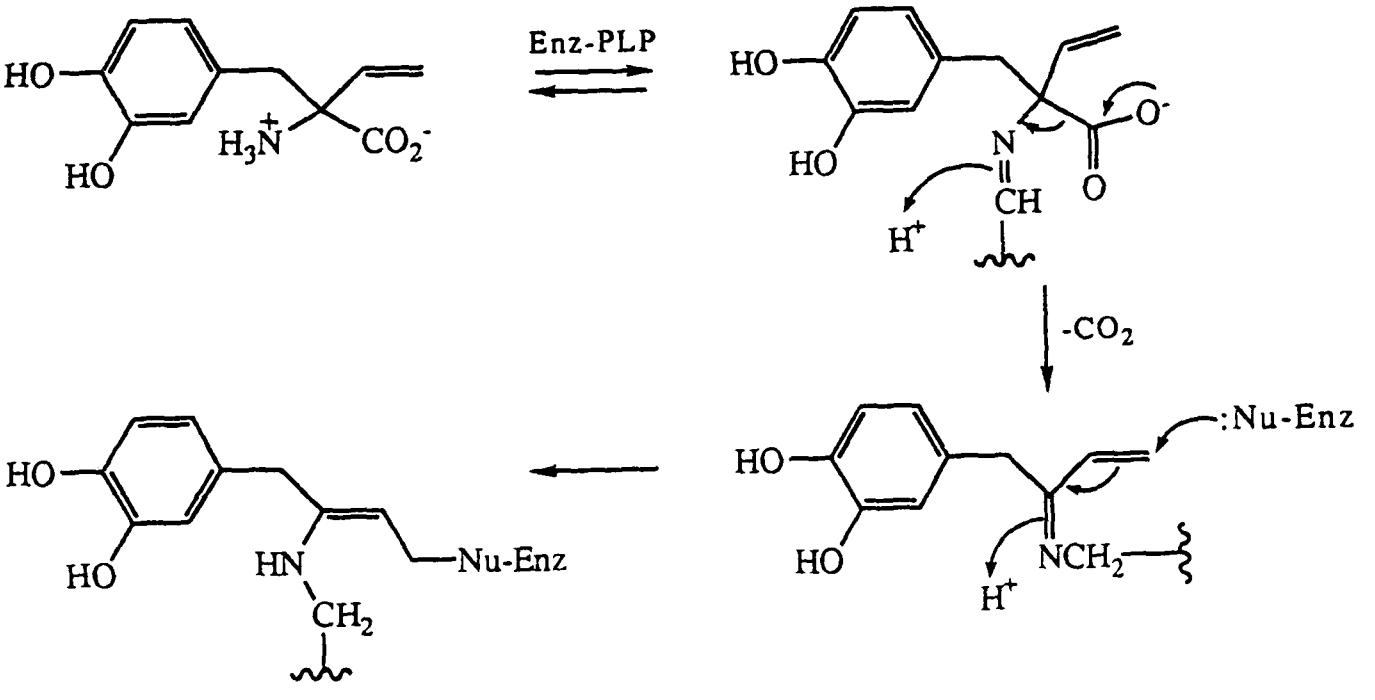


(129)



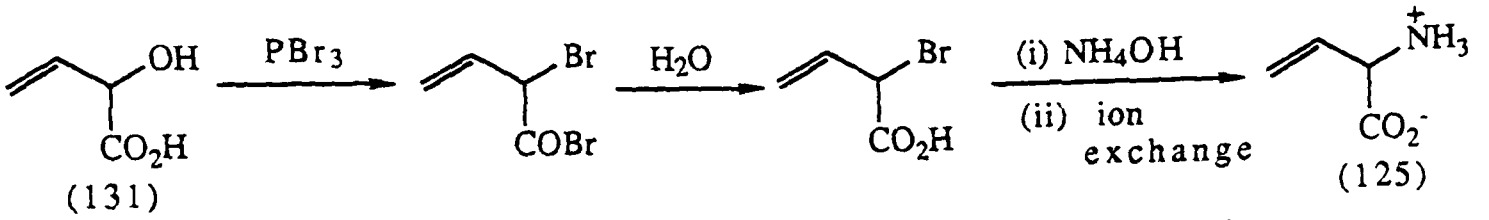
(130)

The proposed mechanism of inhibition of such species is shown in Scheme 4.5.

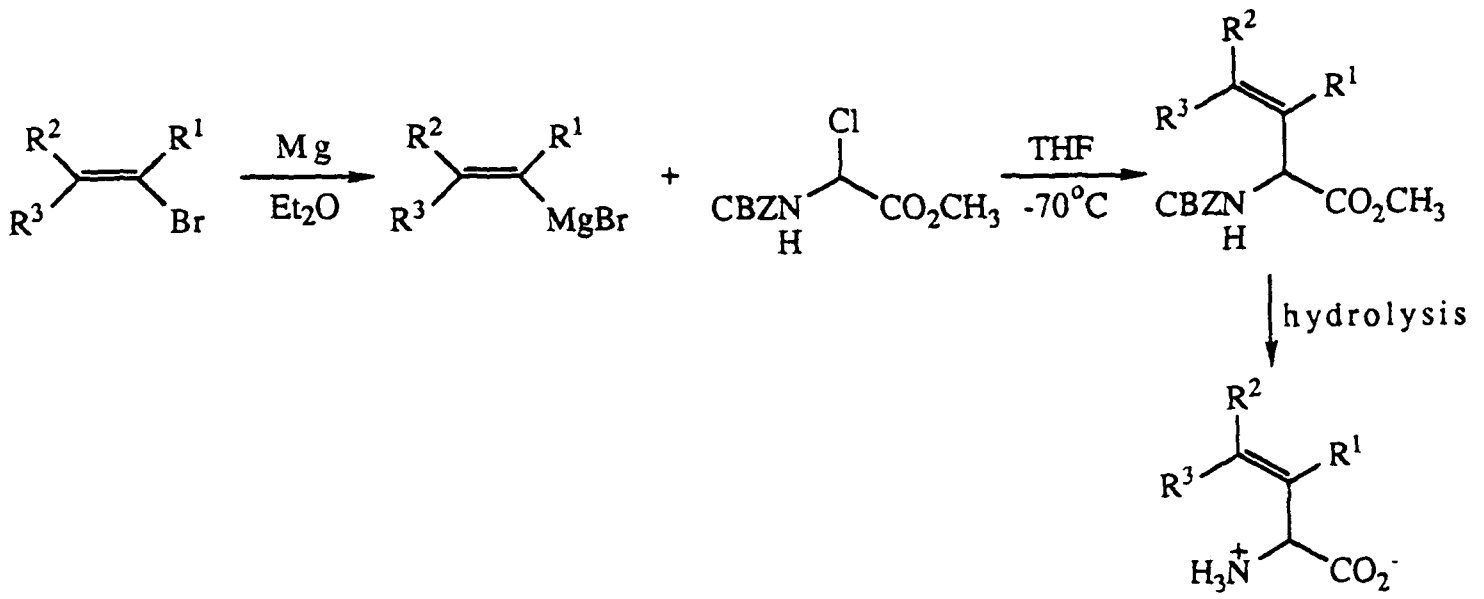


Scheme 4.5

Baldwin *et al*[114] developed a synthesis of vinylglycine (125) from 2-hydroxy-3-butenoic acid (131) (Scheme 4.6), but more versatile procedures have since been developed by Hanessian[115], Rapoport[116], and Castelhano and Krantz (Scheme 4.7).[127]



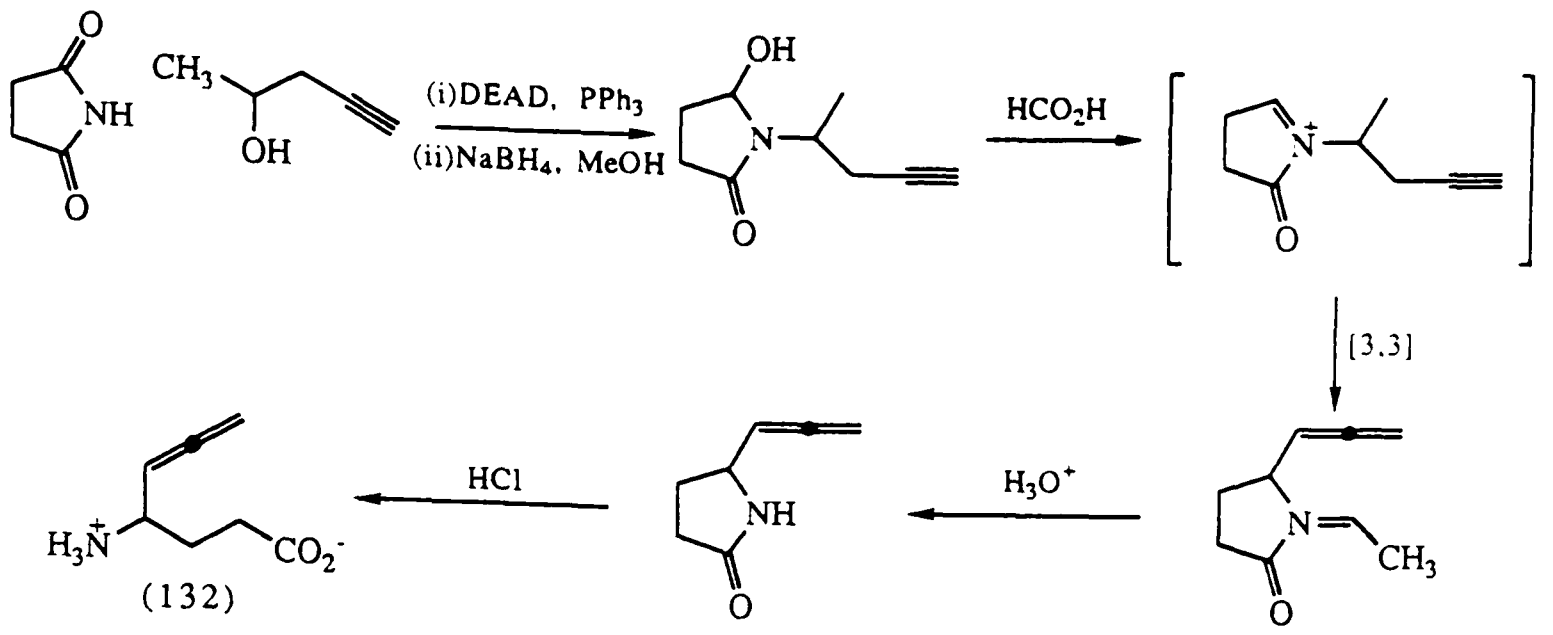
Scheme 4.6



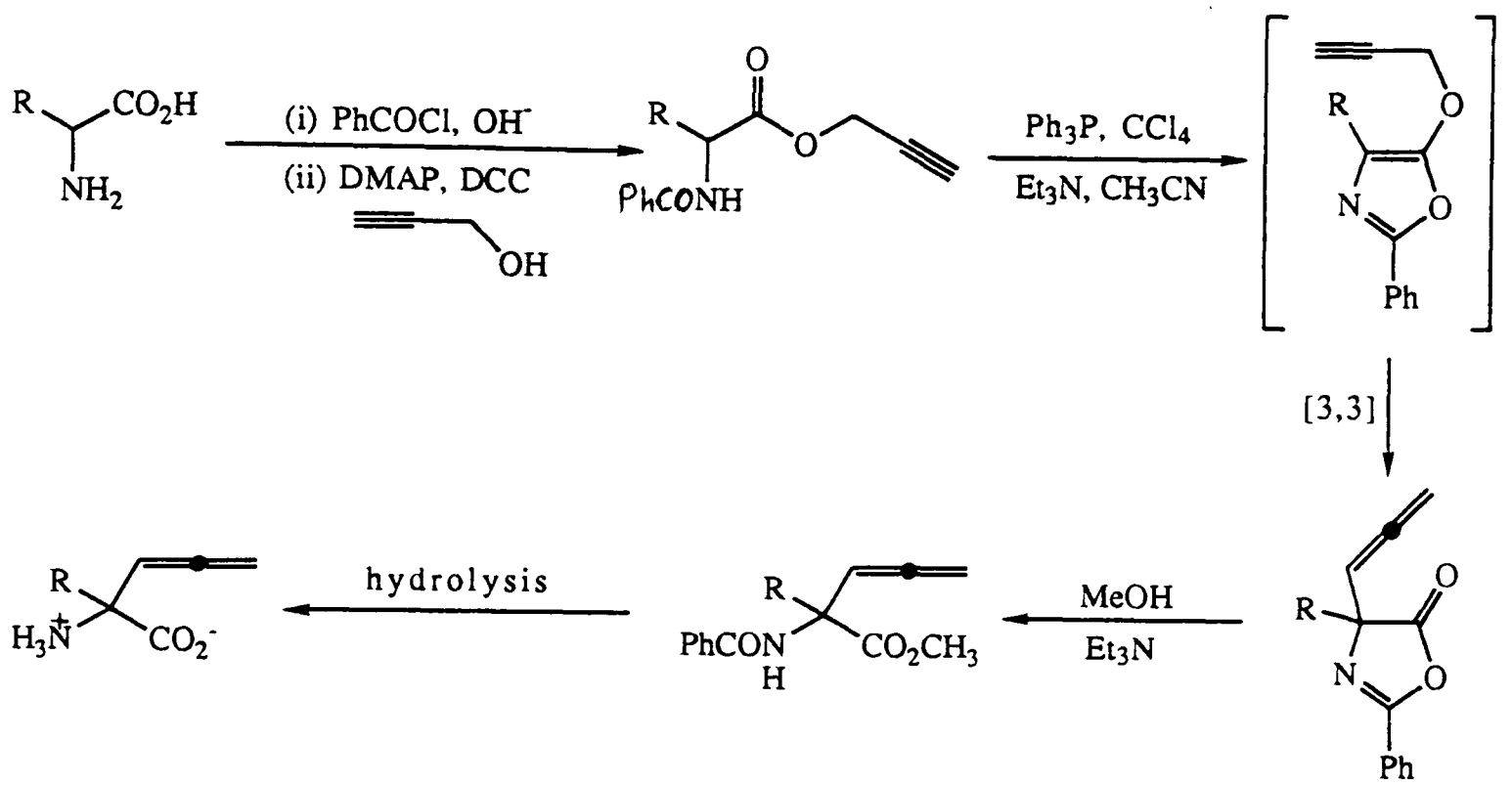
Scheme 4.7

The latter workers have also prepared a parent allenic amino acid, 4-amino-5,6-heptadienoic acid (γ-allenyl GABA) (132),[118] a potent and specific inhibitor of mammalian GABA transaminase, *via* a novel aza-Cope rearrangement (Scheme 4.8).

The Claisen rearrangement[119] has also been used *en route* to the synthesis of α-functionalised α-allenylglycine derivatives (Scheme 4.9)[112]



Scheme 4.8

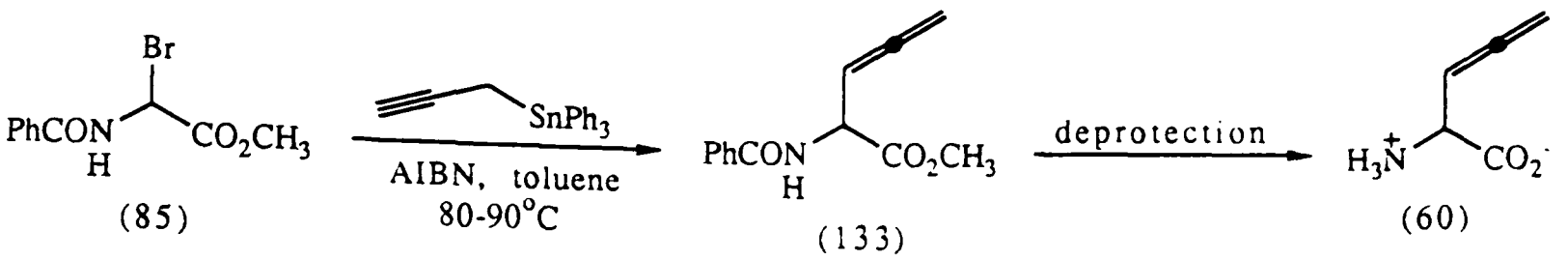


Scheme 4.9

Despite these efforts, there had been no literature report of the synthesis of the parent α-amino acid, α-allenylglycine (60). We thus envisaged the use of the 'allene transfer reaction' discussed in Section 2.4, to prepare (60) from the α-glycidyl radical.

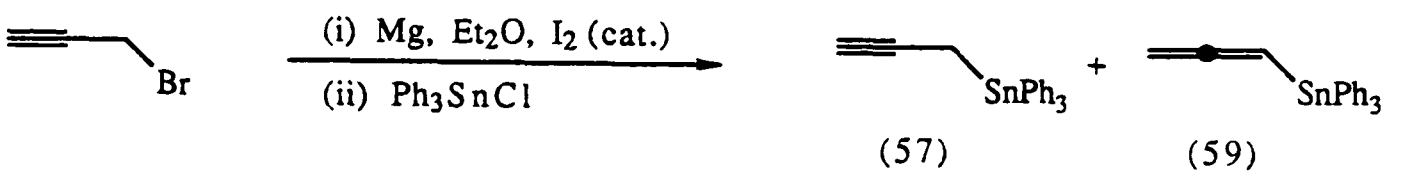
4.3. Synthesis of α-Allenylglycine

The envisaged synthetic scheme is shown below (Scheme 4.10).



Scheme 4.10

Propargyltriphenylstannane (57)[120] was synthesised by adding propargyl bromide to a vigorously stirred mixture of magnesium turnings and a catalytic amount of iodine in dry ether. Triphenyltin chloride was then slowly added to the Grignard reagent and the reaction mixture stirred at room temperature for 2.5 hours, before cautiously quenching the reaction with aqueous ammonium chloride. Extraction and purification of the product by chromatography furnished (57) in 53% yield, with allenyltriphenylstannane (59) (16%) formed as a by-product (Scheme 4.11).

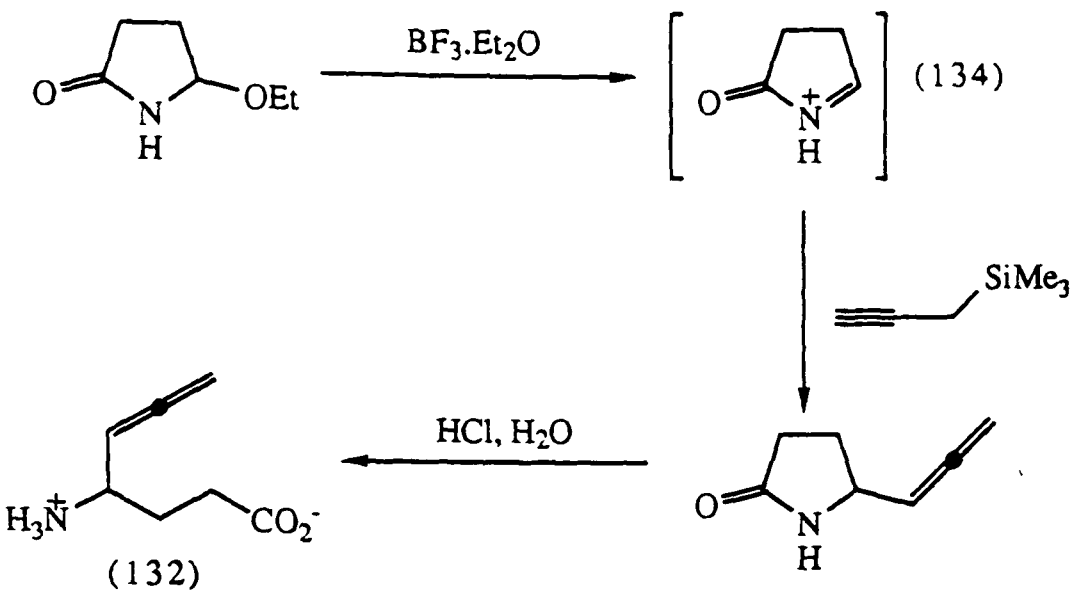


Scheme 4.11

However, the reactions of (57) (2 equiv.) with (85) under standard radical conditions using both photolytic and chemical (AIBN) initiation gave rise to a complex mixture of products. Purification of the crude reaction mixtures by chromatography furnished only negligible amounts of (133). Treatment of (85) with 2 equivalents of (57) initially, followed by a further 2 equivalents every 4 hours for 30 hours (catalytic quantities of AIBN were also added at 4 hourly intervals), produced no identifiable products.

Therefore, the basic strategy had to be rethought. Attention was turned to a publication by Speckamp *et al*[121] outlining the synthesis of γ-allenyl GABA

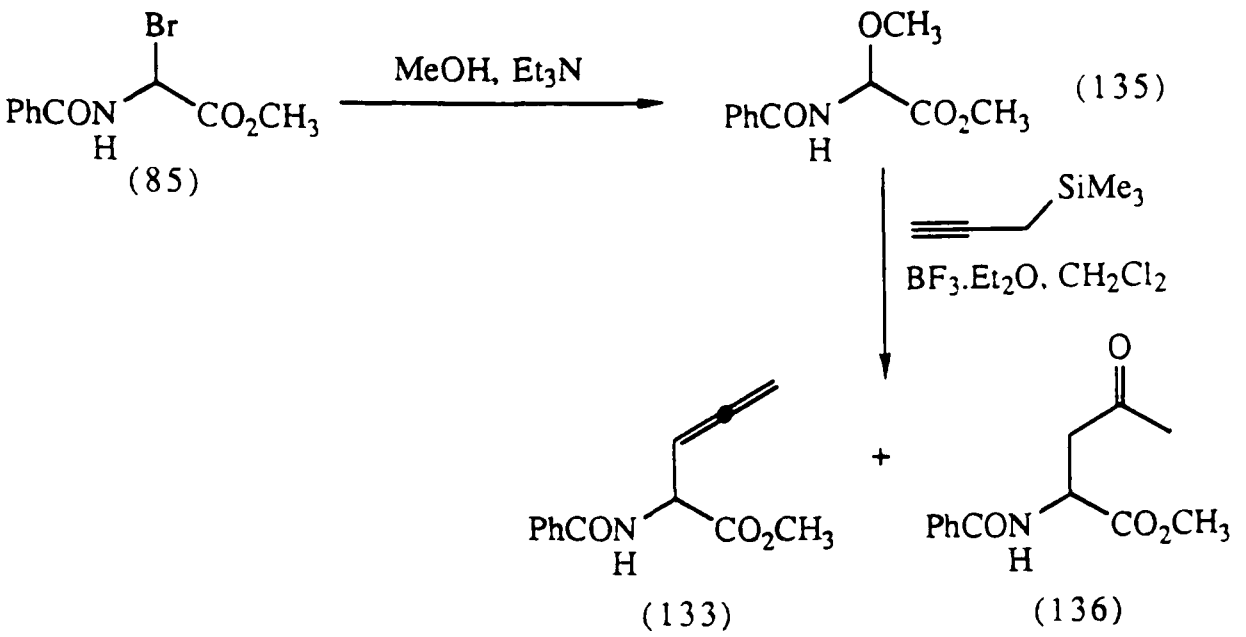
(132) using Lewis acid induced reactions of propargyltrimethylsilane, believed to proceed *via* the acyliminium ion (134) (Scheme 4.12).



Scheme 4.12

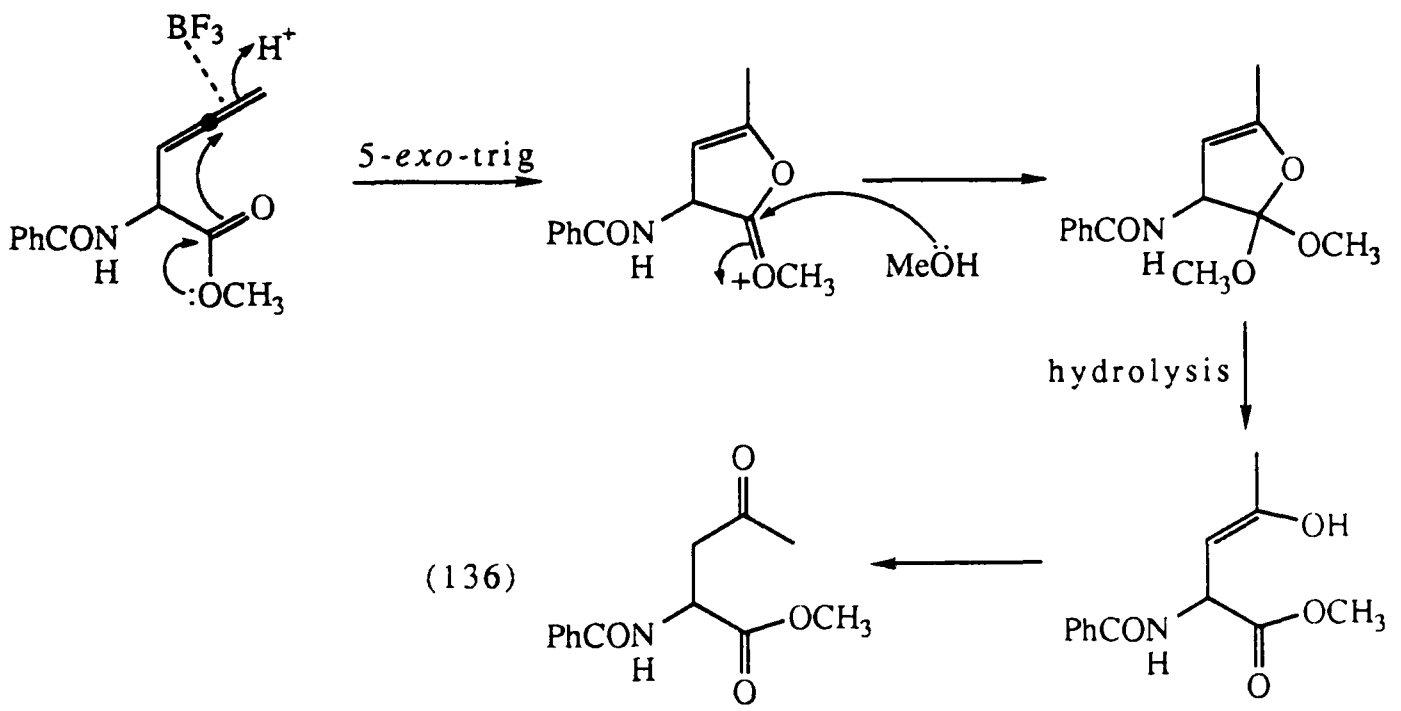
Treatment of (85) with propargyltrimethylsilane (3 equiv.) using boron trifluoride etherate as a Lewis acid in dichloromethane at 0°C failed to produce the desired α-allenylglycine derivative; consequently the α-methoxyglycine derivative (135) was prepared to comply more closely with Speckamp's procedure.

Triethylamine was added to a solution of (85) in methanol at room temperature. Evaporation of the solvent after 3 hours and purification of the residue by chromatography gave (135) in 92% yield (Scheme 4.13). Boron trifluoride etherate (3 equiv.) was added dropwise to a stirred solution of (135) (1 equiv.) and propargyltrimethylsilane (3 equiv.) in dichloromethane at 0°C, and the solution was warmed to room temperature over a period of 5 hours. The reaction was quenched by the addition of water. Purification by chromatography gave rise to the desired product (133) in low yield (14%), and the ketone (136) (14%), which gave a positive 2,4-dinitrophenyl-hydrazine test.



Scheme 4.13

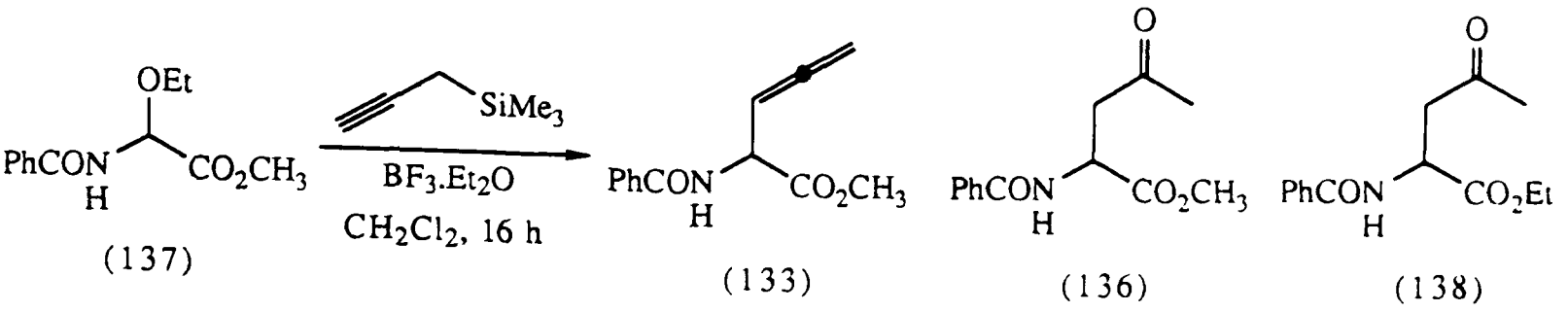
A plausible mechanism for the formation of (136) catalysed by the Lewis acid is shown in Scheme 4.14.



Scheme 4.14

As a complement to (135), and because ethoxide is less nucleophilic than methoxide, the α-ethoxyglycine derivative (137) was prepared in a similar manner by treatment of (85) with ethanol/triethylamine (Scheme 4.15). However, reaction of (137) with propargyltrimethylsilane/boron trifluoride gave rise to three products: (133), (136) and (138) in a ratio of *ca* 5:2:2. As (136) and (138) were produced in essentially equal quantities, a degree of credibility was given to the mechanism postulated in Scheme 4.14.

The alternative explanation is that the Lewis acid merely catalysed both ester exchange and hydrolysis of the allene.

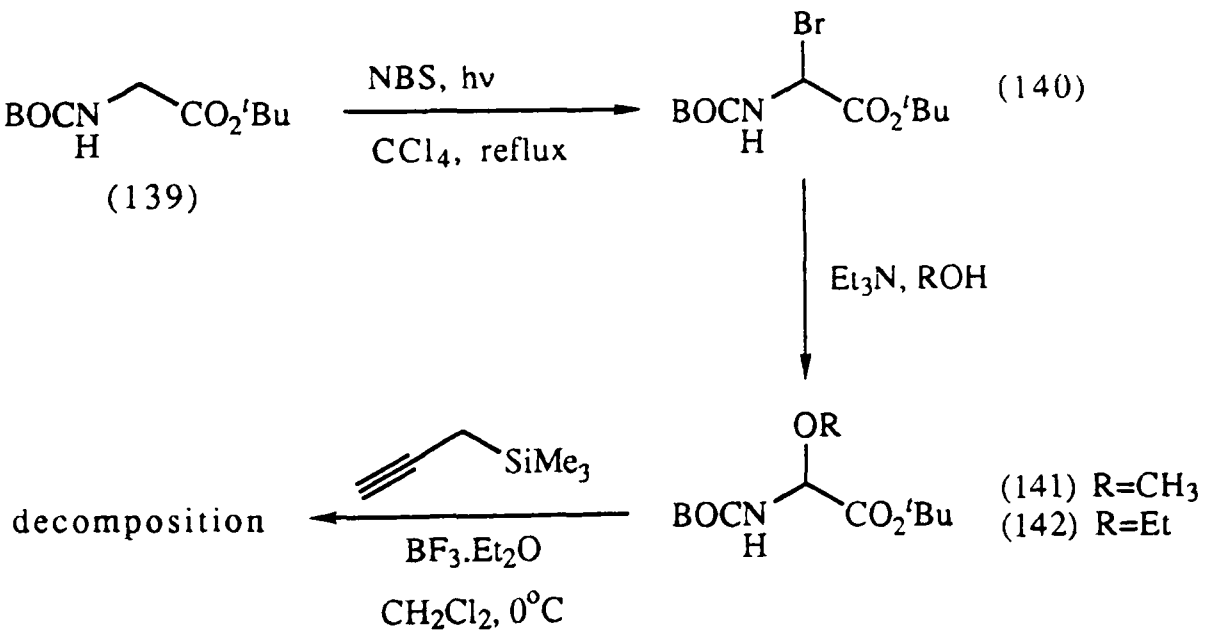


(137) (133) (136) (138)

Scheme 4.15

Bearing in mind the fairly vigorous acidic conditions required to remove the glycine protecting groups, attention was turned to the choice of alternative protecting groups, whose removal would not jeopardise the integrity of the allene. The *t*-butoxycarbonyl urethane protecting group was chosen for the amine, and the *t*-butyl ester for the carbonyl group.

Thus, bromination of (139)[122] under standard conditions produced (140) in quantitative yield. Treatment of (140) with methanol/triethylamine and ethanol/triethylamine, as described above, gave (141) and (142) respectively (Scheme 4.16).

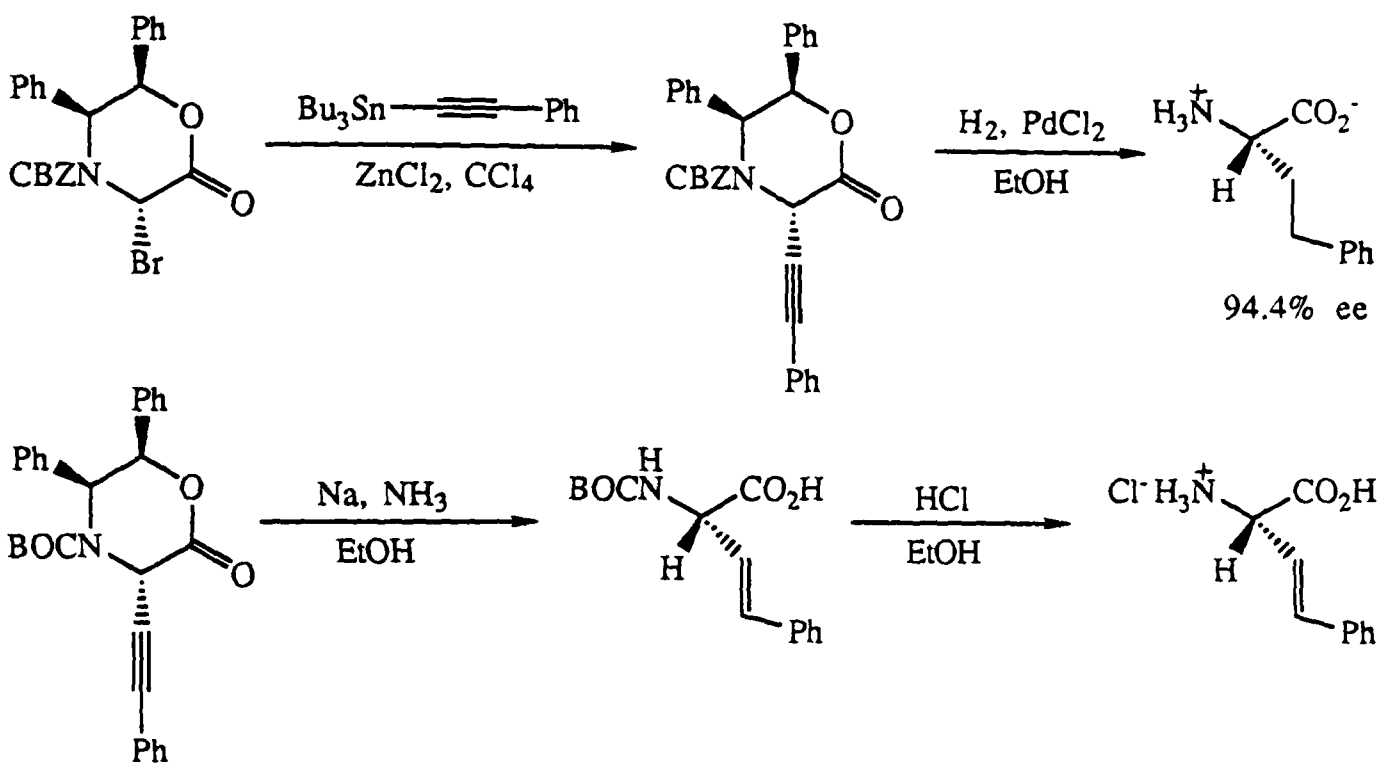


(139)

Scheme 4.16

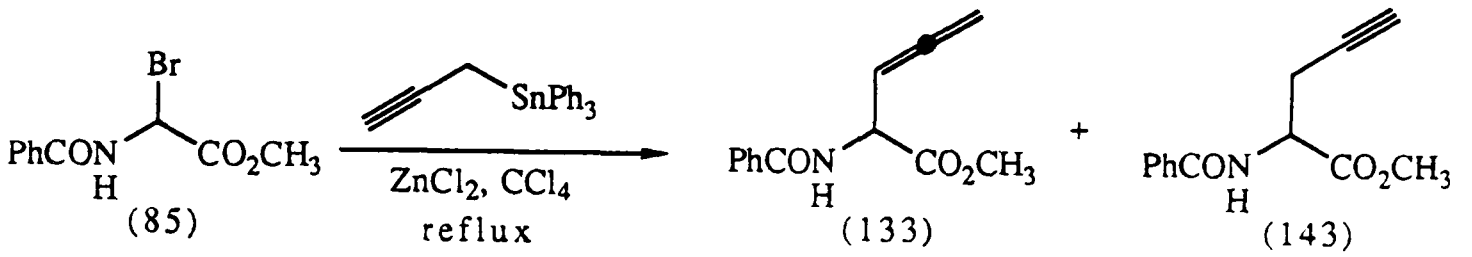
However, reaction of (141) and (142) with propargyltrimethylsilane led only to small quantities of unidentifiable products, the bulk of the reaction mixture being lost in the aqueous work-up. This suggested that the Lewis acid had removed the acid-labile protecting groups.

In a recent disclosure, Williams *et al* reported the use of zinc(II) chloride as a Lewis acid in the preparation of homologated glycines and vinylglycine derivatives from alkynylstannanes (Scheme 4.17).[123,124]



Scheme 4.17

Thus, to a stirred solution of (85) in carbon tetrachloride was added 2 equivalents of propargyltriphenylstannane (57) and 2 equivalents of zinc(II) chloride. Stirring the reaction mixture at room temperature led only to the isolation of allenyltriphenylstannane (59). However, reflux of the reaction mixture for 50 minutes under an inert atmosphere, followed by standard aqueous potassium fluoride work-up, furnished a mixture of (133) and (143) in a ratio of 2:1 respectively (total yield 51%) (Scheme 4.18).

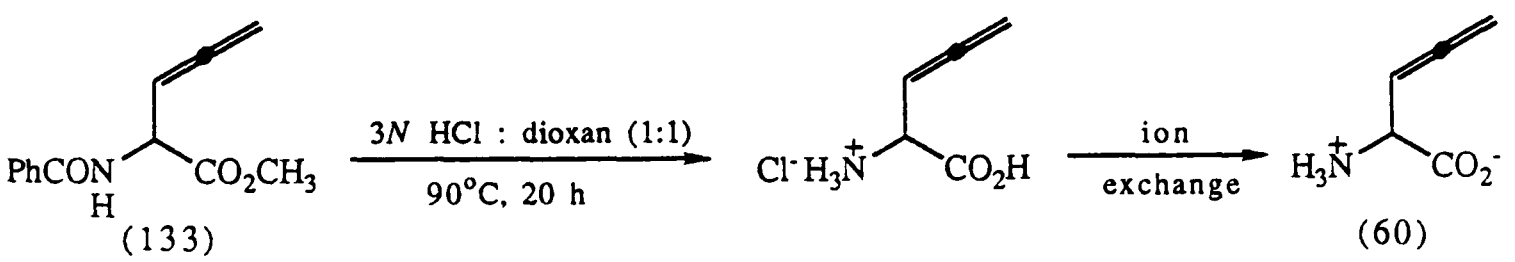


Scheme 4.18

This mixture was inseparable by normal silica gel chromatography, but could be separated on an analytical scale by normal phase HPLC. It was subsequently observed that (143) could be crystallised out of a dichloromethane/petroleum ether mixture, albeit contaminated with (133). However, the mother liquor contained (133) in a pure form.

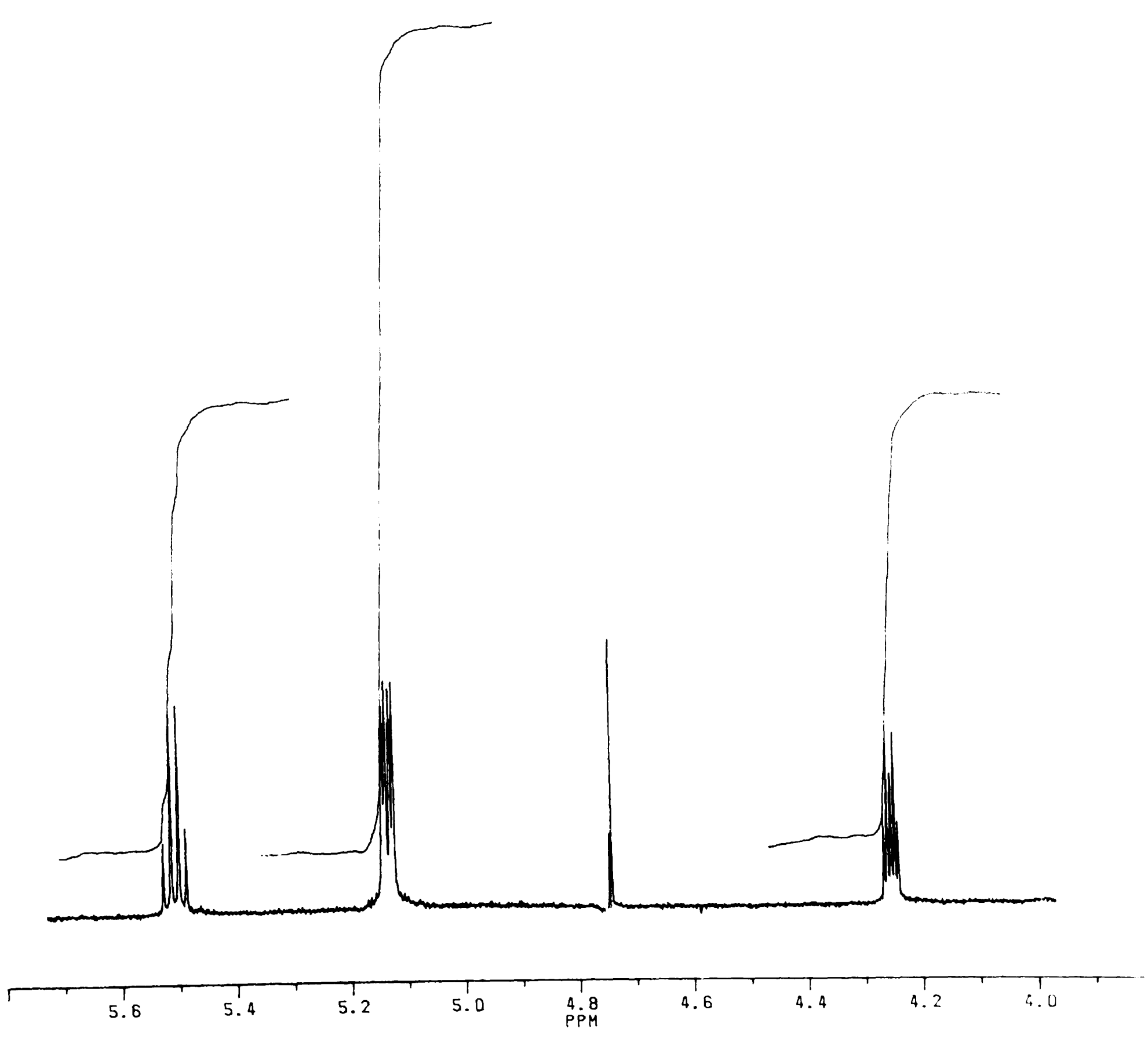
It is interesting to note that treatment of (85) with allenyltriphenyl-stannane (59) under these conditions led only to the isolation of (143) in 47% yield.

Deprotection of (133) could be achieved in a solution of 3*N* aqueous hydrochloric acid : dioxan (1:1) at 90°C for 20 hours (Scheme 4.19). The amino acid was purified by passage of an aqueous solution of the crude product through decolourising charcoal, then by ion-exchange chromatography using Dowex resin, to give α-allenylglycine (60) in 23% yield, which was subsequently fully characterised. The ^1H nmr spectrum (500 MHz, D_2O, referenced to TSP) is shown in Figure 4.1.

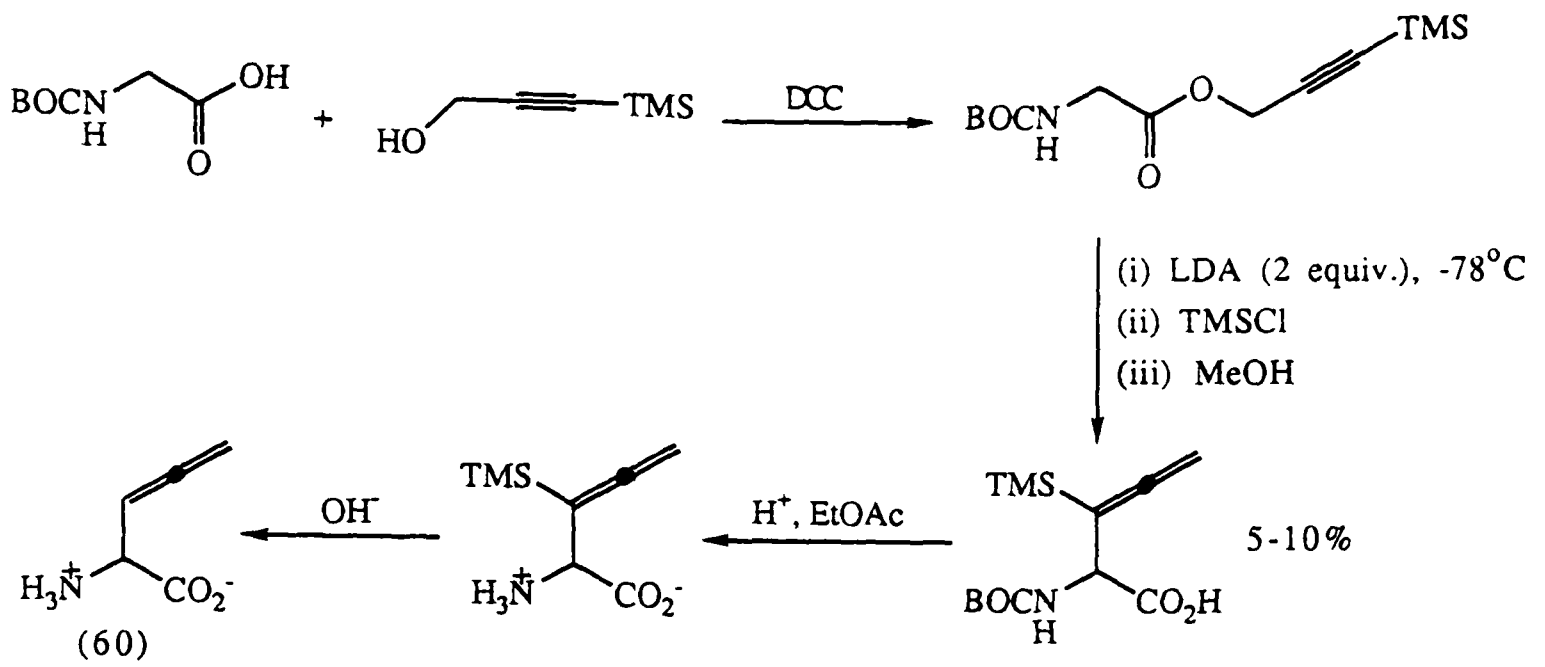


Scheme 4.19

Figure 4.1



Towards completion of this work, a low-yielding synthesis of (60) utilising the Ireland-Claisen rearrangement was reported in the literature (Scheme 4.20).[125]



Scheme 4.20

The overall yield of (60) was not disclosed and the overall process was claimed to be generally irreproducible! Repetition of this route in our laboratory gave <<5% yield.[126] However, the reported characterisation data for (60), though minimal, was in agreement with our data.

5. EXPERIMENTAL

General Experimental

The same general considerations applicable to Part One of this thesis also apply to Part Two.

N-Benzoyl-glycine, methyl ester (84)

Hydrogen chloride gas was bubbled through a suspension of *N*-benzoyl-glycine (3.0g, 16.7mmol) in methanol (30ml) cooled in an ice-water bath, for 1.5 h. A calcium chloride drying tube was then fitted to the reaction vessel, and the clear solution allowed to stir at room temperature for 24 h. The methanol was removed *in vacuo*; the residue was dissolved in ethyl acetate (100ml), washed with water (2 x 150ml), sodium hydrogen carbonate solution (150ml) and brine (100ml). The organic layer was dried (sodium sulphate), filtered and evaporated *in vacuo*. The resulting oil was purified by chromatography [(flash silica, 300g), ethyl acetate] R_f 0.55 to yield the title compound (84) (3.0g, 93%) as a clear oil which crystallised on standing; m.p. 83-84°C, (Lit.[93], 85°C); ν_{max} ($CHCl_3$) 3439 bm, 3013 m, 1747 s, 1662 s, 1604 m, 1581 m, 1521 s, 1488 s, 1440 s, 1373 s; δH (200 MHz, $CDCl_3$) 3.79 (3H, s, $-OCH_3$), 4.24 (2H, d, *J* 5 Hz, $-CH_2-$), 6.86 (1H, bs, -NH-), 7.39-7.56 [3H, m, Ar(*m*-H, *p*-H)], 7.79-7.85 [2H, m, Ar(*o*-H)]; δC (50 MHz, $CDCl_3$) 41.6 (t, $-CH_2-$), 52.3 (q, $-OCH_3$), 127.3, 128.7, 131.9 (3 x d, Ar), 133.7 [s, Ar(*ipso*-C)], 167.9, 170.8 (2 x s, 2 x CO); m/e (NH_3 CI) 211 (MNH_4^+, 25%), 194 (MH^+, 100), 105 (10).

N-Benzoyl-2-bromoglycine, methyl ester (85)

Method 1

N-Benzoyl-glycine methyl ester (84) (2.93g, 15mmol) and *N*-bromosuccinimide (2.75g, 15mmol) were suspended in degassed carbon

tetrachloride (85ml). The solution was then refluxed under illumination from a 300W tungsten lamp for 45 min until the brown colouration had disappeared. The reaction mixture was cooled to 0°C and the insoluble succinimide filtered off. The solvent was removed *in vacuo* to yield (85) (4.06g, 99%) as a cream coloured amorphous solid. The product was stored in the dark at 4°C; ν_{max} ($CHCl_3$) 3421 w, 3020 w, 1747 s, 1688s, 1505 s, 1484 s, 1440 s, 1347 s; δH (500 MHz, $CDCl_3$) 3.93 (3H, s, -OCH_3), 6.69 (1H, d, *J* 10 Hz, 2-H), 7.44 (1H, d, *J* 10 Hz, -NH-), 7.50 [2H, *ca* t, Ar(*m*-H)], 7.60 [1H, t, *J* 7.5 Hz, Ar(*p*-H)], 7.84 [2H, d, *J* 8.5 Hz, Ar(*o*-C)]; δC (50 MHz, $CDCl_3$) 49.5 (d, 2-C), 53.7 (q, -OCH_3), 127.5, 129.0, 133.1 (3 x d, Ar), 133.4 [s, Ar(*ipso*-C)], 166.0, 167.6 (2 x s, 2 x CO).

Method 2

A suspension of *N*-Benzoyl-glycine methyl ester (84) (2.65g, 14.0mmol), *N*-bromosuccinimide (2.51g, 14.0mmol) and AIBN (50mg) in degassed carbon tetrachloride (75ml) was refluxed under argon for 1.5 h, cooled to 0°C, and the insoluble succinimide filtered off. Evaporation of the solvent *in vacuo* furnished (85) (3.65g, 98%); data as in Method 1 above.

Triphenylallylstannane (61)

To magnesium turnings (2.43g, 100mmol) in THF (32ml) was added a small quantity of allyl bromide to initiate reaction. The rapidly stirred mixture was then heated to reflux. A solution of triphenyltin chloride (9.60g, 25.0mmol) and allyl bromide (4.70g, 39.0mmol) in THF (24ml) was added over 2 h, and the reaction mixture refluxed for a further 16 h. After cooling to room temperature, the reaction mixture was cautiously quenched with saturated ammonium chloride solution (100ml). The organic layer was decanted and the aqueous layer extracted with diethyl ether (100ml). The combined organic layers were evaporated *in vacuo* and the solid residue recrystallised from 30-40 petroleum ether to yield (61) (8.40g, 86%) as white

crystals; m.p. 70-71°C, (Lit.[82], 73.5-74.5°C); δH (500 MHz, $CDCl_3$) 2.49 (2H, d, *J* 8.5 Hz, $^2J_{SnH}$ 36 Hz, -$SnCH_2$-), 4.83 (1H, d, J_{cis} 9 Hz, $^4J_{SnH}$ 16 Hz, C<u>H</u>$_2$=CH-), 5.01 (1H, d, J_{trans} 17 Hz, $^4J_{SnH}$ 20 Hz, C<u>H</u>$_2$=CH-), 6.06-6.15 (1H, m, CH_2=C<u>H</u>-), 7.39-7.62 (15H, m, Ar).

<u>2-Benzoylaminopent-4-enoic acid, methyl ester (88)</u>

A solution of (85) (100mg, 0.368mmol) and allyltriphenylstannane (290mg, 0.742mmol) in toluene (2.5ml) was degassed by bubbling argon through the solution for 20 min. AIBN (20mg) was added and the solution heated at 90°C under argon for 2 h. The toluene was removed *in vacuo*; the residue was dissolved in ethyl acetate (20ml) and shaken with 5% aqueous potassium fluoride solution (20ml) to form a precipitate of triphenyltin fluoride, which was filtered off. The organic layer was dried (sodium sulphate), filtered and evaporated *in vacuo*. The crude product was purified by chromatography [(flash silica, 30g), diethyl ether] R_f 0.5 to yield (88) (53mg, 62%) as a colourless oil; ν_{max} 3435 m, 3019 m, 1742 s, 1663 s, 1515 s, 1487 s, 1440 m, 1360 m, 997 m, 929 m; δH (500 MHz, $CDCl_3$) 2.61-2.76 (2H, m, 3-H), 3.79 (3H, s, -OCH_3), 4.88-4.93 (1H, m, 2-H), 5.17, 5.18 (2H, 2 x d, J_{cis} 10.5 Hz, J_{trans} 17.5 Hz, 5-H), 5.72-5.80 (1H, m, 4-H), 6.75 (1H, d, *J* 8.5 Hz, -NH-), 7.42-7.56 [3H, m, Ar(*o*-C, *p*-C)], 7.80 [2H, *ca* d, *J* 9 Hz, Ar(*m*-C)]; δC (125 MHz, $CDCl_3$) 36.6 (t, 3-C), 52.0 (d, 2-C), 52.4 (q, -OCH_3), 119.3 (t, 5-C), 127.0, 128.5, 131.6 (3 x d, Ar), 132.2 (d, 4-C), 133.9 [s, Ar(*ipso*-C)], 166.9, 172.2 (2 x s, 2 x CO); m/e (NH_3 ACE) 251 (MNH_4^+, 5%), 234 (MH^+, 100), 154 (30), 105 (50), 78 (12).

<u>*N*-Benzoyl-2-deuterioglycine, methyl ester (87)</u>

<u>Method 1</u>

A solution of (85) (200mg, 0.73mmol), tri-*n*-butyltin deuteride (595µl, 2.21mmol) and AIBN (20mg, 0.12mmol) in toluene (10ml) was degassed with argon and then heated at 90-95°C for 1.5 h. The toluene was removed *in*

vacuo, and the residue partitioned between hexane (30ml) and acetonitrile (30ml). The hexane layer was washed with acetonitrile (30ml), and the combined acetonitrile layers were dried (sodium sulphate), filtered and evaporated *in vacuo*. The resulting oil was purified by chromatography [(flash silica, 45g), ethyl acetate : 30-40 petroleum ether (1:1)] R_f 0.25 to yield (87) (94mg, 67%) as an oil which crystallised on standing; m.p. 83-84°C; ν_{max} ($CHCl_3$) 3441 m, 3008 m, 2956 m, 1744 s, 1662 s, 1604 m, 1582 m, 1518 s, 1488 s, 1440 m, 1351 s, 1293 m, 1232 m, 1165 m, 1103 m; δH (500 MHz, $CDCl_3$) 3.82 (3H, s, $-OCH_3$), 4.24-4.26 (1H, m, 2-H), 6.66 (1H, bs, -NH-), 7.44-7.48 [2H, m, Ar(*o*-H)], 7.52-7.55 [1H, m, Ar(*p*-H)], 7.82-7.83 [2H, m, Ar(*m*-H)]; δC (50 MHz, $CDCl_3$), 40.5, 40.7, 40.9 (3 x s, 2-C), 52.3 (q, $-OCH_3$), 127.2, 128.7, 131.9 (3 x d, Ar), 133.8 [s, Ar(*ipso*-C)], 167.9, 170.8 (2 x s, 2 x CO); m/e (NH_3 CI) 212 (MNH_4^+, 35%), 195 (MH^+, 100), 105 (10).

Method 2

A solution of (85) (100mg, 0.37mmol), tri-*n*-butyltin deuteride (250µl, 0.9mmol) and AIBN (10mg) in toluene (5ml) was sonic ated in an ultrasound bath at 5-10°C for 4 h under argon. The toluene was removed *in vacuo* and the residue purified by chromatography [(flash silica, 20g), diethyl ether] to yield (87) (30mg, 42%); data as in Method 1 above.

Attempted inhibition of the formation of (87)

To a solution of (85) (50mg, 0.184mmol) in degassed toluene (5ml) was added hydroquinone (40mg, 0.363mmol) and tri-*n*-butyltin deuteride (125µl, 0.464mmol). The solution was heated at 90°C for 5 h, and the toluene was removed *in vacuo*. ^1H nmr analysis of the residue, however, indicated the presence of (87) in good yield.

N-Benzoyl-2-hydroxyglycine, methyl ester (86)

Attempted purification of (85) by chromatography [flash silica, ethyl acetate: 30-40 petroleum ether (3:2)] led to the isolation of the title compound (86) (58% from 84) as a white solid; ν_{max} ($CHCl_3$) 3434 bm, 3018 m, 1749 s, 1663 s, 1517 s, 1487 m, 1442 m, 1099 m; δH (500 MHz, $CDCl_3$) 3.90 (3H, s, $-OCH_3$), 5.83 (1H, d, *J* 7 Hz, 2-H), 7.40 (1H, bs, -NH-), 7.46-7.51 [2H, m, Ar(*o*-H)], 7.55-7.59 [1H, m, Ar(*p*-H)], 7.82-7.86 [2H, m, Ar(*m*-H)]; m/e (NH_3 ACE) 210 (MH^+, 2%), 121 (55), 105 (95), 77 (100), 59 (31).

Ethyl α-(hydroxymethyl) acrylate (63)[75]

To a mixture of triethylphosphonoacetate (45.0g, 0.20mol) and 40% aqueous formaldehyde (65ml) was slowly added (over a period of 35 min) a saturated solution of potassium carbonate (48g, 0.35mol) in water (65ml) with vigorous stirring at room temperature. The mixture was stirred for a further 2 h, before careful addition of saturated ammonium chloride solution (75ml) to quench the reaction. The mixture was extracted with diethyl ether (3 x 75ml), and the combined organic phases dried (sodium sulphate), filtered and evaporated *in vacuo* to yield an oil (27.9g). The crude product was purified by distillation under reduced pressure to yield (63) (17.2g, 66%); b.p. 91-95°C (0.4 mmHg); δH (500 MHz, $CDCl_3$) 1.30 (3H, t, *J* 7 Hz, $-CH_2C\underline{H}_3$), 2.59 (1H, bs, -OH), 4.23 (2H, q, *J* 7 Hz, $-C\underline{H}_2CH_3$), 4.31 (2H, d, *J* 6Hz, $-C\underline{H}_2OH$), 5.82, 6.24 (2 x s, 2 x 1H, CH_2=C-).

Ethyl α-(bromomethyl) acrylate (64)[75]

Phosphorus tribromide (5.75ml, 17.0g, 0.13mol) was added in one portion *via* syringe to a solution of (63) (17.2g, 0.13mol) in diethyl ether (125ml) at -10°C under an atmosphere of argon. The reaction mixture was allowed to warm to 20°C and was stirred for 3 h. The mixture was then cooled to -10°C and water (75ml) was added. After extraction of the mixture with hexane (3

x 25ml), the combined organic phases were dried (sodium sulphate), filtered and evaporated *in vacuo* to give a pale golden liquid which was distilled under reduced pressure to yield (64) (20.7g, 82%) as a colourless liquid; b.p. 53-58°C (0.4 mmHg); δH (500 MHz, $CDCl_3$) 1.33 (3H, t, *J* 7 Hz, -$OCH_2C\underline{H}_3$), 4.18 (2H, s, -CH_2Br), 4.27 (2H, q, *J* 7 Hz, -$OC\underline{H}_2CH_3$), 5.94, 6.33 (2 x 1H, 2 x s, CH_2=C-).

Ethyl α-(4-tolylsulphonylmethyl) acrylate (65)[75]

A solution of (64) (19.0g, 0.10mol) and sodium 4-tolylsulphinate dihydrate (21.0g, 0.10mol) in methanol (75ml) was refluxed for 16 h. The solvent was removed *in vacuo* and the resulting oil was purified by chromatography [(flash silica, 400g), gradient elution, diethyl ether : 30-40 petroleum ether (1:1 - 4:1)] to yield (65) (15.5g, 60%) as a viscous oil which crystallised on standing; R_f 0.2 [diethyl ether : 30-40 petroleum ether (1:1)]; δH (500 MHz, $CDCl_3$) 1.18 (3H, t, *J* 7 Hz, -$OCH_2C\underline{H}_3$), 2.43 (3H, s, Ar$C\underline{H}_3$), 4.03 (2H, q, *J* 7 Hz, -$OC\underline{H}_2CH_3$), 4.14 (2H, s, -CH_2SO_2-), 5.90, 6.50 (2 x 1H, 2 x s, CH_2=C-), 7.32, 7.73 (4H, ABq, *J* 8 Hz, Ar); m/e (NH_3 CI) 286 (MNH_4^+, 100%), 269 (MH^+, 15).

Ethyl α-(tri-*n*-butylstannylmethyl) acrylate ()

Method 1[75]

A solution of (65) (2.0g, 7.5mmol), tri-*n*-butyltin hydride (4.0ml, 15.0mmol) and AIBN (60mg) in degassed toluene was heated at 85-90°C for 40 min. The reaction mixture was concentrated *in vacuo* to give a viscous oil which was purified by chromatography [(flash silica, 400g), 30-40 petroleum ether : diethyl ether (9:1)] R_f 0.6 to yield the stannane (56) (2.17g, 76%) as a colourless liquid; b.p. 150°C (0.3 mmHg); ν_{max} ($CHCl_3$) 2959 s, 2927 s, 2872 s, 2855 m, 1704 s, 1613 m, 1465 m, 1185 s; δH (500 MHz, $CDCl_3$) 0.81-0.92 [18H, m, -$Sn(CH_2)_2C\underline{H}_2C\underline{H}_3$, -$CO_2CH_2C\underline{H}_3$], 1.26-1.52 (12H, m, -$SnC\underline{H}_2C\underline{H}_2C_2H_5$), 1.98 [2H, s, -$C\underline{H}_2Sn(n\text{-}Bu)_3$, ($^2J_{SnH}$ 30 Hz)], 4.19 (2H, q, -$CO_2C\underline{H}_2CH_3$), 5.28 [1H, d, *J* 1 Hz, CH_2=C-, ($^4J_{SnH}$ 5 Hz)], 5.82 [1H, d, *J* 1 Hz, CH_2=C-, ($^4J_{SnH}$ 8 Hz)]; m/e (EI) 404

(MH^+ ^{120}Sn, 8%), 402 (MH^+ ^{118}Sn, 7), 400 (MH^+ ^{116}Sn, 6), 347 (^{120}Sn, 50), 345 (^{118}Sn, 35), 343 (^{116}Sn, 20), 235 (40), 233 (45), 231 (35), 179 (90), 177 (100).

Method 2

A solution of the sulphone (65) (2.00g, 7.50mmol), tri-*n*-butyltin hydride (4ml, 15.0mmol) and AIBN (50mg) in toluene (30ml) was sonic ated in an ultrasound bath at 5-10°C for 4 h under argon. The toluene was removed *in vacuo*, and the residue purified as above to yield (56) (2.46g, 86%); data as in Method 1 above.

2-Benzoylamino-4-methylene-4-carboethoxypentanoic acid, methyl ester (89)

A solution of (85) (640mg, 2.35mmol), the stannane (56) (1.80g, 4.46mmol) and AIBN (50mg) in degassed toluene (12ml) was heated at 90°C for 1.5 h. The toluene was removed *in vacuo* and the residue partitioned between ethyl acetate (20ml) and 5% aqueous potassium fluoride solution (20ml). After shaking, the resulting white precipitate was filtered off. The organic layer was dried (sodium sulphate), filtered and evaporated *in vacuo*, and the residue was purified by chromatography [(flash silica, 250g), diethyl ether] R_f 0.45 to yield (89) (480mg, 67%) as an oil; ν_{max} ($CHCl_3$) 3020 s, 1740 s, 1711 s, 1663 s, 1519 s, 1488 s, 1439 m, 1373 m; δH (500 MHz, $CDCl_3$) 1.30 (3H, t, *J* 7 Hz, $-OCH_2C\underline{H}_3$), 2.87-2.91, 2.93-2.96 (2H, 2 x 4 line multiplets, AB part of ABX system, 3-H), 3.76 (3H, s, $-OCH_3$), 4.23 (2H, q, *J* 7 Hz, $-OC\underline{H}_2CH_3$), 4.86-4.90 (1H, m, 2-H), 5.72, 6.29 [2 x 1H, 2 x s, CH_2=C-, ($J_{allylic}$ 1 Hz)], 7.29 (1H, bd, *J* 7 Hz, -NH-), 7.42-7.45 [2H, m, Ar(*o*-H)], 7.49-7.52 [1H, m, Ar(*p*-H)], 7.81-7.83 [2H, m, Ar(*m*-H)]; δC (50 MHz, $CDCl_3$) 13.9 (q, $-OCH_2\underline{C}H_3$), 34.0 (t, 3-C), 52.3 (q, $-OCH_3$), 52.9 (d, 2-C), 61.3 (t, $-O\underline{C}H_2CH_3$), 127.2, 128.6, 131.8 (3 x d, Ar), 129.2 (t, $\underline{C}H_2$=C-), 133.8 [s, Ar(*ipso*-C)], 136.1 (s, 4-C), 167.2, 167.7, 172.1 (3 x s, 3 x CO); m/e (NH_3 CI) 323 (MNH_4^+, 5%), 306 (MH^+, 100), 199 (20).

α-(Hydroxymethyl)acrylonitrile (67)

A mixture of diethylcyanomethylphosphonate (35.5g, 0.20mol) and 40% aqueous formaldehyde (65ml, 0.80mol) was stirred vigorously at 18°C, followed by a slow addition over 30 min of a saturated solution of potassium carbonate (48g, 0.35mol) in water (65ml). The reaction mixture was then stirred at room temperature for a further 2 h, then saturated aqueous ammonium chloride solution (75ml) was added carefully. The mixture was extracted with diethyl ether (3 x 75ml) and the combined organic layers were dried (sodium sulphate), filtered and evaporated *in vacuo*. The crude product was purified by distillation under reduced pressure to yield (67) (5.0g, 30%) as an oil; b.p. 76-80°C (0.4 mmHg); νmax (thin film) 3440 s, 2940 s, 2330 s, 1625 m, 1230 m, 950 m; δH (500 MHz, $CDCl_3$) 2.44 (1H, t, *J* 6 Hz, -OH), 4.26 (2H, *ca* d, *J* 6 Hz, $J_{allylic}$ 1 Hz, -C<u>H</u>$_2$OH), 6.03, 6.06 [2 x 1H, 2 x s, CH_2=C-, ($J_{allylic}$ 1 Hz)]; m/e (EI) 84 (MH^+, 30%), 83 (M^+, 11).

α-(Bromomethyl)acrylonitrile (68)

To a solution of (67) (4.25g, 0.051mol) in diethyl ether (30ml) cooled to -10°C under argon was added phosphorus tribromide (4.25g, 0.016mol) in one portion. After stirring at room temperature for 3.5h, the mixture was cooled to -10°C and water (20ml) was added. The reaction mixture was extracted with hexane (3 x 10ml); the combined organic layers were dried (sodium sulphate), filtered and evaporated *in vacuo*. The residue was distilled under reduced pressure to yield the title compound (68) (2.02g, 27%); b.p. 55-58°C (0.4 mmHg); ν_{max} (thin film) 2945 m, 2455 m, 1620 w, 1460 s, 915 m, 734 s; δH (500 MHz, $CDCl_3$) 4.01 (2H, s, -CH_2Br), 6.03, 6.06 (2 x 1H, 2 x s, CH_2=C-).

α-(4-Tolylsulphonylmethyl)acrylonitrile (69)

A solution of (68) (1.9g, 13.0mmol) and sodium 4-tolylsulphinate dihydrate (2.78g, 13.0mmol) in methanol (10ml) was refluxed for 16 h. The solvent was removed *in vacuo* and the resulting white solid was partitioned between ethyl acetate (50ml) and water (50ml). The aqueous layer was washed with further ethyl acetate (50ml) and the combined organic layers were dried (sodium sulphate), filtered and evaporated *in vacuo*. The crude product was purified by chromatography [(flash silica, 200g), diethyl ether] R_f 0.4 to yield the sulphone (69) (2.21g, 77%) as a white solid; m.p. 87-88°C; ν_{max} ($CHCl_3$) 2933 m, 2232 w, 1598 m, 1410 m, 1330 s; δH (500 MHz, $CDCl_3$) 2.48 (3H, s, Ar$C\underline{H}_3$), 3.91 (2H, s, -CH_2SO_2-), 6.01, 6.22 (2 x 1H, 2 x s, CH_2=C-), 7.41, 7.80 (4H, ABq, *J* 8 Hz, Ar); m/e (EI) 221 (M^+, 18%), 155 (52), 91 (100), 65 (28); [Found C, 59.45%; H, 4.99%; N, 6.26%. $C_{11}H_{11}NO_2S$ requires C, 59.70%; H, 5.01%; N, 6.33%].

α-(Tri-*n*-butylstannylmethyl) acrylonitrile (70)

A solution of the sulphone (69) (1.65g, 7.46mmol), tri-*n*-butyltin hydride (4.0ml, 15.0mmol) and AIBN (60mg) in degassed toluene (28ml) was heated at 85-90°C for 50 min. The solution was cooled and the toluene removed *in vacuo*. The residue was purified by chromatography [(flash silica, 350g), diethyl ether : 30-40 petroleum ether (1:19)] R_f 0.55 to yield (70) (2.01g, 76%) as a colourless liquid; ν_{max} ($CHCl_3$) 3020 s, 2958 s, 2928 s, 2873 m, 2855 m, 1602 m, 1466 m, 1269 m; δH (500 MHz, $CDCl_3$) 0.90-1.01 [15H, m, -$Sn(CH_2)_2C\underline{H}_2C\underline{H}_3$], 1.29-1.56 (12H, m, -$SnC\underline{H}_2C\underline{H}_2C_2H_5$), 1.92 [2H, s, $^2J_{SnH}$ 26 Hz, -$C\underline{H}_2Sn$(n-Bu)$_3$], 5.39 (1H, s, $^4J_{SnH}$ 8.5 Hz, CH_2=C-), 5.48 (1H, s, $^4J_{SnH}$ 8 Hz, CH_2=C-); δC (50 MHz, $CDCl_3$) 13.5 [q, -$Sn(CH_2)_3\underline{C}H_3$], 9.4, 17.3, 27.1, 28.8 (4 x t, alkyl chain -CH_2-), 119.8 (s, CH_2=$\underline{C}$-), 120.1 (s, -CN), 123.8 (t, $\underline{C}H_2$=C); m/e (EI) 358 (M^+ ^{120}Sn, 25%), 356 (M^+ ^{118}Sn, 20), 354 (M^+ ^{116}Sn, 15), 291 (50), 289 (35), 287 (20), 235 (55), 233 (40), 231 (25), 179 (100), 177 (100), 175 (75), 121 (60), 119 (50), 117

(33). [Found C, 54.03%; H, 8.90%; N, 3.85%. $C_{16}H_{31}NSn$ requires C, 53.78%; H, 8.68%; N, 3.92%].

2-Benzoylamino-4-cyano-4-methylenepentanoic acid, methyl ester (90)

Prepared in an analogous manner to (89), using (85) (570mg, 2.09mmol), the stannane (70) (1.50g, 4.2mmol) and AIBN (40mg), except the solution was heated at 85°C for 4 h. Purification of the crude product by chromatography [(flash silica, 200g), diethyl ether] R_f 0.35 gave (90) (398mg, 74%) as an oil; ν_{max} ($CHCl_3$) 3430 bw, 2957 m, 2927 m, 1743 s, 1665 s, 1514 s, 1486 s, 1440 m, 1360 m; δH (500 MHz, $CDCl_3$) 2.87-2.91, 3.02-3.06 (2H, m,2 x 4 line multiplets, AB part of ABX system, 3-H), 3.84 (3H, s, -OCH_3), 4.97-5.01 (1H, m, 2-H), 5.83, 6.00 (2 x 1H, 2 x s, CH_2=C-), 6.98 (1H, d, *J* 7.5 Hz, -NH-), 7.43-7.46 [2H, m, Ar(*o*-H)], 7.51-7.53 [1H, m, Ar(*p*-H)], 7.81-7.83 [2H, m, Ar(*m*-H)]; δC (125 MHz, $CDCl_3$) 36.7 (t, 3-C), 51.8 (q, -OCH_3), 52.9 (d, 2-C), 117.8, 118.1 (2 x s, -CN, CH_2=C-), 127.1, 128.7, 132.0 (3 x s, Ar), 133.5 [s, Ar(*ipso*-C)], 134.6 (t, CH_2=C-), 167.1, 170.9 (2 x s, 2 x CO); m/e (NH_3 DCI) 276 (MNH_4^+, 27%), 259 (MH^+, 100), 194 (35), 173 (17), 122 (32), 105 (50).

Inhibition of allyl transfer by hydroquinone

A solution of (85) (32mg, 0.12mmol), the stannane 56 (100mg, 0.25 mmol) and hydroquinone (40mg, 0.36mmol) in toluene (1ml) was stirred at 90°C in the dark for 6 h. The toluene was removed *in vacuo* and the residue analysed by ^1H nmr (500 MHz, $CDCl_3$), which showed a complete absence of the allyl transfer product (89).

Attempted reactions of 3-tri-*n*-butylstannylacrolein (99) with (85).

Method 1

A solution of tri-*n*-butylstannylacrolein (99) (45mg, 0.13mmol), (85) (20mg, 0.065mmol) and AIBN (2mg) in degassed toluene (250µl) was heated at 85-

90°C and the reaction monitored by tlc. After 2 h, the toluene was removed *in vacuo*, and the residue partitioned between ethyl acetate (5ml) and 5% aqueous potassium fluoride solution (5ml). The precipitate was filtered off, and the organic layer was dried (sodium sulphate), filtered and evaporated *in vacuo*. Analysis of the residue by ^{1}H nmr suggested a complex mixture of products.

Method 2

A solution of the stannane (99) (23mg, 0.067mmol) and (85) (13mg, 0.044mmol) in degassed toluene (275µl) was heated at 80°C with illumination from a 300W tungsten lamp for 18 h. Upon evaporation of the solvent *in vacuo*, standard potassium fluoride work up and purification by PLC, no identifiable products were observed by ^{1}H nmr analysis.

Method 3

A solution of the stannane (99) (23mg, 0.067mmol), (85) (13mg, 0.044mmol) and benzoyl peroxide (21mg, 0.088mmol) in degassed toluene (175µl) was heated at 65-70°C over 16 h. The toluene was removed *in vacuo* and the residue subjected to a potassium fluoride work up. Purification by PLC [diethyl ether : 30-40 petroleum ether (1:1)] yielded an oil corresponding to the benzoylated product (102).

N-Benzoyl-2-benzoyloxy-glycine methyl ester (102)

A solution of (85) (240mg, 0.88mmol) and benzoyl peroxide (420mg, 1.76mmol) in degassed toluene (4ml) was heated at 80°C for 7 h. The toluene was removed *in vacuo* and the residue purified by chromatography [(flash silica, 60g), gradient elution, diethyl ether : 30-40 petroleum ether (1:1 - 2:1)] to yield (102) (176mg, 64%) as an oil; t.l.c. [diethyl ether : 30-40 petroleum ether (1:1)] R_f0.15; ν_{max} ($CHCl_3$) 3443 bw, 3020 s, 1757 s, 1720 s, 1683 s, 1603 m, 1512 s, 1489 s, 1453 m, 1440 m, 1337 s, 1289 s, 1261 s, 1167 m, 1087 s, 1068 s, 1002 s; δH (500 MHz, $CDCl_3$) 3.86 (3H, s, -OCH_3), 6.86 [1H, d, *J* 9

Hz, 2-H], 7.44-7.48 (4H, m, Ar), 7.54-7.61 (2H, m, Ar), 7.72 (1H, bd, *J* 9Hz, -NH-), 7.86, 8.08 (4H, ABq, *J* 8.5 Hz, Ar); δC (125 MHz, $CDCl_3$) 53.3 (q, $-OCH_3$), 73.2 (d, 2-C), 126.7-134.4 (6 x d, Ar), 166.0, 166.7, 167.4 (3 x s, 3 x CO); m/e (NH_3 CI) 314 (MH^+, 4%), 194 (50), 139 (18), 122 (100), 105 (68). [Found C, 65.30%; H, 5.02%; N, 4.55%. $C_{17}H_{15}NO_5$ requires C, 65.17%; H, 4.83%; N, 4.47%.]

4-Methylene-glutamic acid (94)

The allyl transfer product (89) (116mg, 0.38 mmol) was refluxed in 3*N* hydrochloric acid : dioxan (1:1, 10ml) for 45 h. The solvent was removed *in vacuo*; the residue was dissolved in water (10ml) and washed with ethyl acetate (3 x 10ml). The aqueous layer was freeze-dried to yield (94) (47mg, 52%) as its hydrochloride salt, identical by ^1H nmr to the natural product[95]; δH (500 MHz, D_2O) 2.66-2.71, 2.82-2.87 (2H, m, 8 lines, 3-H), 4.09 (1H, dd, *J* 7, 5.5 Hz, 2-H), 5.77, 6.25 (2 x 1H, 2 x s, CH_2=C-).

3-(4-Tolylsulphonyl)prop-1-yne (74)[87]

To a stirred solution of propargyl bromide (80% w/v, 9.18g, 61.7mmol) in THF (55ml) was added a solution of tetra-*n*-butylammonium 4-toluenesulphinate (44.43g, 61.7mmol, as a 1:1 mixture with tetra-*n*-butylammonium bromide) in THF (50ml), and the solution stirred at 20°C for 16 h. A saturated aqueous solution of ammonium chloride (30ml) was added and the resulting mixture was extracted with chloroform (2 x 50ml). The combined organic layers were dried (sodium sulphate), filtered and evaporated *in vacuo* to yield (74) as a pale yellow solid; δH (250 MHz, $CDCl_3$) 2.42 (1H, t, *J* 3 Hz, 1-H), 2.51 (3H, s, $ArC\underline{H}_3$), 3.92 (2H, d, *J* 3 Hz, 3-H), 7.33, 7.77 (4H, ABq, *J* 8 Hz, Ar).

2-Acetoxy-3-(4-tolylsulphonyl)prop-1-ene (75)[87]

To a stirred solution of (74) (2.08g, 10.7mmol) in DMSO (10ml) was added glacial acetic acid (0.65ml, 11.3mmol) and triethylamine (1.4ml, 10mmol), and the reaction mixture was stirred at room temperature for 4 h. Diethyl ether (60ml) was then added; the mixture was washed with water (2 x 75ml) and 5% aqueous sodium hydrogen carbonate solution (2 x 25ml). The organic layer was dried (sodium sulphate), filtered and evaporated *in vacuo*. The residue was purified by chromatography [(flash silica, 200g), diethyl ether: 30-40 petroleum ether (2:3)] R_f 0.5 to yield (75) (574mg, 21%) as a white solid; δH (250 MHz, $CDCl_3$) 1.96 (3H, s, -$OCOCH_3$), 2.42 (3H, s, Ar$C\underline{H}_3$), 3.98 (2H, s, 3-H), 4.81, 5.03 (2 x 1H, 2 x d, *J* 2 Hz, 1-H), 7.34, 7.76 (4H, ABq, *J* 8 Hz, Ar).

2-Acetoxy-3-triphenylstannylprop-1-ene (76)[83]

A solution of (75) (574mg, 2.25mmol), triphenyltin hydride (1.59g, 4.50mmol) and AIBN (10mg) in degassed toluene (10ml) was refluxed for 15 h under an inert atmosphere. The solvent was removed *in vacuo* and the residue partitioned between 30-40 petroleum ether (10ml) and acetonitrile (10ml). The acetonitrile layer was dried (sodium sulphate), filtered and evaporated *in vacuo* to yield an oil, which was recrystallised from methanol to yield (76) (625mg, 62%) as a white solid; δH (500 MHz, $CDCl_3$) 1.60 (3H, s, -$OCOCH_3$), 2.61 (2H, s, $^2J_{SnH}$ 30 Hz, 3-H), 4.58 (1H, s, $^4J_{SnH}$ 5 Hz, 1-H), 4.68 (1H, s, $^4J_{SnH}$ 8 Hz, 1-H), 7.35-7.73 (15H, m, Ar).

Attempted allyl transfer reaction of (76)

A solution of (76) (67mg, 0.15mmol), (85) (20mg, 0.075mmol) and AIBN (*ca* 2mg) in degassed toluene (0.4ml) was heated at 85°C under an inert atmosphere for 2 h. The solvent was removed *in vacuo*, and after normal work up procedure a crude oil (44mg) was obtained. ^{1}H nmr analysis

indicated traces of the desired product (91); δH (500 MHz, $CDCl_3$) 2.88-2.95 [8 lines, -C<u>H</u>$_2$-C(OAc)-]; however, purification by PLC (diethyl ether) yielded only trace amounts of the hydrolysed product (136); δH (250 MHz, $CDCl_3$) 3.13-3.35 (8 lines, -C<u>H</u>$_2$-$COCH_3$).

<u>(2*S*, 5*R*) and(2*S*, 5*S*)-2,5-Dihydro-3,6-dimethoxy-2-isopropyl-5-phenylselenopyrazine (104,105)</u>

To a solution of 2*S*-(+)-2,5-dihydro-3,6-dimethoxy-2-isopropylpyrazine () (260mg, 253µl, 1.41mmol) in THF (3ml), cooled to -78°C under argon was added *n*-butyllithium (1.70*M* in hexanes, 890µl, 1.5mmol) and the solution was stirred at -70°C for 10 min. A solution of phenylselenyl chloride (287mg, 1.5mmol) in THF (3ml) was added rapidly, causing instantaneous decolourisation to a pale yellow solution which did not change colour over the next hour at -70°C. Saturated ammonium chloride solution (5ml) was added and the mixture allowed to warm to room temperature. The mixture was partitioned between diethyl ether (30ml) and water (30ml). The aqueous layer was extracted with further diethyl ether (2 x 30ml) and the combined organic layers were dried (sodium sulphate), filtered and evaporated *in vacuo*. The crude product was purified by chromatography [(flash silica, 25g), dichloromethane] to give two major products:

(105) (83mg, 17%); R_f 0.30 (dichloromethane); δH (500 MHz, $CDCl_3$) 0.53, 0.91 [2 x 3H, 2 x d, *J* 7 Hz, (C<u>H</u>$_3$)$_2$CH-], 2.07-2.18 [1H, m, $(CH_3)_2$C<u>H</u>-], 2.78 [1H, *ca* t, *J* 3 Hz, 2-H (exhibiting weak homoallylic coupling)], 3.68, 3.75 (2 x 3H, 2 x s, 2 x -OCH_3), 5.45 (1H, d, *J* 3 Hz, $^2J_{SeH}$ 32 Hz, 5-H), 7.24-7.52 (5H, 3 x m, Ar); m/e (NH_3 DCI) 341 (M^+ ^{81}Se, 100%), 339 (M^+ ^{79}Se, 40), 337 (M^+ ^{77}Se, 15), 185 (60), 183 (55), 141 (32).

(104) (258mg, 54%); R_f 0.25 (dichloromethane); δH (500 MHz, $CDCl_3$) 0.86, 1.06 [2 x 3H, 2 x d, *J* 7 Hz, (C<u>H</u>$_3$)$_2$CH-], 2.04-2.18 [1H, m, $(CH_3)_2$C<u>H</u>-], 3.72, 3.73 (2 x 3H, 2 x s, 2 x -OCH_3), 3.94 [1H, dd, *J* 5, 3.5 Hz, 2-H (exhibiting strong

homoallylic coupling)], 5.49 (1H, d, *J* 3.5 Hz, 5-H), 7.30-7.31, 7.72-7.74 (5H, 2 x m, Ar); m/e (NH_3 DCI) 341 (M^+ ^{81}Se, 100%), 339 (M^+ ^{79}Se, 60), 337 (M^+ ^{77}Se, 30), 183 (25), 141 (22).

(2*S*)-2,5-Dihydro-3,6-dimethoxy-2-isopropyl-5-deuteriopyrazine (107)

To a solution of (104) (100mg, 0.295mmol) in dry toluene (5ml) under argon was added AIBN (10mg) and tri-*n*-butyltin deuteride (240µl, 0.885mmol). The solution was degassed with argon for 15 min and then heated at 90°C for 1.5 h under an atmosphere of argon. The toluene was removed *in vacuo* and the residue partitioned between acetonitrile (20ml) and hexane (20ml). The acetonitrile layer was evaporated *in vacuo* and the crude product purified by chromatography [(flash silica, 40g), diethyl ether : 30-40 petroleum ether (1:4)] R_f 0.35 to yield (107) (20mg, 37%) as an oil; δH (500 MHz, $CDCl_3$) 0.75, 1.03 [2 x 3H, 2 x d, *J* 7 Hz, ($C\underline{H}_3$)$_2$CH-], 2.21-2.28 [1H, m, (CH_3)$_2C\underline{H}$-], 3.68, 3.72 (2 x 3H, 2 x s, 2 x -OCH_3), 3.98-4.03 (2H, complex, 2-H, 5-H).

N-Benzoylglycyl-*L*-valine, methyl ester (108)

L-Valine methyl ester hydrochloride (500mg, 3.0mmol), *N*-benzoyl glycine (538mg, 3.0mmol), dicyclohexylcarbodiimide (618mg, 3.3mmol), 4-dimethylaminopyridine (*ca* 10mg), triethylamine (418µl, 3.0mmol) and anhydrous sodium sulphate (*ca* 15mg) were stirred together in ethyl acetate (5ml) for 16 h at room temperature under an inert atmosphere. The resulting mixture was diluted with ethyl acetate (30ml) and washed with hydrochloric acid (2*N*, 30ml) and 5% aqueous sodium hydrogen carbonate solution (30ml). The organic layer was dried (sodium sulphate), filtered and evaporated *in vacuo*. The residue was purified by chromatography [(flash silica, 45g), gradient elution, diethyl ether : ethyl acetate (9:1)] to yield (108) (320mg, 37%) as a white solid; R_f 0.1 (diethyl ether); ν_{max} ($CHCl_3$) 3421 bw, 2969 w, 1740 s, 1653 bs, 1514 s, 1485 s, 1439 m, 1212 m; $[\alpha]_D$ +9.05° (c = 1,

$CHCl_3$); δH (500 MHz, $CDCl_3$) 0.93, 0.96 [2 x 3H, 2 x d, *J* 7 Hz, $(C\underline{H}_3)_2CH$-], 1.73-2.22 [1H, m, $(CH_3)_2C\underline{H}$-], 3.74 (3H, s, $-OCH_3$), 4.24 (2H, d, *J* 5 Hz, $-NHC\underline{H}_2CO$-), 4.56 (1H, dd, *J* 8.5, 5 Hz, $-C\underline{H}CO_2CH_3$), 6.92 (1H, d, *J* 8.5 Hz, $-CH_2CON\underline{H}$-), 7.19 (1H, bs, PhCON<u>H</u>-), 7.43-7.46, 7.50-7.54, 7.83-7.85 (5H, 3 x m, Ar); δC (50 MHz, $CDCl_3$), 17.7, 18.9 [2 x q, $(\underline{C}H_3)_2CH$-], 30.9 [d, $(CH_3)_2\underline{C}H$-], 43.7 (t, PhCONH$\underline{C}H_2$-), 52.1 (q, $-CO_2\underline{C}H_3$), 57.5 (d, $-\underline{C}HCO_2CH_3$), 127.4, 128.7, 132.0 (3 x d, Ar), 133.7 [s, Ar(*ipso*-C)], 168.3, 169.8, 172.5 (3 x s, 3 x CO); m/e (NH_3 CI) 310 (MNH_4^+, 2%), 293 (MH^+, 100), 132 (30), 105 (25), 72 (35).

<u>*N*-Benzoyl-2-(*R*,*S*)-bromoglycyl-*L*-valine, methyl ester (109)</u>

The dipeptide (108) (150mg, 0.51mmol) and *N*-bromosuccinimide (90mg, 0.51mmol) were refluxed in carbon tetrachloride (6ml) with illumination from a 300W tungsten lamp. Decolourisation occurred after 10 min, and refluxing was continued for a further 40 min. The mixture was cooled to room temperature, the insoluble succinimide was filtered off, and the solvent was removed *in vacuo* to yield (109) (206mg, 99%) as a 60:40 ratio of diastereoisomers; δH (500 MHz, $CDCl_3$) 0.95-1.08 [6H, 4 x d, $(C\underline{H}_3)_2CH$-], 2.23-2.30, 2.31-2.36 [1H, 2 x m, $(CH_3)_2C\underline{H}$-], 3.79, 3.82 (3H, 2 x s, $-CO_2CH_3$), 4.60, 4.65 (1H, 2 x dd, *J* 8.5, 5 Hz, $-C\underline{H}CO_2CH_3$), 6.87 [1H, d, *J* 9.5 Hz, PhCONHC<u>H</u>(Br)-], 6.80, 6.92 [1H, 2 x bd, J 5 Hz, -CH(Br)CON<u>H</u>-], 7.45-7.50, 7.56-7.59, 7.85-7.88 (5H, 3 x m, Ar), 7.81 (1H, bd, J 9 Hz, PhCON<u>H</u>-).

<u>*N*-Benzoyl-2-(*R*,*S*)-allylglycyl-*L*-valine, methyl ester ()</u>

The brominated dipeptide (109) (100mg, 0.27mmol), allyltriphenylstannane (250mg, 0.64mmol) and AIBN (20mg) were dissolved in toluene (2ml), and the solution heated at 90°C for 15 h. After standard work up procedure and purification by chromatography [(flash silica, 40g), diethyl ether] R_f 0.35, the title compound (110) (11.3mg, 13%) was obtained as a 55:45 ratio of diastereoisomers; ν_{max} ($CHCl_3$) 3424 bw, 2970 w, 1740 s, 1684 s, 1653 s, 1510 s,

1484 s, 1438 m, 1272 w, 1212 m; δH (500 MHz, $CDCl_3$) 0.91-1.00 [6H, 4 x d, $(C\underline{H}_3)_2CH$-], 2.17-2.22 [1H, m, $(CH_3)_2C\underline{H}$-], 2.64-2.71 (2H, m, CH_2=CH-$C\underline{H}_2$-), 3.70, 3.78 (3H, 2 x s, -OCH_3), 4.51-4.58 (1H, 2 x dd, -$C\underline{H}CO_2CH_3$), 4.74-4.82 (1H, m, PhCONHC$\underline{H}$-), 5.91-5.24 (2H, m, $C\underline{H}_2$=CH-CH_2-), 5.83-5.92 (1H, m, CH_2=$C\underline{H}$-CH_2-), 6.76 (1H, d, *J* 8.5 Hz, -CH-CON$\underline{H}$-CH-), (1H, d, PhCON$\underline{H}$-), 7.42-7.47, 7.51-7.55, 7.78-7.81 (5H, 3 x m, Ar); δC (125 MHz, $CDCl_3$) 17.7, 19.0 [2 x q, $(\underline{C}H_3)_2CH$-], 31.1 [d, $(CH_3)_2\underline{C}H$-], 36.8 (t, CH_2=CH-$\underline{C}H_2$-), 52.1 (q, -OCH_3), 52.8 (d, PhCONH$\underline{C}$H-), 57.4 (d, -$\underline{C}HCO_2CH_3$), 119.2 (t, $\underline{C}H_2$=CH-CH_2-), 127.0, 128.7, 131.8 (3 x d, Ar), 133.1 (d, CH_2=$\underline{C}$H-CH_2-), 133.9 [s, Ar(*ipso*-C)], 167.4, 170.9, 172.0 (3 x s, 3 x CO); m/e (NH_3 CI) 333 (MH^+, 100%), 132 (40), 105 (70).

<u>*N*-Benzoyl-2-deuterioglycyl-*L*-valine methyl ester (111)</u>

A solution of the brominated dipeptide (109) (100mg, 0.27mmol), tri-*n*-butyltin deuteride (182ml, 0.68mmol) and AIBN (20mg) in toluene (5ml) was heated at 85°C for 4 h. After standard work up procedure and purification by chromatography [(flash silica, 25g), ethyl acetate : 30-40 petroleum ether (1:1)], the title compound (111) (42.4mg, 54%) was obtained as an oil; R_f 0.15 (diethyl ether); ν_{max} ($CHCl_3$) 3416 bm, 2969 m, 1740 s, 1653 bs, 1517 s, 1485 s, 1439 m, 1374 m, 1314 m, 1272 m, 909 s; δH (500 MHz, $CDCl_3$) 0.93, 0.96 [6H, 2 x d, *J* 7 Hz, $(C\underline{H}_3)_2CH$-] , 2.18-2.22 [1H, m, $(CH_3)_2C\underline{H}$-], 3.74 (3H, s, -OCH_3), 4.21-4.23 [1H, m, PhCONH-C$\underline{H}$(D)-], 4.55-4.58 (1H, m, -$C\underline{H}$-CO_2CH_3), 6.98 (1H, bd, *J* 8 Hz, -CH-CON$\underline{H}$-CH-), 7.23 (1H, bs, PhCON$\underline{H}$-), 7.43-7.46, 7.50-7.53, 7.82-7.85 (5H, 3 x m, Ar); m/e (NH_3 CI) 294 (MH^+, 100%), 132 (35), 105 (38), 72 (40). [Found C, 61.48%; H, 7.13%; N, 9.11%. $C_{15}H_{19}DN_2O_4$ requires C, 61.41%; H, 7.22%; N, 9.54%.]

(5*S*, 6*R*)-4-Benzyloxycarbonyl-5,6-diphenyl-2,3,5,6-tetrahydro-4*H*-1,4-oxazine-2-one (28a)

δH (300 MHz, DMSO-d_6, 373K) 4.62, 4.67 (2H, ABq, *J* 18 Hz, 3-H), 5.05, 5.11 (2H, ABq, *J* 14 Hz, PhC$\underline{H}_2$O-), 5.30 (1H, d, *J* 3 Hz, 5-H), 6.23 (1H, d, *J* 3 Hz, 6-H), 6.67, 6.69 (2 x 1H, 2 x s, Ar), 7.05-7.30 (13H, m, Ar).

(3*S*, 5*S*, 6*R*) and (3*R*, 5*S*, 6*R*)-4-Benzyloxycarbonyl-3-bromo-5,6-diphenyl-2,3,5,6-tetrahydro-4*H*-1,4-oxazine-2-one (29a, 29c)

A suspension of (28a) (200mg, 0.52mmol) in carbon tetrachloride (15ml) was brought to reflux. Upon complete dissolution of the oxazinone, *N*-bromosuccinimide (100mg, 0.56mmol) was added and the mixture refluxed for an additional 45 min. The mixture was cooled to 0°C, filtered, and the filtrate concentrated *in vacuo* to yield the title compounds (240mg, 99%) as a mixture of diastereoisomers (29a:29c *ca* 3:1);

one diastereomer; δH (300 MHz, DMSO-d_6, 373K) 5.03, 5.08 (1H, $^1/_2$ ABq, *J* 14 Hz, PhC$\underline{H}_2$O-), 5.19, 5.25 (1H, $^1/_2$ ABq, *J* 14 Hz, PhC$\underline{H}_2$O-), 5.25 (1H, d, *J* 3 Hz, 5-H), 6.09 (1H, s, 3-H), 6.51 (1H, d, *J* 3 Hz, 6-H), 6.58, 6.62 (2 x 1H, 2 x s, Ar), 6.95-7.45 (13H, m, Ar).

other diastereoisomer; data as above, except δH (300 MHz, DMSO-d_6, 373K) 5.51 (1H, d, *J* 3 Hz, 5-H), 5.99 (1H, s, 3-H), 6.16 (1H, d, *J* 3 Hz, 6-H).

(3*S*, 5*S*, 6*R*) and (3*R*, 5*S*, 6*R*)-4-Benzyloxycarbonyl-3-deuterio-5,6-diphenyl-2,3,5,6-tetrahydro-4*H*-1,4-oxazine-2-one (112)

A solution of (29a,c) (100mg, 0.214mmol), tri-*n*-butyltin deuteride (156μl, 0.536mmol) and AIBN (15mg) in toluene (10ml) was degassed with argon for 20 min, then heated at 85°C for 3.5 h. The solvent was evaporated *in vacuo*, and the crude product purified by chromatography [(flash silica, 50g), diethyl ether] R_f 0.45 to yield (112) (53mg, 63%) as a white powder; δH (300 MHz, DMSO-d_6, 393K) 4.51-4.67 (1H, m, 3-H), 5.03, 5.11 (2H, ABq, *J* 14 Hz,

$PhC\underline{H}_2O$-), 5.32 (1H, d, *J* 3.5 Hz, 5-H), 6.22 (1H, d, *J* 3.5 Hz, 6-H), 6.70, 6.73 (2 x 1H, 2 x s, Ar), 7.07-7.50 (13H, m, Ar); m/e (NH_3 DCI) 389 (MDH^+, 100%), 388 (MHH^+, 30), 345 (42), 191 (40), 119 (38), 91 (39).

Attempted preparation of (3*S*, 5*S*, 6*R*)-4-Benzyloxycarbonyl-3-allyl-5,6-diphenyl-2,3,5,6-tetrahydro-4*H*-1,4-oxazine-2-one

A solution of (29a,c) (100mg, 0.214mmol), allyltriphenylstannane (210mg, 0.537mmol) and AIBN (10mg) in toluene (10ml) was degassed with argon for 30 min, then heated at 85°C. After 6 h, analysis by tlc revealed a complex mixture of products. Purification by PLC (diethyl ether) and analysis by ^1H nmr yielded none of the desired allyl transfer product.

2-(*R*,*S*)-2-*t*-Butyl-3-methylimidazolidin-4-one (114)

Glycine methyl ester hydrochloride (5.0g, 40mmol) was added to ice-cooled methylamine [33% in industrial methylated spirits (8*M*), 15ml, 120mmol) with stirring. The suspension was stirred at room temperature for 20 h, then concentrated *in vacuo*. A slurry of the resulting viscous material in dichloromethane (10ml) was prepared and concentrated *in vacuo*, three times. A solution of the residue, trimethylacetaldehyde (9.4ml, 7.45g, 86.5mmol) and triethylamine (8.4ml, 6.1g, 60.3mmol) in dichloromethane (40ml) was heated at reflux for 24 h with water separation using a modified Dean-Stark apparatus. After cooling to room temperature, the mixture was filtered and the filter cake washed with diethyl ether (50ml). Concentration of the filtrate *in vacuo* yielded a slurry, which was dissolved in dry methanol (12ml), cooled to 0°C and combined with methanol (24ml) which had been saturated with hydrogen chloride gas. After stirring at 0°C for 25 min and at 18°C for 2 h, the solvent was removed *in vacuo* and the residue dissolved in dichloromethane (40ml), washed with aqueous sodium hydroxide solution (3*M*, 55ml) and concentrated *in vacuo* to give (114)

(3.44g) as a yellow oil which solidified on cooling; δH (500 MHz, $CDCl_3$) 0.98 [9H, s, $(CH_3)_3C$-], 2.95 (3H, s, CH_3N-), 3.43, 3.50 (2H, ABq, *J* 16 Hz, 5-H), 4.12 (1H, s, 2-H). This was used without further purification in the next reaction.

2-(*R*,*S*)-1-Benzoyl-2-*t*-butyl-3-methylimidazolidin-4-one (115)

Benzoyl chloride (2.60ml, 3.15g, 22.4mmol) and triethylamine (6.25ml, 44.8mmol) were simultaneously added at 0°C to a stirred solution of (114) (3.44g, 22.0mmol) in dichloromethane (20ml) under argon. The reaction mixture was stirred at room temperature for 6 h, then diluted with diethyl ether (80ml). The mixture was washed with aqueous sodium carbonate solution (2*M*, 2 x 40ml) and water (40ml). The organic phase was dried (sodium sulphate), filtered and evaporated *in vacuo* to yield the crude product (6.26g). This was purified by chromatography [(flash silica, 300g), diethyl ether] R_f 0.2 to yield (116) (4.34g, 42% from glycine methyl ester hydrochloride) as a white solid; δH (500 MHz, $CDCl_3$) 1.09 [9H, s, $(CH_3)_3C$-], 3.05 (3H, s, CH_3N-), 3.85, 4.13 (2H, ABq, *J* 16 Hz, 5-H), 5.60 (1H, s, 2-H), 7.43-7.58 (5H, m, Ar); δC (125 MHz, $CDCl_3$) 26.0 [q, $(\underline{C}H_3)_3C$-], 31.6 (q, CH_3N-), 39.9 [s, $(CH_3)_3\underline{C}$-], 53.0 (t, 5-C), 80.9 (d, 2-C), 127.8, 129.0, 131.5 (3 x d, Ar), 134.3 [s, Ar(*ipso*-C)], 169.1, 170.7 (2 x s, 2 x CO).

(2*R*, 5*R*) and (2*S*, 5*S*)-1-Benzoyl-2-*t*-butyl-5-bromo-3-methylimidazolidin-4-one (116a, 116b)

To a solution of (115) (500mg, 1.92mmol) in degassed carbon tetrachloride (20ml) was added *N*-bromosuccinimide (343mg, 1.92mmol) and AIBN (10mg). The solution was refluxed for 1 h, cooled to 0°C, filtered, and the solvent removed *in vacuo* to yield (116a,b) (650mg, 99%) as a pale straw coloured foam; δH (500 MHz, $CDCl_3$) 1.24 [9H, s, $(CH_3)_3C$-], 3.11 (3H, s, CH_3N-), 5.70 (1H, s, 2-H), 5.96 (1H, s, 5-H), 7.45-7.61 (5H, m, Ar).

Reaction of (116) with tri-*n*-butyltin deuteride

A solution of (116) (85mg, 0.25mmol), tri-*n*-butyltin deuteride (200µl, 0.75mmol) and AIBN (5mg) in toluene (2ml) was degassed with argon for 30 min, then heated at 90°C under argon for 2 h. The toluene was removed *in vacuo*, and after standard aqueous potassium fluoride work up, the crude product was purified by chromatography [(flash silica, 35g), diethyl ether] to give the following mixture of products, quoted with selected data:

(2*R*, 5*R*) and (2*S*, 5*S*)-1-Benzoyl-2-*t*-butyl-5-deuterio-3-methylimidazolidin-4-one (117) (16%); δH (500 MHz, $CDCl_3$) 3.82 (1H, bs, 5-H).

(2*R*, 5*S*) and (2*S*, 5*R*)-1-Benzoyl-2-*t*-butyl-5-deuterio-3-methylimidazolidin-4-one (118) (8%); δH (500 MHz, $CDCl_3$) 4.11 (1H, bs, 5-H).

^2H nmr analysis indicated the ratio of (117):(118) to be 2:1.

2-(*R*,*S*)-1-Benzoyl-2-*t*-butyl-3-methylimidazolidin-4-one (115) (12%); data as above.

The β-lactam compound (120) (16%); ν_{max} ($CHCl_3$) 1735 m; δH (500 MHz, $CDCl_3$) 1.10 [9H, s, $(CH_3)_3C$-], 3.02 (3H, s, CH_3N-), 4.83, 5.06 (2H, ABq, *J* 5.5 Hz, β-lactam ring-H).

Attempted allyl transfer reactions of (116)

A solution of (116) (85mg, 0.25mmol), allyltriphenylstannane (61) (250mg, 0.64mmol) and AIBN (10mg) in degassed toluene (5ml) was heated at 85-90°C for 2 h, then subjected to standard work up procedure. Analysis of the residue by tlc revealed a complex mixture of products. Attempted purification by flash chromatography yielded unidentifiable products by ^1H nmr analysis (500 MHz, $CDCl_3$).

A similar procedure using ethyl α-(tri-*n*-butylstannylmethyl) acrylate (56) yielded a similarly complex mixture of unidentifiable products.

Propargyltriphenylstannane (57)[120]

To a flask containing magnesium turnings (920mg, 38.4mmol) in diethyl ether (50ml) under argon was added propargyl bromide (80%w/v, 4.63g, 31.0mmol) and iodine (catalytic amount). The mixture was stirred vigorously at room temperature. After 10 min, an exothermic reaction set in, the reaction mixture being kept at room temperature by occasional cooling. Stirring was continued for 30 min. Aliquots of triphenyltin chloride (9.60g, 24.9mmol) were then added over *ca* 15 min and the mixture stirred at room temperature for a further 2.5 h. The reaction was cautiously quenched with aqueous ammonium chloride solution (100ml), then extracted with diethyl ether (2 x 250ml). The organic phase was dried (sodium sulphate), filtered and evaporated *in vacuo*. The crude product was purified by chromatography [(flash silica, 850g), gradient elution, diethyl ether : 30-40 petroleum ether (1:12 - 1:8)] to yield (57) (5.07g, 53%) as a white solid; R_f 0.4 [diethyl ether : 30-40 petroleum ether (1:10)]; δH (500 MHz, $CDCl_3$) 1.94 (1H, t, *J* 3 Hz, H-C≡C-, $^4J_{SnH}$ 10 Hz), 2.24 (2H, d, *J* 3 Hz, $-CH_2Sn-$, $^2J_{SnH}$ 35 Hz), 7.40-7.69 (15H, m, Ar).

Also obtained was allenyltriphenylstannane (59) (1.52g, 16%); R_f 0.5 [diethyl ether : 30-40 petroleum ether (1:10)]; ν_{max} ($CHCl_3$) 1927 s, 1430 s, 1076 m; δH (500 MHz, $CDCl_3$) 4.40 (2H, d, *J* 7 Hz, CH_2=C=, $^4J_{SnH}$ 22 Hz), 5.42 (1H, t, *J* 7 Hz, -Sn-CH=), 7.41-7.68 (15H, m, Ar); m/e (FI) 390 (M^+ ^{120}Sn, 100%), 388 (M^+ ^{118}Sn, 75), 386 (M^+ ^{116}Sn, 40).

N-Benzoyl-2-methoxyglycine, methyl ester (135)

To a solution of (85) (800mg, 2.94mmol) in methanol (10ml) was added triethylamine (410µl, 2.94mmol), and the solution stirred at room temperature for 3 h. The solvent was removed *in vacuo* and the residue partitioned between ethyl acetate (20ml) and water (20ml). The organic layer was dried (sodium sulphate), filtered and evaporated *in vacuo* to yield

(135) (600mg, 92%) as an oil which crystallised on standing; R_f 0.4 (diethyl ether); δH (500 MHz, $CDCl_3$) 3.54 (3H, s, $C\underline{H}_3O$-CH-), 3.85 (3H, s, $-CO_2CH_3$), 5.78 (1H, d, *J* 9 Hz, 2-H), 7.21 (1H, bd, *J* 9 Hz, -NH-), 7.46-7.57, 7.85-7.86 (5H, m, Ar); m/e (NH_3 CI) 241 (MNH_4^+, 18%), 224 (MH^+, 77), 122 (100), 105 (62).

Reaction of (135) with propargyltrimethylsilane

To a stirred solution of (135) (110mg, 0.49mmol) and propargyltrimethylsilane (220µl, 1.48mmol) in dichloromethane (1.5ml) at 0°C was added boron trifluoride etherate (180µl, 1.5mmol) dropwise. The solution was stirred at 0°C under argon for 1 h, then allowed to warm to room temperature over *ca* 4 h. The reaction was quenched by the addition of water (5ml), then extracted with chloroform (2 x 10ml). The combined organic layers were dried (sodium sulphate), filtered and evaporated *in vacuo* to give a golden oil which was purified by chromatography [(flash silica, 30g), diethyl ether], yielding *N*-Benzoylamino-3,4-pentadienoic acid, methyl ester (133) (15mg, 14%) as an oil; R_f 0.5 (diethyl ether); ν_{max} ($CHCl_3$) 3440 m, 2957 m, 1959 m (allene), 1746 s, 1666 s, 1514 s, 1486 s, 1439 m, 1349 m, 1210 m; δH (500 MHz, $CDCl_3$) 3.80 (3H, s, $-OCH_3$), 5.01 (2H, dd, *J* 6.5, 4 Hz, $C\underline{H}_2$=C=CH-), 5.30-5.34 (1H, m, 2-H), 5.50 (1H, q, *J* 6.5 Hz, CH_2=C=C$\underline{H}$-), 6.83 (1H, bd, -NH-), 7.42-7.54, 7.80-7.85 (5H, m, Ar); δC (50 MHz, $CDCl_3$) 50.6 (d, 2-C), 52.7 (q, $-CO_2\underline{C}H_3$), 80.0 (t, $\underline{C}H_2$=C=CH-), 88.5 (d, CH_2=C=$\underline{C}$H-), 127.2, 128.7, 132.0 (3 x d, Ar), 133.8 [s, Ar(*ipso*-C)], 167.0, 171.1 (2 x s, 2 x CO), >200 (CH_2=$\underline{C}$=CH-); m/e (NH_3 CI) 232 (MH^+, 100%), 122 (35), 105 (52).

Also obtained was 2-Benzoylamino-4-oxopentanoic acid, methyl ester (136) (15mg, 14%) as an oil; R_f 0.35 (diethyl ether); ν_{max} ($CHCl_3$) 1749 s, 1715 s, 1663 s, 1517 s, 1486 s, 1441 m; δH (500 MHz, $CDCl_3$) 2.20 (3H, s, 5-H), 3.14-3.23, 3.25-3.33 (2H, 2 x 4 line multiplets, J_{AB} 18 Hz, 3-H), 5.00 (1H, dt, *J* 8, 4 Hz, 2-H), 7.25 (1H, bd, *J* 8 Hz, -NH-), 7.42-7.58, 7.79-7.84 (5H, m, Ar); m/e (NH_3 CI) 250 (MH^+, 100%), 122 (84), 105 (30).

N-*t*-Butyloxycarbonyl-2-bromoglycine, *t*-butyl ester (140)

Prepared in an analogous manner to (85) using (139) (1.60g, 6.92mmol), *N*-bromosuccinimide (1.23g, 6.92mmol) and carbon tetrachloride (40ml), to yield (140) (2.15g, 99%) as an oil which crystallised on standing; δH (500 MHz, $CDCl_3$) 1.50, 1.52 [2 x 9H, 2 x s, 2 x $(CH_3)_3C$-], 5.90 (1H, bs, 2-H), 6.21 (1H, bs, -NH-).

N-*t*-Butyloxycarbonyl-2-methoxyglycine, *t*-butyl ester (141)

Triethylamine (224µl, 1.61mmol) was added to a solution of (140) (500mg, 1.61mmol) in methanol (8ml) and the reaction mixture was then stirred for 3 h at room temperature. The solvent was removed *in vacuo* and the residue partitioned between ethyl acetate (20ml) and water (20ml). The organic layer was dried (sodium sulphate), filtered and evaporated *in vacuo* to yield (141) (393mg, 93%) as an oil; R_f 0.65 (diethyl ether); δH (500 MHz, $CDCl_3$) 1.48, 1.51 [2 x 9H, 2 x s, 2 x $(CH_3)_3C$-], 3.43 (3H, s, $-OCH_3$), 5.13 (1H, d, *J* 9 Hz, 2-H), 5.59 (1H, bs, -NH-).

Similarly prepared was *N*-*t*-butyloxycarbonyl-2-ethoxyglycine, *t*-butyl ester (142); δH (500 MHz, $CDCl_3$) 1.23 (3H, t, *J* 7 Hz, $C\underline{H}_3CH_2O$-), 1.46, 1.50 [2 x 9H, 2 x s, 2 x $(CH_3)_3C$-], 3.66 (2H, q, *J* 7 Hz, $CH_3C\underline{H}_2O$-), 5.18 (1H, d, *J* 9 Hz, 2-H), 5.60 (1H, bs, -NH-); m/e (GCMS NH_3 CI) 293 (MNH_4^+, 10%), 276 (MH^+, 10), 130 (100).

Similarly prepared was *N*-benzoyl-2-ethoxyglycine, methyl ester (137); R_f 0.5 (diethyl ether); δH (500 MHz, $CDCl_3$) 1.25 (3H, t, *J* 7 Hz, $C\underline{H}_3CH_2O$-), 3.78 (2H, q, *J* 7 Hz, $CH_3C\underline{H}_2O$-), 3.83 (3H, s, $-CO_2CH_3$), 5.86 (1H, d, *J* 9 Hz, 2-H), 7.25 (1H, bd, *J* 9 Hz, -NH-), 7.44-7.56, 7.84-7.86 (5H, m, Ar).

Reaction of (137) with propargyltrimethylsilane/boron trifluoride

Boron trifluoride etherate (720µl, 6.00mmol) was added dropwise to a stirred solution of (137) (440mg, 1.85mmol) and propargyltrimethylsilane (880µl,

5.92mmol) in dichloromethane (6ml) at 0°C under argon. The mixture was allowed to warm to room temperature (10°C) over *ca* 16h. The reaction mixture was diluted with diethyl ether (20ml) and washed with water (2 x 20ml). The organic layer was dried (sodium sulphate), filtered and evaporated *in vacuo*. The residue was purified by chromatography [(flash silica, 60g), diethyl ether] to yield three major products: (133) (44mg, 11%), data as above; (136) (16mg, 4%), data as above; and 2-benzoylamino-4-oxopentanoic acid ethyl ester (138) (19mg, 5%); δH (300 MHz, $CDCl_3$) 1.58 (3H, t, *J* 6.5 Hz, $C\underline{H}_3CH_2O$-), 2.20 (3H, s, 5-H), 3.14-3.23, 3.25-3.33 (2H, 2 x 4 line multiplets, J_{AB} 18 Hz, 3-H), 4.25 (2H, q, *J* 6.5 Hz, $CH_3C\underline{H}_2O$-), 4.95 (1H, dt, *J* 9, 4 Hz, 2-H), 7.25 (1H, bs, -NH-), 7.40-7.55, 7.78-7.85 (5H, m, Ar); m/e (GCMS NH_3 CI) 264 (MH^+, 35%), 122 (100).

Reaction of (85) with propargyltriphenylstannane/zinc(II) chloride at 18°C

To a stirred solution of (85) (50mg, 0.18mmol) in carbon tetrachloride (20ml) was added propargyltriphenylstannane (150mg, 0.37mmol) and a solution of anhydrous zinc(II) chloride in THF (0.5ml). The solution was stirred under argon at room temperature for 4.5 h, then the solvent was removed *in vacuo*. The residue was purified by chromatography [(flash silica, 100g), diethyl ether : 30-40 petroleum ether]. The only identifiable product was allenyltriphenylstannane (59) (70mg, 47%), obtained as a white amorphous solid; data as above.

2-Benzoylamino-3,4-pentadienoic acid, methyl ester (133)

To a stirred solution of (85) (1.50g, 5.51mmol) in degassed carbon tetrachloride (400ml) was added propargyltriphenylstannane (4.30g, 11.05mmol) and a solution of anhydrous zinc(II) chloride (1.50g, 11.01mmol) in THF (15ml). The solution was then refluxed for 50 min under an inert

atmosphere. After aqueous potassium fluoride work up, the crude product was purified by chromatography [(flash silica, 300g), diethyl ether : 30-40 petroleum ether (2:1)] R_f 0.35 to yield the title compound (133) and <u>*N*-Benzoyl-2-propargylglycine, methyl ester (143)</u> as an inseparable mixture (650mg, 51%) in a ratio of 2:1 respectively. Small quantities of this mixture could be separated by HPLC [silica, dichloromethane : ethyl acetate (99:1), retention time *ca* 22 min]. However (143) was crystallised out of the mixture from dichloromethane/30-40 petroleum ether, though contaminated with (133). The mother liquor contained pure (133) (225mg); data as above.

<u>*N*-Benzoyl-2-propargylglycine, methyl ester (143)</u>

To a solution of (85) (25mg, 0.09mmol) in carbon tetrachloride (10ml) was added allenyltriphenylstannane (59) (70mg, 0.17mmol) and a solution of anhydrous zinc(II) chloride (25mg, 0.18mmol) in THF (0.25ml). The solution was refluxed for 45 min under an inert atmosphere. After aqueous potassium fluoride work up, the crude product was purified by chromatography [(flash silica, 25g), diethyl ether] R_f 0.5 to yield the title compound (143) (10mg, 47%) as a clear oil; ν_{max} ($CHCl_3$) 3309 w, 3071 m, 2249 s, 1746 m, 1666 m, 1516 m, 1483 s, 1431 s, 1076 s, 998 s; δH (500 MHz, $CDCl_3$) 2.08 (1H, t, *J* 3 Hz, H-C≡C-), 2.87-2.98 (2H, m, $-CH_2-C\equiv C-$), 3.86 (3H, s, $-OCH_3$), 4.96-5.00 (1H, m, 2-H), 6.97 (1H, *ca* d, *J* 9 Hz, -NH-), 7.40-7.75 (15H, m, Ar); m/e (NH_3 CI) 232 (MH^+, 100%), 154 (24), 122 (18), 105 (17), 78 (20).

<u>2-Amino-3,4-pentadienoic acid (60)</u>

A solution of the diprotected allenylglycine (133) (230mg, 1.0mmol) in 3*N* aqueous hydrochloric acid : dioxane (1:1, 50ml) was heated at 90°C for 20 h. The solvent was removed *in vacuo* and the residue partitioned between water (50ml) and dichloromethane (50ml). The aqueous layer was washed

with further dichloromethane (50ml), passed through decolourising charcoal, then applied to clean, activated ion-exchange resin ['Dowex' 50W-X8 (H), 100-200 mesh, 60mm x 20mm diameter column]. The column was eluted with water (300ml), followed by aqueous ammonia solution (2*M*, 250ml). The ammoniacal washings were freeze-dried to yield (60) (25mg 23%) as a white fluffy solid; ν_{max} (KBr disc) 2954 bs, 1962 m (allene), 1652 s 1587 s, 1524 s, 1351 s, 1121 m, 859 m; δH (500 MHz, D_2O) 4.26 (1H, dt, *J* 6.5, 3 Hz 2-H), 5.14 (2H, dd, *J* 6.5, 3 Hz, C<u>H</u>$_2$=C=CH-), 5.51 (1H, q, *J* 6.5 Hz, CH$_2$=C=C<u>H</u>-); δC (125 MHz, D_2O) 53.7 (d, 2-C), 80.9 (t, <u>C</u>H$_2$=C=CH-), 87.2 (d, CH$_2$=C=<u>C</u>H-), 173.8 (s CO), 208.7 (s, CH$_2$=C=CH-); m/e (NH_3 DCI) 114 (M^+, 100%), 68 (22).

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