The Synthesis of Modified Peptide Isosteres as Potential Anti-parasite Drugs

A thesis submitted for the degree of Ph.D.

Stuart C. Niven

Department of Chemistry
University of Glasgow
May 1998

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

Summary

Protease enzymes are responsible for the catalytic hydrolysis of peptides and therefore play vital roles in the biochemistry of organisms. Serine proteases, which achieve proteolytic cleavage by a mechanism involving an activated serine residue, are numerous in mammalian systems while only a limited number of the analogous cysteine proteases are to be found. Cysteine protease enzymes are however, widespread in parasitic species and often essential to the functioning of the organism. Thus the use of inhibitors for the treatment of parasitic infestation may be possible if the subtle differences between these two enzyme families can be exploited.

The opportunity exists in using modified substrates (peptides) to effect the inhibition of protease enzymes. In this case the required modification must induce the irreversible binding of the peptide to the active site of cysteine, but not serine, proteases. Indeed, *N*-protected dipeptides bearing the fluoromethyl ketone or diazomethyl ketone modifications of the carboxy terminus have been shown to be particularly effective in this respect. Unfortunately, little is known about the substrate specificity of individual parasite cysteine protease enzymes.

The therapeutic use of dipeptide based inhibitors is limited by the vulnerability of the peptide linkage itself to proteolytic cleavage. Pseudo peptides containing the non-hydrolysable (*E*)-ethene linkage in place of the amide group have been shown to possess biological activities similar to those of the parent compounds in some systems. This concept has not, however, been applied to the field of parasite cysteine protease inhibition.

The aim of this study was to synthesise a selection of *N*-protected pseudo peptides bearing the (*E*)-ethene isosteric linkage and a modified carboxy terminus (preferably as the more selective fluoromethyl ketone). The biological activity of these compounds could then be evaluated against a battery of cysteine protease containing parasitic species. Thus further clues as to the

substrate selectivity of these enzymes may be obtained in addition to the assessment of the suitability of the chosen isosteric linkage.

A selection of *N*-phthaloyl protected amino acids was formed and used as model systems to test the carboxy terminus modification methodology. While the generation of the diazomethyl ketone derivatives of these *via* an intermediate mixed carbonate was successful, the subsequent conversion to the fluoromethyl ketone was found to be inefficient and impractical. The use of bromomethyl ketones as intermediates was therefore investigated.

The problems initially experienced during the attempted formation of bromomethyl ketones were overcome by the use of dry hydrogen bromide gas as a reagent. Thus it was shown that these materials could be formed in excellent yield. It was also found that the subsequent halogen exchange reaction could be performed successfully with triethylamine trishydrofluoride to give the desired fluoromethyl ketones.

Despite several important questions concerning this synthesis remaining unanswered and alternative methods untested, studies in this area were halted due to recent developments in end group technology. It was decided that the less selective, but more easily prepared, diazomethyl ketones would suffice as end groups for initial testing of the isostere concept with respect to parasite cysteine proteases.

Studies were initiated with the aim of synthesising the (*E*)-ethene isostere of *N*-BOC-(L)-Phe-(L)-Ala. Preparation of the *N*-BOC protected (L)-phenylalaninal required for the first route chosen was effected using literature procedure. The subsequent addition of vinylmagnesium bromide to this aldehyde resulted in the generation of a pair of diastereomers from which the less abundant product was required. As the isolation of this material was found to be inefficient, studies in this area were not continued and an alternative route to the target compound was investigated. This strategy, which would utilise the Julia olefin synthesis as

the key synthetic step, required the prior generation of a sulfone derived from the appropriate *N*-BOC protected (L)-amino acid. The four step sequence for the formation of this species was carried out successfully and reliably, on both large and small scales, on *N*-BOC protected (L)-phenylalanine, (L)-valine and (L)-leucine. Purification of the intermediate compounds involved was found to be straightforward.

The chiral aldehyde required for the generation of a position-2 alanine mimic was formed, after some degree of experimentation, by the action of diisobutylaluminium hydride on the corresponding methyl ester. In contrast, for a glycine mimic, ozonolysis of an olefin was employed as the key aldehyde generating reaction.

The sulfone to aldehyde coupling reaction was initially the source of some problems. Conditions were eventually developed where the coupling could be effected and a quantitative estimate made on the extent of product formation. The β-hydroxysulfones produced by this coupling were converted into the corresponding *trans* olefins by reductive elimination. Removal of the *C*-terminus protection and generation of the required carboxylic acid was then effected in a single step by the use of Jones' reagent. Thus the (*E*)-ethene isosteres of *N*-BOC-(L)-Phe-(L)-Ala, *N*-BOC-(L)-Val-(L)-Ala and *N*-BOC-(L)-Val-Gly were successfully synthesised and shown to possess the desired stereochemistry.

With a view to the synthesis of further intermediate aldehydes, studies on 3-pentenoic acid indicated that α-alkylation could be carried out without inducing a shift in the position of the double bond. This result also indicated that the synthesis of 4-methyl-3-pentenoic acid, a starting material employed in the literature route to alternative aldehydes, would be superfluous. Investigations into the generation of this material were therefore halted.

The standard method for the synthesis of diazomethyl ketones, as tested earlier, was applied to one of the completed isosteres but met with failure. A full scale

investigation into the factors influencing this result and possible alternative methods for activation of the carboxylic acid, allowed a plausible explanation for the reaction failure to be proposed. A technique was then developed, using 3-pentenoic acid as a model system, which allowed the successfull generation of diazomethyl ketones from βγ-unsaturated carboxylic acids. The application of this methodology to the three completed isosteres allowed the synthesis of the desired diazomethyl ketones with the corresponding methyl esters being produced as the main by-products. An investigation into the use of methyl esters as starting materials in diazomethyl ketone syntheses proved to be fruitful with methyl 3-pentenoate. Unfortunately the application of this methodology to an (*E*)-ethene isostere methyl ester resulted in destruction of the olefin.

Thus the diazomethyl ketones of the *N*-BOC-(L)-Phe-(L)-Ala, *N*-BOC-(L)-Val-(L)-Ala and *N*-BOC-(L)-Val-Gly (*E*)-ethene isosteres were successfully synthesised. Molecular mechanics calculations were carried out on the diazomethyl ketone of both amide and (*E*)-ethene linked *N*-BOC-(L)-Val-(L)-Ala, the results of which suggested the isosteric nature of these groups.

The three isostere diazomethyl ketones, the corresponding methyl esters and also the diazomethyl ketone of the amide linked dipeptide *N*-CBZ-(L)-Phe-(L)-Ala, were screened for activity against a variety of parasitic species. Results indicate that the diazomethyl ketone of *N*-BOC-(L)-Phe-(L)-Ala possesses some form of growth inhibiting activity against *Leishmania mexicana*. This effect was not observed with either the isostere methyl ester or the diazomethyl ketone of the amide linked material. Similarly the diazomethyl ketone of *N*-BOC-(L)-Val-(L)-Ala caused some degree of growth inhibition on several species of *Trichomonas*. It was not proven however, that these observed effects were due to cysteine protease inhibition.

Surprisingly, the methyl ester of *N*-BOC-(L)-Val-Gly proved to be a powerful anti-protease. Both this compound and the corresponding diazomethyl ketone showed the ability to inhibit parasitic growth.

Thus it can be concluded that (*E*)-ethene isosteres of *N*-protected dipeptide diazomethyl ketones are capable of restricting the growth of specific parasitic species. It is as yet unknown if this effect is due to cysteine protease inhibition.

This thesis is dedicated to
Mrs Marion Niven.

"...in battle, use the normal force to engage;
use the extraordinary to win."
(translation)

- Sun Tzu

Acknowledgements

I would like to thank Dr E. W. Colvin, Prof. J. D. Connolly and Prof. G. H. Coombs for their unending support throughout the course of this study.

I would also like to thank the following people for their advice and assistance; Kim Wilson (microanalysis), Tony Ritchie (mass spectrometry), George M^{C}Culloch (infra-red spectroscopy), Alex Burns (technical support), Jim Gall, Jim M^{C}Ivor and Dr D. S. Rycroft (N.M.R. spectroscopy).

Full credit must be given to those who have contributed their time to the experimental work involved in this study; Colin Macleod for small scale investigations into the generation of sulfones from protected leucine and valine, Richard Tyers for the attempted synthesis of *trans*-4-methylpent-3-enoic acid, Kirsty M^{C}Cormack for isostere molecular modelling and David Laughland for product biological evaluation.

Thanks are also due to my family, friends and fellow students for their support and understanding throughout the past three years.

This work was funded by the World Health Organisation.

Contents

General Preamble 1

Introduction

Chapter 1: Protease Enzymes 5

1.1 The Serine Proteases 5

1.2 The Cysteine Proteases 9

1.3 Cysteine Proteases and Disease States 9

1.4 Cysteine Proteases and Parasitic Diseases 10

1.5 Protease Enzyme Inhibition 13

1.6 Concepts Inferred from the Serine Protease Model 14

1.7 Cysteine Protease Inhibition 15

Chapter 2: Aldehyde Based Inhibitors of Cysteine Proteases 17

2.1 Natural Aldehyde Based Inhibitors 17

2.2 NMR Spectroscopic Studies on Papain Inhibition 21

2.3 Fluorescence Studies on Peptidyl Aldehyde Inhibitors 23

2.4 Limitations of Aldehyde Based Inhibitors 24

Chapter 3: E-64, a Natural Irreversible Inhibitor 25

3.1 Investigations into the Mode of Action of E-64 25

3.2 Limitations of Epoxide Based Inhibitors 28

Chapter 4: Halomethyl Ketone Based Inhibitors 29

4.1 The Chloromethyl Ketones 30

4.2 NMR Spectroscopic Studies on Chloromethyl Ketone Induced Inhibition 31

4.3 X-Ray Crystallographic Studies on Inhibited Serine Proteases 34

4.4 Kinetic Studies on Chloromethyl Ketone Induced Inhibition 35

4.5 Chloromethyl Ketones with Cysteine Proteases 37

4.6 Limitations of Chloromethyl Ketones 37

Chapter 5: Diazomethyl Ketones 39

5.1 Diazomethyl Ketones with Protease Enzymes 40

5.2 Limitations of Diazomethyl Ketones 43

Chapter 6: Peptidyl Fluoromethyl Ketones 44

6.1 Inhibition by Fluoromethyl Ketones 44

6.2 Fluoromethyl Ketones in Biological Systems 46

6.3 The Unsuitability of Peptidyl Fluoromethyl Ketones as Drugs 47

Chapter 7: The Concept of the (*E*)-Ethene Isostere 49

Chapter 8: The History and Development of (*E*)-Ethene Isostere Synthesis 51

8.1 The Cox Approach 51

8.2 Biological Activity of Isostere Containing Compounds 53

8.3 The Sammes Approach 54

8.4 Thompson Methodology 58

8.5 The Hanson Synthesis 59

8.6 The Hopkins Organosulfur Route 63

Chapter 9: Aims and Objectives 66

Results and Discussion

Chapter 10: Fluoromethyl Ketone End Group Investigations 67

10.1 Literature Fluoromethyl Ketone Syntheses 67

10.2 Experimental Investigations into the Shaw Route 69

10.3 Characteristic Spectroscopic Details of Diazomethyl Ketones 72

10.4 Attempted Direct Fluorination by Shaw's Procedure 72

10.5 Studies Concerning Bromomethyl Ketone Synthesis 74

10.6 Fluoromethyl Ketones by Halogen Exchange 76

10.7 Features Requiring Further Investigation 77

10.8 Recent Advances in the Field of Cysteine Protease Inhibitors 80

Chapter 11: Initial Studies Concerning (*E*)-Ethene Isostere Synthesis 84

11.1 Synthetic Test of the Hanson Route 85

Chapter 12: The Formation of Precursors for an Alternative Strategy 88

12.1 Formation of the Sulfone Synthon 88

12.2 Advantages of the Employed Route for Sulfone Generation 92

12.3 Sulfones from Alternative Starting Materials 92

12.4 Synthesis of the Aldehyde Component 94

12.5 Alternative Aldehydes 96

12.6 Aldehyde Precursor to a Glycine Mimic 99

Chapter 13: The Coupling Reaction and Isostere Generation 101

13.1 Literature Methodology 101

13.2 Experimental Findings 102

13.3 Application of the Coupling Modification 104

13.4 Reductive Elimination of the β-Hydroxysulfone 105

13.5 The Coupling of Other Materials 106

13.6 Generation of the Carboxylic Acid 108

Chapter 14: Diazomethyl Ketone Formation 110

14.1 Theory 1: Wolff Rearrangement 110

14.2 Theory 2: Reagent Problems 112

14.3 Theory 3: Problems Associated with the Extended Chain 113

14.4 Alternative Activation of the Carboxylic Acid 114

14.5 Further Acid Chloride Investigations 115

14.6 The Use of an Alternative Base in Mixed Carbonate Formation 118

14.7 Potassium Trimethylsilanolate Method of Salt Formation 120

14.8 Isostere Diazomethyl Ketone and Methyl Ester Formation 122

Chapter 15: Anti-parasite Evaluation 125

15.1 Results of Biological Evaluation 126

Chapter 16: Conclusions 129

16.1 Synthesis of the Fluoromethyl Ketone End Group 129

16.2 (*E*)-Ethene Isostere Synthesis - Route #1 130

16.3 (*E*)-Ethene Isostere Synthesis - Route #2 131

16.4 Coupling and Isostere Generation 132

16.5 Diazomethyl Ketone Formation 133

Chapter 17: Avenues for Further Investigation 136

17.1 Further Work on Completed (*E*)-Ethene Isostere Diazomethyl Ketones 136

17.2 Further Work Concerning End Group Synthesis 137

17.3 Further Work Concerning Isostere Synthesis 138

Experimental Preamble 141

Experimental

Part 1: *N*-Phthaloyl Protection of Amino Acids 145

Part 2: Diazomethylation of Protected Amino Acids *via* the Mixed Carbonate 151

Part 3: Further Reaction of Diazomethyl Ketones and Subsequent Halogen Exchange 162

Part 4: Synthesis of Sulfone Coupling Components 177

Part 5: Synthesis of Aldehyde Coupling Components 200

Part 6: Coupling and Isostere Generation 209

Part 7: Evaluation of Alternative Diazomethyl Ketone Formation Methods 224

Part 8: Further Reaction of Completed Isosteres 231

References 242

Appendices

Appendix 1: Molecular Mechanics Calculations 249

Appendix 2: Initial Biological Results 251

Appendix 3: Second Round Parasite Growth Studies 257

General Preamble

Terminology

Amino Acids

Throughout this study amino acids may be referred to by the recognised three letter abbreviations.

Alanine	Ala	Leucine	Leu
Arginine	Arg	Lysine	Lys
Asparagine	Asn	Methionine	Met
Aspartic acid	Asp	Phenylalanine	Phe
Cysteine	Cys	Proline	Pro
Glutamine	Gln	Serine	Ser
Glutamic acid	Glu	Threonine	Thr
Glycine	Gly	Tryptophan	Trp
Histidine	His	Tyrosine	Tyr
Isoleucine	Ile	Valine	Val

In addition to these, the abbreviation Xxx will be used to represent an unspecified amino acid.

Peptides

The composition of peptides constructed from the above amino acids will be described using the recognised amino to carboxy terminus convention. For example, the peptide formed by the condensation of the carboxyl group of phenylalanine with the amino group of alanine would be given the abbreviation Phe-Ala.

Stereochemistry

The stereochemistry of each amino acid will be indicated by either an (L)- or (D)- prefix with (L)- being the natural configuration. Racemic centres will be assigned

the prefix (DL)-. For example, the dipeptide formed by the condensation of natural phenylalanine with racemic alanine would be given the abbreviation (L)-Phe-(DL)-Ala.

Amino Acid Position

The position of each amino acid in a peptide may be indicated by a numbering system which follows the amino to carboxy terminus convention. Thus, in the dipeptide (L)-Phe-(DL)-Ala, phenylalanine would be described as occupying position 1. The associated benzyl side chain may also be described as the position 1 or *N*-terminal amino acid side chain. Similarly, alanine would be described as occupying position 2 with the associated methyl side chain termed the position 2 or *C*-terminal amino acid side chain.

Amide Link Replacement

In cases where the amide link of a dipeptide has been replaced by an isosteric group this will be stated. For example, the variant of (L)-Phe-(DL)-Ala where the central amide link has been replaced by an (*E*)-ethene group would be termed as the (*E*)-ethene isostere of (L)-Phe-(DL)-Ala.

N-Terminus Protection

The *N*-, or amino, terminus of a peptide may possess a protecting group such as (abbreviations indicated in brackets) benzyloxycarbonyl (CBZ), *tert*-butoxycarbonyl (BOC), phthaloyl (phth) or tosyl (Tos). For example, the dipeptide (L)-Phe-(DL)-Ala bearing an *N*-terminus *tert*-butoxycarbonyl protection would be shown as *N*-BOC-(L)-Phe-(DL)-Ala. Modifications to amino acid side chains will be indicated by a suffix enclosed in square brackets. For example, a benzyl protection of the hydroxyl group of tyrosine would be indicated by Tyr[*O*-Bz].

C-Terminus Modifications

Modifications to the carboxy terminus will be indicated in the following manner. In cases where the modification results in the generation of a new carbon to

carbon bond, this will be indicated by the placing of the modifying group directly after the amino acid designation. For example, the compound produced by the conversion of the carboxyl group of alanine into a diazomethyl ketone would be shown as AlaCHN_2. Similarly, the corresponding chloromethyl ketone would be given the designation AlaCH_2Cl. However, in cases where the modification does not result in the generation of a new carbon to carbon bond, this will be indicated by inclusion of a hyphen. For example, the methyl ester of alanine would be given the designation Ala-OMe. Similarly, a *C*-terminal amide would be indicated by Ala-NH_2.

Exception to the Above Rules

When illustrating the mechanism of enzyme action, functional groups or part side chains of interest will be shown linked to the parent amino acid in the peptide sequence which constitutes the enzyme. For example, Ser_{95}-OH may be used to indicate the side chain hydroxyl function of a serine residue which occupies position 95 in the linear amino acid sequence of an enzyme.

Additional Abbreviations

The following abbreviations may also be used throughout this study.

B	base
cat.	catalytic
Diazald®	*N*-methyl-*N*-nitroso-*p*-toluenesulphonamide
DIBAL-H	Diisobutylaluminium hydride
DMAP	4-Dimethylaminopyridine
DME	Dimethoxyethane
DMF	*N*,*N*-Dimethylformamide
DMSO	Dimethylsulfoxide (deuteriated form indicated by d_6-)
E or Enz	Enzyme
EI	Enzyme-inhibitor complex
G.	*Giardia*
HPLC	High pressure liquid chromatography
I	Inhibitor
L	Ligand
L.	*Leishmania*
LDA	Lithium diisopropylamide
Ms	Mesyl
NMO	*N*-Methylmorpholine *N*-oxide
Nu	Nucleophile
T.	*Trichomonas*
THP	Tetrahydropyranyl

Any other abbreviations used will be defined as required within the main text.

INTRODUCTION

Chapter 1: Protease Enzymes

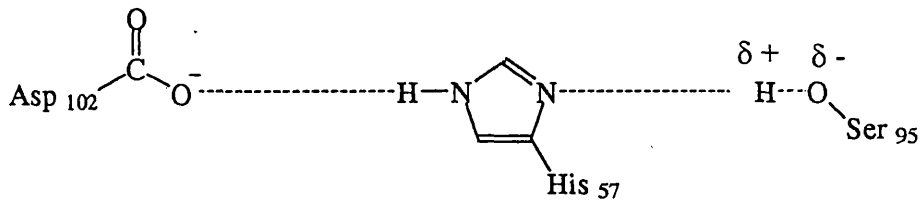
Four main classes of protease enzymes have been detected, aspartic, metallo-, cysteine and serine proteases, the common feature of which is that they all possess the ability to catalyse the hydrolysis of amide links within peptides and proteins. These enzymes therefore play vital roles in the biochemistry of organisms.

It is the aim of this study to develop compounds which will disrupt the biochemical processes of parasitic organisms by inhibiting essential protease enzymes. Since proteolytic cleavage is also a vital part of the biochemistry of the host, it is imperative that the inhibiting action is highly selective. In order to gain an insight as to how this effect can be achieved, it is first essential that the general mechanism by which the serine and cysteine proteases achieve amide cleavage is illustrated.

1.1 The Serine Proteases

Chymotrypsin is a digestive serine protease which is involved in the hydrolysis of proteins in the small intestine.[1] The enzyme selectively cleaves amide links on the carboxyl side of aromatic and other large hydrophobic side chains. This preference is caused by the the presence of a large hydrophobic pocket in the tertiary structure of the enzyme which, once filled by suitable side chains, places the following amide link close to specific active residues. As with others of this class the enzyme employs an unusually reactive serine side chain to initiate peptide link cleavage. A closer analysis of the tertiary structure of the enzyme indicates how this hydroxyl group, normally unreactive under physiological conditions, is activated.

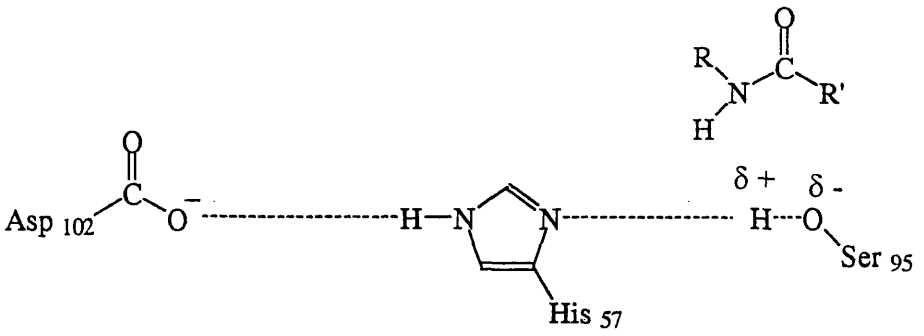
The specific folding of the enzyme results in three residues, distant from each other on the protein chain, being sited within close spatial proximity to each other (figure 1.1-1).



(figure 1.1-1)

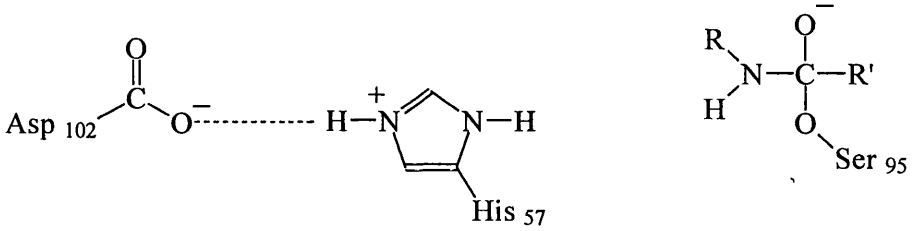
The three side chains of aspartate, histidine and serine occupy positions 102, 57 and 95 respectively in the linear amino acid sequence of the enzyme. However, as indicated, the close arrangement of the side chains in space allows a complex sequence of hydrogen bonding to occur. The net result of this interaction is a partial deprotonation of the serine hydroxyl oxygen thus imparting an increase in nucleophilicity. The following sequence of events can now occur:

Stage 1: The peptide substrate is aligned close to the active serine side chain (figure 1.1-2).



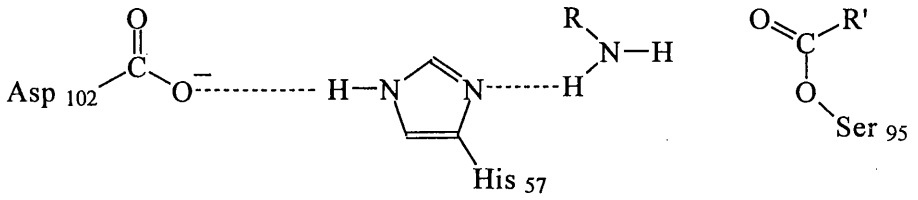
(figure 1.1-2)

Stage 2: Nucleophilic attack by the active serine on the amide carbonyl results in the formation of a tetrahedral intermediate (figure 1.1-3). This intermediate is stabilised by hydrogen bonding from amide NH groups in the enzyme main chain.



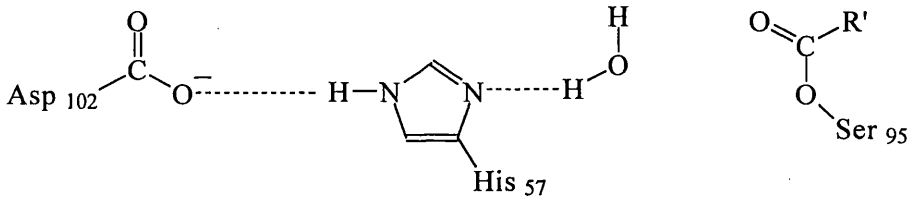
(figure 1.1-3)

Stage 3: The proton held by the histidine side chain is donated to the nitrogen atom of the intermediate facilitating its collapse (figure 1.1-4). Release of the product amine can now occur. The product acid remains bound to the serine residue by an ester linkage.



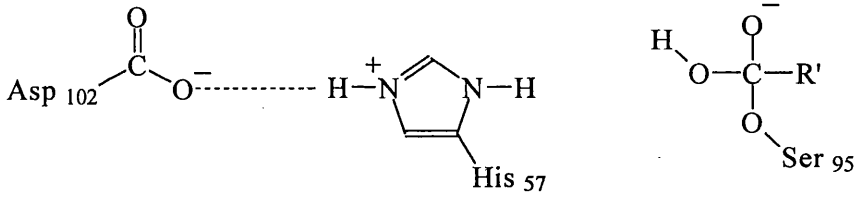
(figure 1.1-4)

Stage 4: With the product amine now replaced by water, the process of activation can be repeated with this molecule (figure 1.1-5).



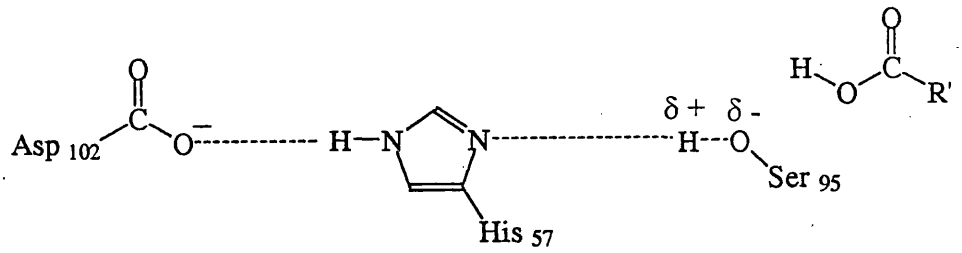
(figure 1.1-5)

Stage 5: A second tetrahedral intermediate results from the attack of water on the acyl-enzyme species (figure 1.1-6). This intermediate is stabilised in a similar manner to that formed in stage 2.



(figure 1.1-6)

Stage 6: Donation of the proton from the histidine side chain to the tetrahedral intermediate facilitates the collapse of this species thus giving the carboxylic acid product and returning the serine residue to its original state (figure 1.1-7).



(figure 1.1-7)

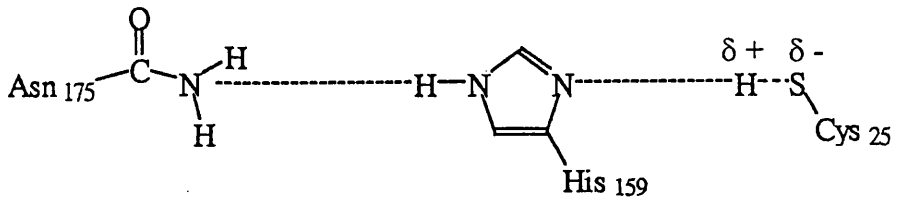
From this illustration, the catalytic behaviour of the enzyme in this case can be readily appreciated and, as such, the three key residues involved in the cycle are often referred to as the catalytic triad of chymotrypsin.

Other members of the serine protease family share this arrangement of residues in the active site. Where each member differs however, is in the selection of substrates.[2] [3] While with chymotrypsin the large hydrophobic pocket selects aromatic and similar bulky non-polar side chains, the selectivity of trypsin is different due to a slight modification in this region. A serine residue at the base of the pocket is absent and in its place exists aspartate. Thus trypsin is found to cleave peptide links which follow an arginine or lysine residue due to the favourable electrostatic interaction these species form. In the case of elastase the hydrophobic pocket is partially blocked by the replacement of two glycine residues with valine and threonine. This enzyme is therefore found to select substrates containing small non-polar side chains such as the methyl group of alanine.

Other enzymes of this class include thrombin, an enzyme involved in blood clotting and acetylcholinesterase which plays a key role in nerve function.

1.2 The Cysteine Protease

Cysteine proteases (or thiol proteases) utilise a thiol-imidazolyl catalytic system, analogous to that employed by the serine proteases (figure 1.2-1).[4] In this case the unusually reactive serine residue is replaced by a similarly activated cysteine. A family of cysteine proteases similar in structure to each other can be found in plants including papain, from the papaya fruit, and fiacin from figs.[5]



The papain catalytic triad

(figure 1.2-1)

Most mammalian tissues contain only three major cysteine proteases, cathepsins B, H, and L. As these proteases are involved in protein turnover they are generally located within the lysosomes of cells.[6] Sub-cellular lysosomes are responsible for the breakdown of cellular protein and thus play a key role in the maintenance and renewal (turnover) of this material.

1.3 Cysteine Proteases and Disease States

Extralysosomal cysteine proteases are believed to have a causitive role in a number of disease states. During the sequence of steps resulting in the spread of cancer from one tissue to another (metastasis), the release and extracellular activity of cysteine proteases has been noted.[7] Cathepsin B has also been found to be present in elevated amounts in cell variants of high metastatic potential. Thus the inhibition of such enzymes may result in the limitation or elimination of this process with obvious therapeutic advantages. Similarly, non-lysosomal cysteine proteases have been implicated as being involved in extracellular collagen degradation in conditions such as arthritis suggesting a further possible application of selective inhibitors.[8]

In the realm of parasitic diseases the role of cysteine proteases has been the subject of much research.[6] The proteases of parasites are crucially involved in many aspects of host-parasite interaction in addition to their general role in protein turnover. Cysteine proteases have been detected in most groups of parasitic protozoa (unicellular eukaryotes which are unable to derive energy from photosynthesis or inorganic chemicals and therefore require to feed on plants or animals). Indeed many species contain relatively large numbers of distinct enzymes unlike mammalian tissues. These enzymes tend to have high activity in at least one stage in the life cycle of the parasite, often a stage which takes place within the mammalian host. Since parasite cysteine proteases have been noted as being different from their mammalian cousins (for example many appear to be larger and/or have a higher stability to alkaline conditions), the opportunity for selective inhibition of either general life processes or specific host-parasite interactions may exist. Unfortunately, for the majority of these enzymes, little is known about substrate specificity.

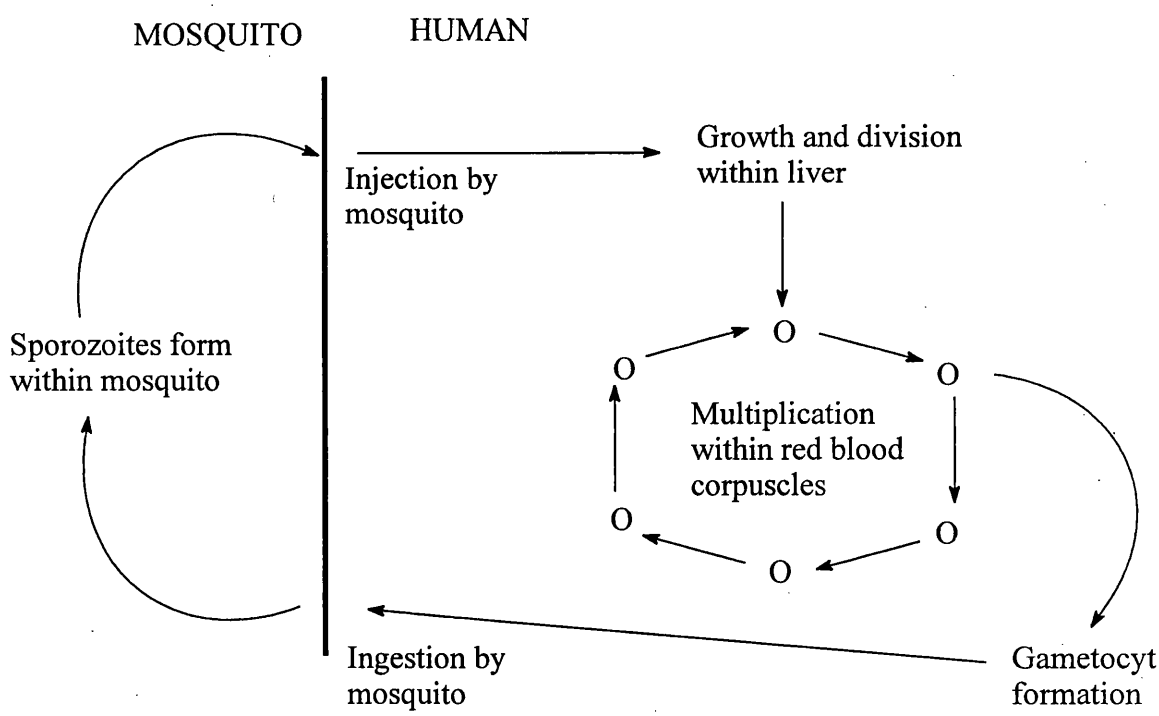
1.4 Cysteine Proteases and Parasitic Diseases

In order to fully appreciate the crucial role played by parasite cysteine protease enzymes, in addition to standard protein turn-over, one must first discuss typical parasite life cycles and their resulting impact on the human host. From this, a measure of the importance of research in this area can also be gauged.

Malaria[9] [10]

Approximately 250 million people are affected by this disease at any one time and between 2 and 4 million of these die each year as a result. This disease is caused by sporozoans (non-motile, amoeba-like, spore forming parasites) of the genus *Plasmodium* which are transmitted by the female *Anopheles* mosquito. When the *Anopheles* mosquito takes a blood meal from from a human it injects saliva in order to inhibit clot formation. It is from this saliva that the *Plasmodium* cells of the sporozoite stage enter the host where they are carried by the blood

stream to the liver (figure 1.4-1). In this organ the sporozoites grow for between 5 and 15 days after which they divide producing a large number of cells known as merozoites. These are released from the liver and penetrate red blood corpuscles (erythrocytes) within which they divide rapidly. This process causes enlargement and eventual rupture of the corpuscle resulting in the release of the fresh merozoites which enter further corpuscles continuing the process. Eventually some merozoites develop into gametocytes which have the potential to become either male or female sex cells (gametes). These cells will not mature as gametes while they remain within the human host but must pass, during a blood meal, back into a female *Anopheles* mosquito. Within the gut of such a mosquito these cells complete their development and reproduce sexually. The product of this process divides to ultimately produce new sporozoites which migrate to the salivary glands of the mosquito and are then ready to infect further human hosts.



(figure 1.4-1)

Cysteine protease enzymes are believed to be involved in the invasion of red blood corpuscles by merozoites and the earlier maturation of these within the

liver.[11] [6] Work on the species *Plasmodium falciparum* has also suggested that the parasite cysteine proteases are responsible for the digestion of the host corpuscle haemoglobin.[12] The breakdown of host haemoglobin is essential in order to provide the developing parasite with amino acids for protein synthesis.[13] Indeed it has been shown that inhibition of cysteine protease enzymes prevents intraerthyrocytic development and causes the enlargement of parasite food vacuoles (analogous organelle to mammalian lysosomes).[11]

Trypanosoma and *Leishmania*

These parasitic protozoa belong to the phylum zoomastigina.[14] The distinctive feature of the zoomastigotes is that they achieve motility by the use of one or more long flagella. Thousands of species of zoomastigotes exist, many of which are parasitic.

Trypanosoma cruzi affects 11-12 million people in Central and South America.[15] The vector for this disease are bugs belonging to the Reduviidae family. As with malaria the vector species becomes infected by feeding on mammalian blood containing the organism. Multiplication occurs within the insect host with the infective species passed out in the insect faeces. The human host becomes infected if these faeces come into contact with the insect bite or other wounds or the conjunctiva of the eye. The parasites then enter a variety of tissues particularly macrophages (mobile cells of the immune system which engulf and digest invading bacteria, fungi, other microorganisms and general cellular debris), muscle and nerve cells where they multiply and develop. The life cycle of the parasite continues upon ingestion by the insect vector during a blood meal. In the most serious cases of Chagas disease, as caused by this parasite, cardiac failure or loss of alimentary canal nervous control can result.

The cystein protease cruzain (also known as cruzipain) has been shown to be responsible for the major proteolytic activity of this organism. Cysteine protease enzymes may also play a role in the penetration of the host cell.[16]

The African variants *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense* are responsible for the disease commonly known as sleeping sickness.[15] The vector in this case being the tsetse fly of the Glossinidae family. The closely related *Trypanosoma brucei brucei*, which similarly affects horses, camels and cattle, is often used as a model system for the study of these species. At least four cysteine protease enzymes are believed to be present in *Trypanosoma brucei brucei*.[6]

Stages in the life cycle of the Central American *Leishmania mexicana* (transmitted by the sand fly *Lutzomyia*) take place within the macrophages of a human host. Cysteine proteases, of which at least seven are present, are probably required for the survival of the parasite within these cells of the immune system.[15] [6]

1.5 Protease Enzyme Inhibition

The parasite cysteine protease enzymes are a major target for the development of specific inhibitors in order to combat the widespread diseases discussed above. The apparent differences between these enzymes and their mammalian counterparts suggests that effective inhibition may be possible with minimal side effects.

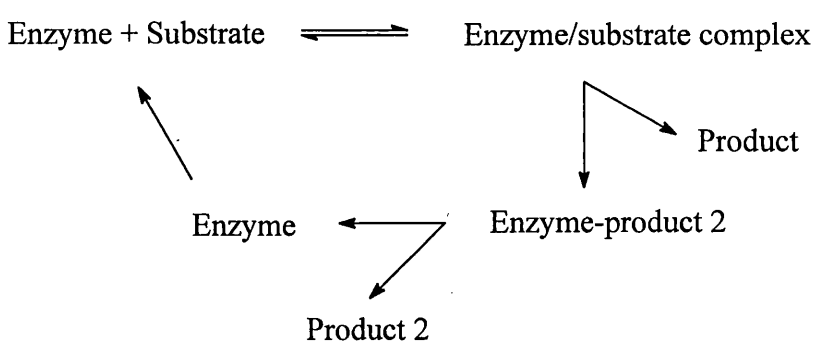
To inhibit an enzyme the potential inhibitor must bind in such a way that the active site is either blocked or altered in structure. In effect the active site or its associated catalytic machinery must no longer exist in a functional form. The inhibitor molecule may bind directly to the active site of the enzyme, possibly by mimicking the general structure of the true substrate. If the interaction between this material and the enzyme is weak, and the binding therefore reversible, the degree of inhibition will depend on the concentrations of both inhibitor and substrate. This is inevitable as both materials will, in effect, be in competition for the same site.

An alternative to this is the binding of the inhibitor to a region remote from the active site. Such a material relies on the gross conformational change inflicted on the enzyme by its attachment to disrupt the active site arrangement. In such a system the inhibitor molecule need not compete for the active site with the substrate.

For irreversible inhibition the enzyme-inhibitor interaction must be extremely tight or permanent. Once again the binding of the inhibitor may be competitive or non-competitive. In both cases the net result will be the same; complete and permanent inactivation of the enzyme. With competitive inhibitors however, this may take some time in the presence of the true substrate.

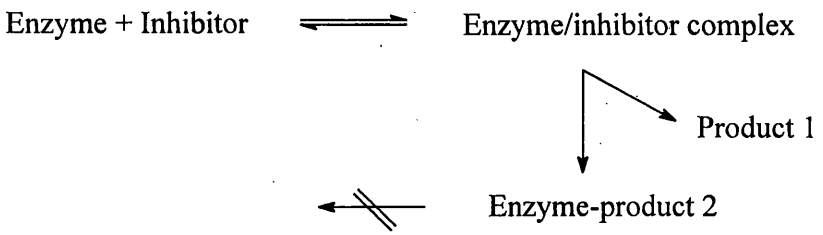
1.6 Concepts Inferred from the Serine Protease Model

From the small selection of serine protease enzymes discussed earlier it can be clearly seen that the variation of one or more residues in the region of the active site can change substrate selectivity markedly. It may therefore be possible to design substrate-like inhibitors which have the ability to reduce the activity of one specific enzyme. In theory this inhibition could take place in the presence of other protease enzymes without seriously affecting their performance. Another important feature giving scope for inhibition is the formation of a covalent bond between the enzyme and the substrate during the catalytic cycle. This cycle can be descibed by the following simple diagram (figure 1.6-1).



(figure 1.6-1)

If the covalent bond formed between the enzyme and product 2 can be made permanent not only will the substrate site be blocked but the catalytic machinery will also be effectively destroyed (figure 1.6-2).



(figure 1.6-2)

1.7 Cysteine Protease Inhibition

As very little is known about the exact structure of most of the cysteine proteases a selective non-competitive inhibitor may be difficult to develop. The scope for active site directed inhibition is, however, significant. With proteases the opportunity exists in using pseudo-peptides as competitive inhibitors. These peptide-like species could be fine tuned to mimic the exact amino acid sequence found in the preferred substrate. As the activity of each protease varies with each substrate sequence, a degree of selectivity may then be possible.

The desire also exists for inhibition to be irreversible. This could be satisfied by the formation of a covalent link from the inhibitor to the active site cysteine. The agent or group chosen to effect the formation of this link should ideally possess the following qualities:

1. Only the active site cysteine should be attacked. The thiol groups of other biological molecules should be unaffected. The inhibitor must not react with non-activated cysteine residues which may be found in other enzymes, proteins and free cysteine.

2. The activated hydroxyl group of the serine proteases must be left unharmed by the attention of the molecule. As indicated earlier, the serine proteases are a numerous and diverse family which play a variety of vital roles in the biochemistry of organisms. This becomes even more important when the same pseudo-peptide may perfectly mimic a serine protease substrate.

Thus, with the information so far available, the ideal inhibitor would appear to be a peptide consisting of residues which would normally be part of the natural substrate of the parasite cysteine protease. These residues would preferrably also reject or limit the attentions of host cysteine proteases such as the cathepsin family. The peptide would also contain a reactive group which, once directed to the heart of the enzyme, would be able to irreversibly modify the activated cysteine residue. This group would however, have no effect on the serine protease catalytic triad.

Chapter 2: Aldehyde based Inhibitors of Cysteine Proteases

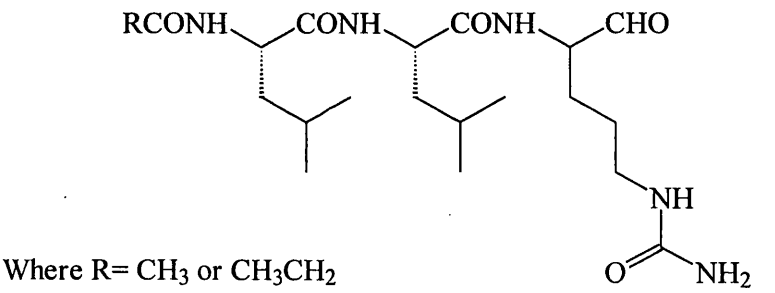
The history and development of a selection of protease inhibitors will now be discussed in order to expand and illustrate the basic concepts discussed in the previous chapter. Such a study will also allow the indication of which inhibitors show the greatest potential for development into anti-parasite drugs.

2.1 Natural Aldehyde Based Inhibitors

A number of peptide derivatives possessing reversible activity against serine and cysteine proteases have been isolated from a range of *Streptomyces.*[17] [18]

Leupeptin

Isolated from the culture filtrates of over eleven species of streptomyces, the leupeptin group of compounds were observed to have an inhibiting effect on trypsin, plasmin and papain. The parent form of leupeptin is illustrated in figure 2.1-1.

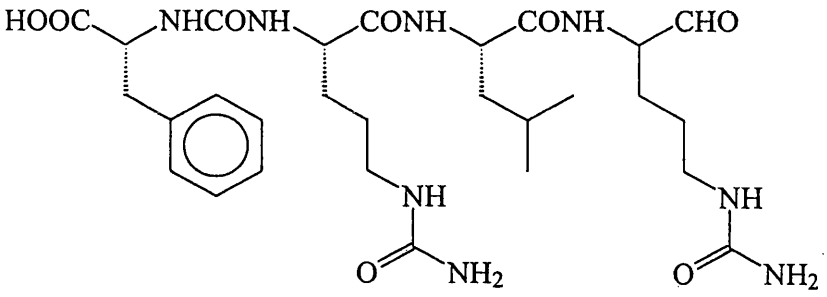


(figure 2.1-1)

The analogues of these compounds where leucine is replaced by isoleucine or valine, also possess similar activity.

Antipain

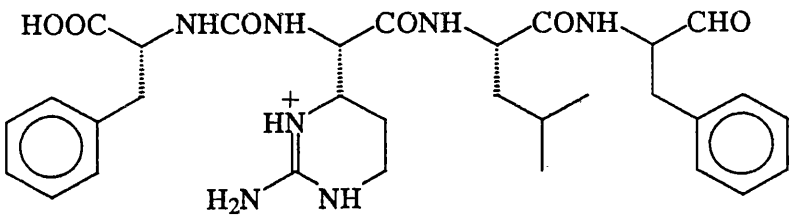
Isolated from *Streptomyces michigaensis*, *Actinomyces violasceus, Streptomyces mauvecolor* and *Streptomyces yokosukaensis*, antipain (figure 2.1-2) shows inhibiting action on papain, trypsin and cathepsin B.



(figure 2.1-2)

Chymostatin

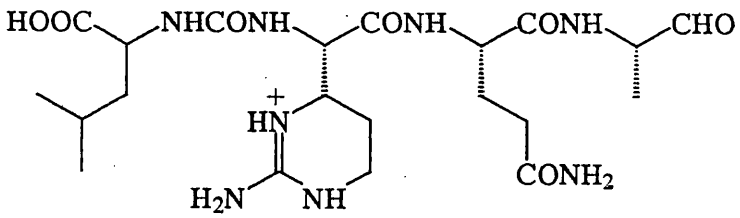
Isolated from *Streptomyces hygroscopicus* and *Streptomyces lavendulae*, this compound (figure 2.1-3) has shown activity against the serine protease chymotrypsin.



(figure 2.1-3)

Elastatinal

This compound (figure 2.1-4) was isolated from *Streptomyces griseoruber*, and found to inhibit pancreatic elastase.

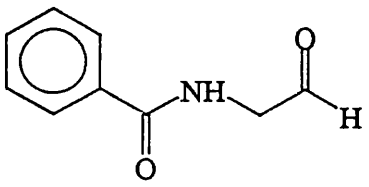


(figure 2.1-4)

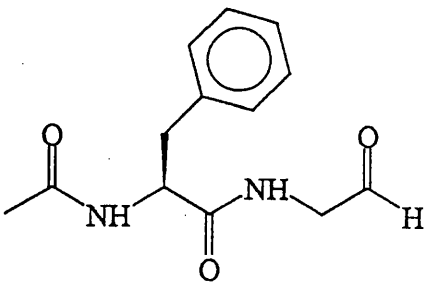
Much information can be obtained from a comparative analysis of the structure/activity relationship for these compounds. The peptide nature of this selection of compounds is readily apparent but a closer look reveals another key feature. In each case the terminal residue, adjacent to the aldehyde, is identical to that normally recognised by the enzyme as part of the natural substrate (see

section 1.1). Indeed, the aldehyde is located at the position normally occupied by the peptide link selected for cleavage. One can therefore suggest that this group plays an active role in the inhibition mechanism. This postulate is backed up by the fact that the corresponding acid, alcohol and dibutyl acetal of leupeptin do not show such pronounced activity.[18]

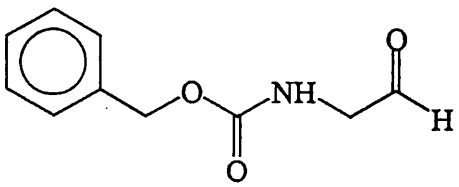
Aldehydes structurally related to the acyl portion of natural substrates have been synthesised and shown to be potent inhibitors of papain.[19] The necessity of both aldehyde and peptide portions for effective inhibition can be illustrated with reference to the compounds shown in figure 2.1-5.



Benzoylamino acetaldehyde



N-Acetyl-(L)-phenylalanylamino acetaldehyde



Benzyloxycarbonylamino acetaldehyde

(figure 2.1-5)

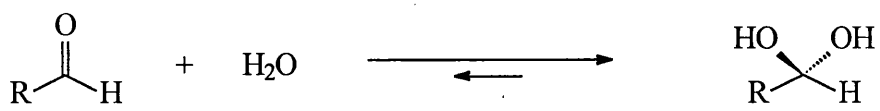
These three materials show reversible inhibiting activity against papain. The strength of this inhibition was observed to be greatest for acetyl-(L)-phenylalanylamino acetaldehyde and weakest for benzoylamino acetaldehyde. The presence of a longer peptide-like portion therefore increases the effectiveness of the inhibition in some way. Indeed acetaldehyde and propionaldehyde are relatively ineffective in comparison to these three compounds. Thus it can be postulated that initial selection by the enzyme takes place by recognition of the substrate mimicking portion.

Considering the aldehyde group, it was shown that the corresponding nitrile, carboxylic acid, amide and methyl ester of benzoylamino acetaldehyde are much less tightly held. From this result it can also be postulated that some form of strong but non-permanent enzyme-aldehyde interaction takes place causing greater reversible inhibition.

Further experimental results add weight to these theories. The observed enzyme/inhibitor binding ratio (approximately 1:1) is as expected for competitive, substrate-like recognition. Papain has also been shown to be reversibly protected from the action of *N*-ethylmaleimide, a known active site thiol modifier, in the presence of these peptidyl aldehydes indicating their active site directed nature.

While at this stage of discussion we can conclude that the peptidyl aldehydes are reversible and competitive inhibitors, the actual role of the aldehyde is still unclear. There are two possible explanations for the increased binding power conferred by this group.

1. In aqueous solution the aldehyde function will exist mainly as the hydrate (figure 2.1-6).



(figure 2.1-6)

This hydrate closely resembles the tetrahedral intermediate formed during peptide hydrolysis. The significant hydrogen bonding employed by the enzyme to stabilise this intermediate may therefore be responsible for the tighter binding of the hydrated aldehyde inhibitor.

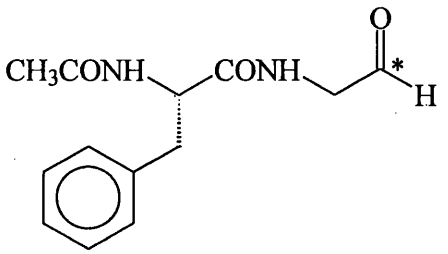
2. The enzyme itself may attack the aldehyde, as it would a peptide link carbonyl, forming a hemiacetal (serine proteases) or hemithioacetal (cysteine

proteases). The inhibitor would therefore be covalently bound to the enzyme for the lifetime of the tetrahedral adduct. Once again the hydrogen bonding abilities of the enzyme may be utilised to stabilise this normally labile structure. This inhibitor-enzyme complex however, must not be permanent if the reversible nature of the inhibition is to be maintained.

2.2 NMR Spectroscopic Studies on Papain Inhibition

The true role of the aldehyde in papain inhibition was uncovered using a ^{13}C enriched inhibitor and NMR spectroscopy.[20]

[1-^{13}C] *N*-Acetyl-(L)-phenylalanylglycinal (figure 2.2-1) was prepared from [1-^{13}C] glycine.



where * indicates enrichment position

(figure 2.2-1)

The ^{13}C NMR spectrum of this material in water, in the absence of enzyme, shows two main sets of signals. The stronger of these occurs at 88.2 ppm and corresponds to the hydrate of the aldehyde. Ninety-five percent of the material was observed to exist in this form in aqueous solution. Smaller signals for the unhydrated aldehyde were also present at 200.9 ppm.

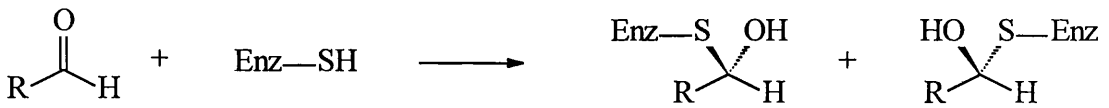
On the addition of excess papain both sets of signals were found to disappear and be replaced by a new set at 74.9 ppm. Addition of a three-fold excess of inhibitor to this solution resulted in enhancement of the new set and reappearance of the hydrate signals. These new signals were postulated as

being due to the presence of a hemithioacetal which was confirmed by the synthesis of model compounds.

Further evidence for the reversible participation of the thiol in the inhibition mechanism was obtained by the addition of 2,2'-dipyridyl disulfide at pH 4.1. This active site thiol modifying reagent caused the disappearance of the hemithioacetal signal resulting in the corresponding increase in hydrate peak intensity.

This combined evidence confirms that free papain and the hemithioacetal containing inhibited complex are in equilibrium in an aqueous solution of peptidyl aldehyde (i.e. reversible competitive inhibition *via* hemithioacetal formation).

Closer examination of the hemithioacetal peak revealed that it was actually composed of two broad signals. Incubation of the complex with 2,2'-dipyridyl disulfide at pH 7 preferentially removed the downfield peak and caused the corresponding increase in hydrate signals. This can be explained as follows: The binding of the inhibitor to the enzyme *via* a hemithioacetal (figure 2.2-2) will result in the formation of a new chiral centre. As both enzyme and inhibitor are chiral and enantiomerically pure, binding will result in diastereomer formation.

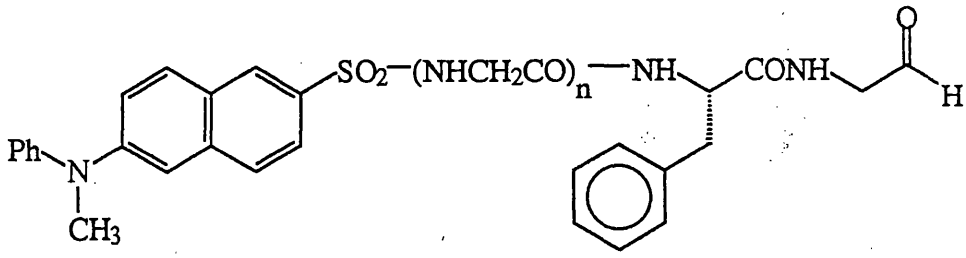


(figure 2.2-2)

It is conceivable that one of these diastereomers will be significantly more stable than the other. The less stable form can therefore be induced to collapse, leaving the thiol group free for the attentions of 2,2'-dipyridyl disulfide, under less acidic conditions. The more stable configuration is likely to be that which best fits the available hydrogen bonding framework used to stabilise the substrate hydrolysis intermediate. Which configuration is more stable, however, is still unclear.

2.3 Fluorescence Studies on Peptidyl Aldehyde Inhibitors

A fluorescence study on a series of mansyl (6-[*N*-methylanilino]-2-naphthalene sulfonyl) protected peptidyl aldehydes (figure 2.3-1) highlighted a further, potentially useful, feature regarding inhibitor structure.[21]



mansyl-(Gly)$_n$-(L)-Phe-glycinal n = 0, 1, 2

(figure 2.3-1)

For all three structures (n = 0, 1 and 2) the observed intensity of fluorescence from the mansyl group was found to increase on the binding of the inhibitor to the enzyme. The extent of this increase was found to be dependent on the glycine chain length with n = 0 showing the greatest change. Chain length however had little influence on binding ability as all three were shown to be equally effective inhibitors.

The increase in fluorescence was assigned to energy transfer from a papain tryptophan residue to the mansyl group. The strength of this transfer would obviously diminish as the distance between the two groups increased. It was therefore proposed that the mansyl group protruded further into the solvent (water) as the chain length of the inhibitor increased. The important point to be taken from this study is that bulky *N*-terminus protecting groups may be used without adversely affecting the inhibitor binding. In the three case studied here the large mansyl group had little influence on the inhibiting ability of the compounds, even when close to the active site (n = 0). If indeed the *N*-terminus

protrudes into the solvent as suggested here then protecting group selection will not be limited by enzyme steric restrictions.

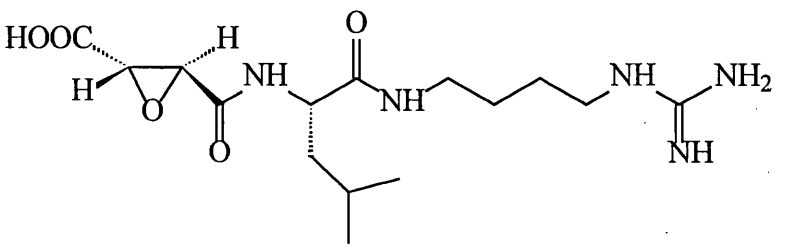
2.4 Limitation of Aldehyde Based Inhibitors

A possible problem with aldehyde based inhibitors was reported by Sutherland and Greenbaum in 1983.[22] Leupeptin, in adequate concentrations *in vivo*, was shown to be a potent stimulator of cathepsin B activity in striated muscle, heart, liver and kidney tissues of the mouse. The mechanism of this action was unclear but what the result did illustrate was that leupeptin, or its metabolites, has complex effects on cells beyond that of an antiprotease. The *in vivo* application of these materials may, as a result, be limited.

The lack of selectivity for cysteine proteases exhibited by members of this group presents further limitations for *in vivo* use. The reversible nature of the inhibition mechanism may exacerbate this problem as relatively large and repeated doses may be required in order to achieve a sufficient effect.

Chapter 3: E-64, a Natural Irreversible Inhibitor

In 1978 a compound of the formula $C_{15}H_{27}O_5N_5$ was reported as being isolated from a solid culture of the fungus *Aspergillus japonicus* (TPR-64) and named E-64.[23] Initial structural assignments were made on the basis of infra-red spectroscopy and chemical reactivity. Degradation studies in conjunction with proton NMR and a range of partial syntheses later confirmed the structure as being that shown in figure 3-1.[24]



N-[*N*-(L-3-*trans*-carboxyoxiran-2-carbonyl)-L-leucyl]-agmatine
(or L-*trans*-epoxysuccinyl leucylamido[4-guanido]butane)

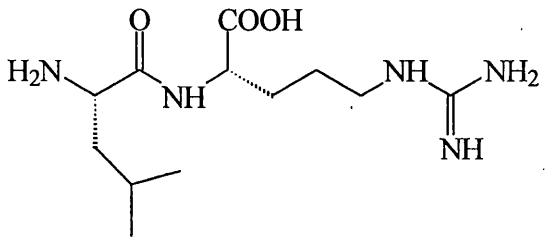
(figure 3-1)

Two years later, E-64 was reported to bind to an equimolar quantity of active cysteine residues in isolated cathepsin B (under physiological conditions) but not simple unactivated thiols.[25] Further work in this area showed that E-64 inhibited cathepsins B, L and papain irreversibly. The selectivity for such cysteine proteases, while better than that achieved with aldehyde based inhibitors, was found not to be absolute with the serine proteases trypsin and thrombin affected in a similar manner.[26]

3.1 Investigations into the Mode of Action of E-64

The site of inhibition was confirmed as being the same as that used by peptidyl aldehydes by the protection of cathepsin B and papain with the reversible competative inhibitor leupeptin.[27] One can therefore assume that E-64 is initially selected by the enzyme on the basis of its substrate mimicking peptide

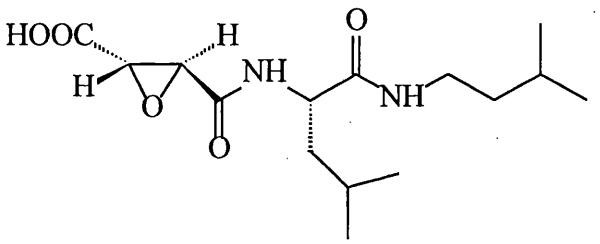
chain. The resemblance of the E-64 structure to the peptide (L)-leucine-(L)-arginine (figure 3.1-1) is readily apparent.



(L)-leucine-(L)-arginine

(figure 3.1-1)

If the site of binding is the same as that for the natural substrate, one would expect some degree of selectivity with respect to the structure of the peptide portion of the inhibitor. Indeed, the rate of E-64 induced inhibition on a series of closely related proteases was found to vary. Similarly the analogue Ep-475 (figure 3.1-2), where only the peptide portion has been varied, was shown to inhibit cathepsins B and L faster than the parent compound.[27]



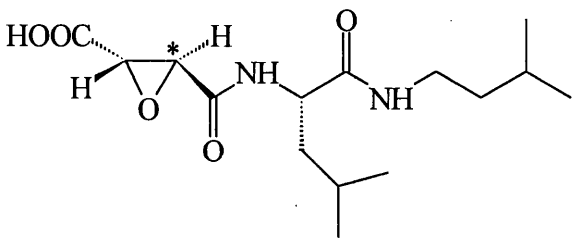
Ep-475

(L-*trans*-epoxysuccinylleucylamido[3-methyl]butane)

(figure 3.1-2)

Considering the mechanism of inhibition, the initial isolators of E-64 found that destruction of the epoxide, by the use of hydrochloric acid, resulted in the complete loss of irreversible inhibition activity.[28] It can therefore be postulated that after initial recognition of the peptide fragment by the enzyme, irreversible inhibition takes place by covalent binding of the epoxide to the active site. By comparison with the aldehyde inhibitor mechanism, it appears plausible that the epoxide binding position is the active site cysteine.

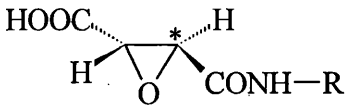
By 1988 direct observation of such an alkylated cysteine had not yet been reported. In this year Yake, Guillaume and Rich reported the results of ^{13}C NMR spectroscopic studies on enriched Ep-475 (figure 3.1-3). [29]



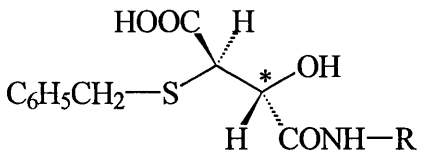
Ep-475 where * indicates enrichment site

(figure 3.1-3)

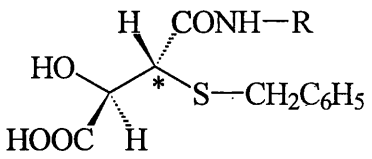
Non-enzymic thiols at high pH were shown, by ^{13}C NMR spectroscopy in water, to attack both epoxide carbons giving two products in a 4:1 ratio. The chemical shifts of the ^{13}C (C-2) enrichment following this reaction are indicated in figure 3.1-4.



benzyl mercaptan/NaOH
pH 10



4:1



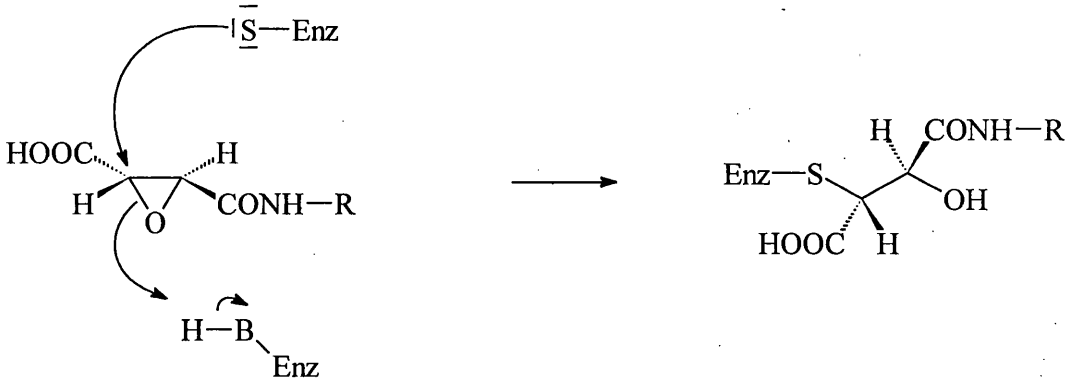
chemical shift * ~73 ppm
C-3 ATTACK

chemical shift * ~53 ppm
C-2 ATTACK

(figure 3.1-4)

With papain, buffered at pH 6.8, the only ^{13}C enriched signal observed was located at 76.5 ppm. No new signal in the 50 ppm area was apparent. Thus the

activated cysteine of papain attacks the epoxide at C-3 exclusively, resulting in irreversible inhibition by covalent bond formation (figure 3.1-5).



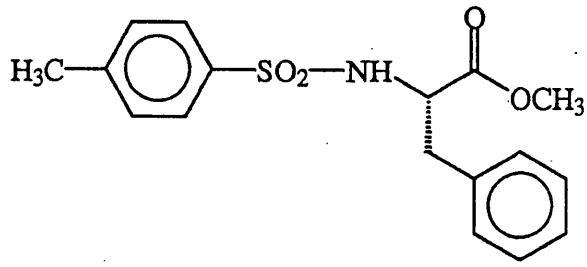
(figure 3.1-5)

3.2 Limitations of Epoxide Based Inhibitors

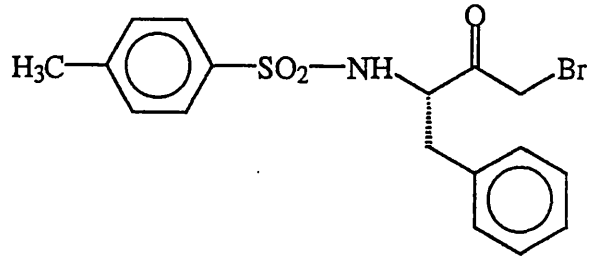
The irreversible activity of these compounds represents a significant improvement over aldehyde based inhibitors. However, the selectivity for cysteine proteases, though better than that achieved with previous compounds, is not absolute. A further problem yet to be overcome is the rate at which these compounds are eliminated from the body. This was illustrated by tracer studies on tritium labelled E-64 in rats.[26] On initial administration the material was observed to diffuse rapidly from the injection site. This process was, as expected, accompanied by a rapid rise in blood radioactivity. An equally rapid fall in blood radioactivity was then noted and assigned as being due to the uptake of E-64 by the internal organs. The liver and kidneys were identified as being the main recipients of the radioactivity suggesting that metabolism and excretion are early fates for the injected material. Indeed, within 24 hours, 74.9% of the administered dose of radioactivity was found to be excreted in the urine.

Chapter 4: Halomethyl Ketone Based Inhibitors

In 1962 Schoellmann and Shaw reported the use of an amino acid derivative (figure 4-1) as a labelling agent for the active centre of chymotrypsin.[30] Designed to mimic a known substrate of this enzyme, *N*-tosyl-(L)-phenylalanyl bromomethyl ketone was observed to cause the irreversible loss of enzymic activity.



N-tosyl-(L)-phenylalanine methyl ester
(Substrate for hydrolysis by chymotrypsin)



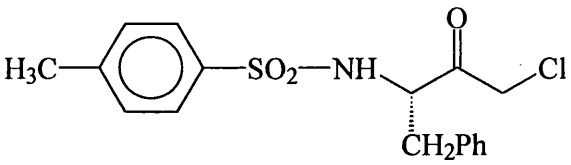
N-tosyl-(L)-phenylalanyl bromomethyl ketone
[L-(1-tosylamido-2-phenyl)ethyl bromomethyl ketone]

(figure 4-1)

The active site directed nature of the material, due to its substrate similarity, was confirmed by the protection of the enzyme with hydrocinnamic acid, a known reversible and competitive inhibitor of chymotrypsin. These results triggered research into a range of halomethyl ketones that would prove to be powerful tools for the study of the biological function of proteases both *in vitro* and *in vivo*.

4.1 The Chloromethyl Ketones

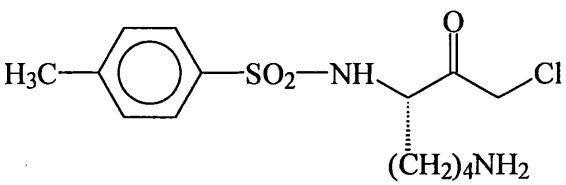
Initially investigated as more stable variants of the bromine containing compounds, chloromethyl ketones were found to exert a similar biological action. For example *N*-tosyl-(L)-phenylalanyl chloromethyl ketone (figure 4.1-1) was observed to completely inactivate chymotrypsin in a specific and stoichiometric manner.[31] Irreversible inhibition in this manner suggested that the compound was binding to one specific residue in the enzyme active site. In order to verify this, the enzyme-inhibitor complex was digested and the resulting short chain peptides separated allowing the isolation of a decapeptide bearing a modified histidine residue. This peptide fragment was shown to be non-artefactual by comparison of the amino acid sequence with the known primary structure of chymotrypsin. The histidine residue was therefore implicated as being the active site alkylation target of the chloromethyl ketone.



N-tosyl-(L)-phenylalanyl chloromethyl ketone

(figure 4.1-1)

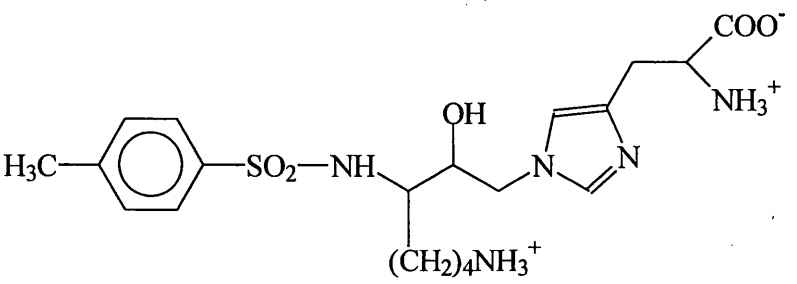
In the same manner, trypsin inactivated with *N*-tosyl-(L)-lysyl chloromethyl ketone (figure 4.1-2) yielded an octapeptide fragment bearing a modified histidine residue.[32]



N-tosyl-(L)-lysyl chloromethyl ketone

(figure 4.1-2)

In this case a derivative of the modified residue was also isolated and shown to be of the structure shown in figure 4.1-3 (note that the generation of the alcohol function is an artefact of the technique used to allow isolation).[33]



Inhibitor-histidine derivative
(stereochemistry not specified)

(figure 4.1-3)

Notice how in both cases above the inhibitors are derived from the amino acids normally selected as part of the natural substrate. Indeed, chloromethyl ketones unrelated to the natural substrate for a particular serine protease tend to react only very slowly or not at all with that enzyme.[34] [35] Thus the same substrate-mimic selection observed with the aldehyde and epoxide bearing compounds is also found with these competitive, irreversible inhibitors. The inhibitor must therefore bind to the enzyme active site in such a fashion as to place the alkylating group close to the histidine residue of the catalytic triad.

4.2 NMR Spectroscopic Studies on Chloromethyl Ketone Induced Inhibition

In 1983 Malthouse, Mackenzie, Boyd and Scott reported NMR spectroscopic evidence to suggest that the binding between the chloromethyl ketone and the enzyme is more complex than the accepted single bond to histidine.[36]

For this study *N*-benzyloxycarbonyl-(L)-lysyl chloromethyl ketone, ^{13}C enriched at the ketone carbonyl, was used to inhibit trypsin. The carbon spectrum of the pure compound in DMSO consisted of a single large peak at 200.6 ppm. In

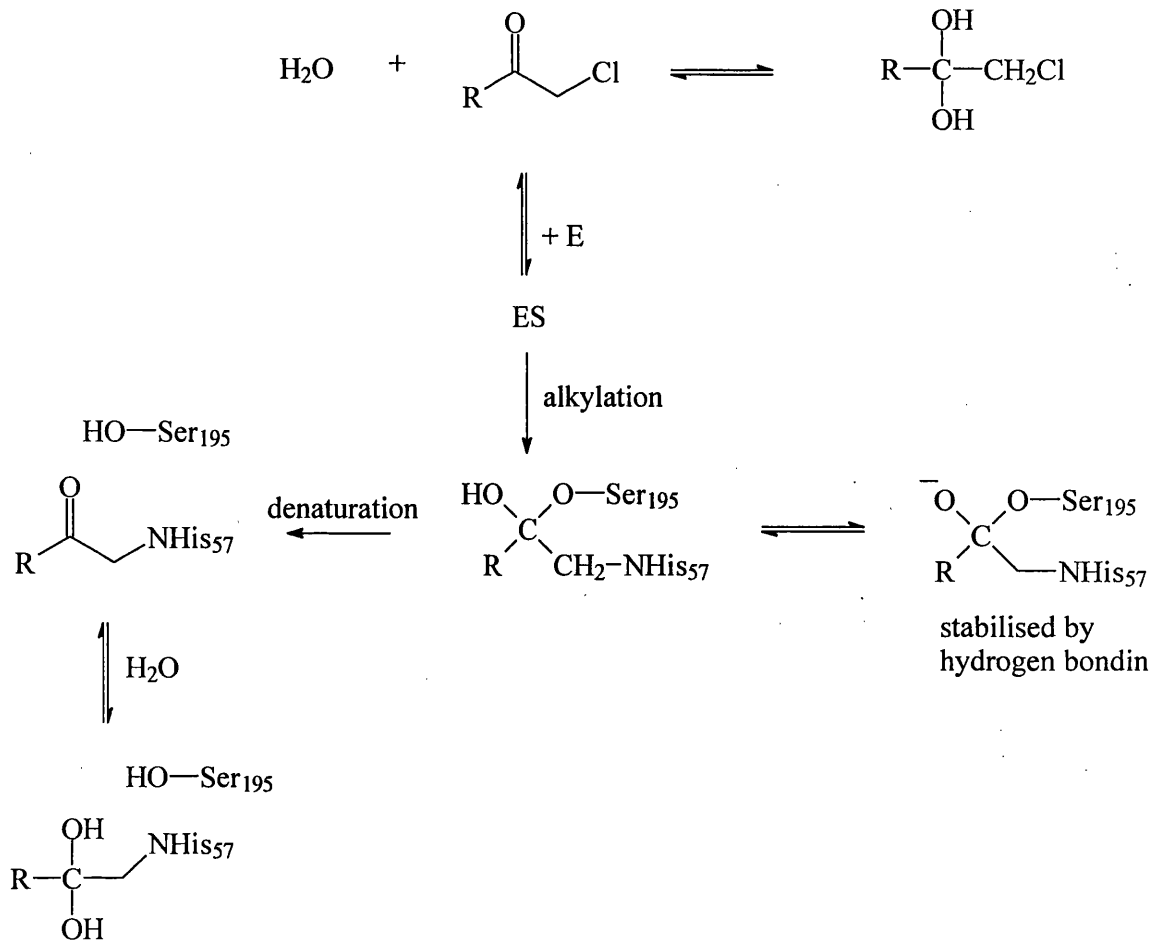
water however, hydrate formation was significant and two peaks were observed; 204.7 ppm (ketone) and 95.4 ppm (hydrate).

Addition of the enzyme to the aqueous solution of the inhibitor at pH 3.2 resulted in no change to the enriched signals and no loss of enzyme activity. At pH 6.9 however, both enriched peaks disappeared and were replaced by a single new peak at 98.0 ppm while enzyme activity was significantly reduced. By sequentially adding excess inhibitor and enzyme to this solution it was possible to show that the new peak was indeed due to some form of stoichiometric enzyme-inhibitor interaction. As gel filtration and the addition of a competitive inhibitor (benzamidine) were unable to remove the 98.0 ppm signal, it was concluded that covalent binding between the labelled inhibitor carbonyl and enzyme active site had taken place.

A tetrahedral state formed from attack of the active site serine residue on the carbonyl was proposed as being responsible for the 98.0 ppm peak. Further evidence for this was obtained from the alkali induced denaturation of the enzyme which resulted in new hydrate and ketone signals at 95.1 ppm and 205.5 ppm respectively. These occur as the inhibitor in the denatured enzyme remains attached only to histidine. The residues of the catalytic triad are no longer in close proximity therefore the tetrahedral enzyme-inhibitor complex cannot reform and the inhibitor carbonyl is left free for the attention of water.

The results observed in this study bear a significant degree of similarity to those found with aldehyde based inhibitors as discussed earlier. Thus it can be postulated that, in the absence of the chlorine moiety, these compounds would cause reversible inhibition in a similar manner to the aldehyde based inhibitors.

The binding of chloromethyl ketones to serine proteases can now be represented by the following diagram (figure 4.2-1, stereochemistry not specified).



(figure 4.2-1)

Using heavy water ($H_2{}^{18}O$) as an NMR solvent it was shown that a hemiketal was indeed formed between the enzyme and the inhibitor.[37] In a dilute heavy water solution, as the ketone is in equilibrium with the hydrate one would expect both oxygen atoms of the hydrate to be ^{18}O enriched. With a hemiketal however, one of the two oxygen atoms will always originate from the serine hydroxyl group and therefore always be ^{16}O. The effect of substituting ^{18}O for ^{16}O directly bonded to ^{13}C is to shift the NMR signal of this species slightly upfield (e.g. 0.05 ppm movement for the inhibitor ketone carbonyl).[38] This shift is quantifiable from model systems and is additive (*i.e.* replacing two ^{16}O with two ^{18}O doubles the magnitude of the ^{13}C shift). When bound to trypsin it is possible to show that only one solvent derived oxygen is present. Thus the inhibition is not due to the simple binding of the hydrate in conjunction with histidine alkylation.

4.3 X-Ray Crystallographic Studies on Inhibited Serine Proteases

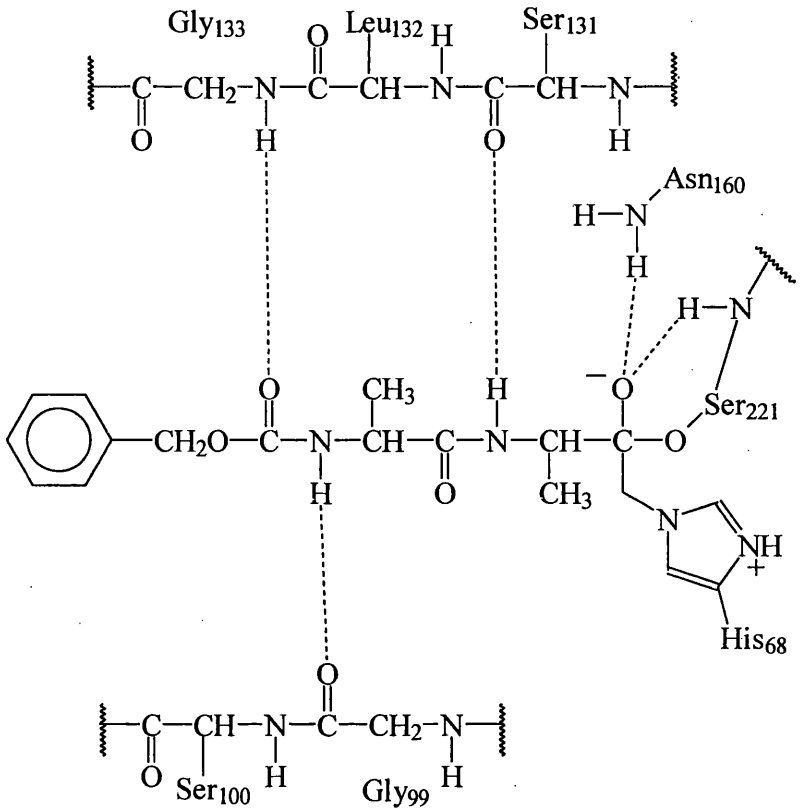
The presence of more than one bond between *N*-benzyloxycarbonyl-(L)-lysyl chloromethyl ketone and trypsin, as suggested by the NMR spectroscopic data, was confirmed by X-ray studies.[37] Continuous electron density was found between the inhibitor ketone carbonyl carbon and serine 195 in which the carbonyl carbon to serine oxygen distance was roughly that expected for a carbon-oxygen bond of standard length. The geometry around this carbon was also virtually identical to that expected for a purely tetrahedral species. The expected stabilisation of the tetrahedral state was also clearly seen with two relatively long hydrogen bonds from the amide protons of glycine 193 and serine 195 to the carbonyl oxygen.

These results however, represent only the solid state of the enzyme-inhibitor complex. In solution this may not be the case. Solid state NMR spectroscopy was therefore used to find the ^{13}C chemical shift of the enriched carbonyl carbon of the bound inhibitor. This signal was observed at 98.0 ppm, identical to that found in solution for the postulated tetrahedral adduct.

The inhibitor therefore clearly binds to the trypsin catalytic triad *via* two covalent bonds with the normally labile tetrahedral state thus produced, stabilised by hydrogen bonding from the enzyme main chain. A further interesting feature noted was that no accurate electron density could be obtained for the benzyl group of the inhibitor *N*-protection. This indicates that the group is not held in any specific geometry by the enzyme. As suggested earlier, variation of this bulky protecting group should therefore have little effect on the binding ability of the molecule.

In the same year the crystal structure of proteinase K, a serine protease isolated from the fungus *Tritirachium album*, with a bound chloromethyl ketone was reported.[39] In this case the inhibitor [*N*-CBZ-(L)-Ala-(L)-AlaCH_2Cl] was again found to bind to both the histidine and serine residues of the catalytic triad

(figure 4.3-1). Extensive hydrogen bonding between the enzyme main chain and the rest of the inhibitor molecule was also observed resulting in a triple anti-parallel pleated sheet structure.



(figure 4.3-1)

An important point which should be noted from this study is how the longer inhibitor chain participates in the binding to the enzyme. What is not evident from this structure however, are the substrate/inhibitor side chain selection sites. It is these peptide and side chain interactions that are considered to represent the initial complexation between enzyme and inhibitor.

4.4 Kinetic Studies on Chloromethyl Ketone Induced Inhibition

The peptidyl chloromethyl ketone (D)-Phe-(L)-Pro-(L)-ArgCH_2Cl inhibits the serine protease thrombin and has been shown to prevent intravascular coagulation in rabbits.[40] A study of the kinetics of this inhibition indicated that there was indeed a reversible complex rapidly formed prior to the slower

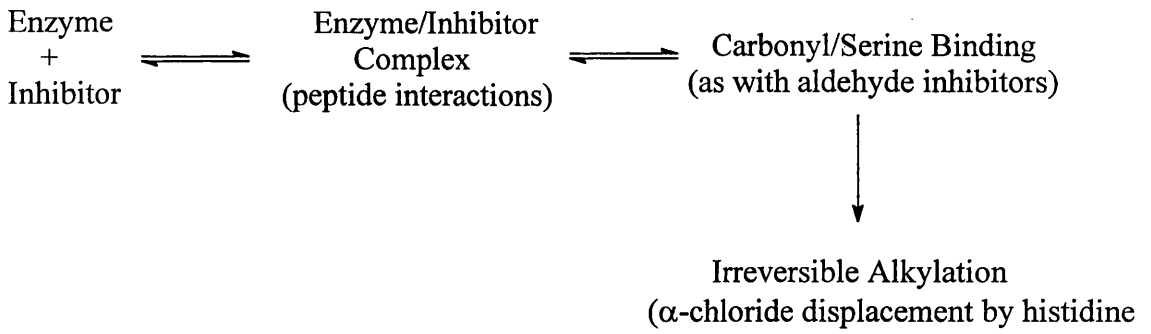
irreversible binding. Four years after this, Stein and Trainor reported the formation and accumulation of two complexes within this reversible step for the inhibition of human leukocyte elastase with a peptidyl chloromethyl ketone.[41] The dissociation constant (K_i) of the enzyme-inhibitor reversible complex (figure 4.4-1) was also shown to be ten times lower than those of substrates and other reversible inhibitors lacking the chloromethyl ketone end group.

$$E + I \rightleftharpoons EI \qquad K_i = \frac{[E]\,[I]}{[EI]}$$

(figure 4.4-1)

Thus kinetic evidence suggests that the peptide to enzyme complexation is indeed followed by a further reversible interaction prior to irreversible alkylation. Further kinetic evidence for the formation of a hemiketal intermediate after initial complexation was provided by McMurray and Dyke from their study on trypsin inhibition by α-substituted peptidyl ketones.[42] In this series of experiments the stength of binding of (L)-Lys-(L)-Ala-(DL)-LysCH_2X was noted as X was varied (X = H, CH_2COOCH_3, $COCH_3$, $OCOCH_3$ and F, racemisation of the modified lysine was due to the method employed for the attachment of the end group and will be discussed in chapter 10.1) It was found that the binding strength for the reversible stage increased as the electron withdrawing power of X increased. This effect would be expected if nucleophilic attack at the adjacent carbonyl was involved in the binding mechanism. The reversible binding constant for the corresponding chloride was also shown to fit the relationship calculated from these experiments.

By combining the results from the X-ray, NMR spectroscopic and kinetic studies discussed here, it can be confidently stated that the mechanism of inhibition proceeds as indicated in figure 4.4-2.



(figure 4.4-2)

4.5 Chloromethyl Ketones With Cysteine Proteases

As one would expect, peptidyl chloromethyl ketones were observed to have a similar effect on cysteine proteases. The initial selection of the inhibitor by the enzyme follows the same basic substrate-mimic requirements as found with serine proteases. For example, *N*-benzyloxycarbonyl-(L)-phenylalanyl chloromethyl ketone was shown to have a second order rate constant for the inhibition of papain 150 times greater than the tosyl protected variant.[43] This effect is presumably due to the greater peptide-like character of the former protecting group.

The inactivation of papain with chloromethyl ketones, however, was shown to result in no drop in the amount of histidine residues observable by amino acid analysis.[44] Only the quantity of cysteine showed any significant change and was therefore identified as being the target residue for alkylation.[45] The rate of alkylation was also shown to increase rapidly with increasing pH. For example the rate of alkylation of papain by *N*-Tos-(L)-PheCH_2Cl increased 34 fold in the pH range 6.5 to 9.[44] Thus the anionic form of the active centre thiol must be the species involved in the displacement of the inhibitor chlorine.

4.6 Limitations of Chloromethyl Ketones

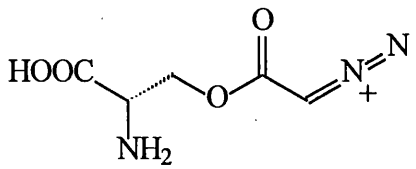
As has been shown here, chloromethyl ketones have been widely used in the investigation of serine protease mechanisms. The α-choro ketone arrangement

makes these compounds strong alkylating agents and any desired selectivity for cysteine proteases must be sought from the amino acid sequence of the inhibitor. With such a great variety of serine proteases present in organisms this selectivity may be difficult to achieve.

The alkylating power of chloromethyl ketones also leads to further problems. These highly electrophilic species are vulnerable to the attentions of non-activated thiols.[46] [7] Indiscriminate alkylation would therefore be expected *in vivo* thus limiting the therapeutic use of this group of compounds.

Chapter 5: Diazomethyl Ketones

Interest in diazomethyl ketones was initiated in 1973 with the isolation of a natural antibiotic, named (L)-azaserine (figure 5-1) , from broths of *Streptomyces.*[47]

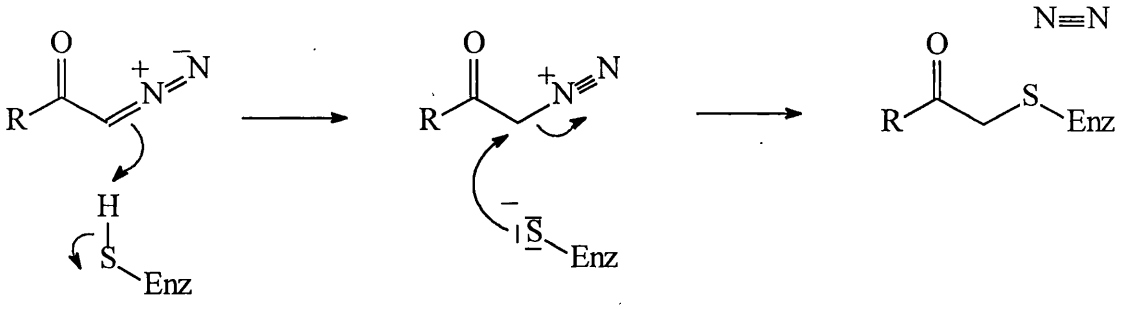


(L)-azaserine

(figure 5-1)

This compound was observed to cause inactivation of an amido transferase enzyme. The biological role of enzymes of this type is to catalyse the transference of the side chain nitrogen of (L)-glutamine (figure 5-3) to other substrates thus forming a new carbon-nitrogen bond. In order to do this the enzyme utilises an optimally placed cysteine side chain to attack the γ-amide of glutamine and effect hydrolysis.

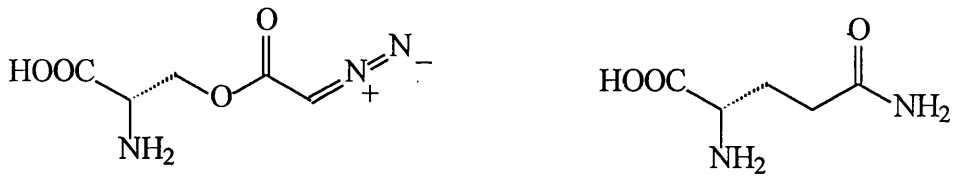
(L)-Azaserine was observed to compete with glutamine for the active site of the enzyme and cause irreversible inhibition. Digestion of a sample of enzyme inactivated with ^{14}C labelled (L)-azaserine indicated that cysteine was indeed the target for alkylation. It was then proposed that alkylation occurs by protonation of the diazomethyl group followed by nucleophilic attack expelling molecular nitrogen (figure 5-2).



Amido transferase with (L)-azaserine

(figure 5-2)

The structural similarity of (L)-azaserine to the natural substrate (L)-glutamine is clearly evident (figure 5-3). The possibility may therefore exist for inhibition of cysteine proteases by the use of a substrate related short chain peptide bearing a diazomethyl ketone end group.



(L)-azaserine (L)-glutamine

(figure 5-3)

5.1 Diazomethyl Ketones with Protease Enzymes

In 1977 it was reported that *N*-CBZ-(L)-PheCHN_2 and *N*-CBZ-(L)-Phe-(L)-PheCHN_2 could be used to inactivate papain in a competitive, irreversible and stoichiometric manner.[43] This inactivation took place at the active centre thiol and was observed to decrease in rate rapidly as the pH was increased above 6.5. Thus the protonated form of the active site cysteine is involved in the mechanism as suggested above. This is in contrast with chloromethyl ketone inhibitors where the anionic thiolate is required and rate increases sharply with increasing pH.

The participation of the "extended binding site" in substrate/inhibitor selection was also well illustrated by this case where the action of *N*-CBZ-(L)-Phe-(L)-$PheCHN_2$ was shown to be 200 times more rapid than *N*-CBZ-(L)-$PheCHN_2$.[48] In a more extensive study with cathepsin B this substrate-mimic theory was backed up as inhibitors bearing unnatural D amino acids were found to be poor inhibitors.[49] Interestingly enough, tri-peptides were also shown to be of no greater effectiveness than related dipeptides.

A feature of great importance noted during these studies was the retention of chymotrypsin activity in the presence of *N*-CBZ-(L)-$PheCHN_2$.[49] Under conditions which would give rapid and total inactivation of cathepsin B, chymotrypsin was unaffected. The characteristic 277 nm absorbance of the diazomethyl ketone group in aqueous solution was however, observed to slowly diminish in the presence of this serine protease at pH 7. Similarly, when pre-incubated with chymotrypsin, *N*-CBZ-(L)-Phe-(L)-$AlaCHN_2$ was observed to lose its papain inactivating ability. This catalytic destruction of the diazomethyl ketone was not observed with chymotrypsin previously inactivated with a chloromethyl ketone, implying the participation of the active site. This process alone cannot account for the relative immunity of the serine protease however, due to its slow rate. For example, 25% decomposition of diazomethyl ketones has been observed in 3 hours under conditions where complete cysteine protease inactivation is measured in minutes.

Kinetic studies showed that with papain, cathepsin B and chymotrypsin an initial competitive, reversible inhibition complex is formed with peptidyl diazomethyl ketones. The complexed inhibitor molecule would then, in theory, pick up a proton to form the unstable diazonium ion. Only in the case of papain and cathepsin B does alkylation rapidly follow, chymotrypsin remains unharmed. In the active site of cysteine proteases the diazomethyl ketone end group must therefore be held in close proximity to the reactive cysteine anion. With serine proteases the inhibitor must not be so ideally oriented or the activity of the nucleophilic species (histidine) must be lower. Considering the highly reactive

nature of the protonated diazomethyl ketone the former explanation seems more plausible.

In order to test the serine protease immunity further, Green and Shaw synthesised a series of diazomethyl ketones based on amino acid sequences normally present in serine protease substrates.[50] Indeed resistance was found to be general. Some discrimination between cysteine proteases was also achieved as reagents not satisfying a particular specificity either did not inhibit or did so only very slowly. For example, considering the cathepsins B, L and H; *N*-CBZ-(L)-Phe-(L)-AlaCHN$_2$ reacted 2000 times faster with L than B but 1000 times faster with B than H.[51]

In most cases the diazomethyl ketones were observed to be less reactive towards the enzyme than the corresponding chloromethyl ketone.[46] This would be expected if initial hemithioketal formation at the active site were still important. The low carbonyl infra-red absorption frequency of diazomethyl ketones (typically 1615-1645 cm^{-1}) indicates the presence of conjugation with the group. Thus the carbonyl would be slightly inactivated towards nucleophilic attack. For chloromethyl ketones (carbonyl infra-red absorption frequency typically 1725-1745 cm^{-1}) one would expect the carbonyl to be activated towards nucleophilic attack due to the inductive effect of chlorine.

While this decrease in rate of inhibition is significant, the increase in specificity of these compounds for cysteine proteases more than compensates. Furthermore the reagents were observed not to alkylate non-activated thiols. For example; diazomethyl ketones were shown to be stable to high concentrations of dithiothreitol [(R,R)-1,4-dimercapto-2,3-butane diol] while chloromethyl ketones were rapidly destroyed.[46] Similarly, no reaction was observed with the cysteine residue of glutathione (γ-(L)-Glu-(L)-Cys-Gly).[50] Glyceraldehyde-3-phosphate dehydrogenase, a non-protease enzyme possessing an active site thiol, was also unable to induce any reaction further highlighting the essential nature of accurate complex formation.[50]

5.2 Limitations of Diazomethyl Ketones

The specificity of action of these species suggests that widespread application in living systems would be possible. Unfortunately the instability of diazomethyl ketones under acidic conditions prevents their *in vivo* use. This is well illustrated by the decomposition catalysed by serine proteases. Thus, in any environment where there are loosely held protons, modification of the inhibitor molecule would be expected and further interaction with the biological system a distinct possibility. This has indeed been shown to be the case with diazomethyl ketones having drastic effects on protein synthesis and also possessing mutagenic properties.[52] [8]

Chapter 6: Peptidyl Fluoromethyl Ketones

A logical step in the attempt to moderate the reactivity of chloromethyl ketones would be to replace chlorine with fluorine. This would give an inhibitor with a stronger halogen-carbon bond and result in the formation of a poorer leaving group during alkylation. In addition to this the inductive effect of fluorine should also result in a stronger hemithioacetal complex.[42]

6.1 Inhibition by Fluoromethyl Ketones

First synthesised in 1985 by Rasnick, the dipeptide fluoromethyl ketone *N*-CBZ-(L)-Phe-(DL)-AlaCH_2F was observed to be 30 times more potent in the inhibition of cathepsin B than the corresponding (L)-(L)-diazomethyl ketone (note once again that the racemisation of the alanine stereocentre was due to the early technique used for the introduction of the fluoromethyl ketone end group as will be discussed in chapter 10.1).[46] This increased potency was assigned to the tighter reversible binding of fluoromethyl ketones to the enzyme. By the theory discussed in chapter 5.1 this could have been predicted from the observed infra-red absorption frequency for the carbonyl of fluoromethyl ketones (typically 1750 cm^{-1}). One would therefore expect the initial reversible binding displayed by a fluoromethyl ketones to also be stronger than that of the corresponding chloromethyl ketone.

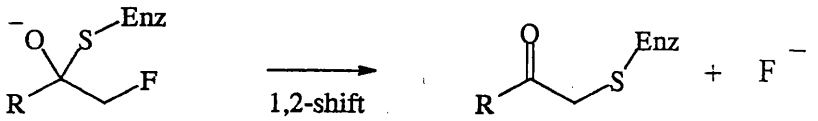
Fluoromethyl ketones are, however, less reactive than chloromethyl ketones. For example, as also noted with diazomethyl ketones, fluoromethyl ketones are unaffected by non-activated thiols such as dithiothreitol.[46] In more detail, chlorinated inhibitors were shown to be as much as 500 times more active in the alkylation of glutathione at 37°C and pH 7.4.[53]

Considering the reactivity towards protease enzymes, the work of Rauber, Angliker, Walker and Shaw suggests much scope for selective inhibition.[54] The rate of inhibition of chymotrypsin by *N*-CBZ-(L)-PheCH_2F was found to be 1/40th

of that noted for the corresponding chloromethyl ketone. The site of alkylation by the fluorinated inhibitor on this serine protease however, remained the same with the modification of the active centre histidine being cofirmed by amino acid analysis and radiolabelling experiments.

With the cysteine protease cathepsin B, the difference between chlorinated and fluorinated inhibitor activity was found to be much less pronounced. Typically the fluoromethyl ketones were observed to be about 35-40% as effective as the chlorinated forms. In this case the lower rate of alkylation displayed by the fluorinated inhibitors (usually around one quarter that of the chloromethyl ketones) was partially offset by tighter reversible binding to the enzyme.

It is considered that the mechanism of cysteine protease inactivation by fluoromethyl ketones involves two main stages as the formation of an intermediate complex has once again been noted prior to irreversible alkylation.[54] [55] The first of these stages is therefore the formation of the reversible hemithioketal. Collapse of this complex then results in a 1,2-shift of the cysteine residue with a nucleophilic displacement of fluorine (figure 6.1-1). Such a mechanism is also believed to take place during chloromethyl ketone induced inhibition (see section 4.5).



(figure 6.1-1)

This mechanism is in contrast with that involving the histidine side chain as used by the serine proteases (see section 4.2). Thus it appears that the vital difference between serine and cysteine protease action has been uncovered. It is also apparent that this difference can be exploited by appropriate selection of the inhibitor end group.

To summarise, it is now possible to distinguish between serine and cysteine proteases by the mechanism of alkylation with halomethyl ketone bearing inhibitors. It has also been found by experiment that the rate of irreversible inhibition caused by fluoromethyl ketones compared with chloromethyl ketones is far less depressed with cysteine proteases than with serine proteases. The extent of this difference is around one order of magnitude making the *in vivo* inhibition of cysteine proteases, with a minimum of side effects, a real possibility.

6.2 Fluoromethyl Ketones in Biological Systems

The ability of *N*-CBZ-(L)-Phe-(DL)-AlaCH_2F to curtail the development of acute rheumatoid arthritis in a rat model, by the inhibition of cathepsin B, has been investigated. The LD_{50} value of this compound (360 mg/kg mice) was found to be 3000 times greater than the effective dose for *in vitro* inhibition and 500 times greater than the oral dose for a similar effect *in vivo*.[7]

Thus the fluoromethyl ketones are effective and reasonably selective cysteine protease inhibitors in mammalian systems. One would therefore hope for a similar effect on parasitic organisms.

In 1990 Ashall, Angliker and Shaw reported a significant result on the application of the fluoromethyl ketone *N*-CBZ-(L)-Ala-(L)-PheCH_2F to intact trypanosomes.[56] The compound was observed to have a lethal effect that was limited to the infectious phase of the parasitic growth cycle. The use of a radiolabelled derivative also indicated that this effect corresponded to the labelling of a protein which was therefore deduced as being an essential protease. Taking this concept one step further, the administration of small doses (1-2 mg per day) of a dipeptide fluoromethyl ketone has been shown to be sufficient to protect mice from a lethal infection of *Trypanosoma cruzi*.[16]

The isolation and crystallisation of the main cysteine protease (cruzain) of *Trypanosoma cruzi* has been discussed in a review by M^{c}Kerrow, M^{c}Grath and

Engel.[16] X-Ray analysis of this enzyme after inhibition by *N*-CBZ-(L)-Phe-(L)-AlaCH_2F confirmed that the site of attachment of the irreversible inhibitor was indeed the active site cysteine. Also, as illustrated earlier, the *N*-terminus protecting group of such compounds was observed to make no contact with the protein and in fact protrudes from the active site. Derivatisation of this group should therefore have little effect on the binding properties of the inhibitor.

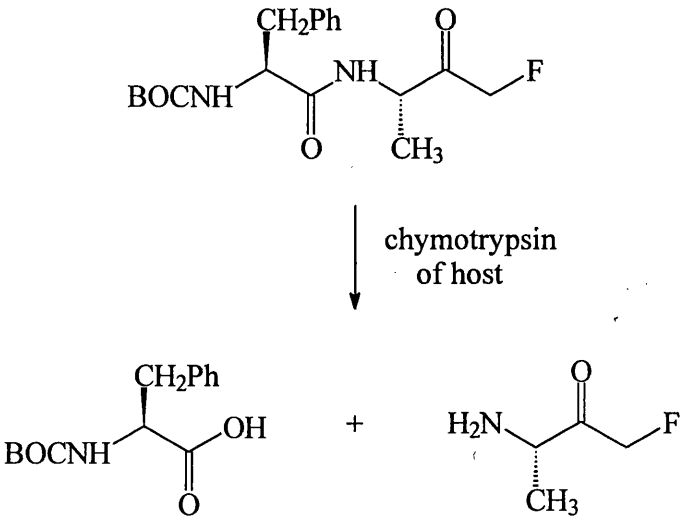
Plasmodium falciparum trophozoite cysteine protease (TCP) has been implicated as being involved in the degradation of host haemoglobin by intraerythrocytic malaria parasites. By the use of a panel of peptidyl fluoromethyl ketones, it was possible to show that the effectiveness of inhibiting this enzyme correlated with the effectiveness at blocking haemoglobin degradation and killing cultured parasites.[12] In this system *N*-CBZ-(L)-Phe-(DL)-ArgCH_2F proved to be the most potent. In picomolar concentrations this compound proved capable of inhibiting TCP while at nanomolar concentrations the blocking of haemoglobin degradation and death of the parasite resulted. Micromolar concentrations were also shown to be non toxic to cultured mammalian cells.

The fine tuning of these compounds for antiparasite action in the presence of mammalian cysteine proteases, by informed choice of amino acid sequence, is also a distinct possibility. It has been reported that cathepsin L is 10 times more susceptible to an inhibitor containing the *N*-CBZ-(L)-Phe-(L)-Ala sequence than *N*-CBZ-(L)-Leu-(L)-Tyr.[11] On the other hand, fluoromethyl ketones bearing the former sequence are 50 times more effective at inhibiting the intraerythrocytic development of *Plasmodium falciparum*.

6.3 The Unsuitability of Peptidyl Fluoromethyl Ketones as Drugs

It appears feasible that *N*-protected peptides, bearing a suitable amino acid sequence and the fluoromethyl ketone end group, can be developed which selectively disrupt the biochemical processes of parasites. It is also possible that this effect can be achieved with only a limited effect on the host organism.

However, one serious problem remains: protease inhibitors derived from peptides are themselves vulnerable to the actions of proteolytic enzymes. These enzymes may be the proteases of the host or indeed those of the parasite itself. As a result, not only will the rapid destruction of the inhibitor be observed, but an increase in side effects will be observed due to the presence of fluorinated amino acid derivatives. This is illustrated in figure 6.3-1 for the chymotrypsin catalysed cleavage of *N*-BOC-(L)-Phe-(L)-AlaCH_2F.

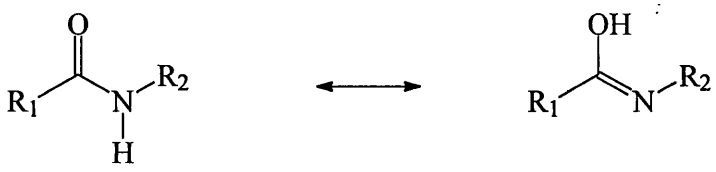


(figure 6.3-1)

In order to prevent this it may be possible to replace the vulnerable amide link with a non-hydrolysable alternative. Such a linkage, while being immune to the attentions of proteolytic enzymes, must mimic the appearance of the amide in order to allow the compound to perform its designated role.

Chapter 7: The Concept of the (*E*)-Ethene Isostere

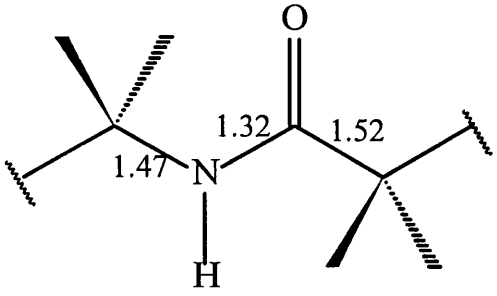
X-Ray studies of peptides and proteins indicate that the peptide link is in fact rigid and planar.[57] There is no free rotation around the amide bond joining the two amino acid residues. This is due to the significant double bond character of this linkage (around 50%) resulting from amide-iminol tautomerism (figure 7-1).



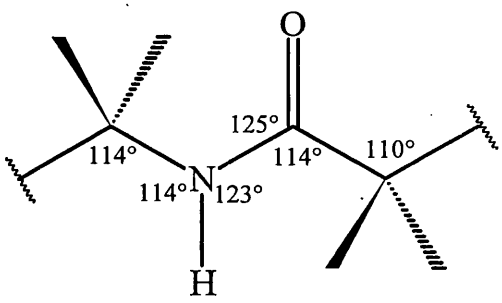
Amide-Iminol Tautomerism

(figure 7-1)

As a result of this double bond character the observed C-N bond length lies between that expected for single and double bonds.[58] [59] Furthermore the bond angles around nitrogen have values close to those expected for a purely trigonal system (figure 7-2).



Main Chain Bond Lengths (Angstroms

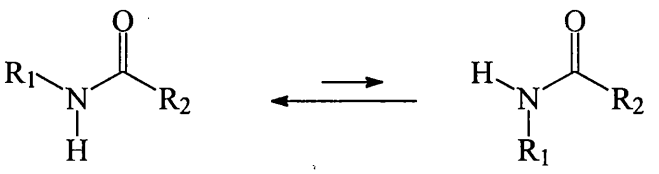


Selected Bond Angles

(figure 7-2)

Note however that the single bonds between the components of the amide link and the α-carbons of the joined amino acids, are of standard length. A significant amount of conformational freedom therefore exists on either side of this rigid link.

An important result of the partial double bond nature of the amide link is that only two conformations are generally observed (figure 7-3).



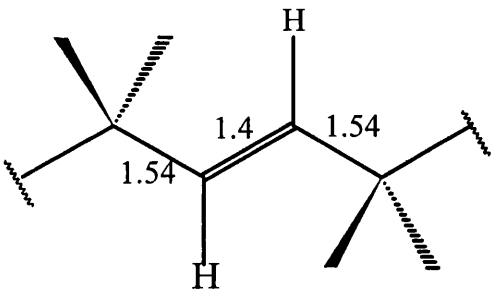
trans *cis*

(where R_1 and R_2 represent the continuation of the peptide chain

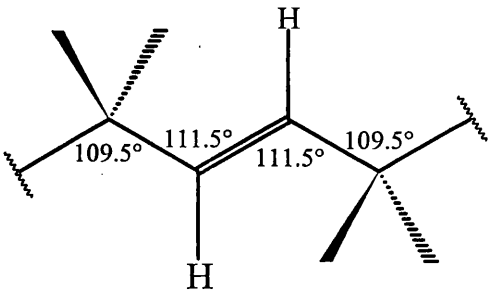
(figure 7-3)

In most systems the amide link is found to adopt the more thermodynamically stable *trans* geometry.

Considering these factors as a whole, it is clear to see the viability of using the *trans*-ethene group as a structural replacement for the amide function (figure 7-4).[58] [59]



Main Chain Bond Lengths (Angstroms



Selected Bond Angles

(figure 7-4)

Such a group is isosteric with the amide linkage and also possesses the desired rigidity and geometry while being immune from enzymic hydrolysis.[60]

Chapter 8: The History and Development of (*E*)-Ethene Isostere Synthesis

Early attempts at (*E*)-ethene incorporation into biologically active molecules were focused on the synthesis of more stable forms of the enkephalins (figure 8-1). These naturally occuring peptides and their analogues display agonist activity at opiate receptors.

(L)-Tyr-Gly-Gly-(L)-Phe-(L)-Met
Methionine enkephalin

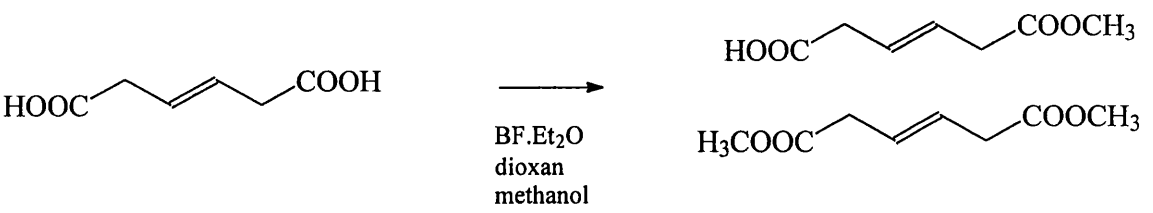
(L)-Tyr-Gly-Gly-(L)-Phe-(L)-Leu
Leucine enkephalin

(figure 8-1)

However, these compounds have a very short half life *in vivo* presumably due to enzymic hydrolysis.[61] In theory, the replacement of one or more amide links may increase the resilience of the compound. An added bonus of amide bond replacement would also be an increase in lipophilicity thus facilitating passage through the blood/brain barrier.

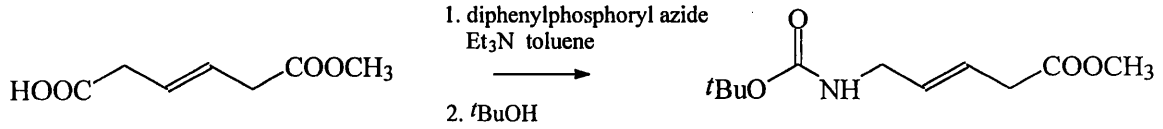
8.1 The Cox Approach

The synthetic approach adopted by Cox and colleages was basically composed of a series of functional group manipulations and α-alkylations of *trans*-hex-3-enedioic acid.[62] Esterification of the diacid produced a mixture of mono and diester (figure 8.1-1), both of which were utilised to produce a range of isosteres.



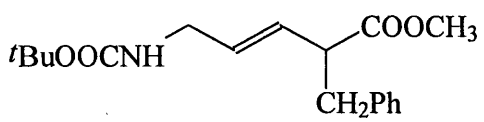
(figure 8.1-1)

The acid group of the monoesterified material was converted into the *tert*-butoxycarbonyl protected amine *via* a modified Curtius reaction (figure 8.1-2).[63]



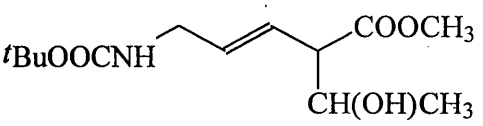
(figure 8.1-2)

Treating this compound with lithium diisopropylamide followed by benzyl bromide produced the protected, racemic Gly-Phe (*E*)-ethene isostere (figure 8.1-3a). Similarly, replacing benzyl bromide with acetaldehyde allowed the isolation of the Gly-Thr variant (figure 8.1-3b).



protected Gly-Phe isostere

(a)



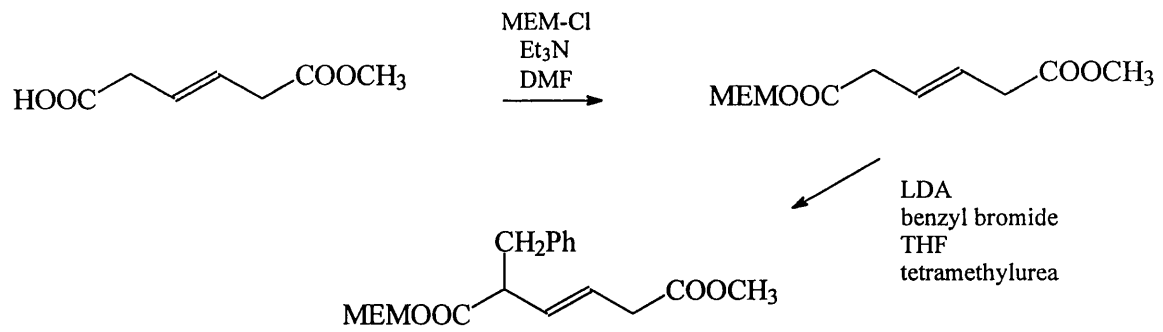
protected Gly-Thr isostere

(b)

(figure 8.1-3)

It is worthy of note that with these alkylations the βγ-unsaturated products, and not their structural isomers containing an αβ-unsaturation, were isolated when the reaction temperature was maintained below -50°C.

Using the same monoester starting material, a similar route was employed to effect α-alkylation to what would become the *N*-terminus. In this case the methoxyethoxymethyl (MEM) ester was employed to induce regioselective α-alkylation, presumably due to internal lithium chelation (figure 8.1-4).

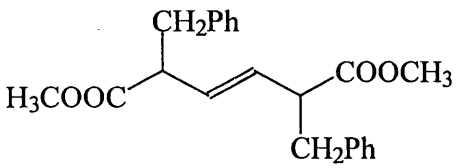


(figure 8.1-4)

Selective removal of the MEM ester, using titanium tetrachloride, followed by the modified Curtius procedure gave the protected, racemic Phe-Gly isostere. With

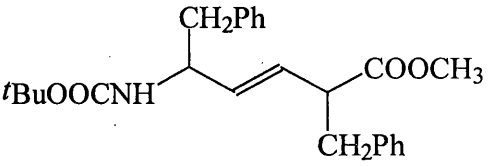
a little additional protecting group manipulation the same synthetic route was extended to allow preparation of the tyrosine-glycine analogue. In this case *para-tert*-butoxybenzyl bromide was used in the alkylation step.

Carrying out dialkylation on the diester from figure 8.1-1 with benzyl bromide resulted in the production of the 2,5-dialkylated compound (figure 8.1-5a). Partial ester hydrolysis, using iodotrimethylsilane, on this compound followed by the modified Curtius procedure gave the protected Phe-Phe isostere (figure 8.1-5b) as two pairs of enantiomers.



dialkylation product

(a)



protected Phe-Phe isostere

(b)

(figure 8.1-5)

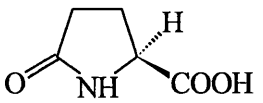
Obviosly the routes described here bear no consideration to stereochemistry. They do, however, allow the rapid synthesis of a wide range of isostere analogues.

8.2 Biological Activity of Isostere Containing Compounds

The Gly-Gly and racemic Tyr-Gly isosteres were separately incorporated into varients of the enkephalin analogue (L)-Tyr-Gly-Gly-(L)-Phe-(L)-Pro-NH_2.[64] In the case of the Gly-Gly isostere, the resulting pseudopeptide exhibited just 0.1% of the activity of the parent pentapeptide. Better results were achieved with the Tyr-Gly isostere where elaboration of this material gave a pseudopeptide which showed up to three times the activity of the parent compound on the tissues tested despite the inclusion of a racemic centre.

Using solid phase techniques, the Phe-Gly and Phe-Phe isosteres were elaborated into mimics of the peptide pyroGlu-(L)-Phe-(L)-Phe-Gly-(L)-Leu-(L)-

Met-NH_2. The use of pyroglutamic acid (figure 8.2-1) as an *N*-terminus blocking group in this peptide is also worthy of note.



pyroglutamic acid

(figure 8.2-1)

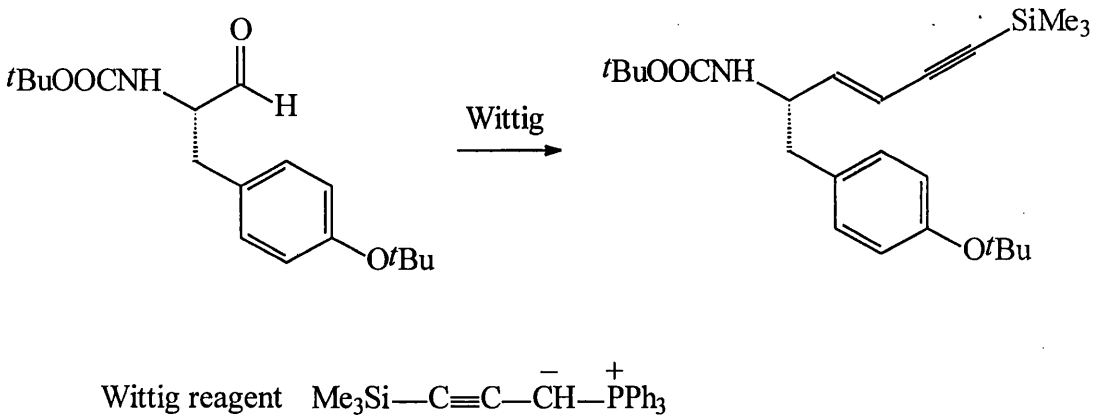
These derivatives possessed 24% and 100% activity respectively when compared to the parent peptide. It is also worthy of note that for the Phe-Phe isostere containing compound (displaying 100% activity of the parent peptide) the corresponding saturated analogues exhibited much lower activity (0.35% for the SS/RR enantiomeric mixture and 40% for SR/RS).

Thus, it is apparent that the Gly-Gly amide link of enkepalin analogues has some important biological role. This bond may, for example, induce a specific conformation on the rest of the molecule by intramolecular hydrogen bonding or may be instrumental in binding to the receptor. The Tyr-Gly amide bond, on the other hand, appears non-essential but the maintenance of amide geometry enhances activity. The results obtained from this study however, remain ambiguous due to the use of peptides bearing mimics of the non-natural (D)-amino acids.

8.3 The Sammes Approach

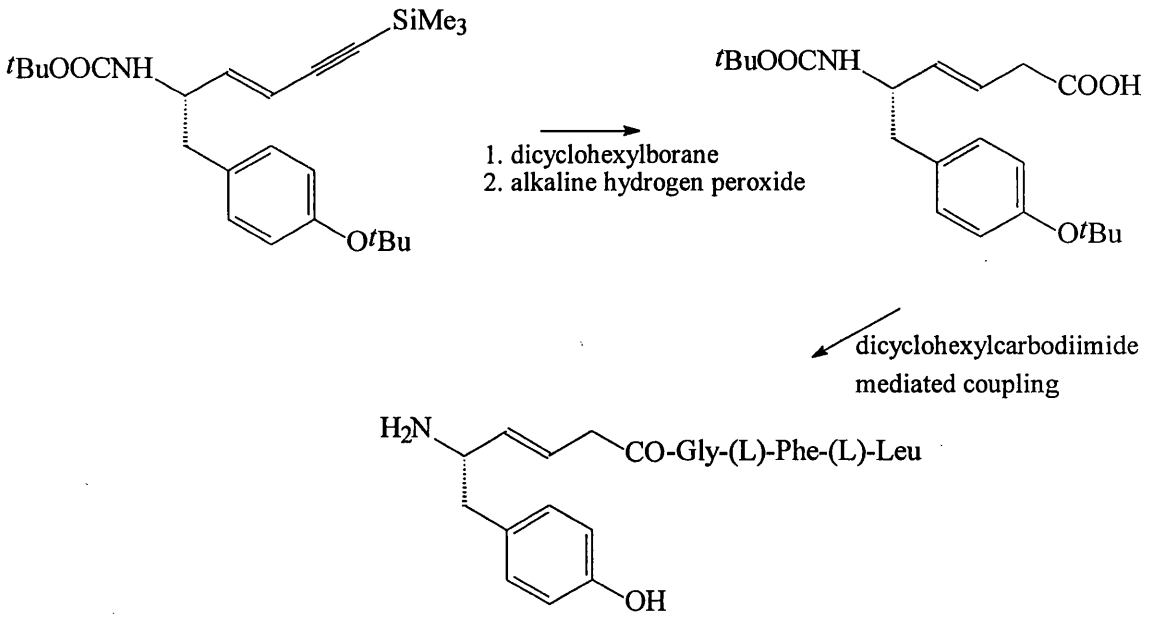
The initial step in the breakdown of the enkephalins was identified as enzymic hydrolysis of the Tyr-Gly amide link.[61] The product of this hydrolysis appeared to be far more resilient than the parent compound but without the biological activity. It was therefore considered that replacement of just the Tyr-Gly bond would result in a longer active lifetime *in vivo*. The initial target of Sammes and co-workers was therefore the Tyr-Gly isostere of leucine enkephalin [(L)-Tyr-Gly-Gly-(L)-Phe-(L)-Leu].[58] [59]

In this synthesis the stereochemistry at the *N*-terminus (tyrosine mimic) was defined by the use of natural (L)-tyrosine as a starting material. The synthesis then centred on the application of Wittig methodology. A stabilised ylide, synthesised from prop-2-yn-1-ol, was condensed with the aldehyde obtained from diisobutylaluminium hydride reduction of *O*,*N*-protected (L)-tyrosine methyl ester (figure 8.3-1).[65]



(figure 8.3-1)

Hydroboration of the *trans*-enyne thus produced followed by oxidation yielded the desired βγ-unsaturated acid. This compound was coupled with the short peptide Gly-(L)-Phe-(L)-Leu-OMe then deprotected to give the leucine-enkephalin analogue with totally defined stereochemistry (figure 8.3-2).



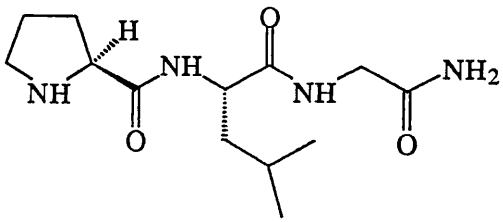
leucine enkephalin containing the Tyr-Gly isostere

(figure 8.3-2)

This compound was observed to have an activity virtually identical to the natural pentapeptide. It was also noted that the isostere containing material remained effective on prolonged incubation under conditions which would induce the rapid proteolysis of the parent peptide.

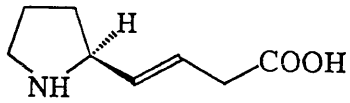
Thus, once again we can conclude that the Tyr-Gly amide link is non-essential in this system and functions merely as a steric spacer. The *trans* disposition is, however, vital as the fully reduced hydrocarbon analogue showed negligible opiate receptor binding. Attempts to synthesise the corresponding *cis*-βγ-unsaturated variant for comparative testing proved fruitless due to low stability. With this compound rearrangement to the more stable *trans*-αβ-unsaturated isomer was noted during an attempted ester hydrolysis.

Following the early success, attempts were made to expand the methodology to allow the introduction of a second substituent by direct alkylation.[66] The target chosen for study was Melanocyte Stimulating Hormone Release Inhibiting Factor. (MIF) This peptide (figure 8.3-3a) is rapidly inactivated in biological systems by enzymic hydrolysis of the Pro-Leu amide link.



MIF (L)-Pro-(L)-Leu-Gly-NH_2

(a)



Pro-Gly isostere

(b)

(figure 8.3-3)

Firstly, using a sequence similar to that previously described, the *N*-protected Pro-Gly isostere (figure 8.3-3b) was synthesised from (L)-proline.

The work of Rathke and Sullivan indicated that lithium enolates of αβ-unsaturated esters react by alkylation at the α position giving βγ-unsaturated products.[67] It was therefore suggested that α-alkylation of the Pro-Gly isostere methyl ester would occur without the double bond moving into conjugation. As expected, treating this compound with lithium diisopropylamide and isobutyl iodide (for the Pro-Leu isostere) or benzyl bromide (for the Pro-Phe isostere) resulted in α-alkylation in reasonable yield.

Unfortunately the diastereomers produced could not be separated by standard chromatographic methods. A degradative procedure was therefore employed to investigate the stereochemistry of this alkylation and results showed that while the stereochemical integrity of proline was retained, no chiral induction had occurred at the site of alkylation. Similar results were observed in the attempted synthesis of the (D)-Ala-(D)-Ala isostere.

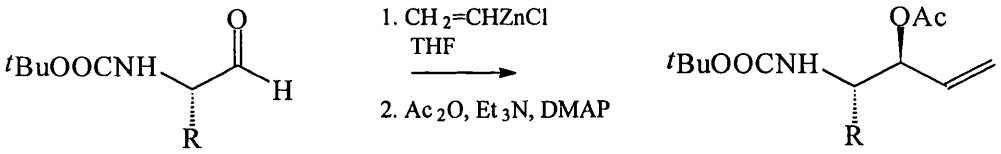
Therefore by the use of chiral amino acids as starting materials, the Sammes method allows the stereochemistry at only one position to be defined. The chiral nature of the starting material is not sufficient to induce further stereoselectivity during alkylation.

A very similar route was employed by Johnson in order to incorporate Leu-Gly (*E*)-ethene isosteres into substrate-like renin inhibitors.[68] In this case, where the chirality of the leucine mimic was defined by the choice of (L)-leucine as a starting material, the isosteres were shown to have an inhibitory activity very similar to the parent peptides. It was therefore proposed that the amide link replaced held a *trans* conformation while bound to the active site of the enzyme.

8.4 Thompson Methodology

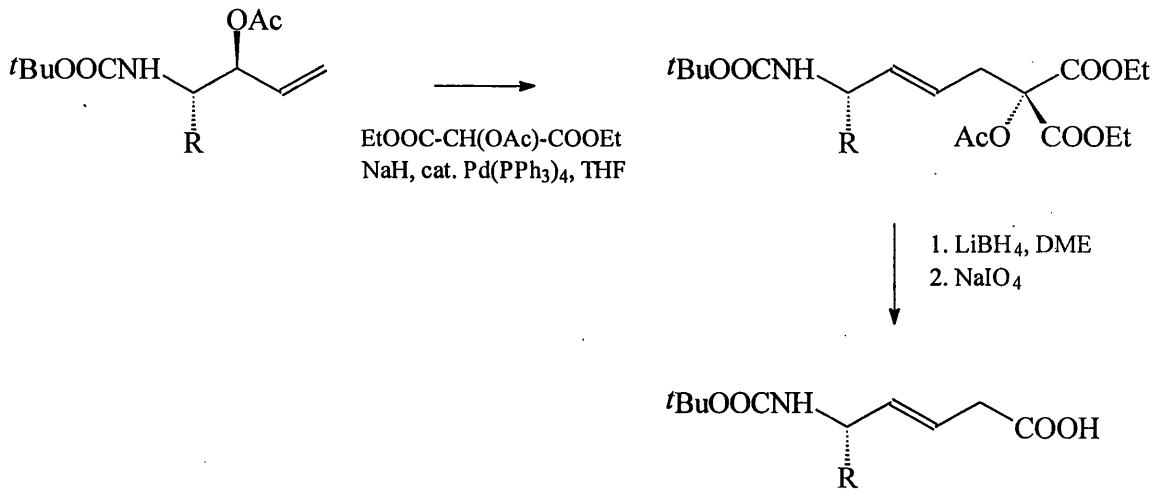
A more direct approach to (*E*)-ethene isosteres was reported by Thompson and co-workers.[69] This route involved the application of the Trost alkylation to allylic acetates.

The desired allylic acetates were produced in good yield by the reaction of the corresponding amino aldehyde with vinylzinc chloride followed by acetylation of the resulting alcohol (figure 8.4-1). The diastereoselectivity for the *threo* product in such systems (typically 8:1 *threo:erythro*) is worthy of note.



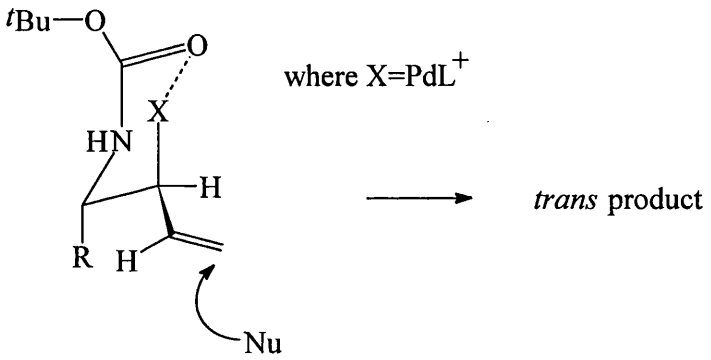
(figure 8.4-1)

Palladium catalysed coupling (Trost alkylation) of these compounds was then carried out with acetylated diethyl tartronate and the resulting 1,3-diesters further reacted in order to yield the Xxx-Gly isosteres (figure 8.4-2).[70]



(figure 8.4-2)

This method therefore provides a more rapid route to chiral dipeptide ethene isosteres but is limited to the incorporation of a glycine mimic at the carboxyl terminus. The achievement of the *trans* double bond geometry (typically 99:1 *trans:cis*) in this case can be explained by the participation of the BOC group in the allylic acetate/palladium complex. It is thought that, due to the involvement of this group, the complex holds a chair-like conformation. In the more stable of these, of which an example is shown in figure 8.4-3, nucleophilic attack on the complex would result in the observed *trans* product.

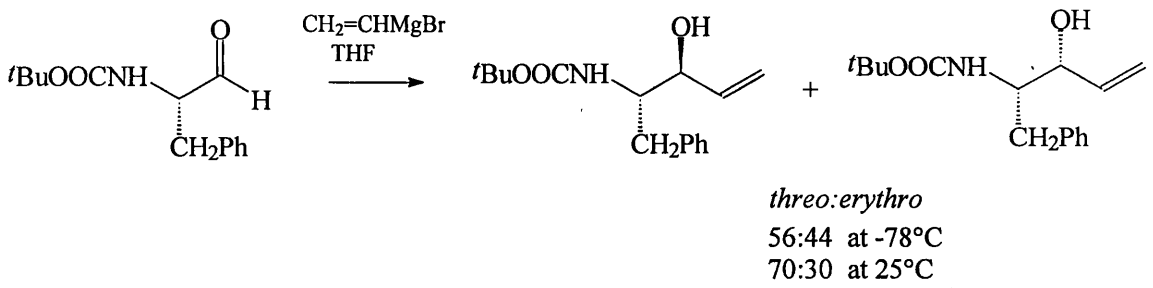


(figure 8.4-3)

8.5 The Hanson Synthesis

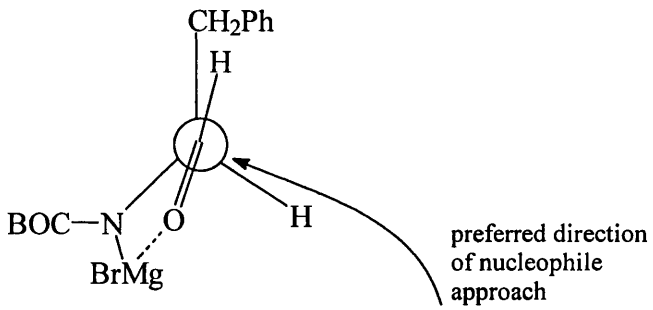
The reaction between vinylzinc chloride and protected amino aldehydes in the Thompson method illustrated selectivity for the formation of the *threo* product. Earlier work by Hanson *et al* showed that this selectivity was not as pronounced

with vinylmagnesium bromide (figure 8.5-1).[71] It was also shown that the proportion of *threo* product obtained could be increased by carrying out the reaction at a higher temperature.



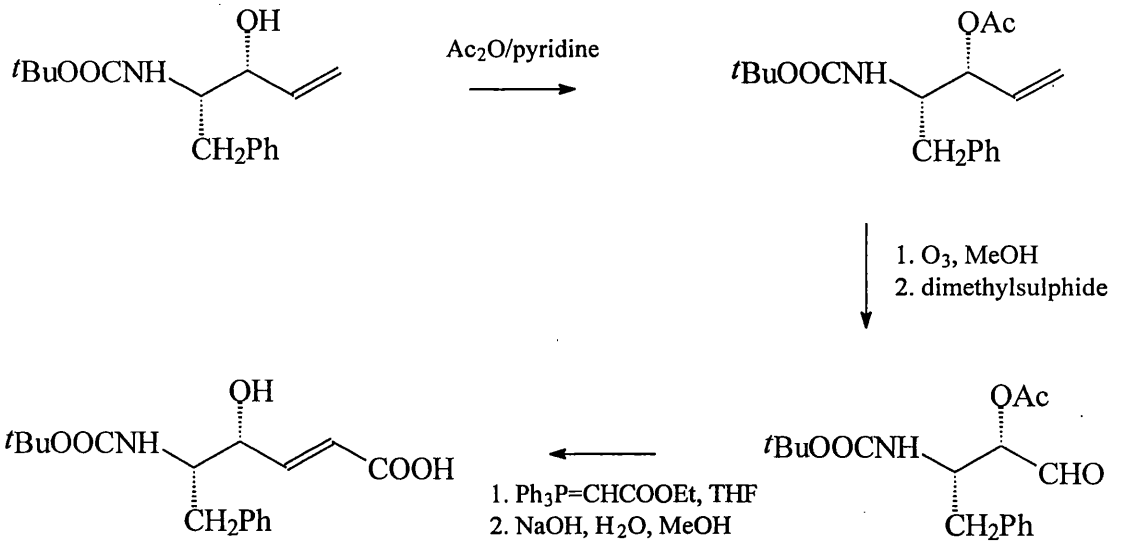
(figure 8.5-1)

It was suggested that, once again, the *N*-terminus plays a role in the reaction mechanism. Complexation of magnesium bromide between the aldehyde oxygen and carbamate nitrogen enforces a conformation which favours the formation of the *threo* product (figure 8.5-2). At higher temperatures it was assumed that a greater proportion of the carbamate groups would be deprotonated, thus allowing the formation of this complex and giving higher *threo* selectivity.



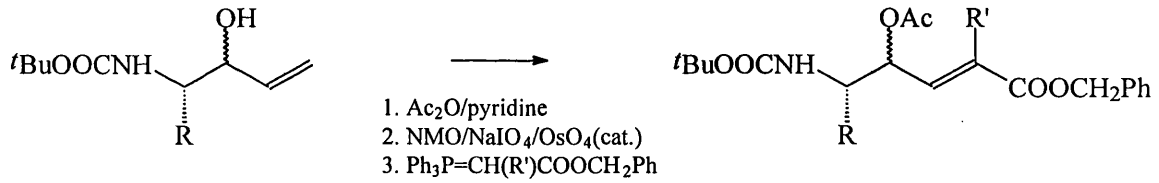
(figure 8.5-2)

The mixture of diastereomers produced by this reaction was separated by HPLC and individually elaborated as illustrated in figure 8.5-3 for the minor diastereomer.



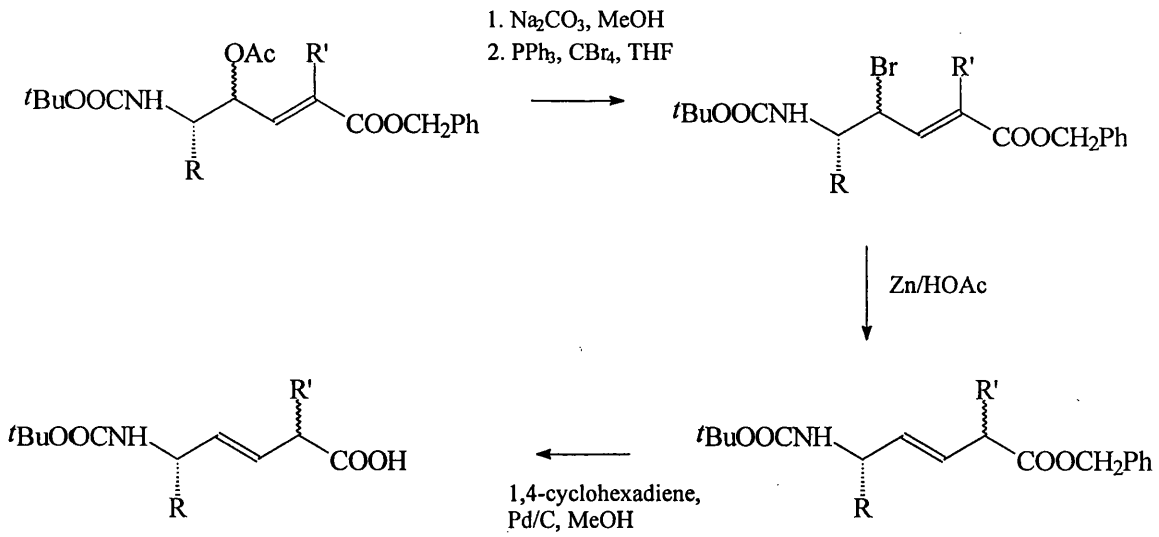
(figure 8.5-3)

A route very similar to this was employed by Shue and co-workers to synthesise a range of dipeptide isosteres.[72] [73] In this case the mixture of diastereomeric allylic alcohols, produced by the reaction of vinylmagnesium bromide on the amino aldehyde, was used directly without separation. Acetylation, followed by an analogous process of olefin cleavage, produced an aldehyde which was condensed with an alternative Wittig reagent (figure 8.5-4).



(figure 8.5-4)

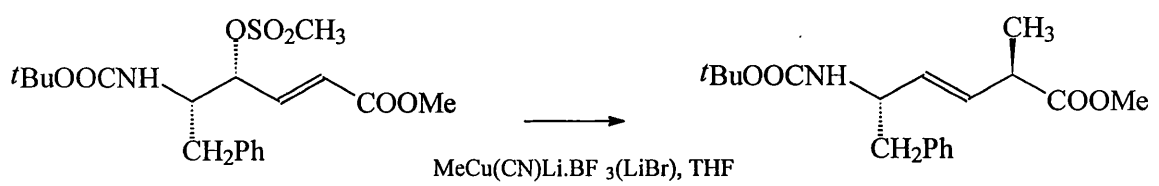
Careful methanolysis of the acetyl protecting group, followed by mild bromination yielded the corresponding allylic bromide. Treating this material with zinc metal in acetic acid effected a double bond shift giving the protected (*E*)-ethene isostere as a pair of diastereomers (figure 8.5-5). Separation of these diastereomers at this stage, or as the free acids produced on mild hydrogenation, was found not to be possible.



(figure 8.5-5)

A number of isosteres were synthesised bearing various side chains (R and R'). As can be seen however, the problem of undefined stereochemistry at R' still exists. Since difficulty was experienced in the separation of these diastereomers, a further stereoselective stage needs to be incorporated into the synthesis.

The stereochemical problem inherent in this route was finally overcome by the application of methodology developed by Ibuka, Fujii, Yamamoto and colleagues.[74] The enantiomerically pure γ-hydroxy-αβ-unsaturated carboxylic acid, produced by the Hanson route, was esterified and converted into the γ-mesyloxy variant. Treating this material with $MeCu(CN)Li.BF_3(LiBr)$, produced by the sequential addition of MeLi/LiBr (Et_2O) and $BF_3.Et_2O$ to a THF slurry of CuCN, resulted in alkylation with clean 1,3-chirality transfer (figure 8.5-6).

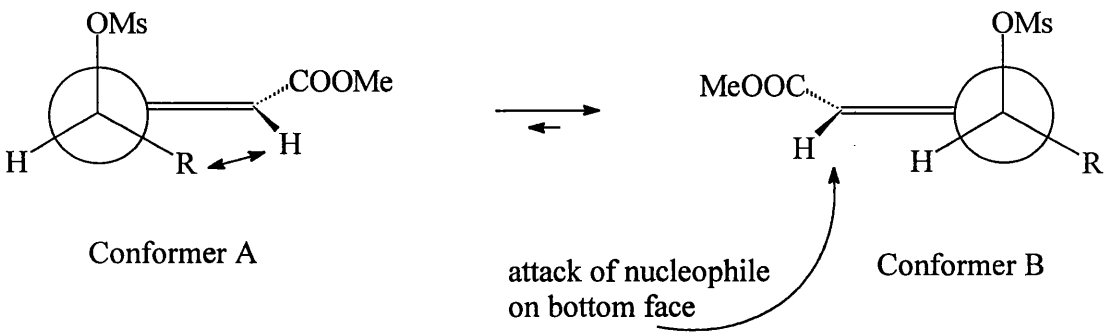


(figure 8.5-6)

The observed olefinic product was found to be exclusively of the (*E*) geometry. The level of diastereoselectivity for the desired product, achieved in this

alkylation, was also extremely high. In this case reaction yields were of the order of 98% with a diastereoselectivity greater than 99:1.

While the controlling factors in this reaction remain unclear, an explanation for the observed (*E*) product has been postulated.

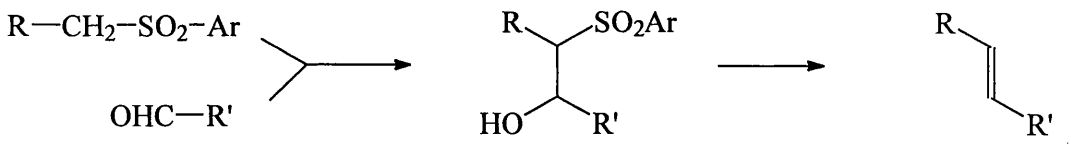


(figure 8.5-7)

Conformer A (figure 8.5-7), which would lead to the (*Z*) product, is destabilised by the steric interaction between the bulky R group (CH_2Ph in this case) and the olefinic hydrogen. Conformer B is therefore more favourable with nucleophilic attack resulting in the observed (*E*) product geometry. Thus displacement of the mesylate leaving group only takes place when it and the attacking nucleophile are in a mutually antiperiplanar disposition. The reaction can therefore be described as proceeding by an *anti*-S_N2' mechanism.

8.6 The Hopkins Organosulfur Route

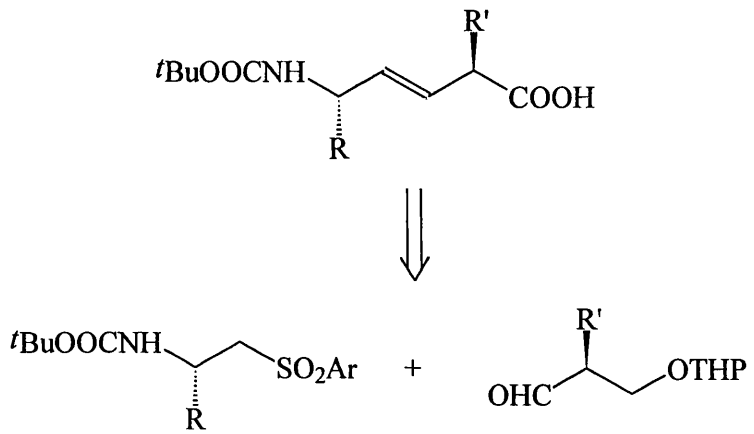
Stereocontrol at both chiral centres and *trans* double bond geometry was also achieved by Hopkins and colleagues by a convergent route incorporating the Julia Olefin Synthesis (figure 8.6-1).[75] [76] [77]



Julia Olefin Synthesis

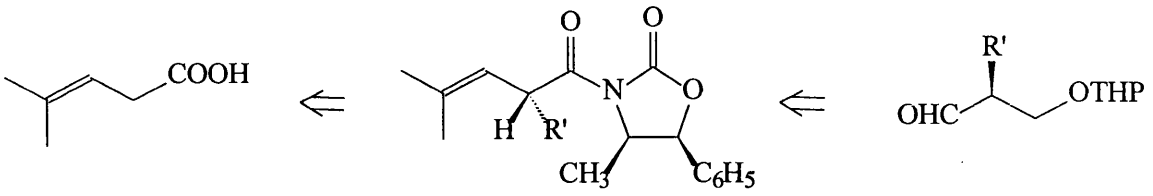
(figure 8.6-1)

In the previously described synthetic routes, chiral amino aldehydes were used in order to define the stereochemistry at the *N*-terminal side chain in the product peptide mimic. Unfortunately, in this application, the coupling of these compounds with sulfones provided only traces of the desired olefinic product. Better results were however achieved with the sulfone as the amino acid derived species (figure 8.6-2).



(figure 8.6-2)

In general, the aldehydes required for coupling to these sulfones were synthesised from 4-methylpent-3-enoic acid. Evans' method for asymmetric alkylation was employed in this sequence to introduce the R' groups with defined chirality (figure 8.6-3). These reactions will be discussed in more detail in later chapters.



(figure 8.6-3)

Thus the synthesis of dipeptide isosteres with fully defined chirality is possible. However, the application of such methodology to peptidyl cysteine protease inhibitors has not yet been reported.

Chapter 9: Aims and Objectives

Literature synthetic routes to dipeptide (*E*)-ethene isosteres with fully defined stereochemistry are available as illustrated above. Such a stereoselective synthesis may be essential as the adequate separation of dipeptide isosteres may be difficult to achieve (see section 8.5). In order to prove the concept of the isostere with regard to anti-parasitic activity, it was decided to synthesise a series of such compounds, with defined chirality, bearing the fluoromethyl ketone end group.

The compounds produced in the first round of synthetic work would be tested for activity against an initial battery of parasitic species. From the results of this study, clues as to the ideal selection of side chains for effective inhibition of each species would hopefully be obtained. Promising compounds could then be employed in more extensive testing incuding investigations into the comparative *in vivo* stability of active isosteres and the corresponding amides.

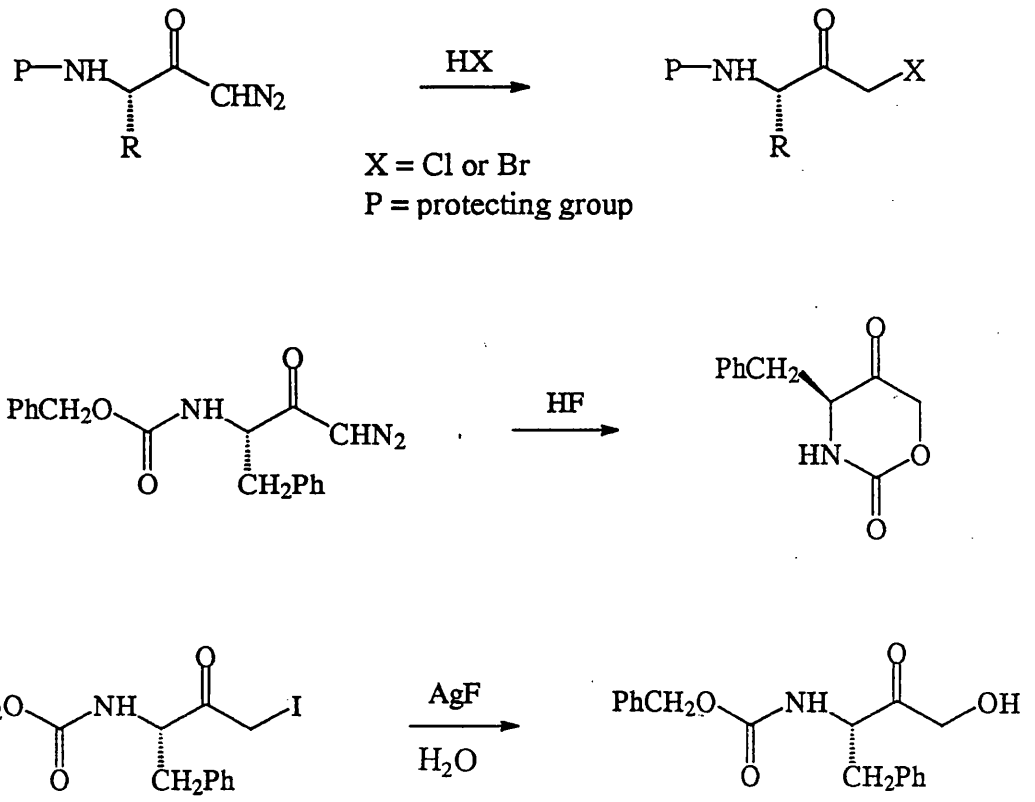
Results and Discussion

Chapter 10: Fluoromethyl Ketone End Group Investigations

Classically, the fluoromethyl ketone end group has been formed by the direct conversion of *N*-protected amino acids or peptides. Methodology therefore exists for the synthesis of this group from carboxylic acids and its application to completed (*E*)-ethene isosteres should be possible. Prior complete formation of isosteres may be essential as in the viable routes to these compounds selected for further study, generation of the carboxylic acid terminus occurs late in the synthetic sequence. It was therefore considered wise to test the potential end group elaboration method as it would eventually be employed in the closing stages of non-trivial syntheses.

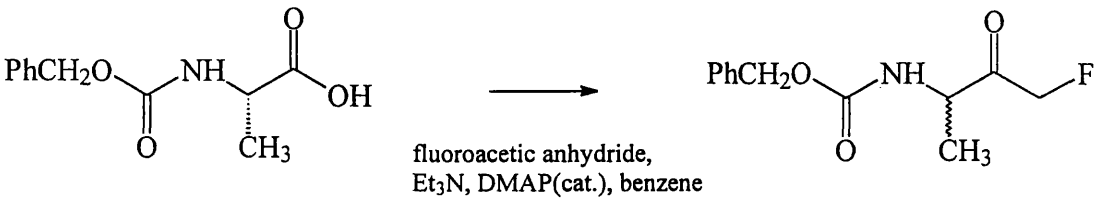
10.1 Literature Fluoromethyl Ketone Syntheses

Traditional methodology for the formation of *N*-protected peptidyl halomethyl ketones involved reacting the corresponding diazomethyl ketone with hydrogen halides.[78] While being effective for chloromethyl and bromomethyl ketone formation, the route fails for the insertion of fluorine (figure 10.1-1). Upon treatment of *N*-CBZ-(L)-PheCHN$_2$ with hydrofluoric acid, a cyclised product is produced. This intramolecular cyclisation reaction dominates in this situation due to the lower nucleophilicity of fluoride. This factor also explains the isolation of hydroxymethyl ketones rather than the fluorinated product during attempted halogen exchange (note that in the failed fluorination cases reported in the literature, product stereochemistry was not considered and is therefore assumed to be unaffected by the reagents). In contrast, iodomethyl ketones are readily prepared from chloromethyl ketones by halogen exchange using sodium iodide in acetone.



(figure 10.1-1)

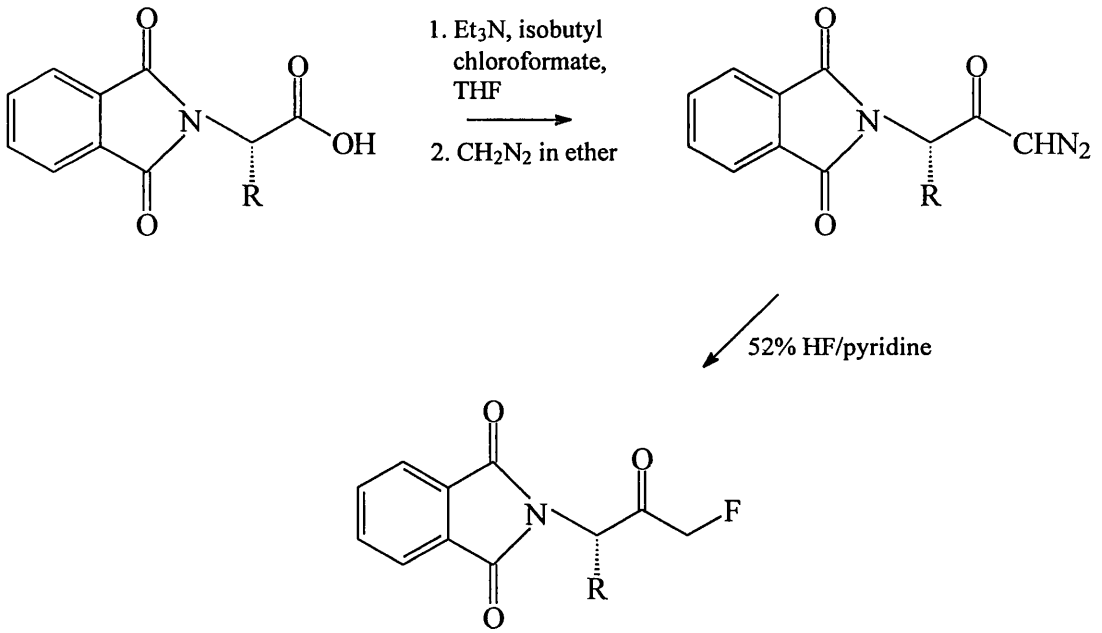
This problem of C-F bond formation was indirectly overcome by the use of a modified Dakin-West reaction (figure 10.1-2).[79] In this reaction the entire carboxyl group of the amino acid is replaced thus forming a new C-C bond.



(figure 10.1-2)

The synthetic utility of this reaction is limited as racemisation is inherent, yields are generally poor and purification is tedious due to the large number of by-products formed. Despite this, much early work has been carried out on fluoromethyl ketones formed by this method (see sections 4.4, 6.1 and 6.2).[46][80][81]

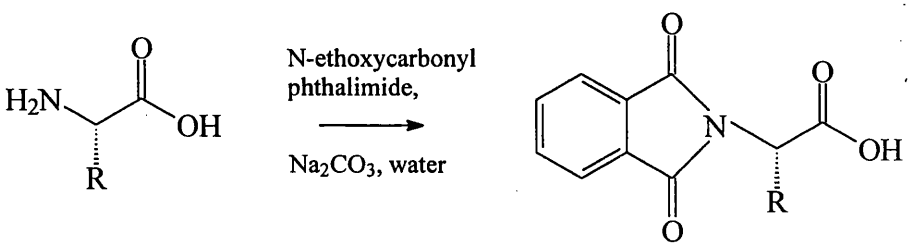
The direct, racemisation free conversion of peptidyl diazomethyl ketones to fluoromethyl ketones was achieved by Shaw and colleagues using HF/pyridine, the problem of the competing cyclisation reaction being solved in this case by the use of the *N*-phthaloyl protecting group (figure 10.1-3).[54] It was this synthetic sequence that was selected for further study with the aim of being incorporated into the synthesis of *N*-protected dipeptide isostere fluoromethyl ketones. However, scope for improvement of this method existed as the yields reported for the fluorination step were both low and variable. For example, from *N*-phthaloyl-(L)-phenylalanyl diazomethyl ketone (R = $PhCH_2$) a 33% yield was reported while with *N*-phthaloyl-(L)-alanyl diazomethyl ketone (R=CH_3) only 5% of the fluorinated product was obtained.



(figure 10.1-3)

10.2 Experimental Investigations into the Shaw Route

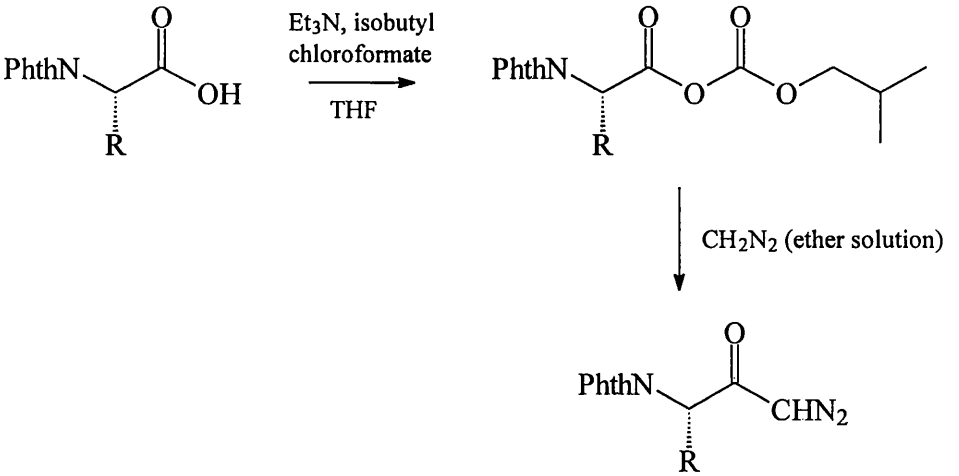
N-Phthaloyl protected amino acids were formed according to the method of Nefkens, Tesser and Nivard.[82] (L)-Phenylalanine, (L)-alanine and glycine were protected in consistently good yield (>70%) and high purity (figure 10.2-1).



R = CH_2Ph, CH_3 or H

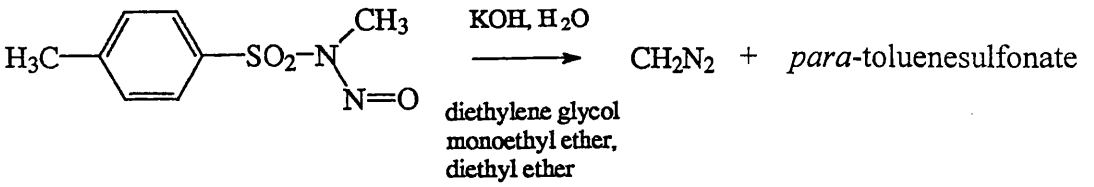
(figure 10.2-1)

Treatment of these compounds with triethylamine and isobutyl chloroformate, in dry THF at -20°C, resulted in the formation of the corresponding mixed carbonate (figure 10.2-2). In such a system, the carbonyl originating from the carboxylic acid is activated towards nucleophilic attack. The carbonate carbonyl is less receptive in comparison, due to the presence of flanking oxygen atoms. Thus, on the addition of diazomethane, formation of a diazomethyl ketone occurs accompanied by fragmentation of the mixed carbonate.



(figure 10.2-2)

The ethereal solution of diazomethane required for this step was prepared by alkali induced decomposition of Diazald® (*N*-methyl-*N*-nitroso-*p*-toluenesulfonamide) using the procedure defined by Hudlicky (figure 10.2-3).[83] Quantities of diazomethane between 0.06 and 0.3 moles were produced and reacted without any problems as the standard precautions for the safe handling of this material were observed throughout its generation, storage and use.



(figure 10.2-3)

The ethereal diazomethane solution was prepared regularly and, preferably, used while fresh. In this situation the 90% Diazald® to diazomethane conversion, as achieved by Hudlicky, could be assumed. Storage at under 5°C for short periods of time (2-3 weeks) was found to be possible as long as the possible reduction in concentration, by decomposition, was accounted for by the use of a greater volume. Obviously some degree of inaccuracy results from these estimations but the intended use of at least a three to one molar ratio of diazomethane to carboxylic acid, in diazomethyl ketone synthesis, should partially allow for this. By taking this factor into account, variable product yields due to diazomethane concentration inaccuracy and decomposition were not observed. A method for the determination of diazomethane solution concentration, by the back titration of excess benzoic acid in dry THF, was in fact tested but considered to be superfluous and impractical for regular use.[84]

Thus, the addition of a three fold excess of diazomethane to the mixed carbonate of *N*-phthaloyl-(L)-phenylalanine allowed the reliable production of the desired diazomethyl ketone, with purity being attained by a simple recrystallisation process. Similarly, application of the procedure to *N*-phthaloyl-(L)-alanine gave the diazomethyl ketone containing product as a yellow oil. Solidification and recrystallisation of this compound was not achieved by the original authors but was found to be possible here by storage of the material at 0°C. During this reaction, for simplicity and to test the resilience of the method, far greater than required quantities of reagents were used. No significant ill effects were noted but racemisation was however, unchecked for. *N*-Phthaloylglycyl diazomethyl ketone, not discussed by the authors of the original method, was also synthesised in crystalline form.

10.3 Characteristic Spectroscopic Details of Diazomethyl Ketones

As a significant amount of later work concentrated on the formation of more complex diazomethyl ketones, the spectroscopic details of these simple model compounds are worthy of discussion.

The most striking feature of the infra-red spectra of these compounds is an extremely strong and sharp peak in the normally clear area around 2100 cm^{-1}. This absorption is due to the C=N=N grouping of the diazomethyl ketone and as a result can be used to identify the presence of such compounds in mixtures or crude products.

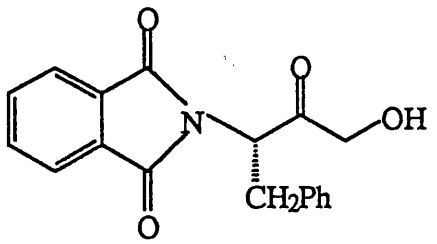
The single hydrogen of the diazomethyl ketone group appears as a broad signal, typically between 5 and 5.5 ppm, in the ^1H NMR spectrum of these compounds in deuteriochloroform. Since such a peak can be easily mistaken for a carbamate NH or partially obscured by components of olefinic signals, its diagnostic use can be limited with *N*-protected peptides and (*E*)-ethene isosteres. Better results can be achieved when the spectrum is run in d_6-DMSO. In this solvent the diazomethyl ketone hydrogen can be observed as a sharp peak in the much clearer region around 6.5 ppm.

A common feature observed in the mass spectrum of diazomethyl ketones is the early loss of N_2. The parent molecular ion, if seen at all, is usually found at very low intensity. The product of this nitrogen loss is often the highest molecular ion peak of significance observed.

10.4 Attempted Direct Fluorination by Shaw's Procedure

Attempted conversion of *N*-phthaloylphenylalanyl diazomethane to the corresponding fluoromethane, using HF/pyridine, gave what appeared to be two distinct products (Rf 0.61 and 0.43, 2:1 chloroform:ethyl acetate). These were separated by dry flash chromatography using a 2:1 chloroform:ethyl acetate

solvent system as suggested by Shaw *et al.* The later eluted material showed no ^{19}F signal and was considered to be hydroxymethyl ketone (figure 10.4-1) formed as a by-product. This was later confirmed by the deliberate synthesis of this material.



N-Phthaloylphenylalanyl hydroxymethyl ketone

(figure 10.4-1)

Avoiding the production of such a material would be difficult due to the highly hygroscopic nature of HF/pyridine. This problem is further compounded by the unsuitability of standard glassware for the handling of this reagent.

The earlier material to be eluted was identified by ^1H NMR spectroscopy (in d_6-DMSO) as being partly, but not exclusively, starting material. A clean ^{19}F NMR signal coupled to two identical species was observed for this sample (-232.7 ppm, 46.1 Hz) suggesting the presence of some of the desired product. Subtraction of the diazomethyl ketone ^1H NMR spectrum from that of the fluorine containing product suggested that the CH_2-F connection was indeed present as a coupling of approximately 46 Hz was observed for a plausible CH_2-X signal. Isolation of this small amount of material was not attempted but it could be clearly seen, however, that product yields by this route were unacceptably low.

In an attempt to reduce the amount of unreacted starting material recovered and limit moisture uptake by the reagent, slightly stronger conditions involving less reagent manipulation were investigated (note that the use of 52% HF/pyridine required the addition of pyridine to the commercially available 70% reagent). Once again the major fluorine-free by-product observed in the previous reaction was isolated. A small sample of the partially purified reaction product was

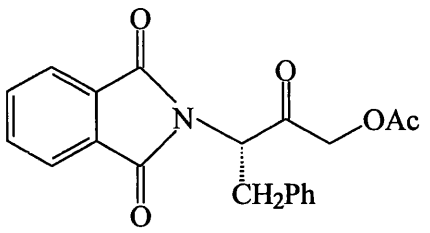
observed to contain an ^{19}F NMR signal at the desired chemical shift. This signal was, however, dwarfed by a variety of additional peaks suggesting that uncontrolled fluorination had taken place.

Under the same conditions, the reaction was attempted with a milder reagent. No desired fluorination was observed from the reaction in neat triethylamine trishydrofluoride, the major product in this case being the unreacted starting material.

Direct conversion of diazomethyl ketones to fluoromethyl ketones with HF/pyridine, though possible, was considered to be highly inefficient from these results. The unreliability of this method was also illustrated by the varying yields obtained by Shaw. For our purposes the possibility of obtaining a mere 5% yield, as suggested by Shaw for *N*-phthaloyl-(L)-alanyl fluoromethyl ketone, is totally unacceptable. It was therefore decided to investigate the use of bromomethyl ketones as intermediates.

10.5 Studies Concerning Bromomethyl Ketone Synthesis

The rather vague procedure described by Pattabiraman and Lawson, using lithium bromide in acetic acid, was tested as a potentially mild route to bromomethyl ketones.[85] The physical characteristics of this mixture made crude product manipulation and isolation difficult but, after some degree of experimentation, conditions were developed where at least partial reaction of *N*-phthaloylphenylalanyl diazomethyl ketone could be observed. With this reagent, and with 45% aqueous HBr, several by-products were also formed. The most significant of these was identified by ^{13}C NMR spectroscopy as being the α-acetate (figure 10.5-1).

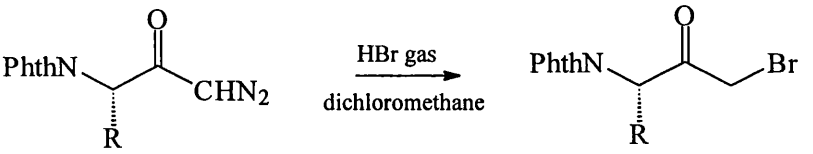


(figure 10.5-1)

^{1}H NMR spectroscopy was found to be extremely useful in assessing the number and approximate relative quantities of products resulting from these reactions. This could be gauged by analysing the appearance of the α-CH signal in the relatively clear 5 to 5.5 ppm ($CDCl_3$) region.

The search for an improved method by which hydrobromic acid could be utilised free from moisture and alternative nucleophiles, uncovered the synthesis of triphenylphosphonium bromide.[86] Easily prepared from triphenylphosphine and aqueous hydrobromic acid, this crystalline material can be thermally decomposed to release hydrogen bromide gas.

Solid triphenylphosphonium bromide, prepared and purified by this very simple method, was dissolved in dry xylene and brought to reflux. The gaseous hydrogen bromide thus produced was carried in a stream of dry nitrogen through a dichloromethane solution of *N*-phthaloylphenylalanyl diazomethyl ketone. Immediate reaction of this material was noted by the evolution of further gas (nitrogen) and the loss of the characteristic yellow diazomethyl ketone solution colouration. As a result, excellent yields (around 90%) of the corresponding bromomethyl ketone (figure 10.5-2) were obtained following product purification by a simple recrystallisation. The purified product compound was found to be strongly optically active suggesting that racemisation had not taken place. Repeating this process with *N*-phthaloylalanyl and *N*-phthaloylglycyl diazomethyl ketones also achieved the desired conversion.

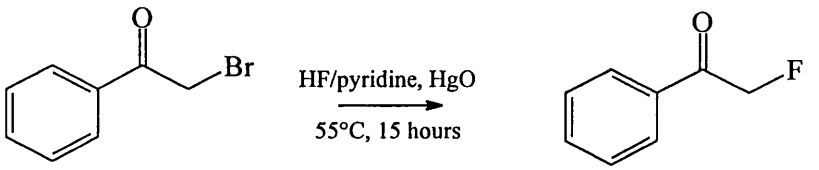


(figure 10.5-2)

With *N*-phthaloyl protected materials, the HBr gas method is far superior to the other bromination procedures investigated here. It is also considered that the practical yield attainable could be brought close to 100% with further work.

10.6 Fluoromethyl Ketones by Halogen Exchange

Using HF/pyridine and yellow mercuric oxide, Olah reported a 68% yield in the halogen exchange reaction of α-bromoacetophenone (figure 10.6-1).[87]



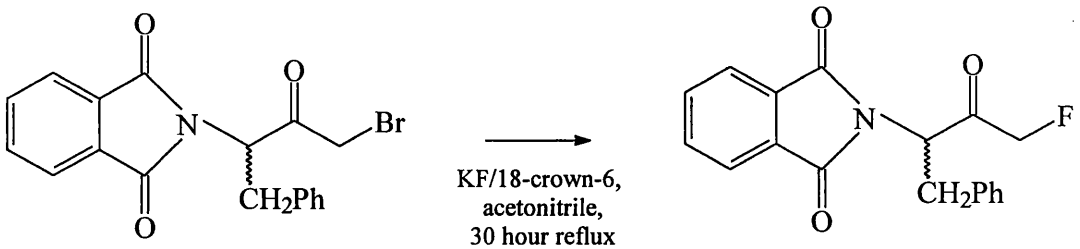
(figure 10.6-1)

An attempt to apply this methodology to *N*-phthaloyl-(L)-phenylalanyl bromomethyl ketone however, resulted in gross decomposition of the starting material. Studies involving this method were not continued as a concurrently run experiment with an alternative fluorinating agent showed greater signs of promise.

It has been reported that fluoroacetone can be formed by the reaction of bromoacetone in triethylamine trishydrofluoride at 110-115°C.[88] The effects of this reagent on *N*-phthaloylphenylalanyl bromomethane were therefore investigated. One of the potential advantages of the use of triethylamine trishydrofluoride, as opposed to standard HF reagents, is that regular glass apparatus may be employed without problems. The handling characteristics of this reagent are also far superior.

Initial experiments using approximately three molar equivalents of this reagent in a chloroform solution showed that some fluoromethyl ketone was formed when the reaction mixture was heated to 80°C. During further studies the course of the reaction was carefully followed by thin layer chromatography allowing the successful production of the desired fluoromethyl ketone to be achieved. This compound was purified by precipitation from dichloromethane by the addition of hexane, giving a 78% yield. Repeating the procedure with *N*-phthaloylalanyl and *N*-phthaloylglycyl bromomethyl ketones also achieved success. It is predicted that, with further experimentation, the yields obtainable by this method could be significantly improved.

Had the triethylamine trishydrofluoride route proved not to be successful, it was intended that the next attempted halogen exchange would be carried out using potassium fluoride and 18-crown-6 as described by Liotta and Harris.[89] It was later discovered that this method has been used on racemic *N*-phthaloylphenylalanyl bromomethyl ketone by Kolb *et al*, giving a 45% product yield (figure 10.6-2).[90] [91] Note however that this yield is inferior to that obtained here with the developed $Et_3N.3HF$ route.



(figure 10.6-2)

10.7 Features Requiring Further Investigation

Recent developments in the field of cysteine protease inhibitors, which will be discussed in due course, caused the premature termination of studies in the direction of fluoromethyl ketone synthesis. Unfortunately, at this stage of the

investigation, several important questions remain unanswered and methods untested.

Application of the Developed Route to *N*-BOC Protected Compounds

The use of HBr gas in the developed route suggests that the diazomethyl to bromomethyl ketone conversion may cause problems with *tert*-butoxycarbonyl protected compounds. In order to assess this problem *N*-BOC-(L)-phenylalanyl diazomethyl ketone was synthesised. As expected, treatment of this compound with HBr gas caused the destruction of the protecting group. It is unknown if moderation of the conditions employed, by the use of low temperatures for example, would allow the successful reaction of the diazomethyl ketone group in the presence of a *N*-BOC protection.

A variation of the HBr/acetic acid method was employed by Zimmerman, Bissell and Smith to allow the formation of bromomethyl ketones in the presence of *tert*-butoxycarbonyl or benzyloxycarbonyl protecting groups.[92] In this case the HBr/acetic acid was significantly diluted with dichloromethane prior to its use on a solution of the starting material. A product yield of 75% was claimed for this methodology.

An analogous moderation process was employed by Kolb *et al* where a 94% yield of *N*-phthaloylphenylalanyl bromomethyl ketone was obtained using 47% aqueous HBr in THF.[90] [91] The use of these procedures should therefore overcome the side-product formation problems experienced in more concentrated systems (see section 10.5).

Scope and Limitations of the Triethylamine Trishydrofluoride Halogen Exchange

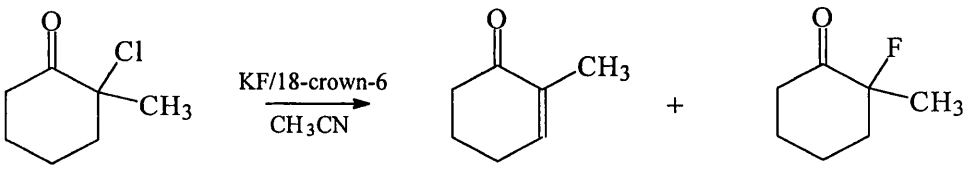
The factors governing this reaction have not been fully investigated. For example, the reaction times required (typically greater than 25 hours) for complete halogen exchange were considered to be excessive. The effects of

increased reagent concentration on this have not been investigated. Similarly the use of HgO, as employed by Olah in the HF/pyridine induced exchange, may also reduce the necessary reaction time or temperature. Such moderation may be essential to allow the selective reaction of *N*-BOC or *N*-CBZ protected materials. The stability of such groups with triethylamine trishydrofluoride has not been tested.

Possible Racemisation of *N*-Phthaloylphenylalanyl Fluoromethyl Ketone

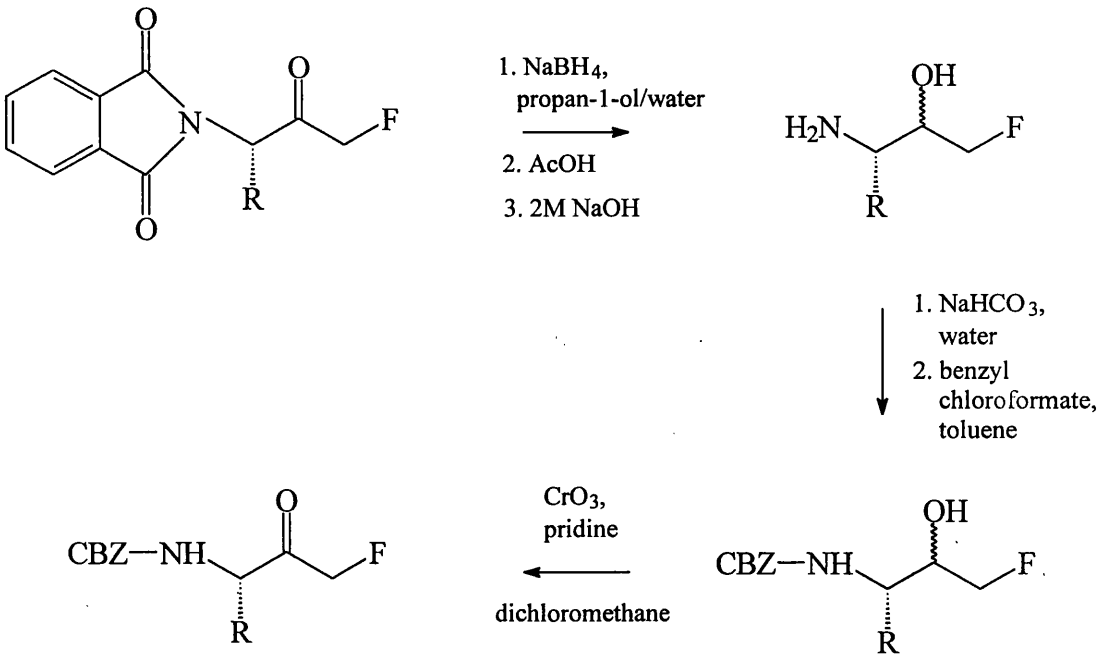
The optical rotation of the synthesised *N*-phthaloylphenylalanyl fluoromethyl ketone was observed to be small. As no reference standard existed for this compound it could not be established to what extent, if at all, racemisation had occurred. It was intended that a sample of fluoromethyl ketone would be prepared, from the optically active bromomethyl ketone, by the Kolb KF/crown ether procedure for comparative purposes.

Racemisation by the KF/crown ether procedure however, may also be possible as Kolb reported a competing elimination reaction in a cyclic system (figure 10.7-1).[90] In this situation the fluoride ion is clearly acting as a base.



(figure 10.7-1)

A literature value exists for the optical rotation of *N*-CBZ-(L)-phenylalanyl fluoromethyl ketone.[54] Changing the protecting group, by the method employed by Shaw (figure 10.7-2), could therefore be considered if stereochemical ambiguity with the fluorinated material still existed.



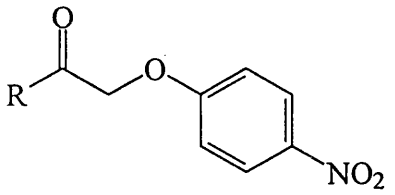
Protecting Group Exchange

(figure 10.7-2)

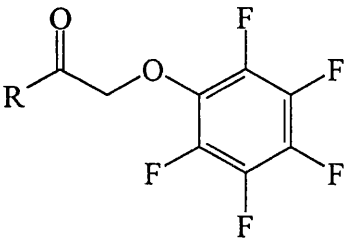
10.8 Recent Advances in the Field of Cysteine Protease Inhibitors

Work on the fluoromethyl ketone end group was halted at this stage as alternative leaving groups have recently been developed and used in preference to fluorine.[92] [93]

A vast range of aryloxymethyl and arylacyloxymethyl ketones have been synthesised and shown to possess activity against cysteine proteases.[94] The theoretical basis of this study originates from the fact that the electronegativity of oxygen most closely approaches that of fluorine, particularly when the former atom is bonded to an aromatic ring. Typical leaving groups tested include those bearing further electron withdrawing substituents on the aromatic ring such as those shown in figure 10.8-1.



4-nitrophenoxymethyl
ketone end group

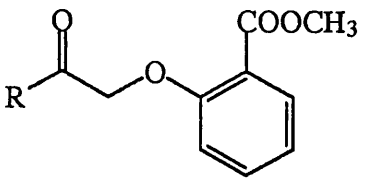


pentafluorophenoxymethyl
ketone end group

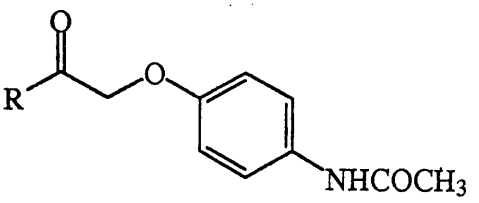
(figure 10.8-1)

The main advantage of the use of such end groups appears to be that the activity of the resulting compound can be modified by alteration of the aromatic substitution pattern. Thus, a large degree of reactivity fine tuning is now possible as the selection of electron withdrawing leaving groups is no longer limited to halogens.

Leaving groups derived from aspirin, *p*-acetaminophenol and similar anti-inflammatories have also been suggested (figure 10.8-2). The release of such compounds directly at the site of anti-parasite drug action would obviously be of benefit.



methyl salicylate releasing end group



p-acetaminophenol releasing end group

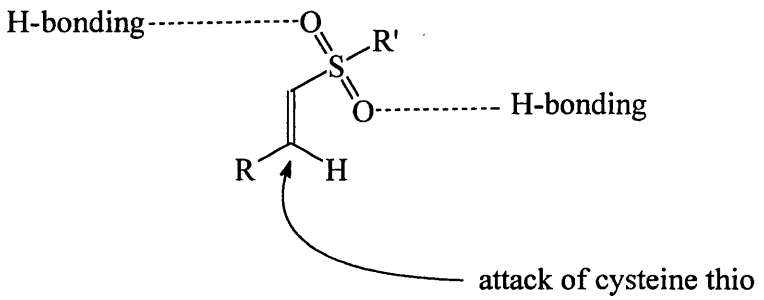
(figure 10.8-2)

It may be possible that investigations concerning alternative leaving groups were initiated in order to minimise the toxic effects of drug metabolites. Since the incorporation of isosteres into peptidyl fluoromethyl ketones is not yet common practice, undesirable side-effects would be expected during the use of such compounds as amide link hydrolysis is still possible. This process would result in the production of unprotected amino acids bearing the fluoromethyl ketone end group. It may be possible that the metabolic products of compounds

bearing the alternative end groups are less toxic or are metabolised further due to their additional functionality.

The activity of such compounds also indicates the absence of steric constraints in this area of the active site or the protrusion of the leaving group from the enzyme.

More recently, work has been carried out on end groups unrelated to α-substituted methyl ketones. Peptidyl vinyl sulfones have been synthesised which show activity against cysteine proteases but not serine proteases or unactivated thiols.[95] In these compounds the sulfone is thought to hydrogen bond to the enzyme active site thus compensating for the absence of the carbonyl group and its associated interaction with the enzyme (figure 10.8-3).



(figure 10.8-3)

The application of the Fehrentz and Castro procedure has been specified in order to produce the chiral amino aldehydes from which these compounds can be formed.[96] Thus the application of this methodology, which will be described in section 11.1, to completed isosteres may be informative.

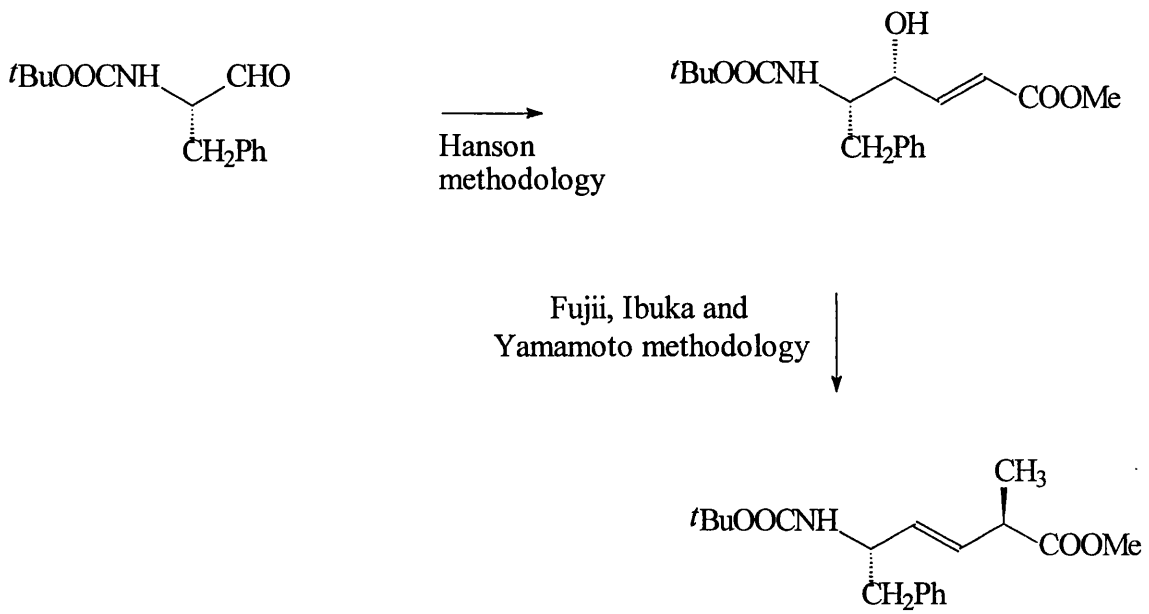
End group technology is advancing rapidly and as the pool of potential groups continues to grow, it seemed wise to concentrate on proving the concept of the isostere. Such testing can be carried out using any active end group on a battery of parasites. If (*E*)-ethene isosteres are shown to possess activity, the more complicated process of synthesising the compound of highest, and most specific, activity can begin.

It was therefore decided to test the completed isosteres initially bearing the diazomethyl ketone end group. The most promising of these, indicated by the first round of biological tests, could then be taken further by incorporating the latest end-group technology.

Chapter 11: Initial Studies Concerning (*E*)-ethene Isostere Synthesis

Much biological work has been carried out on the fluoromethyl ketone of the dipeptide *N*-BOC-(L)-Phe-(L)-Ala. Because of this and the possible absence of complications arising from side chain functionality, it was decided that the (*E*)-ethene isostere of this compound would be a suitable first synthetic target.

An initial, rapid study was carried out concerning the possibility of using the Hanson route to hydroxyethylidene compounds followed by the 1,3 chirality transfer reaction as developed by Fujii, Ibuka and Yamamoto.[71] [74] (figure 11-1) One of the main attractions of this route is the prospect of stereocontrol at both chiral centres and exclusive synthesis of the *trans* double bond (see section 8.5).

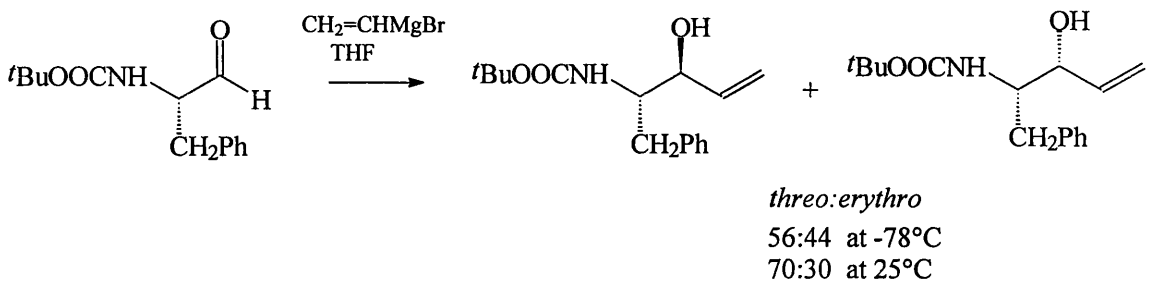


(figure 11-1)

As the insertion of the carboxyl terminus side chain occurs in the final stages of the synthesis, extensive variation at this position may be possible without the need to alter reaction conditions or purification procedures for the majority of the route. Another feature worthy of mention is the use of *N*-protected chiral amino aldehydes derived from the corresponding amino acids as starting materials. A

wide variety of such *N*-protected amino acids are commercially available and inexpensive.

The addition of vinyl magnesium bromide to *N*-BOC protected phenylalaninal, as described in the Hanson procedure, results in the production of a pair of diastereomeric vinyl alcohols (figure 11-2). For the synthesis of (*E*)-ethene isosteres bearing the same chirality as the natural dipeptide, the elaboration of the minor *erythro* diastereomer would be required. Hanson *et al* specify the use of HPLC to effect the separation of these diastereomers and, in their situation, both were isolated and used in further studies.

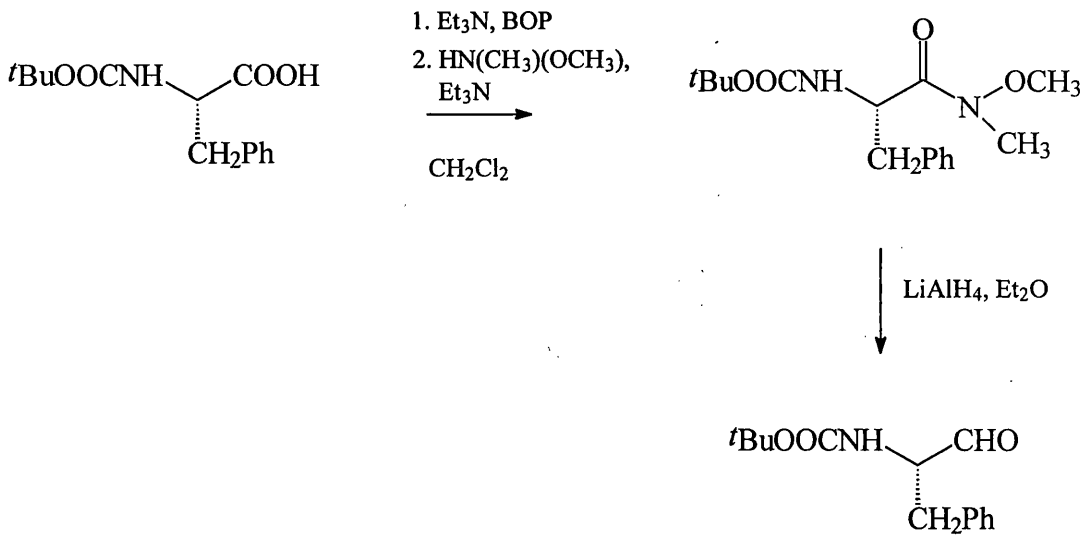


(figure 11-2)

As preparative HPLC was unavailable, it was decided to undertake a rapid test synthesis of this diastereomeric mixture to investigate if efficient separation could be achieved by standard silica gel column chromatography.

11.1 Synthetic Test of the Hanson Route

A sample of *N*-BOC protected (L)-phenylalaninal was produced from *N*-BOC-(L)-phenylalanine by the method of Fehrentz and Castro (figure 11.1-1).[96]



(figure 11.1-1)

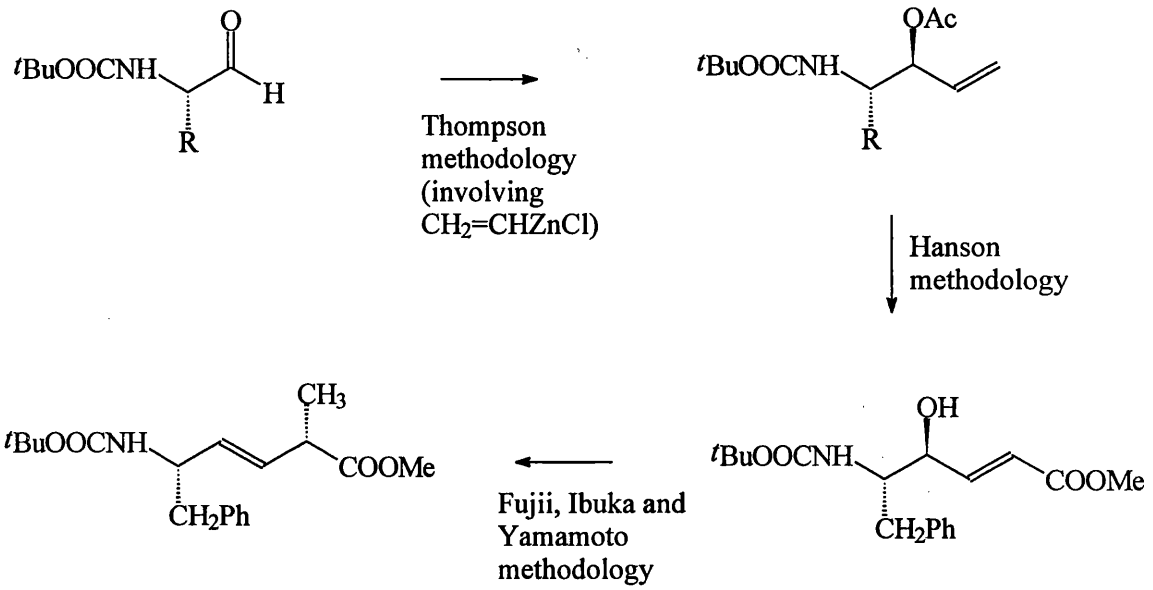
This method was chosen as it was claimed by the original authors that, in general, the crude aldehyde products contained minimal impurities thus avoiding the need for further purification. Such a result would be beneficial as column chromatography of optically active α-amino aldehydes often leads to decomposition or racemisation.

The ^{1}H NMR spectrum of the crude amino aldehyde compared well to that quoted by Fehrentz and Castro. This material was therefore used directly without further purification or analysis.

As expected, the reaction of vinyl magnesium bromide with *N*-BOC protected phenylalaninol produced two main compounds with close retention factors on thin layer chromatography (typical Rf difference 0.05, eluent 1:4 ethyl acetate:hexane). Purification was attempted by dry flash, positive pressure and gravity column chromatography but efficient separation of the two diastereomers could not be achieved.

This route therefore no longer represents an efficient option for the synthesis of the (L)-Phe-(L)-Ala isostere. The efficient synthesis of isosteres mimicking peptides bearing the unnatural (D)-amino acid at the C-terminus may however, still be possible by this route. In this situation vinylmagnesium bromide would be

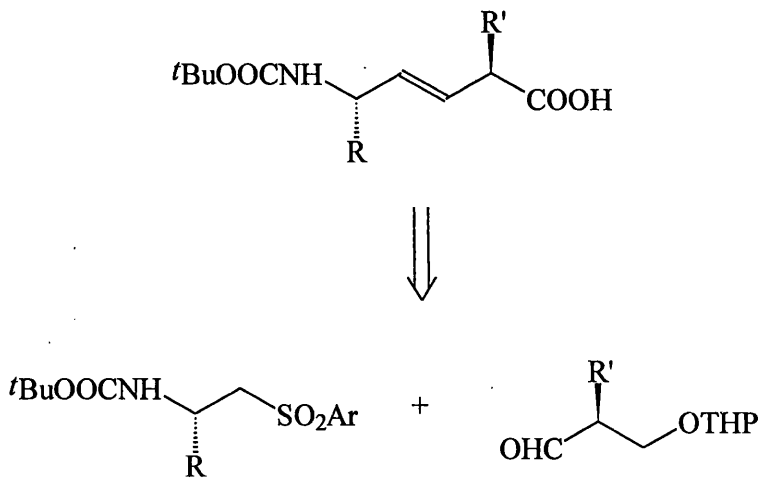
replaced with vinylzinc chloride as this reagent was shown by Thompson to increase the proportion of the major (*threo*) isomer formed (see section 8.4).[69] The methodology combination required for synthesis of the *N*-BOC-(L)-Phe-(D)-Ala isostere methyl ester is outlined in figure 11.1-2.



(figure 11.1-2)

Chapter 12: The Formation of Precursors for an Alternative Strategy

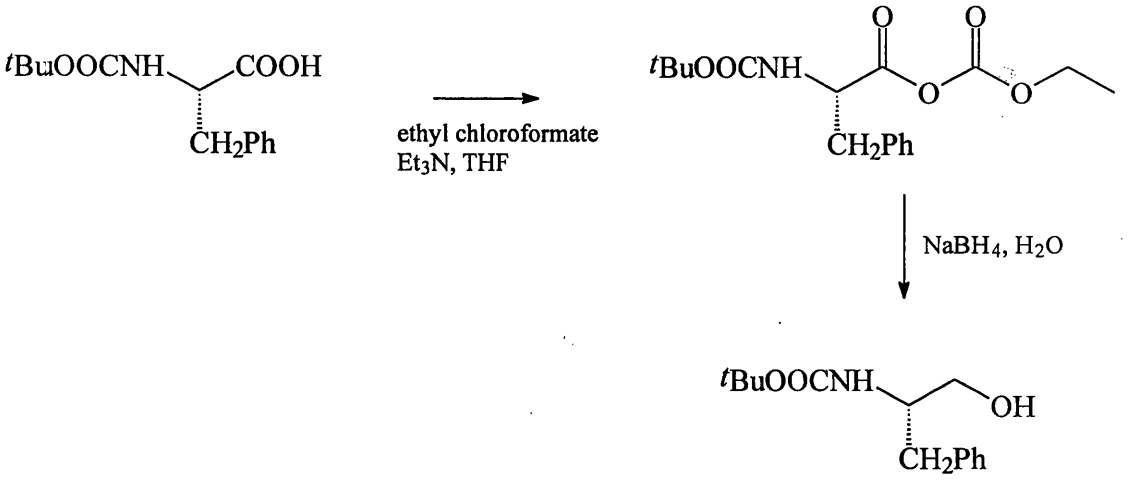
The Hopkins approach to the synthesis of (*E*)-ethene isosteres involved the formation of the olefin by the coupling of a sulfone with an aldehyde (figure 12-1).[75] The synthetic routes employed here to obtain these precursors will be discussed separately and in turn.



(figure 12-1)

12.1 Formation of the Sulfone Synthon

Initial studies on the synthesis of sulfones were carried out using *N*-BOC protected (L)-phenylalanine as a starting material. The carboxyl group of this compound was activated by the formation of a mixed carbonate then reduced to the corresponding alcohol by the action of sodium borohydride (figure 12.1-1).

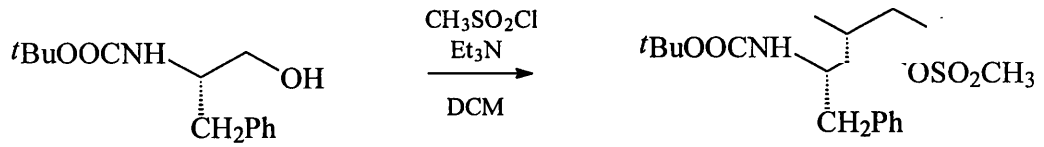


(figure 12.1-1)

A significant quantity of white solid, later identified as triethylamine hydrochloride, was removed from the mixed carbonate solution by filtration before addition of the solution to a sodium borohydride/water slurry. The use of water as a co-solvent is apparently essential in this situation as sodium borohydride is poorly soluble in THF alone and thus would give rise to low yields.[97]

Prior activation of the carboxylic acid, as a mixed carbonate, allows the reduction to proceed very smoothly under mild conditions. However, extreme care was found to be necessary during the course of the reaction as the breakdown of this activated system resulted in the generation of significant quantities of carbon dioxide and heat. The crude product alcohol, identifiable by the loss of the carboxylic acid infra-red absorbance (1714 cm^{-1}, KBr disc), was shown as being virtually pure by ^1H NMR spectroscopy. Absolute purity was easily attained by a simple recrystallisation from ethyl acetate/hexane.

Conversion of the *N*-BOC-(L)-phenylalaninol to the corresponding mesylate was carried out in order to increase the susceptibility of the alcohol function to displacement by S_N2 attack.[98] This was carried out using methanesulfonyl chloride and triethylamine in dichloromethane (figure 12.1-2).

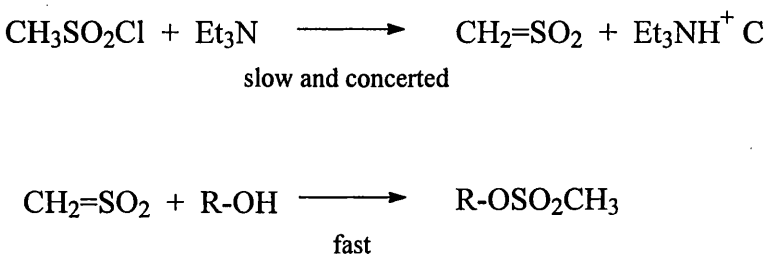


(figure 12.1-2)

Product yields in excess of 95%, following recrystallisation, were regularly obtained for this reaction. Mesylates were generally used within three days of synthesis and during this time the compounds were stored at 0°C under a nitrogen atmosphere in order to minimise decomposition.

The mechanism of this reaction is worthy of discussion. Direct nucleophilic displacement, by the attack of the alcohol on sulfur, was ruled out by Crossland and Servis as the nucleophilicity of the alcohol appeared unimportant to the progression of the reaction. This was illustrated by the successful mesylation of 2,2,2-trifluoroethanol and 1,1,1,3,3,3-hexafluoro-2-propanol. Indeed, King and Lee showed that the kinetics of the reaction were independent of alcohol concentration with the observed reaction rate being first order with respect to both the sulfonyl chloride and tertiary amine. [99]

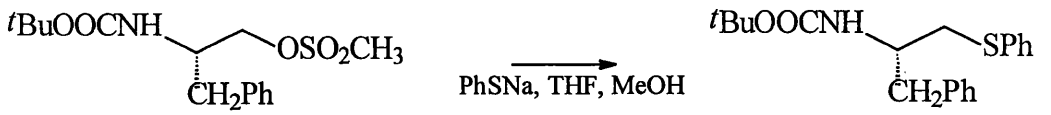
Model studies carried out by this group showed that the reaction was most likely to proceed by an E2 mechanism where the base-induced elimination of hydrogen chloride leads to the formation of a sulfene (figure 12.1-3). This highly reactive intermediate then reacts rapidly with the available alcohol thus forming the product mesylate.



(figure 12.1-3)

The next stage in the synthetic route involved the displacement of the mesylate by thiophenol. In order to effect this, the thiol was added to a previously

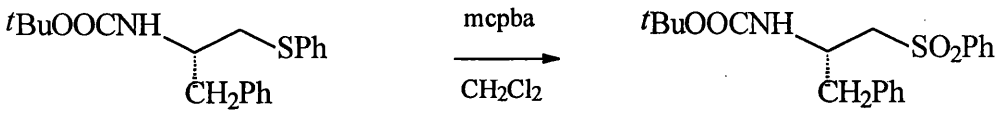
prepared solution of sodium methoxide. In this base, one would expect virtually complete formation of the thiolate anion due to the lower basicity of this species. S_N2 attack of the thiolate anion with concurrent displacement of the mesyloxy group was found to be effective and efficient under the conditions described (figure 12.1-4).[75] Dilute sodium hydroxide solution was then added to the reaction mixture prior to work-up. Maintaining the basic nature of this mixture would prevent the inclusion of methanesulfonic acid into the product solution during extraction. The quantity of excess thiophenol similarly incorporated may also be limited.



(figure 12.1-4)

Recrystallisation was found to be sufficient to remove the small number of by-products, observed by thin layer chromatography, from the product thioether. Excess thiophenol was indeed identified as being the major contaminant.

meta-Chloroperoxybenzoic acid oxidation of the thioether, to give the desired sulfone (figure 12.1-5), initially gave some product purification problems.[75] These were eventually identified as being due to the incomplete removal of *meta*-chlorobenzoic acid by extraction into sodium hydroxide. This by-product was also found to persist as a major contaminant during recrystallisation of the crude sulfone from methanol. Slow recrystallisation in several crops from hot 1:1 ethyl acetate:hexane, although appearing visually to give a less pure product, was found to be more effective at keeping the by-product within the mother liquor. However, careful monitoring of the extensive nature and relative integral of the ^{1}H NMR aromatic region was still essential for each crop to ensure the absence of this impurity. Typical yields of pure sulfone around 90% were achieved by this method.



(figure 12.1-5)

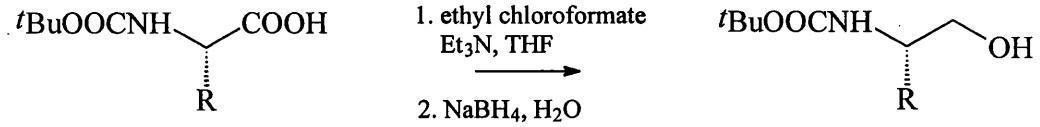
12.2 Advantages of the Employed Route for Sulfone Generation

An obvious advantage of the route employed for sulfone synthesis is the absence of the necessity for chromatographic purification. In each step a recrystallisation process was sufficient to obtain material of high purity. Indeed, in many cases the high yielding reactions furnished crude products of sufficient quality to be used directly in the following stage. Obviously this ease of crystallisation may not be generally extended to sequences involving amino acids other than phenylalanine but the high yields experienced and the ability to continue with crude products should minimise problems.

The above reactions were carried out a number of times with similar success in each case. Variation of the scale of the process, typically from 100 mg to 30 g, did not prove to be in any way detrimental to the course of the reaction.

12.3 Sulfones From Alternative Starting Materials

Using the reaction procedure described above, *N*-BOC protected (L)-valine and (L)-leucine were reduced to the corresponding alcohols in crude yields greater than 80% (figure 12.3.1). In both cases the crude products were viscous oils but, after storage under vacuum for several days, purity was indicated by ^{1}H NMR spectroscopy and microanalysis.



$R = CH(CH_3)_2$ or $CH_2CH(CH_3)_2$

(figure 12.3-1)

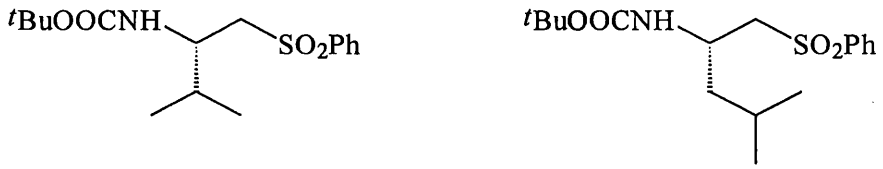
Note that in the case of *N*-BOC-(L)-leucine, the monohydrate was used as a starting material. The quantity of ethyl chloroformate employed was therefore doubled in order to give comparable results to the *N*-BOC-(L)-valine reaction.

Conversion of *N*-BOC-(L)-valinol and *N*-BOC-(L)-leucinol to the corresponding mesylates proceded smoothly. After some degree of experimentation, crystallisation conditions were developed for both these products. As expected, isolated yields for this stage were found to be good (97% and 87% respectively). The slightly lower yield for the mesylate derived from *N*-BOC-(L)-leucinol may be explained by the greater reluctance of this material to crystallise.

Displacement of the valine derived mesylate with thiophenol gave rise to a solid product which was partially purified by precipitation from 1:9 dichloromethane:hexane. The resulting material was considered to be of sufficient purity for further reaction but for full analysis a small quantity was passed though a short column of silica giving a clean solid. In the case of the leucine derived sulfide, as crystallisation could not be achieved, the whole product oil was purified by chromatography in a similar manner.

The non-polar impurity present in both these compounds was identified by mass spectrometry, ^{13}C and ^{1}H NMR spectroscopy as being diphenyl disulfide. It was presumed that this material exists as a contaminant in the reagent thiophenol. Alternatively, this material may have been formed if addition of the thiol to the reaction mixture had taken place before the complete formation of sodium methoxide by the reaction of sodium with methanol.

Following the oxidation of both sulfides to sulfones, extensive extraction of the crude product solutions was carried out using aqueous sodium hydroxide for the following reason. Since crystallisation of these materials was expected to be difficult, it was considered that prior removal of as much by-product *meta*-chlorobenzoic acid as possible would be essential. Crystallisation of the pure sulfones (figure 12.3-2) was then achieved after some degree of experimentation.



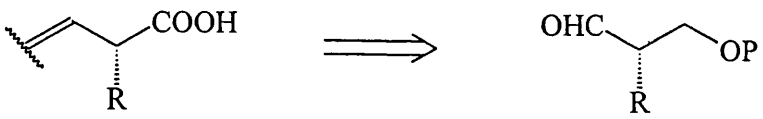
(figure 12.3-2)

Thus, to summarise, sulfones derived from *N*-BOC protected (L)-phenylalanine, (L)-valine and (L)-leucine have been successfully synthesised by a highly efficient and convenient route. These compounds were also observed to be optically active with rotations comparable to literature values.[75] Melting points, optical rotations and overall yields for the four step conversion are indicated in the following table.

N-BOC-(L)-Amino Acid	Sulfone Yield	Melting Point (lit. [75])	Optical Rotation (lit. [75])
Phenylalanine	38%	204-206°C (215-216°C)	-25.95 (-32.7)
Valine	56%	108-110°C (103-105°C)	+9.2 (+11.02)
Leucine	27%	95-97°C (95-98°C)	-9.65 (-9.87)

12.4 Synthesis of the Aldehyde Component

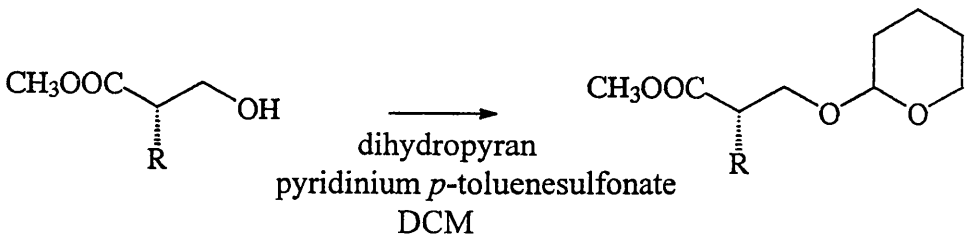
The coupling synthon required in order to mimic the carboxy terminus amino acid of a dipeptide can be defined as a chiral three carbon aldehyde bearing a protected β-alcohol and an appropriate α-side chain (figure 12.4-1).



R = desired amino acid side chain
P = protecting group

(figure 12.4-1)

Fortunately, for R = CH_3 (synthon required for an alanine mimic) a commercially available precursor exists bearing the desired stereochemistry. This compound, methyl 3-hydroxy-2(S)-methylpropionate, was therefore purchased and the alcohol function quantitatively protected as a tetrahydropyranyl ether (figure 12.4-2).[75]



(figure 12.4-2)

Attempts were then made to reduce the methyl ester to the desired aldehyde using diisobutylaluminium hydride as described by Hopkins.[75] Initial reactions illustrated that a virtually clean sample of crude aldehyde could be obtained using DIBAL-H (1.0 M solution in hexane) in dry dichloromethane. Severe problems were then experienced with the reproducibility of these results as further reactions produced, at best, only traces of the desired product. A number of experiments were carried out with meticulously dried and purified starting material and solvents, without any improvement in yield, before the problem was finally identified as being due to the lability of the reagent to decomposition.

After first use, the reagent was found to become inactive very rapidly despite being stored in a sealed (Aldrich Sure-Seal® system), nitogen filled vessel at less than 5°C. Thus, once purchased, DIBAL-H was used as rapidly as possible as the fresh reagent gave excellent yields of the desired product.

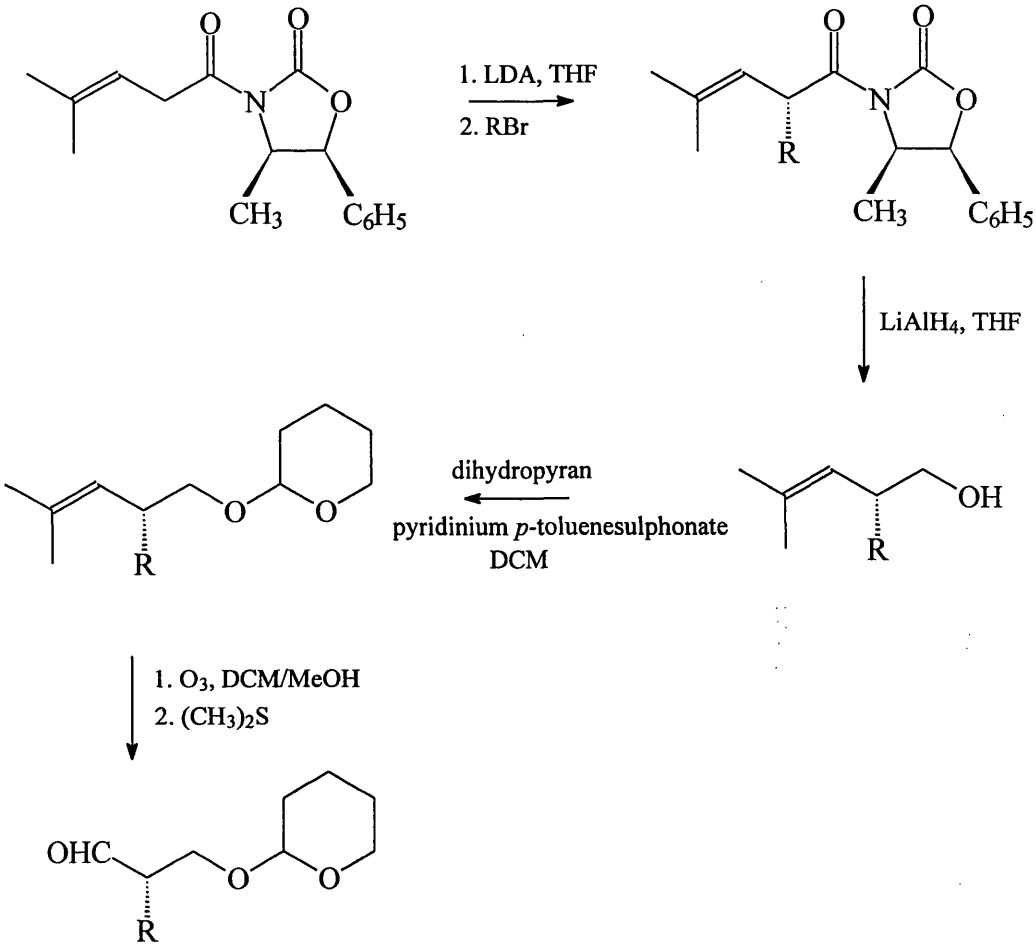
^{1}H NMR spectroscopy was found to be extremely useful for the estimation of the success and extent of this reaction. Apart from the obvious loss of the methyl ester and appearance of aldehyde signals, the chemical shift of the α-methyl peaks were also highly characteristic of the compound in question. These signals could be used to gauge the amount of product, starting material and other α-methyl bearing impurities, such as the corresponding carboxylic acid, in crude mixtures. The diastereomeric nature of THP protected chiral compounds was also indicated by these peaks.

The problem of rapid reagent decomposition was compounded by the instability of the product aldehyde. Indeed, a sample of this material was observed to give almost complete carboxylic acid by air oxidation when stored at room temperature for approximately one week. During initial experiments on the aldehyde-sulfone coupling, the severe problems experienced (which will be discussed in section 13.2) were at first considered to be due to aldehyde impurity or decomposition. Thus, during further studies, the aldehyde was synthesised regularly on a small scale immediately prior to its use. This material was also carefully purified by distillation.

Once the true source of the coupling problem had been identified, the aldehyde was used without purification as the small amount of contaminants present were observed not to interfere with the reaction. Storage of the aldehyde in a sealed, nitrogen flushed vessel at 0°C was found to be effective at minimising decomposition.

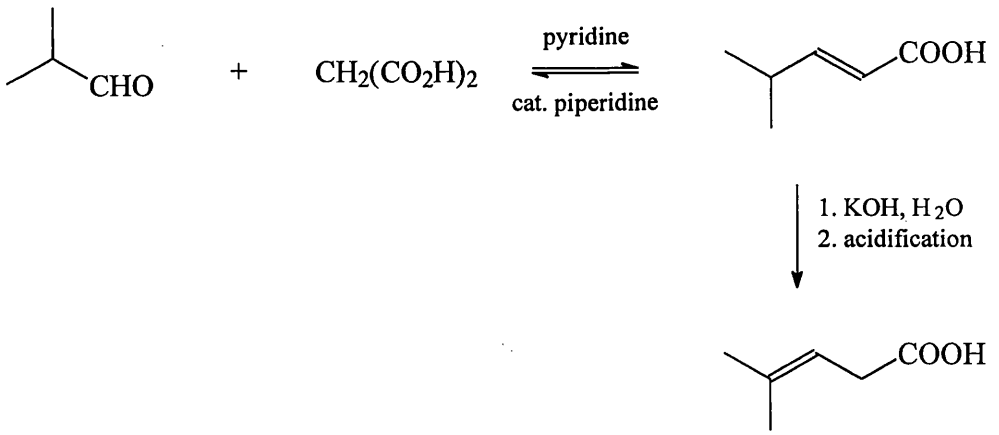
12.5 Alternative Aldehydes

The route defined by Hopkins *et al* [75] for the synthesis of alternative aldehydes (figure 12.5-1) utilised Evans' alkylation for the introduction of defined chirality. [100]



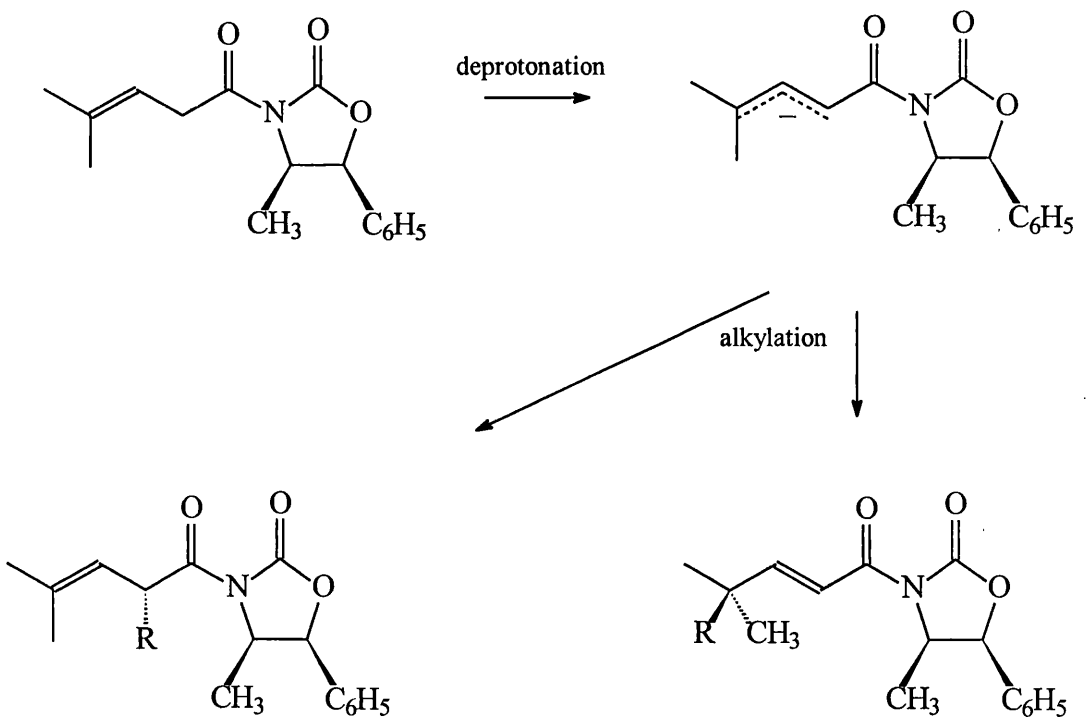
(figure 12.5-1)

A study concerning the synthesis of *trans*-4-methylpent-3-enoic acid, required as a starting material for this route, was initiated (figure 12.5-2). A Knoevenagel condensation with decarboxylative dehydration was investigated in order to synthesise the 2,3-unsaturated carboxylic acid.[101] Treating this compound with base would result in a double bond migration giving the 3,4-unsaturated product.



(figure 12.5-2)

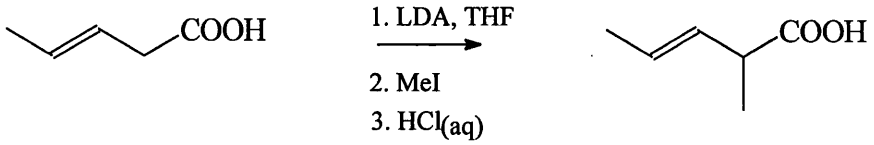
It is presumed that the trisubstituted olefin was employed by Hopkins as this would be less susceptible to the reverse double bond migration and γ-alkylation taking place during the attempted α-alkylation. The products of both α- and γ-alkylation are illustrated in figure 12.5-3.



(figure 12.5-3)

A simple test methylation of commercially available *trans*-3-pentenoic acid indicated that the synthesis of *trans*-4-methyl-pent-3-enoic acid may be

superfluous. In this case the α-methyl-βγ-unsaturated acid was shown by ^{1}H NMR spectroscopy to be the main product formed on alkylation (figure 12.5-4).



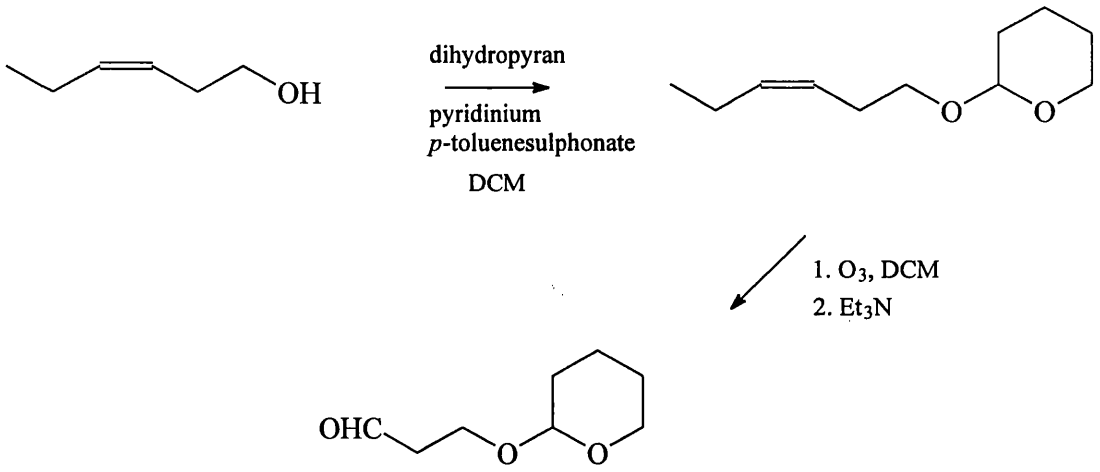
(figure 12.5-4)

This result is in complete agreement with the findings of Rathke and Sullivan who stated that the α-carbon of the intermediate delocalised anion, formed in such systems, is kinetically the most reactive.[102] [103] This also suggests that, if the delocalised anion is indeed formed, one would expect identical results in the alkylations of both αβ- and βγ-unsaturated 4-methylpentenoic acid

Unfortunately, studies in this area were not taken further due to time constraints.

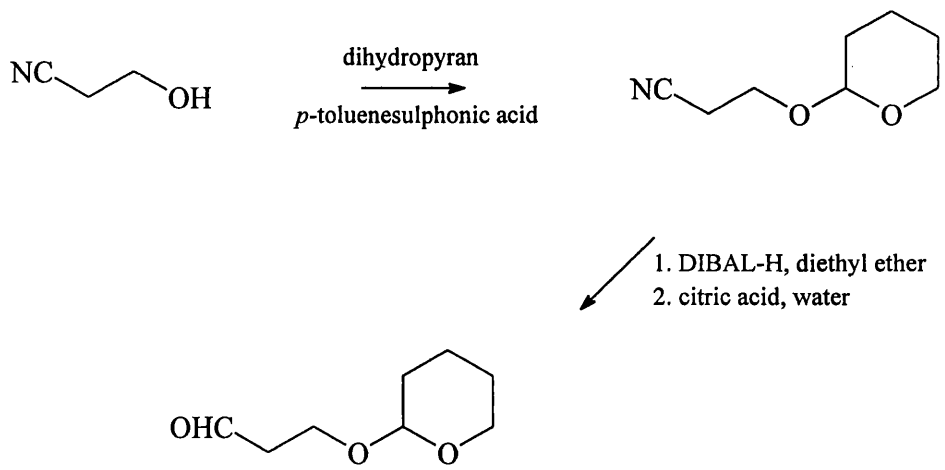
12.6 Aldehyde Precursor to a Glycine Mimic

A simple route to an aldehyde precursor for a glycine mimic was devised using *cis*-3-hexenol as a starting material (figure 12.6-1). The alcohol function was protected as a THP ether as before and attempts were made at cleaving the double bond by ozonolysis. Initial reactions using several variations of the ozonolysis procedure employed by Hopkins (dichloromethane/methanol mixed solvent, dimethylsulfide quench) yielded only traces of the desired aldehyde. It was suggested by ^{1}H NMR spectroscopy that the main product was in fact the methyl ester of THP protected β-hydroxypropanoic acid, possibly formed as a result of methanol attack on the intermediate ozonide. The reaction was therefore attempted in pure dichloromethane where reasonable quantities of the desired aldehyde were observed by ^{1}H NMR spectroscopy following a triethylamine quench. This crude product was used in subsequent reactions after only partial purification as the main residual impurity was identified as being triethylamine. Full purification was however, possible by short path distillation giving an estimated 28% yield.



(figure 12.6-1)

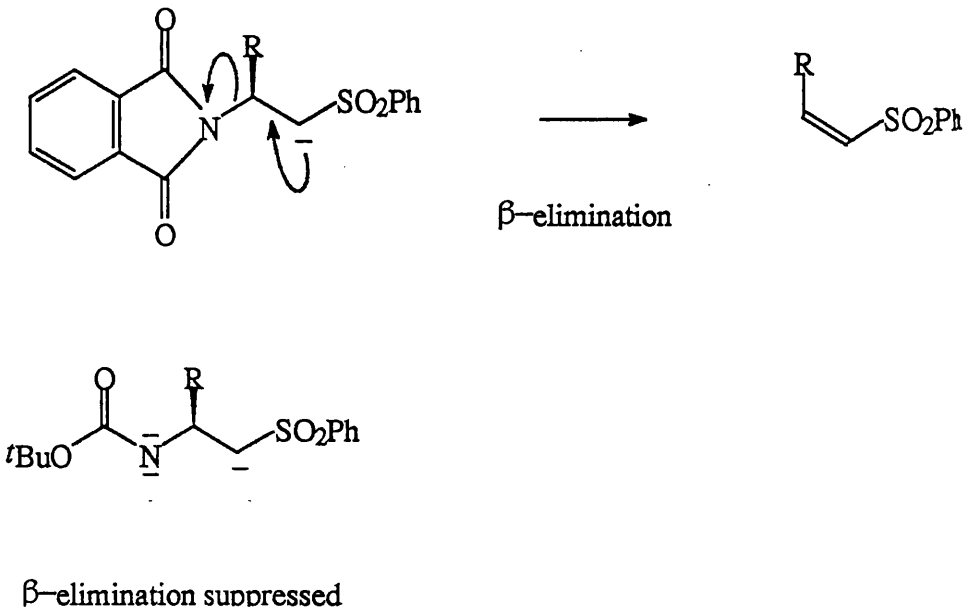
This route is therefore a suitable alternative to that preferred by Kozikowski and Stein (figure 12.6-2) which utilised 3-hydroxypropionitrile as an inexpensive starting material.[104]



(figure 12.6-2)

Chapter 13: The Coupling Reaction and Isostere Generation

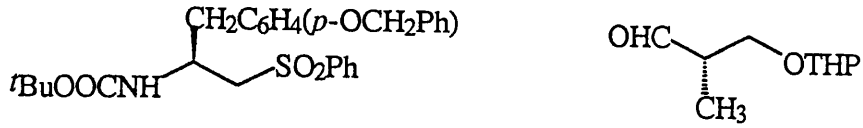
The procedure commonly referred to as Julia Olefin Synthesis was employed by Hopkins to effect the sulfone to aldehyde coupling and double bond formation.[75] [77] Studies carried out by this group showed that the starting *N*-BOC protected amino sulfone must be reacted with two equivalents of base before the coupling is attempted. This is essential as deprotonation of the carbamate nitrogen would be expected in addition to sulfone α-anion formation. Furthermore, the generation of such a dianion is required in order to prevent a β-elimination reaction from taking place. One would predict the decomposition of *N*-phthaloyl protected amino sulfones during attempted coupling (figure 13-1). The presence of a *N*-BOC or *N*-CBZ protection is therefore essential for successful coupling.



(figure 13-1)

13.1 Literature Methodology

Hopkins defined methodology for the coupling of a sufone produced from *O,N*-protected (L)-tyrosine, and the (L)-alanine mimic aldehyde synthon (figure 13.1-1).

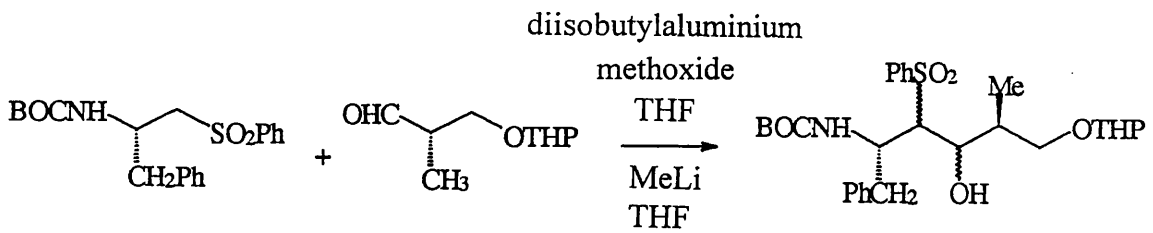


(figure 13.1-1)

Poor coupling yields were initially experienced by this group. Trapping of the product β-hydroxysulfone by derivatisation of the hydroxyl group, in order to inhibit the reverse reaction, was attempted without any improvement in results. The problem was therefore considered by Hopkins to be due to the lack of progress of the forward reaction. After some degree of experimentation it was found that prior complexation of the aldehyde component with an aluminium ion allowed the effective generation of the coupled product.

13.2 Experimental Findings

Following the procedure prescribed by Hopkins, a sample of the sulfone derived from (L)-phenylalanine was taken up in dry THF to produce what appeared to be a milky white solution. A strong yellow colour was noted as being formed in this vessel during the addition of just over two equivalents of methyl lithium. In a separate vessel, two equivalents of aldehyde in THF were treated with diisobutylaluminium methoxide (formed by the addition of methanol to a 1.0 M solution of DIBAL-H in THF) then transferred dropwise *via* cannula to the sulfone solution. This method was expected to yield, after aqueous work-up, a crude mixture of β-hydroxysulfone diastereomers (figure 13.2-1).



(figure 13.2-1)

However, initial attempts at the production of the coupled material resulted in the bulk recovery of unreacted starting sulfone. A further reaction was carried out where the crude product was used directly in a reductive elimination reaction. In

this situation no noticeable quantity of olefinic product was observed thus indicating that the recovery of starting sulfone was not due to the reverse reaction occurring during the isolation process.

As previous problems were noted with DIBAL-H reactivity and aldehyde stability, these factors were initially blamed for the failure of the coupling reaction. The aldehyde/aluminium complex was also considered by Hopkins to be highly unstable, even at -78°C. Extensive experimentation was therefore carried out where the aldehyde was rigorously purified, aluminium complex formation techniques varied and fresh DIBAL-H (1.0 M solution in THF) employed. No improvement in results was noted for any of these experiments.

In situ formation of aldehyde, from the methyl ester, was attempted using DIBAL-H (1.0 M solution in THF) and DIBAL-H (1.0 M solution in hexane) with the direct transferrance of these crude reaction mixtures to the sulfone dianion solution. Again no improvement was noted.

It has been reported that the use of lithio derivatives of sulfones can give poor coupling results due to α-proton abstraction from highly enolisable aldehyde carbonyls.[105] In such situations, the use of ethylmagnesium bromide in place of methyl lithium gives improved yields. By the method of Lythgoe *et al*, the coupling was therefore attempted using this alternative base.[105] While mainly starting sulfone and β-elimination products were yielded by this method an interesting feature was also noted. At the higher temperatures employed in this procedure (0°C as opposed to -78) the reaction mixture of ethyl magnesium bromide and the starting sulfone in THF was observed to form a clear, colourless solution. To determine whether this was indicative of the decomposition of the sulfone, the solubility of this material at various temperatures was investigated. It was found that, by the action of heat, a completely clear and colourless solution of the sulfone in THF could be achieved. It was also noted that, if this solution was sufficiently dilute, the previously noted colloidal nature would not return on cooling to -78°C.

Thus it was postulated that the previous coupling reaction failures were not due to aldehyde problems, but instead caused by the poor performance of the sulfone as a fine suspension. The next stage was therefore to test this hypothesis by reacting the sulfone solution pre-warmed, at high dilution. Obviously reaction times would also have to be extended to compensate for the lower concentration.

Though the modification discussed here may appear simple, there is reason to believe that this problem was not defined or overcome by the Hopkins group as will be discussed in section 13.5.

13.3 Application of the Coupling Modification

On application, the coupling modification showed immediate signs of improvement. No permanent yellow colouration was formed in the sulfone solution until after one full equivalent of methyl lithium had been fully added. Addition of the second equivalent rapidly increased the intensity of the colouration suggesting that the dianion was responsible for the presence of this.

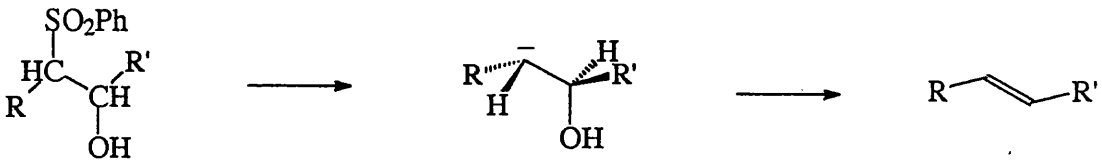
The ^{1}H NMR spectrum of the crude product formed by completing the coupling process with this solution gave an indication of at least partial success as the *N*-BOC group for the starting sulfone was joined by a second similar peak approximately 0.03 ppm further upfield. No other worthwhile information could be obtained from this spectrum due to the highly diastereomeric nature of the β-hydroxysulfone and the presence of unreacted starting materials and their decomposition products.

It was discovered that from the crude oily product mixture a quantity of clean white solid could be precipitated by the addition of ether. This material was identified as being the starting sulfone and, as the associated *N*-BOC group was no longer evident in the ^{1}H NMR spectrum of the material obtained by

evaporation of the mother liquor, the quantity isolated was used to gauge the extent of the coupling reaction. A rough calculation suggested that 78% of the starting sulfone had either reacted or been modified in some way. It was decided that the best test of β-hydroxysulfone formation would be the reductive elimination of this material to form the desired olefin. As this process would result in the removal of undefined chirality at two centres, effective product purification should then also be possible.

13.4 Reductive Elimination of the β-Hydroxysulfone

Reductive elimination of β-hydroxysulfones is thought to occur by initial electron transfer to the sulfone group which induces the expulsion of the benzenesulfinate anion or radical (figure 13.4-1).[106] [107] The intermediate carbanion thus formed is sufficiently long lived to seek the conformation bearing a minimum of steric interaction. *Anti*-periplanar elimination of this conformation would then result in the formation of the *trans*-olefin.



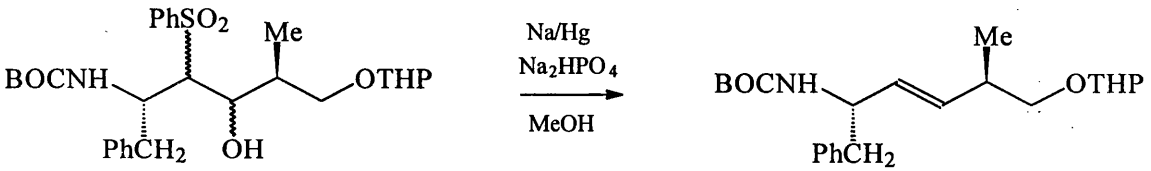
β-hydroxysulfone optimal conformation *trans*-product

(figure 13.4-1)

In systems where the premier carbons of R and R' are further substituted, as in the case under consideration, the selectivity for the *trans* product is expected to be absolute.

Using the crude β-hydroxysulfone mixture, reductive elimination was successfully carried out with 2% sodium/mercury amalgam by the method of Trost (figure 13.4-2).[108] In this procedure, the addition of four equivalents of disodium hydrogen phosphate is specified in order to suppress the base induced retro-aldol decomposition reaction.

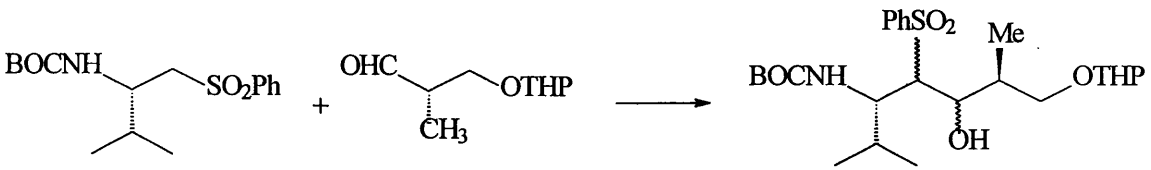
The crude product obtained by following the Trost procedure was purified by chromatography to give the desired olefin in an overall yield of 64% for the two step process from the protected amino sulfone. Analysis by ^{13}C NMR spectroscopy illustrated the diastereomeric nature of this material as pairs of signals (of similar intensity) were observed for carbon atoms close to the stereocentre of the THP protecting group. No other such double signals were observed. Any further diastereomeric nature, resulting from the racemisation of either aldehyde or sulfone, would hopefully have been evident at this stage.

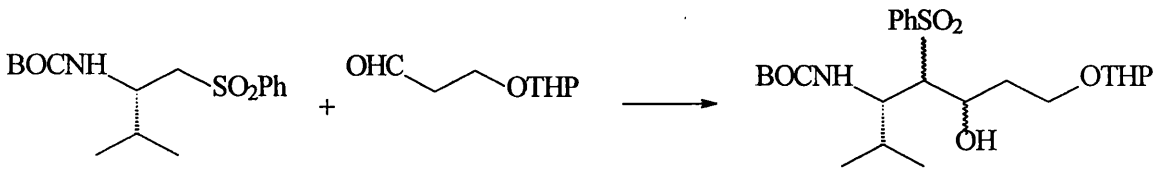


(figure 13.4-2)

13.5 The Coupling of Other Materials

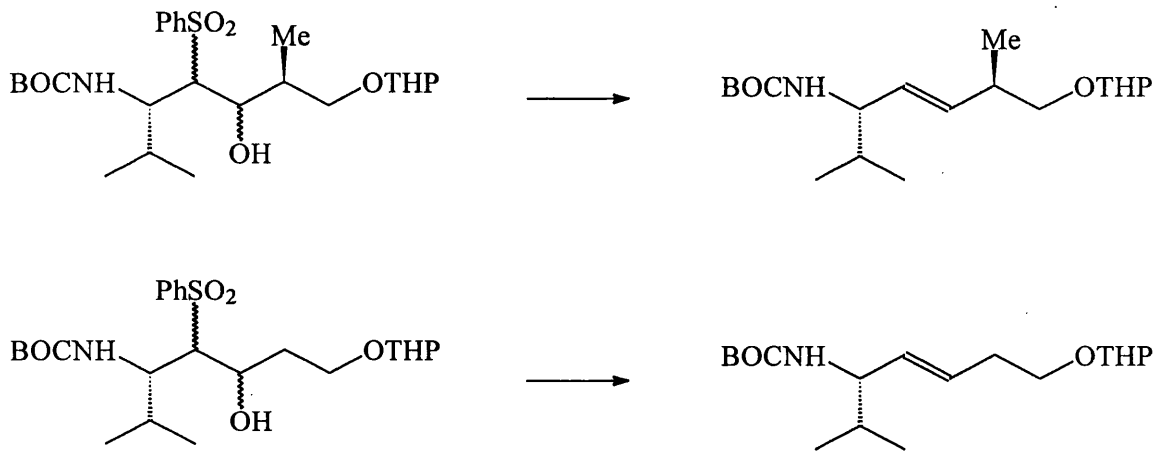
The sulfone derived from *N*-BOC-(L)-valine was individually coupled to both the alanine and glycine mimic precursor aldehydes (figure 13.5-1). In these cases the additional dilution and warming of the sulfone vessel was not required as the valine derived sulfone was found to be sufficiently soluble under the standard conditions. The precipitation of unreacted sulfone after coupling was also found not to be possible. The crude product mixtures were therefore used directly in the reductive elimination step.





(figure 13.5-1)

Reductive elimination of these materials still proceeded smoothly with column chromatography giving the Val-Ala and Val-Gly olefins in yields of 58% and 25% respectively (figure 13.5-2). The lower yield in the Val-Gly case is considered to be mainly due to the higher level of impurity present in the starting aldehyde as, with products bearing such a substitution pattern, high yields and an approximately 90% stereochemical preference for the *trans* olefin are still to be expected.[106] [107]



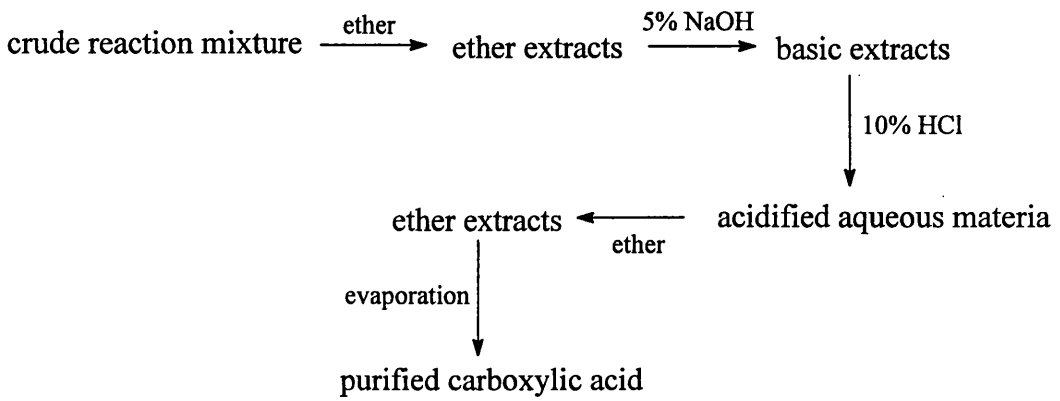
(figure 13.5-2)

It is worthy of note that the yield experienced here for the two stage formation of the (L)-Phe-(L)-Ala olefin (64%) is far higher than that achieved by Hopkins for the (L)-Phe-(L)-Phe analogue (37%). While direct comparison between these two results cannot be made, the fact that Hopkins achieved a 65% yield using the sulfone derived from *O,N*-protected tyrosine, giving the (L)-Tyr[*O*-Bz]-(L)-Phe mimic, suggests that solvation problems of the phenylalanine derived sulfone were not overcome by this group. It is also worthy of note that none of the combinations described here were reported as being formed by Hopkins and co-workers.

Unfortunately, a lack of time prevented the coupling of the leucine derived sulfone.

13.6 Generation of the Carboxylic Acid

Removal of the THP protection and oxidation of the alcohol thus released was effected in one step by the use of Jones' reagent in acetone.[109] [110] Purification of the resulting carboxylic acid was carried out by a sequence of extraction processes as outlined in figure 13.6-1.



(figure 13.6-1)

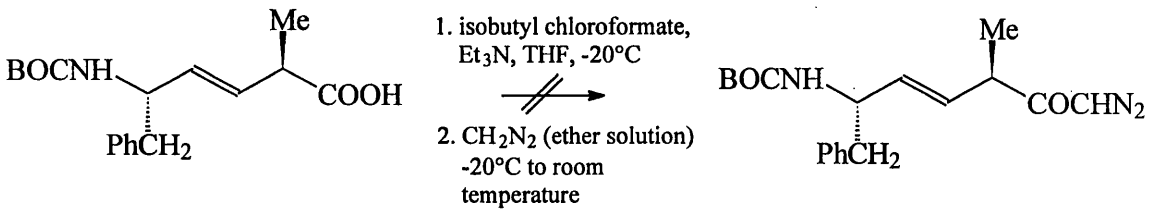
Initial attempts to purify the carboxylic acid of the *N*-protected (L)-Phe-(L)-Ala mimic highlighted an unexpected problem. Retention of chromium by the carboxylic acid was thought to be resulting in extremely poor NMR spectra being obtained for the "purified" product. This problem was solved, after some experimentation, by significantly reducing the volume of the initial organic extracts by evaporation. Separation of a quantity of chromium (III) containing aqueous solution resulted which could then be easily removed thus preventing this material being carried into the aqueous stages of the purification process.

For ease of analysis, a small quantity of this oily material was partially esterified with diazomethane. Column chromatography of the product of this process allowed the isolation of a small amount of pure carboxylic acid in addition to the ester.

Carboxylic acids of the Val-Ala and Val-Gly analogues were similarly produced with, in the case of the former material, further purification being effected by recrystallisation.

Chapter 14: Diazomethyl Ketone Formation

Initial attempts at the conversion of the *N*-BOC-(L)-Phe-(L)-Ala isostere carboxylic acid into the corresponding diazomethyl ketone met with failure (figure 14-1). ^{1}H NMR spectroscopy indicated that the standard method for this conversion had not yielded the desired compound.[54] Instead, the main product of the reaction appeared to be a compound which contained some form of attached isobutanol.

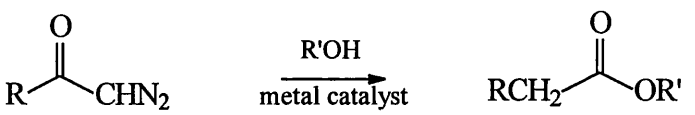


(figure 14-1)

In order to prove that the observed product was not simply due to the failure of the intermediate mixed carbonate to react further, a similar reaction was carried out with phthaloyl protected alanine but without the addition of diazomethane. As expected, the results of this experiment showed that such simple mixed carbonate systems do not survive the aqueous work-up conditions employed.

14.1 Theory 1: Wolff Rearrangement

It is known that diazomethyl ketones can undergo a Wolff rearrangement in the presence of an alcohol and a metal ion catalyst (figure 14.1-1).[111] [112] Such a process could possibly explain the observed bound isobutanol.



(figure 14.1-1)

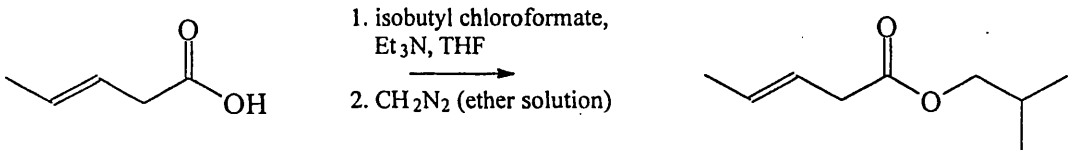
This rearrangement seemed plausible as the presence of residual chromium (III) in the carboxylic acid was a distinct possibility (as outlined in section 13.6). In

order to test this hypothesis, the reaction was carried out using commercial 3-pentenoic acid as a chromium free model compound. However, with this system similar results were experienced. This compound was therefore considered to be a suitable model material and used in further test reactions.

It has been reported that the Wolff rearrangement can be initiated by heat or light without the presence of a metal catalyst.[113] [114] A series of experiments were therefore carried out, using the chromium free model compound, in the absence of light and also at a lower temperature than previously employed (-78°C as opposed to -20°C). In all these cases however, no improvement in results was noted. While it is known that increased conjugation can lower the temperature necessary to initiate the Wolff rearrangement, the conditions required are still far more harsh than those employed in the original diazomethyl ketone formation method.[115] Nevertheless, the modifications employed here to counter such a transformation were still considered to be a reasonable starting point to begin more general investigations into the cause of the reaction failure.

As it was considered possible that some form of transformation may be taking place during product isolation, direct derivatisation of the crude post-reaction material was attempted with both HBr/acetic acid and aqueous HCl. No derivatives of the diazomethyl ketone appeared to have been formed in these reactions.

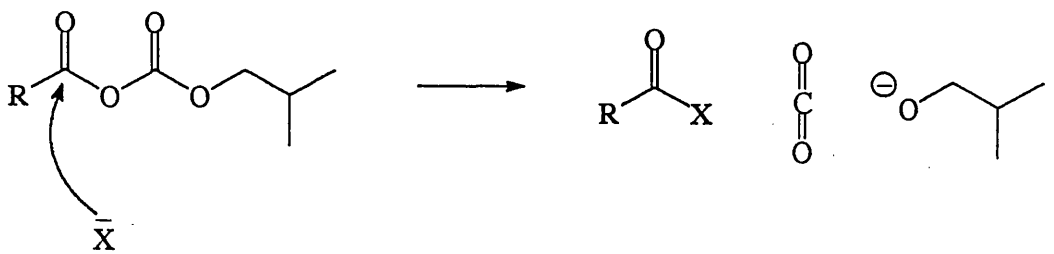
Mass spectroscopic analysis of the common product of these reactions indicated that a Wolff rearrangement had not taken place. The results of this test indicated that the isobutyl ester of the starting acid had been formed (figure 14.1-2) and no incorporation of an additional carbon atom from diazomethane had taken place. After this result, work began on an alternative avenue of investigation.



(figure 14.1-2)

14.2 Theory 2: Reagent Problems

Under standard conditions, the diazomethyl ketone generation reaction was attempted using fresh isobutyl chloroformate. No improvement in results was noted but an interesting feature was observed in a concurrently run reaction employing what appeared to be an old and impure sample of ethyl chloroformate. As with previous diazomethyl ketone formation failures, but not successes, a faint green/yellow colouration was evident in the reaction mixture before the addition of diazomethane. In the case of ethyl chloroformate this colouration was stronger and its evolution more rapid. It was therefore postulated that catalytic decomposition of the mixed carbonate was taking place, initiated by the attack of an impurity nucleophile.[116]



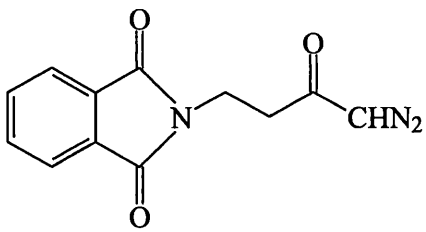
(figure 14.2-1)

The initiation stage in the catalytic decomposition of the mixed carbonate formed from isobutyl chloroformate is shown in figure 14.2-1. Note that once initiation has taken place, the process can be repeated by the generated isobutoxide. Such a chain reaction would also explain the isobutyl peaks observed in the product ^{1}H NMR spectrum. Note also that in the ethyl chloroformate case, incorporation of an ethyl group into the product occurred as would be expected by this hypothesis.

Following this observation, reactions were attempted with a 5% molar equivalent of acetyl chloride either pre-mixed with the reagent or added to the reaction system prior to the reagent. In theory this compound may remove the initiating nucleophile without generating a similarly active species. A reaction was also attempted at high dilution where the propagation steps of the chain reaction may be more limited. In all these reactions the triethylamine employed (see figure 14-1) was freshly dried, distilled and used promptly without being stored over further drying agents in order to eliminate the possibility of the contamination of this material. However, no change in the observed product was noted for any of these reactions with the isobutyl ester being the main compound yielded.

14.3 Theory 3: Problems Associated With the Extended Chain

In order to prove that the standard method for the formation of diazomethyl ketones from α-amino acids was applicable to compounds with a longer carbon chain, the outcome of the reaction with β-alanine was investigated. As expected, this compound was *N*-phthaloyl protected and converted into the diazomethyl ketone (figure 14.3-1) by the method outlined in figure 14-1 with no significant problems.[54] [82]

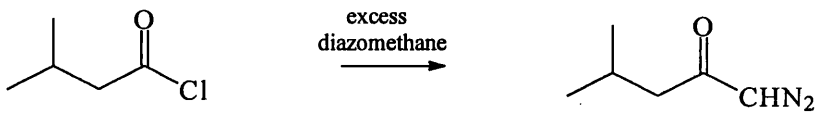


(figure 14.3-1)

It was decided that the next logical step in the investigation would be an attempted synthesis of the diazomethyl ketone using an alternative strategy for the activation of the carboxylic acid.

14.4 Alternative Activation of the Carboxylic Acid

Classically, diazomethyl ketones were synthesised from the corresponding acid chloride.[117] Indeed, such methodology has been successfully applied to *N*-phthaloyl protected amino acids.[118] [112] [119] The main drawback of using this route is the production of hydrochloric acid in the reaction of the acid chloride with diazomethane. Such a by-product may react with the product diazomethyl ketone giving the corresponding chloromethyl ketone which also possesses biological activity (as discussed in chapter 4).[90] The extent of chloromethyl ketone formation can be limited by the presence of a large excess of diazomethane. Despite this, careful product purification may still be required. A rapid test reaction with isobutyryl chloride indicated that this route could be used to produce samples of diazomethyl ketones (figure 14.4-1).



(figure 14.4-1)

Studies were therefore initiated with the aim of producing the acid chloride of 3-pentenoic acid.

In 1966 Lee reported the formation of acid chlorides by heating the carboxylic acid with triphenylphosphine in carbon tetrachloride (figure 14.4-2).[120] This conversion procedes with no generation of highly acidic by-products.

$$RCOOH + CCl_4 + (C_6H_5)_3P \longrightarrow RCOCl + (C_6H_5)_3PO + HCCl_3$$

(figure 14.4-2)

This reaction was attempted several times with 3-pentenoic acid but direct treatment of the product with diazomethane did not yield the desired diazomethyl ketone. Although infra-red analysis was hampered by the presence of by-products, this technique was employed in a more detailed study which suggested that formation of the acid chloride had not taken place.

On repeating this reaction with phthaloyl protected alanine, a small quantity of diazomethyl ketone was formed. The main product in this case was the methyl ester indicating incomplete formation of the acid chloride. Such simple identification of the complex by-products formed in the 3-pentenoic acid situation was however, not possible.

As stated earlier, reacting acid chlorides with diazomethane generates hydrochloric acid in addition to the diazomethyl ketone. Were this by-product not to be rapidly and efficiently removed from the system further reaction of the diazomethyl ketone would occur. In this situation formation of the chloromethyl ketone would be expected. In order to obtain a typical value for the ^1H NMR chemical shift of a chloromethyl ketone group, a residual sample of phthaloylalanyl diazomethyl ketone was deliberately treated with hydrochloric acid. The generation of the chloromethyl ketone was confirmed by full analysis of the product with the ^1H NMR chemical shift of the CH_2Cl group being observed at 4.20 ppm. No signal in this area was observed in the 3-pentenoic acid reactions indicating that such derivatisation of the diazomethyl ketone had not taken place. The problem was therefore considered to reside in the formation of the activated species.

14.5 Further Acid Chloride Investigations

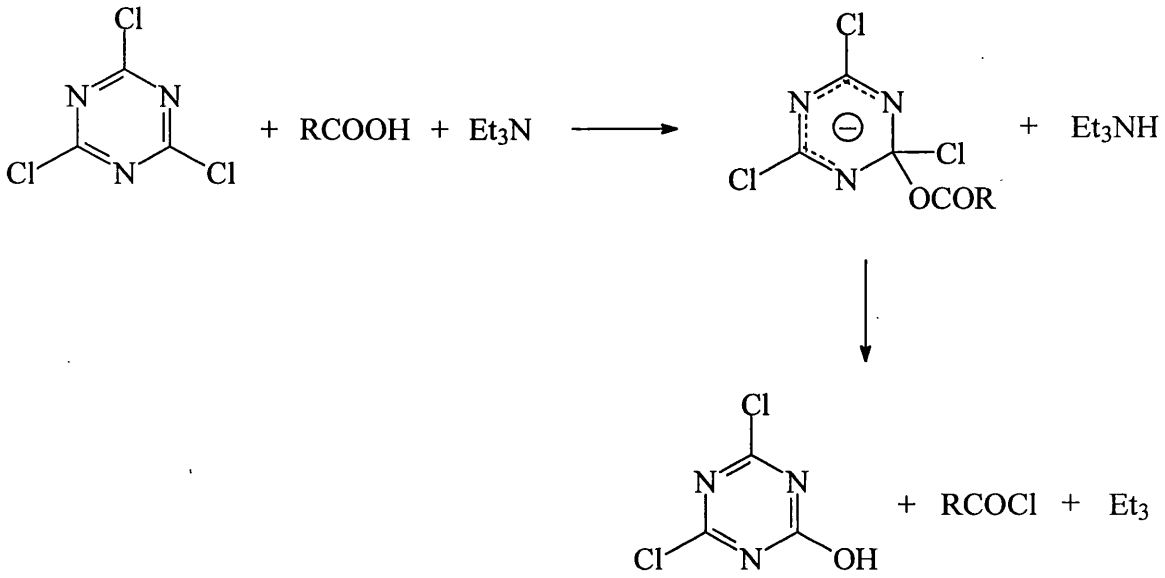
The use of oxalyl chloride as a reagent for the formation of acid chlorides is generally limited by the accompanying generation of acidic by-products as shown in figure 14.5-1.[121]

$$RCOOH + (COCl)_2 \longrightarrow (RCOOCO)_2 + 2HCl$$
$$(RCOOCO)_2 \longrightarrow (RCO)_2O + CO_2 + CO$$
$$(RCO)_2O + (COCl)_2 \longrightarrow 2RCOCl + CO_2 + CO$$

(figure 14.5-1)

As expected, carrying out this reaction by the standard method on *N*-BOC protected (L)-phenylalanine resulted in starting material decomposition. Attempts were then made to reduce the severity of the conditions by increasing dilution and reducing the reaction temperature to under 0°C. No improvement in results was noted for these modifications but the incorporation of triethylamine into the reaction mixture showed some signs of promise with reduced decomposition. The extent of the acid chloride formation was however, not investigated. At this stage, studies in this area were halted due to an important discovery which will be discussed in section 14.6.

In 1979 Venkataraman and Wagle reported the generation of acid chlorides in high yield by the use of cyanuric chloride (figure 14.5-2).[122]



(figure 14.5-2)

In general, this mild reaction is carried out at room temperature in acetone with 0.5 molar equivalents of the reagent. An added bonus arises from the precipitation of the by-product 2,4-dichloro-6-hydroxy-1,3,5-triazine during the reaction thus reducing the need for purification.

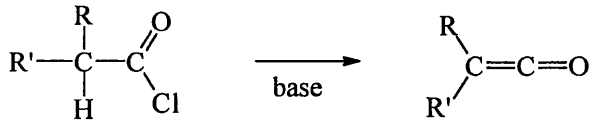
This conversion was attempted with *N*-BOC-phenylalanine and the product treated directly with diazomethane. The resulting crude mixture was shown, by

comparative thin layer chromatography in several solvent systems, to contain the desired diazomethyl ketone. Partial purification by column chromatography followed by ^1H NMR analysis in both $CDCl_3$ and d_6-DMSO allowed the presence of this compound to be confirmed. It was also indicated however, that purification would not be as straightforward as predicted.

The reaction was repeated with IR analysis in order to gauge the success of acid chloride formation. Unfortunately the spectra obtained from the crude reaction mixture were extremely complex and therefore ambiguous.

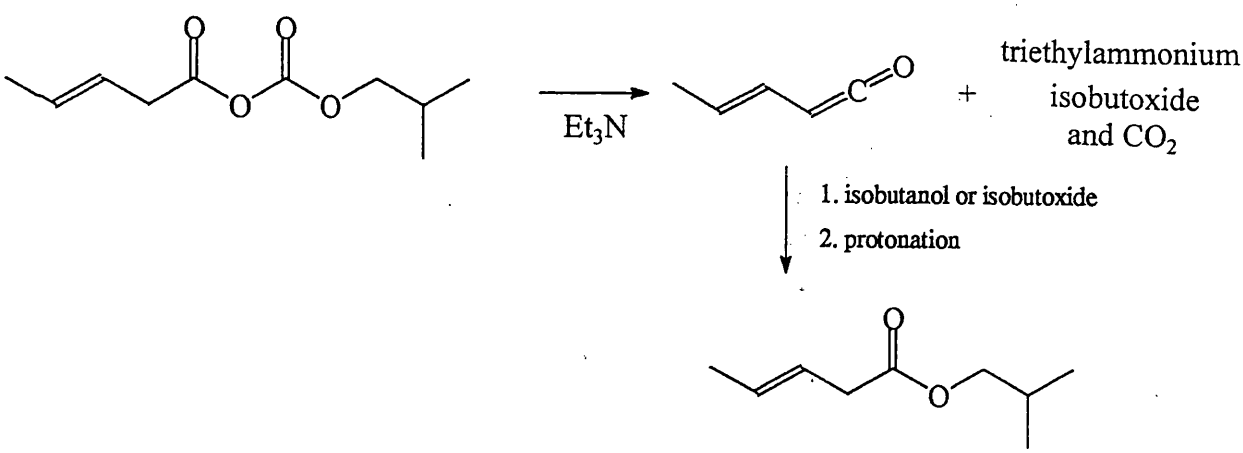
As with the oxalyl chloride method, studies in this area were terminated at this stage for reasons which will now be explained.

The search for further acid chloride formation methods revealed a problem which may be more generally applicable to the system under study. In the presence of a suitable base, the α-hydrogen of an acid chloride may be extracted allowing the formation of a ketene (figure 14.5-3).[123] Note that this mechanism can be considered as analogous to that of methanesulfonyl chloride with triethylamine as described in section 12.1.



(figure 14.5-3)

It is therefore postulated that in βγ-unsaturated systems triethylamine would be a sufficiently powerful base to promote this reaction. Indeed, diphenylketene has been isolated following the addition of triethylamine to diphenylacetyl chloride at 0°C.[124] Were this reaction to occur during attempted βγ-unsaturated acid chloride formation, the observed products may be extremely complex but in the case of mixed carbonate activation, the corresponding ester would conceivably be formed as illustrated in figure 14.5-4.



(figure 14.5-4)

The deliberate formation of ketenes in the presence of trapping species has been carried out.[123] Furthermore, pyridine has been reported as being a suitable catalyst for the acetylation of glucose with ketene.[125] Notice that in the process illustrated in figure 14.5-4 the base employed can be considered as catalytic since it may be reformed in the final stage of the reaction. It is also possible that the deprotonation process may be continued by the generated isobutoxide.

The formation of such a ketene may also explain the colouration noted during unsuccessful mixed carbonate reactions (see section 14.2).

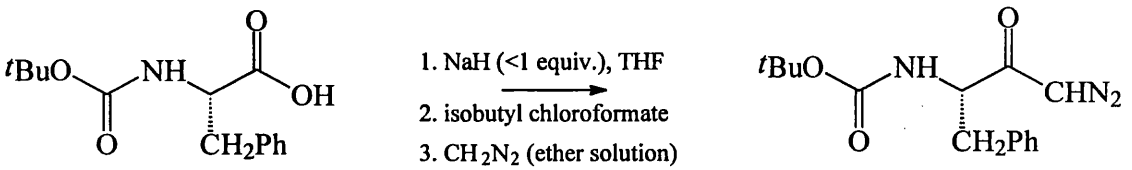
14.6 The Use of an Alternative Base in Mixed Carbonate Formation

Racemisation, by C-terminal α-hydrogen exchange, during the mixed carbonate method of peptide synthesis has been the subject of an extensive study.[126] It was reported that, if racemisation were a problem it could be reduced to zero by the use of *N*-methylmorpholine as a base and a 5% excess of isobutyl chloroformate. This modification was therefore incorporated into the standard method for diazomethyl ketone synthesis. However, carrying out the procedure with 3-pentenoic acid once again resulted in the formation of the isobutyl ester.

According to Anderson *et al* interaction between the base (normally triethylamine) and chloroformate prior to coupling is essential for high yields.[126]

Despite this it was decided to investigate the use of sodium hydride in this reaction with the following reasoning. With this reagent, rapid neutralisation of the carboxylic acid would result in the generation of gaseous hydrogen which will not interfere with the further progress of the reaction. Such an assurance cannot be given with triethylamine as, despite the precipitation of the hydrochloride salt, the potential remains for the reformation of a small amount of the free base. This catalytic quantity of base may initiate the decomposition of the mixed carbonate.

A reaction was therefore attempted with the slow addition of just under one equivalent of sodium hydride (suspended in THF) to a THF solution of *N*-BOC-(L)-phenylalanine (figure 14.6-1). With this stoichiometry complete reaction of all hydride would be expected. Addition of isobutyl chloroformate and diazomethane followed in the usual manner and resulted in the formation of the desired diazomethyl ketone.

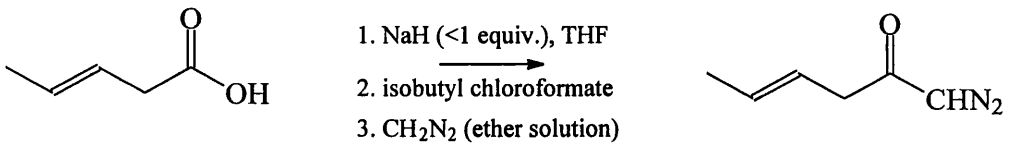


(figure 14.6-1)

The yield (27%) and product purity were, as expected, lower than those achieved by the standard route. At this stage it could not be deciphered if this was due to the lack of a base-chloroformate complex or lower than expected assay of sodium hydride.

Following the success of this reaction, the same conditions were employed with the aim of effecting a similar conversion on 3-pentenoic acid (figure 14.6-2). Analysis of the product by ^{1}H NMR spectroscopy in both $CDCl_3$ and d_6-DMSO indicated the presence of the desired diazomethyl ketone end group. In order to simplify the procedure, the reaction was repeated where the effects of adding the solution of the acid to a hydride suspension were investigated. Results

indicated that the combining of these two materials could be carried out in either order at -78°C without a detrimental effect on the course of the reaction.



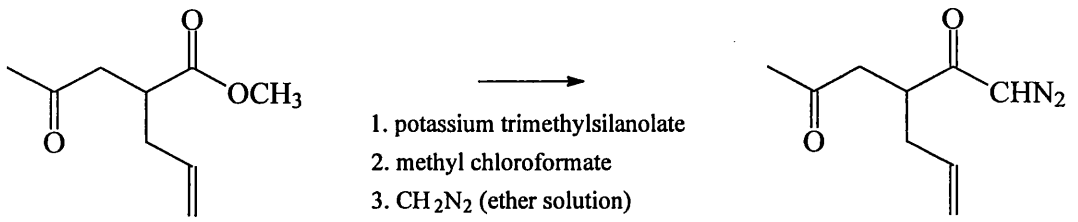
(figure 14.6-2)

After some product manipulation, samples of 3-butenyl diazomethyl ketone were isolated and characterised. The product yields obtained for this conversion were, as expected, not high (typically 15%).

This methodology was next tested on the *N*-BOC-(L)-Phe-(L)-Ala isostere. Extensive thin-layer chromatographic anaysis of the product indicated that two main compounds were present. As the more polar of these was observed to be more active to UV irradiation it was presumed that this material would be the desired diazomethyl ketone. Repeating this chromatographic study with a mixture of the reaction product and a known sample of isostere methyl ester indicated that this compound may be the main by-product. ^{1}H NMR analysis of the product mixture, in both $CDCl_3$ and d_6-DMSO, confirmed these findings with it being possible to show by integral comparison (NH, CHN_2, OCH_3) that a ratio of approximately 60% diazomethyl ketone to 40% methyl ester had been obtained. Unfortunately an insufficient quantity of this material remained for further purification to be attempted.

14.7 Potassium Trimethylsilanolate Method of Salt Formation

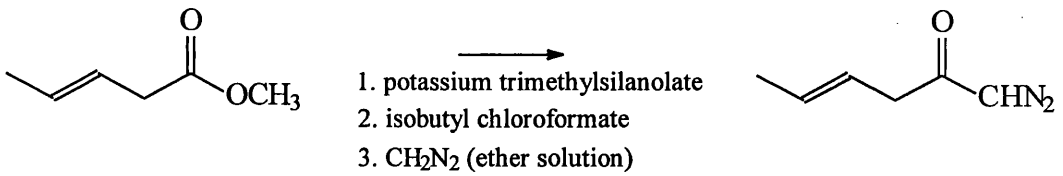
In 1984 Laganis and Chenard reported the use of potassium trimethylsilanolate as a reagent for the formation of carboxylate salts from the corresponding methyl esters.[127] This methodology was later employed by Padwa to give a 61% yield of a diazomethyl ketone (figure 14.7-1) by a route which can be considered as analagous to the sodium hydride method discussed in section 14.6.[128]



(figure 14.7-1)

The potential use of the methyl esters of isosteres as starting materials made this route highly attractive as these compounds are much more readily purified than carboxylic acids. With simplified isostere purification in mind, it was decided to investigate the use of this reagent on the methyl ester of 3-pentenoic acid.

Prior to the addition of the chloroformate, good cleavage of the methyl ester was indicated by ^{1}H NMR spectroscopy after acidification of a small portion of reaction mixture. Continuing the reaction with isobutyl chloroformate resulted in the formation of the desired diazomethyl ketone (figure 14.7-2) as shown by IR and NMR spectroscopic analysis. The isolated material compared well with that obtained by the sodium hydride route. Once again however, product yield was low but tolerable (23%).



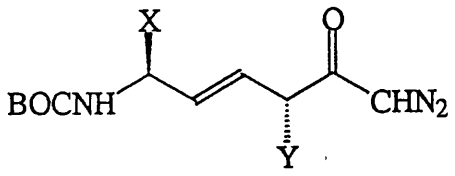
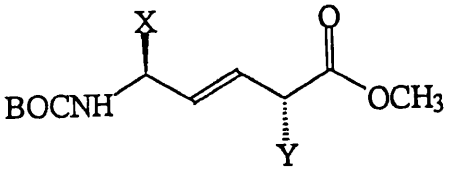
(figure 14.7-2)

Following this success, a quantity of the Phe-Ala isostere was esterified with diazomethane. An attempt was then made at converting this material into the corresponding diazomethyl ketone which resulted in the generation of at least six reaction products. After partial purification by column chromatography, ^{1}H NMR spectroscopy was used to show that none of these compounds were the desired diazomethyl ketone. It is worthy to note that destruction of the olefin appeared to be a very important, and still unexplained, side reaction.

14.8 Isostere Diazomethyl Ketone and Methyl Ester Formation

With the failure of the potassium trimethylsilanolate test reaction it was decided to form the isostere diazomethyl ketones using the sodium hydride/isobutyl chloroformate methodology. It has been shown that in this reaction the main by-product is the corresponding methyl ester which may also be used as a control material in comparative biological testing. Such a control material was required as it was expected that active diazomethyl ketones would cause some degree of parasite growth inhibition by reversibly blocking the substrate binding site of the protease enzyme. Such a process may still occur if the reactive diazomethyl ketone end group is not ideally placed for irreversible binding. The methyl ester on the other hand, lacking the active site modifying group, can exert an inhibitory effect only in the reversible manner. Thus, by comparing the results obtained for both compounds, the degree of growth inhibition due to irreversible activity can be deduced. By using the hydride methodology it may therefore be possible to produce both the potentially active compound and a relevant control material in a single reaction.

Application of the developed methodology to the three isosteres gave, as expected, low yields of diazomethyl ketones and reasonable yields of methyl esters as shown in figure 14.8-1. The poor yields of diazomethyl ketone are considered to be due mainly to the inherant difficulties in handling small quantities of sodium hydride accurately. As even a tiny excess of base may be sufficient to initiate catalytic decomposition of the activated isostere it was thought wise to err on the side of caution and tolerate such low yields.

Backbone Structure	X	Y	Yield (%)
	$-CH_2Ph$	$-CH_3$	25
	$-CH(CH_3)_2$	$-CH_3$	17
	$-CH(CH_3)_2$	-H	5
	$-CH_2Ph$	$-CH_3$	56
	$-CH(CH_3)_2$	$-CH_3$	49
	$-CH(CH_3)_2$	-H	52

(figure 14.8-1)

Compound purity was not absolute in the case of the Phe-Ala diazomethyl ketone. Repeated attempts at removing the tiny impurity remaining gave limited results and, as the quantity of material remaining was approaching the critical level necessary for biological evaluation, purification was therefore halted. A sample containing elevated levels of this impurity was also produced for use in biological testing in order to clarify results if required.

It is worthy to note at this stage that characterisation of these compounds indicated that the desired *trans* double bond was indeed present. Furthermore, no signs of diastereomer formation by racemisation of either chiral centre, were observed by ^1H NMR spectroscopy. All six compounds also proved to be optically active.

Molecular mechanics calculations were carried out on the diazomethyl ketones of both the *N*-BOC-(L)-Val-(L)-Ala dipeptide and (*E*)-ethene isostere. The results of this study (shown in appendix 1) confirmed that the (*E*)-ethene group is a reasonable structural replacement for the amide link in this compound. Note

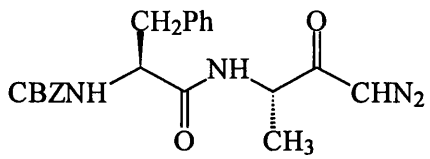
however that this simple calculation does not take into account features such as hydrogen bonding or solvation.

Chapter 15: Anti-parasite Evaluation

The initial test battery of parasites employed in biological evaluation comprised three strains of *Trichomonas*, (*T. foetus* F2, *T. vaginalis* G3 and *T. augusta*) *Leishmania mexicana* (wild type and the genetic modifications N53, ΔN8m and ΔC10), *Giardia lamblia* and *Giardia intestinalis*.

Such a selection of parasites should allow a reasonable screening of compounds for activity. With *Trichomonas vaginalis* at least 11 distinct cysteine protease activities have been detected. Similarly *Leishmania mexicana* possesses at least seven cysteine proteases while two major such enzymes are found in *Giardia Lamblia*.[6] In the case of *Leishmania mexicana*, genetically modified strains where the genes coding for specific cysteine protease enzymes are disrupted, were also selected for initial testing. The absence of activity in only a selection but not all of these modified variants would confirm the action of the compound under investigation as a cysteine protease inhibitor.

It is also worthy to note that, in addition to the synthesised compounds, a sample of ZPA (*N*-CBZ protected, amide linked (L)-Phe-(L)-Ala peptide bearing a diazomethyl ketone end group, figure 15-1) was obtained and used in testing.



(figure 15-1)

Biological evaluation of the compounds was carried out in two ways:

1. Protease enzyme inhibition (SDS-PAGE)

In order to test for protease enzyme inhibition, the parasites were incubated in the presence of each compound and the cell extracts separated by gel

electrophoresis. As the strongly coloured gel on which the extracts are separated is composed of an amide linked polymer, destruction of this material can be observed at positions occupied by protease enzymes. Thus a visual fingerprint of the protease activity of the cell can be obtained. Inhibition of such activity, by the presence of the test compound, would be indicated by the absence of one or more such zones of destruction. Obviously a control gel is run concurrently using cells incubated in the absence of the compound under evaluation.

2. *In vitro* parasite growth reduction

As protease activity is essential for parasite cellular function, one would expect an inhibition of the growth of such species in the presence of an active compound. Isolated parasites were therefore incubated in the presence of various concentrations of test compound. A control sample containing no such potential inhibitor was treated similarly. In each case the number of parasite cells in a specific volume of culture were counted visually after incubation. From this it is possible to gauge the minimum dose of each active compound required for 100% lethality.

15.1 Results of Biological Evaluation

The results of first and second round biological evaluation are shown in appendices 2 and 3 respectively.

Testing indicated that the *N*-BOC-(L)-Phe-(L)-AlaCHN_2 isostere is a highly capable inhibitor of the growth of *Leishmania mexicana*. Since the corresponding ester was shown to have no such effect it can be concluded this action is due to the presence of the diazomethyl ketone function. It is also interesting to note that the amide link containing *N*-CBZ-Phe-AlaCHN_2 did not exhibit this inhibiting ability. As the replacement of a *N*-CBZ protection for a similar carbamate based group (*N*-BOC) should have little effect on the action of

the inhibitor (see section 6.2), one can assume that the evolution of activity is due to the presence of the isostere. The lack of performance of the amide linked compound, which is known to be a potent inhibitor of isolated cysteine protease enzymes (see section 5.1), may be due to the hydrolysis of this group by the battery of proteases possessed by the organism. If this were indeed to be the case, the suitability of (*E*)-ethene as an isosteric replacement for the amide link would be implied. This result does not however, indicate what level of inhibitor activity towards the isolated target enzyme is retained following such a structural replacement. It also has not been proven that growth reduction was indeed achieved by cysteine protease inhibition. No major impact on the visual proteolytic fingerprint was observed and the genetic modifications of the parasite proved to be similarly susceptible to the attentions of the inhibitor.

It is therefore suggested that studies on the *N*-BOC-(L)-Phe-(L)-AlaCHN$_2$ isostere are continued. Initially, the activity of the molecule against isolated cysteine proteases must be evaluated and compared with that of the amide linked *N*-CBZ-(L)-Phe-(L)-AlaCHN$_2$. The Cathepsins B, H and L would be sutable enzymes for use in this study (see section 5.1).[51] Should this work prove to be fruitful, attempts must then be made at the conversion of the *N*-BOC-(L)-Phe-(L)-AlaCHN$_2$ isostere to the corresponding fluoromethyl ketone or substituted phenoxymethyl ketone (see section 10.8), for further testing in intact biological systems. The methodology required to effect these conversions has, however, yet to be tested.

Prior to further work on the isostere it is also considered wise that similar biological testing be carried out on the impurity noted as being present in the sample of the *N*-BOC-(L)-Phe-(L)-AlaCHN$_2$ isostere (see section 14.8). It is worthy to note that this impurity was not observed as being present in the mixed sample of ester and diazomethyl ketone produced during preliminary studies (see section 14.6). It is therefore suggested that a simple rapid test be carried out on *Leishmania mexicana* using this early mixed material. A sufficient quantity of the mixture should be available for such a study and as the ester has

now been shown to be inactive, clarification of the diazomethyl ketone test results should be possible.

Biological evaluation of the *N*-BOC-(L)-Val-(L)-AlaCHN$_2$ isostere indicated that a limited degree of growth inhibition could be achieved on the three species of *Trichomonas* employed. Such an activity was not noted for the corresponding ester. Later testing showed both diazomethyl ketone and ester to be slightly active on *Leishmania mexicana*. In this situation, however, adequate discrimination between the activity of these two materials was not achieved. Despite this slight activity neither compound had a noticeable impact on the visual proteolytic fingerprint of the parasites.

An unexpected result was obtained on the evaluation of the *N*-BOC-(L)-Val-Gly isostere based pair of compounds. While the diazomethyl ketone had no noticeable effect on the proteolytic activity of the organisms tested, this was not the case with the "control" methyl ester. This compound reduced the activity of enzymes from *T. foetus*, *T. vaginalis* and *T. augusta* to some extent. In addition to this, the ester proved capable of completely halting the activity of at least one very powerful protease of wild type *L. mexicana*.

Parasite growth studies in the presence of the *N*-BOC-(L)-Val-Gly isostere based compounds partially confirmed these findings. Relatively low concentrations of the ester proved to be extremely toxic on both wild type and genetically modified *L. mexicana.* A significant degree of toxicity was also noted on *T. foetus*, *T. vaginalis* and *Giardia Intestinalis*.

Since in many cases the corresponding diazomethyl ketones caused some reduction in growth without an obvious effect on the proteolytic fingerprint, it can be postulated that both compounds may show a more general toxicity. Such a toxicity may be explained by a reduction in selectivity due to the absence of a position 2 side chain. It is expected that this factor could be tested further on isolated enzyme systems.

Chapter 16: Conclusions

Protease enzymes can be selectively inhibited by *N*-protected dipeptides bearing a modified carboxy terminus. The diazomethyl ketone and fluoromethyl ketone modifications of the carboxy terminus are known to be effective in this respect with the fluoromethyl ketone being more selective and efficacious. However, as little is known about individual protease enzymes, the most appropriate side chains for the dipeptides has not yet been defined.

In addition to this, the amide link of a dipeptide based inhibitor is itself vulnerable to the action of protease enzymes. The use of amide bond isosteres, which may alleviate this problem, has not yet been reported in this particular application. Studies on other biologically active peptides have shown however, that the (*E*)-ethene linkage can be used as a replacement for the amide group without causing a reduction in activity. The suitability of this group has also been suggested by crystallographic data and molecular mechanics calculations.

16.1 Synthesis of the Fluoromethyl Ketone End Group

The amino acids (L)-phenylalanine, (L)-alanine, glycine and β-alanine were *N*-phthaloyl protected and successfully converted into the corresponding diazomethyl ketones using literature procedures. The synthesis of the diazomethyl ketone derived from *N*-BOC-(L)-phenylalanine was also shown to proceed efficiently by the same methodology. For these experiments the required diethyl ether solution of diazomethane was safely produced by the alkali induced decomposition of Diazald®.

As literature methodology for the direct formation of fluoromethyl ketones from diazomethyl ketones proved to be unreliable, the use of bromomethyl ketones as intermediates was investigated. Using dry hydrogen bromide gas, liberated from triphenylphosphonium bromide, bromomethyl ketones were successfully formed from the diazomethyl ketones derived from *N*-phthaloyl protected (L)-

phenylalanine, (L)-alanine and glycine. High yields were recorded for these conversions and analysis of the (L)-phenylalanine derived product indicated that stereochemical integrity had been maintained.

While the application of this methodology to the *N*-BOC protected material resulted in the destruction of this group, it is considered that optimisation of the reaction conditions would allow the selective generation of the desired bromomethyl ketone in the presence of such an amino terminus protection.

Reaction conditions were developed in order to allow the high yielding conversion of bromomethyl ketones into fluoromethyl ketones using triethylamine trishydrofluoride. Whether this process gives rise to a mainly racemic product has not been ascertained.

The techniques employed here for the synthesis of bromomethyl ketones and fluoromethyl ketones can, at this stage, be considered as complimentary to literature methodology. Studies in this area were not pursued as it has recently been reported that a variety of phenoxymethyl ketone based end groups have been developed and used in preference to fluoromethyl ketones.[94] Methodology for the production of such end groups from diazomethyl ketones has been identified.

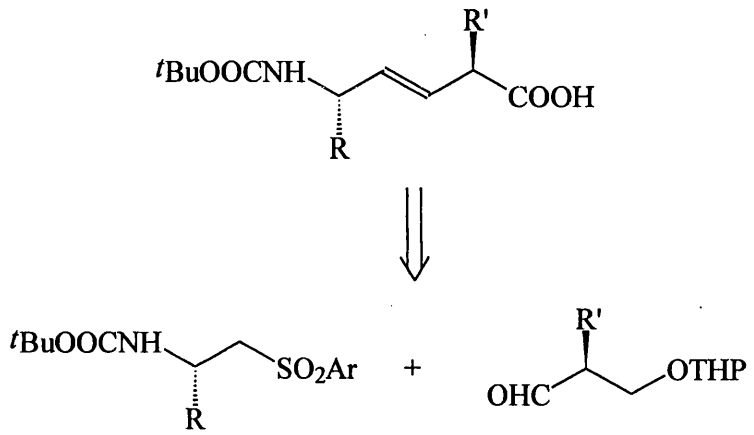
16.2 (*E*)-Ethene Isostere Synthesis - Route #1

The initial route chosen for the synthesis of the *N*-BOC-(L)-Phe-(L)-Ala (*E*)-ethene isostere required the use of *N*-BOC-(L)-phenylalaninal as a starting material. This material was produced efficiently *via* the *N*-methoxy-*N*-methylcarboxamide. The addition of vinylmagnesium bromide to this aldehyde resulted in the generation of a pair of diastereomers of which the minor product was required for further elaboration. Effective separation of these diastereomers was not achieved.

Since it has been reported that the replacement of vinylmagnesium bromide by vinylzinc chloride in the above reaction significantly increases the proportion of the major isomer formed, the use of this reagent may make viable the production of the *N*-BOC-(L)-Phe-(D)-Ala (*E*)-ethene isostere and associated analogues.

16.3 (*E*)-Ethene Isostere Synthesis - Route #2

The second route chosen for study utilised the Julia olefin synthesis as the key synthetic step. The two main groups of precursors required for this convergent isostere synthesis are shown in figure 16.3-1.



(figure 16.3-1)

The *N*-protected β-amino sulfones required for this reaction were produced from *N*-BOC-(L)-phenylalanine, *N*-BOC-(L)-valine and *N*-BOC-(L)-leucine by a four step sequence. This route was found to be extremely reliable with high yields of the desired product being obtained in both small and large scale studies. Purification of both intermediate and final products was found to be straightforward. Optical rotations comparable to literature values were obtained for the product sulfones.

The initial aldehyde (R' = CH_3) employed in the coupling reaction was produced from methyl 3-hydroxy-2(S)-methylpropionate using diisobutylaluminium hydride. It was found that the use of fresh reagent was essential for the successful generation of the desired aldehyde. Poor results were obtained using material

which had been stored for any length of time after first use. It was also found that oxidation of the product aldehyde could be limited by storage at 0 °C under a nitrogen atmosphere.

Studies on commercially available *trans*-3-pentenoic acid indicated that α-alkylation may be carried out without inducing a shift in the position of the unsaturation. From this result it can be concluded that 4-methyl-3-pentenoic acid, as employed in literature syntheses of alternative aldehydes ($R' \neq CH_3$), would not be required.

For an aldehyde precursor to a position 2 glycine mimic (R' = H), a route involving the ozonolysis of *O*-protected *cis*-3-hexenol was devised. After some experimentation this approach proved to be successful and can be considered as complimentary to the literature methodology for the formation of this precursor.

16.4 Coupling and Isostere Generation

Initial problems with sulfone to aldehyde coupling were identified as being due to poor sulfone solubility. Thus the incorporation of suitable modifications was required to enable literature methodology to effect this reaction. Procedures were developed by which the extent of β-hydroxysulfone formation could be estimated and, in the case of the *N*-BOC-(L)-phenylalanine derived material, recovery of the unreacted sulfone achieved. It is worthy to note that, for the successful reaction of the sulfone, the formation of a dianion by proton abstraction from the carbamate nitrogen in addition to abstraction α to the sulfone is essential to prevent an elimination reaction from taking place. Thus the selection of an appropriate base is essential. For example, while good yields of product were obtained by the use of methyl lithium, a method employing ethylmagnesium bromide resulted in the gross decomposition of the starting sulfone.

Reductive elimination of the crude β-hydroxysulfones was shown to result in the formation of the desired *trans* olefins. It is recommended that the products obtained at this stage are thoroughly purified in order to remove any residual quantities of the many β-hydroxysulfone diastereomers.

Production of the *C*-terminus carboxyl group was effected by the use of Jones' reagent. Initial purification of the product carboxylic acids was achieved by a sequence of extraction processes with a modification incorporated in order to limit chromium ion retention.

Thus the (*E*)-ethene isosteres of *N*-BOC-(L)-Phe-(L)-Ala, *N*-BOC-(L)-Val-(L)-Ala and *N*-BOC-(L)-Val-Gly were successfully synthesised bearing the desired chirality and double bond geometry.

16.5 Diazomethyl Ketone Formation

The literature methodology, as tested earlier, for the formation of the diazomethyl ketone end group was found to fail with the synthesised isosteres. After much experimentation it was deduced that the observed product was formed by the further reaction of a transient ketene, generated by α-proton abstraction from the intermediate βγ-unsaturated mixed carbonate. A technique was therefore developed by which the formation of this ketene could be prevented and thus the successful synthesis of the desired diazomethyl ketones achieved.

While the yields of diazomethyl ketones obtained by this route were not high, the main by-products were found to be the corresponding methyl esters which were also isolated and used as control materials in biological evaluation. A method of utilising the easily purified methyl ester, as opposed to the carboxylic acid, as a starting material in diazomethyl ketone synthesis was investigated but despite success being achieved with a model system, decomposition of the (*E*)-ethene isostere was found to occur.

It is believed that the modified procedure for the successful generation of diazomethyl ketones from βγ-unsaturated carboxylic acids has not yet been reported in the literature. It is also believed that the three isostere diazomethyl ketones thus formed are novel compounds. While suitable literature methodology for the synthesis of (*E*)-ethene isostere carboxylic acids and methyl esters is available, the application of this to the specific compounds synthesised here (with the exception of the methyl ester of the *N*-BOC-(L)-Phe-(L)-Ala isostere which was synthesised by Yamamoto and colleages[74]) has not been reported.

The diazomethyl ketones of the *N*-BOC-(L)-Phe-(L)-Ala, *N*-BOC-(L)-Val-(L)-Ala and *N*-BOC-(L)-Val-Gly (*E*)-ethene isosteres, together with the corresponding methyl esters and the diazomethyl ketone of the amide linked dipeptide *N*-CBZ-(L)-Phe-(L)-Ala, were screened for activity against a variety of parasitic species. It is believed that the use of (*E*)-ethene isosteres in such an application has not been reported in the literature.

Results indicate that the diazomethyl ketone of *N*-BOC-(L)-Phe-(L)-Ala possesses some form of growth inhibiting activity against *Leishmania mexicana*. This effect was not observed with either the isostere methyl ester or the diazomethyl ketone of the amide linked material. Similarly, the diazomethyl ketone of the *N*-BOC-(L)-Val-(L)-Ala isostere caused a higher degree of growth inhibition than the corresponding methyl ester on several species of *Trichomonas*. Surprisingly however, the methyl ester of the *N*-BOC-(L)-Val-Gly isostere proved to be a powerful anti-protease. Both this compound and the corresponding diazomethyl ketone showed the ability to inhibit parasite growth thus implying the more general toxicity of peptide mimic based inhibitors bearing no position 2 side chain.

Thus it can be concluded that dipeptide isostere diazomethyl ketones show potential as selective parasite growth inhibitors. It is not clear however, if the observed effect is due to cysteine protease inhibition.

Chapter 17: Avenues for Further Investigation

17.1 Further Work on Completed (*E*)-Ethene Isostere Diazomethyl Ketones

While the (*E*)-ethene dipeptide isostere diazomethyl ketones have shown some degree of selective anti-parasite activity, their exact mode of action remains open to question. Furthermore, as intact organisms were used in this study, a comparison with the protease inhibiting performance of amide link containing dipeptides cannot be made. It is therefore suggested that the synthesised (*E*)-ethene isostere diazomethyl ketones and methyl esters are tested on isolated cysteine protease enzymes. The results of this study may then be compared with published data for the amide linked variants. Cysteine proteases such as papain or the cathepsin family should be suitable for such a study as the activity of amide linked *N*-CBZ-(L)-Phe-(L)-AlaCHN$_2$ against these enzymes has been well documented (see section 5.1).

If the above study were to show the selective anti-protease activity of the synthesised diazomethyl ketones, one could then suggest the suitability of dipeptide (*E*)-ethene isosteres, albeit with a more suitable end group than that employed here (see section 10.8), for use against disease states caused by irregularities in cysteine protease action (see section 1.3). Furthermore, the success of the (*E*)-ethene isostere of *N*-BOC-(L)-Phe-(L)-AlaCHN$_2$ against an isolated enzyme would imply that amide hydrolysis was responsible for the failure of amide linked *N*-CBZ-(L)-Phe-(L)-AlaCHN$_2$ *in vivo* (note the observations discussed in sections 2.3 and 6.2 concerning *N*-terminus protection).

Comparative work on isolated enzymes would also require the prior synthesis of amide link bearing diazomethyl ketones of the (L)-Val-(L)-Ala and (L)-Val-Gly structures. By the method discussed in section 10.2, the generation of diazomethyl ketones from the parent *N*-protected dipeptide should be straightforward.

Prior to commencing work on isolated enzymes it is advised that the small sample of the (*E*)-ethene isostere of *N*-BOC-(L)-Phe-(L)-AlaCHN$_2$ highly enriched in the unidentified impurity be tested for anti-parasite activity (see section 14.8). If this impurity, present in tiny amount in the original sample of diazomethyl ketone employed, is in fact responsible for the observed effect, a massive increase in activity would be expected for the enriched sample. Such a result is not anticipated; nevertheless testing must be carried out in order to clarify results.

A small quantity of mixed *N*-BOC-(L)-Phe-(L)-Ala diazomethyl ketone and methyl ester was prepared during initial investigations into product generation (see section 14.6). As this sample of material was not observed to contain the unidentified impurity and as the ester has been shown to be inactive, biological evaluation of this mixture is now an additional option.

Since the result obtained for the isosteric *N*-BOC-(L)-Val-Gly derived pair of compounds appears to be the opposite of that expected, it seems wise to repeat the study with fresh samples of both materials. Growth inhibition studies on a wider variety of organisms, particularly those known to contain few cysteine proteases, should be carried out in order to evaluate the general toxicity of the pair of isosteres. Such a study should indeed be carried out using all six synthesised compounds. Similarly, testing all compounds for inhibiting activity against isolated serine proteases, for which negative results are expected, would be worthwhile.

17.2 Further Work Concerning End Group Synthesis

Literature methodology for the preparation of phenoxymethyl ketones from the corresponding bromomethyl ketones has been uncovered (see section 10.8). Indeed, this methodology may be applied to *N*-BOC protected dipeptides where a yield of 85% can be expected. The bromomethyl ketones employed in this

literature synthesis were generated from the corresponding diazomethyl ketones using 30% hydrogen bromide in acetic acid. Here the reaction was moderated by the use of low temperature and an additional solvent (dichloromethane). While only mediocre yields (typically 40%) were claimed for this process the result suggests that, following further investigation into temperature and dilution effects, the method involving dry hydrogen bromide gas (section 10.5) may be applied successfully to such systems. It is also expected that the increased backbone length of dipeptide isosteres compared with protected amino acids, should reduce the likelihood of competing cyclisation reactions occurring during diazomethyl ketone manipulations.

Thus, it is suggested that the methods for the synthesis of bromomethyl ketones and phenoxymethyl ketones be tested and applied to the completed isostere diazomethyl ketones. Compounds formed during such a study may be tested in biological systems and the results compared with those obtained for the diazomethyl ketones.

Despite phenoxymethyl ketones replacing fluoromethyl ketones as the materials of choice for further study, the synthesis of the latter group may still be of interest. It is unknown if the methodology used in this study for the synthesis of fluoromethyl ketones (section 10.6) gave racemic products. It is therefore suggested that the fluoromethyl ketone generation be repeated in parallel with a study using KF/18-crown-6 in acetonitrile as an alternative. A small quantity of the target fluoromethyl ketone may be obtained by the reaction of the corresponding diazomethyl ketone with HF/pyridine (see section 10.4) for use as a comparison. It has been reported that the chiral nature of the starting material is unaffected during conversion by the latter route.

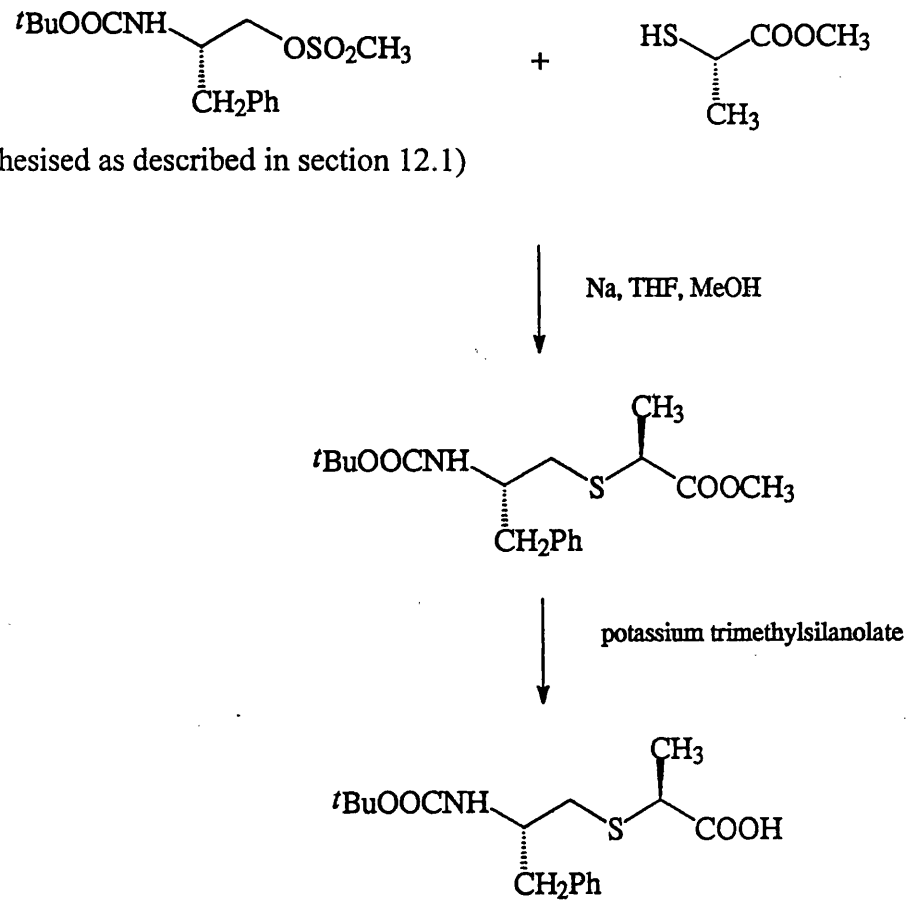
17.3 Further Work Concerning Isostere Synthesis

The method employed here for the synthesis of (*E*)-ethene isosteres may be expanded to encompass a greater variety of side chains. Indeed, literature

methodology which begins to address the problem of incorporating side chains bearing functional groups is available.[76]

In the case of parasite enzyme inhibition, the synthesis of (*E*)-ethene isosteres bearing the non-natural (D)-configuration of the position 2 side chain would be of greater interest. For example, the effect of the (*E*)-ethene isostere of *N*-BOC-(L)-Phe-(D)-AlaCHN$_2$ on parasite growth should be investigated in comparison with the *N*-BOC-(L)-Phe-(L)-AlaCHN$_2$ isostere. It is anticipated that, if the material indeed inactivates an enzyme, the effect would be altered by the incorporation of an amino acid mimic of the non-natural configuration. The methodology discussed in sections 11 and 11.1 may be suitable for such a synthesis. Additional steps would be required, however, as the product isostere would be yielded as the methyl ester.

Fundamental results may be obtained by the synthesis and testing of peptide mimics containing an alternative amide group isostere. Improved results may be obtained, for example, by use of an isostere which is capable of some degree of hydrogen bonding (see section 4.3). The suitability of thioether based groups as amide link isosteres has been suggested by Spatola.[60] Furthermore, the possibility of oxidising such an isostere to generate sulfoxide and sulfone based mimics also exists. A proposed route for the synthesis of thioether based peptide mimics is outlined in figure 17.3-1. Note that the methodology required is, in many cases, similar to that tried and tested during this study.



(synthesised as described in section 12.1)

(figure 17.3-1)

Experimental Preamble

Materials

Solvents for chromatogrophic and general laboratory use were reagent grade or better (BDH General Purpose Reagent, Fischer Scientific Specified Laboratory Reagent, AnalaR Reagent or HPLC Grade).

Solvents and materials for use in reactions were dried in the following ways.

Tetrahydrofuran (THF), diethyl ether and xylene were dried by storage over sodium wire overnight followed by reflux over, and distillation from, sodium wire/benzophenone under a nitrogen atmosphere.

Dichloromethane (DCM) and chloroform were dried by reflux over, and distillation from, diphosphorus pentoxide under a nitrogen atmosphere.

Ethanol and methanol were dried by reflux over, and distillation from, magnesium/iodine under a nitrogen atmosphere.

Acetone was dried by reflux over, and distillation from, activated 4Å molecular sieves.

Carbon tetrachloride was dried by storage over dry calcium chloride.

Triethylamine and *N*-methylmorpholine were dried prior to use by reflux over, and distillation from, potassium hydroxide under a nitrogen atmosphere. These materials were stored for short periods of time over potassium hydroxide. Note that in some cases direct use of the freshly distilled material, without the incorporation of additional potassium hydroxide, was preferred (see section 14.2).

Starting materials and reagents were purchased from the following suppliers.

Aldrich Chemical Company Ltd., Gillingham, Dorset.
Sigma Chemical Company Ltd., Gillingham, Dorset.
Fluka Chemicals Ltd., Gillingham, Dorset.
Avocado Research Chemicals Ltd., Heysham, Lancashire.
Lancaster Synthesis Ltd., Morecambe, Lancashire.

Thin layer chromatography was performed on Merck Silica Gel 60 F_{254} TLC plates with a 0.25 mm coating thickness. Dry flash chromatography (negative pressure) was performed on Fluka Silica Gel H (5 - 40 μm mesh) and positive pressure chromatography on Prolabo Sorbsil Silica Gel 60-H (40 - 60 μm mesh).

Equipment

Melting points were measured using a Gallenkamp 7/MF-370 melting block and are uncorrected.

Elemental analyses were performed on a Carlo Erba Strumentazione elemental analyser MOD 1106 with a Hewlett Packard 3394A integrator.

Optical rotations were measured using an Optical Activity Ltd. AA10 polarimeter operating at 589 nm.

Infra-red spectra were obtained using a Philips PU9800 Fourier transform spectrophotometer.

Mass spectrometry was carried out using electron impact conditions on an AEI/Kratos MS12 instrument for low resolution and an AEI/Kratos MS902S instrument for accurate mass investigations.

Nuclear magnetic resonance spectra were obtained using either a Bruker AM200 or a Bruker WP200 instrument running at 200 MHz for hydrogen, 50 MHz for carbon and 188 MHz for fluorine nuclei.

Spectroscopic and Analytical Data

Spectroscopic and analytical data are listed for each compound in the style prescribed by the Journal of the Chemical Society, Perkin Transactions.

Melting points (m.pt.) and boiling points (b.pt.) are noted in degrees centigrade (°C) with a literature (lit.) value given where appropriate. In the case of vacuum distillation, the relevant pressure is given in millimetres of mercury (mmHg).

Chromatographic retention factors (Rf) are expressed as a fraction of the distance travelled by the solvent front.

Elemental analysis results are recorded for each element as a percentage.

Optical rotations ($[\alpha]_D$) are given in degrees (°) with the ambient temperature, in degrees centigrade (°C), noted as a superscript. Solution concentrations are given in milligrammes (mg) of sample per millilitre (ml) of the specified solvent.

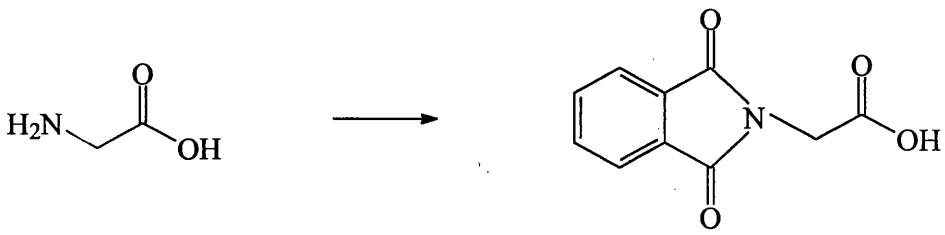
Infra-red (IR) spectroscopic data is given with wavenumbers of maximum absorbance (ν_{max}) expressed in reciprocal centimetres (cm^{-1}). The abbreviations s, m and br are used for strong, medium and broad respectively.

Mass spectroscopic data is recorded as the mass to charge ratio (m/z) with M^+ being the parent molecular ion. Fragments may be identified as the product resulting from the loss of a particular group from the parent ion. Relative signal intensities are given as a percentage of the largest observed peak.

Nuclear magnetic resonance (NMR) spectroscopic data is given with chemical shifts (δ) expressed in parts per million (ppm) with respect to tetramethylsilane (TMS) as an internal standard. The abbreviations s, d, t, q, quin and m are used for singlet, doublet, triplet, quartet, quintet and multiplet respectively. Diastereotopic protons displaying geminal coupling are assigned the abbreviation AB_q. Coupling constants (J) are recorded in hertz (Hz). When indicating the species responsible for each signal *italics* will be used in cases where ambiguity may still exist from the fragment given.

EXPERIMENTAL PART 1: *N*-Phthaloyl Protection of Amino Acids

N-Phthaloylglycine from glycine



Materials

Glycine	0.50 g (6.67 mmol)
Sodium carbonate decahydrate	1.93 g (6.75 mmol)
N-Ethoxycarbonyl phthalimide	1.50 g (6.85 mmol)
Distilled water	15 ml

Method

In a 100 ml conical flask equipped with a magnetic stirring bar, glycine and sodium carbonate decahydrate were placed. Distilled water was added and the mixture stirred until total solution was achieved. *N*-Ethoxycarbonyl phthalimide was added and stirring continued for 1 hour at room temperature. The small amount of undissolved material was then removed by filtration. The resulting clear filtrate was acidified slowly, with stirring, using concentrated hydrochloric acid (35% min.) giving a thick white precipitate. Heating this mixture to boiling gave a clear colourless solution which on slow cooling yielded long thin colourless needles. These needles were isolated by filtration and washed with distilled water. Analysis confirmed the identity of this material as *N*-phthaloylglycine (0.98 g, 71.7% yield, m.pt. 193-197°C, lit. 193-196°C [129]).

Found: C, 58.61; H, 3.49; N, 6.84. $C_{10}H_7N_1O_4$ requires C, 58.53; H, 3.41; N, 6.82%

ν_{max}(KBr)/cm^{-1} 3063-2701br (acid OH), 1775m (imide), 1725s (imide and acid C=O)

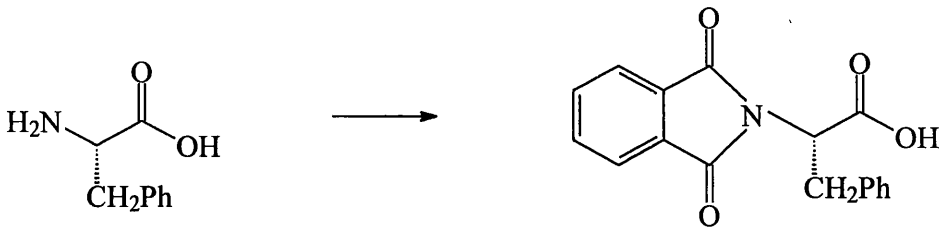
m/z 205 (M^+, 1.3%), 160 (M - COOH, 100), 132 (4.8), 104 (21.7), 76 (27.5)

m/z 205.0383 (M^+, $C_{10}H_7N\,O_4$ requires 205.03755)

δ_H(200MHz d_6-DMSO) 4.34 (2H, s, CH_2), 7.88-7.99 (4H, m, phth.)

δ_C(50MHz d_6-DMSO) 39.0 (t), 123.5 (d), 131.5 (s), 134.9 (d), 167.3 (s, phth C=O), 169.0 (s, COOH)

N-Phthaloyl-(L)-phenylalanine from (L)-phenylalanine



Materials

(L)-Phenylalanine	2.00 g (12.1 mmol)
Sodium carbonate decahydrate	3.48 g (12.2 mmol)
N-Ethoxycarbonyl phthalimide	2.64 g (12.1 mmol)
Distilled water	60 ml

Method

In a 100 ml conical flask equipped with a magnetic stirring bar, (L)-phenylalanine and sodium carbonate decahydrate were suspended in distilled water. After stirring for 20 minutes at room temperature complete solution was almost achieved. *N*-Ethoxycarbonyl phthalimide was added and stirring continued for a further 30 minutes. Filtration of this mixture gave a clear solution which was acidified slowly with concentrated (35% min.) hydrochloric acid. The resulting thick precipitate was found to be only partially soluble when the mixture was heated to boiling. After being allowed to cool the solid material was removed by vacuum filtration, crushed manually and washed with distilled water.

The resulting white solid was dried under vacuum after which the crystalline nature of the product could be more clearly observed. Analysis showed the crystalline solid to be *N*-phthaloyl-(L)-phenylalanine (2.73 g, 77.1% yield, m.pt. 177-184°C, lit. 178°C for racemate [82]).

Found: C, 67.62; H, 4.32; N, 4.75. $C_{17}H_{13}N_1O_4$ requires C, 69.15; H, 4.41; N, 4.74%

ν_{max}(KBr)/cm^{-1} 3272br (acid OH), 1773m (imide), 1750s (acid C=O), 1698s (imide C=O)

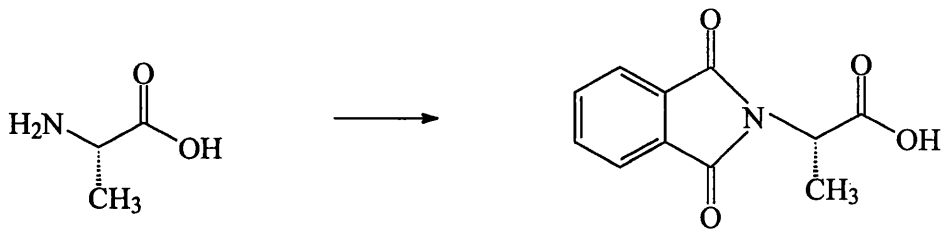
m/z 295 (M^+, 5.5%), 250 (M - COOH), 77 (19.1), 91 (31.7), 76 (20.2)

m/z 295.0821 (M^+, $C_{17}H_{13}NO_4$ requires 295.08441)

δ_H(200MHz $CDCl_3$) 3.58 (2H, dd, J 7.5 and 9.2, CH_2), 5.22 (1H, dd, J 7.5 and 9.2, NCH), 6.82 (1H, br s, COOH), 7.16 (5H, m, Ph), 7.63-7.81 (4H, m, phth)

δ_C(50MHz $CDCl_3$) 34.3 (t, Ph*C*H_2), 53.0 (d, NCH), 123.5 (d), 126 (d), 128.6 (d), 128.8 (d), 131.4 (s), 134.2 (d), 136.4 (s), 167.5 (s, phth C=O), 174.0 (s, COOH)

N-Phthaloyl-(L)-alanine from (L)-alanine



Materials

(L)-Alanine	10.00 g (112.4 mmol)
Sodium carbonate decahydrate	32.05 g (112.1 mmol)
N-Ethoxycarbonyl phthalimide	24.63 g (112.5 mmol)
Distilled water	250 ml

Method

Using the same basic procedure as that utilised for the production of *N*-phthaloyl-(L)-phenylalanine, *N*-phthaloyl-(L)-alanine was obtained as fine white needles (18.52 g, 75.5% yield, m.pt. 143-146°C, lit. 145-147°C [130]).

Found: C, 60.31; H, 4.20; N, 6.34. $C_{11}H_9N_1O_4$ requires C, 60.27; H, 4.11; N, 6.39%

ν_{max}(KBr)/cm^{-1} 3264br (acid OH), 1777m (imide), 1757s (acid C=O), 1694s (imide C=O)

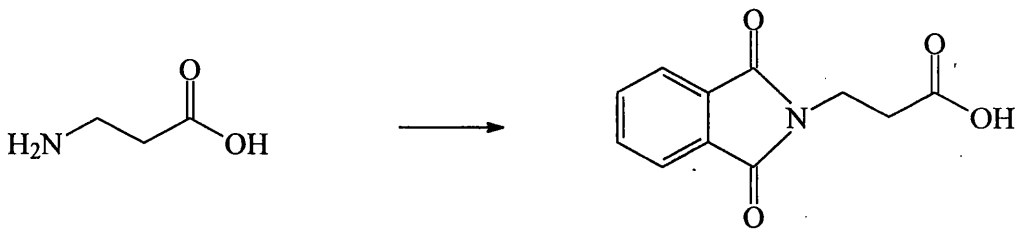
m/z 219 (M^+, 2.1%), 174 (M - COOH, 100), 146 (1.7), 132 (1.4)

m/z 219.0532 (M^+, $C_{11}H_9N\,O_4$ requires 219.05312)

δ_H(200MHz d_6-DMSO) 1.57 (3H, d, J 7.3, CH_3), 4.89 (1H, q, J 7.3, CH_3), 2.82-7.95 (4H, m, phth)

δ_C(50MHz d_6-DMSO) 14.9 (q), 47.0 (d, NCH), 123.3 (d), 131.3 (s), 134.8 (d), 167.2 (s, phth C=O), 171.1 (s, COOH)

N-Phthaloyl-β-alanine from β-alanine



Materials

β-Alanine	2.00 g (22.5 mmol)
Sodium carbonate decahydrate	6.50 g (22.7 mmol)
N-Ethoxycarbonyl phthalimide	4.93 g (22.5 mmol)
Water (non distilled)	40 ml

Method

Using the same basic procedure as that utilised to produce *N*-phthaloylglycine, *N*-phthaloyl-β-alanine was obtained as well formed white plates (3.22g, 66.8% yield, m.pt. 149-152°C, lit. 151.5°C [82]).

Found: C, 60.23; H, 4.11; N, 6.40. $C_{11}H_9N_1O_4$ requires C, 60.27; H, 4.11; N, 6.39%

ν_{max}(KBr)/cm^{-1} 3206br (acid OH), 1770m (imide), 1722s (acid C=O), 1702s (imide C=O)

m/z 219 (M^+, 6.0%), 202 (M - OH, 1.7), 174 (M - COOH, 19.1), 160 (86.1), 146 (10.6), 132 (7.0), 104 (45.7), 76 (100)

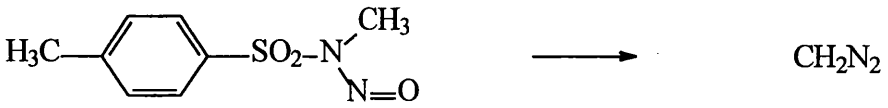
m/z 219.0537 (M^+, $C_{11}H_9N\,O_4$ requires 219.05312)

δ_H(200MHz $CDCl_3$) 2.80 (2H, t, J 7.3, CH_2CO), 4.00 (2H, t, J 7.3, CH_2N), 7.77-7.81 (4H, m, phth), 10.10 (1H, br s, COOH)

δ_C(50MHz $CDCl_3$) 35.5 (t), 33.3 (t), 123.4 (d), 131.9 (s), 134.1 (d), 167.8 (s, phth C=O), 176.4 (s, COOH)

EXPERIMENTAL PART 2: Diazomethylation of Protected Amino Acids *Via* the Mixed Carbonate

Diazomethane from *p*-toluenesulfonylmethylnitrosamide (Diazald®)



Materials

Potassium hydroxide	18.12 g (32.4 mmol)
Water	30 ml
Carbitol (diethylene glycol monoethyl ether)	105 ml
Diethyl ether	30 ml
Diazald®	63.18 g (29.5 mmol)
Diethyl ether	380 ml

Method

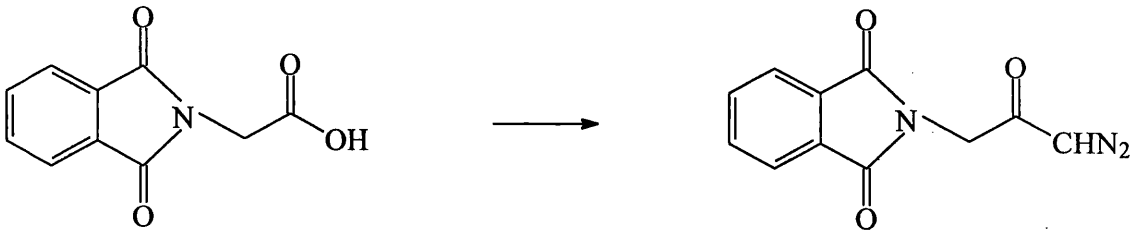
The synthesis of diazomethane was carried out using the Aldrich large scale Diazald® kit.[131] This kit consists of a 500 ml pressure equilibriated dropping funnel with a Teflon® stopper, a 500 ml two neck round bottom flask, a pressure equilibrated dry ice condenser and a 500 ml receiver vessel. The dry ice condenser is constructed such that the condensate is directed through a stopcock and U-tube before reaching the receiver vessel. All apparatus connections and joints are ground glass free. The kit is also equipped with Teflon® stopcocks.

The 500 ml two neck flask was equipped with a Teflon® coated magnetic stirring bar and charged with carbitol and diethyl ether. Potassium hydroxide was predissolved in water and added to this mixture and the vessel immersed in an oil bath. Diazald® was dissolved as far as possible in diethyl ether and placed in the dropping funnel. The receiver vessel was immersed in ice and the condenser pocket filled with acetone/dry ice. The oil bath was then heated to

60°C, the magnetic stirring started and the introduction of the Diazald® initiated giving rise to yellow vapours. The rate of Diazald® solution addition was carefully controlled to ensure that all vapours produced were fully condensed. This condensate initially filled the U-tube, where it could be observed as a yellow solution, before slowly passing into the chilled receiver vessel. Complete addition of Diazald® was carried out over approximately 1.5 hours after which 30 ml diethyl ether was added to the reaction vessel via the dropping funnel. The temperature of the oil bath was raised to 70°C and heating continued until the condensate in the U-tube appeared colourless. The receiver vessel was removed and its position replaced with an aqueous acetic acid bath. Aqueous acetic acid was also introduced slowly to the reaction vessel in order to neutralise any remaining potassium hydroxide and destroy residual diazomethane in the system.

A total of approximately 440 ml yellow diazomethane solution was obtained which, if complete conversion is assumed, contains 0.67 mmol/ml.

N-Phthaloylglycyl diazomethyl ketone from *N*-phthaloylglycine



Materials

N-Phthaloylglycine	0.15 g (0.73 mmol)
THF	5 ml
Triethylamine	0.3 ml (2.2 mmol)
Isobutyl chloroformate	0.2 ml (1.5 mmol)
Diazomethane (ether solution)	*c.* 7.8 mmol

Method

The ground glass joints of a scratch free 100 ml three neck flask were thoroughly greased. This vessel was flame dried after being equipped with a nitrogen balloon, magnetic stirring bar (Teflon® coated) and septum caps. A solution of *N*-phthaloylglycine in THF was added by syringe. After being cooled to -20°C triethylamine was added slowly. Isobutyl chloroformate was then added in a similar manner giving a white precipitate in the reaction mixture. This mixture was stirred for 20 minutes at -20°C after which the vessel was temporarily opened to admit the diazomethane solution under a nitrogen stream. The funnel used for this process was completely free of ground glass and surface imperfections. The resulting mixture was stirred for 1 hour at -20°C then allowed to warm to room temperature and stirred for a further hour. The reaction was quenched by addition of an equal volume of water and stirred vigorously for at least 1 hour before any manipulations were attempted.

The crude reaction mixture was transferred to a separatory funnel where the clear, strongly yellow, organic layer was separated from the clear, very mildly yellow, aqueous layer. The aqueous layer was extracted with ethyl acetate, the organic layers were combined and washed with saturated aqueous sodium

bicarbonate, dried with magnesium sulfate and filtered then evaporated under reduced pressure. The resulting yellow oily solid was recrystallised from ethyl acetate to give fine needles with a similar colouration (0.07 g, 42% yield, m.pt. 177-179°C, lit. 168°C [118], Rf 0.47 eluent 1:1 ethyl acetate:hexane).

Found: C, 57.61; H, 3.20; N, 18.17. $C_{11}H_7N_3O_3$ requires C, 57.64; H, 3.05; N, 18.34%

ν_{max}(KBr)/cm^{-1} 3075m (diazo CH), 2132s (diazo), 1775m (imide), 1736s (imide), 1628s (diazo C=O)

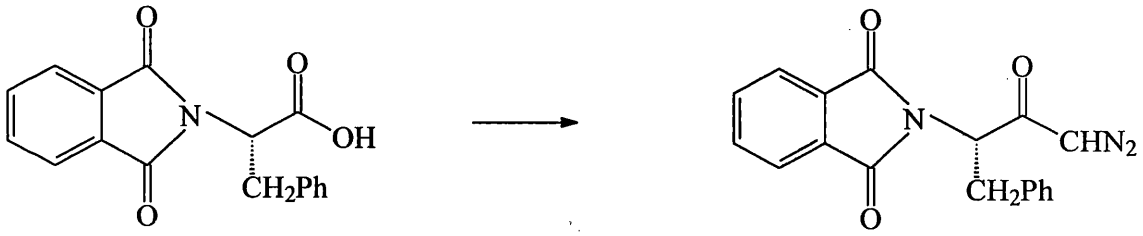
m/z 201 (M - N_2, 48.6%), 160 (M - $COCHN_2$, 100), 146 (3.2), 132 (5.5), 104 (40.5), 76 (59.3)

m/z 201.0413 (M - N_2, $C_{11}H_7NO_3$ requires 201.04256)

δ_H(200MHz d_6-DMSO) 4.45 (2H, s CH_2), 6.39 (1H, s, CHN_2), 7.84-7.79 (4H, m, phth)

δ_C(50MHz d_6-DMSO) 44.2 (t, CH_2), 53.6 (d, CHN_2), 123.4 (d), 131.5 (s), 134.7 (d), 167.4 (s, phth C=O), 188.2 (s, ketone)

N-Phthaloyl-(L)-phenylalanyl diazomethyl ketone fom *N*-phthaloyl-(L)-phenylalanine



Materials

N-Phthaloyl-(L)-phenylalanine	14.49 g (49.1 mmol)
THF	250 ml
Triethylamine	7.5 ml (53.9 mmol)
Isobutyl chloroformate	7.0 ml (54.0 mmol)
Diazomethane (ether solution)	*c.* 147 mmol

Method

Using the same basic method as that utilised to produce *N*-phthaloylglycyl diazomethyl ketone, *N*-phthaloyl-(L)-phenylalanyl diazomethyl ketone was obtained as a yellow oily solid. Product recrystallisation in this case was achieved using ethyl acetate/hexane (8.92 g, 57% yield, m.pt. 133-136°C, lit. 142-144°C [54]).

Found: C, 67.62; H, 4.16; N, 13.16. $C_{18}H_{13}N_3O_3$ requires C, 67.71; H, 4.07; N, 13.16%

$[\alpha]_D^{22}$ -221.3 (16.5 mg/1 ml $CHCl_3$)

ν_{max}(KBr)/cm^{-1} 3106w (diazo CH), 2101s (diazo), 1779w (imide), 1709s (imide C=O), 1651m (diazo C=O)

m/z 319 (M^+, 0.5%), 291 (M - N_2, 9.0), 250 (73.2), 91 (25.4), 77 (49.7), 76 (54.9)

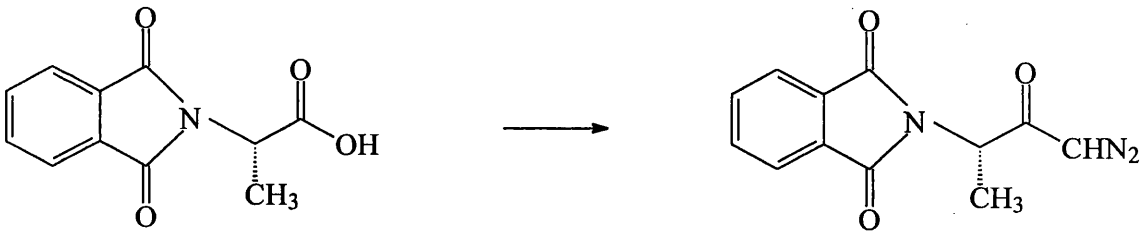
m/z 319.0970 (M^+, $C_{18}H_{13}N_3O_3$ requires 319.09573)

δ_H(200MHz d_6-DMSO) 5.12 (1H, d of AB_q, J 4.9(AB), 11.5 and 13.8, NCH), 6.58 (1H, s, CHN_2), 7.13 (5H, m, Ph), 7.83 (4H, s, phth)

δ_C(50MHz d_6-DMSO) 33.2 (t, Ph*C*H_2), 53.7 (d, CHN_2), 57.9 (d, CHN), 123.4 (d), 126.6 (d), 128.3 (d), 128.8 (d), 130.9 (s), 134.8 (d), 137.1 (s), 167.2 (s, phth C=O), 190.2 (s, ketone C=O)

δ_H(200MHz $CDCl_3$) 3.52 (2H, pair of d, J 6.8 and 9.6, PhC*H*$_2$), 5.09 (1H, dd, J 6.8 and 9.6, CHN), 5.42 (1H, s, CHN_2), 7.14 (5H, m, Ph), 7.65-7.81 (4H, m, phth)

N-Phthaloyl-(L)-alanyl diazomethyl ketone from *N*-phthaloyl-(L)-alanine



Materials

N-Phthaloyl-(L)-alanine	3.65 g (16.7 mmol)
THF	85 ml
Triethylamine	2.6 ml (18.7 mmol)
Isobutyl chloroformate	2.4 ml (18.5 mmol)
Diazomethane	*c.* 50 mmol

Method

Using the same basic method as that employed to produce *N*-phthaloylglycyl diazomethyl ketone, *N*-phthaloyl-(L)-alanyl diazomethyl ketone was obtained as a yellow oil (3.86 g, 94% crude yield with ethyl acetate being indicated as the main impurity by ^{1}H NMR spectroscopy). Thin layer chromatography indicated the presence of a small amount of a further impurity (Rf 0.57 [product], 0.73 [impurity], eluent 1:1 ethyl acetate:hexane).

Upon storage at 0°C for several days this material solidified. A slow process of recrystallisation from ethyl acetate/hexane was found to be possible when the saturated solution was stored at the aforementioned temperature (product m.pt. 60-63°C).

Found: C, 59.19; H, 3.89; N, 17.23. $C_{12}H_9N_3O_3$ requires C, 59.25; H, 3.70; N, 17.28%

ν_{max}(KBr)/cm^{-1} 3092m (diazo CH), 2103s (diazo), 1775m (imide), 1705s (imide C=O), 1647m (diazo C=O)

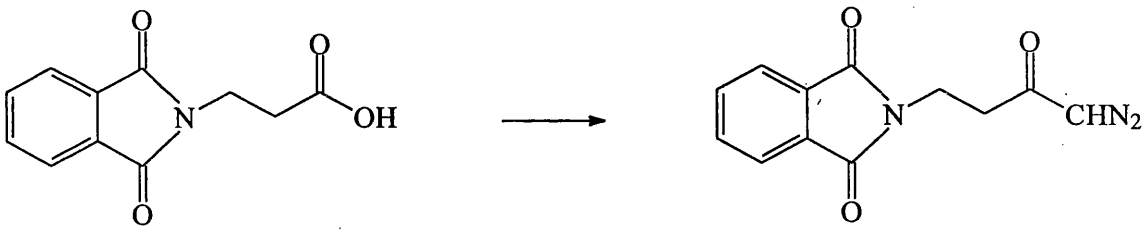
m/z 215 (M - N_2, 20.7), 174 (M - $COCHN_2$, 100), 146 (2.3) 132 (1.9)

m/z 215.0574 (M - N_2, $C_{12}H_9NO_3$ requires 215.05828)

δ_H(200MHz $CDCl_3$) 1.60 (3H, d, J 7.3, CH_3), 4.83 (1H, q, J 7.3, CHN), 5.38 (1H, s, CHN_2), 7.63-7.83 (4H, m, phth)

δ_C(50MHz $CDCl_3$) 14.5 (q), 52.1 and 53.7 (both d, CHN and CHN_2), 123.4 (d), 131.7 (s), 134.2 (d), 167.5 (s, phth C=O), 190.1 (s, ketone C=O)

N-Phthaloyl-β-alanyl diazomethyl ketone from *N*-phthaloyl-β-alanine



Materials

N-Phthaloyl-β-alanine	1.095 g (5.00 mmol)
THF	30 ml
Triethylamine	0.8 ml (5.8 mmol)
Isobutyl chloroformate	0.7 ml (0.7 mmol)
Diazomethane	*c.* 20 mmol

Method

Using the same basic method as that employed to produce *N*-phthaloylglycyl diazomethyl ketone, *N*-phthaloyl-β-alanyl diazomethyl ketone was obtained as a white solid. Recrystallisation from hot ethyl acetate/hexane yielded fine needles with a very mild yellow-green colouration (0.37 g, 30% yield, m.pt. 119-122°C).

Found: C, 59.49; H, 3.96; N, 17.01. $C_{12}H_9N_3O_3$ requires C, 59.26; H, 3.70; N, 17.28%

ν_{max}(KBr)/cm^{-1} 3088m (diazo CH), 2116s (diazo), 1770m (imide), 1716s (imide), 1610s (diazo C=O)

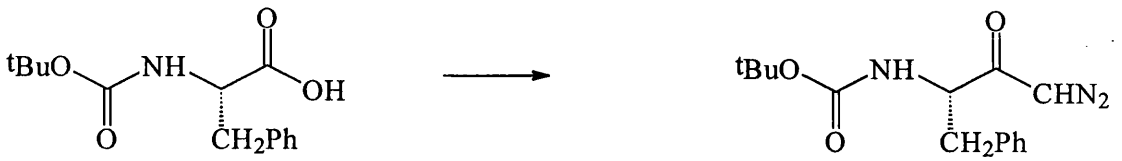
m/z 215 (M - N_2, 22.5%), 202 (M - CHN_2, 1.3), 174 (10.3), 160 (100), 146 (4.5), 132 (8.8), 104 (52.4), 76 (72.8)

m/z 243.0633 (M^+, $C_{12}H_9N_3O_3$ requires 243.06437)

δ_H(200MHz $CDCl_3$) 2.77 (2H, t, J 7.2, CH_2CO), 4.01 (2H, t, J 7.2, CH_2N), 5.36 (1H, s, CHN_2), 7.68-7.89 (4H, m, phth)

δ_C(50MHz $CDCl_3$) 33.6 (t), 38.4 (t), 55.0 (d, CHN_2), 123.2 (d), 131.8 (s), 133.9 (d), 167.9 (s, phth C=O), 191.9 (s, ketone C=O)

N-tert-Butoxycarbonyl-(L)-phenylalanyl diazomethyl ketone from *N-tert*-butoxycarbonyl-(L)-phenylalanine



Materials

N-tert-Butoxycarbonyl-(L)-phenylalanine	3.50 g (13.2 mmol)
THF	65 ml
Triethylamine	2.0 ml (14.4 mmol)
Isobutyl chloroformate	1.8 ml (13.9 mmol)
Diazomethane	*c.* 80 mmol

Method

Using the same basic procedure as that employed to produce *N*-phthaloylglycyl diazomethyl ketone, *N-tert*-butoxycarbonyl-(L)-phenylalanyl diazomethyl ketone was obtained as a yellow oil which solidified on standing. Recrystallisation from hot ethyl acetate/hexane yielded the desired compound as clear yellow crystals (2.54 g, 68% yield, m.pt. 95-97°C, Rf 0.74, eluent 1:1 ethyl acetate:hexane).

Found: C, 62.01; H, 6.67; N, 14.36. $C_{15}H_{19}N_3O_3$ requires C, 62.28; H, 6.57; N, 14.53%

ν_{max}(KBr)/cm^{-1} 3328m (carbamate NH), 3127m (diazo CH), 2105s (diazo), 1686s (carbamate C=O), 1642s (ketone C=O), 1526s (carbamate)

m/z 233 (M - $[CH_3]_2C{=}CH_2$, 0.2%), 220 (M - $COCHN_2$, 9.1) 205 (3.7)

m/z 220.1329 (M - $COCHN_2$, $C_{13}H_{18}N\,O_2$ requires 220.13372)

δ_H(200MHz $CDCl_3$) 1.32 (9H, s, $[CH_3]_3C$), 2.93 (2H, d of AB_q, J 13.8(AB), 6.6 and 6.7, PhCH_2), 4.35 (1H, m, CHN), 5.20 (1H, br d, J 8.5, NH), 5.23 (1H, s, CHN_2), 7.08-7.26 (5H, m, Ph)

δ_C(50MHz $CDCl_3$) 28.1 (q), 38.3 (t), 54.2 and 58.4 (both d, CHN and CHN_2), 79.8 (s, $[CH_3]_3C$), 126.8 (d), 128.4 (d), 129.2 (d), 136.3 (s), 155.1 (s, carbamate NH), 193.4 (s, ketone C=O)

δ_H(200MHz d_6-DMSO) 1.32 (9H, s, $[CH_3]_3C$), 2.89 (2H, d of AB_q, J 13.8(AB), 4.4 and 10.5), 4.19 (1H, m, CHN), 6.13 (1H, s, CHN_2), 7.17-7.33 (5H, m, Ph)

EXPERIMENTAL PART 3: Further Reaction of Diazomethyl Ketones and Subsequent Halogen Exchange

Triphenylphosphonium bromide from triphenylphosphine

$$HBr_{(aq)} + PPh_{3(s)} \longrightarrow PPh_3H^+Br^-_{(s)}$$

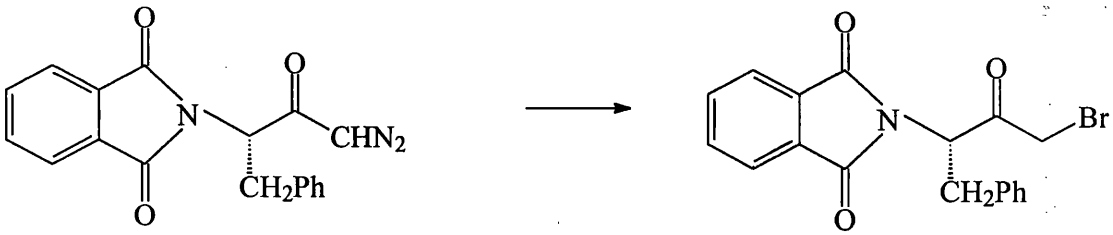
Materials

Hydrobromic acid (48% aqueous solution)	70 ml (0.41 mol)
Triphenylphosphine	26.20 g (0.10 mol)

Method

To a 250 ml conical flask equipped with a magnetic stirring bar, hydrobromic acid was added and heated to 70°C. Triphenylphosphine was then added and the mixture stirred for 10 minutes at this temperature. After being allowed to cool the solution was transferred to a separatory funnel and extracted four times with 30 ml portions of chloroform. The combined organic layers were dried with sodium sulfate, filtered and evaporated. The resulting solids were transferred to a Buchner funnel where they were purified by washing with warm ethyl acetate. The desired product was isolated as a clean white solid and stored below 5°C (32.42 g, 95% yield, m.pt. 184-197°C, lit. 185-195°C [86]).

N-Phthaloyl-(L)-phenylalanyl bromomethyl ketone from *N*-phthaloyl-(L)-phenylalanyl diazomethyl ketone



Materials

N-Phthaloyl-(L)-phenylalanyl diazomethyl ketone	0.50 g (1.57 mmol)
Dichloromethane	30 ml
Triphenylphosphonium bromide	11.0 g (32.1mmol)
Xylene	40 ml

Apparatus

The following were connected in series via plastic tubing:

1. Nitrogen gas cylinder
2. Drying vessel containing calcium chloride.
3. 250ml three necked flask equipped with a glass inlet tube and water condenser from the top of which the series connection was continued.
4. Reaction vessel bubbler.
5. Empty catchment trap.
6. Bubbler filled with an aqueous solution of sodium bicarbonate.

The 250 ml three neck flask was charged with xylene and triphenylphosphonium bromide and the reaction vessel with dichloromethane and *N*-phthaloyl-(L)-phenylalanyl diazomethyl ketone. Nitrogen gas was passed through the system at such a rate as to give steady bubbling through all three solutions. The xylene vessel was heated to reflux at which point white fumes of hydrogen bromide were observed in the nitrogen stream. Effervescence in the reaction vessel began almost immediately and the characteristic yellow colouration of the starting solution was rapidly lost. Reflux was continued for 30 minutes after the

effervescence in the reaction vessel ceased and began in the bicarbonate trap. The xylene vessel was then allowed to cool and nitrogen gas used to purge the system.

Addition of further dichloromethane followed by drying with magnesium sulfate, filtration and evaporation gave 0.60 g of a buff coloured solid. Recrystallisation of 0.40 g of this material, from ethyl acetate/hexane, yielded colourless needles (0.35 g, 0.94 mmol, 87.5% yield from recrystallisation which extrapolates to 90% yield for full reaction, m.pt. 108-112°C, Rf 0.86, eluent 1:1 ethyl acetate:hexane, Rf 0.75, eluent 10:1 chloroform:ethyl acetate).

Evaporation of the xylene allowed recovery of triphenylphosphine which was generally used for the production of further triphenylphosphonium bromide without purification.

Found: C, 57.92; H, 3.94; N, 3.69; Br, 21.47. $C_{18}H_{14}NO_3Br$ requires C, 58.08; H, 3.76; N, 3.76; Br, 21.50%

$[\alpha]_D^{22}$ -185.9 (12.1 mg/1 ml $CHCl_3$)

ν_{max}(KBr)/cm^{-1} 1775m (imide), 1744s (ketone C=O), 1713s (imide C=O)

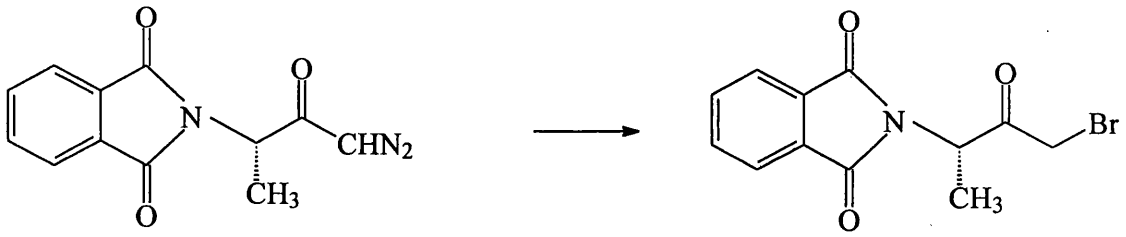
m/z 373 (M^+, 1.3%), 371 (M^+, 1.3), 292 (M - Br, 8.6), 278 (0.4), 250 (100)

m/z 373.0146 (M^+, $C_{18}H_{14}NO_3Br$ requires 373.01381), m/z 371.0144 (M^+, $C_{18}H_{14}NO_3Br$ requires 371.01571)

δ_H(200MHz $CDCl_3$) 3.42 (2H, d of AB_q, J 10.9(AB), 5.1 and 14.1, PhCH_2), 3.93 (2H, s, CH_2Br), 5.31 (1H, dd, J 5.1 and 10.9), 7.06 (5H, m, Ph), 7.58-7.74 (4H, m, phth)

δ_C(50MHz $CDCl_3$) 31.3 (t), 33.9 (t), 57.6 (d, CHN), 123.6 (d), 127.0 (d), 128.6 (d), 128.9 (d), 131.2 (s), 134.0 (d), 136.1 (s), 167.4 (s, phth C=O), 196.8 (s, ketone C=O)

N-Phthaloyl-(L)-alanyl bromomethyl ketone from *N*-phthaloyl-(L)-alanyl diazomethyl ketone



Materials

N-Phthaloyl-(L)-alanyl diazomethyl ketone	0.50 g (2.06 mmol)
Dichloromethane	30 ml
Triphenylphosphonium bromide	14.0 g (40.8 mmol)
Xylene	45 ml

Method

Using the same basic procedure as that employed to form *N*-phthaloyl-(L)-phenylalanyl bromomethyl ketone, crude *N*-phthaloyl-(L)-alanyl bromomethyl ketone was produced as a yellow oil (0.54 g, 87% crude yield). Thin layer chromatography indicated the presence of only a small amount of impurity (Rf 0.81 [product] and 0.03 [impurity], eluent 1:1 ethyl acetate:hexane).

Storage for two weeks at 0°C effected solidification of this material. Recrystallisation was found to be possible from ethyl acetate/hexane by storage of a saturated solution of the material at the aforementioned temperature. Clean white crystals of the desired product were obtained (0.39 g, 64% yield, m.pt. 75-78°C, lit. 66°C [132], Rf 0.74, eluent 10:1 chloroform:ethyl acetate).

In this case reflux of the xylene vessel was halted before the generation of hydrogen bromide gas was complete. Once cool, further triphenylphosphonium bromide and xylene were added to the reflux vessel. The reaction vessel was removed, its site thoroughly cleaned, and the remaining apparatus used directly in further bromination reactions.

Found: C, 48.76; H, 3.52; N, 4.73; Br, 27.00. $C_{12}H_{10}NO_3Br$ requires C, 48.65; H, 3.38; N, 4.73; Br, 27.03%

ν_{max}(KBr)/cm^{-1} 1781m (imide), 1721s (ketone C=O), 1713 (imide C=O)

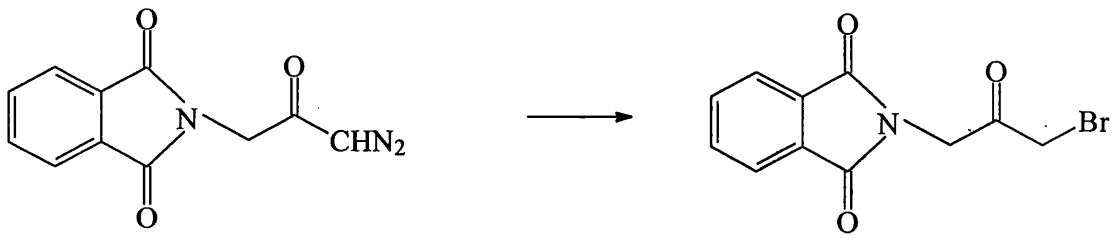
m/z 216 (M - Br, 0.3%), 174 (M - $COCH_2Br$, 100), 146 (1.2)

m/z 216.0680 (M - Br, $C_{12}H_{10}NO_4$ requires 216.06601)

δ_H(200MHz $CDCl_3$) 1.58 (3H, d, J 7.2, CH_3), 3.93 (2H, s, CH_2Br), 5.13 (1H, q, J 7.2, CHN), 7.62-7.83 (4H, m, phth)

δ_C(50MHz $CDCl_3$) 14.4 (q), 30.9 (t), 51.6 (d, CHN, 123.6 (d), 131.6 (d), 134.4 (d), 167.3 (s , phth C=O), 197.4 (s, ketone C=O)

_N_-Phthaloylglycyl bromomethyl ketone from _N_-phthaloylglycyl diazomethyl ketone



Materials

_N_-Phthaloylglycyl diazomethyl ketone	0.50 g (2.18 mmol)
Dichloromethane	30 ml
Triphenylphosphonium bromide	10.0 g additional
Xylene	30 ml additional

Method

Additional xylene and triphenylphosphonium bromide were added to the apparatus used for the formation of _N_-phthaloyl-(L)-alanyl bromomethyl ketone. The same basic procedure as that utilised to form _N_-phthaloyl-(L)-phenylalanyl bromomethyl ketone was employed to yield _N_-phthaloylglycyl bromomethyl ketone as buff coloured solid (0.63 g). Recrystallisation of 0.40 g of this material from ethyl acetate/hexane gave colourless needles (0.33 g, 82% crystallisation yield which extrapolates to 84% yield for reaction, Rf 0.75, eluent 1:1 ethyl acetate:hexane, Rf 0.61, eluent 10:1 chloroform:ethyl acetate, m.pt. 144-148°C, lit. 145-146°C [133] and 147°C [134]).

Found: C, 46.88; H, 2.87; N, 4.86; Br, 28.25. $C_{11}H_8NO_3Br$ requires C, 46.81; H, 2.84; N, 4.96; Br, 28.37%

ν_{max}(KBr)/cm^{-1} 1775m (imide), 1732s (ketone C=O), 1721s (imide C=O)

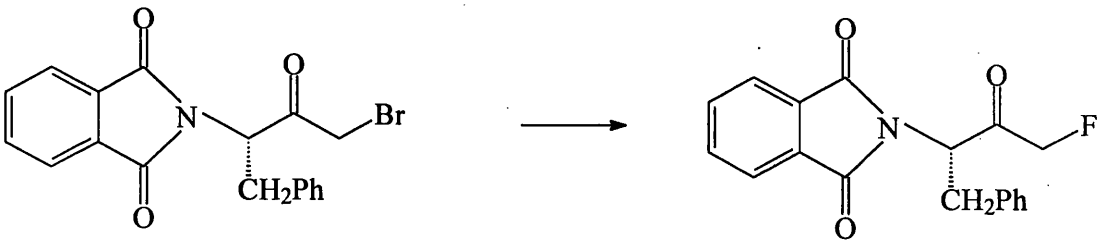
m/z 283 (M^+, 0.6%), 281 (M^+, 0.6), 202 (M - Br, 1.7), 188 (0.1), 160 (100)

m/z 202.0523 (M - Br, $C_{11}H_8NO_3$ requires 202.05040)

δ_H(200MHz $CDCl_3$) 3.93 (2H, s, CH_2Br), 4.66 (2H, s, CH_2N), 7.59-7.78 (4H, m, phth)

δ_C(50MHz $CDCl_3$) 30.9 (t, CH_2Br), 44.4 (t, CH_2N), 123.5 (d), 132.0 (s), 134.1 (d), 167.2 (s, phth C=O), 194.4 (s, ketone C=O)

N-Phthaloyl-(L)-phenylalanyl fluoromethyl ketone from N-phthaloyl-(L)-phenylalanyl bromomethyl ketone



Materials

N-Phthaloyl-(L)-phenylalanyl bromomethyl ketone	0.200 g (0.538 mmol)
Chloroform	5 ml
Triethylamine trishydrofluoride	0.5 ml (3.1 mmol)

Method

A 10 ml round bottom flask with a side arm was equipped with a magnetic stirring bar and a water condenser topped with a nitrogen balloon. To this flask was added *N*-phthaloyl-(L)-phenylalanyl bromomethyl ketone and chloroform. Triethylamine trishydrofluoride was added, the flask flushed with nitrogen and the side arm sealed with a septum cap. Using an oil bath held at 80°C, the solution was taken to gentle reflux. Carefull monitoring of the reaction by thin layer chromatography indicated complete conversion of the material after 31 hours at this temperature (product Rf 0.62, starting material Rf 0.75, eluent 10:1 chloroform:ethyl acetate). The reaction was quenched by addition of saturated aqueous sodium bicarbonate at room temperature.

The mixture was transferred to a separatory funnel with the aid of 100 ml diethyl ether and 100 ml saturated aqueous sodium bicarbonate. The organic layer was separated and the aqueous layer extracted with three 100 ml portions of diethyl ether. The combined organic layers were washed sequentially with 400 ml distilled water, 200 ml hydrochloric acid (2 M), 400 ml distilled water and 200 ml saturated aqueous sodium bicarbonate. Following this the organic layer was dried with magnesium sulfate, filtered and evaporated to give an off-white solid. This material was purified by precipitation from a dichloromethane solution by

the addition of hexane. Clean white solids were obtained which analysis showed to be the desired product (130 mg, 78% yield, m.pt. 110-112°C, lit. 113-114°C [90]).

Found: C, 69.20; H, 4.60; N, 4.24. $C_{18}H_{14}NO_3F$ requires C, 69.45; H, 4.50; N, 4.50%

$[\alpha]_D^{22}$ -2.5 (10.1 mg/1 ml $CHCl_3$)

ν_{max}(KBr)/cm^{-1} 1780m (imide), 1752s (ketone C=O), 1714s (imide C=O)

m/z 311 (M^+, 1.1%), 292 (M - F, 0.4), 278 (0.8), 250 (100)

m/z 311.0942 (M^+, $C_{18}H_{14}NO_3F$ requires 311.09461)

δ_H(200MHz $CDCl_3$) 3.43 (2H, d of AB_q, J 14.1(AB), 5.2 and 10.6, PhCH_2), 4.92 (2H, d of AB_q, J 15.9(AB) and 47.2, CH_2F), 5.21 (1H, ddd, J 1.5, 5.2 and 10.6, CHN), 7.11 (5H, m, Ph), 7.62-7.75 (4H, m, phth)

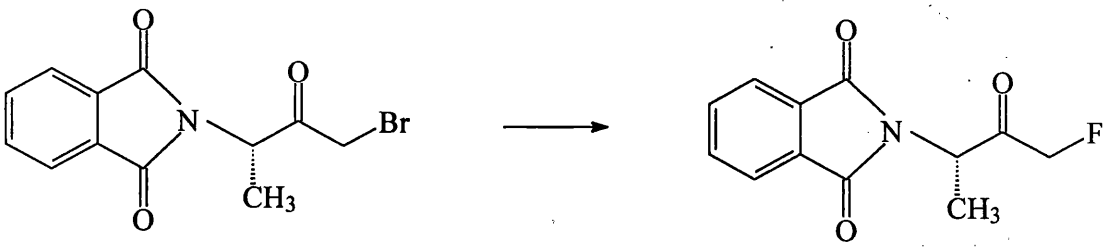
δ_C(50MHz $CDCl_3$) 33.3 (PhCH_2, 57.8 (d, CHN), 82.6 and 86.3 (both t, CH_2F, 123.6 (d), 127.0 (d), 128.6 (d), 129.0 (d), 131.3 (s), 134.3 (d), 136.1 (s), 167.4 (s, phth C=O) , 199.9 and 200.3 (both s, $COCH_2F$)

δ_F(188MHz $CDCl_3$) -230.93 (t, J 47.2)

δ_H(200MHz d_6-DMSO) 3.23 (2H, d of AB_q, J 13.9(AB), 11.2 and 4.9, PhCH_2), 5.37 (1H, dd, J 11.2 and 4.9, CHN), 5.39 (2H, d of AB_q, J 16.8(AB) and 46.1, CH_2-F), 7.15 (5H, m, Ph), 7.85 (4H, m, phth)

δ_F(188MHz d_6-DMSO) -232.7 (t, J 46.1)

N-Phthaloyl-(L)-alanyl fluoromethyl ketone from *N*-phthaloyl-(L)-alanyl bromomethyl ketone



Materials

N-Phthaloyl-(L)-alanyl bromomethyl ketone	100 mg (0.338 mmol)
Chloroform	3 ml
Triethylamine trishydrofluoride	0.32 ml (1.96 mmol)

Method

The same general procedure as that used to produce *N*-phthaloyl-(L)-phenylalanyl fluoromethyl ketone was employed to yield crude *N*-phthaloyl-(L)-alanyl fluoromethyl ketone as a yellow oil (70 mg, 88% crude yield).

Complete conversion was indicated by thin layer chromatography after a reaction time of 48 hours (product Rf 0.60, starting material Rf 0.74, eluent 10:1 chloroform:ethyl acetate).

Purification by precipitation was found to be possible but inefficient. The desired compound precipitated as an off white solid upon storage of a saturated ethyl acetate/hexane solution at 0°C (20 mg, 25%yield).

ν_{max}(KBr)/cm^{-1} 1777m (imide), 1745s (ketone C=O), 1713s (imide C=O)

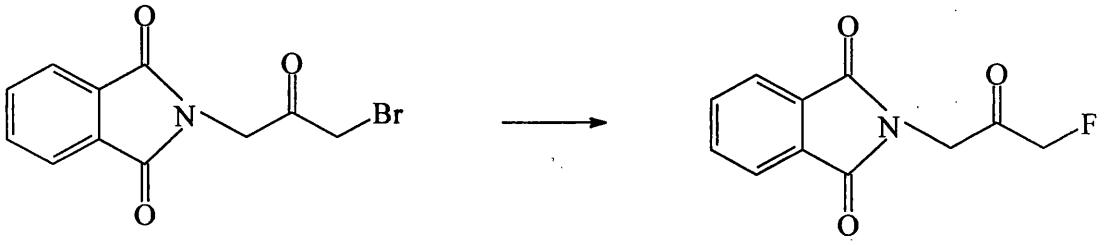
m/z 235 (M^+, 0.1%), 216 (M - F, 0.3), 174 (100), 146 (1.5)

m/z 235.0660 (M^+, $C_{12}H_{10}N O_3F$ requires 235.06446)

δ_H(200MHz $CDCl_3$) 1.67 (3H, d, J 7.2, CH_3), 5.08 (1H, dq, J 1.4(d) and 7.2(q), CHN), 5.00 (2H, d, J 47.1, CH_2F), 7.75-7.91 (4H, phth)

δ_F(188MHz $CDCl_3$) -231.21 (t, J 47.1)

N-Phthaloylglycyl fluoromethyl ketone from *N*-phthaloylglycyl bromomethyl ketone



Materials

N-Phthaloylglycyl bromomethyl ketone	100 mg (0.355 mmol)
Chloroform	3 ml
Triethylamine trishydrofluoride	0.35 ml (2.15 mmol)

Method

The same general procedure as that used to produce *N*-phthaloyl-(L)-phenylalanyl fluoromethyl ketone was employed to yield crude *N*-phthaloylglycyl fluoromethyl ketone as a buff coloured solid.

Complete conversion was indicated by thin layer chromatography after a reaction time of 28 hours (product Rf 0.53, starting material Rf 0.61, eluent 10:1 chloroform:ethyl acetate).

Off-white crystals were obtained on recrystallisation from ethyl acetate/hexane (40 mg, 51% yield).

ν_{max}(KBr)/cm^{-1} 1778w (imide), 1759m (ketone C=O), 1716s (imide C=O)

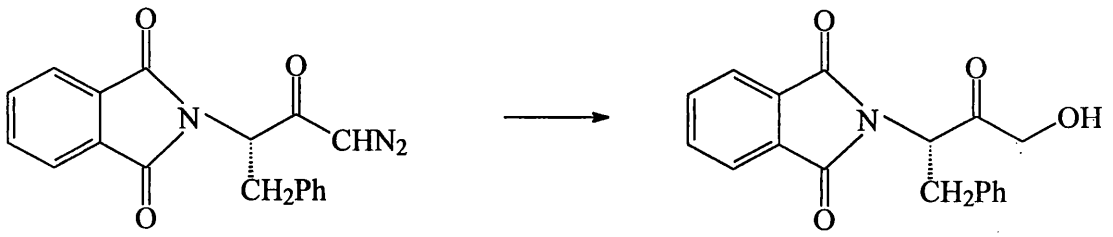
m/z 221 (M^+, 4.3%), 188 (M - CH_2F, 0.7), 160 (100)

m/z 221.0485 (M^+, $C_{11}H_8NO_3F$ requires 221.04878)

δ_H(200MHz $CDCl_3$) 4.77 (2H, d, J 2.0, CH_2N), 5.03 (2H, d, J 47.2, CH_2F), 7.27-7.92 (4H, m, phth)

δ_F(188MHz $CDCl_3$) -233.76 (t, 47.2)

N-Phthaloyl-(L)-phenylalanyl hydroxymethyl ketone from *N*-phthaloyl-(L)-phenylalanyl diazomethyl ketone



Materials

N-Phthaloyl-(L)-phenylalanyl diazomethyl ketone	0.50 g (1.57 mmol)
THF	30 ml
Sulfuric acid (2 M)	25 ml

Method

N-Phthaloyl-(L)-phenylalanyl diazomethyl ketone was suspended as a solid in aqueous sulfuric acid. Addition of THF effected partial solution and initiated gas evolution. The mixture was heated to boiling by which time solution was total and gas evolution had ceased. The mixture was then allowed to cool and extracted three times with a total of 90 ml diethyl ether. The combined ether layers were dried with magnesium sulfate, filtered and evaporated to give a white oily solid. This solid was dissolved in a minimal amount of ethyl acetate and slow precipitation effected by addition of hexane thus allowing the isolation of the desired compound as a white solid (310 mg, 64% yield, m.pt. 95-105°C, lit. 108-112°C [135]).

Found: C, 69.59; H, 4.99; N, 4.26. $C_{18}H_{15}N\,O_4$ requires C, 69.90; H, 4.85; N, 4.53%

ν_{max}(KBr)/cm^{-1} 3427m (OH), 1779m (imide), 1746m (ketone C=O), 1711s (imide C=O)

m/z 309 (M^+, 0.7%), 292 (M - OH, 0.9), 280 (0.9), 250 (100)

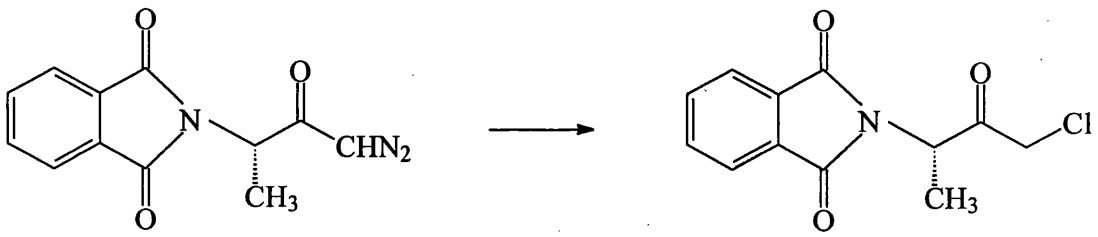
m/z 309.1000 (M^+, $C_{18}H_{15}N\,O_4$ requires 309.10010)

δ_H(200MHz $CDCl_3$) 3.02 (1H, br s, OH), 3.52 (2H, d of AB_q, J 14.1(AB), 5.5 and 10.9, PhCH_2), 4.39 (2H, s, CH_2O), 5.15 (1H, dd, J 5.5 and 10.9, CHN), 7.15 (5H, m, Ph), 7.69-7.82 (4H, m, phth)

δ_C(50MHz $CDCl_3$) 33.5 (t, $PhCH_2$), 57.8 (d, CHN), 66.4 (t, CH_2OH), 123.7(d), 127.1(d), 128.7(d), 128.8(d), 131.2(s), 134.4 (d), 135.8 (s), 167.5 (s, phth C=O), 204.7 (s, ketone C=O)

δ_H(200MHz d_6-DMSO) 3.43 (2H, d of AB_q, J 13.6(AB), 4.8 and 11.4, PhCH_2), 4.37 (2H, d of AB_q, J 18.3(AB) and 6.0, CH_2OH), 5.35 (1H, dd, J 4.8 and 11.4, CHN), 7.19 (5H, m, Ph), 7.88 (4H, m, phth)

N-Phthaloyl-(L)-alanyl chloromethyl ketone from *N*-phthaloyl-(L)-alanyl diazomethyl ketone



Materials

N-Phthaloyl-(L)-alanyl diazomethyl ketone — *c.* 2.5 g (0.01 mol)

Concentrated hydrochloric acid (35% min.)

Method

Concentrated hydrochloric acid was slowly added to a neat sample of *N*-phthaloyl-(L)-alanyl diazomethyl ketone until no further gas evolution or change in physical properties was noted. Distilled water and diethyl ether were added to the reaction vessel and the whole transferred to a separatory funnel. The organic layer was separated, washed with saturated aqueous solutions of sodium carbonate and sodium chloride. Following this the organic layer was dried with magnesium sulfate, filtered and evaporated to give a yellow oily solid. Recrystallisation from diethyl ether/light petroleum yielded a clean white solid which, on analysis, was shown to be the desired product (*c.* 1g, 40% yield, m.pt. 74-77°C).

Found: C, 57.07; H, 4.06; N, 5.42. $C_{12}H_{10}N\,O_3Cl$ requires C, 57.25; H, 3.97; N, 5.56%

ν_{max}(KBr)/cm^{-1} 1775m (imide), 1752s (ketone C=O), 1709s (imide C=O)

m/z 216 (M - Cl, 0.5%), 174 (M - $COCH_2Cl$, 55.7), 146 (2.2)

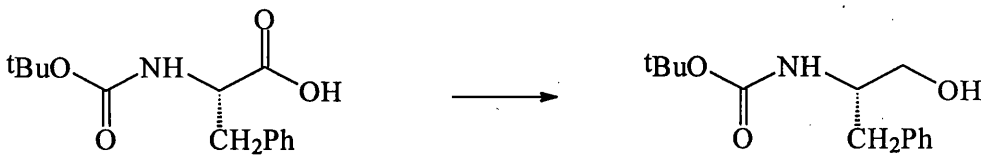
m/z 253.0305 (M^+, $C_{12}H_{10}N\,O_3Cl$ requires 253.03196), m/z 251.0373 (M^+, $C_{12}H_{10}N\,O_3Cl$ requires 251.03491)

δ_H(200MHz $CDCl_3$) 1.67 (3H, d, J 7.2, CH_3), 4.20 (2H, s, CH_2), 5.13 (1H, q, J 7.2, CHN), 7.74-7.92 (4H, m, phth)

δ_C(50MHz $CDCl_3$) 14.4 (q), 45.7 (t), 52.0 (d, CHN), 123.7 (d), 131.7 (s), 134.5 (d), 167.5 (phth C=O), 198.0 (s, ketone C=O)

EXPERIMENTAL PART 4: Synthesis of Sulfone Coupling Components

2(S)-[(*tert*-Butoxycarbonyl)amino]-3-(phenyl)propanol from *N-tert*-butoxycarbonyl-(L)-phenylalanine



Materials

N-tert-Butoxycarbonyl-(L)-phenylalanine	11.25 g (42.45 mmol)
THF	53 ml
Triethylamine	5.9 ml (42 mmol)
Ethyl chloroformate	4.1 ml (43 mmol)
Sodium borohydride	4.02 g (106 mmol)
Water	53 ml

Method

A 250 ml three necked round bottom flask was equipped with a magnetic stirring bar and nitrogen balloon. The flask was sealed with septum caps and flame dried. To this vessel *N-tert*-butoxycarbonyl-(L)-phenylalanine and triethylamine were added, predissolved in THF, and cooled to -5°C. Ethyl chloroformate was added dropwise via syringe resulting in the formation of a white precipitate. After being stirred at -5°C for 30 minutes the mixture was filtered by suction and the filtered solid washed with a further 40ml THF. The combined filtrates were added slowly to a 500ml conical flask containing a slurry of sodium borohydride in water at 0°C which resulted in the vigorous evolution of gas. The mixture was then stirred for 4 hours at 0°C before being transferred to a separatory funnel where extraction was carried out several times with diethyl ether giving a total organic volume of 1.5 L. The multiple extraction process was repeated with a total of 1.5 L dichloromethane. The diethyl ether and dichloromethane extracts were dried separately with magnesium sulfate, filtered and evaporated. A thick colourless oil, which solidified as a white solid on standing at room temperature,

was obtained from the diethyl ether extracts. A further small quantity of white solid was obtained from the dichloromethane extracts.

Recrystallisation from ethyl acetate/hexane yielded the desired compound in several crops which were stored in an evacuated desiccator overnight (5.54 g, 52% yield, m.pt. 81-88°C, lit. 94-96°C [136]).

Found: C, 66.96; H, 8.24; N, 5.58. $C_{14}H_{21}NO_3$ requires C, 66.93; H, 8.37; N, 5.58%

ν_{max}(KBr)/cm^{-1} 3357br,s (OH and NH), 1686s (C=O), 1528, (carbamate)

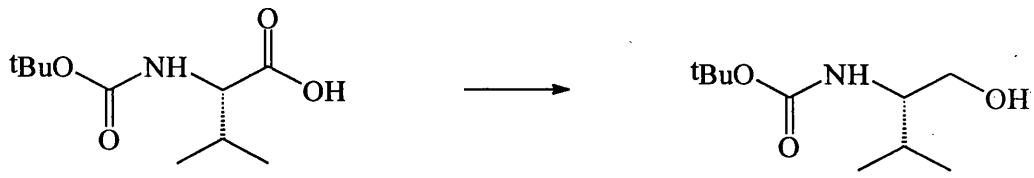
m/z 251 (M^+, 0.4%), 220 (M - CH_2OH, 3.5), 195 (6.0), 164 (12.3), 160 (45.4)

m/z 251.1516 (M^+, $C_{14}H_{21}NO_3$ requires 251.15217)

δ_H(200MHz $CDCl_3$) 1.40 (9H, s, $[CH_3]_3C$), 2.83 (2H, d, J 7, CH_2Ph), 3.03 (1H, m, CHN), 3.58 (2H, d of AB_q, J 11(AB), 3.6 and 5.1, CH_2O), 4.93 (1H, br s, NH), 7.17-7.33 (5H, m, Ph)

δ_C(50MHz $CDCl_3$) 28.2 (q), 37.3 (t, CH_2Ph, 53.6 (d, CHN), 63.7 (t, CH_2O) 79.5 (s, $[CH_3]_3C$), 126.3 (d), 128.4 (d), 129.2(d), 137.9 (s), 156.1 (s, C=O)

2(S)-[(*tert*-Butoxycarbonyl)amino]-3-(methyl)butanol from *N-tert*-butoxycarbonyl-(L)-valine



Materials

N-tert-Butoxycarbonyl-(L)-valine	9.50 g (43.7 mmol)
THF	54 ml
Triethylamine	6.1 ml (44 mmol)
Ethyl chloroformate	4.2 ml (44 mmol)
Sodium borohydride	4.14 g (109 mmol)
Water	50 ml

Method

The procedure used to produce 2(S)-[(*tert*-butoxycarbonyl)amino]-3-(phenyl)propanol was employed with only small alterations in washing and extraction solvent volumes (THF wash 50 ml, diethyl ether extraction 1 L in total, dichloromethane extraction 1 L in total). The desired compound was obtained as a viscous oil (7.62 g, 86% yield). Analysis indicated purity and the material was therefore used directly in further reactions.

Found: C, 58.92; H, 10.29; N, 6.99. $C_{10}H_{21}NO_3$ requires C, 59.11; H, 10.34; N, 6.89%

ν_{max}(film)/cm^{-1} 3354br,s (OH and NH), 2968s (CH_3-), 1691s (C=O), 1517s (carbamate)

m/z 172 (M - CH_2OH, 6.0%), 160 (M - $[CH_3]_2CH$, 1.5), 130 (6.3), 116 (15.2), 87 (6.4), 57 (100)

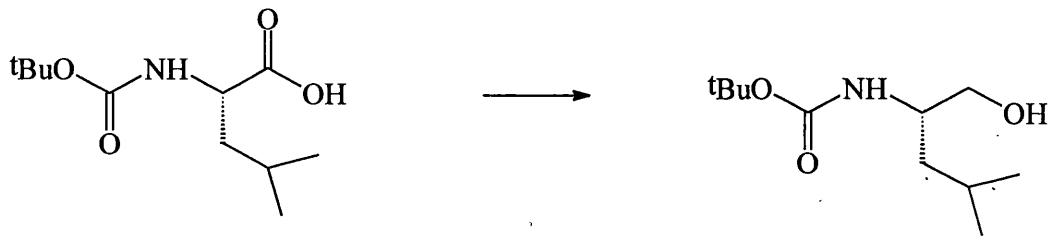
m/z 203.1516 (M^+, $C_{10}H_{21}NO_3$ requires 203.15217)

δ_H(200MHz $CDCl_3$) 0.93 and 0.95 (3H and 3H, both d, J 6.8, $[CH_3]_2CH$), 1.45 (9H, s, $[CH_3]_3C$), 1.83 (1H, m, J possible 6.8, $CH[CH_3]_3$), 3.28 (1H, br s, OH),

3.42 (1H, m, CHN), 3.64 (2H, d of AB_q, J 11.2(AB), 4.3 and 5.8, CH_2O), 4.90 (1H, br d, J 8.1, NH)

δ_C(50MHz $CDCl_3$) 18.4 and 19.3 (both q, $[CH_3]_2CH$), 28.3 (q, $[CH_3]_3C$), 29.1 (d, $[CH_3]_2CH$), 57.9 (d,CHN), 63.7 (t), 79.3 (s, $[CH_3]_3C$), 156.7 (s, C=O)

2(S)-[(*tert*-Butoxycarbonyl)amino]-4-(methyl)pentanol from *N-tert*-butoxycarbonyl-(L)-leucine monohydrate



Materials

N-tert-Butoxycarbonyl-(L)-leucine monohydrate	9.5 g (38 mmol)
THF	50 ml
Triethylamine	5.5 ml (40 mmol)
Ethyl chloroformate	7.4 ml (77 mmol)
Sodium borohydride	3.60 g (95.2 mmol)
Water	50 ml

Method

The procedure used to produce 2(S)-[(*tert*-butoxycarbonyl)amino]-3-(phenyl)propanol was employed with only small alterations in washing and extraction solvent volumes (THF wash 50 ml, diethyl ether extraction 1 L in total, dichloromethane extraction 1 L in total). The desired compound was obtained as a viscous oil (6.71 g, 81% yield). Analysis indicated purity and the material was therefore used directly in further reactions.

Found: C, 60.66; H, 10.45; N, 6.51. $C_{11}H_{23}NO_3$ requires C, 60.82; H, 10.59; N, 6.45%

ν_{max}(film)/cm^{-1} 3350br,s (OH and NH), 2958s (CH_3-), 1692s (C=O), 1524s (carbamate)

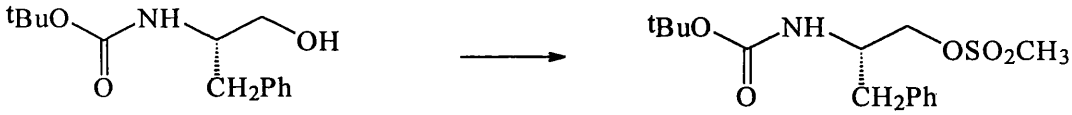
m/z 186 (M - CH_2OH, 4.8), 160 (M - $[CH_3]_2CHCH_2$ or M - $[CH_3]_3C$, 0.3), 144 (4.8), 101 (1.3)

m/z 217.1698 (M^+, $C_{11}H_{23}NO_3$ requires 217.16776)

δ_H(200MHz $CDCl_3$) 0.93 (6H, d, J 6.5, $[CH_3]_2CH$), 1.32 (2H, m, $CH_2CH[CH_3]_2$), 1.45 (9H, s, $[CH_3]_3C$), 1.67 (1H, m, $CH[CH_3]_2$), 3.46-3.78 (3H, m, CH_2O and CHN), 4.80 (1H, br d, J 7.5, NH)

δ_C(50MHz $CDCl_3$) 22.1 and 23.0 (both q, $[CH_3]_2CH$), 24.7 (d, $CH[CH_3]_2$), 28.3 (q, $[CH_3]_3C$), 40.5 (t, $CH_2CH[CH_3]$), 50.8 (d, CHN), 66.1 (t, CH_2O), 79.4 (s, $[CH_3]_3C$), 156.5 (s, C=O)

2(S)-[(*tert*-Butoxycarbonyl)amino]-1-[(methylsulfonyl)oxy]-3-(phenyl)propane from 2(S)-[(*tert*-butoxycarbonyl)amino]-3-(phenyl)propanol



Materials

2(S)-[(*tert*-Butoxycarbonyl)amino]-3-(phenyl)propanol

	20.00 g (0.0797 mmol)
Dichloromethane	500 ml
Triethylamine	32 ml (0.23 mol)
Methanesulfonyl chloride	15 ml (0.19 mol)

Method

A 1 L three necked round bottom flask was equipped with a magnetic stirring bar and nirogen balloon, sealed with septum caps and flame dried. To this 2(S)-[(*tert*-butoxycarbonyl)amino]-3-(phenyl)propanol was added predissolved in dichloromethane. Triethylamine was added with stirring and the mixture taken to 0°C where methanesulfonyl chloride was added dropwise. The resulting yellow solution was stirred at 0°C for a further 25 minutes before being quenched by the addition of 300 ml water. The mixture was transferred to a separatory funnel where the organic layer was separated and the aqueous layer extracted several times with dichloromethane. The combined organic layers were dried with magnesium sulfate, filtered and evaporated to give a yellow solid. Recrystallisation from ethyl acetate/hexane yielded the desired compound in several crops (25.17 g, 96% yield, m.pt. 110-112°C). The material was stored at 0°C in a sealed vessel flushed with nitrogen.

$\nu_{max}(CHCl_3)/cm^{-1}$ 3441m (NH), 3028s (CH_3-), 1709s (C=O), 1500s (carbamate), 1366s (-SO_2-O-)

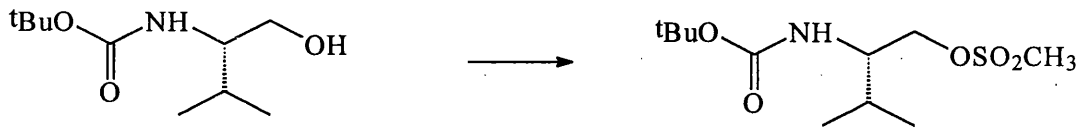
m/z 273 (M - $[CH_3]_2C{=}CH_2$, 1.6%), 257 (M - $[CH_3]_3CO$, 0.3), 178 (2.6)

m/z 273.0670 (M - $[CH_3]_2C{=}CH_2$, $C_{11}H_{15}N_1O_5S_1$ requires 273.0671)

δ_H(200MHz $CDCl_3$) 1.41 (9H, s, $[CH_3]_3C$), 2.86 (2H, m, CH_2Ph), 3.02 (3H, s, SO_2CH_3), 4.13 (2H, m, CH_2O), 4.23 (1H, m, CHN), 4.89 (1H, br d, J 7.2, NH), 7.20-7.36 (5H, m, Ph)

δ_C(50MHz $CDCl_3$) 28.2 (q, $[CH_3]_3C$), 37.2 (q, SO_2CH_2), 46.1 (t, CH_2Ph, 50.8 (d, CHN), 69.8 (t, CH_2O), 79.9 (s, $[CH_3]_3C$), 126.9 (d), 128.7 (d), 129.2 (d), 136.6 (s), 155.0 (s, C=O)

2(S)-[(*tert*-Butoxycarbonyl)amino]-3-methyl-1-[(methylsulfonyl)oxy]butane from 2(S)-[(*tert*-butoxycarbonyl)amino]-3-(methyl)butanol



Materials

2(S)-[(*tert*-Butoxycarbonyl)amino]-3-(methyl)butanol	20.05 g (0.099 mol)
Dichloromethane	700 ml
Triethylamine	42 ml (0.30 mol)
Methanesulfonyl chloride	19 ml (0.26 mol)

Method

The general procedure to 2(S)-[(*tert*-butoxycarbonyl)amino]-1-[(methylsulfonyl)oxy]-3-(phenyl)propane was repeated giving crude 2(S)-[(*tert*-butoxycarbonyl)amino]-3-methyl-1-[(methylsulfonyl)oxy]butane as a yellow solid. Recrystallisation was performed from dichloromethane/hexane yielding the desired product in two crops (27.11 g, 97% yield, m.pt. 64-66°C).

ν_{max}(KBr)/cm^{-1} 3396s (NH), 2978m (CH_3-), 1686s (C=O), 1519s (carbamate), 1377s (-SO_2-O-)

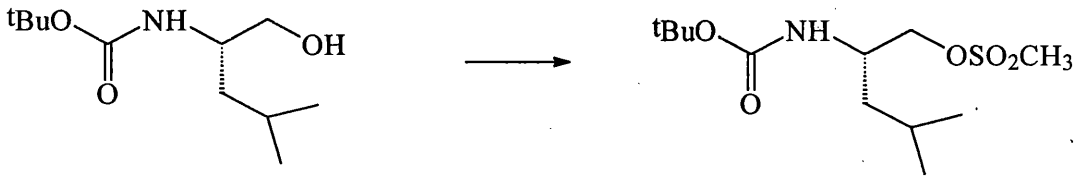
m/z 281 (M^+, 0.1%), 266 (M - CH_3, 0.7), 238 (4.8), 208 (4.8), 172 (19.6), 116 (47.3)

m/z 266.1071 (M - CH_3, $C_{10}H_{20}N O_5S$ requires 266.10622)

δ_H(200MHz $CDCl_3$) 0.96 (3H, d, J 6.8, [C*H*$_3$][CH_3]CH), 0.99 (3H, d, J 6.7, [CH_3][C*H*$_3$]CH,) 1.45 (9H, s, [CH_3]$_3$C), 1.85 (1H, m, [CH_3]$_2$C*H*), 3.04 (3H, s , SO_2CH_3), 3.63 (1H, m, CHN), 4.27 (2H, d, J 4.4, CH_2O), 4.79 (1H, br d, J 9.1, NH)

δ_C(50MHz $CDCl_3$) 18.3 and 19.2 (both q, [CH_3]$_2$CH), 28.2 (q, [CH_3]$_3$C), 28.2 (d, [CH_3]$_2$CH), 37.2 (q, SO_2CH_3), 54.7 (d, CHN), 69.7 (t, CH_2O), 79.5 (s, [CH_3]$_3$*C*), 155.5 (s, C=O)

2(S)-[(*tert*-Butoxycarbonyl)amino]-4-methyl-1-[(methylsulfonyl)oxy]pentane from 2(S)-[(*tert*-butoxycarbonyl)amino]-4-(methyl)pentanol



Materials

2(S)-[(*tert*-Butoxycarbonyl)amino]-4-(methyl)pentanol	5.01 g (23.1 mmol)
Dichloromethane	165 ml
Triethylamine	9.6 ml (69 mmol)
Methanesulfonyl chloride	4.4 ml (57 mmol)

Method

The general procedure to 2(S)-[(*tert*-butoxycarbonyl)amino]-1-[(methylsulfonyl)oxy]-3-(phenyl)propane was repeated giving crude 2(S)-[(*tert*-butoxycarbonyl)amino]-4-methyl-1-[(methylsulfonyl)oxy]pentane as yellow oil. Slow recrystallisation was achieved upon storage of a saturated 1:9 ethyl acetate:hexane solution of this material at 0°C. The desired product was obtained in two crops (5.90 g, 87% yield, m.pt. 74-76°C).

Found: C, 48.56; H, 8.57; N, 4.74. $C_{12}H_{25}NO_5S$ requires C, 48.81; H, 8.47; N, 4.74%

ν_{max}(KBr)/cm^{-1} 3384 (NH), 2978 (CH_3-), 1659 (C=O), 1521 (carbamate), 1369 (-SO_2-O-)

m/z 280 (M - CH_3, 0.1%), 238 (M - $[CH_3]_2CHCH_2$ or M - $[CH_3]C$, 0.1), 222 (1.1), 186 (5.6), 116 (0.5)

m/z 295.1428 (M^+, $C_{12}H_{25}NO_5S$ requires 295.14534)

δ_H(200MHz $CDCl_3$) 0.93 (3H, d, J 6.5, $[CH_3][CH_3]CH$), 0.94 (3H, d, J 6.6, $[CH_3][CH_3]CH$), 1.32-1.53 (2H, m, $CHCH_2CH$), 1.44 (9H, s, $[CH_3]_3C$), 1.67 (1H, m, $[CH_3]_2CH$], 3.04 (3H, s, SO_2CH_3), 3.92 (1H, m, CHN), 4.21 (2H, d of AB_q, J 9.9(AB), 3.7 and 4.0, CH_2O), 4.68 (1H, br d, J 8.3, NH)

δ_C(50MHz $CDCl_3$) 21.9 and 22.8 (both q, $[CH_3]_2CH$), 24.5 (d, $[CH_3]_2CH$), 28.2 (q, $[CH_3]C$), 37.1 (q, SO_2CH_3), 39.9 ($CHCH_2CH$), 47.7 (d, CHN), 71.6 (t, CH_2O), 79.6 (s, $[CH_3]C$), 155.2 (s, C=O)

2(S)-[(*tert*-Butoxycarbonyl)amino]-3-phenyl-1-(phenylthio)propane from 2(S)-[(*tert*-butoxycarbonyl)amino]-1-[(methylsulfonyl)oxy]-3-(phenyl)propane

Materials

2(S)-[(*tert*-Butoxycarbonyl)amino]-1-[(methylsulfonyl)oxy]-3-(phenyl)propane	5.09 g (0.0155 mol)
Sodium	1.14 g (0.0496 mol)
Methanol	9.4 ml (0.23 mol)
Thiophenol	5.4 ml (0.053 mol)
THF	37 ml

Method

A 250 ml three necked round bottom flask was equipped with a magnetic stirring bar and a water condenser topped with a nitrogen balloon. The vessel was sealed with septum caps and flame dried. The vessel was temporarily opened to admit sodium pieces under a flow of nitrogen. By syringe, THF was added and the condenser water flow initiated. Methanol was added slowly, with stirring, resulting in heat generation. Once no traces of solid sodium remained, thiophenol was added rapidly and the mildly yellow solution stirred for 15 minutes at 25°C. The reaction vessel was opened to admit 2(S)-[(*tert*-butoxycarbonyl)amino]-1-[(methylsulfonyl)oxy]-3-(phenyl)propane as a solid, then purged with nitrogen and resealed. Continual nitrogen flow during addition was not employed as the starting solid was very light in nature. The reaction was stirred at 50°C for 2.5 hours then overnight at room temperature. After being diluted with 10% aqueous sodium hydroxide (100 ml), the mixture was transferred to a separatory funnel where extraction with dichloromethane was carried out several times. The combined organic layers were washed with brine, dried with magnesium sulfate, filtered and evaporated to give a yellow oil which slowly solidified on standing. This solid was recrystallised thermally from 1:9

ethyl acetate:hexane to give the desired product as a white solid in several crops (4.50 g, 85% yield, m.pt. 81-84°C, Rf 0.88 eluent 1:1 ethyl acetate:hexane).

Found: C, 69.87; H, 7.46; N, 3.92; S, 9.15. $C_{20}H_{25}N\,O_2S$ requires C, 69.97; H, 7.29; N, 4.08; S, 9.33%

ν_{max}(KBr)/cm^{-1} 3374 (NH), 1694s (C=O), 1526s (carbamate)

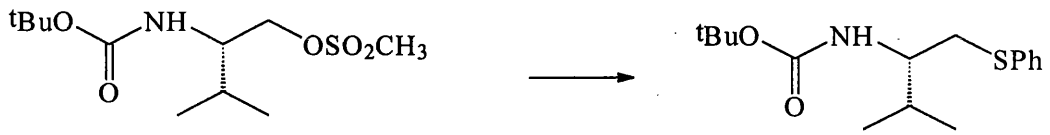
m/z 343 (M^+, 4.8%), 287 (0.4), 270 (4.9), 252 (18.0), 220 (6.0), 109 (17.9), 77 (13.0) 91 (53.5), 57 (100), 56 (4.2)

m/z 343.1596 (M^+, $C_{20}H_{25}N\,O_2S$ requires 343.16060)

δ_H(200MHz $CDCl_3$) 1.39 (9H, s, $[CH_3]_3C$), 2.91 (2H, d, J 6.7, CH_2Ph), 3.03 (2H, d, J 5.6, CH_2S), 4.04 (1H, m, CHN), 4.67 (1H, br d, NH), 7.15-7.37 (10H, m, Ph and SPh)

δ_C(50MHz $CDCl_3$) 28.2 (q, $[CH_3]_3C$, 37.6 and 39.4 (both t, CH_2S and CH_2Ph) , 51.2 (d, CHN), 79.3 (s, $[CH_3]_3C$), 126.2 (d), 126.5 (d), 128.4 (d), 128.9 (d), 129.3 (d), 129.5 (d), 136.0 (s), 137.4 (s), 155.0 (s, C=O)

2(S)-[(*tert*-Butoxycarbonyl)amino]-3-methyl-1-(phenylthio)butane from 2(S)-[(*tert*-butoxycarbonyl)amino]-3-methyl-1-[(methylsulfonyl)oxy]butane



Materials

2(S)-[(*tert*-Butoxycarbonyl)amino]-3-methyl-1-[(methylsulfonyl)oxy]butane 6.2g (22.1mmol)

Sodium	1.61g (70.0 mmol)
Methanol	18ml (0.45 mol)
Thiophenol	7.46ml (72.7 mmol)
THF	53ml + 40ml

Method

The procedure employed to produce 2(S)-[(*tert*-butoxycarbonyl)amino]-3-phenyl-1-(phenylthio)propane was repeated using 2(S)-[(*tert*-butoxycarbonyl)amino]-3-methyl-1-[(methylsulfonyl)oxy]butane as a starting material. In this case an extra 40 ml THF was added to the reaction vessel shortly after the starting material to alleviate stirring problems caused by the consistency of the mixture.

2(S)-[(*tert*-Butoxycarbonyl)amino]-3-methyl-1-(phenylthio)butane was produced as a golden oil which solidified on standing. This material was initially precipitated from hot 1:9 dichloromethane:hexane as a white solid (5.84 g, 90% yield) and used without further purification as recrystallisation was not achieved. A small amount (0.50 g) of material was loaded onto a short column of silica gel and washed with 7x15 ml 6:4 dichloromethane:hexane in order to remove the impurity (Rf 0.73 with 6:4 dichloromethane:hexane as eluent and 0.85 with pure dichloromethane). Flushing the column with 150ml dichloromethane then allowed the isolation of a highly pure sample of the desired product (0.45 g, 90% column yield, m.pt. 65-67°C, Rf 0.27, eluent 6:4 dichloromethane:hexane, and Rf 0.33, eluent dichloromethane).

Found: C, 64.96; H, 8.68; N, 4.65. $C_{16}H_{25}N\,O_2S$ requires C, 64.86; H, 8.78; N, 4.72%

ν_{max}(KBr)/cm^{-1} 3302s (NH), 2974s (CH_3-), 1692s (C=O), 1576s (carbamate)

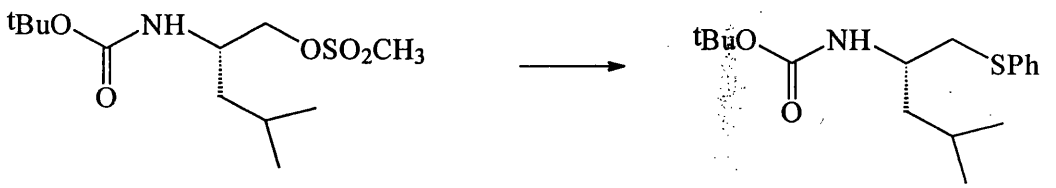
m/z 295 (M^+, 6.4%), 218 (M - Ph, 7.1), 186 (0.5), 179 (3.8), 172 (19.9), 123 (31.5), 116 (52.1)

m/z 295.1594 (M^+, $C_{16}H_{25}N\,O_2S$ requires 295.16063)

δ_H(200MHz $CDCl_3$) 0.89 (3H, d, J 6.7, [C*H*$_3$][CH_3]CH), 0.92 (3H, d, J 6.7, [CH_3][C*H*$_3$]CH), 1.43 (9H, s, [CH_3]$_3$C), 1.92 (1H, m, [CH_3]$_2$C*H*), 3.07 (2H, d, J 5.7, CH_2), 3.64 (1H, m, CHN), 4.59 (1H, br d, J 9.4, NH), 7.34 (5H, m, Ph)

δ_C(50MHz $CDCl_3$) 17.7 and 19.3 (both q, [CH_3]$_2$CH), 28.2 (q, [CH_3]$_3$C), 30.7 (d, [CH_3]$_2$*C*H), 37.2 (t, CH_2S), 55.0 (d, CHN), 78.9 (s, [CH_3]*C*), 126.0 (d), 129.8 (d), 129.4 (d), 136.3 (s), 155.5 (s, C=O)

2(S)-[(*tert*-Butoxycarbonyl)amino]-4-(methyl)-1-(phenylthio)pentane from 2(S)-[(*tert*-butoxycarbonyl)amino]-4-methyl-1-[(methylsulfonyl)oxy]pentane



Materials

2(S)-[(*tert*-Butoxycarbonyl)amino]-4-methyl-1-[(methylsulfonyl)oxy]pentane	5.89 g (20.0 mmol)
Sodium	1.46 g (63.5 mmol)
Methanol	22 ml (0.53 mol)
Thiophenol	6.8 ml (66.2 mmol)
THF	48 ml

Method

The procedure utilised to produce 2(S)-[(*tert*-butoxycarbonyl)amino]-3-phenyl-1-(phenylthio)propane was repeated to give 2(S)-[(*tert*-butoxycarbonyl)amino]-4-(methyl)-1-(phenylthio)pentane as a thick yellow oil.

The short column procedure used to purify 2(S)-[(*tert*-butoxycarbonyl)amino]-3-methyl-1-(phenylthio)butane was employed here to obtain a highly pure sample of 2(S)-[(*tert*-butoxycarbonyl)amino]-4-(methyl)-1-(phenylthio)pentane as a clear colourless oil (2.51 g, 41% yield, Rf 0.27 (product), 0.76 (by-product), eluent 6:4 dichloromethane:hexane and Rf 0.41 (product), 0.85 (by-product), eluent pure dichloromethane).

Found: C, 66.08; H, 8.92; N, 4.44. $C_{17}H_{27}N\,O_2S$ requires C, 66.02; H, 8.74; N, 4.53%

ν_{max}(KBr)/cm^{-1} 3348m (NH), 2957s (CH_3-), 1698s (C=O), 1584s (carbamate)

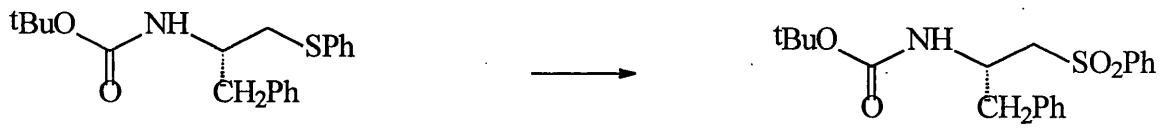
m/z 309 (M^+, 8.1%), 252 (M - $[CH_3]_2CHCH_2$ or M - $[CH_3]_3C$, 0.5), 236 (3.4), 208 (0.6), 193 (6.1), 186 (21.2)

m/z 309.1744 (M^+, $C_{17}H_{27}N\,O_2S$ requires 309.17626)

δ_H(200MHz $CDCl_3$) 0.86 (3H, d, J 6.2, [C*H*$_3$][CH_3]CH), 0.90 (3H, d, J 6.4, [CH_3][C*H*$_3$]CH), 1.41 (2H, m, CHC*H*$_2$CH) 1.41 (9H, s, [CH_3]$_3$C), 1.62 (1H, m, [CH_3]$_2$C*H*), 3.08 (2H, d, J 4.6, CH_2S), 3.90 (1H, m, CHN), 4.56 (1H, br d, J 8.8, NH), 7.26 (5H, m, Ph)

δ_C(50MHz $CDCl_3$) 22.0 and 22.9 (both q, [*C*H$_3$]$_2$CH), 22.9 (d, [CH_3]$_2$*C*H), 28.2 (q, [*C*H$_3$]$_3$C), 39.6 (t, [CH_3]$_2$CH*C*H$_2$), 42.8 (t, CH_2S), 48.2 (d, CHN), 79.0 (s, [CH_3]*C*), 125.9 (d), 128.0 (d), 129.0 (d), 136.3 (s), 155.1 (s, C=O)

2(S)-[(*tert*-Butoxycarbonyl)amino]-3-phenyl-1-(phenylsulfonyl)pentane from 2(S)-[(*tert*-butoxycarbonyl)amino]-3-phenyl-1-(phenylthio)propane



Materials

2(S)-[(*tert*-Butoxycarbonyl)amino]-3-phenyl-1-(phenylthio)propane	1.00 g (2.92 mmol)
Dichloromethane	22.5 ml
3-Chloroperoxybenzoic acid (57-86%)	1.61 g (min. 5.32 mmol)

Method

In a 100 ml round bottom flask equipped with a magnetic stirrer, 2(S)-[(*tert*-butoxycarbonyl)amino]-3-phenyl-1-(phenylthio)propane was placed and dissolved in dichloromethane. The solution was taken to 0°C where 3-chloroperoxybenzoic acid was added as a solid with stirring. The resulting slurry was stirred for 1 hour at room temperature before being transferred to a separatory funnel where it was shaken carefully with a saturated aqueous solution of sodium bisulfite. After being allowed to stand for 1 hour and 20 minutes the aqueous layer was removed and the organic layer washed several times with a total of 70 ml 10% sodium hydroxide aqueous solution. The dichloromethane layer was removed and the combined sodium hydroxide washings extracted with a further 75 ml dichloromethane. The combined organic layers were washed with a saturated aqueous solution of sodium chloride, dried with magnesium sulfate and filtered. Evaporation of the resulting dichloromethane solution gave a white solid which was slowly recrystallised from hot 1:1 ethyl acetate:hexane giving the desired product in two crops (0.99 g, 90% yield, m.pt. 204-206°C, lit. 215-216°C [75], Rf 0.13 eluent 100% dichloromethane).

$[\alpha]_D^{22}$ -25.95 (5.5 mg/1 ml DMSO) lit. -32.7 (5.2 mg/1 ml DMSO at 20°C) [75]

Found: C, 64.07; H, 6.69; N, 3.62; S, 8.25. $C_{20}H_{25}N\,O_4S$ requires C, 64.00; H, 6.66; N, 3.73; S, 8.53%

ν_{max}(KBr)/cm^{-1} 3383s (NH), 1693s (C=O), 1521s (carbamate)

m/z 319 (M - $[CH_3]_2[CH_2]C$, 1.0), 284 (M - $PhCH_2$, 7.6), 141 (M - $PhSO_2$,141)

m/z 319.0869 (M^+, $C_{16}H_{17}N\,O_4S$ requires 319.08780)

δ_H(200MHz $CDCl_3$) 1.38 (9H, s, $[CH_3]_3C$), 2.99 (2H, d of AB_q, J 13.5(AB), 6.4 and 7.2, CH_2Ph), 3.34 (1H d of AB_q, J 14.4(AB), 4.7 and 7.4, CH_2SO_2), 4.11 (1H, m, CHN), 4.90 (1H, br s, NH), 7.13-7.90 (10H, m, Ph and SPh)

δ_H(200MHz d_6-DMSO) 1.19 (9H, s, $[CH_3]_3C$), 2.75 (2H, m, CH_2Ph), 3.44 (2H, d of AB_q, J 14.7(AB), 3.8 and 8.5, CH_2SO_2), 4.03 (1H, m, CHN), 7.09-7.87 (10H, m, Ph and SPh)

δ_C(50MHz d_6-DMSO) 28.1 (q, $[CH_3]_3C$), 40.3 (t, CH_2Ph), 47.6 (d, CHN), 58.2 (t, CH_2SO_2), 77.7 (s, $[CH_3]_3C$), 126.3(d), 127.8 (d), 128.2 (d), 129.2 (d), 129.3 (d), 133.7 (d), 137.7 (s), 139.5 (s), 154.3 (s, C=O)

2(S)-[(*tert*-Butoxycarbonyl)amino]-3-methyl-1-(phenylsulfonyl)butane from 2(S)-[(*tert*-butoxycarbonyl)amino]-3-methyl-1-(phenylthio)butane

Materials

2(S)-[(*tert*-Butoxycarbonyl)amino]-3-methyl-1-(phenylthio)butane	23.1 g (78.3 mmol)
Dichloromethane	600 ml
3-Chloroperoxybenzoic acid (57-86%)	43.22 g (min. 142.8 mmol)

Method

The procedure employed to form 2(S)-[(*tert*-butoxycarbonyl)amino]-3-phenyl-1-(phenylsulfonyl)pentane was repeated to give a white solid. Initial recrystallisation from 1:2 ethyl acetate:hexane yielded a white solid which was shown to contain mainly 3-chlorobenzoic acid. The mother liquors were therefore washed with further quantities of 10% sodium hydroxide aqueous solution. Separation of the organic layer was followed by extraction of the aqueous layer with dichloromethane. The combined organic layers were dried with magnesium sulfate, filtered and evaporated to give an off-white solid. Recrystallisation was initiated by dissolving the material in hot 1:2 ethyl acetate:hexane and slowly adding further hexane to the cooled solution. The desired product was isolated in this way in two crops (18.9 g, 74% yield, m.pt. 108-110°C, lit. 103-105°C [75]).

$[\alpha]_D^{22}$ +9.2 (23 mg/1 ml $CHCl_3$) lit. +11.02 (23 mg/ml DMSO at 20°C)[75]

Found: C, 58.63; H, 7.78; N, 4.13. $C_{16}H_{25}N\,O_4S$ requires C, 58.71; H, 7.64; N, 4.28%

ν_{max}(KBr)/cm^{-1} 3388m (NH), 2970m (CH_3-), 1688s (C=O), 1576s (carbamate)

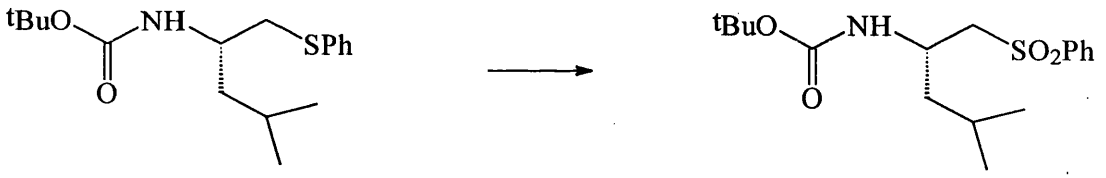
m/z 284 (M - $[CH]_2CH$, 7.0%), 250 (M - Ph, 0.2), 223 (0.2), 186 (3.2), 141 (12.1)

m/z 284.0944 (M - $[CH]_2CH$, $C_{13}H_{18}N\,S\,O_4$ requires 284.09565)

δ_H(200MHz $CDCl_3$) 0.86 (3H, d, J 6.3, [C*H*$_3$][CH_3]CH), 0.89 (3H, d, J 6.1, [CH_3][C*H*$_3$]CH), 1.42 (9H, s, [CH_3]$_3$C), 2.01 (1H, m, [CH_3]$_2$C*H*), 3.27 (2H, d of AB_q, J 14.5(AB), 8.6 and 2.7, CH_2), 3.80 (1H, m, CHN), 4.75 (1H, br d, J 8.7, NH), 7.74 (5H, m, Ph)

δ_C(50MHz $CDCl_3$) 17.6 and 18.6 (both q, [*C*H$_3$]$_2$CH), 28.1 (q, [*C*H$_3$]$_3$C), 31.9 (d, [CH_3]$_2$*C*H), 51.6 (d, CHN), 57.3 (t, CH_2SO_2), 79.1 (s, [CH_3]*C*), 127.8 (d), 129.0 (d), 133.5 (d), 139.2 (s), 155.0 (s, C=O)

2(S)-[(*tert*-Butoxycarbonyl)amino]-4-methyl-1-(phenylsulfonyl)pentane from 2(S)-[(*tert*-butoxycarbonyl)amino]-4-(methyl)-1-(phenylthio)pentane



Materials

2(S)-[(*tert*-Butoxycarbonyl)amino]-4-(methyl)-1-(phenylthio)pentane	2.1 g (6.80 mmol)
Dichloromethane	53 ml
3-Chloroperoxybenzoic acid (57-86%)	3.73 g (min. 12.3 mmol)

Method

The general procedure employed to produce 2(S)-[(*tert*-butoxycarbonyl)amino]-3-phenyl-1-(phenylsulfonyl)pentane was repeated to give a white solid (1.94 g, 84% crude yield). A small quantity (0.50 g) of this was purified by slow recrystallisation from hot 1:9 ethyl acetate:hexane for full analysis which confirmed the product identity as being the desired material (0.46 g, 92% recrystallisation yield, m.pt. 95-97°C, lit. 95-98°C [75]).

$[\alpha]_D^{22}$ -9.65 (24 mg/1 ml $CHCl_3$) lit. -9.87 (24 mg/1 ml $CHCl_3$ at 20°C) [75]

Found: C, 59.67; H, 7.98; N, 4.01. $C_{17}H_{27}NO_4S$ requires C, 59.82; H, 7.92; N, 4.10%

ν_{max}(KBr)/cm^{-1} 3387m (NH), 2974m (CH_3-), 1689s (C=O), 1588s (carbamate)

m/z 284 (M - $[CH_3]_2CHCH_2$ or M - $[CH_3]_3C$, 1.6%), 268 (M - $[CH_3]_3CO$, 1.2), 240 (1.1), 225 (0.5), 186 (2.6)

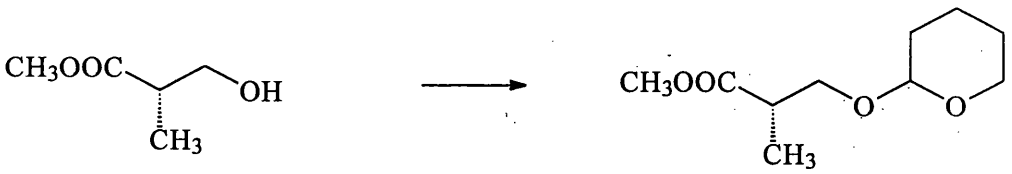
m/z 341.1660 (M^+, $C_{17}H_{27}NO_4S$ requires 341.16660)

δ_H(200MHz $CDCl_3$) 0.88 (6H, d, J 6.1, $[CH_3]_2CH$), 1.40 (9H, s, $[CH_3]_3C$), 1.59 (3H, m, $CHCH_2CH$) and $[CH_3]_2CH$), 3.35 (2H, d of AB_q, J 14.3(AB), 6.2 and 4.5, CH_2S), 4.00 (1H, m, CHN), 4.86 (1H, br d, J 8.1, NH), 7.79 (5H, m, Ph)

δ_C(50MHz $CDCl_3$) 21.7 and 22.6 (both q, $[CH_3]_2CH$), 24.6 (d, $[CH_3]_2CH$), 28.2 (q, $[CH_3]_3C$), 43.1 (t, $[CH_3]_2CHCH_2$), 45.5 (d, CHN), 59.6 (t, CH_2SO_2), 78.4 (s, $[CH_3]C$), 127.7 (d), 129.2 (d), 133.6 (d), 139.9 (s), 154.8 (s, C=O)

EXPERIMENTAL PART 5: Synthesis of Aldehyde Coupling Components

Methyl 3-[(2-tetrahydropyranyl)oxy]-2(S)-methylpropionate from (S)-methyl-3-hydroxy-2-methylpropionate



Materials

(S)-Methyl-3-hydroxy-2-methylpropionate	3.80 g (32.2 mmol)
3,4-Dihydropyran	3.54 g (42.1 mmol)
Dichloromethane	50 ml
Pyridinium *p*-toluenesulfonate	0.16 g (0.64 mmol)

Method

(S)-Methyl-3-hydroxy-2-methylpropionate and 3,4-dihydropyran were weighed directly into a 250 ml round bottom flask, dissolved in dichloromethane and a magnetic stirring bar added. The vessel was immersed in a water bath held at approximately 25°C, stirring initiated and pyridinium *p*-toluenesulfonate added as a solid. After being stirred for 3 hours at this temperature the water bath was removed and stirring continued for a further 1 hour and 20 minutes. With the aid of a little additional dichloromethane the solution was transferred to a separatory funnel. In this vessel the solution was washed sequentially with saturated aqueous solutions of sodium bicarbonate and sodium chloride. Following this the organic layer was dried with magnesium sulfate, filtered and evaporated yielding a clear colourless oil (6.51 g, 100% yield).

Found: C, 59.26; H, 8.71. $C_{10}H_{18}O_4$ requires C, 59.40; H, 8.91%

ν_{max}(film)/cm^{-1} 2945s (CH_3- and -CH_2-), 2876m (O-CH-O), 1742s (C=O)

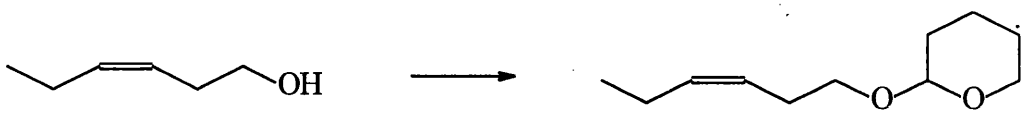
m/z 202 (M^+, 0.1%), 187 (M - CH_3,0.5), 171 (0.4), 117 (0.8), 85 (100)

m/z 202.1194 (M^+, $C_{10}H_{18}O_4$ requires 202.1251)

δ_H(200MHz $CDCl_3$) 1.19 and 1.20 (3H, both d, J 7.1, ratio 1:1, CHC*H*$_3$), 1.47-1.83 (6H, m, J possible 7.1, C*H*$_2$C*H*$_2$C*H*$_2$), 2.77 (1H, m, C*H*CH_3), 3.40-3.96 (4H, m, 2xCH_2O), 3.70 (3H, 2xs, OCH_3), 4.61 (1H, m, OCHO)

δ_C(50MHz $CDCl_3$) 13.6 (q, $CHCH_3$, 18.9 and 19.1 (t, $CH_2CH_2CH_2$), 25.2 (t, $CH_2CH_2CH_2$), 30.2 and 30.3 (t, $CH_2CH_2CH_2$), 39.8 and 40.0 (d, *C*HCH_3), 51.4 (q, OCH_3), 61.5 and 61.8 (t, OCH_2), 68.8 and 69.1 (t, OCH_2), 98.1 and 98.6 (d, OCHO), 175.1 (s, C=O)

1-[(2-Tetrahydropyranyl)oxy]-(*cis*)-3-hexene from (*cis*)-3-hexen-1-ol



Materials

(*cis*)-3-Hexen-1-ol	10.08 g (0.101 mol)
Dihydropyran	11.09 g (0.132 mol)
Dichloromethane	140 ml
Pyridinium *p*-toluenesulfonate	0.49 g (1.95 mmol)

Method

The general procedure employed to produce methyl 3-[(2-tetrahydropyranyl)oxy]-2(S)-methylpropionate was repeated to give 1-[(2-tetrahydropyranyl)oxy]-(*cis*)-3-hexene as a clear colourless oil (17.56 g, 94% yield).

Found: C, 71.82; H, 10.82. $C_{11}H_{20}O_2$ requires C, 71.74; H, 10.87%

ν_{max}(film)/cm^{-1} 2942s (CH_3- and -CH_2-), 2872s (O-CH-O), 1652w (C=C)

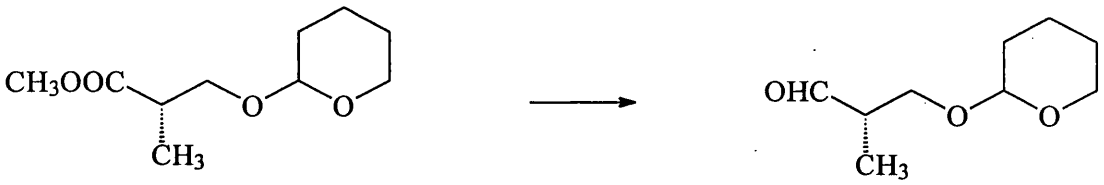
m/z 184 (M^+, 0.1%), 155 (M - CH_3CH_2, 0.1), 115 (3.6), 101 (8.3), 100 (1.1), 99 (0.4), 85 (100), 84 (3.7), 83 (12.5)

m/z 184.1466 (M^+, $C_{11}H_{20}O_2$ requires 184.14630)

δ_H(200MHz $CDCl_3$) 0.96 (3H, t, J 7.4, C*H*$_3$CH$_2$), 1.47-1.85 (6H, m, $CH_2CH_2CH_2$), 2.07 (2H, quin, J 7.4, CH_3CH_2), 2.35 (2H, q, J 6.7, CHC*H*$_2$CH$_2$O), 3.88-3.34 (4H, m, 2xCH_2O) 4.60 (1H, br t, J 3.4, OCHO), 5.29-5.61 (2H, m, CHCH)

δ_C(50MHz $CDCl_3$) 14.2 (q), 19.5 (t), 20.5 (t), 25.4 (t), 27.7 (t), 30.6 (t), 62.1 (t, CH_2O), 67.0 (t, CH_2O), 98.6 (d, OCHO), 124.8 and 133.5 (both d, CH=CH)

2(S)-Methyl-3-[(2-tetrahydropyranyl)oxy]propionaldehyde from methyl 3-[(2-tetrahydropyranyl)oxy]-2(S)-methylpropionate



Materials

Methyl 3-[(2-tetrahydropyranyl)oxy]-2(S)-methylpropionate	1.01 g (5.00 mmol)
Dichloromethane	18 ml
Diisobutylaluminium hydride (1.0 M in hexane)	5.3 ml (5.3 mmol)

Method

A 50 ml round bottom flask with a side arm was equipped with a magnetic stirring bar and nitrogen balloon. After being flame dried the vessel was charged with methyl 3-[(2-tetrahydropyranyl)oxy]-2(S)-methylpropionate predissolved in dichloromethane. The vessel and its contents were taken to -78°C where diisobutylaluminium hydride was added dropwise, with stirring, via syringe. Stirring was continued at -78°C for a further 10 minutes after which the reaction was quenched at this temperature by the addition of saturated aqueous ammonium chloride. The mixture was allowed to warm to room temperature where it was filtered with the aid of a little additional dichloromethane and saturated aqueous ammonium chloride. The filtrate was transferred to a separatory funnel where the organic layer was separated and washed with saturated aqueous sodium chloride. The organic layer was then dried with magnesium sulfate, filtered and evaporated to give a clear colourless oil (0.80 g, 93% crude yield). This material was of sufficient purity to be used directly in further reactions. Small amounts of highly pure material could be produced by short path distillation (b.pt. 45°C/0.4 mm Hg).

This material was shown to slowly oxidise in air at room temperature to the corresponding carboxylic acid. When stored under nitrogen at 0°C this process could be prevented.

ν_{max}(film)/cm^{-1} 2942s (CH_3- and -CH_2-), 2873s (O-CH-O), 2728w (aldehyde CH), 1727s (C=O)

m/z 172 (M^+, 0.2%), 153, (M - CH_3, 0.1) 143 (0.2), 101 (21.8), 85 (100)

δ_H(200MHz $CDCl_3$) 1.14 and 1.15 (3H, 2xd, J 7.1, ratio 1:1, C*H*$_3$CH), 1.51-1.83 (6H, m, $CH_2CH_2CH_2$), 2.68 (1H, m, CH_3C*H*), 3.48-3.65 and 3.78-4.01 (4H, m, 2xCH_2O), 4.62 (1H, m, OCHO), 9.75 (1H, m, CHO)

δ_C(50MHz $CDCl_3$) 10.5 and 10.5 (q), 18.9 and 19.1 (t, $CH_2CH_2CH_2$), 25.2 and 25.3 (t, $CH_2CH_2CH_2$), 30.2 and 30.4 (t, $CH_2CH_2CH_2$), 46.4 and 46.5 (d, *C*HCH_3), 61.8 and 61.9 (t, CH_2O), 67.0 and 67.3 (t, CH_2O), 98.4 and 98.9 (d, OCHO), 203.6 and 203.7 (d, C=O)

3-[(2-Tetrahydropyranyl)oxy]propionaldehyde from 1-[(2-tetrahydropyranyl)oxy]-(*cis*)-3-hexene

Materials

1-[(2-Tetrahydropyranyl)oxy]-(*cis*)-3-hexene	11.82 g (6.42 mmol)
Dichloromethane	150 ml
Ozone	
Triethylamine	17 ml (122 mmol)

Method

In a 250 ml round bottom flask equipped with a magnetic stirring bar, 1-[(2-tetrahydropyranyl)oxy]-(*cis*)-3-hexene was placed and dissolved in dichloromethane. The solution was cooled to -78°C and a steady stream of ozone bubbled through until a mild blue colouration was observed (*c.* 3 hours). After the system was purged with nitrogen triethylamine was added to the blue solution at -78°C with stirring. The resulting colourless solution was allowed to warm to room temperature where it was flushed with nitrogen, sealed with a nitrogen balloon and stirred overnight. With the aid of additional dichloromethane the solution, which was now slightly yellow in colouration, was washed through a pad of silica and evaporated giving a cloudy yellow oil (13.51 g, main product Rf 0.60, eluent 100% ethyl acetate). This material was adsorbed onto a short column of silica and eluted with ethyl acetate. The appropriate ethyl acetate fractions were combined, dried with magnesium sulfate, filtered and evaporated giving a clear golden liquid (7.75 g, 76% crude yield).

This material was used directly in further reactions as triethylamine was identified by ^{1}H NMR spectroscopy as being the main impurity. For full analysis

a small quantity (0.55 g) was purified by short path distillation (40°C, 0.3 mm Hg) giving 0.20 g of the desired product.

ν_{max}(film)/cm^{-1} 2944s (-CH_2-), 2874m (O-CH-O), 1728s (C=O)

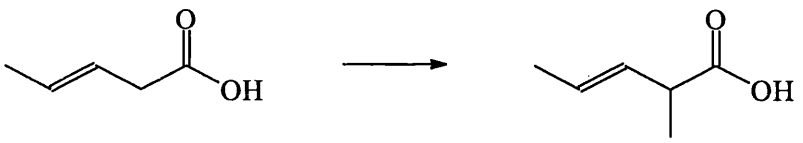
m/z 157 (M - H, 1.2%), 101 (41.1), 85 (100)

m/z 157.0878 (M - H, $C_8H_{13}O_3$ requires 157.08644)

δ_H(200MHz $CDCl_3$) 1.51-1.95 (6H, m, $CH_2CH_2CH_2$), 2.70 (2H, dt, J 1.9(d) and 6.0(t), CH_2CHO), 3.47-3.58 and 3.71-3.90 and 4.04-4.15 (4H, m, 2xCH_2O), 4.63 (1H, m, OCHO), 9.82 (1H, t, J 1.9, CHO)

δ_C(50MHz $CDCl_3$) 19.2 (t, $CH_2CH_2CH_2$), 25.2 (t, $CH_2CH_2CH_2$), 30.3 (t, $CH_2CH_2CH_2$), 43.7 (t, CH_2CHO), 61.0 and 62.1 (both t, 2xCH_2O) , 98.6 (d, OCHO), 201.3 (s, C=O)

(±) 2-Methyl-*trans*-3-pentenoic acid from *trans*-3-pentenoic acid



Materials

Diisopropylamine	1.7 ml
n-Butyl lithium (1.47 M in hexane)	7.9 ml (11.6 mmol)
Methyl iodide	0.7 ml (11.2 mmol)
THF	10 ml
trans-3-Pentenoic acid	0.50 g (5.00 mmol)
THF	1 ml

Method

A 100 ml round bottom flask with side arm was equipped with a magnetic stirring bar, fitted with a nitrogen balloon and sealed with a septum cap. This vessel was flame dried then charged with diisopropylamine and THF. Stirring was initiated, the solution cooled to 0°C and *n*-butyl lithium added slowly by syringe. After being allowed to stir at 0°C for 30 minutes the solution was taken to -78°C where *trans*-3-pentenoic acid was added, predissolved in THF, by syringe. The reaction was stirred at -78°C for 50 minutes before being allowed to warm to room temperature where stirring was continued for a further 30 minutes. The now yellow solution was taken back to -78°C where methyl iodide was added and the resulting mixture stirred for 30 minutes. Over two hours the mixture was brought slowly to room temperature where, after a further half hour, the reaction was quenched by the addition of a 5% aqueous solution of hydrochloric acid. The mixture was extracted several times with diethyl ether, the combined organic layers dried with magnesium sulfate, filtered and evaporated yielding a brown oil. Dry flash chromatography allowed the isolation of a quantity of reasonably pure yellow oil which was shown to be the desired product (Rf 0.39, eluent 1:1 ethyl acetate:hexane).

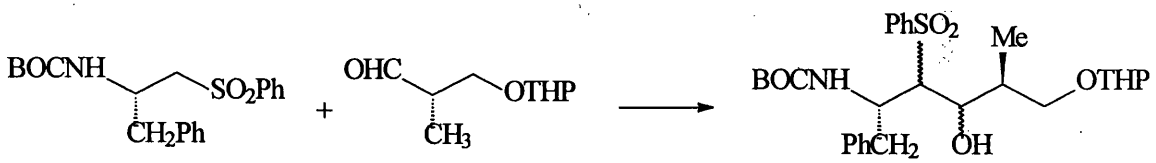
m/z 114 (M^+, 19.3%), 99 (M - CH_3, 7.5), 73 (2.8), 69 (83.7), 41 (100)

δ_H(200MHz $CDCl_3$) 1.26 (3H, d, J 7.0, αCH_3), 1.69 (3H, d, J 5.0, terminal CH_3), 3.12 (1H, dq, J 7.0, C*H*COOH), 5.54 (2H, m, CH=CH), 10.59 (1H, br s, COOH)

δ_C(50MHz $CDCl_3$) 17.1 (q, αCH_3), 17.8 (q, CH_3CH=CH), 42.7 (*C*HCOOH), 127.5 and 129.2 (both d, CH=CH), 181.7 (s, COOH)

EXPERIMENTAL PART 6: Coupling and Isostere Generation

5(S)-[(*tert*-Butoxycarbonyl)amino]-2(R)-methyl-6-phenyl-4-(phenylsulfonyl)-1-[(2-tetrahydropyranyl)oxy]-3-hexanol from 2(S)-[(*tert*-butoxycarbonyl)amino]-3-phenyl-1-(phenylsulfonyl)propane and 2(S)-methyl-3-[(2-tetrahydropyranyl)oxy]propionaldehyde



Materials

2(S)-[(*tert*-Butoxycarbonyl)amino]-3-phenyl-1-(phenylsulfonyl)propane	4.36 g (11.6 mmol)
THF	330 ml
Methyl lithium (1.4 M in ether)	18.3 ml (25.6 mmol)
Diisobutylaluminium hydride (1.0 M in THF)	23.5 ml (23.5 mmol)
Methanol	0.95 ml (23.5 mmol)
2(S)-Methyl-3-[(2-tetrahydropyranyl)oxy]propionaldehyde	4.00 g (23.3 mmol)
THF	28 ml

Method

A 1 L three necked round bottom flask (vessel A) was equipped with a magnetic stirring bar and nitrogen balloon. A 100 ml round bottom flask with a side arm (vessel B) was similarly equipped. Both vessels were sealed with septum caps and flame dried.

2(S)-[(*tert*-Butoxycarbonyl)amino]-3-phenyl-1-(phenylsulfonyl)propane was added to vessel A, as a solid, under a flow of nitrogen. THF (330 ml) was added by syringe and the suspension heated until complete solution was achieved. The solution was then taken to -78°C where methyl lithium was added dropwise

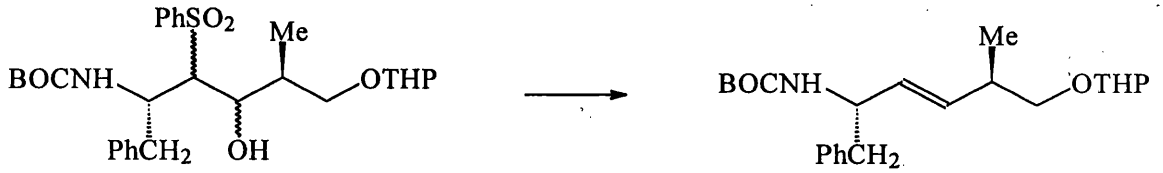
with stirring. The yellow colouration observed on addition of this material became permanent once approximately half the specified volume had been added. This solution was stirred at -78°C for 30 minutes during which time the vessel B reaction was prepared.

In vessel B diisobutylaluminium hydride was taken to 0°C where methanol was added dropwise with stirring. After the effervescence ceased the solution was taken to -78°C where 2(S)-methyl-3-[(2-tetrahydropyranyl)oxy]propionaldehyde was added, predissolved in THF, and stirring continued for a further 2 minutes.

With both solutions at -78°C the contents of vessel B were transferred *via* cannula to the yellow solution in vessel A with stirring. A gradual colour loss in vessel A was noted. Once addition was complete the reaction mixture was stirred for a further 1 hour at -78°C before being quenched at this temperature by the addition of saturated aqueous ammonium chloride. After being allowed to warm to room temperature the mixture was filtered and the filtrate extracted several times with diethyl ether (total extraction volume 600 ml). The ether extracts were then dried with magnesium sulfate and evaporated giving an off-white oily solid. The extraction procedure was repeated with dichloromethane but no further material was obtained. Addition of diethyl ether (200 ml) to the oily solid allowed the isolation of a clean white solid which was shown to be unreacted 2(S)-[(*tert*-butoxycarbonyl)amino]-3-phenyl-1-(phenylsulfonyl)propane (0.56 g, 1.5 mmol, 13% recovery). Evaporation of the diethyl ether yielded a yellow oil which was considered to contain the many possible diastereomers of the desired product (7.19 g.) This material was used directly in the next step without purification.

δ_H(200MHz $CDCl_3$) 1.35 ppm ($[CH_3]_3C$, desired product), 1.38 ppm ($[CH_3]_3C$, recovered starting sulfone).

5(S)-[(*tert*-Butoxycarbonyl)amino]-2(R)-methyl-6-(phenyl)-1-[(2-tetrahydropyranyl)oxy]-*trans*-3-hexene from 5(S)-[(*tert*-butoxycarbonyl)amino]-2(R)-methyl-6-phenyl-4-(phenylsulfonyl)-1-[(2-tetrahydropyranyl)oxy]-3-hexanol



Materials

5(S)-[(*tert*-Butoxycarbonyl)amino]-2(R)-methyl-6-phenyl-4-(phenylsulfonyl)-1-[(2-tetrahydropyranyl)oxy]-3-hexanol	7.19 g (13.14 mmol)
Disodium hydrogen phosphate	7.52 g (52.96 mmol)
Methanol	132 ml
Sodium/mercury amalgam (2% sodium)	59.31 g (51.57 mmol Na)

Method

5(S)-[(*tert*-Butoxycarbonyl)amino]-2(R)-methyl-6-phenyl-4-(phenylsulfonyl)-1-[(2-tetrahydropyranyl)oxy]-3-hexanol was dissolved in methanol in a 500 ml three necked round bottom flask. A magnetic stirring bar was introduced, the vessel flushed with nitrogen and sealed with a nitrogen balloon. The solution was taken to 0°C where disodium hydrogen phosphate was added followed by sodium/mercury amalgam. After being stirred at 0°C for 4 hours and 10 minutes the reaction was quenched at this temperature by the addition of water. The resulting mixture was allowed to stir overnight at room temperature then carefully decanted, with the aid of a little dichloromethane and water, from the residual mercury. The mixture was extracted several times with dichloromethane (total organic solvent volume 1200 ml) which was then dried with magnesium sulfate, filtered and evaporated yielding a virtually colourless clear oil. Dry flash chromatography (eluent 3:7 ethyl acetate:hexane) gave a colourless clear oil which analysis showed to be the desired product (2.87 g, product Rf 0.75, eluent 3:7 ethyl acetate:hexane).

This quantity of product represented a 64% overall yield from 2(S)-[(*tert*-butoxycarbonyl)amino]-3-phenyl-1-(phenylsulfonyl)pentane for the two step coupling process.

Found: C, 70.83; H, 9.16; N, 3.77. $C_{23}H_{35}N\,O_4$ requires C, 70.95; H, 9.00; N, 3.60%

$[\alpha]_D^{26}$ +0.75 (14.3 mg/1 ml $CHCl_3$)

ν_{max}(film)/cm^{-1} 3442m (NH), 2947s (CH_3- and -CH_2-), 2873m (O-CH-O), 1706s (C=O), 1496s (carbamate)

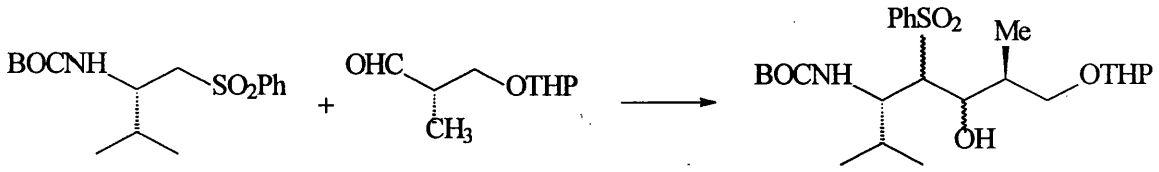
m/z 298 (M - $PhCH_2$, 1.7%), 214 (M - dihydropyran, 2.9), 158 (13.4)

m/z 298.1996 (M - $PhCH_2$, $C_{16}H_{18}N\,O_4$ requires 298.20182)

δ_H(200MHz $CDCl_3$) 0.99 (3H, 2xd, J 6.7, CHC*H*$_3$), 1.19-1.43 (6H, m, $CH_2CH_2CH_2$), 1.40 (9H, s, $[CH_3]_3C$), 2.41 (1H, m, C*H*CH_3), 2.81 (2H, d, J 6.4, PhC*H*$_2$), 3.18-3.82 (4H, m, 2xCH_2O), 4.36 (1H, m, NHC*H*), 4.54 (2H, m, NH and OCHO), 5.45 (2H, m, J possible 15.6, CH=CH), 7.14-7.31 (5H, m, Ph)

δ_C(50MHz $CDCl_3$) 16.9 (q, CH_3CH), 19.3 (t, $CH_2CH_2CH_2$), 25.3 (t, $OCH_2CH_2CH_2CH_2$), 28.2 (q, $[CH_3]_3C$), 30.4 (t, $CH_2CH_2CH_2CHO_2$), 36.4 (d, $CHCH_3$), 41.8 (t, CH_2Ph), 53.0 (d, CHN), 61.8 (t, CH_2O), 71.8 and 71.9 (both t, CH_2O), 79.0 (s, $[CH_3]_3C$), 98.4 and 98.6 (d, OCHO), 126.1, 128.0, 129.5, 133.4, 133.5 (all d, CH=CH and Ph), 137.5 (s, Ph), 154.9 (s, C=O)

5(S)-[(*tert*-Butoxycarbonyl)amino]-2(R)-methyl-6-methyl-4-(phenylsulfonyl)-1-[(2-tetrahydropyranyl)oxy]-3-heptanol from 2(S)-[(*tert*-butoxycarbonyl)amino]-3-methyl-1-(phenylsulfonyl)butane and 2(S)-methyl-3-[(2-tetrahydropyranyl)oxy]propionaldehyde



Materials

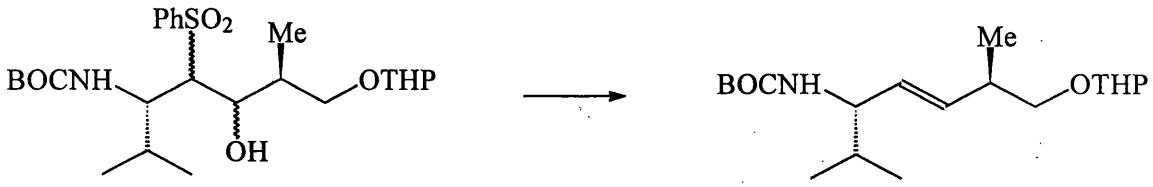
(S)-[(*tert*-Butoxycarbonyl)amino]-3-methyl-1-(phenylsulfonyl)butane	5.70 g (17.4 mmol)
THF	270 ml
Methyl lithium (1.4 M in ether)	27.5 ml (38.5 mmol)
Diisobutylaluminium hydride (1.0 M in THF)	35 ml (35 mmol)
Methanol	1.4 ml (34.6 mmol)
2(S)-Methyl-3-[(2-tetrahydropyranyl)oxy]propionaldehyde	6.00 g (34.9 mmol)
THF	40 ml

Method

The general reaction procedure used to form 5(S)-[(*tert*-butoxycarbonyl)amino]-2(R)-methyl-6-phenyl-4-(phenylsulfonyl)-1-[(2-tetrahydropyranyl)oxy]-3-hexanol was repeated using the above starting materials. In this case the initial warming of vessel A to obtain complete solution was not necessary and the vessel A reaction time was increased to 40 minutes before the transference of the vessel B material. The crude product was obtained as a yellow oil with a mild yellow colouration (11.60 g). The precipitation of unreacted (S)-[(*tert*-butoxycarbonyl)amino]-3-methyl-1-(phenylsulfonyl)butane was not achieved.

δ_H(200MHz $CDCl_3$) 1.42, 1.44(x2) ($[CH_3]_3C$, crude product), 1.42 ($[CH_3]_3C$, starting sulfone)

5(S)-[(*tert*-Butoxycarbonyl)amino]-2(R)-methyl-6-(methyl)-1-[(2-tetrahydropyranyl)oxy]-*trans*-3-heptene from 5(S)-[(*tert*-butoxycarbonyl)amino]-2(R)-methyl-6-methyl-4-(phenylsulfonyl)-1-[(2-tetrahydropyranyl)oxy]-3-heptanol



Materials

5(S)-[(*tert*-Butoxycarbonyl)amino]-2(R)-methyl-6-methyl-4-(phenylsulfonyl)-1-[(2-tetrahydropyranyl)oxy]-3-heptanol	11.60 g (32.25 mmol)
Disodium hydrogen phosphate	13.2 g (92.96 mmol)
Methanol	230 ml
Sodium/mercury amalgam (2% sodium)	104.5 g (90.87 mmol Na)

Method

The general procedure used to form 5(S)-[(*tert*-butoxycarbonyl)amino]-2(R)-methyl-6-(phenyl)-1-[(2-tetrahydropyranyl)oxy]-*trans*-3-hexene was repeated. With the quantity of sodium/mercury amalgam employed here, a mechanical stirrer was found to be preferable to a magnetic stirring bar. Positive pressure flash chromatography (eluent 1:9 ethyl acetate:hexane) yielded the desired product as a colourless clear oil (3.46 g, Rf 0.31, eluent 1:9 ethyl acetate:hexane). This quantity of material represents an overall yield of 58% from (S)-[(*tert*-butoxycarbonyl)amino]-3-methyl-1-(phenylsulfonyl)butane for the two step coupling process.

ν_{max}(film)/cm^{-1} 3456m (NH), 2957s (CH_3- and -CH_2-), 2872s (O-CH-O), 1702s (C=O), 1519s (carbamate)

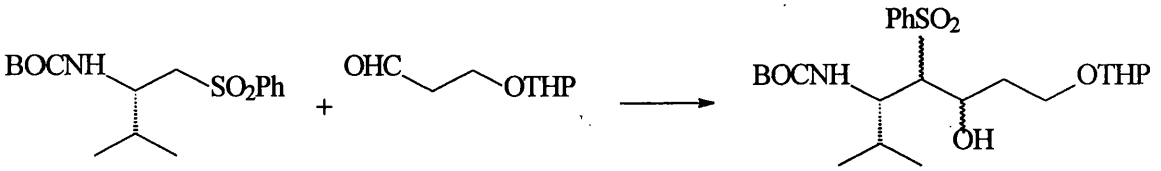
m/z 341 (M^+, <0.1%), 298 (M - $[CH_3]_2CH$, 3.8), 285 (0.2), 268 (0.1), 256 (0.2), 240 (0.1), 225 (0.3)

m/z 298.1993 (M - $CH[CH_3]_2$, $C_{16}H_{27}NO_4$ requires 298.20183)

δ_H(200MHz $CDCl_3$) 0.88 (6H, d, J 6.8, $[CH_3]_2CH$), 1.03 and 1.04 (3H, 2xd, J 6.8, CH_3CH), 1.44 (9H, s, $[CH_3]_3C$), 1.53-1.84 (7H, m, $CH_2CH_2CH_2$ and $[CH_3]_2CH$), 2.46 (1H, m, CH_3CH), 3.24 (1H, m, CHN), 3.46-3.88 (4H, m, $2xCH_2O$), 4.58 (2H, m, NH and OCHO), 5.44 (2H, m, CH=CH)

δ_C(50MHz $CDCl_3$) 17.1, 18.1 and 18.1, 18.2 (all q, CH_3CH and $[CH_3]_2CH$), 19.2 and 19.3 (both t, $CH_2CH_2CH_2$), 25.4 (CH_2CH_2O), 28.3 (q, $[CH_3]_3C$), 30.5 (CH_2CHO_2), 32.4 (d, $[CH_3]_2CH$), 36.6 and 36.7 (both d, $CHCH_3$), 56.9 (d, CHN), 61.8 and 61.9 , 72.0 (all t, $2xCH_2O$), 75.7 (s, $[CH_3]_3C$), 98.5 (d, OCHO), 128.5, 133.5 (both d, CH=CH), 154.8 (s, C=O)

5(S)-[(*tert*-Butoxycarbonyl)amino]-6-methyl-4-(phenylsulfonyl)-1-[(2-tetrahydropyranyl)oxy]-3-heptanol from 2(S)-[(*tert*-butoxycarbonyl)amino]-3-methyl-1-(phenylsulfonyl)butane and 3-[(2-tetrahydropyranyl)oxy]propionaldehyde



Materials

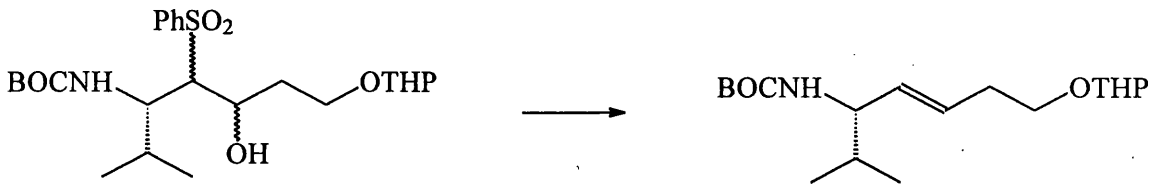
(S)-[(*tert*-Butoxycarbonyl)amino]-3-methyl-1-(phenylsulfonyl)butane	5.70 g (17.4 mmol)
THF	270 ml
Methyl lithium (1.6 M in ether)	24 ml (38.4 mmol)
Diisobutylaluminium hydride (1.0 M in THF)	35 ml (35 mmol)
Methanol	1.4 ml (34.58 mmol)
3-[(2-Tetrahydropyranyl)oxy]propionaldehyde	5.52 g (34.9 mmol)
THF	40 ml

Method

The general reaction procedure used to form 5(S)-[(*tert*-butoxycarbonyl)amino]-2(R)-methyl-6-phenyl-4-(phenylsulfonyl)-1-[(2-tetrahydropyranyl)oxy]-3-hexanol was repeated with the above starting materials. In this case the initial warming of vessel A to obtain complete solution was not necessary and the vessel A reaction time was increased to 35 minutes before the transference of vessel B material. The crude product was obtained as a clear golden oil (10.12 g). The precipitation of unreacted (S)-[(*tert*-butoxycarbonyl)amino]-3-methyl-1-(phenylsulfonyl)butane was not achieved.

δ_H(200MHz $CDCl_3$) 1.42 and 1.44 ($[CH_3]_3C$, crude product), 1.44 ($[CH_3]_3C$, starting sulfone)

5(S)-[(*tert*-Butoxycarbonyl)amino]-6-(methyl)-1-[(2-tetrahydropyranyl)oxy]-*trans*-3-heptene from 5(S)-[(*tert*-butoxycarbonyl)amino]-6-methyl-4-(phenylsulfonyl)-1-[(2-tetrahydropyranyl)oxy]-3-heptanol



Materials

5(S)-[(*tert*-Butoxycarbonyl)amino]-2(R)-6-methyl-4-(phenylsulfonyl)-1-[(2-tetrahydropyranyl)oxy]-3-heptanol	10.12 g (20.87 mmol)
Disodium hydrogen phosphate	12.62 g (88.87 mmol)
Methanol	220 ml
Sodium/mercury amalgam (2% sodium)	100.07 g (87.02 mmol)

Method

The general procedure used to form 5(S)-[(*tert*-butoxycarbonyl)amino]-2(R)-methyl-6-(phenyl)-1-[(2-tetrahydropyranyl)oxy]-*trans*-3-hexene was repeated. With the quantity of sodium/mercury amalgam employed here, a mechanical stirrer was found to be preferable to a magnetic stirring bar. Positive pressure flash chromatography (eluent 3:7 ethyl acetate:hexane) yielded the desired product as a colourless clear oil (1.44 g, Rf 0.58, eluent 3:7 ethyl acetate:hexane). This quantity of material represents an overall yield of 25% from (S)-[(*tert*-butoxycarbonyl)amino]-3-methyl-1-(phenylsulfonyl)butane for the two step process.

ν_{max}(film)/cm^{-1} 3345m (NH), 2958s (CH_3- and -CH_2-), 2872s (O-CH-O), 1702s (C=O), 1518s (carbamate)

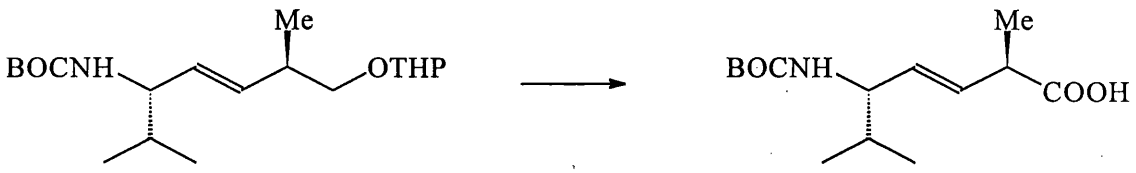
m/z 284 (M - $[CH_3]_2C$, 1.2%), 172 (1.4), 129 (1.3), 115 (1.5), 101 (9.1)

δ_H(200MHz $CDCl_3$) 0.89 (6H, 2xd, J 6.8, $[CH_3]_2CH$), 1.44 (9H, s, $[CH_3]_3C$), 1.51-1.85 (7H, m, $CH_2CH_2CH_2$ and $[CH_3]_2CH$), 2.34 (2H, q, J 6.7, $CH{=}CHCH_2$), 3.26-3.55 and 3.70-3.93 (5H, m, 2xCH_2O and CHN), 4.52 (1H, br, NH), 4.59 (1H, t, J

3.2, OCHO), 5.40 (1H, dd, J 15.5 and 6.1, CHC*H*=CH) , 5.55 (1H, dt, J 15.5(d) and 6.8(t), CH=C*H*CH_2)

δ_C(50MHz $CDCl_3$) 18.1 and 18.5 (both q, $[CH_3]_2CH$), 19.4 (t, $CH_2CH_2CHO_2$) 25.4 (t, $CH_2CH_2CH_2O$), 28.3 (q, $[CH_3]_3C$), 30.6 (t, CH_2CHO_2), 32.4 (d, $[CH_3]_2CH$), 32.7 (t, CH=CHCH_2), 57.5 (d, CHN), 62.1 and 66.8 (both t, 2xCH_2O), 78.8 (s, $[CH_3]C$), 98.6 (d, OCHO), 127.8 (d, CHCH=CH), 130.8 (d, CH=CHCH_2), 151.7 (s, C=O)

5(S)-[(*tert*-Butoxycarbonyl)amino]-2(R)-methyl-6-(methyl)-*trans*-3-heptenoic acid from 5(S)-[(*tert*-butoxycarbonyl)amino]-2(R)-methyl-6-(methyl)-1-[(2-tetrahydropyranyl)oxy]-*trans*-3-heptene



Materials

5(S)-[(*tert*-Butoxycarbonyl)amino]-2(R)-methyl-6-(methyl)-1-[(2-tetrahydropyranyl)oxy]-*trans*-3-heptene	2.99 g (8.77 mmol)
Acetone	550 ml
Chromic acid (2 M)	15 ml (30 mmol)

Method

5(S)-[(*tert*-Butoxycarbonyl)amino]-2(R)-methyl-6-(methyl)-1-[(2-tetrahydropyranyl)oxy]-*trans*-3-heptene was dissolved in acetone and placed in a 1 L round bottom flask equipped with a magnetic stirring bar. The solution was cooled to 0°C where chromic acid was added dropwise with stirring. The vessel was flushed with nitrogen, sealed with a nitrogen balloon and the solution stirred at 0°C for 3 hours. The mixture was then diluted with water (200 ml) and extracted five times with diethyl ether. The combined ether extracts were dried with magnesium sulfate, filtered and evaporated giving an oily material still containing a quantity of water. This mixture was taken up in diethyl ether (200 ml) and transferred to a separatory funnel. The organic layer was decanted and the small aqueous layer extracted five times with diethyl ether. (total volume 100 ml) The combined organic layers were then extracted five times with aqueous sodium hydroxide (5%) giving approximately 1.5 L of basic solution. After being cooled to 0°C this solution was carefully acidified to pH 2 with hydrochloric acid (10%) then extracted twice with diethyl ether. The combined final ether extracts were dried with magnesium sulfate, filtered and evaporated yielding the crude carboxylic acid product as a colourless clear oil.

On standing at room temperature, the crude carboxylic acid solidified. An initial attempt at recrystallisation from ethyl acetate/hexane resulted in the precipitation of a white solid impurity (90 mg). Evaporation of the mother liquors yielded a solid which was identified as the desired product. For ease of manipulation this material was taken up in hot ethyl acetate/hexane and allowed to precipitate on cooling (0.68 g, m.pt. 68-74°C). Evaporation of the solvent allowed recovery of the non-precipitated material (0.59 g). Both samples were of sufficient purity for use in further reactions (total 1.27 g, 53% yield). A small amount of the precipitated product was recrystallised, from hot hexane containing a minimal quantity of ethyl acetate, allowing the isolation of highly pure material.

Found: C, 61.96; H, 9.26; N, 5.01. $C_{14}H_{25}NO_4$ requires C, 61.99; H, 9.22; N, 5.17%

$[\alpha]_D^{22}$ -16.6 (6.5 mg/1 ml $CHCl_3$)

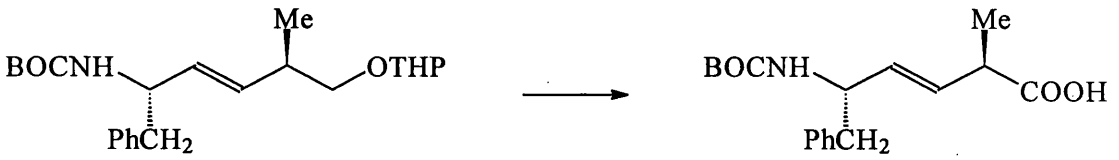
ν_{max}(KBr)/cm^{-1} 3308m (NH), 2584br (acid OH), 1728s (acid C=O), 1654s (carbamate C=O)

m/z 228 (M - $[CH_3]_2CH$, 5.9%), 172 (41.9), 155 (4.9), 116 (1.6), 99 (1.8)

m/z 228.1228 (M - $CH[CH_3]_2$, $C_{11}H_{18}NO_4$ requires 228.15353)

δ_H(200MHz $CDCl_3$) 0.89 (6H, d, J 6.8, $[CH_3]_2CH$), 1.29 (3H, d, J 7.0, $CHCH_3$), 1.44 (9H, s, $[CH_3]_3C$), 1.76 (1H, m, $[CH_3]_2CH$), 1.96 (1H, q, J possible 7.3, CHCOOH), 3.16 (1H, q, J possible 7.1, CHN), 4.61 (1H, br, NH), 5.46 (1H, dd, J 15.2 and 4.8, CH=CHCH[CH_3]) , 5.68 (1H, dd, J 15.2 and 7.3, CH=CHCH[CH_3]), 11.61 (1H, br s, COOH)

5(S)-[(*tert*-Butoxycarbonyl)amino]-2(R)-methyl-6-(phenyl)-*trans*-3-hexenoic acid from 5(S)-[(*tert*-butoxycarbonyl)amino]-2(R)-methyl-6-(phenyl)-1-[(2-tetrahydropyranyl)oxy]-*trans*-3-hexene



Materials

5(S)-[(*tert*-Butoxycarbonyl)amino]-2(R)-methyl-6-(phenyl)-1-[(2-tetrahydropyranyl)oxy]-*trans*-3-hexene	6.63 g (17.0 mmol)
Acetone	1060 ml
Chromic acid (2 M)	28.5 ml (57 mmol)

Method

5(S)-[(*tert*-Butoxycarbonyl)amino]-2(R)-methyl-6-(phenyl)-1-[(2-tetrahydropyranyl)oxy]-*trans*-3-hexene was dissolved in acetone and placed in a 2 L round bottom flask equipped with a magnetic stirring bar. The solution was cooled to 0°C where chromic acid was added dropwise with stirring. The vessel was flushed with nitrogen, sealed with a nitrogen balloon and the solution stirred at 0°C for 3 hours. The mixture was diluted with water (500 ml) and extracted three times with diethyl ether (total volume 4.5 L). The ether extracts were reduced to 600 ml by evaporation resulting in the separation of more aqueous material. The mixture was transferred to a separatory funnel, the organic layer separated and the aqueous layer extracted with diethyl ether. The combined organic layers (total volume now 1L) were then extracted five times with aqueous sodium hydroxide (5%) giving approximately 5 L of basic solution. After being cooled to 0°C this solution was carefully acidified to pH 2 with aqueous hydrochloric acid (10%) then extracted twice with diethyl ether (total volume 10 L). The combined final ether extracts were dried with magnesium sulfate, filtered and evaporated yielding the crude carboxylic acid as a golden oil (3.91 g, 72% crude yield).

A small amount of material was partially esterified using diazomethane (ether solution). Dry flash chromatography (hexane/ethyl acetate polarity gradient) was carried out on the product of this reaction and allowed the isolation of a small quantity of pure carboxylic acid.

Found: C, 67.66; H, 7.98; N, 4.35. $C_{18}H_{25}NO_4$ requires C, 67.71; H, 7.84; N, 4.38%

$\nu_{max}(CHCl_3)/cm^{-1}$ 3440m (NH), 1708s (carbamate C=O and acid C=O), 1496s (carbamate)

m/z 263 (M - $[CH_3]_2[CH_2]C$, 0.1%), 228 (M - $PhCH_2$, 6.3), 203 (0.7), 116 (1.1)

m/z 228.1237 (M - $PhCH_2$, $C_{11}H_{18}NO_4$ requires 228.12360)

δ_H(200MHz $CDCl_3$) 1.25 (3H, d, J 6.9, CH_3CH), 1.37 (9H, s, $[CH_3]_3C$), 2.81 (2H, d, J 6.4, CH_2), 3.12 (1H, q, J possible 6.9, CH_3CH), 4.39 (1H, m, CHN), 4.61 (1H, br, NH), 5.58 (2H, d of AB_q, J 15.6(AB), 6.3 and 4.9, CH=CH), 7.12-7.48 (5H, m, Ph), 10.38 (1H, br, COOH)

5(S)-[(*tert*-Butoxycarbonyl)amino]-6-(methyl)-*trans*-3-heptenoic acid from 5(S)-[(*tert*-butoxycarbonyl)amino]-6-(methyl)-1-[(2-tetrahydropyranyl)oxy]-*trans*-3-heptene

Materials

5(S)-[(*tert*-Butoxycarbonyl)amino]-6-(methyl)-1-[(2-tetrahydropyranyl)oxy]-*trans*-3-heptene	1.34 g (4.10 mmol)
Acetone	254 ml
Chromic acid (2 M)	6.9 ml (13.8 mmol)

Method

The general procedure employed to produce crude 5(S)-[(*tert*-butoxycarbonyl)amino]-2(R)-methyl-6-(methyl)-*trans*-3-heptenoic acid was repeated. Crude 5(S)-[(*tert*-butoxycarbonyl)amino]-6-(methyl)-1-[(2-tetrahydropyranyl)oxy]-*trans*-3-heptene was obtained as an almost colourless clear oil (0.74 g, 70% crude yield).

ν_{max}(film)/cm^{-1} 1712s (carbamate and acid C=O), 1515m (carbamate)

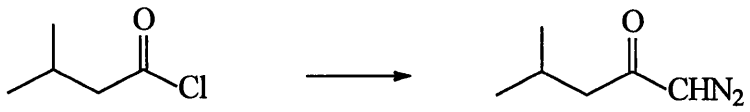
m/z 214 (M - $[CH_3]_2CH$, 4.0%), 201 (M - $[CH_3]_2[CH_2]C$, 1.1), 156 (0.8), 141 (3.4), 85 (2.0)

m/z 214.1101 (M - $[CH_3]_2CH$, $C_{10}H_{16}NO_4$ requires 214.10788)

δ_H(200MHz $CDCl_3$) 0.89 (6H, d, J 6.7, $[CH_3]_2CH$), 1.44 (9H, s, $[CH_3]_3C$), 1.76 (1H, m, $[CH_3]_2CH$), 3.11 (2H, d, J 6.6, CH_2), 3.98 (1H, m, CHN), 4.69 (1H, br, NH), 5.50 (1H, dd, J 15.6 and 5.5, CHCH=CH), 5.67 (1H, dt, J 15.6 and 6.6, $CH=CHCH_2$)

EXPERIMENTAL PART 7: Evaluation of Alternative Diazomethyl Ketone Formation Methods

Isobutyryl diazomethyl ketone from isobutyryl chloride



Materials

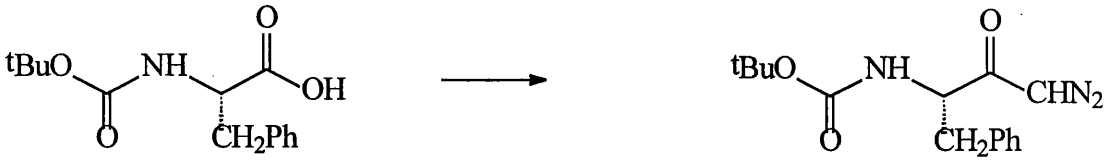
Isovaleryl chloride	*c.* 0.25 ml (2.05 mmol)
Diethyl ether	12 ml
Diazomethane (diethyl ether solution)	*c.* 9.6 mmol

Method

A scratch free 150 ml round bottom flask was equipped with a Teflon® coated magnetic stirring bar. The ground glass connection of this vessel was well greased to allow the later fitting of a nitrogen balloon. To this flask diazomethane (diethyl ether solution) was carefully added and cooled to -5°C. At this temperature isovaleryl chloride, predissolved in diethyl ether, was added dropwise. After complete addition, the flask was topped with a nitrogen balloon and the yellow solution stirred for two hours. The reaction was quenched by the addition of 50 ml water followed by rapid stirring for at least one hour before further manipulations were attempted. After being transferred to a separatory funnel the organic layer was separated and the aqueous layer extracted with diethyl ether. The combined organic layers were then washed sequentially with saturated aqueous solutions of sodium bicarbonate and sodium chloride, dried with magnesium sulfate, filtered and evaporated. The resulting light yellow liquid was characterised by ^{1}H NMR spectroscopy.

δ_H(200MHz $CDCl_3$) 0.96 (6H, d, J 6.5, $[CH_3]_2CH$), 2.02-2.18 (3H, m, CH_2 and CH), 5.24 (1H, s, CHN_2)

N-tert-Butoxycarbonyl-(L)-phenylalanyl diazomethyl ketone from *N-tert*-butoxycarbonyl-(L)-phenylalanine



Materials

Sodium hydride (55-65% in oil)	0.175 g (max. 4.74 mmol)
THF	4 ml
N-tert-Butoxycarbonyl-(L)-phenylalanine	1.33 g (5.02 mmol)
THF	25 ml
Isobutyl chloroformate	0.7 ml (5.4 mmol)
Diazomethane (diethyl ether solution)	*c.* 14.54 mmol

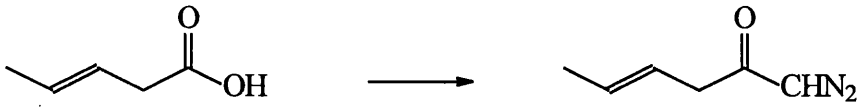
Method

A 25 ml round bottom flask with a side arm was equipped with a Teflon® coated magnetic stirring bar and a nitrogen balloon. After being flushed with nitrogen and sealed with a septum cap the vessel was flame dried. A 250 ml three necked round bottom flask was similarly treated. With this vessel however, the ground glass joints were thoroughly greased. Sodium hydride (55-65% in oil) was admitted, as a solid, to the 25 ml vessel under a flow of nitrogen. Pentane (2 ml) was added by syringe and stirring initiated. After 2 minutes the pentane was removed by suction leaving clean sodium hydride. THF was then added to this vessel and the sodium hydride suspended. The 250 ml vessel was charged with *N-tert*-butoxycarbonyl-(L)-phenylalanine dissolved in THF, then taken to -78°C. The sodium hydride suspension was transferred to this vessel by syringe and the resulting mixture allowed to warm slowly to 0°C. As warming occurred the solution became more turbid. The reaction mixture was then taken to -20°C where isobutyl chloroformate was added dropwise. After being stirred at -20°C for 20 minutes diazomethane (diethyl ether solution) was added to the reaction mixture. Stirring was continued at -20°C for 45 minutes after which the reaction

was allowed to warm slowly to room temperature where it was stirred for a further 1 hour. The reaction was then quenched by the addition of water (50 ml) with vigorous stirring. The resulting mixture was extracted twice with diethyl ether, the combined organic layers washed with saturated aqueous sodium bicarbonate, dried with magnesium sulfate, filtered and evaporated. A yellow oil was initially obtained which solidified on long term evacuation. The resulting yellow solid (0.97 g) was recrystallised from ethyl acetate/hexane at 0°C giving a sample of the desired product contaminated with a very small quantity of methyl ester (0.39 g, 27% yield, m.pt. 81-85°C).

ν_{max}(KBr)/cm^{-1} 3327m (NH), 3126m (diazo CH), 2104s (diazo), 1686s (carbamate C=O), 1640s (ketone C=O), 1526s (carbamate)
m/z 233 (M - $[CH_3]_2[CH_2]C$, 0.1%), 220 (M - $COCHN_2$, 8.6), 205 (2.6), 116 (3.6)
m/z 220.1365 (M - $COCHN_2$, $C_{13}H_{18}N\,O_2$ requires 220.13376)
δ_H(200MHz DMSO) 1.34, (9H, s, $[CH_3]_3C$), 2.89 (2H, d of AB_q, J 13.9(AB), 10.5 and 4.6), 4.21 (1H, s, CHN), 6.14 (1H, s, CHN_2), 7.20-7.43 (5H, m, Ph)
δ_H(200MHz $CDCl_3$) 1.41 (9H, s, $[CH_3]_3C$), 3.06 (2H, m, CH_2), 4.42 (1H, m, CHN), 5.12 (1H, br d, J 7.4, NH), 5.23 (1H, s, CHN_2), 7.17-7.36 (5H, m, Ph)

trans-3-Butenyl diazomethyl ketone from *trans*-3-pentenoic acid



Materials

Sodium hydride (55-65% in oil)	0.74 g (max. 20.04 mmol)
THF	80 ml
trans-3-Pentenoic acid	1.99 g (19.9 mmol)
THF	20 ml
Isobutyl chloroformate	2.75 ml (21.2 mmol)
Diazomethane (diethyl ether solution)	*c.* 58.16 mmol + 7.27 mmol

Method

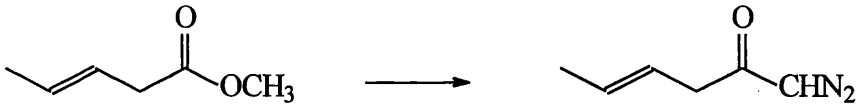
A 500 ml three necked round bottom flask was equipped with a Teflon® coated magnetic stirring bar and a nitrogen balloon. The ground glass joints were thoroughly greased, the flask sealed with septum caps and then flame dried. Under a flow of nitrogen sodium hydride (55-65% in oil) was added to the vessel as a solid. Pentane (2 ml) was then added by syringe and stirring initiated. After 2 minutes the pentane was removed by suction leaving clean sodium hydride. THF (80 ml) was added to the vessel and the resulting suspension taken to -78°C. At this temperature *trans*-3-pentenoic acid in THF (20 ml) was added slowly by syringe. Once addition was complete the reaction was allowed to warm to -20°C where it was stirred for 20 minutes before the dropwise addition of isobutyl chloroformate. After a further 10 minutes diazomethane (diethyl ether solution, 58.16 mmol) was added and stirring continued at -20°C for 40 minutes. The reaction was then allowed to warm slowly to room temperature where stirring was continued for a further 1 hour. Water (100 ml) was added with vigorous stirring to quench the reaction and the mixture transferred to a separatory funnel where the organic layer was removed and the aqueous layer extracted twice with diethyl ether. The combined organic layers were then

washed with saturated aqueous sodium bicarbonate, dried with magnesium sulfate, filtered and evaporated yielding a yellow oil.

Dry flash chromatography (hexane-ethyl acetate gradient) allowed isolation of a small amount of the desired material (0.19 g, 8% yield, Rf 0.53, eluent 1:1 ethyl acetate:hexane). Fractions containing impure samples of the desired product were combined, evaporated, then taken up in diethyl ether. This ether solution was filtered into a round bottom flask equipped with a Teflon® coated magnetic stirring bar. The ground glass connection of this vessel was well greased and the solution treated with diazomethane (7.27 mmol) in diethyl ether. The vessel was sealed with a nitrogen balloon and the mixture stirred for two hours at room temperature before the addition of water. The previously described extraction process was repeated after which dry flash chromatography (eluent pure dichloromethane) allowed isolation of a further small quantity of the desired material (0.18 g, further 7% yield, Rf 0.13, eluent pure dichloromethane).

ν_{max}(film)/cm^{-1} 3088m (diazoCH), 2105s (diazo), 1634s (C=O)
m/z 124 (M^+, <0.1%), 96 (M - N_2, 43.9), 83 (1.3), 55 (45.3), 41 (61.9)
δ_H(200MHz $CDCl_3$) 1.71 (3H, d, J 4.8, CH_3), 3.01 (2H, d, J 5.2, CH_2), 5.38 (1H, s, CHN_2), 5.42-5.70 (2H, m, CH=CH)
δ_C(50MHz $CDCl_3$) 17.7 (q), 44.6 (t), 53.9 (d, CHN_2), 123.0 and 130.1 (CH=CH), 193.4 (s, C=O)
δ_H(200MHz d_6-DMSO) diazomethyl ketone signal 6.02 (s)

trans-3-Butenyl diazomethyl ketone from methyl *trans*-3-pentenoate



Materials

Methyl *trans*-3-pentenoate	1.00 g (8.77 mmol)
THF	44 ml
Potassium trimethylsilanolate	1.68 g (13.1 mmol)
Isobutyl chloroformate	2.8 ml (21.6 mmol)
Diazomethane (diethyl ether solution)	*c.* 33.5 mmol

Method

A 250 ml three necked round bottom flask was equipped with a Teflon® coated magnetic stirring bar and nitrogen balloon. The ground glass connections of this vessel were thoroughly greased, the vessel sealed with septum caps then flame dried. The vessel was charged with a solution of methyl *trans*-3-pentenoate in THF and magnetic stirring initiated. Under a flow of nitrogen potassium trimethylsilanolate was added as a solid. The resulting yellow cloudy solution was stirred at room temperature for 2 hours and 20 minutes. Isobutyl chloroformate was then added dropwise at room temperature and stirring continued for a further 4 hours. After being cooled to 0°C, diazomethane (diethyl ether solution) was added and the reaction allowed to warm slowly to room temperature and stirred overnight. The reaction was then quenched by careful addition of water with vigorous stirring. The resulting mixture was extracted three times with diethyl ether and the combined organic layers washed with saturated aqueous sodium bicarbonate, dried with magnesium sulfate, filtered and evaporated. Positive pressure flash chromatography (eluent pure dichloromethane) was employed to isolate the desired material as a yellow oil (0.25 g, 23% yield, Rf 0.18, eluent pure dichloromethane, Rf 0.54, eluent 1:1 ethyl acetate:hexane).

ν_{max}(film)/cm^{-1} 3089m (diazo CH), 2105s (diazo), 1640s (C=O)

m/z 124 (M^+, 4.8%), 96 (M - N_2, 12.0), 83 (19.2), 69 (26.3), 55 (90.1), 41 (71.9)

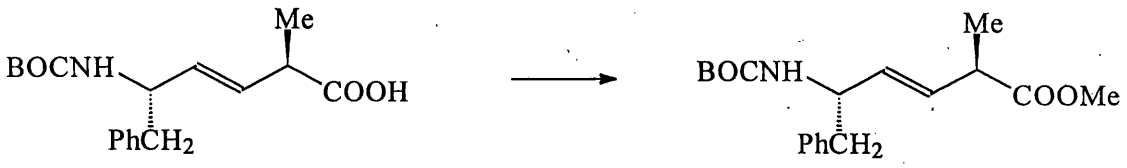
m/z 124.0628 (M^+, $C_6H_8N_2O$ requires 124.06370)

δ_H(200MHz $CDCl_3$) 1.71 (3H, d, J 5.8, CH_3), 3.02 (2H, d, J 5.3, CH_2), 5.35 (1H, s, CHN_2), 5.42-5.70 (2H, m, CH=CH)

δ_C(50MHz $CDCl_3$) 17.8 (q), 44.7 (t), 54.0 (d, CHN_2), 123.0 and 130.3 (both d, CH=CH), 193.5 (s)

EXPERIMENTAL PART 8: Further Reaction of Completed Isosteres

Methyl 5(S)-[(*tert*-butoxycarbonyl)amino]-2(R)-methyl-6-(phenyl)-*trans*-3-hexenoate from 5(S)-[(*tert*-butoxycarbonyl)amino]-2(R)-methyl-6-(phenyl)-*trans*-3-hexenoic acid



Materials

5(S)-[(*tert*-Butoxycarbonyl)amino]-2(R)-methyl-6-(phenyl)-*trans*-3-hexenoic acid	1.00 g (3.13 mmol)
Diethyl ether	10 ml
Diazomethane (ether solution)	*c.* 10.05 mmol

Method

A 100 ml conical flask was equipped with a Teflon® coated magnetic stirring bar and charged with a previously filtered impure solution of 5(S)-[(*tert*-butoxycarbonyl)amino]-2(R)-methyl-6-(phenyl)-*trans*-3-hexenoic acid in diethyl ether. Diazomethane (ether solution) was carefully added to this with stirring and the resulting yellow solution allowed to stir at room temperature for a further 45 minutes. The reaction was quenched by the addition of water (25 ml) and stirred for 30 minutes before being transferred to a separatory funnel. The organic layer was separated and the aqueous layer extracted twice with diethyl ether. The combined organic layers were dried with magnesium sulfate, filtered and evaporated. Positive pressure flash chromatography (eluent 4:6 ethyl acetate:hexane) allowed the isolation of a sample of the desired product as an off-white solid (0.71 g, 68% crude yield, Rf 0.62 eluent 4:6 ethyl acetate:hexane). Further purification was achieved by crushing this material and washing thoroughly with hexane giving a clean white solid (0.37 g, m.pt. 63-68°C).

Found: C, 68.46; H, 8.10; N, 4.12. $C_{19}H_{27}N\,O_4$ requires C, 68.47; H, 8.11; N, 4.20%

$[\alpha]_D^{26}$ -11.24 (10.2 mg/1 ml $CHCl_3$)

ν_{max}(KBr)/cm^{-1} 3359s (NH), 1738s (ester C=O), 1678s (carbamate C=O), 1524s (carbamate)

m/z 242 (M - $PhCH_2$, 5.8%), 232 (0.2), 217 (1.1), 116 (1.1), 91 (32.6)

m/z 242.1414 (M - $PhCH_2$, $C_{12}H_{20}N\,O_4$ requires 242.13926)

δ_H(200MHz $CDCl_3$) 1.21 (3H, d, J 6.9, C*H*$_3$CH), 1.40 (9H, s, $[CH_3]_3C$), 2.82 (2H, d, J 6.2, CH_2), 3.11 (1H, q, J possible 6.9, CH_3CH), 3.65 (3H, s, OCH_3), 4.44 (2H, m, C*H*N*H*), 5.54 (2H, d of AB_q, J 15.6(AB), 6.3 and 9.4, CH=CH), 7.12-7.33 (5H, m, Ph)

δ_C(50MHz $CDCl_3$) 17.1 (q, *C*H_3CH), 28.3 (q, $[CH_3]_3C$), 41.7 (t, *C*H_2Ph), 42.3 (d, *C*H_3CH), 51.8 (q, OCH_3), 52.8 (d, CHN), 79.4 (s, $[CH_3]_3C$), 126.4, 128.2, 129.5, 129.6, 131.4 (all d, Ph and CH=CH), 137.3 (s, Ph), 155.0 (s, CONH), 174.8 (s, *C*$OOCH_3$)

5(S)-[(*tert*-Butoxycarbonyl)amino]-2(R)-methyl-6-(methyl)-*trans*-3-heptenoyl diazomethane from 5(S)-[(*tert*-butoxycarbonyl)amino]-2(R)-methyl-6-(methyl)-*trans*-3-heptenoic acid

Materials

Sodium hydride (95%)	0.0459 g (max. 1.82 mmol)
THF	4 ml
5(S)-[(*tert*-Butoxycarbonyl)amino]-2(R)-methyl-6-(methyl)-*trans*-3-heptenoic acid	0.590 g (2.17 mmol)
THF	9 ml
Isobutyl chloroformate	0.3 ml (2.3 mmol)
Diazomethane (diethyl ether solution)	13.4 mmol

Method

The ground glass connections of a 100 ml three necked round bottom flask were thoroughly greased. The vessel was then equipped with a Teflon coated magnetic stirring bar and a nitrogen balloon, sealed with septum caps and flame dried. Under a flow of nitrogen, sodium hydride (95%) was added to the vessel as a solid, THF (4 ml) injected and stirring initiated. The resulting suspension was taken to -70°C where 5(S)-[(*tert*-butoxycarbonyl)amino]-2(R)-methyl-6-(methyl)-*trans*-3-heptenoic acid in THF (9 ml) was added by syringe. The solution was then warmed to -20°C where stirring was continued for a further 20 minutes. Isobutyl chloroformate was added dropwise at this temperature and following a 45 minute stir, diazomethane (diethyl ether solution) was also added. After being stirred at -20°C for 1 hour the reaction was allowed to slowly warm to room temperature where stirring was continued for a further 1 hour. The reaction was quenched by the addition of water (25 ml) with vigorous stirring. The mixture was then transferred to a separatory funnel where it was extracted three times with diethyl ether. The combined organic layers from this process

were washed with saturated aqueous sodium chloride, dried with magnesium sulfate, filtered and evaporated yielding a yellow oil (0.78 g).

Using 0.57 g of the crude material, dry flash chromatography (polarity gradient hexane to ethyl acetate) allowed the isolation of the desired compound as a thick yellow oil (0.08 g, Rf 0.43, eluent 1:1 ethyl acetate:hexane). Also isolated was a sample of the corresponding methyl ester as a colourless oil which solidified as a clean white solid on standing (0.22 g, m.pt. 56-63°C, Rf 0.57, eluent 1:1 ethyl acetate:hexane). These quantities of material suggest an overall reaction yield of 17% for the desired product and 49% for the methyl ester by-product.

Diazomethyl ketone: $[\alpha]_D^{22}$ -33.3 (5.2 mg/1 ml $CHCl_3$)
ν_{max}(KBr)/cm^{-1} 3358s (NH), 3078s (diazo CH), 2106s (diazo), 1684s (carbamate C=O), 1618s (ketone C=O), 1520s (carbamate)
m/z 252 (M - $[CH_3]_2CH$, 2.2%), 239 (M - $[CH_3]_2[CH_2]C$, 0.5) 224 (0.4), 211 (0.4), 179 (0.8), 172 (0.7), 123 (0.6), 116 (11.0)
m/z 252.1322 (M - $CH[CH_3]_2$, $C_{12}H_{18}N_3O_3$ requires 252.13478)
δ_H(200MHz $CDCl_3$) 0.83 (6H, 2xd, J 6.8, $[CH_3]_2CH$), 1.16 (3H, d, J 7.0, CH_3CH), 1.37 (9H, s, $[CH_3]_3$), 1.68 (1H, m, $[CH_3]_2CH$), 3.00 (1H, m, $CHCOCHN_2$), 3.87 (1H, m, CHNH), 4.59 (1H, br d, J 7.6, NH), 5.35 (1H, s, CHN_2), 5.38-5.58 (2H, m, J possible 15.5, CH=CH)
δ_C(50MHz $CDCl_3$) 16.6 (q, CH_3CH), 18.1 and 18.6 (both q, $[CH_3]_2CH$), 28.2 (q, $[CH_3]_3C$), 32.2 (d, $[CH_3]_2CH$), 47.9 (d, $CHCH_3$), 53.6 and 57.2 (both d, CHNH and CHN_2), 79.2 (s, $[CH_3]_3C$), 130.1 and 131.9 (both d, CH=CH), 155.8 (s, CONH), 196.8 (s, $COCHN_2$)

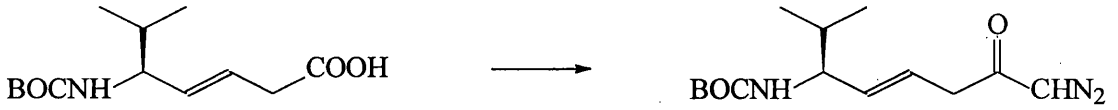
Ester: $[\alpha]_D^{22}$ -23.4 (10.8 mg/1 ml $CHCl_3$)
ν_{max}(KBr)/cm^{-1} 3382s (NH), 1733s (ester C=O), 1684s (carbamate C=O), 1520s (carbamate)
m/z 242 (M - $[CH_3]_2CH$, 5.1%), 229 (M - $[CH_3]_2[CH_2]C$, 1.2), 186 (28.7), 172 (0.3), 169 (4.1), 116 (0.9), 113 (0.7)

m/z 242.1400 (M - $[CH_3]_2CH$, $C_{12}H_{20}NO_4$ requires 242.13919)

δ_H(200MHz $CDCl_3$) 0.81 (6H, d, J 6.7, $[CH_3]_2CH$), 1.18 (3H, d, J 7.0, CH_3CH), 1.37 (9H, s, $[CH_3]_3C$), 1.68 (1H, m, $[CH_3]_2CH$), 3.08 (1H, m, CH_3CH), 3.59 (3H, s, OCH_3), 3.87 (1H, m, NHCH), 4.65 (1H, br d, J 9.2, NH), 5.38, (1H, dd, J 15.5 and 6.0, CH=CHCH[CH_3])5.58 (1H, ddd, J 15.5, 7.4, 1.0, CH=CHCH[CH_3])

δ_C(50MHz $CDCl_3$) 17.1 (q, CH_3CH), 18.0 and 18.5 (both q, $[CH_3]_2CH$), 28.2 (q, $[CH_3]_3C$), 32.4 (d, $[CH_3]_2CH$), 42.3 (d, $CHCH_3$), 51.6 (q, OCH_3), 57.0 (d, CHN), 79.0 (s, $[CH_3]_3C$), 129.7 and 130.7 (both d, CH=CH), 155.3 (s, CONH), 174.8 (s, $COCHN_2$)

5(S)-[(*tert*-Butoxycarbonyl)amino]-6-(methyl)-*trans*-3-heptenoyl diazomethane from 5(S)-[(*tert*-butoxycarbonyl)amino]-6-(methyl)-*trans*-3-heptenoic acid



Materials

Sodium hydride (95%)	0.0522 g (max. 2.066 mmol)
THF	4 ml
5(S)-[(*tert*-Butoxycarbonyl)amino]-6-(methyl)-*trans*-3-heptenoic acid	0.56 g (2.18 mmol)
THF	7 ml
Isobutyl chloroformate	0.350 ml (2.699 mmol)
Diazomethane (diethyl ether solution)	16.75 mmol

Method

The ground glass connections of a 100 ml three necked round bottom flask were thoroughly greased. The vessel was then equipped with a Teflon® coated magnetic stirring bar and a nitrogen balloon, sealed with septum caps and flame dried. Under a flow of nitrogen, sodium hydride (95%) was then added as a solid, THF (4 ml) injected and stirring initiated. The resulting suspension was taken to -70°C where 5(S)-[(*tert*-butoxycarbonyl)amino]-6-(methyl)-*trans*-3-heptenoic acid in THF (7 ml) was added by syringe. The solution was warmed to -20°C where stirring was continued for a further 15 minutes. Isobutyl chloroformate was then added dropwise at this temperature and the mixture stirred for 30 minutes before the addition of diazomethane (diethyl ether solution). After being stirred at -20°C for 1 hour the reaction was allowed to warm slowly to room temperature where stirring was continued for a further 1 hour. The reaction was quenched by the addition of water (50 ml) with vigorous stirring. The mixture was then transferred to a separatory funnel where it was extracted three times with diethyl ether. The combined organic layers were dried with magnesium sulfate, filtered and evaporated yielding a yellow oil (0.69 g).

Positive pressure flash chromatography (eluent 1:5 ethyl acetate:hexane) yielded a clear yellow liquid which was identified as being the desired compound (0.03 g, 5% yield, Rf 0.46, eluent 1:1 ethyl acetate:hexane). Also isolated was the corresponding methyl ester as a clear colourless liquid (0.31 g, 52% yield, Rf 0.72, eluent 1:1 ethyl acetate:hexane).

Diazomethyl ketone: $[\alpha]_D^{18}$ -9.85 (19.6 mg/1 ml $CHCl_3$)

$\nu_{max}(CHCl_3)/cm^{-1}$ 3448m (NH), 2109s (diazo), 1709s (carbamate C=O), 1652m (ketone C=O), 1500s (carbamate)

m/z 281 (M^+, 0.5%), 238 (M - $[CH_3]_2CH$, 7.9), 225 (1.6), 210 (0.6)

m/z 281.1737 (M^+, $C_{14}H_{23}N_3O_3$, requires 281.17393)

δ_H(200MHz $CDCl_3$) 0.83 and 0.84 (6H, both d, J 6.8, $[CH_3]_2CH$), 1.37, (9H, s, $[CH_3]_3C$), 1.71(1H, m, $[CH_3]_2CH$), 2.99 (2H, d, J 6.7, CH_2), 3.85 (1H, m, CHN), 4.50 (1H, br d, J 7.2, NH), 5.36 (1H, s, CHN_2), 5.42, (1H, dd, J 15.5 and 5.9, CH=CHCH[CH_3]), 5.58 (1H, ddt, J 15.4, 6.7(t) and 0.8, CH=CHCH[CH_3])

δ_C(50MHz $CDCl_3$) 16.2 and 16.7 (both q, $[CH_3]_2CH$), 28.3 (q, $[CH_3]_3C$), 32.2 (d, $[CH_3]_2CH$), 44.4, (t, CH_2), 54.3 and 57.5 (both d, CHN and CHN_2), 79.3 (s, $[CH_3]_3C$), 123.4 and 134.5 (both d, CH=CH), 155.5 (s, CONH), 193.1 (s, $COCHN_2$)

Ester: $[\alpha]_D^{18}$ -8.59 (17.7 mg/1 ml $CHCl_3$)

$\nu_{max}(CHCl_3)/cm^{-1}$ 3448m (NH), 1732s (ester C=O), 1718s (carbamate C=O), 1500s (carbamate)

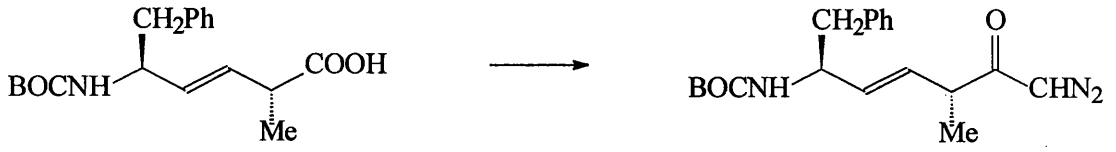
m/z 228 (M - $[CH_3]_2CH$, 17.4%), 215 (M - $[CH_3]_2[CH_2]C$, 3.6), 172 (86.4), 155 (10.6)

m/z 228.1234 (M - $[CH_3]_2CH$, $C_{11}H_{18}NO_4$ requires 228.12357)

δ_H(200MHz $CDCl_3$) 0.81 and 0.82 (6H, both d, J 6.8, $[CH_3]_2CH$), 1.37 (9H, s, $[CH_3]_3C$), 1.68 (1H, m, $[CH_3]_2CH$), 3.01 (2H, d, J 6.7, CH_2), 3.60 (3H, s, OCH_3), 3.88 (1H, m, CHN), 4.63 (1H, br d, J 8.9, NH), 5.41 (1H, dd, J 15.5 and 5.8, CHCH=CH), 5.61 (1H, ddt, J 15.5, 6.7(t) and 1.0, CH=C$H$$CH_2$)

δ_C(50MHz $CDCl_3$) 17.9 and 18.4 (both q, $[CH_3]_2CH$), 28.1 (q, $[CH_3]_3C$), 32.2 (d, $[CH_3]_2CH$), 32.7 and 37.3 (both d, CH_2), 51.3 and 51.5 (both q, OCH_3), 57.0 (d, CHN), 78.8 (s, $[CH_3]_3C$), 122.5 and 133.1 (both d, CH=CH), 155.3 (s, CONH), 171.8 and 173.1 (both s, $COOCH_3$)

5(S)-[(*tert*-Butoxycarbonyl)amino]-2(R)-methyl-6-(phenyl)-*trans*-3-hexenoyl diazomethane from 5(S)-[(*tert*-butoxycarbonyl)amino]-2(R)-methyl-6-(phenyl)-*trans*-3-hexenoic acid



Materials

Sodium hydride (55-65% in oil)	0.0440 g (max. 1.192 mmol)
THF	2 ml
5(S)-[(*tert*-butoxycarbonyl)amino]-2(R)-methyl-6-(phenyl)-*trans*-3-hexenoic acid	0.38 g (1.19 mmol)
THF	4 ml
Isobutyl chloroformate	0.16 ml (1.23 mmol)
Diazomethane (diethyl ether solution)	*c.* 13.4 mmol

Method

A 100 ml three necked round bottom flask was equipped with a Teflon® coated magnetic stirring bar and a nitrogen balloon. The ground glass joints were thoroughly greased, the flask sealed with septum caps then flame dried. Under a flow of nitrogen sodium hydride (55-65% in oil) was added to the vessel as a solid. Pentane (2 ml) was added via syringe and stirring initiated. After 2 minutes the pentane was removed by suction leaving clean sodium hydride. THF (2 ml) was then added and the resulting suspension taken to -78°C where 5(S)-[(*tert*-butoxycarbonyl)amino]-2(R)-methyl-6-(phenyl)-*trans*-3-hexenoic acid in THF (4 ml) was added by syringe. The solution was stirred at -78°C for two minutes then slowly warmed to -20°C. Isobutyl chloroformate was added dropwise at this temperature and, following a 30 minute stir, diazomethane (diethyl ether solution) was also added. After being stirred at -20°C for 1 hour the reaction was allowed to warm to room temperature where stirring was continued for a further 1 hour. The reaction was quenched by the addition of water (50 ml) with vigorous stirring. Following this, the mixture was transferred to a separatory funnel where the organic layer was separated and the aqueous

layer extracted twice with diethyl ether. The combined organic layers were dried with magnesium sulfate, filtered and evaporated yielding a yellow oil (0.44 g).

Dry flash chromatography (polarity gradient, pure hexane to 1:1 ethyl acetate:hexane) yielded a slightly impure sample of the desired product as a yellow oil (0.10 g, 25% yield, Rf 0.53 eluent 1:1 ethyl acetate:hexane). A sample of the corresponding methyl ester was also isolated as white solid (0.22 g, 56% yield, Rf 0.61 eluent 1:1 ethyl acetate:hexane). Recrystallisation attempts, from diethyl ether/hexane, gave a small quantity of highly pure ester for analysis (0.03 g). Purification of the desired diazo compound by precipitating the small impurity from diethyl ether/hexane was only partially successful. Removal of the tiny amount of remaining impurity by dry flash chromatography (hexane to ethyl acetate gradient) gave limited results. Further purification was not attempted as only 16.5 mg of product remained.

Diazomethyl ketone: $[\alpha]_D^{22}$ -14.9 (17.0 mg/1 ml $CHCl_3$)
$\nu_{max}(CHCl_3)/cm^{-1}$ 3441m (NH), 2107s (diazo), 1707s (carbamate C=O), 1636m (ketone C=O), 1496s (carbamate)
m/z 315 (M - N_2, 0.2%), 299 (M - $[CH_3]_2[CH_2]C$, 0.1), 298 (0.6), 282 (0.5), 266 (0.4), 252 (2.5), 239 (0.1), 220 (0.9)
δ_H(200MHz $CDCl_3$) 1.17 (3H, d, J 7.0, CH_3CH), 1.42 (9H, s, $[CH_3]C$), 2.85 (2H, d of AB_q, J 13.4(AB), 7.7 and 6.1, CH_2) , 4.35 (1H, m, CHN), 4.55 (1H, br d, J 8.2, NH), 4.97 (1H, s, CHN_2), 5.46 (2H, m, J possible 15.4, CH=CH), 7.15-7.34 (5H, m, Ph)
δ_H(200MHz d_6-DMSO) 5.88 (1H, s, CHN_2)
δ_C(50MHz $CDCl_3$) 16.5 (q, CH_3CH), 28.3 (q, $[CH_3]_3C$), 41.5 (t), 42.4 (d, CH_3CH), 48.0 (d, CHN_2), 53.5 (d, CHN), 79.6 (s, $[CH_3]_3C$), 126.5 (d, Ph), 128.3 (d, CH=CH), 128.9 (d, Ph), 129.6 (d, Ph), 132.5 (d, CH=CH), 137.3 (s, Ph), 155.1 (s, CONH), 195.9 (s, $COCHN_2$)

Ester: ν_{max}(KBr)/cm^{-1} 3358s (NH), 1738s (ester C=O), 1678s (carbamate C=O), 1524s (carbamate)

m/z 277 (M - $[CH_3]_2[CH_2]C$, 0.1%), 242 (M - $PhCH_2$,11.2), 232 (0.2), 217 (1.5), 91 (32.6)

m/z 242.1398 (M - $PhCH_2$, $C_{12}H_{20}N O_4$ requires 242.13919)

δ_H(200MHz $CDCl_3$) 1.12 (3H, d, J 7.0, CH_3CH), 1.31 (9H, s, $[CH_3]_3C$), 2.72 (2H, d, J 6.9, CH_2), 3.01 (1H, m, CH_3CH), 3.54 (3H, s, OCH_3), 4.30 (1H, m, CHN), 4.61 (2H, br d, J 8.7, NH), 5.45 (2H, d of AB_q, J 15.5(AB), 6.3 and 4.2, CH=CH), 7.03-7.22 (5H, m, Ph)

δ_C(50MHz $CDCl_3$) 17.0 (q, CH_3CH), 28.2 (q, $[CH_3]_3C$), 41.6 (t, CH_2), 42.2 (d, CH_3CH), 51.7 (q, OCH_3), 52.6 (d, CHN), 79.3 (s, $[CH_3]_3C$), 126.3 (d, Ph), 128.1 (d, Ph), 129.4 (d, CH=CH), 129.5 (d, Ph) and 131.5 (d, CH=CH), 137.2 (s, Ph), 155.0 (s, CONH), 174.7 (s, $COOCH_3$)

References

[1] L. Stryer, *Biochemistry*, W. H. Freeman, New York, 3rd ed., 1988, ch. 9, p. 220.

[2] T. Palmer, *Understanding Enzymes*, Prentice Hall/Ellis Horwood, London, 4th ed., 1995, p. 76.

[3] ref. 1, p. 226.

[4] ref. 1, p. 227.

[5] ref. 2, p. 79.

[6] M. J. North, J. C. Mottram and G. H. Coombs, *Parasitol. Today*, 1990, **6**, 270.

[7] R. E. Smith, D. Rasnick, C. O. Burdick, K. Cho, J. C. Rose and A. Vahratian, *Anticancer Research*, 1988, **8**, 525.

[8] C. J. F. Van Noorden and V. Everts, *Biochem. Biophys. Res. Commun.*, 1991, **178**, 178.

[9] P. H. Raven and G. B. Johnson, *Understanding Biology*, Times Mirror/Mosby College, St Louis, int. ed., 1988, 503.

[10] W. T. Keeton and J. L. Gould, *Biological Science*, W. W. Norton, London, 4th ed., 1986, 1048.

[11] K. A. Rockett, J. H. L. Playfair, F. Ashall, G. A. T. Targett, H. Angliker and E. Shaw, *FEBS lett.*, 1990, **259**, 257.

[12] P. J. Rosenthal, W. S. Wollish, J. T. Palmer and D. Rasnick, *J. Clin. Inv.*, 1991, **88**, 1467.

[13] I. R. Sherman, *Micobiol. Rev.*, 1979, **43**, 453.

[14] ref. 9, p. 501.

[15] F. E. G. Cox (ed.), *Modern Parasitology*, Blackwell Scientific Publications, 2nd ed., 1993, ch.1, p. 2.

[16] J. H. McKerrow, M. E. McGrath and J. C. Engel, *Parasitol. Today*, 1995, **11**, 279.

[17] H. Umezawa, *Ann. Rev. Microbiol.*, 1982, 36, 75.

[18] A. J. Barret (ed.), *Proteinases in Mammalian Cells and Tissues*, Elsevier/North Holland Biomedical Press, 1977, 639.

[19] J. O'Connor Westerk and R. Wolfended, *J. Biol. Chem.*, 1972, **247**, 8195.

[20] M. P. Gamcsik, J. P. G. Malthouse, W. Primrose, N. E. Mackenzie, A. S. F. Boyd, R. A. Russell and A. I. Scott, *J. Am. Chem. Soc.*, 1983, **105**, 6324.

[21] J. B. Henes, J. A. Mattis and J. S. Fruton, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 1131.

[22] J. H. R. Sutherland and L. M. Greenbaum, *Biochem. Biophys. Res. Commun.*, 1983, **110**, 332.

[23] K. Hanada, M. Tamai, M. Yamagishi, S. Ohmura, J. Sawada and I. Tanaka, *Agric. Biol. Chem.*, 1978, **42**, 523.

[24] K. Hanada, M. Tamai, S. Ohmura, J. Sawada, T. Seki and I. Tanaka, *Agric. Biol. Chem.*, 1978, **42**, 529.

[25] S. Hashida, T. Towatari, E. Kominami and N. Katunuma, *J. Biochem.*, 1980,**88**, 1805.

[26] S. Hashida, E. Kominami and N. Katunuma, *J. Biochem.*, 1982, **91**, 1373.

[27] A. J. Barret, A. A. Kembhavi, M. A. Brown, H. Kirschke, C. G. Knight, M. Tamai and K. Hanada, *Biochem. J.*, 1982, **201**, 189.

[28] K. Hanada, M. Tamai, S. Morimoto, T. Adachi, S. Ohmura, J. Sawada and I. Tanaka, *Agric. Biol. Chem.*, 1978, **42**, 537.

[29] Y. Yake, D. Guillaume and D. H. Rich, *J. Am. Chem. Soc.*, 1988, **110**, 4043.

[30] G. Schoellmann and E. Shaw, *Biochem. Biophys. Res. Commun.*, 1962, **7**, 36.

[31] E. B. Ong, E. Shaw and G. Schoellmann, *J. Biol. Chem.*, 1965, **240**, 694.

[32] E. Shaw and S. Springhorn, *Biochem. Biophys. Res. Commun.*, 1967, **27**, 391.

[33] P. H. Petra, W. Cohen and E. N. Shaw, *Biochem. Biophys. Res. Commun.*, 1965, **21**, 612.

[34] G. Schoellmann and E. Shaw, *Biochemistry*, 1963, **2**, 252.

[35] E. Shaw, M. Mares-Guia and W. Cohen, *Biochemistry*, 1965, **4**, 2219.

[36] J. P. G. Malthouse, N. E. Mackenzie, A. S. F. Boyd and A. I. Scott, *J. Am. Chem. Soc.*, 1983, **105**, 1685.

[37] A. I. Scott, N. E. Mackenzie, J. P. G. Malthouse, W. U. Primrose, P. E. Fagerness, A. Brisson, Le Zong Qi, W. Bode, C. M. Carter and Yi Jin Jang, *Tetrahedron*, 1986, **42**, 3269.

[38] J. C. Vederas and T. T. Nakashima, *J. Chem. Soc. Chem. Commun.*, 1980, 183.

[39] C. Betzel, G. P. Pal, M. Struck, K. D. Jany and W. Saenger, *FEBS lett.*, 1986, **197**, 105.

[40] D. Collen, O. Matsuo, J. M. Stassen, C. Kettner and E. Shaw, *J. Lab. Clin. Med.*, 1982, **99**, 76.

[41] R. L. Stein and D. A. Trainer, *Biochemistry*, 1986, **25**, 5414.

[42] J. S. McMurray and D. F. Dyckes, *Biochemistry*, 1986, **25**, 2298.

[43] R. Leary, D. Larsen, H. Watanabe and E. Shaw, *Biochemistry*, 1977, **16**, 5857.

[44] M. L. Bender and L. J. Brubacher, *J. Am. Chem. Soc.*, 1966, **88**, 5880.

[45] J. Perez-Villasenor, *Arch. Biochem. Biophys.*, 1968, **124**, 70.

[46] D. Rasnick, *Anal. Biochem.*, 1985, **149**, 461.

[47] J. M. Buchanan, *Advances In Enzymology*, A. Meister (ed.), 1973, **39**, 91.

[48] R. Leary and E. Shaw, *Biochem. Biophys. Res. Commun.*, 1977, **79**, 926.

[49] H. Watanabe, G. D. J. Green and E. Shaw, *Biochem. Biophys. Res. Commun.*, 1979, **89**, 1354.

[50] G. D. J. Green and E. Shaw, *J. Biol. Chem.*, 1981, **256**, 1923.

[51] H. Kirschke and E. Shaw, *Biochem. Biophys. Res. Commun.*, 1981, **101**, 454.

[52] B. Grinde, *Biochim. Biophys. Acta.*, 1983, **757**, 15.

[53] H. Angliker, P. Wikstrom, P. Rauber and E. Shaw, *Biochem. J.*, 1987, **241**, 871.

[54] P. Rauber, H. Angliker, B. Walker and E. Shaw, *Biochem. J.*, 1986, **239**, 633.

[55] USA 4 518 528/1985 (*Chem. Abstr.*, 1985, **103**, 22931b).

[56] F. Ashall, H. Angliker and E. Shaw, *Biochem. Biophys. Res. Commun.*, 1990, **170**, 923.

[57] M. A. Robin, F. A. Bovey and H. Basch, *The Chemistry of Amides*, ed. J. Zabicky, Interscience, 1970, p. 2.

[58] M. H. Hann and P. G. Sammes, *J. Chem. Soc. Chem. Commun.*, 1980, 234.

[59] M. M. Hann, P. G. Sammes, P. D. Kennewell and J. B. Taylor, *J. Chem. Soc. Perkin Trans. 1*, 1982, 307.

[60] A. Spatola, *Chemistry and Biochemistry of Amino Acids, Peptides and Proteins*, ed. B. Weinstein, Marcel-Dekker, New York, 1983, vol. 7, p. 267.

[61] J. M. Hambrook, B. A. Morgan, M. J. Rance and C. F. C. Smith, *Nature*, 1976, **262**, 782.

[62] M. T. Cox, D. W. Heaton and J. Horbury, *J. Chem. Soc. Chem. Commun.*, 1980, 799.

[63] T. Shioiri, K. Ninomiya and S. Yamada, *J. Am. Chem. Soc.*, 1972, **94**, 6203.

[64] M. T. Cox, J. J. Gormley, C. F. Hayward and N. N. Petter, *J. Chem. Soc. Chem. Commun.*, 1980, 800.

[65] M. Ahmed, G. C. Barley, M. T. W. Hearn, E. R. H. Jones, V. Thaller and J. A. Yates, *J. Chem. Soc. Perkin Trans. 1*, 1974, 1981.

[66] N. J. Miles, P. G. Sammes, P. D. Kennewell and R. Westwood, *J. Chem. Soc. Perkin Trans. 1*, 1985, 2299.

[67] M. W. Rathke and D. Sullivan, *Tetrahedron Lett.*, 1972, 4249.

[68] R. L. Johnson, *J. Med. Chem.*, 1984, **27**, 1351.

[69] W. J. Thompson, T. J. Tucker, J. E. Schwering and J. L. Barnes, *Tetrahedron Lett.*, 1990, **31**, 6819.

[70] J. Davoll and D. H. Laney, *J. Chem. Soc.*, 1956, 2124.

[71] G. J. Hanson and T. Lindberg, *J. Org. Chem.*, 1985, **50**, 5399.

[72] Youe-Kong Shue, G. M. Carrera Jr. and A. M. Nadzan, *Tetrahedron Lett.*, 1987, **28**, 3225.

[73] Youe-Kong Shue, M. D. Tufano, G. M. Carrera Jr., H. Kopecka, S. L. Kuyper, M. W. Holladay, C. W. Lin, D. G. White, M. Stashko and A. M. Nadzan, *Bioorg. Med. Chem.*, 1993, **1**, 161.

[74] T. Ibuka, H. Habashita, S. Funakoshi, N. Fujii, Y. Oguchi, T. Uyehara and Y. Yamamoto, *Angew. Chem. Int. Ed. Engl.*, 1990, **29**, 801.

[75] A. Spaltenstein, P. A. Carpino, F. Miyake and P. B. Hopkins, *J. Org. Chem.*, 1987, **52**, 3759.

[76] L. S. Lehman dè Gaeta, M. Czarniecki and A. Spaltenstein, *J. Org. Chem.*, 1989, **54**, 4004.

[77] M. Julia and J.M. Paris, *Tetrahedron Lett.*, 1973, 4833.

[78] J. C. Powers, *Chemistry and Biochemistry of Amino Acids, Peptides and Proteins*, ed. B. Weinstein, Marcel-Dekker, New York, 1977, vol. 4, p. 65.

[79] W. Steglich and G. Hofle, *Angew. Chem. Int. Ed. Engl.*, 1969, **8**, 981.

[80] H. Angliker, P. Wikstrom, P. Rauber, S. Stone and E. Shaw, *Biochem. J.*, 1988, **256**, 481.

[81] H. Angliker, P. Wikstrom, H. Kirschke and E. Shaw, *Biochem. J.*, 1989, **262**, 63.

[82] G. H. L. Nefkens, G. I. Tesser and R. J. F. Nivard, *Rec. Trav. Chim. Pays Bas*, 1960, **79**, 688.

[83] M. Hudlicky, *J. Org. Chem.*, 1980, **45**, 5377.

[84] L. F. Fieser and M. Fieser, *Reagents for Organic Synthesis*, John Wiley and Sons, New York, 1967, vol. 1, p. 191.

[85] T. N. Pattabiraman and W. B. Lawson, *Biochim. Biophys. Acta,* 1972, **258**, 548.

[86] A. Hercouet and M. Le Corre, *Synthesis*, 1988, 157.

[87] G. A Olah, J. T. Welch, Y. D. Vankar, M. Nojima, I. Kerekes and J. A. Olah, *J. Org. Chem.*, 1979, **44**, 3872.

[88] R. Franz, *J. Fluor. Chem.*, 1980, **15**, 423.

[89] C. L. Liotta and H. P. Harris, *J. Am. Chem. Soc.*, 1974, **96**, 2250.

[90] M. Kolb, B. Neises and F. Gerhart, *Liebigs Ann. Chem.*, 1990, 1.

[91] M. Kolb, J. Barth and B. Neises, *Tetrahedron Lett.*, 1986, 1579.

[92] WO 94 04 172/1994 (*Chem. Abstr.* 1994, **120**, 293020c)

[93] R. E. Dolle, J. Singh, J. Rinker, D. Hoyer, C. V. C. Prasad, T. L. Graybill, R. E. Miller and M. A. Ator, *J. Med. Chem.*, 1994, **37**, 3863.

[94] EP 272 671/1988 (*Chem. Abstr.* 1989, **110**, 39372n)

[95] J. T. Palmer, D. Rasnick, J. L. Klaus and D. Brömme, *J. Med. Chem.*, 1995, **38**, 3193.

[96] J. A. Fehrentz and B. Castro, *Synthesis*, 1983, 676.

[97] K. Ishizumi, K. Koga and S. I. Yamada, *Chem. Pharm. Bull.*, 1968, **16**, 492.

[98] R. K. Crossland and K. L. Servis, *J. Org. Chem.*, 1970, **35**, 3195.

[99] J. F. King and T. W. S. Lee, *J. Am. Chem. Soc.*, 1969, **91**, 6524.

[100] D. A. Evans, M. D. Ennis and D. J. Mathre, *J. Am. Chem. Soc.*, 1982, **104**, 1737.

[101] K. L. Mikolajczak and C. R. Smith Jr., *J. Org. Chem.*, 1978, **43**, 4762.

[102] M. W. Rathke and D. Sullivan, *Tetrahedron Lett.*, 1972, 4249.

[103] N. Petragnani and M. Yonashiro, *Synthesis*, 1982, 521.

[104] A. P. Kozikowski and P. D. Stein, *J. Org. Chem.*, 1984, **49**, 2301.

[105] P. J. Kocienski, B. Lythgoe and S. Ruston, *J. Chem. Soc. Perkin Trans. 1*, 1978, 829.

[106] P. J. Kocienski, *Chem. Ind.*, 1981, 548.

[107] N. S. Simpkins, *Sulphones in Organic Synthesis*, Pergamon, Oxford, 1st. ed., 1993, p. 254.

[108] B. M. Trost, H. C. Arndt, P. E. Strege and T. R. Verhoeven, *Tetrahedron Lett.*, 1976, 3477.

[109] S. Hanessian and R. Frenette, *Tetrahedron Lett.*, 1979, 3391.

[110] L. F. Fieser and M. Fieser, *Reagents for Organic Synthesis*, John Wiley and Sons, New York, 1967, vol. 1, p. 142.

[111] H. Meier and K. P. Zeller, *Angew. Chem. Int. Ed. Engl.*, 1975, **14**, 32.

[112] A. H. Beckett and A. F. Casy, *J. Chem. Soc.*, 1955, 900.

[113] M. P. Cava and R. J. Spangler, *J. Am. Chem. Soc.*, 1967, **89**, 4550.

[114] T. Gibson and W. F. Erman, *J. Org. Chem.*, 1966, **31**, 3029.

[115] W. Jugelt and D. Schmidt, *Tetrahedron*, 1969, **25**, 969.

[116] N. F. Albertson, *Org. React.*, 1962, **12**, 157.

[117] W. Bradley and R. Robinson, *J. Chem. Soc.*, 1928, 1310.

[118] K. Balenovic, N. Bregant, D. Cerar and M. Tkalcic, *J. Org. Chem.*, 1951, **16**, 1308.

[119] K. Balenovic, D. Cerar and Z. Fuks, *J. Chem. Soc.*, 1952, 3316.

[120] J. B. Lee, *J. Am. Chem. Soc.*, 1966, **88**, 3440.

[121] R. Adams and L. H. Ulich, *J. Am. Chem. Soc.*, 1920, **19**, 599.

[122] K. Venkataraman and D. R. Wagle, *Tetrahedron Lett.*, 1979, 3040.

[123] F. I. Lukinitskii and B. A. Vovsi, *Russ. Chem. Rev.*, 1969, **38**, 487.

[124] E. C. Taylor, A. McKillop and G. H. Hawks, *Org. Syn.*, 1972, **52**, 36.

[125] C. D. Hurd, S. M. Cantor and A. S. Roe, *J. Am. Chem. Soc.*, 1939, **61**, 426.

[126] G. W. Anderson, J. E. Zimmerman and F. M. Callahan, *J. Am. Chem. Soc.*, 1967, **89**, 5012.

[127] E. D. Laganis and B. L. Chenard, *Tetrahedron Lett.*, 1984, **25**, 5831.

[128] A. Padwa, D. J. Austin and S. F. Hornbuckle, *J. Org. Chem.*, 1996, **61**, 63.

[129] The Aldrich Chemical Company, *Catalogue Handbook of Fine Chemicals*, 1996-1997, p. 1129.

[130] A. K. Bose, F. Greer and C. C. Price, *J. Org. Chem.*, 1958, **23**, 1335.

[131] ref. 129, p. T215.

[132] K. Balenovic, D. Cerar and L. Filipovic, *J. Org. Chem.*, 1953, **18**, 868.

[133] R. G. Jones, E. C. Kornfeld and K. C. McLaughlin, *J. Amer. Chem. Soc.*, 1950, **72**, 4526.

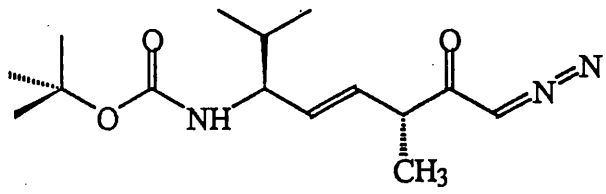
[134] K. Balenovic and N. Bregant, *J. Org. Chem.*, 1952, **17**, 1328.

[135] K. E. B. Parkes, D. J. Bushnell, P. H. Crackett, S. J. Dunsdon, A. C. Freeman, M. P. Gunn, R. A. Hopkins, R. W. Lambert, J. A. Martin, J. H. Merrett, S. Redshaw, W. C. Spurden and G. J. Thomas, *J. Org. Chem.*, 1994, **59**, 3656.

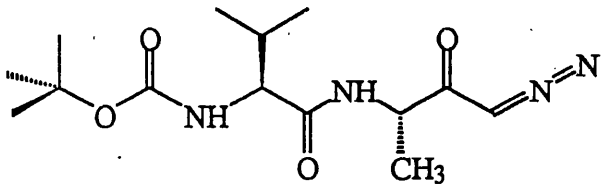
[136] ref. 129, p. 266.

Appendix 1: Molecular Mechanics Calculations

Molecular mechanics calculations (Chem-X software, Chemical Design Ltd. 1994) were carried out on both the amide link and (*E*)-ethene isostere bearing variants of *N*-BOC-(L)-Val-(L)-Ala diazomethyl ketone in order to obtain their minimum energy conformations.

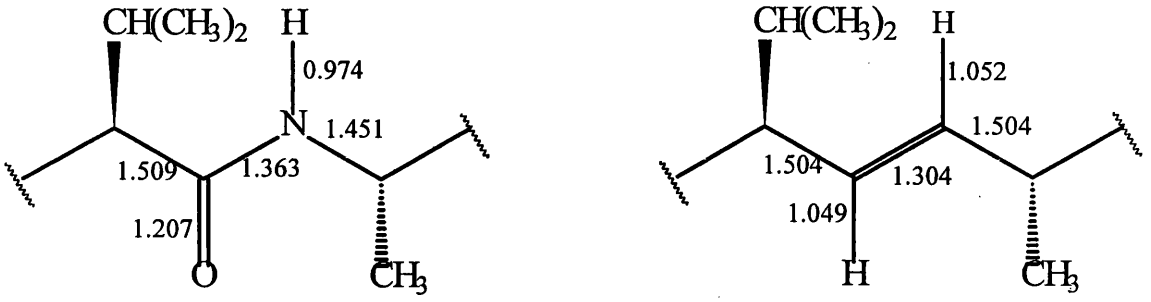


N-BOC-(L)-Val-(L)-AlaCHN$_2$
(*E*)-ethene isostere

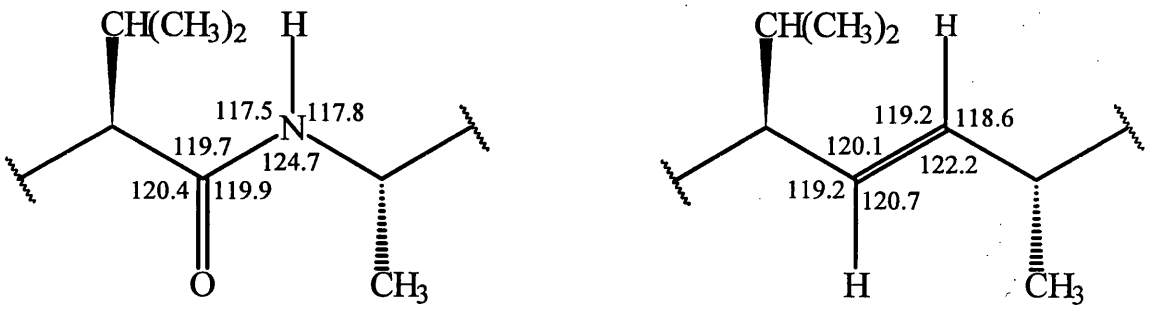


Amide linked
N-BOC-(L)-Val-(L)-AlaCHN$_2$

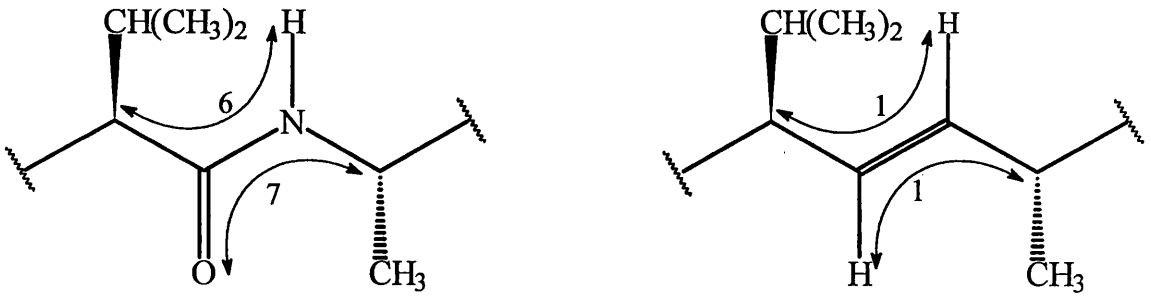
The results of this study, for the region around the linking groups, are indicated on the following diagrams.



Bond Lengths (Angstroms)



Bond Angles (degrees)



Torsion Angles (degrees)

Appendix 2: Initial Biological Results

For simplicity, the compounds under investigation are assigned the following abbreviations:

(E)-Ethene isosteres	
N-BOC-(L)-Phe-(L)-Ala diazomethyl ketone	PAD
N-BOC-(L)-Phe-(L)-Ala methyl ester	PAE
N-BOC-(L)-Val-(L)-Ala diazomethyl ketone	VAD
N-BOC-(L)-Val-(L)-Ala methyl ester	VAE
N-BOC-(L)-Val-Gly diazomethyl ketone	VGD
N-BOC-(L)-Val-Gly methyl ester	VGE
Peptide linked compound	
N-CBZ-(L)-Phe-(L)-Ala diazomethyl ketone	ZPA

1. Protease Enzyme Inactivation (SDS-PAGE)

	T. foetus F2	*T. vaginalis* G3	*T. augusta*	*G. lamblia*	*L. mexicana* (1)*	*L. mexicana* (2)*
PAD	no effect	no effect	no effect	no effect	no effect	
PAE	no effect	no effect	no effect	no effect	no effect	
VAD	no effect	no effect	no effect	no effect	no effect	
VAE	no effect	no effect	no effect	no effect	no effect	
VGD	no effect	no effect	no effect	no effect	no effect	no effect
VGE	some effect	some effect	some effect	no effect	strong effect	no effect

* (1) *L. mexicana* (wild type) stationary phase promastigotes

(2) *L. mexicana* (wild type) amastigotes

2. *In vitro* Growth Inhibition

Test compound solutions (in water with 2.5% added DMSO) were prepared at the following concentrations; 100, 50, 25, 10, 5, 2.5 and 1 μg/ml. Note, however, that solubility problems with VGD and VGE prevented testing at concentrations greater than 50 μg/ml. Note that with the compound ZPA a test solution at 250 μg/ml was also prepared.

The experiments were carried out by treating 180 μl of culture with 20 μl of each test compound solution. These mixtures were incubated under the conditions specified above each of the following tables of results. A control mixture, which consisted of 180 μl culture and 20 μl water/2.5% DMSO with no added test compound, was treated similarly.

All mixtures contained, on initiation, 10^5 parasites/ml as standard.

After incubation, cell counts in 0.1 μl of mixture were taken and compared to those of the control. Results are tabulated as a percentage of the control cell count. The values quoted are for the maximum concentration of inhibitor solution used in cases where growth inhibition was only slight. In cases where the effect of the test compound was significant, the concentration giving complete inhibition is noted together with the results obtained for the weaker solution next in sequence. It should also be noted that all experiments were carried out in duplicate and the figures quoted are for the averaged results of these.

T. augusta

Incubated anaerobically for 48 hours at 25°C.

test compound	compound concentration µg/ml	average cell count (%)
PAD	100	92
PAE	100	100
VAD	100	20
VAE	100	104
VGD	50	87
VGE	50	111

***T. foetus* F2**

Incubated anaerobically for 24 hours at 37°C.

test compound	compound concentration µg/ml	average cell count (%)
PAD	100	57
PAE	100	79
VAD	100	67
VAE	100	102
VGD	50	48
VGE	50	34

***T. vaginalis* G3**

Incubated anaerobically for 24 hours at 37°C.

test compound	compound concentration µg/ml	average cell count (%)
PAD	100	78
PAE	100	92
VAD	100	81
VAE	100	101
VGD	50	83
VGE	50	49

***L. mexicana* (wild type)**

Incubated aerobically for 5 days at 25°C.

test compound	compound concentration µg/ml	average cell count (%)
PAD	100	0
	50	81
PAE	100	109
VAD	100	115
VAE	100	113
VGD	10	53
VGE	10	0
ZPA	250	99

***L. mexicana* (ΔC10)**

Incubated aerobically for 5 days at 25°C.

test compound	compound concentration μg/ml	average cell count (%)
PAD	100	0
	50	4
PAE	100	103
VAE	100	116
ZPA	250	104

***L. mexicana* (N53)**

Incubated aerobically for 5 days at 25°C.

test compound	compound concentration μg/ml	average cell count (%)
PAD	100	0
	50	1
PAE	100	120
VAD	100	66
VAE	100	93
ZPA	250	104

Appendix 3: Second Round Parasite Growth Studies

G. intestinalis

Incubated anaerobically for 48 hours at 37°C

test compound	compound concentration µg/ml	average cell count (%)
VGD	50	52
VGE	100	0
	50	1

L. mexicana (wild type)

Incubated aerobically for 5 days at 25°C.

test compound	compound concentration µg/ml	average cell count (%)
PAD	100	0
	50	2
PAE	100	112
VAD	100	<2
	50	15
VAE	100	7
VGD	50	102
VGE	50	0
	10	7

***L. mexicana* (ΔN8m)**

Incubated aerobically for 5 days at 25°C.

test compound	compound concentration μg/ml	average cell count (%)
PAD	100	<1
	50	32
VGD	50	48
VGE	25	0
	10	4
ZPA	25	108

***L. mexicana* (N53)**

Incubated aerobically for 5 days at 25°C.

test compound	compound concentration μg/ml	average cell count (%)
PAD	50	0
	25	18
PAE	100	127
VAD	100	0
	50	8
VAE	100	2
	50	49
VGD	50	99
VGE	10	0
	5	104
ZPA	25	88

***L. mexicana* (ΔC10m)**

Incubated aerobically for 5 days at 25°C.

test compound	compound concentration µg/ml	average cell count (%)
PAD	100	2
	50	77
VAD	100	7
VAE	100	21
VGD	50	93
VGE	10	0
	5	106
ZPA	25	131