The allenyl bis-amino acids: A novel mimic for the disulphide bond

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THE ALLENYL *BIS*-AMINO ACIDS

A NOVEL MIMIC FOR THE DISULPHIDE BOND

submitted by JONATHAN PAUL SWIFT

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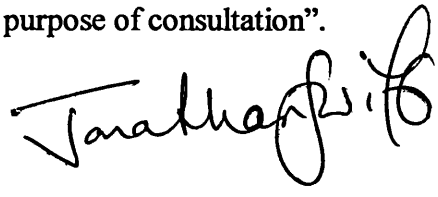

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To my Parents

Acknowledgements

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Abbreviations

AA	Unassigned amino acid
Ac	Acetyl
Asp	Aspartic acid
Ar	Aromatic group
BCNU	1,3-Bis-(2-chloroethyl)-1-nitroso urea
Bn	Benzyl
Boc	*tert*-Butyloxycarbonyl
b.p.	Boiling point
Bu	Butyl
CAT	Catalase
C.I.	Chemical ionisation
Cys	Cysteine
dba	Dibenzylideneacetone
DCM	Dichloromethane
DFAM	Diethyl formylaminomalonate
Dibal-H	Diisobutyl aluminium hydride
DMDO	Dimethyl dioxirane
DNP	2,4-Dinitrophenyl
E.I.	Electron impact
Et	Ethyl
F.A.B.	Fast atom bombardment
FAD	Flavin adenine dinucleotide
Gly	Glycine
Gpase	Glutathione peroxidase
GSH	Glutathione
GSSG	Oxidised glutathione
GR	Glutathione reductase
h	Hour
HIV	Human Immunodefficiency Virus
Hz	Hertz

i.r.	Infrared
J	Coupling constant
kD	Kilodalton
L	Ligand
LDA	Lithium diisopropylamine
LG	Leaving group
LHDMS	Lithium hexamethyldisilazide
M	Molar
m-CPBA	*meta*-Chloroperbenzoic acid
Me	Methyl
mg	Milligram
MHz	Megahertz
mmol	Millimole
m.p.	Melting point
m.s.	Mass spectrum
m/z	Mass to charge ratio
N	Normal
NADPH	Nicotinamide adenine dinucleotide phosphate
n.m.r.	Nuclear magnetic resonance
Nuc	Generalised nucleophile
Ph	Phenyl
ppm	Parts per million
Prot.	Unassigned protecting group
R	Unassigned organic side chain
R_R	Non-transferable ligand
R_T	Transferable ligand
RT	Room temperature
$S^{\ddagger}$	Entropy of transition state
Ser	Serine
SOD	Superoxide dismutase
t-BDPSi	*tert*-Butyl diphenylSilyl
Th	Thienyl

THF	Tetrahydrofuran
Thr	Threonine
TMS	Trimethylsilyl
$T(SH)_2$	Reduced dihydrotrypanothione
$T(S)_2$	Trypanothione disulphide
TR	Trypanothione reductase
Val	Valine
v/v	Volume per unit volume
WHO	World Health Organisation

CONTENTS

INTRODUCTION

Introduction

Collaboration between biologists and chemists has generated medicines of enormous benefit to society. The resulting contributions to the practice of medicine have reduced morbidity and mortality while increasing the quality of life. Medicinal chemists have advanced rational drug discovery to a degree unthinkable even twenty years ago, and yet have only begun to scratch the surface of possible opportunities. Some of the most remarkable discoveries in medicinal chemistry have been achieved in the area of peptide research[1].

Peptides occur throughout nature in a range of roles, from extracellular messengers in plants and animals, to structural uses such as keratin in hair and nails. Peptides are the primary means of intercellular communication in many diverse biological systems. Acting as neurotransmitters, neuromodulators, and hormones they influence a multitude of physiological processes by interacting with peptide receptors[2]. In addition, over the last twenty years their role in the appearance or maintenance of various diseases has been unequivocally proven[3].

Historically, new lead compounds have been discovered either by serendipity or by mass screening of libraries of natural and synthetic compounds through multiple bioassays. Using mass screening, lead compounds are obtained with low success rates (rarely greater than 1 in 80,000 screened compounds) and then painstakingly modified by chemical synthesis in the search for improved bioactivity and reduced toxicity.

This analogue-based approach to drug development requires large numbers of synthetic analogues in order to develop structure-activity profiles. Over the past 30 years, through a better understanding of disease processes, mechanism-based drug design has evolved and produced compounds that interrupt biochemical pathways (usually by mimicking a transition state).

During the past decade, advances in molecular biology and protein chemistry have provided pure proteins in sufficient quantities to allow structural studies to be carried out. These developments, coupled with advances in X-ray crystallography, n.m.r. spectroscopy, and computing technology, have greatly accelerated the determination of 3D structures of proteins. Visualisation of these structures by sophisticated computer graphics has made receptor-based drug design feasible. Receptor-based drug design, unlike earlier methods of drug discovery, allows the examination of all interactions between the bioactive molecule and a drug target at atomic level; hence, it can facilitate the identification of divergent classes of compounds with correspondingly divergent biological properties. This permits rational development of structurally distinct compounds with different pharmacological profiles.

In the last few years new methods have been established for the treatment and therapy of diseases, and hopes have also been raised for the therapy of other diseases in which peptides have been implicated. However, peptides lack the appropriate physical chemical properties and metabolic stability to be ideally suited to therapeutics[3].

These pharmacological problems include:

(1) Short serum half-life values and high susceptibility to hydrolysis by degradative enzymes present in the bloodstream, gut, and cells.

(2) Poor absorption and oral bioavailability.

(3) Rapid liver clearance and biliary excretion.

(4) Undesired effects caused by interaction with various receptors other than those required, due to flexible conformation of the peptide.

Despite these disadvantages the development of peptide-based therapeutic drugs is, at present, one of the most active areas of medicinal research. In order to solve the problems encountered in using peptides as therapeutic agents peptidomimetics have been synthesised.

Peptidomimetics

A peptidomimetic is a molecule with some or all of its peptide functionality replaced by some other organic functionality, but which retains properties analogous to the peptide, i.e. as the ligand of a receptor it can imitate the biological effect of the native peptide at the receptor level [2,3].

In order to improve the stability and bioavailability of inhibitors various synthetic modifications need to be made. Half-lives might be extended by attaching the compounds to carrier molecules, or by decreasing susceptibility to degradation by sterically protecting or removing amide bonds.

Higher gastrointestinal absorption, better cellular membrane penetration, and lower hepatic clearance might be achieved by using low molecular mass ($M_r < 600$) inhibitors with more appropriate water-lipid solubilities. Smaller inhibitors might only occupy four, three or even two side chain positions in the active site, and will need to be tested for their ability to dissolve in and diffuse through lipid membranes.

From the pharmacological and medicinal viewpoint it is frequently desirable to not only imitate the effect of the peptide at the receptor level (an agonist) but also to block the receptor when required (an antagonist). Agents that can imitate or block the biological functions of bioactive peptides can be considered as aids for the investigation of peptide systems and also as possible new types of treatment and therapy for a series of diseases.

The successes in this area cannot be denied. The marked trend of the last few years is the design of effective , orally active nonpeptide ligands for peptide receptors[3]. Of significance in this regard is the fact that it is possible, in principle, to imitate or to block the function of a relatively large peptide at the receptor level with low molecular mass compounds. In addition to the therapeutic side, modified peptides offer new insights into the nature of peptide and protein chemistry.

In comparison with native peptides these mimetics should show higher metabolic stability, better bioavailability, and longer duration of action. Peptidomimetic research owes its impetus to this desire to discover nonpeptides that bind to peptide receptors, but which have better bioavailability, biostability, and possibly greater selectivity than native, endogenous peptide ligands.

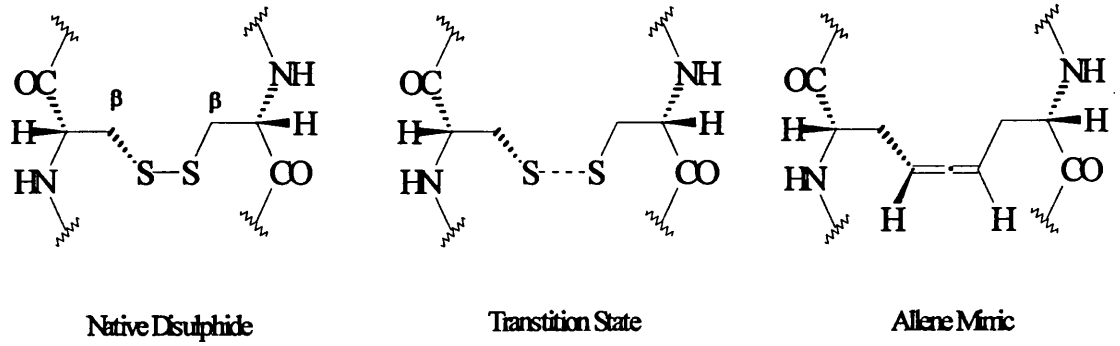
AIMS OF THE PROJECT

Aims of the Project

It was proposed that we should find an effective mimic for the cysteine-cysteine disulphide bond. This peptide functionality was targeted for a number of reasons: It would provide us with a method of altering cyclic peptide structure and conformation, enable us to potentially control the formation and disassembly of discrete disulphide links, and afford peptidomimetics with possible therapeutic properties. To expand on the second point, disulphide bridges are formed systematically during protein folding and disulphide bond formation, breakage, and rearrangement are a useful tool for identifying intermediates in protein folding pathways. Thus, we want a disulphide surrogate that would potentially mimic the transition state that the disulphide bond goes through during the breaking / formation of the S-S bond.

There are many reports in the literature of modifications to cysteine designed to evoke conformational restrictions. An example of these is the replacement of the disulphide bridge (-CH_2-S-S-CH_2 -) by three natural diamino diacids: djenkolic acid (-CH_2-S-CH_2-S-CH_2 -) ; lanthionine (-CH_2-S-CH_2 -) ; and cystathione (-CH_2-S-CH_2-CH_2 -) [4]. The resulting compounds were selective linear competitive inhibitors for trypanothione reductase versus trypanothione, its physiological substrate, and this is discussed in further detail in the chapter on biological targets. The three surrogates were chosen because the resulting compounds did not act as substrates and the distances between both peptidic chains was only slightly modified.

However, these substitutes, and those like them, do not mimic the orthogonality that a disulphide bond elicits on the cysteine β-carbons. A feature of the disulphide bridge is the angle of +90° or -90° subtended by the (β)-C-S bonds in each cysteine, (the barrier of rotation is approximately 10Kcal). A suitable moiety of comparable configuration was therefore sought. It was recognised that the most suitable mimic for this geometry is the allene unit, which exhibits analogous orthogonality, although the distance between $C_{(\alpha)}$ and $C_{(\alpha)}$ is 4.0 Å for the S-S bond but 0.5 Å longer for the allene surrogate. It is not yet known if this will affect the use of allene surrogates in therapeutic peptidomimetics, but it does mimic the transition state the S-S bonds go through on assembly / disassembly, (fig. 1).



Native Disulphide Transition State Allene Mimic

Fig. 1

The central carbon atom of the cumulated 1,2-diene system is sp-hybridised with two sets of orthogonal p-orbitals available for overlap with the remaining p-orbitals of the two terminal sp^2 -carbon atoms of the diene, (fig. 2). For maximum overlap to occur between the p-orbitals, the resulting π-bonds must be orthogonal to each other.

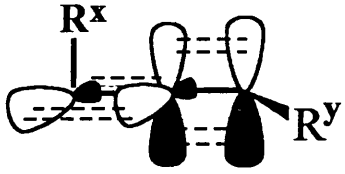


Fig. 2

In the proposed cysteine-mimic, the allene unit can exist in two enantiomeric forms. Thus both (R) and (S) configurations are possible giving +90° and -90° dihedral angle variability. This is of particular value, since conformational restriction is imposed, because it enables further investigation of the effect of the torsion angle orientation on the overall conformation of the peptide.

Allenes have been used previously in medicinal chemistry, where it has been found that their introduction enhanced or modified biological activity [5], and during the last few years allenes have begun to play a crucial role in the development of peptidomimetics. For example, the introduction of the allene function into steroids has proven highly successful and has led to the discovery of pharmacologically very active compounds. In fact, an allenic analogue of the contraceptive norethisterone was found to be 10-20 times more active than the parent compound [6].

Since the biological targets set out below all require the allene unit to be part of a larger peptide sequence it is proposed that protected allenyl *bis*-amino acids, (fig. 3a), will be synthesised as single diastereomers. These will be transformed into the transition state surrogate, (fig. 3b), in which the allenyl unit has appropriate recognition amino acids AA^1 - AA^4. It may be appropriate that in the transition state surrogate, AA^1 and AA^3 should be part of a monocyclic peptide, as is required for the biological target 1 described later.

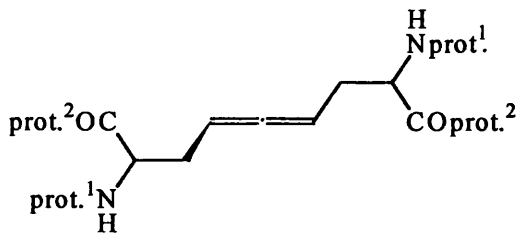


Fig. 3a

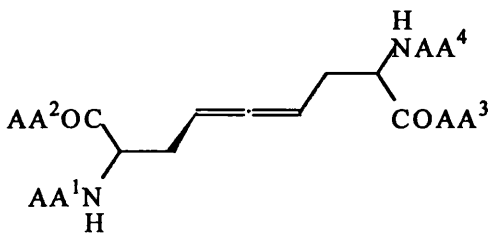


Fig. 3b

Disulphide Bond Formation

With disulphide links playing such an important role in the project it is worth considering the formation of disulphide bonds.

Disulphide bond formation between cysteine residues (Cys) has been studied extensively *in vitro*[7], but how it occurs naturally after biosynthesis is not known. The mechanism of disulphide bond formation *in vivo* is thought to involve thiol-disulphide exchange between the protein synthesised with free SH groups on its Cys residues and small-molecule disulphide compounds. The predominant thiol compound in most cells is glutathione, which exists in both the thiol (GSH) and the disulphide (GSSG) forms. Formation of one disulphide bond in a protein requires two sequential thiol-disulphide exchanges involving the mixed disulphide intermediate, (fig.4). The protein becomes oxidised and the glutathione is reduced. The initial steps in thiol-disulphide interchange are the deprotonation of the dithiol and an S_N2 reaction involving the dithiolate anion, the nucleophilic attack of a thiol on a disulphide has not been observed [7,8].

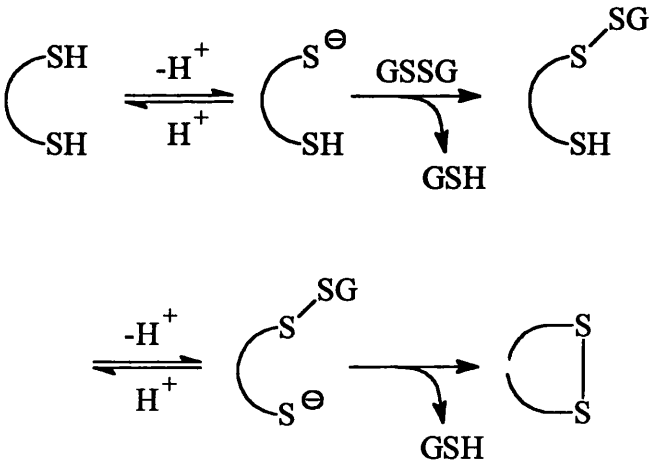


Fig. 4

BIOLOGICAL TARGETS

Biological Targets

There are three main biological targets of the project, and these will be discussed below:

1. Competitive / Suicide inhibitor of Glutathione / Trypanothione reductases.
2. As a hapten for the creation of antibodies with disulphide bond formation / isomerisation / cleavage activity.
3. As a redox stable linkage in C_2 symmetric (HIV) protease inhibitors.

Biological Target No.1:

Competitive / Suicide inhibitors of Glutathione / Trypanothione reductases

Viewed globally, parasitic diseases such as malaria and Chagas' cardiopathy pose an increasing threat to human health and welfare.

Malaria

In the last 25 years malaria has regained its former position as the greatest threat to the health and economic prosperity of mankind. According to figures published by the World Health Organisation, 1500 million people live in areas where malaria is endemic, 300 million cases are recorded each year, and more than two million - mostly children under five years old - die from malaria; an unknown number are left crippled or chronically ill [9]. The development of a vaccine against malaria has so far not fulfilled expectations, although some synthetic peptide vaccines are still in field trials.

Malaria pathogens are most adaptable to chemical changes in their environment. Following the introduction of a new drug, it can be anticipated that *Plasmodeum falciparum*, the protozoa responsible for the disease, will develop resistance within a few years [9].

Chagas' Heart Disease

In 1909 the Brazilian scientist Carlos Chagas discovered the parasite flagellate which causes American Trypanosomiasis, better known as Chagas' disease[10,11]. Chagas named this new protozoa *Trypanosoma cruzi,* and along with the interrelated *Leishmanias* and *Crithidias*, is responsible for many of the severe infectious diseases of man and domestic animals in tropical countries, although *Crithidias* is only pathogenic for insects. Examples along with Chagas' disease are, African sleeping sickness, oriental sore and the devastating cattle disease Nagana. According to the World Health Organisation Chagas' disease affects approximately 24 million people from Southern California to Argentina and Chile, and is after malaria, the most prevalent vector-borne Latin American disease [9-11].

The available treatments for human tropical diseases caused by parasitic trypanosomes and leishmanias lag far behind the enormous advances in the chemotherapy of bacterial diseases. Current drug treatment is either lacking or unsatisfactory, and new drugs for clinical use are too expensive to develop through classical screening methods. Chagas' disease is being fought at present mainly through the elimination of the carrier with insecticides and better housing [12].

Educational campaigns, especially among young people, are becoming a very useful way to combat the disease, but there is still an imperative need to find new molecules which are more specific and more effective against these organisms.

- **Targets for Drug Design** :

Factors such as the metabolic pathways and individual enzymes operative in parasites have been characterised and may be exploited for the design of new drugs. Based on the knowledge of the structure of these molecules, the modelling of enzyme inhibitors as potential chemotherapeutic agents against parasites has become possible. In biochemical terms a common feature of Trypanosomes and malaria parasites is their need for a highly efficient thiol metabolism to protect them from reactive oxygen derivative and other oxidants [9]. These reactive oxygen species, such as $O_2^{\cdot -}$, $H_2O^{\cdot}$, $HO^{\cdot}$, H_2O_2 , are cytotoxic because of their ability to modify nucleic acids, thiol containing proteins and membrane lipids. There are numerous metabolic pathways for the detoxification of reactive oxygen species, and when these antioxidative systems are challenged the cell or tissue is regarded as being under oxidative stress. Without exception, all organisms contain high concentrations of at least one low-molecular weight thiol for maintenance of an intracellular reducing environment. This Achilles heel of parasites, their sensitivity to oxidative stress, is a promising point of attack for drug design.

As drug targets the two disulphide flavoenzymes glutathione reductase (GR), and trypanothione reductase (TR) were chosen.

Glutathione :

Glutathione plays and important role in protecting cells from oxidative damage[9,10]. Due to its free sulphydryl group, GSH may serve as both a nucleophile and a reducing agent, two functions that are facilitated by the action of GSH-dependent enzymes such as the glutathione transferases and glutathione peroxidase. Thus, hazardous electrophilic metabolites may be converted to GSH conjugates through nucleophilic addition or substitution reactions, whereas reactive oxygen species and organic hydroperoxides are reduced to stable products with the concomitant formation of oxidised glutathione (GSSG)[13].

Glutathione peroxidase catalyses the GSH-dependent reduction of H_2O_2 , and is a major pathway of H_2O_2 metabolism in many cells, and one which also catalyses the reduction of other peroxides. Together with superoxide dismutase and catalase these enzymes are important for the protection of membrane lipids against oxidation by reactive oxygen intermediates such as $O_2^{\cdot -}$, $O_2^{\cdot}$, and H_2O_2 , (figure 5).

$$2O_2^{\cdot -} + 2H^+ \xrightarrow{SOD} O_2 + H_2O_2$$

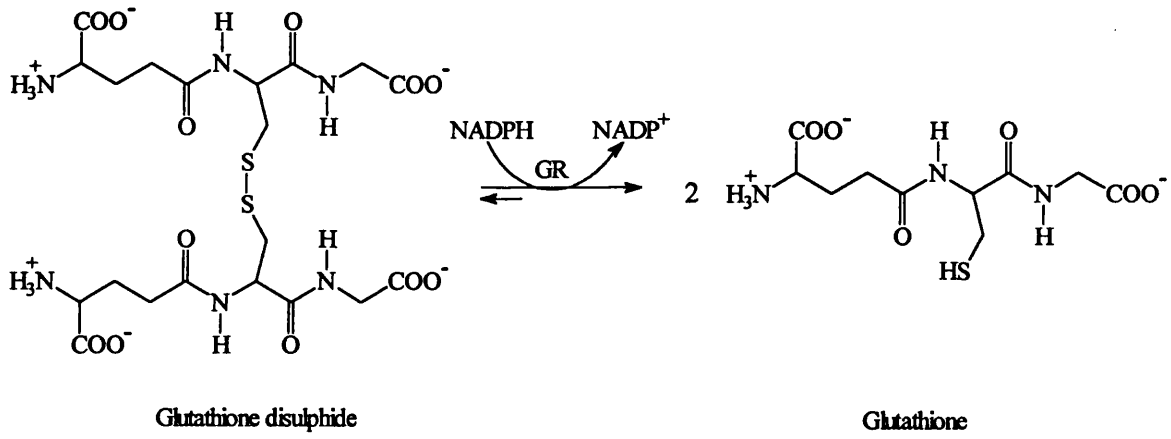
$$2\,H_2O_2 \xrightarrow{CAT} O_2 + 2H_2O$$

$$H_2O_2 + 2GSH \xrightarrow{GPase} GSSG + 2H_2O$$

(Where GSSG is glutathione disulphide.)

Fig. 5

Limiting the quantity of GSH in cells should prevent parasites from overcoming the oxidative stress pathways of the immune system. Human Lymphoid cells depleted of GSH exhibit increased sensitivity to irradiation, while tumour cells depleted of GSH by treatment with buthionine sulphoxime, an inhibitor of GSH synthesis, exhibit increased sensitivity to cytolysis by reactive oxygen intermediates. Also, the treatment of mice infected with trypanosomes with buthionine sulphoxime led to prolonged survival of the animals [14]. Not only can we deplete levels of GSH by restricting its synthesis[9,14,15] but we can also inhibit the reduction of glutathione disulphide (GSSG), which results in GSH formation. Reduction of GSSG to GSH is catalysed by glutathione reductase (GR) as shown in scheme 1.



Glutathione disulphide

Glutathione

Scheme 1

- **Trypanothione:**

Trypanosomes and their relatives exhibit a large number of metabolic reactions not seen in other micro-organisms and higher organisms. In contrast to almost all other organisms whose intracellular redox equilibrium depends on the glutathione / glutathione reductase system, trypanosomatids have no glutathione reductase[9,15-17].

Their most important thiols are compounds in which the sulphur-containing tripeptide glutathione is conjugated to the tripeptide spermidine by an amide bond to form trypanothione, (scheme 2). The reduced disulphide dihydrotrypanothione, $T(SH)_2$, (N^1, N^8-*bis*-glutathionylspermidine), is capable of undergoing rapid nonenzymatic disulphide exchange with intracellular disulphides, including glutathione.

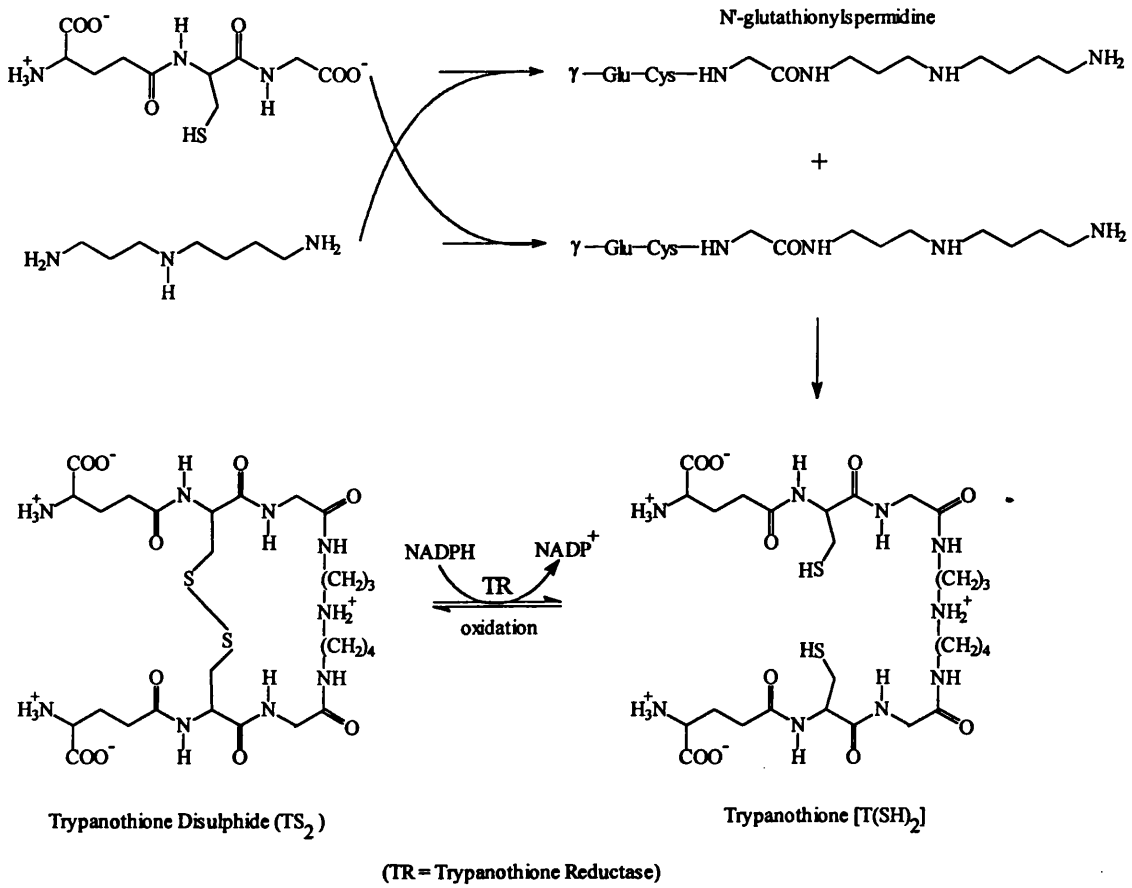
This enzyme, therefore serves the function of glutathione reductase in trypanosomatid parasites and is essential to reduce parasitic GSSG. Thus it represents their main defence against reactive oxygen species. Trypanothione is maintained in the reduced state by the NADPH-dependent enzyme trypanothione reductase[15-17]. Trypanosomes also lack the enzymes catalase and glutathionyl peroxidase, which in other organisms degrade H_2O_2 and other organic peroxidases. The NADPH-dependent flavoprotein trypanothione reductase (TR) reduces $T(S)_2$ to $T(SH)_2$, providing an intracellular reducing environment in these parasites, substituting for the glutathione / GR redox system found in the mammalian host [15-17]. It is thought that trypanothione may function in the trypanosomatid glutathione catalysed reaction as shown in figure 6 [12].

(i) $T(S)_2 + NADPH + H^+ \xrightarrow{TR} T(SH)_2 + NADP^+$

(ii) $T(SH)_2 + GSSG \longrightarrow T(S)_2 + 2\ GSH$

(iii) $GSSG + NADPH + H^+ \longrightarrow 2\ GSH + NADP^+$

Fig. 6



Scheme 2

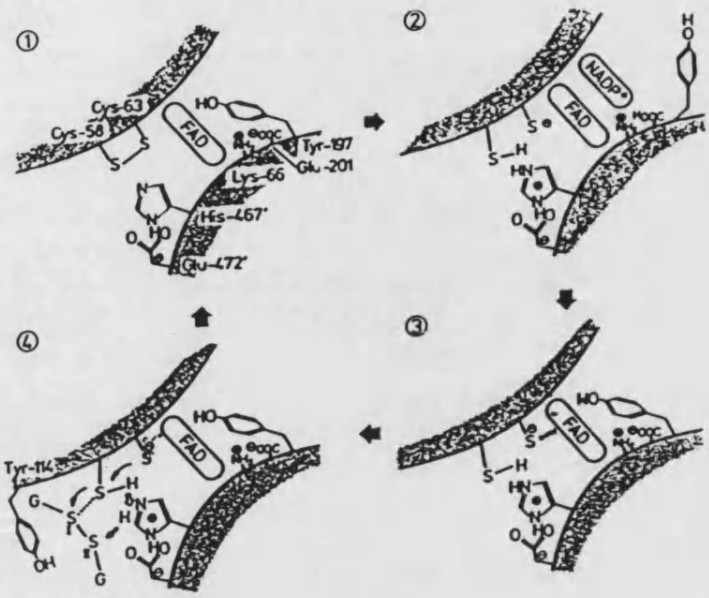
As discussed above, many of the antioxidant functions of trypanothione result in the formation of $T(S)_2$ and so inhibition of the reduction of the disulphide is a principle target for drug design.

- **Mechanism of Action :**

Both GSSG and GSH contain a cysteine residue which undergoes reduction or oxidation during the cycle catalysed by GR. Catalysis takes place in such a way that transfer of reduction equivalents is mediated by the flavin ring[9].

In (1) we have the closed disulphide bridge between residues Cys 58 and Cys 63.

The reduction equivalents are carried to the disulphide bridge of the protein with the result that the dithiol is formed, as shown in (2) of scheme 3. NADPH is released resulting in a charge transfer interaction between the thiolate form of Cys 63 and the flavin ring (3). After binding of GSSG a series of thiol exchanges leads to the release of two molecules of GSH and the reoxidation of the active site dithiol to give the disulphide. This completes the catalytic cycle [9,14].



Scheme 3

TR is also an FAD- and NADPH-dependent disulphide reductase and hence shares many physical and chemical properties with GR, the closely related host enzyme. The most important difference between the two reductases is that TR reduces only the glutathionyl-spermidine conjugate, whereas GR is specific for glutathione disulphide [9]. Apart from this the mechanism of reduction is essentially the same. Alignment and overlay of the primary sequence of trypanothione reductase with that of human erythrocyte glutathione reductase reveal 41% amino acid sequence identity, with essentially all of the catalytically important residues conserved between the two enzymes.

- **Inhibitors of GR and TR** :

There are already various methods of inhibiting the biosynthesis of the two reductases[14], but we are concerned with their inhibition of the action. Many pathogen proteins such as TR, GR, the enzymes of the Aids virus, and ribonucleotide reductase of the herpes simplex virus, are obligatory dimers. Studies have shown that each monomer unit by itself is inactive, and so enzyme inhibitors of TR and GR could be aimed at affecting the dimerization process, and research into this area is covered in several reviews [1,3,18].

It has also been shown that trivalent arsenical drugs, such as melarsen oxide, are potent inhibitors of the NADPH-reduced TR in vitro, it was thought by reaction with the cysteine residues in the active site of the enzyme[19]. However, it has been discovered that the arsenical reacts primarily with intracellular $T(SH)_2$ to form a stable dithioarsane adduct, MelT, and in turn it is this adduct which is a competitive inhibitor of TR. Drugs such as Melarsoprol are still extensively used in the treatment of the latter stages of human African trypanosomiasis, however, the drug itself is thought to be responsible for the deaths of 4 - 8% of patients by causing an arsenical-induced encephalopathy [15,19].

Their are drugs that can inhibit both GR and TR such as the cytostatic agent 1,3-bis-(2-chloroethyl)-1-nitroso urea (BCNU), which covalently inhibits GR but also inhibits TR[15].

As mentioned before, modifications to disulphide bridges have been made before in the design of inhibitors of TR, (page 5), and these compounds were selective competitive inhibitors of the enzyme [4]. It is proposed that we should inhibit GR and TR by synthesising analogues of glutathione disulphide and trypanothione, replacing the S-S bond between cysteine residues with an allene surrogate, (fig 7).

It is hoped that these compounds will be competitive inhibitors of the two respective flavoenzymes by fitting into and blocking the active site of the enzyme. It is also possible that they will act as suicide inhibitors, if the allene is attacked by an enzyme active-site nucleophile forming an extremely long-lasting complex with the enzyme or covalently blocking the active site.

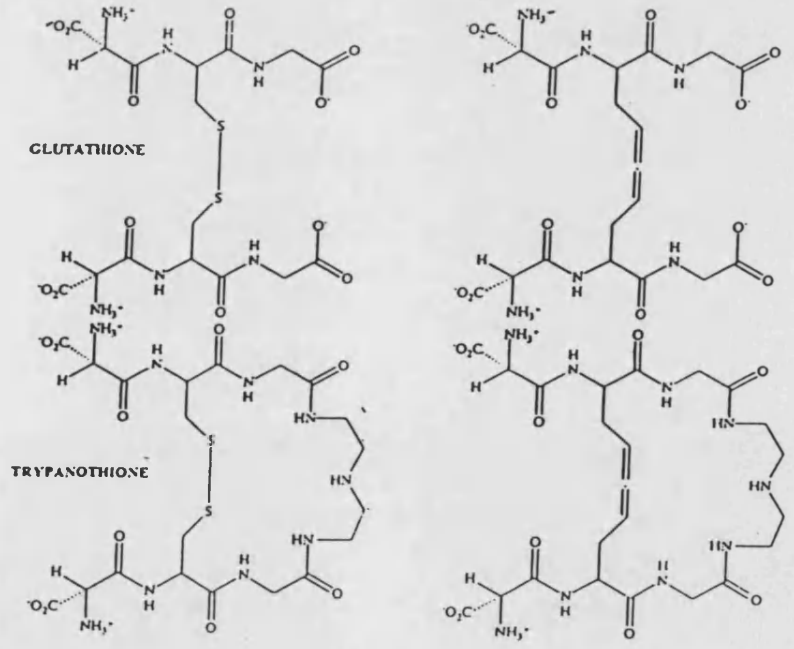


Fig. 7

Biological Target No.2 :

creation of antibodies with disulphide bond formation / isomerisation / cleavage activity

Protein disulphides are present in large concentration in extracellular fluids, yet little is known regarding mechanisms that generate and maintain significant levels of disulphide in the strong reducing environment present in mammalian cells. Further work on the nature of enzymes maintaining cellular thiol:disulphide balance may eventually reveal how this potential is modulated *in vivo*. It is the aim of this part of the project raise antibodies that not only inhibit the action of potentially harmful proteins, but also that catalyse the formation, disassembly and rearrangement of disulphide bonds.

- Introduction to antibodies

Antibodies are the bodies defence mechanism against invading organisms. In most biological systems like hormones and receptors, and enzymes and substrates, recognition usually occurs through fairly accurate complementarity in shape allowing the ligands to approach so close to each other as to permit the normal intermolecular forces to become relatively strong. Selective recognition is achieved through a large number of weak bonding interactions involving hydrogen bonds, van der Waals, electrostatic interactions, and solvent effects that chemists do not yet fully understand and are still far from being able to mimic [8,20,21].

The molecules in the micro-organisms which evoke and react with antibodies are called antigens. Antibodies are formed before antigen is ever seen and they are selected for by the antigen. A lymphocyte makes only one antibody and places it on its outer surface to act as a receptor. When an antigen enters the body it is confronted by a dazzling array of lymphocytes all bearing different antibodies. The antigen will only bind to those receptors with which it makes a good fit.

Lymphocytes whose receptors have bound antigen receive a triggering signal and develop into antibody-forming plasma cells, and since the lymphocytes are programmed to make only one antibody, that secreted by the plasma cell will be identical with that originally acting as the lymphocytes receptor, i.e. it will bind well to the antigen[8,21].

Molecules of <5-kD are rarely antigenic. Yet, when haptens (a well-defined small organic group to which antibodies can be formed), such as the 2,4-dinitrophenyl (DNP) group (fig. 8), are covalently attached to a carrier protein such as bovine serum albumin and then injected into an animal, the animal produces antibodies that bind to the hapten in the absence of the carrier. Without this carrier protein the hapten-antibody complex fails to stimulate antibody production [21].

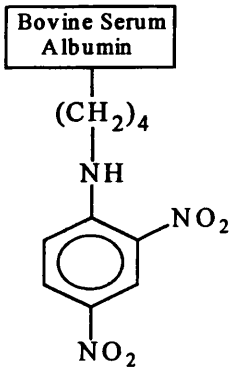


Fig. 8

- **Catalytic Antibodies**

With the aid of mechanistic and synthetic chemistry in combination with the highly evolved synthetic and selection processes of nature, the vast network of molecules and cells of the immune system has been tapped to produce antibodies with a new function - Catalytic Antibodies. Because antibodies can be generated that selectively bind almost any molecule of interest, this new technology offers the potential to tailor-make highly selective catalysts for applications in biology, chemistry, and medicine[21].

Although there was speculation about antibody catalysis it was not until the mid 1980's that catalytic antibodies were discovered. Since that time the number and diversity of antibody catalysed reactions has been growing rapidly as new strategies are being developed for their generation. Catalytic antibodies are characterised by high substrate specificity, share many mechanistic features with enzymes, including catalysis by proximity, transition state stabilisation, and covalent catalysis, and in some cases their rates are comparable to enzymes[21].

One of the obvious advantages of using antibodies as a starting point to make highly selective catalysts is that the problem of engineering substrate specificity is solved by nature. Also, once an approach has been developed the catalytic antibody can be "tuned" by appropriate modifications to hapten structure.

The chemical potential of the immune system was underscored in 1986, when the research groups of Schultz and Lerner showed that antibodies could selectively catalyse the hydrolysis of carbonates and esters, respectively[21]. Since that time, antibodies have been generated that catalyse a wide array of chemical reactions ranging from pericyclic reactions to peptide bond cleavage. Typically the antibody-catalysed reactions proceed with rates 10^3 - 10^6 fold faster than the uncatalysed reactions.

These antibodies catalyse reactions in a number of ways[21]. They can act as entropic traps where the binding energy of the antibody freezes out the rotational and translational degrees of freedom necessary to form the activated complex therefore lowering the $\Delta S^{\ddagger}$ of the reaction. Catalytic antibodies can also stabilise the rate-determining transition state of a reaction, where the antibody combining sites are sterically and electronically complementary to the transition state configuration, again using binding energy to stabilise the unstable transition state. Catalytic antibodies which act in this way can be raised using a hapten which mimics the high energy transition state for a particular reaction therefore generating antibodies with the required antigen-binding site characteristics.

- **Synthetic Targets**

The essential idea underlying antibody catalysis is to anticipate the mechanism of a chemical reaction and then rely on binding energy to lower activation energy along the reaction co-ordinate[21,22]. Such catalysis depends upon the extent to which the hapten models critical features of the rate-determining transition state.

In our case we require a hapten that mimics the transition state that disulphide bonds go through on formation and cleavage, and it is hoped that our allene is just that since it is slightly longer than the native sulphur-sulphur bond. In other words we need to synthesise a peptide with the allene incorporated into its structure in replacement for a disulphide bond. In this respect it was decided to combine biological targets 1 and 2 in that the synthetic targets, i.e. the mimics of glutathione / trypanothione reductases (figure 9), could be used for both aspects of the project.

Once the haptens had been synthesised they could be covalently attached to a carrier such as bovine serum albumin, and used to raise antibodies that selectively bind it. It is then hoped that these antibodies would bind the native peptides in the absence of a carrier and catalyse the reactions of disulphide bond formation and cleavage. Disulphide exchange should occur as well since the activation energy of the disulphide intermediate is lowered by strong binding between the peptide and the antibody. The antibodies would be raised by either injecting the hapten-carrier conjugate into an animal, or by use of combinational antibody libraries. The antibody could then be isolated and then cloned.

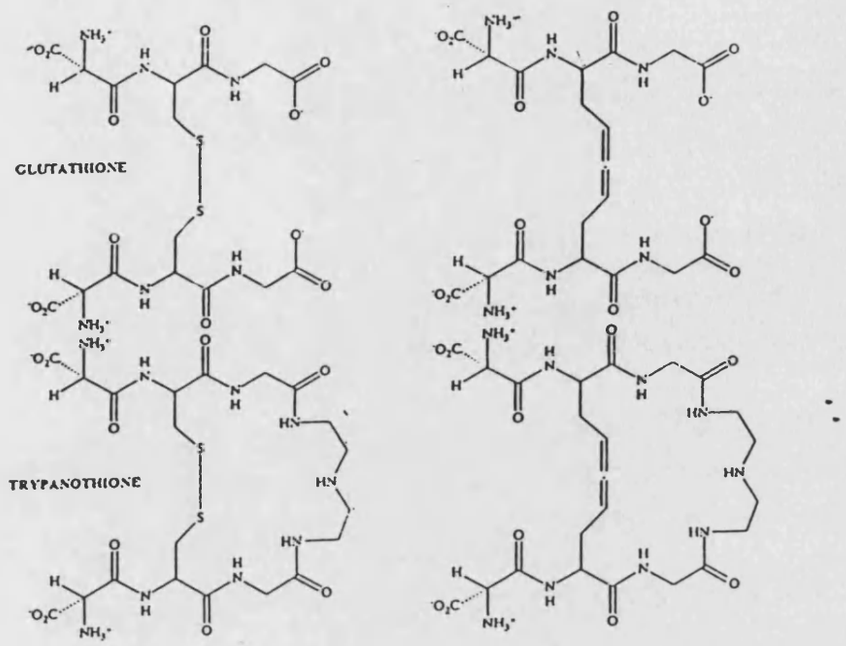


Fig. 9

Biological Target No. 3 :

Use of the allenic substitution as a redox stable linkage in C_2 symmetric inhibitors of HIV protease

- **Introduction :**

The pandemic spread of acquired immunodeficiency syndrome (AIDS) and the discovery of its causative agent human immunodeficiency virus (HIV), a retrovirus, has lead to an unprecedented scientific and clinical effort to understand and combat this lethal disease. HIV is a retrovirus and two genetically distinct subtypes, HIV-1 and HIV-2, have been characterised[23]. Infection by the virus, which targets monocytes expressing surface CD4 receptors, eventually produces profound defects in cell-mediated immunity. Over time infection leads to severe depletion of $CD4^+$ - T-lymphocytes resulting in opportunistic infections, neurological and neoplastic disease, and ultimately death[23]. Original W.H.O. predictions that six million people will be affected with the AIDS virus by the year 2000 were wildly inaccurate and the number is already over 4 times that[24,25].

The replication of numerous plant and animal viruses depends on the specific action of virus encoded proteases. The assembly of infectious progeny virus from these precursors requires proteolysis of specific peptide bonds between the protein domains.

HIV-1 Protease

HIV-1, like other retroviruses, encodes a highly specific protease which is uniquely responsible for the post-translational cleavage of viral gag and gag-pol polyproteins[23-25]. It was recognised that the highly conserved triad Asp-Thr(Ser)-Gly, found in putative retroviral protease sequences was homologous to the catalytic site of proteases belonging to the aspartic acid family and that HIV-1 protease was of this class. While the catalytic triad occurs twice in archetypal aspartic acid proteases like pepsin and renin, it only occurs once in retroviral proteases giving rise to the observation that retroviral proteases exist as homodimers. The homodimeric nature of the HIV-1 protease has been confirmed by X-ray crystallographic and biochemical analysis[25,26]. Assembly of the two 99 amino acid HIV-1 monomers results in a dimer and generates an active site at the interface of the subunits. Each monomer contributes half of the active site which includes two catalytic aspartate residues as well as the threonine/serine and glycine residues[24-26].

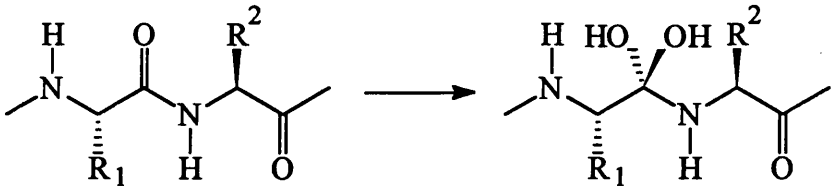
Dimer formation generates not only the catalytic centre but also the extended substrate binding pocket. This substrate binding pocket is formed in a region along the central axis of the symmetric homodimer. Eight individual subsites are formed along the length of the enzyme surface and are designed to accommodate substrate amino acid side chains. Substrate binding in the subsites is governed largely by an ability to form stabilising van der Waals interactions between substrate amino acid side chains and enzyme amino acid side chains lining the subsites[26].

The HIV-1 protease is essential for the assembly and maturation of infection virions, and mutation in the active site renders the protease inactive, resulting in immature, non-infectious virus particles. Replacement of the catalytically active aspartic acid residues by either alanine, asparagine or threonine, by site-directed mutagenesis renders the enzyme inactive[24-26].

The pivotal role of the protease in the post-translational processing of viral proteins makes it a prime target for drug design. Agents that specifically and effectively inhibit the hydrolytic activity of the protease may serve as powerful antiviral pharmaceuticals.

- **Inhibitors** :

The generation of a high energy, tetrahedral diol, (I), from a low energy, trigonal amide constitutes a key step in aspartic protease catalysis, scheme 4 [25,27].

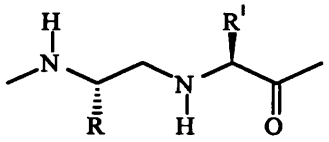


(I)

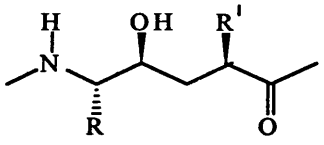
Scheme 4

One of the classical strategies for designing enzyme inhibitors is the incorporation of a transition state mimic into substrate analogues. This can be applied to the design of HIV protease inhibitors by replacing the substrate scissile amide bond with a non-hydrolysable transition state isostere.

To either side of this amide bond are the substrate binding pockets (S,S^1 / S_1 , S_1^1 etc.) that line the substrate-binding groove, and apart from optimising the fitting of inhibitor amino acid side chains into their corresponding binding pockets, inhibitor design must also take into account hydrogen bonding and electrostatic interactions which occur along the groove. A list of possible hydrolytically stable dipeptide isostere replacements for the scissile bond are shown below in table 1 [24,25,28]. These analogues mimic the geometry of the tetrahedral intermediate in peptide hydrolysis.



(II)



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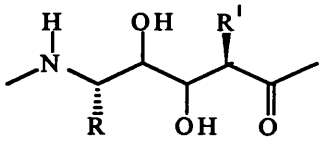

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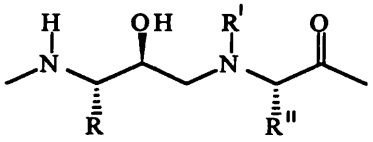
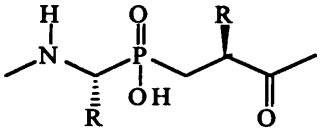
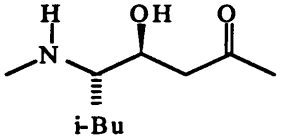


(V)



(VI)



(VII)

Table 1

While many of these peptidomimetic inhibitors posses potent antiviral activity *in vitro* they suffer from the same pharmacological problems that many peptidomimetics suffer from. A major strategy to reduce pharmacological problems is to develop inhibitors which either structurally or functionally mimic bioactive peptides but have either reduced or no peptidic character. An increasing number of reported non-peptidic HIV protease inhibitors which have been discovered through screening (of natural products and libraries of synthetic compounds), or from structural-based design founded upon crystal structures of HIV protease / inhibitor complexes[24]. The distinct features of this series of compounds are the low molecular weight and relatively non-peptide nature, containing only a single amino acid in most cases.

An alternative to transition state mimics is the replacement of the water molecule which bridges the P_2 and P_1' carbonyls of the inhibitor to the NH residues of Ile_{50} from each monomer flap of the enzyme, while the inhibitor hydroxyls bind to the Asp_{25} residues in the active site, (fig.10) [25,27]. The hydrogen binding to this water molecule help the two conformationaly flexible flaps to close around the substrate.

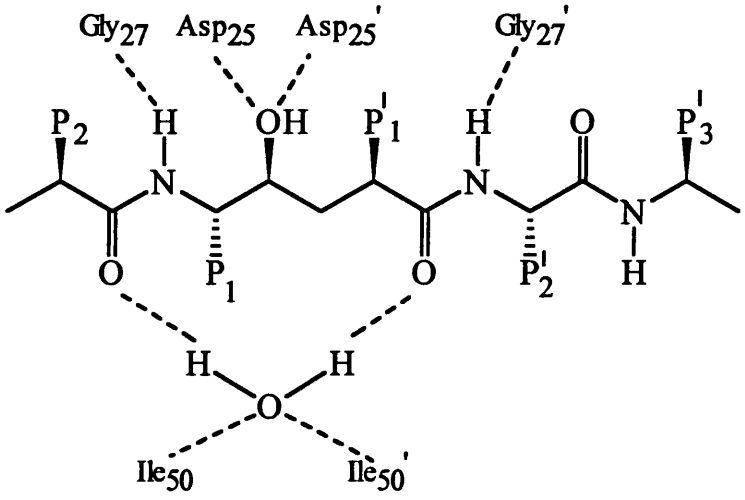


Fig. 10

It has been suggested that there would be a significant entropy gain if this water molecule could be replaced by an inhibitor component, and a number of cyclic inhibitors have been designed using computer modelling techniques [25].

However, one of the most promising reduced-peptide approaches utilises the symmetry characteristics of the protease. As mentioned previously HIV-1 protease was shown by X-ray crystallography to be a homodimer, and this also showed that the native enzyme adopts a conformation in which a two-fold symmetry axis extends through the active site [24,29-32]. It therefore might also be desirable to include this C_2-symmetry through the centre of the inhibitors.

In addition, as symmetric inhibitors are less peptide-like than the peptide-based inhibitors using the natural substrate sequences in conjunction with the transition-state analogue concept, the stability of symmetric inhibitors *in vivo* would be higher than for non-symmetric ones. Moreover, symmetric inhibitors should confer high specificity for retroviral proteases over related mammalian aspartic proteases, whose substrate binding sites are less symmetric.

The enzymes C_2-axis lies between and perpendicular to the two catalytic aspartate residues in the active site. The X-ray crystal structure of HIV-1 protease shows that the so called S_1 and S_1' subsites are related by a C_2-symmetry operation and are thus structurally identical (or nearly identical)[24,29,30]. Some tactics are to divide the design problem into two separate stages, the design of a central unit and the design of the two identical terminal groups. These three units could then be coupled together by amide linkages. This design must satisfy two major constraints.

First, there must be a productive, symmetric interaction between the inhibitor and the enzyme, and for this to occur their C_2-axes must coincide. Second, the inhibitor should be able to fill the hydrophobic subsites that normally interact with the side chains of the asymmetric peptide substrate.

However, careful examination of the substrate binding region indicates that only the P_1 and P_2 substituents would likely be buried in the subsites of the enzyme. The original design of a C_2-symmetric inhibitor from the tetrahedral intermediate for cleavage of the substrate hinged on three operations (figure 11).

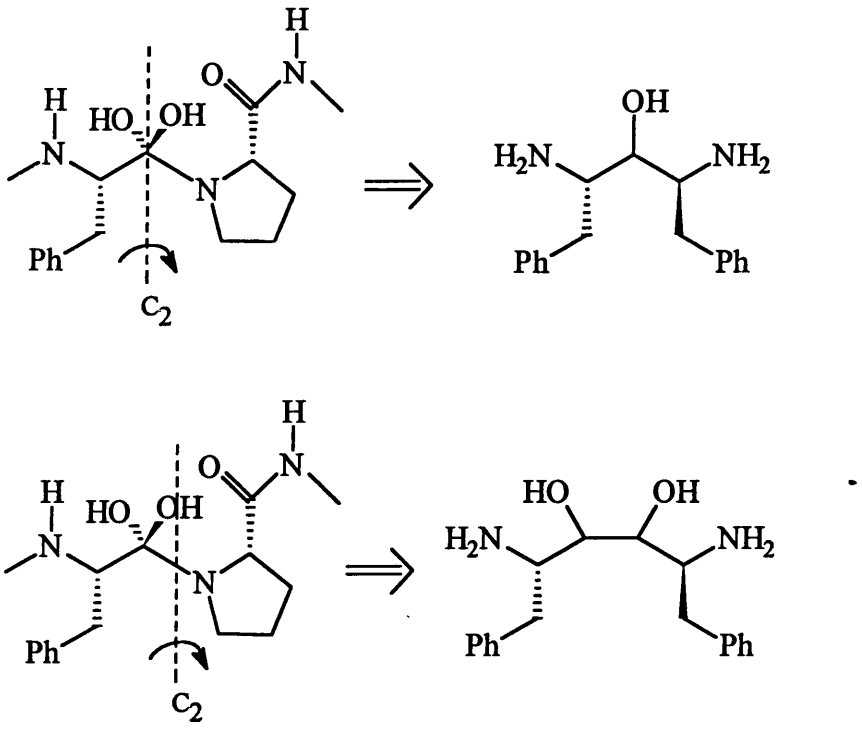
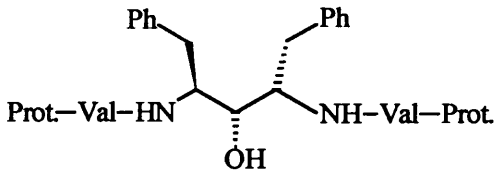


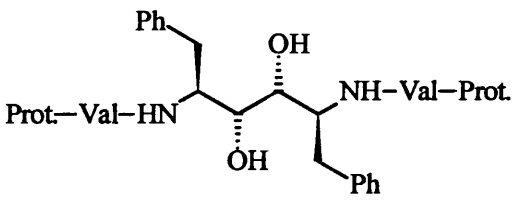
Fig. 11

First, a hypothetical axis of symmetry in the substrate is defined based on the C_2-axis of the enzyme. Modelling experiments and the fact that the catalytically active aspartate residues reside close to the C_2-axis of the enzyme lead to the placement of the symmetry axis either on the carbonyl carbon undergoing cleavage or through the middle of the scissile bond. Second, one "half" of the substrate is arbitrarily deleted. Deletion of the P' region is guided by the greater importance of the P region previously observed for the binding of renin inhibitors[29].

Application of these operations gives rise to such inhibitors as (VIII) and (IX) shown below, both of which were potent inhibitors of HIV-1 protease activity. There are many variations of C_2-symmetric inhibitors, with either different terminal groups or a different core unit, and this are discussed in many reviews[23,24,30-32].



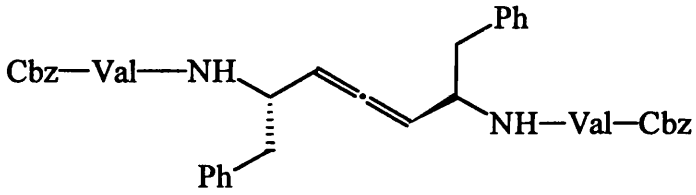
(XIII)



(IX)

Project Proposals :

It is one of the aims of this project to synthesise a C_2-symmetric inhibitor with our allene unit forming the core unit, for example (X).

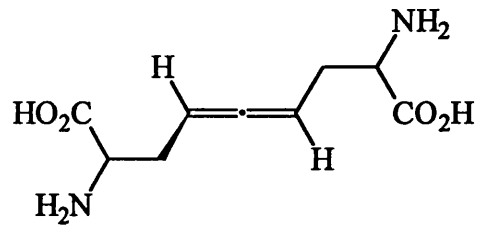


(X)

DISCUSSION

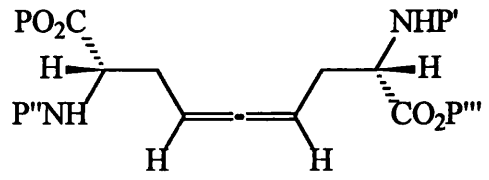
Discussion

To be able to include the allene surrogate into a peptide sequence, for example to enable its use as a hapten, we require a basic subunit that can be used as a building block and then build the peptide around this. It is proposed that the allene *bis*-amino acid (1) be synthesised as a single diastereomer.



(1)

Once we have (1) the amino and carboxylic acid functionalities can be protected, to give (2), and derivatised products can, in turn, be constructed from this using basic amino acid coupling methods.



(2)

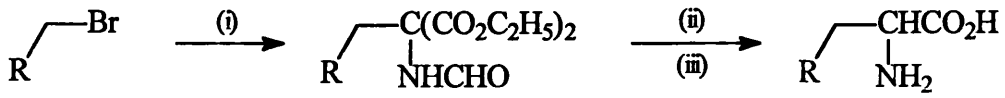
Preparation of allenyl *bis*-amino acids :

In recent years, a steadily increasing demand for sizeable quantities of α-amino acids has developed because of their extensive employment in biochemical, nutritional, microbiological, and tissue culture research, in addition to their widespread use as supplements for natural and processed foods[33]. Other than isolating amino acids from natural sources a number of practical chemical syntheses are presently known which permit ready access to a host of α-amino acids on both an industrial and laboratory scale.

The synthesis of α-amino acids at the bench is an active field because of the demand for specifically labelled, unnatural amino acids. The need is almost always for a homochiral product, so assembly of the target without regard to α-chirality must be followed by resolution; alternatively an asymmetric synthesis may be employed or the conversion of a freely available homochiral compound to the required α-amino acid must be achieved.

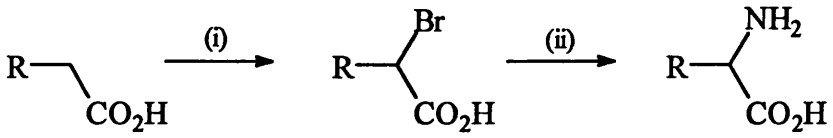
As yet there is no reported synthetic procedure for allenyl *bis*-amino acids. Therefore we have to use known methods for amino acid synthesis coupled with known methods for allene formation. General methods are available for the preparation of racemic α-amino acids. Many of these methods were developed in the early days of amino acid chemistry, but still retain their importance.

These general methods[33-35], shown below, include the DFAM method, scheme 1; displacement reactions, scheme 2; the Strecker synthesis, scheme 3; and synthesis through hydantoins, scheme 4.



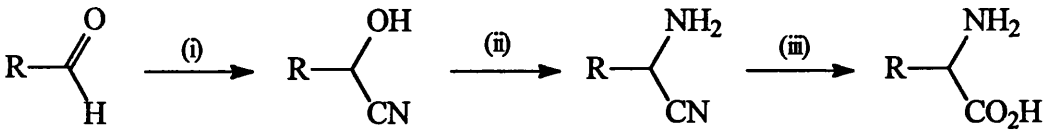
(i) NaH, DFAM ; (ii) NaOH ; (iii) HCl

Scheme 1



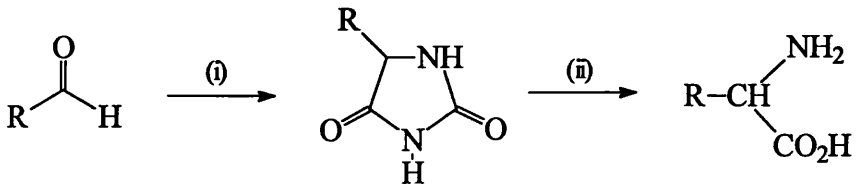
(i) Br_2 / PCl_3 ; (ii) NH_3

Scheme 2



(i) HCN , KCN , 0°C ; (ii) NH_4Cl-NH_4OH , 20°C , 7 days ; (iii) HCl

Scheme 3



(i) KCN, $(NH_4)_2CO_3$; (ii) H_3O^+ or OH^-

Scheme 4

Unfortunately it is highly likely that some of these methods and many of the reagents employed in these approaches are not compatible with the allene unit. For example, the Strecker synthesis requires blocking groups in the 2-position since 3,4-dienals tend to rearrange to 2,4-dienals[34]. The products of these reactions are also racemic, thus calling for tedious and time consuming resolution and separation techniques which can be avoided by the use of more attractive asymmetric and enantiospecific routes.

Schöllkopf and collaborators[36-39] have devised a versatile and useful method for the preparation of a large variety of amino acids based on the metallation and subsequent alkylation of *bis*-lactim ethers. The general protocol involves peptide coupling of two amino acids, piperazinedione formation and *bis*-lactim ether formation with trimethyloxonium tetrafluoroborate. Metallation with n-BuLi in THF at low temperature followed by electrophilic quench furnishes the homologated *bis*-lactim ethers. The electrophile is considered to add anti- (fig.1) to the methyl group in a highly stereoselective fashion with typical diastereomeric excesses of greater than 90%, (see (7) of scheme 5).

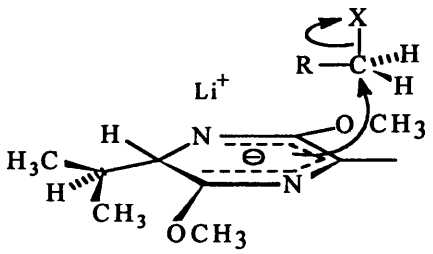
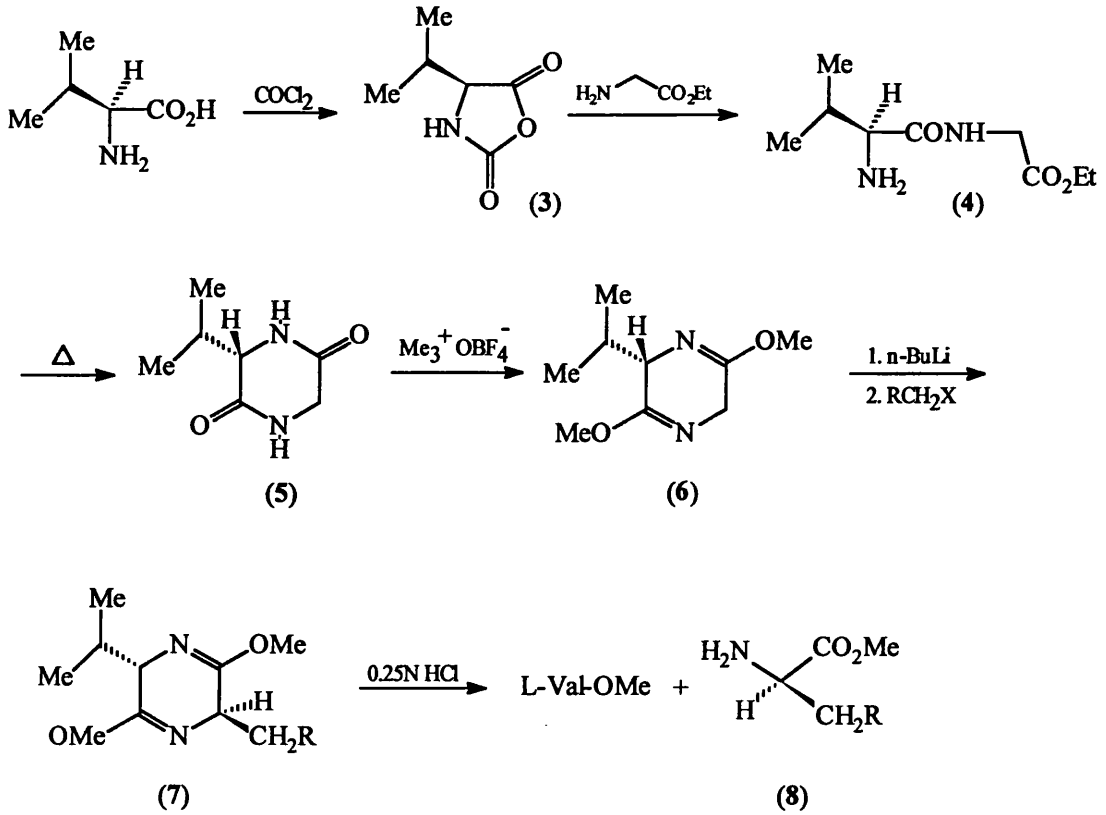


Fig. 1

By far the most extensively studied *bis*-lactim ether is that derived from L-valine and glycine. As shown in scheme 5 below, L-valine is converted into the N-carboxyanhydride (3) and condensed with glycine ethyl ester. Heating the dipeptide (4) furnishes cyclo-L-Val-Gly (5) which is, in turn, converted into the *bis*-lactim ether (6). This reagent has become the most widely used glycine template and is now commercially available in both enantiomeric forms.

As mentioned above, metallation of the *bis*-lactim ether with butyl-lithium in THF at low temperature followed by alkylation with a variety of electrophiles proceeds with a high degree of stereoselectivity furnishing the anti-products (7). Hydrolytic cleavage of the heterocycle with dilute HCl at ambient temperature furnishes the new amino acid methyl ester (8) and L-valine methyl ester which must be separated.



Where RCH_2X = the dimesylated allene

Scheme 5

New methods for the synthesis of enantiomerically pure α-amino acids are constantly being explored and much recent progress has been made in the use of functionalised zinc reagents as alanine β-anion equivalents[40-43], figure 2.

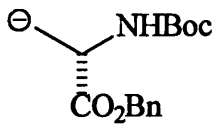


Fig. 2

The organometallic reagent shown in figure 3 is a synthetic equivalent for the nucleophilic alanine synthon shown in figure 2, and couples efficiently with organic electrophiles under catalytic conditions to give enantiomerically pure, protected amino acid derivatives. This area is covered in greater detail later in this report.

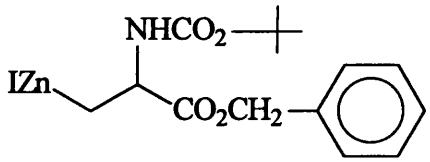


Figure 3

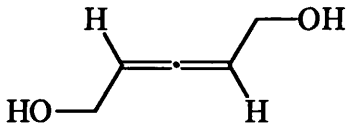
As mentioned earlier there is no reported synthetic procedure for allenyl *bis*-amino acids and that we therefore have to use known methods for amino acid synthesis coupled with known methods for allene formation. This can be done by either assembling the allene structure first and then affixing the amino acid sections, or by attached the amino acid units and then building in the allene. Over the duration of this project three distinct alternatives were attempted.

1. Synthesis of a basic allene unit via elimination from a halo-alkene, then activation of this compound followed by nucleophilic substitution by a suitable amino acid equivalent.
2. Formation of a *bis*-amino acid / alkene compound followed by allene synthesis via a cyclo-propanyl carbene.
3. Formation of an acetylene / amino acid subunit followed again by nucleophilic substitution using by a suitable amino acid equivalent to give the allene.

Each of these methods will now be discussed in turn.

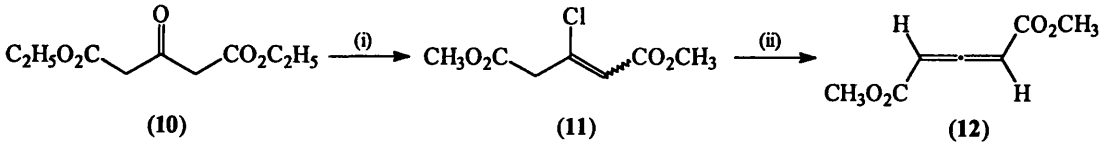
Method 1 - Elimination from halo-alkene followed by nucleophilic substitution :

It was decided that the best precursor for the activation of the allene for nucleophilic substitution was the allenic diol (9).



(9)

A survey of the literature revealed that the allenic diol (9) was an unknown compound. A facile synthesis for this important starting material was therefore required. We initially attempted a known procedure laid out by Bryson *et al*[44] for the synthesis of allenic esters, scheme (6), involving the elimination of HCl from dimethyl-3-chloropent-2-ene-1,5-dioate (11), itself synthesised from the cheap and readily available starting material diethyl pent-3-one-1,5-dioate (10). This method gave easy access, with good yield, to the allene (12). The products (11) and (12) were purified by distillation, with higher yields being obtained by performing the process in small batches. The allene required storage at low temperature to minimise polymerisation processes, detected by increase in viscosity and coloration.



(10) **(11)** **(12)**

(i) PCl_5 , 40^0C , 1 hour ; (ii) NEt_3 , THF, 0^0C

Scheme 6

The strong i.r. absorption detected at 1960 cm^{-1} was characteristic of the allenic antisymmetric stretch. In addition the extreme low field position of the allene central carbon was identified by ^{13}C n.m.r. spectroscopy at δ 220 ppm.

Having obtained (12) in reasonable yield, a suitable method of reduction was sought. Initial attempts by treatment with $LiAlH_4$ were unsuccessful. The reaction was performed in diethyl ether, at various sub-zero temperatures but on each occasion no allene-containing product was detected. However, a strong OH peak was observed in the product and the infra red spectrum showed an absence of any carbonyl bands. Further examination by ^{1}H n.m.r. spectroscopy suggested that a mixture of 1,5-pentanediol and pent-2,4-dienol products had been formed, possibly by the method shown in figure 4. Reduction by borane in THF at 0^0C [45,46] also gave the same results and no allenic products were detected.

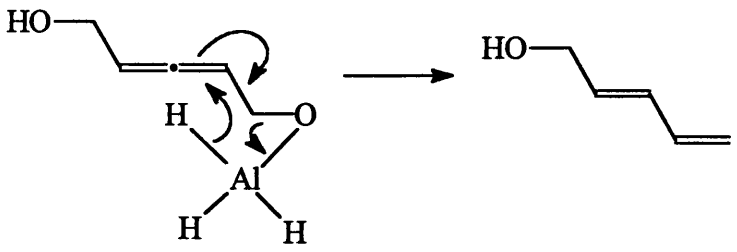
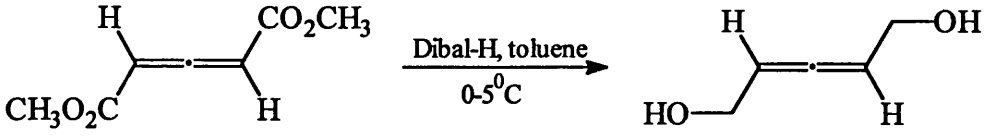


Figure 4

Although it is generally accepted that sodium borohydride is not useful for the reduction of carboxylic esters, cases have been reported in the literature of esters being reduced to primary alcohols [47]. Here it seems a neighbouring group plays some sort of role. Attempts to reduce the ester by using $NaBH_4$ in aqueous ethanol resulted in the successful reduction of the ester groups, but unfortunately the allene unit was also reduced. Successful reduction was finally achieved by using diisobutylaluminium hydride (Dibal-H) as the reducing agent, (scheme 7).

Many different conditions were explored and the best result was obtained when the reaction was carried out in toluene at 0°c. Decomposition of the aluminium salts was best achieved by treatment of the intermediate aluminium complex with potassium sodium tartrate solution, followed by a continuous extraction into diethyl ether to isolate the product.



(12) (9)

Scheme 7

Use of $MeOH/H_2O$ as the initial treatment for the decomposition of the aluminium salts resulted in a lower yield of product. The diol was extremely water soluble and the continuous extraction had to be carried out for as long as a week to obtain a reasonable yield. This was rather tedious, but it proved that the diol was stable in aqueous conditions

Purification was best effected by column chromatography. Use of distillation, even of small batches, resulted in extensive polymerisation and decomposition of the diol; this also occurred on standing at RT, but was suppressed by storage at low temperature.

The identity of (9) was confirmed from spectral data, which showed strong bands in the i.r. spectrum at 3600-3100 cm^{-1} (OH) and 1970 cm^{-1} (central allenic carbon), with no carbonyl absorption detected. In the ^{1}H n.m.r. spectrum no spin-spin coupling was detected from the C-2 to C-4 proton or from C-1 to C-5 protons due to the symmetry of the molecule (figure 5). The coupling constant of C-1 protons to those of the adjacent C-2 and the long range proton-proton coupling, ^{5}J (H-H) to C-4 were identical, giving rise to an apparent triplet at δ-4.16 ppm. The protons of C-2 and C-5 are isochrons and resonate as a triplet at δ 5.51 ppm. The allenic central carbon was evident in the ^{13}C n.m.r. spectrum at δ 202.5 ppm. Furthermore, mass spectrometric analysis by chemical ionisation (C.I.) revealed a weak $(M+1)^{+}$ peak at m/z 101 with an intense signal at m/z 83 $(M+1-H_2O)^{+}$. Due to decomposition of the product a satisfactory elemental analysis was unobtainable.

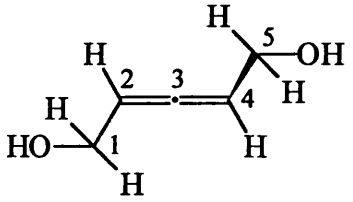
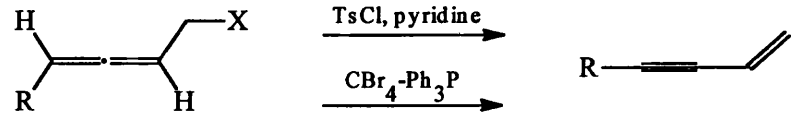


Figure 5

Attempts to Activate the Allene Moiety for Alkylation :

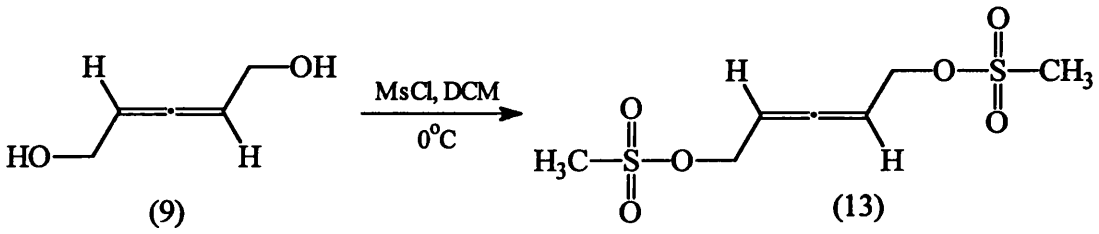
We now required activation of the diol ready for attack by a suitable nucleophile, for example Schöllkopf's reagent, leading to the desired allenic amino acids. An emphasis had recently been placed on accessing α,α–disubstituted amino acids, and on the formal 'double derivatization' of a glycine template [48,49]. Thus it was initially decided to activate both hydroxy groups and displace them both in the same step. Mono-protection would eventually have to be carried out as this would enable us to synthesise allenic compounds with different amino acid sidechains. On the other hand activating both hydroxy groups prior to alkylation would only allow us to make symmetric compounds.

Attempts at direct bromination by treating the diol with triphenylphosphite dibromide resulted in the disruption of the allene unit, while attempts to obtain the ditosylated product, by treatment with tosyl chloride in pyridine, also failed resulting in a mixture of tosylated and vinylic material devoid of any allenic product. It would seem that activation of the alcohol moieties has increased the sensitivity of the allene towards elimination. It has been shown that nucleophilic addition to allenes only takes place readily when there is activation by an electron-withdrawing substituent conjugated with one of the double bonds (Michael type addition) or through a neighbouring group effect as in the reduction of allenic alcohols (figure 4). Indeed, similar work carried out by Trost *et al.* [50] on the activation of β-hydroxy allenes showed that attempts at tosylation and bromination resulted in the exclusive formation of the eliminated product, (scheme 8).



Scheme 8

However, in our hands attempts to activate the allenic diol (9) by formation of the dimesylated adduct (13), via reaction with methanesulphonyl chloride in dry DCM at 0^0C, were successful, (scheme 9), although the resulting compound is very unstable.



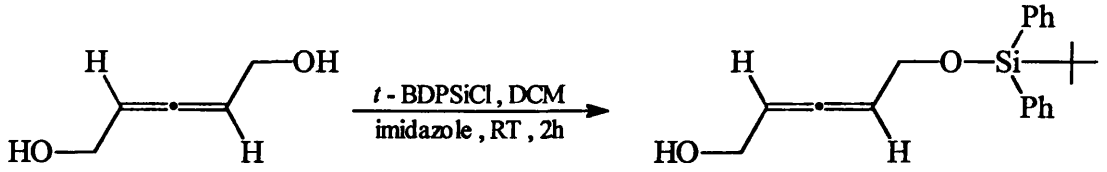
Scheme 9

The i.r. spectrum of the product revealed the absence of the OH band and the appearance of signals at 1340 and 1160 cm^{-1} , due to the sulphonate ester. Presence of this group was further revealed by the methyl singlet at δ 1.05 ppm in the ^{1}H n.m.r. , and at δ 38.3 ppm in the ^{13}C n.m.r. spectra. The allene was identified by the usual characteristic signals in the i.r. and ^{13}C n.m.r. Attempts to purify the product (13) by column chromatography resulted in its decomposition. It is possible that the failure of the tosylation reactions could be due to the differences in mechanism between tosylation and mesylation.

The dimesylated compound (13) was found to be very unstable and decomposition occurred even when stored at low temperature under an inert gas. Due to this instability a satisfactory elemental analysis and a mass spectrum were not obtained. None of the nucleophilic reactions tried so far with this substrate have been successful. With other allenes that do react we consider that attack at the central carbon is inhibited by excess electron density donated from the two terminal carbons of the allene unit. For (13) it is possible that the two mesylate groups are drawing enough electron density away to activate attack on the central carbon and this may be responsible for this compounds lability.

For this reason emphasis was switched to the mono-protected hydroxy compound. The protection of one hydroxy group was carried out using *t*-butyldiphenylsilyl chloride (*t*-BDPSiCl). This reagent was chosen to form the *t*-BDPSi ether over related silyl and butyl ethers because such derivatives are easier to form and show compatibility with a variety of conditions[51]. For example they are moderately stable to acid hydrolysis. This stability is desirable if acid hydrolysis is required to yield the amino acid moiety at the other C-terminus of the allene unit. However, smooth cleavage is effected by treatment with fluoride ion.

The formation of the mono-*t*-BDPSi ether was initially attempted by the treatment of (9) with *t*-BDPSiCl and NaH in THF. However, after several attempts, no allenic product could be detected and hence the procedure was abandoned. However, the desired compound (14) was obtained by use of imidazole in place of NaH according to scheme 10. About 10% disilylated product was also obtained.



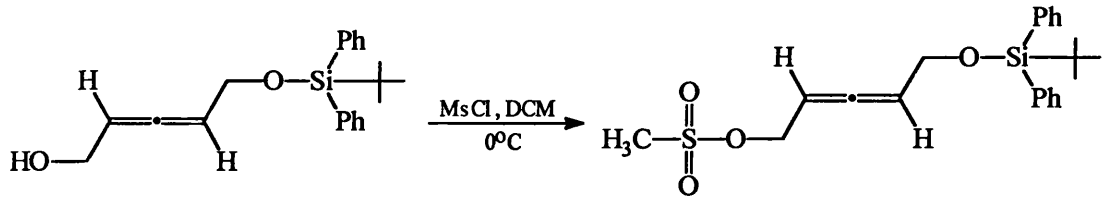
(9) (14)

Scheme 10

All spectral data were consistent with the desired product. A hydroxyl band was present in the i.r. spectrum at 3600-3100 cm^{-1}, with additional bands at 1100 and 820 cm^{-1} (Si-O-C). The presence of the *t*-BDPSi ether was further indicated by *t*-Bu and aromatic signals in the ^{1}H and ^{13}C n.m.r. spectra.

The mono-protection renders loss of symmetry, and this was shown by the ^{1}H n.m.r. spectrum which revealed a double doublet at δ 4.08 and 4.25 ppm for the resonances of the protons of the hydroxy- and silyloxy-methine units respectively, due to ^{3}J (H-H) and ^{5}J (H-H) coupling in each case. Each allenic proton was now differentiated giving rise to a complex signal. Mass spectrometry (C.I.) revealed a major ion at m/z 321 due to loss of H_2O from the molecular species, in addition to a $(M+1)^{+}$ peak at m/z 339. The compound (14) was more stable than the non-functionalised allenic diol and polymerisation no longer took place.

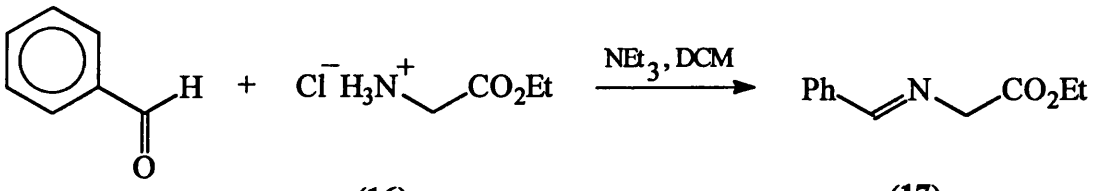
The mono-protected allene (14) was then mesylated in the same manner as the diol to furnish the mono-protected mono-mesylated adduct (15), scheme 11. It was hoped that attack by a suitable nucleophile would then furnish the allenic amino acid.



(14) (15)

Scheme 11

As Schöllkopf's reagent is very expensive it was decided to employ a cheaper nucleophilic agent initially to work out the best possible strategy. Many papers report that the benzylidene derivative of glycine ethyl ester (17) can be used in mono- or sequential reactions leading to a very simple synthesis of α-amino esters and acids[48,49,52-54]. Although the products of such a reaction would be a mixture of diastereomers in our case this should, if successful, validate our methodology prior to using the Schöllkopf procedure. The benzylidene glycine ethyl ester (17) was synthesised from glycine ethyl ester hydrochloride (16) and benzaldehyde, as shown in scheme 12.



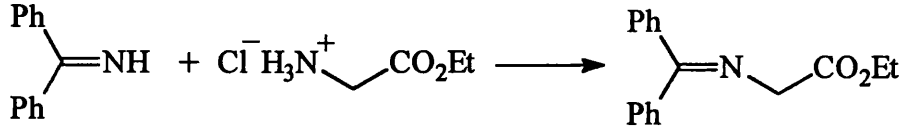
(16) (17)

Scheme 12

The relatively high acidity of (17) permits formation of the corresponding carbanion not only with strong bases like lithium diisopropylamide, which could be detrimental to the allene, but also with weaker bases such as potassium *tert*-butoxide[49].

It is also worth noting that the simple ethyl ester is much more stable than was originally claimed and it can be stored at low temperature for several months[49]. However, it turned out that the benzaldehyde starting material could not be removed from the final product, this meant that the resulting amino acid was impure. All attempts to remove the aldehyde, either by column chromatography or by complexing with sodium bisulphite, resulted in the degradation of the Schiff's base.

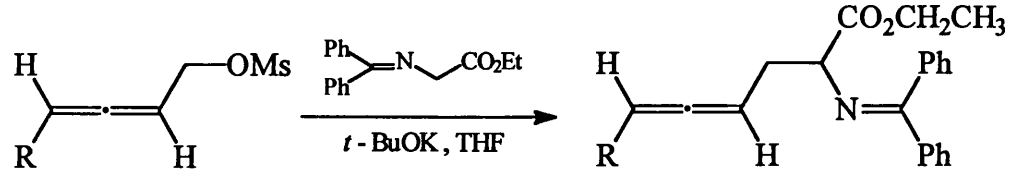
To overcome this problem the diphenyl glycine derivative (18) was synthesised. The first attempt in which benzaldehyde was replaced with benzophenone failed. This is due presumably to the lower reactivity of benzophenone, but when the reaction was carried out by stirring glycine ethyl ester hydrochloride and benzophenone imine together at room temperature for 24 hours, excellent yields of the desired compound were obtained, (scheme 13)[54].



(18)

Scheme 13

Using the benzylidene glycine ethyl ester it should be possible to obtain the mono-protected amino ester (19) by the route shown for the silyl-protected ether in scheme 14. It would then be possible to furnish the amino acid by simple acid or base hydrolysis.

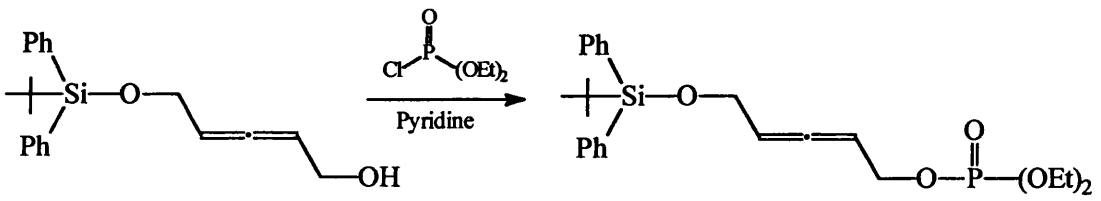


Where R = *t* - BDPSi-O-CH_2 **(19)**

Scheme 14

Unfortunately all attempts to react the allene mesylate with the carbanion of (18) failed. The allene appeared to decompose and only presence of the silyl protection group could be detected by spectroscopic methods. It is possible that elimination is taking place and that volatile products were lost when the solvent was removed under vacuum, or that nucleophilic attack is now focused upon the more electron deficient central carbon of the allene. As an alternative approach we next turned to a palladium catalysed reaction as Cazes and co-workers have developed a palladium catalysed coupling of the glycinate anion of (18) with allenic phosphates [55].

Using this methodology we decided to make the allenic phosphate (20) in a one step reaction from the mono-protected alcohol by reaction with diethyl chlorophosphate in the presence of pyridine, (scheme 15).



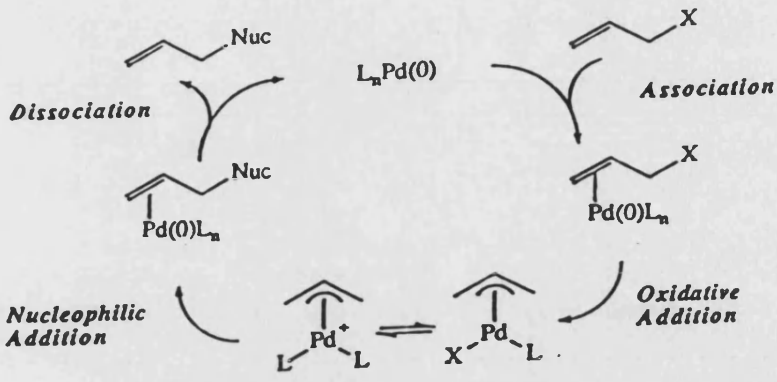
(14) **(20)**

Scheme 15

The structure of the phosphate (20) was confirmed by spectral data. The absorption band in the i.r. spectrum at 3600-3100 cm^{-1} (OH) of the starting material had disappeared but the peak for the central carbon of the allene unit at 1970 cm^{-1} and the bands at 110 and 820 cm^{-1} (Si-O-C) were still present. In addition there were new peaks at 1277 cm^{-1} (P=O) and 1050-1020 cm^{-1} (P-O-CH_2). The presence of the t-BDPSi ether was further confirmed by the t-Bu and aromatic signals in the ^1H- and ^{13}C-spectra. In the ^1H- n.m.r. spectrum the methylene proton resonance next to the phosphate also showed coupling to the phosphorus, and a triplet at δ 1.3 ppm and quartet at δ 4.1 ppm indicated the presence of the ethyl ester groups of the phosphate. Finally mass spectrometry (FAB) revealed the expected $(M+1)^+$ peak at m/z 475.

Transition metals, palladium in particular, have found widespread utility in catalysing a number of important organic chemical processes. Central to all these catalytic reactions is the ease with which organo-palladium compounds can undergo oxidative addition and reductive elimination reactions [56].

The use of palladium in allene chemistry is a type of allylic substitution. It is believed that the mechanism of the palladium catalysed substitution involves the initial co-ordination of palladium (0) to one of the double bonds, followed by an oxidative addition process to afford an intermediate η^3- allyl complex, (scheme 16). In the presence of phosphine, an equilibrium between a neutral and cationic complex results. The cationic complex is favoured by the use of bidentate phosphine ligands to which nucleophilic addition then takes place to one of the termini of the allylic unit to afford the palladium (0) complex of the product. Dissociation of the palladium (0) liberates the product, and regenerates active palladium catalyst [56-60].



Scheme 16

Allenic α-amino acids can be obtained by a three step sequence from the phosphates of α-allenic alcohols:

(i) Palladium catalysed alkylation of a Schiff base derived from glycine ester by the phosphate,

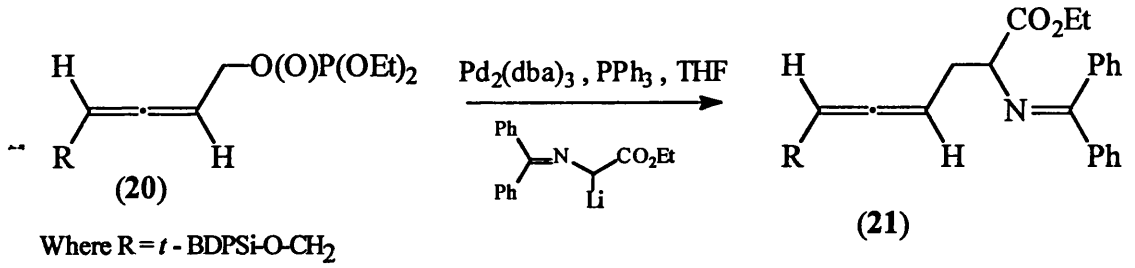
(ii) acidic hydrolysis of the imine functionality

(iii) saponification of the resulting amino ester.

As previously described, the phosphate (20) can be conveniently prepared from the mono-protected allenic alcohol (14), scheme 15, and it has been shown that similar phosphates react smoothly in THF at room temperature with the lithio-derivatives of benzylidene glycine esters. The reaction requires the presence of a 4% catalytic system obtained by addition of six molar equivalents of triphenylphosphine to the stable palladium-bis(dibenzylideneacetone) complex [$Pd(dba)_2$]. Under these conditions the starting material is completely consumed in two hours and the imino esters were obtained in acceptable yields[56].

The imine functionality can be easily removed by reaction with a dilute ethereal solution of hydrochloric acid to provide the amino ester after neutralisation. Treatment at room temperature of these amino esters with 1M methanolic solution of sodium hydroxide, followed by chromatography on an acidic column leads to pure γ-allenic α-amino acids with excellent yields. It appears that the allenic moiety is stable under the acidic or basic conditions used in the last two steps and that intramolecular attack by the amino or carboxylate groups did not occur[56].

Thus, this palladium-catalysed process appears to be a good way for the preparation of diversely substituted γ-allenic α-amino esters and acids from the readily available α-allenic alcohols, and so it was tried upon our substrate (20), as shown in scheme 17.



Where R = *t* - BDPSi-O-CH_2

Scheme 17

The data collected on the product shows that the reaction was a relative success. In the i.r. spectrum signals corresponding to the allenic system and the Si-O-C group are still present, but the phosphate peaks had disappeared. In addition to these, there are strong absorptions at 1730 cm^{-1} (-CO-O-) and 1660 cm^{-1} (C=N). In the ^{1}H-n.m.r. spectrum the two allenic proton resonances are now in different environments to that of the starting material giving rise to two multiplets at δ 5.0 and δ 5.2 ppm.

The silyloxy-methylene resonance now appear as a triplet at δ 4.33 ppm. The quartet for the OCH_2 signals of the ester and the multiplet for the α-proton of the amino acid group overlap to give a complex signal at δ 4.15 ppm. The methylene protons that were adjacent to the phosphate are now no longer so deshielded and they resonate as a double triplet at δ 2.6 ppm. The ^{13}C-n.m.r. spectrum still shows a peak at δ 205 ppm for the central carbon of the allene unit and now also shows signals at δ 196 ppm (-CO-O-) and δ 171 ppm (C=N).

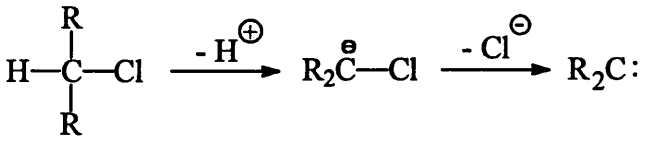
Unfortunately, the best yield obtained for this reaction was only 15%, and changing the catalyst, solvent, or reaction conditions did not raise this yield. This, in conjunction with the poor yield for obtaining the allenic diol, made this route unfeasible as many more steps are still necessary in our proposed synthesis.

It was at this stage that we began to look for alternative routes. One of the main reasons for the failure, or low yield, of the alkylation steps seemed to be the instability of the allene. With this in mind we considered methods that would put the amino acid components into place first and then construct the allenic unit afterwards. Two alternative methods involving either a carbene or an acetylene are discussed below. Of the two alternatives the carbene route looked the most promising as it was simpler, cheaper, and involved the least number of steps. It was thus attempted first.

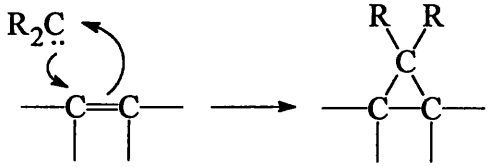
Method 2 - The carbene method :

The conversion of an alkene to an allene through dibromocyclopropane and carbene intermediates, first used in the early 1950's, is probably one of the best general methods for introducing the allene group into an organic molecule [5,62]. An alkene is converted into the allene by what amounts to the insertion of a carbon atom between the two carbon atoms of the double bond. This is achieved in two stages:

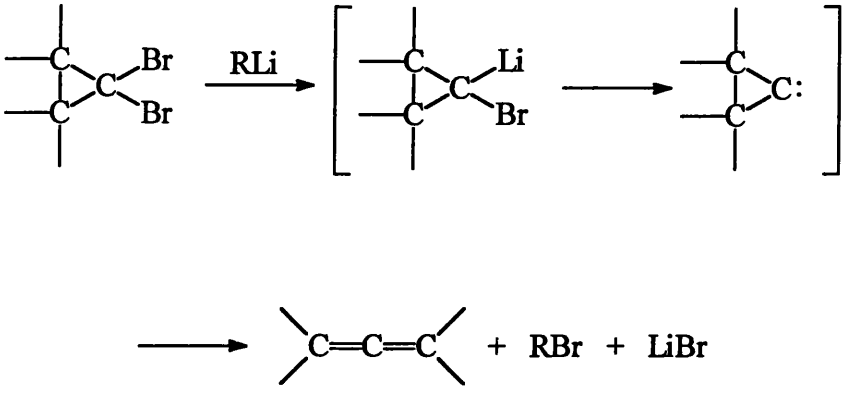
1. Addition of a carbene to an olefin to give a cyclopropane derivative. The carbene is normally a dihalocarbene as halocarbenes are more stable and some side reactions are avoided [5,61,62]. The carbene is usually generated by an α-elimination reaction in which a carbon atom looses a group without its electron pair, usually a proton, then a group with its electron pair, usually a halogen, as shown below [5,61,62].



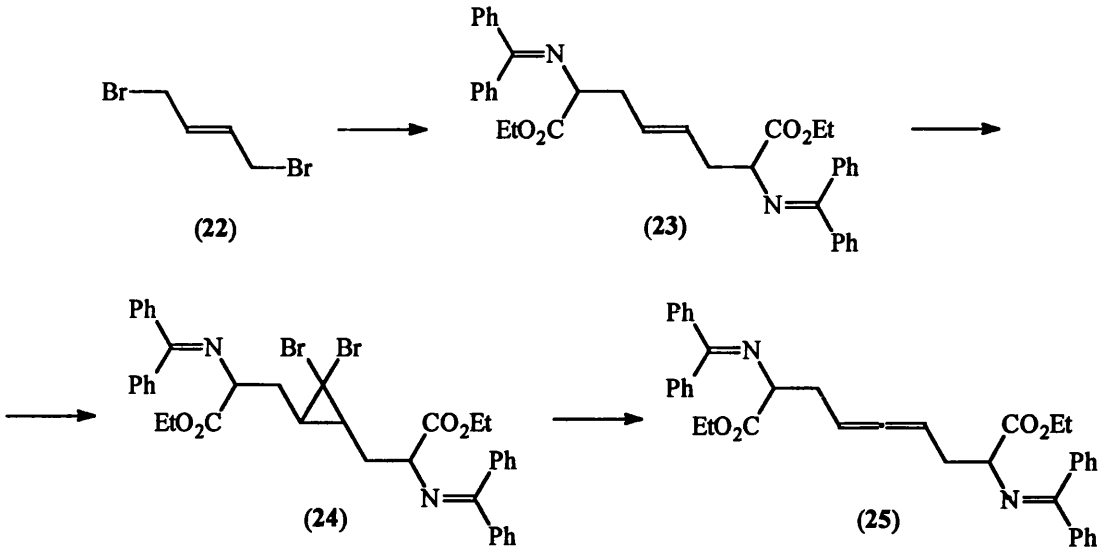
The singlet carbene adds to the double bond via a concerted cycloaddition reaction, and carbenes are so reactive that the reaction is practically simultaneous.



2. The second stage involves the collapse of the cyclopropane to the allene. It was found that the treatment of certain gem-dibromocyclopropanes with either magnesium or sodium resulted in allene formation[62], but the yields were poor (16-35%). Later it was discovered that the yields could be greatly increased, up to 90%, by the treatment of the cyclopropane with alkyllithium reagents[62].



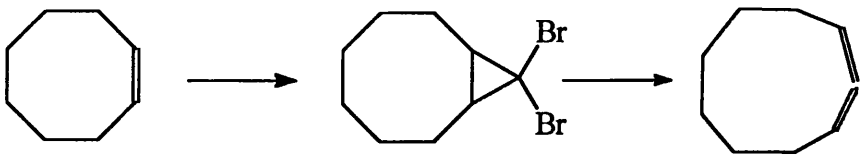
We thus require the allyl bis-amino acid (23), shown in scheme 18. It was decided that the best starting material was the dibromoalkene (22) and that the benzophenone glycine template be used again as the amino acid template to avoid waste of expensive stereoselective reagents. The palladium catalysed reaction between trans-1,4-dibromo-2-butene (22) and the glycine benzophenone imino ester (18) was carried out using the procedure laid out by Nájera and Pedregal[48], scheme 18, except substituting LDA for lithium hexamethyldisilazide (LHMDS). This afforded the masked *bis*-amino acid (23) in good yield. The compound was purified by column chromatography.



Scheme 18

In the i.r spectrum there are strong peaks at 1730 cm^{-1} (-CO-O-) and 1660 cm^{-1} (C=N), the alkene absorption was not evident as would be expected for a symmetric molecule. In the ^{1}H-n.m.r. spectrum the signals of the methylene protons adjacent to the double bond have shifted to δ 2.55 ppm and become a multiplet. In addition the resonances expected for the ethyl protons of the esters are evident as are those for the phenyl groups. Furthermore, a F.A.B. accurate mass spectrum shows an intense $(M+1)^+$ peak at 587, in accord with the required molecular formula: $C_{38}H_{38}N_2O_4$.

There are a number of methods for the formation of dibromocarbene, most involving some form of base and a halide. Early work on the conversion of olefins to allenes was carried out using the conversion of cyclooctene (26) to 1,2-cyclononadiene (28), scheme 19 [62-65].



(26) (27) (28)

Scheme 19

Originally the reaction was carried out over two steps, with the carbene being generated from bromoform using either sodium hydroxide or potassium tert-butoxide, followed by treatment of the cyclopropano derivative with either methyl- or butyl-lithium. Greater overall yield was later achieved by carrying out the synthesis in a one-pot reaction by treating a fourfold excess of cyclooctene with one equivalent of carbon tetrabromide and two equivalents of methyl lithium[64].

The use of alkyl-lithium reagents in dibromocarbene formation or dehalogenation caused us concern over the stability of (23) under such conditions. It is possible that the ester or imine functionalities might be attacked by the alkyllithium reagents, so samples of (23) in THF were treated with methyl- and butyl-lithium at room temperature and stirred for five hours. After work-up it was found that no reaction had taken place and starting material was recovered unaffected.

It was decided to carryout the reaction initially using the two step methodology so that we could isolate and characterise the cyclopropano derivative (24). We also decided to establish the reaction conditions with cyclooctene first, as a standard to prove to ourselves that we were carrying out the procedures correctly. Two different methods of carbene generation were chosen and the formation of the dibromocyclo -propane (24) was attempted using both of these:

(a) A solution of cyclooctene and bromoform was treated dropwise with n-butyl lithium at -78°C under a nitrogen atmosphere. The yield of (27) was 60%.

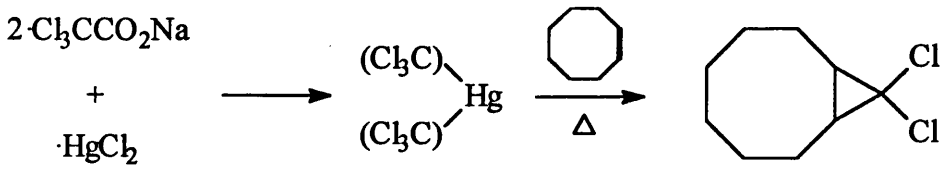
(b) A solution of cyclooctene and bromoform in DCM was treated with aqueous 50% sodium hydroxide solution in the presence of benzyltriethylammonium chloride, a phase-transfer reagent. Compound (27) was isolated in 42% yield.

However, the success of these two methods with cyclooctene did not carry through to the reactions with alkene (23). In both cases no reaction involving the alkene (23) took place and starting material was recovered almost quantitatively.

Intent on forming the allene via the cyclopropano derivative of (23) it was decided to attempt the one-pot olefin to allene conversion following the procedure laid out by Untch, Martin, and Castellucci [64]. The reaction was again carried out using cyclooctene first as a model. A solution of cyclooctene and carbon tetrabromide in diethyl ether, at -78°C under nitrogen atmosphere, was treated with 1.0 N methyl lithium (one equivalent) and the reaction mixture stirred for 30 minutes. The dibromocyclopropano derivative was not isolated but the reaction mixture was maintained at -60°C and another 1.1 equivalents of ethereal methyllithium was added. The solution was stirred for a further 30 minutes before being allowed to warm to room temperature. Work-up gave an oil which exhibited an unresolved n.m.r. spectrum. This was, however shown by infra-red and boiling point to be 1,2-cyclononadiene (28), isolated in 57% yield. Unfortunately, in the 'real experiment' replacing cyclooctene with our alkene (23) and following the reaction conditions exactly as above led only to an almost quantitative recovery of starting alkene.

Since we know that the carbene is being formed from our model reactions with cyclooctene we can only assume that the alkene (23) is unreactive. A possible reason for this failure is that the base used to form the carbene is deprotonating the amino acid α-proton and not attacking the bromoform or carbontetrabromide. It was thought that this was unlikely since the pKa of the bromoform proton is 11.8, while that for the amino acid is about 24 [66].

In our hands adding further equivalents of base had no effect. However, in an attempt to confirm whether or not this was the problem the bromoform / methyl-lithium reaction with (23) was carried out again, but replacing the aqueous work-up with a deuterium oxide work-up. In this way we could detect the incorporation of deuterium into the starting material should deprotonation occur. However, no deuterium was detected by N.M.R. spectroscopy. Nevertheless we attempted to generate dichlorocarbene under neutral conditions, again as a model reaction between sodium trichloroacetate and mercuric chloride and cyclooctene, as shown in scheme 20 [67,68].



(29) **(30)**

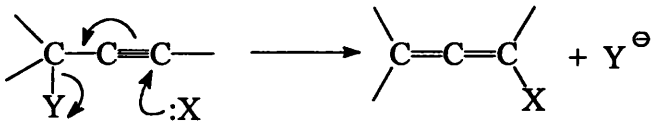
Scheme 20

Mercuric chloride and two equivalents of sodium trichloroacetate were heated in 1,2-dimethoxyethane for one hour. Work-up gave a clean white solid which was recrystallised from chloroform to give bis(trichloromethyl) mercury (29) in 51%. The conditions for the formation of the cyclopropane (30) involve heating the alkene with bis(trichloromethyl) mercury to around 200^{0}C [68], but since cyclooctene boils at 145°C we only heated the two reagents to c.a. 130°C. No cyclopropane product was detected and this method was abandoned without being tried on (23).

At this stage it was decided to abandon the carbene route altogether and start on the third alternative: the nucleophilic substitution of acetylenes.

Method 3 - The acetylene method :

This alternative route utilises the nucleophilic substitution of acetylenes of the general form shown below in scheme 21. It is by far the most versatile type of reaction for the formation of allenes, and often enables the conversion of acetylenes to allenes to be carried out in excellent yields[5]. Most nucleophiles (X) and most of the well known leaving groups (Y) may be applied.



Scheme 21

We yet again require the use of a nucleophilic amino acid template, however, using either Schöllkopf's reagent or benzylidene derivatives would leave the resulting allene one carbon short between the allene unit and the amino acid terminus, and are both therefore inappropriate. We therefore require an alternative nucleophilic equivalent, and here we reverted to the use of the functionalised zinc reagents mentioned earlier in this report. The use of this alanine β-anion equivalent[40-43], shown in figure 6, should overcome the problem of homology.

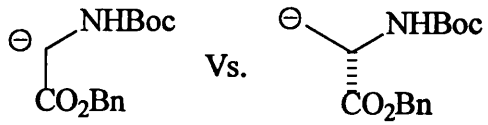
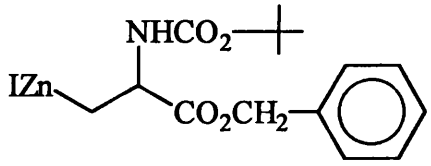


Figure 6

Jackson and his group have devised such a reagent, (31) shown in figure 7, and have used it in nucleophilic 1,3-acetylenic substitutions to give allenes[40-43].

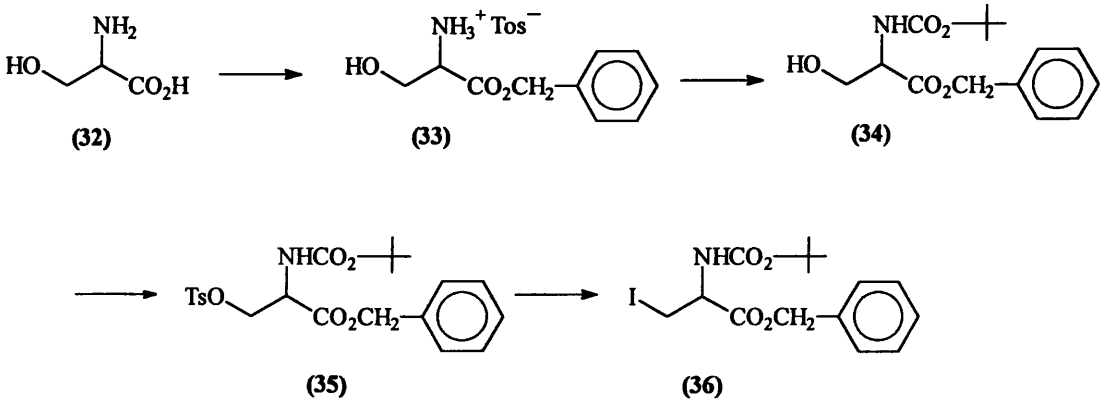


(31)

Figure 7

Normally this compound is activated prior to use by transmetallation since zinc reagents of this nature are relatively unreactive to most electrophiles due to the high covalent character of the carbon-zinc bond[69,70]. The empty low lying p-orbitals of zinc allow many transmetallation reactions with metallic salts to proceed with ease and this excellent transmetallation ability permits the conversion of organozinc reagents into a variety of new organometallic compounds[69].

It was decided to follow the methodology laid out by Jackson's group for the synthesis of (31) and use the reagent in attempts to construct our difunctionalised allene (1).The procedure for the formation of the iodide is shown below in scheme 22.



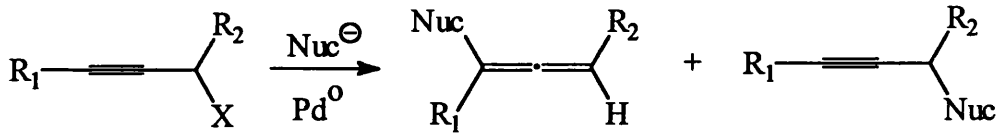
Scheme 22

The starting material L-serine required the protection of both its amino and acid functionalities. α-Carboxy protection was carried out by the 4-toluenesulphonic acid catalysed esterification with benzyl alcohol to give the benzyl ester, isolated as the 4-toluenesulphonate ester (33), in excellent yield. The free base was then generated *in situ* with sodium hydroxide during the α-amino protection step using Boc-anhydride to give (34). The free hydroxy-group was then activated by tosylation to give (35), which was treated with sodium iodide to give the iodide (36).

The organozinc reagent (31) was prepared by the direct insertion of zinc metal into the carbon-iodide bond of the protected iodo-serine compound (36). Direct metallation of an organic halide is known to be difficult, and transmetallation from organo-magnesium or organo-lithium precursors and a zinc halide is usually preferred. However, if zinc dust is activated with 1,2-dibromoethane and trimethylsilyl chloride prior to the addition of the organo-halide, then fast reaction rates are observed[40,43,69]. Studies showed that the rate of zinc insertion depends strongly on the nature of the organic moiety, the halide, the reaction conditions (solvent, temperature, and concentration), and the method of zinc activation[69]. For primary iodides the zinc insertion is complete within 2-3 hours at about 40°C, while secondary iodides react even faster, and a complete conversion to the alkyl zinc iodide is usually observed within an hour at 25°C. The zinc insertion shows remarkable functional group tolerance and most common organic functional groups can be present during the organozinc formation. Relatively acidic protons are tolerated during the preparation of organozinc halides, in strong contrast to the preparation of organo-lithium and magnesium compounds, and remarkably reagent (31) does not show any tendency to undergo β-elimination[69].

Palladium Catalysis :

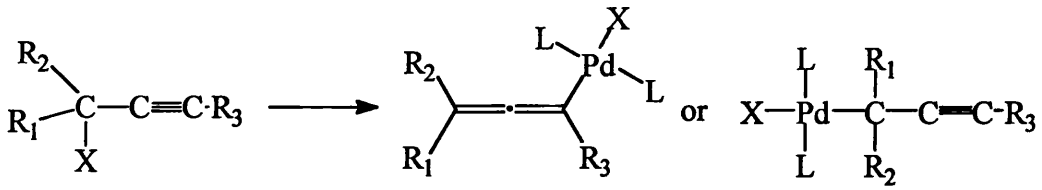
It is well known that various allylic compounds undergo facile palladium catalysed transformations via π-allylpalladium complexes and that nucleophilic attack on these complexes results in newly substituted alkenes[56-58,60,72]. In addition to allylic compounds nucleophilic substitution of propargylic substrates with a palladium catalyst, usually a versatile palladium (0) compound such as $Pd(Ph_3P)_4$, is also possible giving rise to substituted allenes or alkynes, as seen in scheme 23 [58,60,72].



Scheme 23

The chemoselectivity is dependent on the structure of the propargylic compound. The allene formation can be carried out most satisfactorily with a terminal alkyne bond while alkynes with an internal triple bond, or when R_1 is a bulky group, are usually converted to substituted alkynes[40,43,69].

All known palladium catalysed reactions of alkynes are explained by the formation of either a σ-allenyl palladium (II) complex (37) or a σ-propargylpalladium (II) complex (38), resulting from the oxidative addition of palladium (0) to the propargylic substrate. Spectroscopic and microanalytical data provide good evidence for a structure in which an allenyl prop-2-ynylic moiety is σ-bound to palladium[72].



(37) (38)

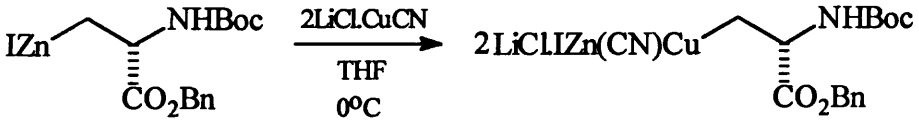
Scheme 24

When the leaving group (X) is a halide and R_3 is hydrogen the reaction gives pure trans-(σ-allenyl) bis (triphenylphosphine) palladium (II) halides (37). The acetylenic isomers (38) are obtained when R_3 is a bulky group such as t-Bu or TMS , and when R_1 and R_2 are both hydrogen. The relative sizes of the R_{1-3} groups is important in deciding the regiochemistry[72].

Leaving groups other than a halide can also be used to prepare the palladium (II) complexes, but salts like zinc chloride or lithium chloride must be present to allow the conversion to proceed[69]. Without these salts no reaction takes place. In the case where the leaving group (X) is an acetate group research showed that no acetate group was present in the σ-allenyl palladium complex[72]. Instead of the acetate group chlorine was present, being delivered by the zinc or lithium salt. Addition of a nucleophile displaces the leaving group (X) from the palladium complex which then undergoes reductive elimination to yield the newly substituted product. It is worth noting that the allene itself can also form complexes with palladium (0). One could imagine that through such complexes racemisation could occur. In some separate experiments it was found that optically active 1,3-diphenylpropadiene suffers from substantial racemisation when treated with 10 mol% of $Pd[Ph_3P]_4$ [69]. It is therefore highly possible that the racemisation of optically active allenes could take place under these reaction conditions.

Copper Catalysis :

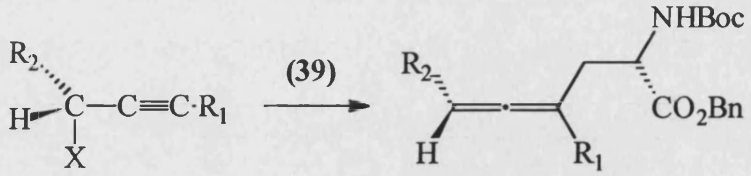
Apart from the use of palladium as the second metal catalyst copper was also considered involving the formation of the zinc / copper reagent (39)[40-43,69], scheme 25. The most efficient method of forming the zinc / copper couple is by an *in situ* reaction by the addition of the THF soluble copper (I) salt CuCN.2LiCl. The reaction of this salt with organozinc iodides is carried out at 0°C and takes about ten minutes. The use of lithium chloride to solubilise copper cyanide was found to facilitate the transmetallation allowing the use of milder conditions[40-43,69].



(31) **(39)**

Scheme 25

Investigations show that these zinc / copper reagents follow the same mechanism of action as that for organocuprates[40,69]. The reaction sequence with propynyl halides and esters is analogous to the known mechanism for the palladium-catalysed alkylation. This type of catalytic reaction also shows high levels of 1,3-substitution. The reaction between the zinc/copper reagent (39) and chiral propargylic derivatives leads to the formation of optically active allenes, with overall anti 1,3 displacement.



In all cases reported so far, organocopper (I) mediated S_N2' reactions with alkynes proceed with anti stereochemistry [69,71]. It is thought that a π-complex between the organocopper (I) fragment and the acetylenic compound occurs first; examples of isolated $Cu^{(I)}$-acetylene complexes are known. Subsequent elimination of the leaving group will take place only when the copper and leaving group moieties are in a mutual antiperiplanar disposition[69,71], figure 8.

In this geometry not only is the interaction of the relevant copper (I) p and d orbitals with the acetylenic π and π^* systems important, but there is also additional donation from the copper $3d_{xz}$ orbital into the carbon-leaving group antibonding σ^* orbital. It is this latter interaction that electronically initiates the decoupling of the leaving group. The directional specificity of this process guarantees that the 1,3-substitution occurs with a very high level of anti stereoselectivity. As a result the unstable σ-allenyl-copper (III) series is generated, which readily collapses by means of reductive elimination of CuCN to give the allene. This latter step is assumed to proceed with complete retention of configuration as is usually observed for such alkyl shifts from transition metal centres. Occurrence of the transient Cu(III) intermediate has been corroborated in the past[69].

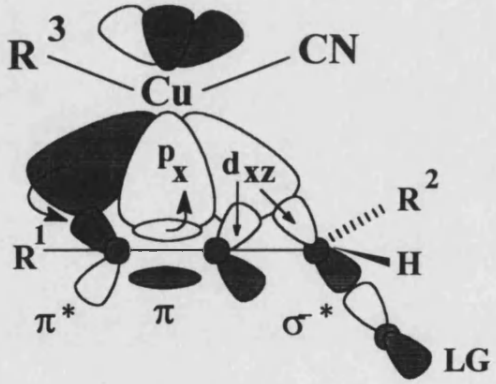
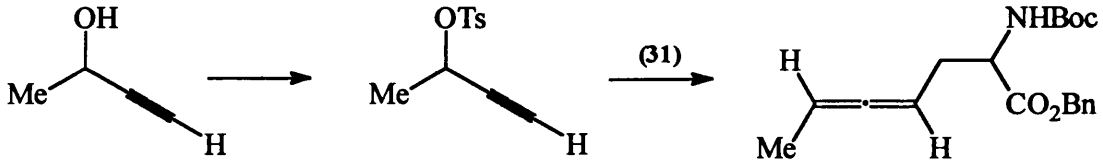


Figure 8

The choice of organometallic, solvent, leaving group and substrate, are all very important factors affecting the enantiomeric purity of the allenes formed. Interestingly there seems to be less, or even no, problems regarding racemisation when the solvent is diethyl ether, whereas with THF fast racemisation of allenes by excess organocuprates has been reported[69]. It was found that higher yields were obtained when reaction time was kept as short as possible, and when better leaving groups where employed. The leaving group has a decisive influence on the stereochemistry of the 1,3-substitution in propargylic systems[69]. It is possible for the reaction mechanism to change with different leaving groups. Studies showed that the best leaving groups were either the methyl- or toluene-sulphonate esters, which reacted in the anti mode at all temperatures[69].

Jackson's group investigated the reaction of the zinc reagent (31) under both palladium and copper catalysed conditions with tosylated 3-butyn-2-ol (41) to give allene (42) [40,43], as shown is scheme 26.



(40) (41) (42)

Scheme 26

We decided to carry out this reaction running the palladium and the copper catalysed reactions side by side as (41) is similar to the terminus of the propargylic substrate that we would use in our coupling reaction, and it would be a good indication of whether or not we were carrying out this methodology correctly. Racemic 3-butyn-2-ol (40) was tosylated in dry pyridine, the product was isolated in excellent yield, and then purified by crystallisation from ethanol.

A suspension of zinc in dry THF was activated with 1,2-dibromoethane and TMS-chloride. The suspension was then treated with a solution of the iodide (36) in THF, followed by a solution of (41) and tetrakis(triphenylphosphine)palladium in THF. After work-up no allenic product could be detected, although the starting material (36) was destroyed.

The copper catalysed reaction involving the *in situ* formation of the zinc / copper couple (39) fared much better. The zinc organometallic was prepared in the same way as for the palladium catalysed reaction, and then treated with a solution of copper cyanide and two equivalents of lithium chloride in THF. The solution was then cooled and treated with the tosylated 3-butyn-2-ol (41). Work-up gave the allenic product (42) as a colourless oil in very good yield. The presence of the allene was confirmed by infra-red and N.M.R. spectroscopy.

Now that we had an established method for the coupling of an alanine β-anion equivalent to an acetylene, we needed to construct the amino acid / acetylene substrate itself. The substrate needs to be of the type shown in figure 9 with the leaving group in the 3-position.

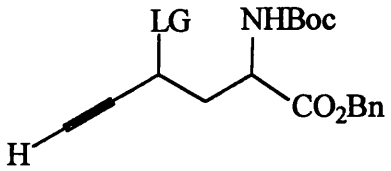
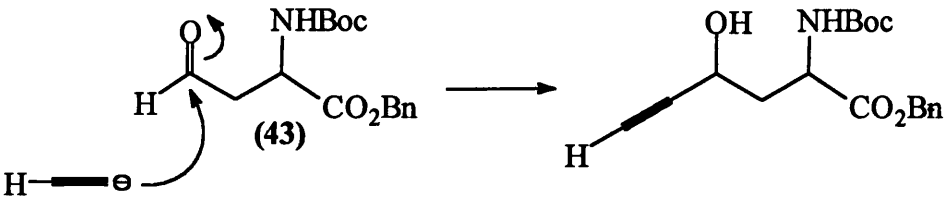


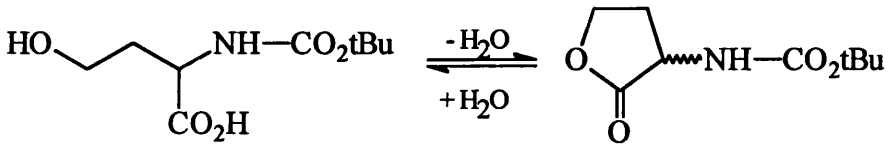
Figure 9

It was decided that this could be most effectively synthesised by the nucleophilic addition of a propargylic anion to the aldehyde (43), aspartic acid β-semialdehyde, as shown in scheme 27. The hydroxyl group could then be converted into a good leaving group, for example a tosylate.



Scheme 27

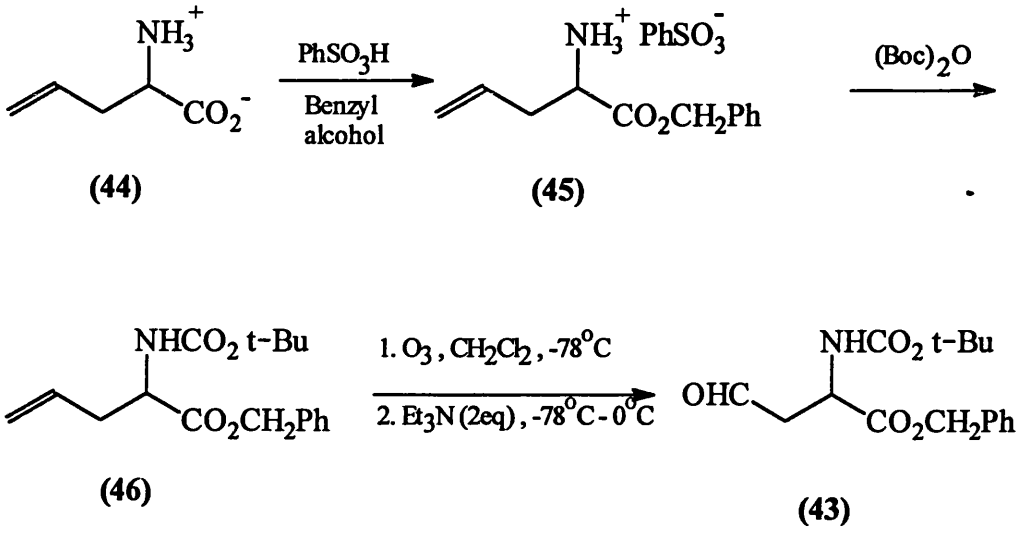
The aldehyde (43) had already been synthesised at Zeneca Agrochemicals where it was an intermediate in the study of DHDP synthase[73]. At the company the initial strategy was the oxidation of protected DL-homoserine to the aldehyde. However, it was found that homoserine readily cyclised under the reaction conditions for amino-protection and that yields were very low due to the formation of the lactone, scheme 28.



Scheme 28

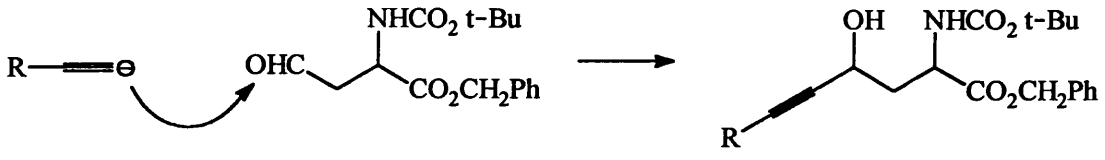
Eventually the company abandoned this method and used a new route starting from allylglycine (44) instead, scheme 29. The starting material was protected in two steps to give N-t-butoxycarbonyl DL-allylglycine benzyl ester (46).

We decided to work out the initial strategy using the cheaper racemic starting material, which could be replaced with chiral allylglycine to give enantiomerically pure product once the methodology had been worked out.



Scheme 29

The doubly protected allylic compound was then subjected to ozonolysis[74,75] at -78 °C in DCM to give the required aldehyde (43) in 50% yield. With the aldehyde available nucleophilic addition with an acetylene unit to give the propargylic amino acid needed to be attempted, scheme 30.



Scheme 30

It is worth noting that during this reaction another chiral centre is created. It should be possible to separate the diastereomers and investigate the difference in reactivity between the two in the 1,3-nucleophilic substitution reaction with the zinc cuprate (31).

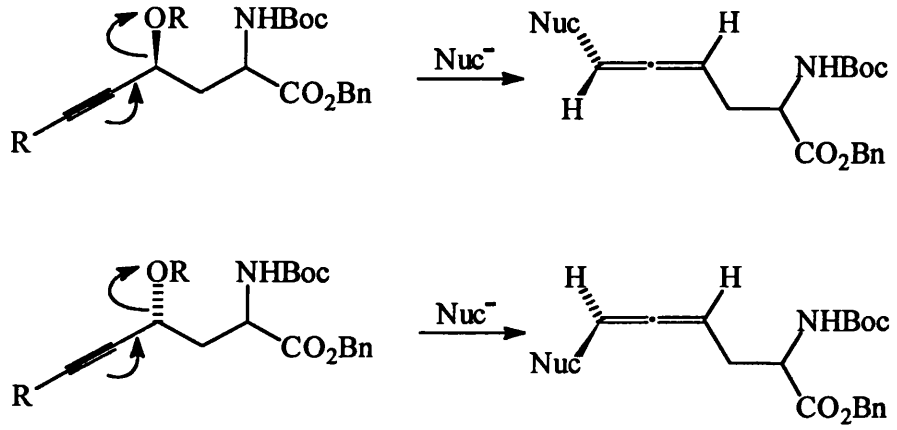
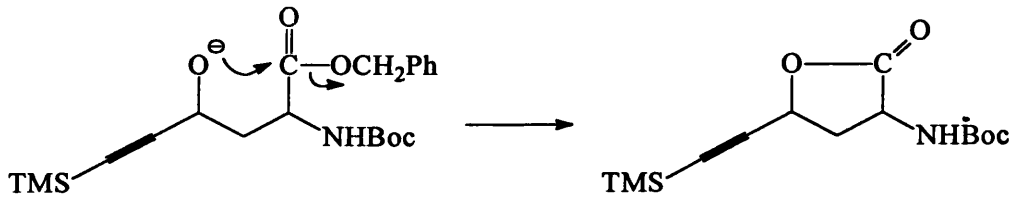


Fig. 10

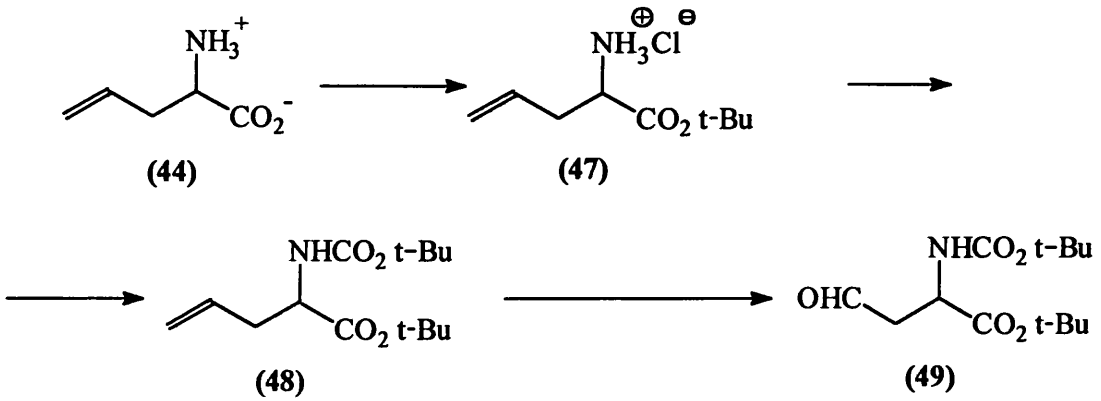
The reaction of Grignard and organolithium reagents with carbonyl compounds to yield alcohols is one of the most fundamental and versatile reactions in organic chemistry and has wide-spread synthetic applications. After consultation with colleagues at Zeneca, who had experience with reactions related to ours, it was decided to attempt the coupling reaction with lithium TMS-acetylide. Ethynyltrimethyl silane was treated with butyllithium at -78°C in THF, under nitrogen atmosphere, after stirring for an hour the lithiated compound was added dropwise to a solution of aldehyde (43) in THF, also at -78°C under nitrogen. Despite several attempts at this reaction no product was detected and only benzyl alcohol was obtained in the pure state. In addition a gummy material was also formed during the reaction but this proved extremely intractable and its i.r. spectrum revealed no discernible functional groups.

A possible problem in this attempt is that the free hydroxyl group formed in the reaction is deprotonated under the basic conditions and caused lactonisation as shown in scheme 31. If this is the case, however, the lactone must undergo further reaction since neither it or any derivatives were isolated.



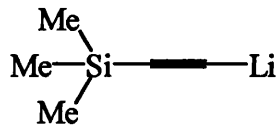
Scheme 31

It was hoped that this problem might be overcome by replacing the relatively labile benzyl protecting group with a ^{t}Bu unit, scheme 32. Boc protection was carried out by bubbling 2-methylpropene gas through a stirred solution of (±)-allylglycine in DCM in the presence of sulphuric acid at sub-zero temperatures. The tert-butyl ester hydrochloride (47) was isolated as a clean white solid in good yield.

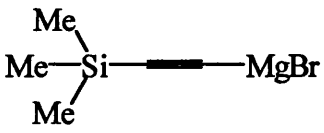


Scheme 32

The reaction with the acetylide was carried out again using the Boc/^{t}Bu-ester protected aldehyde (49) using the same conditions but again none of the desired product was isolated. Only an intractable gum was produced. In another attempt a solution of (49) in THF was treated with an equivalent of lithium (TMS)acetylide (50). Once more this failed, as did a similar reaction with (trimethylsilyl)ethynyl magnesium bromide (51). The coupling was also attempted using stabilised lithium acetylide (52) but with the same result.



(50)

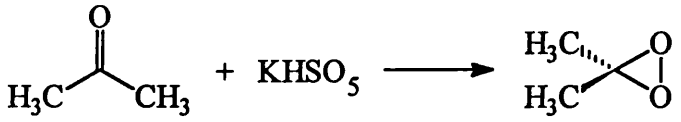


(51)

(52)

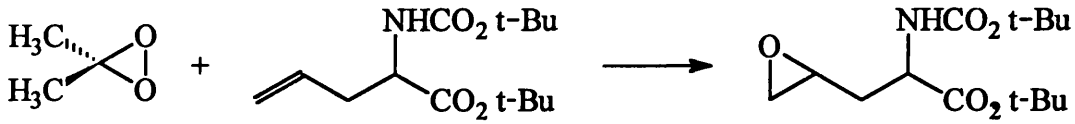
Another reason for the failure might be that the basic conditions effected α-deprotonation of the aldehyde causing aldolisation and hence the inextractable gum. To avoid this we decided to synthesise the epoxide (54) from the doubly protected allyl glycine and to attack this compound with lithium acetylide.

Two reagents were individually used to form the epoxide, m-CPBA[76] and dimethyldioxirane (DMDO)[77-82] (53). The DMDO method was attempted first. This reagent is formed by the reaction between acetone and potassium monoperoxy -sulphonate (caroate), as shown in scheme 33. The yield of DMDO is very low (around 5%), but the advantage is that the only side product of the epoxidation is acetone, making it easy to purify the product, and in our hands the yield of epoxide (54) was high (84%). The epoxidation was also attempted using m-CPBA and, although there was no problem with the BOC-protecting groups, the crude product needed purifying by column chromatography and the yield of epoxide was relatively poor (53%).



(53)

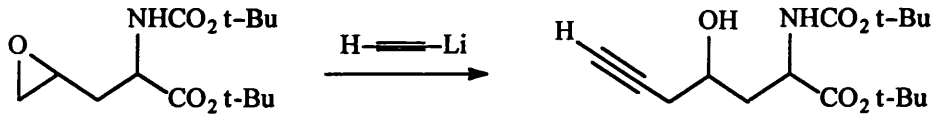
Scheme 33



(48) (54)

Scheme 34

The epoxide was then treated with lithium acetylide in the anticipation that it would afford the alcohol (55), scheme 35. Unfortunately this did not occur, but none of the intractable gum was produced.

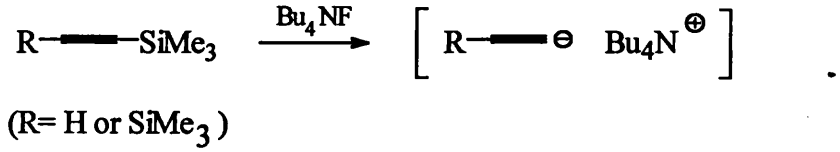


(54) (55)

Scheme 35

This result substantiated our view that the earlier failures were largely due to enolisation of the aldehyde and other attempts were made to overcome this problem. One method for avoiding strongly acidic or basic conditions is the generation of acetylides from trimethylsilylacetylenes using a catalytic amount of tetrabutylammonium fluoride, scheme 36 [83].

The acetylides produced are less reactive than the corresponding lithium species and have been reported to react well with carbonyl compounds to give hydroxy acetylenes in good yields. This fluoride catalysed addition of silylacetylenes to carbonyl compounds was attempted using TMS-acetylene and bis(TMS)acetylene, but the lower basicity of the reagent had little affect and frustratingly a gum formed.



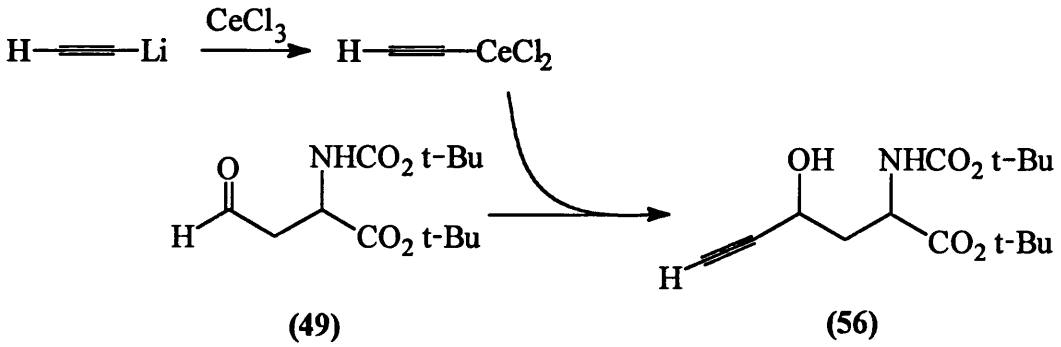
Scheme 36

An alternative and one of the best ways of avoiding unwanted side reactions in carbonyl addition reactions is the use of lanthanides[84-89]. Lanthanide reagents are becoming increasingly important in organic synthesis and many of their applications deal with trivalent lanthanides which are the most common.

Anhydrous cerium chloride is most often the reagent of choice as it is readily obtained by the dehydration of its heptahydrate, it is relatively inexpensive, and shows low toxicity. It has been observed that organocerium (III) reagents, generated by the *in situ* transmetallation from organolithium or Grignard reagents, undergo efficient 1,2-addition to carbonyl groups with remarkable suppression of abnormal reactions such as enolisation, aldol condensation, reduction, and conjugate addition in the case of α,β-unsaturated substrates[84-89]. These characteristic reactivities of organocerium reagents are mainly ascribed to attenuated basicity and high oxophilicity. Trivalent cerium shows a strong affinity for oxygen atoms and is capable of activating carbonyl groups by co-ordination.

This activation may be the most important driving force for cerium chloride promoted carbonyl additions, the other important factor being the low basicity of organocerium reagents compared to their organolithium and Grignard counterparts.

Little is known of the structure of these organoceriums, or the exact nature of the reactive species. Although they have been denoted as trivalent σ-bonded species ("$RCeCl_2$"), other compositions (e.g. $RM\text{-}CeCl_3$, "ate" complexes) cannot be ruled out[84]. Regardless of the true nature of the reactive nucleophiles, they react efficiently with aldehydes and ketones, providing alcohols in yields that are often superior to those reported utilising organolithium or Grignard reagents[84-89]. In fact, some reactions not involving cerium chloride afforded no trace of addition products, while those utilising organo cerium compounds showed yields as high as 95% [84,86]. It was thus decided to form the cerium (III) chloride derivative of lithium acetylide and use this in the coupling reaction with aldehyde (49), scheme 37.



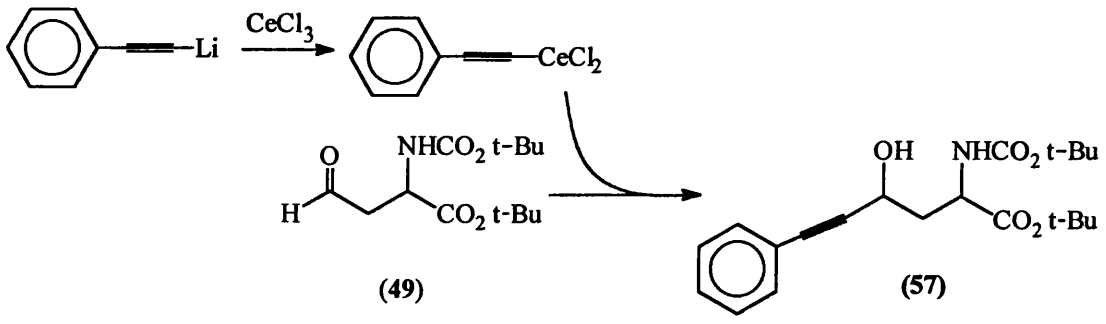
(49) (56)

Scheme 37

Cerium (III) chloride was finely ground and heated *in vacuo* to around 140°C for two hours and protected by a nitrogen atmosphere. The solid was then cooled in an ice bath and dry THF was then added via a syringe and the flask placed in an ultrasonic bath at room temperature for 1 hour.

The resulting white slurry was cooled to -78°C and a solution of an ethylene diamine complex of lithium acetylide in dry THF was added dropwise. After stirring at -78°C for one hour a solution of aldehyde (49) in dry THF was added and the reaction mixture stirred for three hours, maintaining the low temperature. After work-up and several attempts at this reaction starting material was recovered unchanged. It is most likely that since cerium also shows a high affinity for nitrogen that it is binding to the ethylene diamine stabilising agent, possibly causing the degradation of the lithium acetylide in the process, and so preventing reaction. Sodium acetylide was then used, which requires no stabilising agent, but only a resinous gum was obtained from the reaction mixture, suggesting that transmetallation with the cerium chloride was not taking place, while enolisation was. We also tried the reaction with lithium (trimethylsilyl)acetylide but none of the desired product could be isolated.

We then carried out the reaction using lithium phenylacetylide in place of lithium acetylide, which has no stabilising reagent, to make sure that it was not the preparation of the cerium suspension which was the problem, (scheme 38). The same reaction conditions were used as above and the product (57) was isolated in excellent yield, thus showing that the coupling reaction was being carried out correctly and that the problem resides with the nature of the acetylene reagent.



(49) (57)

Scheme 38

These disappointing results prompted us to attempt the coupling using yet another method. We have already seen, in the cases of the palladium and copper catalysed reactions, that transition metals mediate the formation of carbon-carbon α-bonds under much milder conditions than the simple lithium and Grignard organometallics. Organocopper reagents in particular have become popular among organic chemists. The widespread acceptance and use of organocopper reagents is largely attributable to their ease of preparation and their ability to effect transformations difficult or impossible to accomplish with other reagents [90-96]. However, while there is substantial literature on the addition of copper reagents to propargylic species[90-96], there is none on the transfer of acetylenes from copper. The effectiveness of copper-acetylene reagents is yet to be seen.

Organocopper complexes are intrinsically correlated with such major pathways for carbon-carbon bond formation as nucleophilic displacements of halides, sulphonates, and allylic acetates, epoxide ring openings, additions to acetylenes, and conjugate additions to α,β-unsaturated carbonyl compounds[90-92,96]. It has been shown that for conjugate additions there is competition between 1,2-addition and 1,4-addition. Some organocuprates favour one route over the other, and in the cases of non- α,β-unsaturated carbonyl compounds, organocuprates do undergo efficient 1,2-addition[95].

Mono-organocopper compounds (RCu), prepared from organic halides and activated copper metal, are thermally very unstable and decompose readily at temperatures above -15°C [90-92].

Polynuclear structures have been suggested for most of these types of compound, which tends to make them insoluble in conventional organic solvents[90]. They are also considerably less reactive than the corresponding lower order cuprate species (R_2CuLi / R_2CuMgX), often referred to as "Gilman" type reagents[90-96]. These homocuprate reagents are the most widely used of the organocopper reagents, showing much greater reactivity than the mono-organocopper compounds. These lower order organocuprates are usually prepared by a transmetallation reaction between the corresponding organolithium or Grignard reagents and a copper (I) salt. The preparation is easy and the reactions of the resulting reagents are usually characterised by high chemo-, regio-, and stereoselectivity. However, one of the major limitations of most lower order cuprates concerns their thermal stability. While more stable than the mono-organic species they still show a tendency to breakdown[90,92,96].

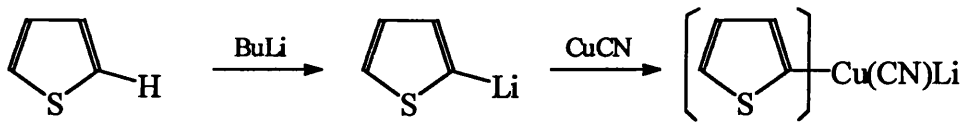
The stability of these reagents can be greatly improved by the formation of lower order heterocuprates (RCu(Z)Li), where enhanced thermal stability is imparted by the presence of a donor molecule, usually a cyano-group, which is incorporated into the molecule itself[90-96]. These lower order heterocuprates are formed using copper cyanide which is the best precursor[97], whereas copper (I) halides often undergo incomplete cuprate formation, leaving free organolithium reagents in solution, something we desperately want to avoid. CuCN is also inexpensive, nonhygroscopic and light insensitive. Another advantage is that only one equivalent of a potentially valuable organolithium is required for the formation of an active reagent[92-95,97].

These mixed ligand lower order cyanocuprates, however, have the disadvantage of being considerably less reactive than the diorgano-homocuprates, or Gilman reagents, prompting chemists to investigate the synthetic potential of higher order, mixed ligand cyanocuprate reagents[92-95]. Higher order cyanocuprates, stoichiometrically represented as "$R_2Cu(CN)Li_2$", are distinctly different from the Gilman reagents by virtue of two distinguishing features:

1. They are believed to be copper (I) dianionic species, implying that three ligands are bound to the copper (I) centre.
2. They contain a cyano-group, which presumably imparts the required π-acidity to the lower order cyanocuprate, RCu(CN)Li, so that another equivalent of an organolithium can be accepted.

It was originally believed that higher order cuprates of the form "R_3CuLi_2" also existed but research has shown that addition of three molar equivalents of an organolithium to a copper (I) halide affords only R_2CuLi and free RLi, rather than"R_3CuLi_2" [90,93-95]. The term higher order cuprates has therefore now come to imply the mixed ligand cyanocuprates. These higher order heterocuprates have proved to be extremely versatile synthetic reagents. They are formed easily, and combine the increased stability of lower order heterocuprates with the reactivity of the Gilman type reagents. In fact, in some cases higher order cyanocuprates show greater reactivity relative to that of the Gilman type reagents, perhaps because of the build-up of negative charge in the cluster (i.e. as dianions rather than as monoanions)[90,93-95]. It was due to this increased stability yet high reactivity that we opted for higher order cyanocuprates in our research

Higher order cyanocuprates containing only one transferable ligand have also been generated by incorporating a second dummy ligand in addition to the cyano-group[90,93-95]. A number of organic moieties have been used as the second residual ligand, including acetylenes themselves which was rather worrying. The ligand of choice though, is the thiophene group (58), shown in scheme 39 [90,93-95,98]. Thiophene (Th) itself is very cheap, and is essentially quantitatively metallated with strong base. Treatment with CuCN then forms the homogeneous, stable lower cuprate (2-Th)Cu(CN)Li (59).



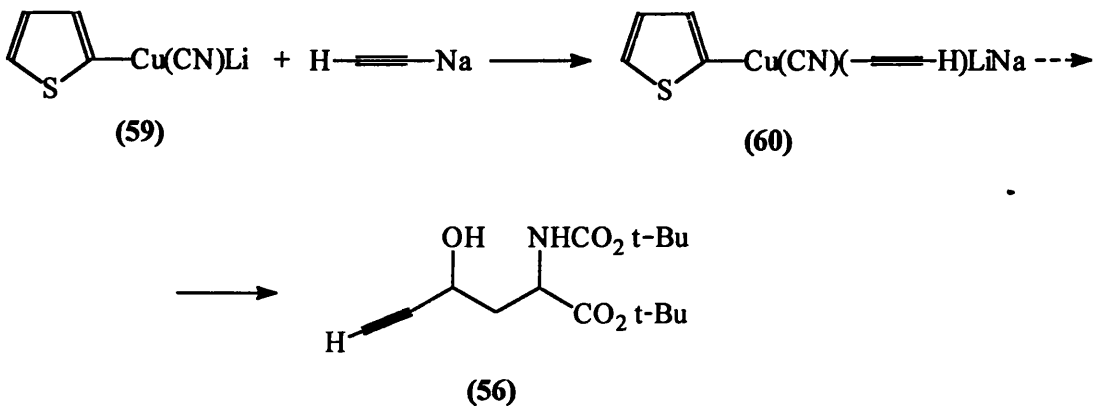
(58) **(59)**

Scheme 39

Subsequent introduction of the lithiated transferable ligand (R_TLi) immediately forms the higher order species, $R_T(2\text{-Th})Cu(CN)Li_2$, which selectively releases the R_T group[90,93-95,98]. The stable lower order thienyl-cyanocuprate is commercially available, allowing chemists to bypass the handling of the copper (I) salt.

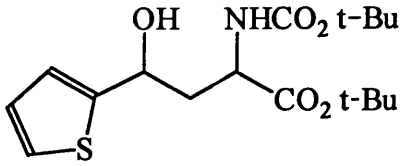
As previously mentioned, one of the distinguishing features between lower order and higher order cuprates is the mono- vs. dianionic lithium cations. It has been shown that the use of mixed metal reagents does not compromise the reactivity of the higher order reagent, so cuprates of the form $R_TR_RCu(CN)LiMgBr$ or $R_TR_RCu(CN)LiNa$ may also be used, thus enabling the use of Grignard or sodium metallated reagents in the formation of the higher order cuprate[95].

With this in mind we proposed to attempt the acetylene coupling using the mixed ligand, mixed metal higher order cyanocuprate (60), formed by the treatment of the lower order thienyl-cyanocuprate (59) with sodium acetylide, scheme 40.



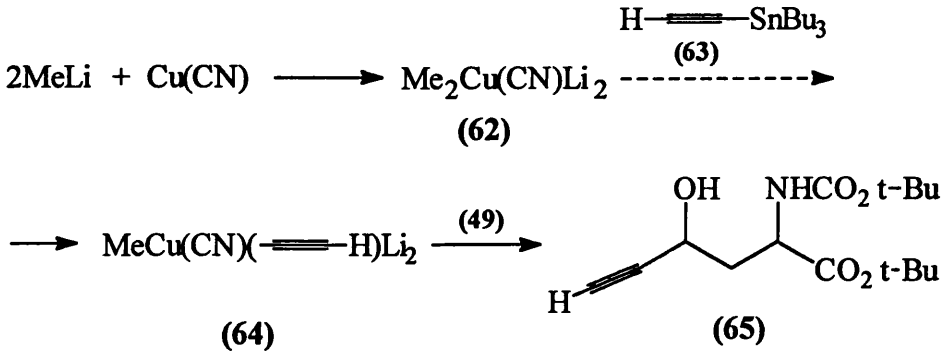
Scheme 40

A 0.25 molar solution of lithium-2-thienyl cyanocuprate (59) in THF, purchased from Aldrich, was treated with an 11% suspension of sodium acetylide in xylene, at -78°C under nitrogen atmosphere. After stirring the solution at -78°C for half an hour a solution of aldehyde (49) in THF was added and the reaction mixture stirred for two hours maintaining the low temperature. After work-up we discovered that the higher order cuprate formed during the reaction had selectively transferred its "non-transferrable" ligand forming (61), which was the sole isolated product. No product resulting from enolisation was detected, so we were certain that no free sodium acetylide was present in solution and the higher order derivative did form. We can only presume, therefore, that the acetylene ligand is too strongly bound to the copper centre.



(61)

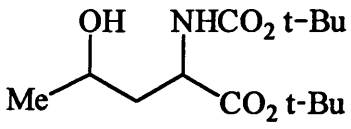
It was then decided to form another higher order cyanocuprate with a different residual ligand, and we opted for the methyl group which has been used with great success for such a purpose. We attempted the formation of this reagent by a transmetallation reaction between the higher order dimethyl species (64), formed by the reaction of two molar equivalents of methyl lithium, copper cyanide and tributyl(ethynyl)tin (63), scheme 41. This method of forming the higher order reagent with the correct R_T has been used for a number of alkenyl species and is was hoped that it would prove equally successful for transferring acetylenes[93,99].



Scheme 41

Copper cyanide in THF was treated with two equivalents of methyl lithium at -78°C under nitrogen, and the solution stirred for half an hour. The reaction mixture was allowed to warm to room temperature and then treated dropwise with tributyl(ethynyl) tin and stirred for one hour.

A colour change from colourless to a deep red was observed, pointing to formation of the acetylenic cuprate. The reaction mixture was cooled back down to -78°C and treated with a solution of aldehyde (49) in THF, then stirred for three hours, monitoring the reaction by tlc. Work-up and isolation of product showed that the reaction had not gone as expected and we speculate that methyl transfer had taken place to give (66), however the N.M.R. was ambiguous.

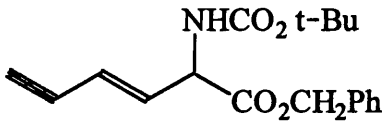


(66)

In several cases in the literature acetylenes are used as the non-transferable ligands in mixed ligand cuprate chemistry[93-95,100-102], and we assumed that the alkyne group is bound too tightly to the metal centre. It is the strength of this attachment that led us to abandon the use of copper altogether.

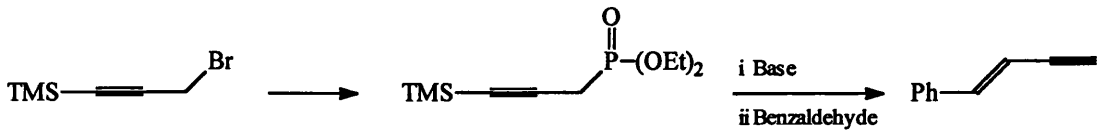
Method 4 - Allenes via terminal conjugated enynes :

Another important way of making allenes, and an alternative to the 1,3-substitution of acetylenes, is 1,4-addition to enynes[103-105]. Terminal conjugated enynes are important intermediates for the synthesis of naturally occurring allenes and have been utilised successfully in laboratory syntheses[103-105]. Consequently, we proposed to synthesise the terminal enyne (67) as an alternative to the activated derivative of acetylene (56).

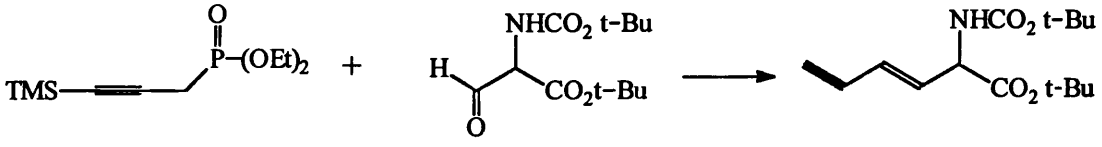


(67)

There are a number of methods for the preparation of terminal enynes but due to the shortage of time only a handful were looked into. It was decided initially to attempt an Emmons-Horner reaction which is often the preferred method. An example where this works well is the synthesis of 4-phenyl-3-buten-1-yne[105].



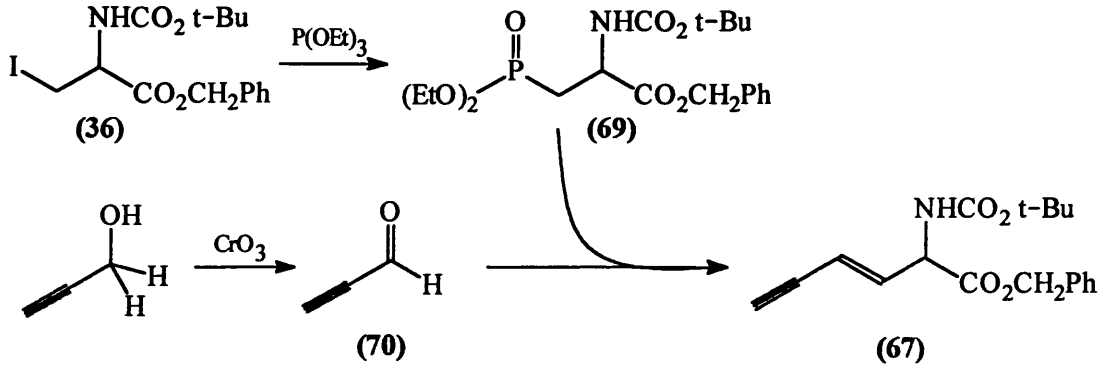
Whereas this method offers the advantages of increased stability of the phosphonate carbanion and water soluble phosphate by-products, it could not be used in the synthesis of enyne (67) as this would involve the aldehyde (68), (scheme 42), which seems to be unstable, as all previous attempts at its synthesis have failed.



(68)

Scheme 42

It was therefore decided to switch strategy and make the ylide of the amino acid component (69) and couple this with propynal (70), Scheme 43. The iodide (36) was made as earlier in scheme 22 where it was used in the synthesis of the alanine β-anion equivalent. It was then treated with triethylphosphite in THF and refluxed overnight. Work up gave the required phosphonate (69) as a white powder in excellent yield.



Scheme 43

Propynal (70) was synthesised by the Jones oxidation of propynol[108]. Unfortunately, the product was contaminated with ethyl methyl ketone, which is recommended as a co-solvent. The presence of this ketone was a nuisance and when a coupling reaction was attempted on the crude product we failed to detect the presence of the required compound (67) in the reaction mixture.

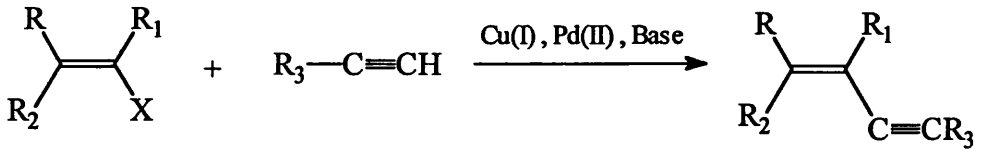
We also tried to find an alternative solvent for the preparation of the aldehyde (70) and we carried out preparative reactions using (a) nitrobenzene and (b) dimethyl sulphoxide, neither were successful.

CONCLUSION

Conclusion

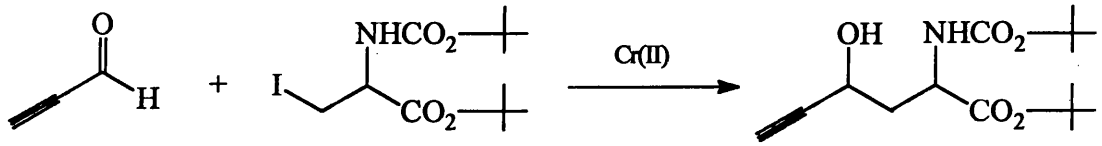
At this point laboratory work had to stop and yet the target compounds had eluded us. Much time has been used up following approaches to them which in our hands reached dead ends. However, the Emmons-Horner synthesis, despite the initial disappointment, still seems to have promise and work remains to be done on finding a suitable solvent which will allow the preparation of propynal (70) in good yield.

Another route to terminal enynes is that involving the Songashira Cu-Pd catalysed alkyne coupling reaction[109]. Under these conditions a terminal alkyne can be coupled with a vinyl halide as shown in scheme 44, although the synthesis of a suitable vinyl halide substrate would have to be looked into.



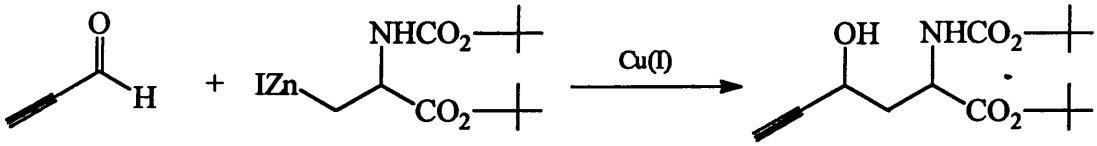
Scheme 44

Alternatively, propynal could be coupled with iodide (36) under chromium (II) conditions [110,111] to give the hydroxy-acetylene compound (56) discussed earlier, which could then be activated and treated with Jackson's organozinc reagent (31), scheme 45, or both the amino acid components could be added under copper catalysed conditions with the organozinc reagent (31), scheme 46.



(70) (36) (56)

Scheme 45



(70) (31) (56)

Scheme 46

Finally, if none of the above routes proved productive, it is felt that it would be worthwhile going back to the palladium catalysed alkylation discussed in the first earlier and shown in scheme 17. Although the reaction was low yielding it was still successful and could be carried out on a large enough scale to produce sufficient quantities of material for incorporation into peptide structures.

EXPERIMENTAL

Experimental

Solvents and Techniques

Petroleum ether (petrol) refers to petroleum spirit, b.p. range 60-80°C, and ether refers to diethyl ether. Reaction solvents were dried and distilled before use, as were solvents used for chromatography. Tetrahydrofuran (THF) was pre-dried over sodium wire, and then refluxed over sodium benzophenone ketyl until dry, and re-distilled prior to use. DCM and triethylamine (NEt_3) were dried by distillation from calcium hydride, and methanol (MeOH) and ethanol (EtOH) were distilled from magnesium turnings.

For reactions performed under dry nitrogen, the glassware and apparatus were dried overnight in an oven at 125°C, then allowed to cool in a dessicator. Flasks were sealed with rubber septa and flame dried before use. All reactants were transferred with syringes and needles.

All yields quoted are of purified products, unless otherwise stated.

Specroscopy

N.m.r. spectra were recorded at 270 Mhz (^1H), and were run in deuteriochloroform ($CDCl_3$) with trimethyl silane (TMS) as the internal standard, unless otherwise stated. Chemical shifts (δ) are expressed as downfield shifts from TMS with multiplicities denoted by s (singlet), d (doublet), t (triplet), q (quartet), p (quintet), dd (doublet of doublets), dt (doublet of triplets), bs (broad singlet), m (multiplet), and bm (broad multiplet).Infrared (i.r.) spectra were recorded as liquid film or nujol mulls, with the absorption frequencies (υ) expressed in cm^{-1}. Mass spectra (m.s.) were recorded using electron impact (E.I.) and/or chemical ionisation (C.I.) techniques, quoting the molecular ion $(M)^+$ and/or protonated molecular ion $(M+1)^+$ respectively. In addition, the base peak and sizeable fragments are denoted with assignments, where possible. Elemental microanalyses were obtained from the Physical and Chemical Measurement Unit (University of Bath).

Dimethyl 3-chloropent-2-ene-1,5-dioate[51] (**11**).- Phosphorus pentachloride (108 g, 520 mmol) was added portionwise to diethyl pentan-3-one-1,5-dioate (95.9g, 474 mmol) under an atmosphere of nitrogen with stirring. After addition was complete, the reaction mixture was warmed to 40°C for 30 min, during which time a red colouration developed. The reaction mixture was then cooled in an ice bath and poured onto ice (200 cm^3) in an ice-cooled flask. A mixture of water and DCM (1:1 v/v, 100cm^3) was used to wash traces of product from the reaction vessel, and the resulting mixture was stirred for 15 min. After separating the two layers, the aqueous phase was extracted with DCM (3 x 80 cm^3) and the combined organic extracts dried (Na_2SO_4) and concentrated under reduced pressure to yield a red oil. This was added to a mixture of concentrated sulphuric acid (26 cm^3) and dry methanol (350 cm^3) and heated under reflux for 18 h. Excess methanol (250 cm^3) was removed by distillation, the residue allowed to cool to RT and then poured into water (150 cm^3). Sodium chloride was added to saturation and the solution extracted with ether (6 x 100 cm^3). The combined ether extracts were washed successively with saturated aqueous $NaHCO_3$ solution (120 cm^3) and saturated aqueous NaCl solution (120 cm^3), dried ($MgSO_4$) and concentrated under reduced pressure to afford a yellow oil. Purification was effected by distillation under reduced pressure to afford the *title compound* (**11**) (46.3 g, 51%) as a colourless liquid, b.p. 70-80°C at 0.1 mmHg, (lit.,[115] b.p. 50-60°C at 0.02 mmHg). The product was determined to be a mixture of stereoisomers (approximately 6:1) by ^1H n.m.r. The spectral data of (**5**) were in accord with published data;[115] υ_{max} (liquid film) 3080 (HC=), 2955, 1719 (CO-O), 1640 (HC=), 1430 cm^{-1}; δ_H ($CDCl_3$) 3.73 (3H, s, $C\underline{H}_3O$-$COCH_2$), 3.75 (3H, s, C=CHCO-$OC\underline{H}_3$), 4.12 (2H, s, CH_3O-$COC\underline{H}_2$), 6.21, 6.28 (1H, 2 x s, stereoisomeric C=CH); δ_C ($CDCl_3$), 41.2 (C=CH$\underline{C}H_2$), 51.6 ($\underline{C}H_3O$-$COCH_2$), 52.3 (C=CHCO-$O\underline{C}H_3$), 121.5 (ClC=$\underline{C}$H), 150.0 (Cl$\underline{C}$=CH), 164.4 (CH_3O-$\underline{C}OCH_2$), 168.1 (C=CH$\underline{C}$O-OCH_3); m/z (C.I.) 193 $(M+1)^+$ (19%), 161 $(M-CH_3O)^+$ (100%).

Dimethyl pent-2,3-dienedioate[51] (**12**). - To a stirred solution of (**11**) (45.0 g, 233 mmol) in dry THF (150 cm^3) at 0°C under a nitrogen atmosphere was added freshly distilled triethylamine (39 cm^3, 280 mmol) portionwise over a 10 min period. The nitrogen inlet/outlet was then replaced by a drying tube ($CaCl_2$) and the mixture stirred for 18 h at 0-5°C. During this time a precipitate of triethylamine hydrochloride formed which was removed by filtration and washed with ether (3 x 25 cm^3). The combined filtrate and washings were washed successively with 0.1 N hydrochloric acid (3 x 20 cm^3) and saturated aqueous NaCl solution (25 cm^3). After drying (Na_2SO_4) the ethereal solution was concentrated under reduced pressure to yield a red / orange oil. The oil was purified by distillation under reduced pressure to afford the *title compound* (**12**) (22.1 g, 60%) as a colourless liquid, b.p. 78-80°C at 0.1 mmHg (lit.,[115] 58°C at 0.02 mmHg). The product became yellow and viscous on standing at RT, due to polymerisation, and was thus stored at -20°C. The spectral data of (**12**) were in accordance with published data;[115] υ_{max} (liquid film) 2960, 1960 (C=C=C), 1710 (CO-O) cm^{-1}; δ_H ($CDCl_3$) 3.78 (6H, s, 2 x CH_3O-CO), 6.07 (2H, s, HC=C=CH); δ_C ($CDCl_3$) 52.3 (2 x $\underline{C}H_3O$-CO), 91.9 ($\underline{C}$=C=$\underline{C}$), 163.5 (CH_3O-$\underline{C}O$), 219.5 (C=$\underline{C}$=C); m/z (E.I.) no $(M)^{+\cdot}$, 125 $(M\text{-}CH_3O)^+$ (77%), 66 (34), 59 (100), (C.I.) 157 $(M+1)^+$ (100%), 125 (64%).

1,5-Dihydroxypent-2,3-diene (**9**).- A 1.5 M solution of Dibal-H in toluene (70 cm^3, 102.4 mmol) was added portionwise over 1 h to a stirred solution of dimethyl penta-2,3-dienedioate (**12**) (4.00 g, 25.6 mmol) in dry toluene (100 cm^3) at 0°C under nitrogen. After 1.5 h of additional stirring at 0-5°C, a saturated solution of sodium potassium tartrate (95 cm^3) was slowly added to the cooled reaction mixture which was then allowed to warm to RT. The toluene was separated off and the aqueous portion extracted by continuous extraction into ether over two consecutive 15 h periods. The combined organic isolates were dried (Na_2SO_4), and then evaporated under reduced pressure to afford an orange oil. Purification of the crude product by column chromatography [silica gel, petrol-EtOAc (9:1)] gave the *title compound* (**9**) (1.23 g, 48%) as a colourless oil.

The product was stored at -20°C as polymerisation occured on standing at RT; υ_{maz} (liquid film) 3600-3100 (OH), 2920, 2860, 1969 (C=C=C) cm^{-1}; δ_H ($CDCl_3$) 2.39 (2H, bs, OH), 4.16 (4H, t, J=4.2 Hz, 2 x $C\underline{H}_2OH$), 5.51 (2H, t, J=4.2 Hz, HC=C=CH); δ_C ($CDCl_3$) 60.1 (2 x CH_2OH), 94.7 ($\underline{C}$=C=$\underline{C}$), 202.5 (C=$\underline{C}$=C); m/z (E.I.) no $(M)^{+\cdot}$, 82 $(M-H_2O)^+$ (99%), 55 (100), (C.I.) 101 $(M+1)^+$ (8), 100 (69), 83 $(M+1-H_2O)^+$ (100%), 56 (26%).

Pent-2,3-diene-1,5-dimesylate (**13**).- Freshly distilled triethylamine (240 mg, 2.2 mmol) was added dropwise to a stirred solution of 1,5-dihydroxy-penta-2,3-diene (**9**) (100 mg, 1.0 mmol) and methanesulphonyl chloride (270 mg, 2.2 mmol) in dry DCM (5 cm^3) at 0°C under nitrogen. The reaction mixture was stirred for 2 h at 0-5°C. During this time a precipitate of triethylamine hydrochloride formed which was removed by filtration and washed with DCM (3 x 10 cm^3). The reaction mixture was then poured onto ice cold 2 N Hydrochloric acid / ice (1:2 v/v 10 cm^3) and the organic layer separated. The aqueous layer was then further extracted with DCM (2 x 20 cm^3), and the DCM isolates combined, dried (Na_2SO_4), and concentrated under reduced pressure to afford the mesylate (**13**). The crude product was then dissolved in dry toluene (20 cm^3) and filtered to remove any remaining triethylamine hydrochloride salts. The toluene was then removed under reduced pressure to afford the *title compound* as a yellow oil (128 mg, 50%). Storage at -20°C was employed to prevent decomposition. The compound was to be used without further purification as decomposition occured during column chromatography; υ_{max} (liquid film) 2957, 2872, 1972 (C=C=C), 1352 and 1173 (SO_2O); δ_H ($CDCl_3$) 2.97 (6H, s, 2 x CH_3SO_2O), 4.71 (4H, dd, J=3.9, 1.5 Hz, 2 x SO_2OCH_2), 5.42 (2H, m, HC=C=CH); δ_C ($CDCl_3$) 37.7 (2 x CH_3OSO_2), 66.4 (2 x SO_2OCH_2), 88.9 (H$\underline{C}$=C=$\underline{C}$H), 207.3 (HC=$\underline{C}$=CH); no m/z due to decomposition.

Pent-2,3-diene-1,5-bis-(ethyl-N-(diphenylmethylene)glycinate) - A solution of (**18**) (191mg, 1.0mmol) in dry THF (3 cm^3) was added to a solution of potassium *tert*-butoxide (113mg, 1.0mmol) in dry THF (5 cm^3) at -78°C under nitrogen atmosphere, followed by a solution of (**13**) (130mg, 1.0mmol) in THF (5 cm^3).

The solution was allowed to warm to room temperature and stirred for a further 2 hours, following the reaction by thin layer chromatography. Work-up involved extraction of the crude product into diethyl ether (2 x 20 cm^3) and washing of the combined organic extracts with saturated aqueous ammonium chloride solution (4 x 10 cm^3). The organic layer was then dried (Na_2SO_4), filtered and concentrated under reduced pressure to give an orange oil (108 mg). None of the data acquired on the crude compound corresponded with the desired product.

5-(t-Butyldiphenylsilyloxy)pent-2,3-diene-1-ol (**14**).- To a stirred solution of 1,5-dihydroxypenta-2,3-diene (**9**) (174 mg, 1.7 mmol) in dry DCM (10 cm^3) containing imidazole (118 mg, 1.7 mmol) under nitrogen atmosphere was added *t*-butyldiphenylsilyl chloride (479 mg, 1.7 mmol) dropwise at RT. After 2 h the reaction mixture was filtered through a celite pad and evaporated under reduced pressure to afford the crude silyl ether which was purified by column chromatography [silica gel, petrol-EtOAc (4:1)] to yield the *title compound* (**14**) (180 mg, 32%) as a colourless oil; υ_{max} (liquid film) 3600-3100 (OH), 3070, 3049 (Ar-H), 2935, 2857, 1966 (C=C=C), 1100 and 820 (SiOC) cm^{-1}; δ_H ($CDCl_3$) 1.05 (9H, s, $C(CH_3)_3$), 1.54 (1H, bs, OH), 4.08 (2H, dd, J= 8.6, 3.2 Hz, $HOC\underline{H}_2$), 4.25 (2H, dd, J= 8.8, 3.1 Hz, CH_2OSi), 5.41 (2H, m, HC=C=CH), 7.35-7.73 (10H, m, H-aromatic); δ_C ($CDCl_3$) 19.3 ($\underline{C}(CH_3)_3$), 26.9 ($C(\underline{C}H_3)_3$), 60.5 ($HOCH_2$), 61.9 (CH_2OSi), 93.6 (HC=C=$\underline{C}$H-CH_2OSi), 94.4 (HOH_2C-H$\underline{C}$=C=CH), 127.7, 129.7, 133.7, 135.6 (C-aromatic) 205.8 (C=$\underline{C}$=C); m/z (FAB) 339 $(M+1)^+$ (5%), 321 $(M+1-H_2O)^+$ (83%), 229 (58%), 199 (100%), 143 (75%); [Found: C, 74.4; H, 7.9. $C_{21}H_{26}O_2Si$ requires C, 74.5; H,7.8%].

5-(t-Butyldiphenylsilyloxy)pent-2,3-diene-1-mesylate (**15**).- Triethylamine (58 mg, 0.53 mmol) was added dropwise to a stirred solution of (**14**) (180 mg, 0.53 mmol) and methanesulphonyl chloride (65 mg, 0.53 mmol) in dry DCM (5 cm^3) at 0°C under nitrogen. After stirring for a further 2 h at 0-5°C the solution was poured into a mixture of 2 N hydrochloric acid and ice (2:1 v/v, 10 cm^3).

The organic layer was isolated and the aqueous layer extracted further with DCM (2 x 10 cm^3). The combined organic layers were washed with saturated aqueous $NaHCO_3$ solution (2 x 10 cm^3) and saturated NaCl solution (10 cm^3), dried (Na_2SO_4) and concentrated under reduced pressure to afford the mesylate (**15**). The crude product was then dissolved in dry toluene (20 cm^3) and filtered to remove any remaining triethylamine hydrochloride salts. The toluene was then removed under reduced pressure to afford the *title compound* as a pale yellow oil (155 mg, 70%). Storage at -20°C was employed to prevent decomposition. The compound was to be used without further purification as decomposition occurred during column chromatography; υ_{max} (liquid film) 3070, 3049 (Ar-H), 2930, 2856, 1968 (C=C=C), 1361 and 1174 (SO_2O), 1090 and 820 (SiOC) cm^{-1}; δ_H ($CDCl_3$) 1.06 (9H, s, $C(CH_3)_3$), 2.93 (3H, s, CH_3SO_2O), 4.26 (2H, dd, J= 8.6, 2.9 Hz, CH_2OSi), 4.65 (2H, dd, J= 9.3, 2.0 Hz, SO_2OCH_2), 5.42 (2H, m, HC=C=CH), 7.37-7.69 (10H, m, H-aromatic); δ_C ($CDCl_3$) 19.2 ($\underline{C}(CH_3)_3$), 26.7 ($C(\underline{C}H_3)_3$), 38.1 (CH_3OSO_2), 61.0 (CH_2OSi), 67.8 (SO_2OCH_2), 87.6 (SO_2OCH_2-$\underline{C}$H=C=CH), 94.3 (HC=C=$\underline{C}$H-CH_2OSi), 127.6, 129.7, 133.3, 135.4 (C-aromatic), 205.7 (C=$\underline{C}$=C); m/z (C.I., NH_3) 434 $(M+NH_4)^+$ (98%), 417 $(M+1)^+$ (21%), 338 $(M+1\text{-}SO_2CH_3)^+$ (13%), 321 $(M\text{-}OSO_2CH_3)^+$ (52%), 143 (74%).

Benzylidene glycine ethyl ester (**17**)[60].- Benzaldehyde (3.79 g, 35.8 mmol) was added dropwise to a solution of glycine ethyl ester hydrochloride (5 .0 g, 35.8 mmol) and triethylamine (7.26 g, 71.8 mmol) and anhydrous magnesium sulphate (3 g) in dry DCM (75 cm^3) at RT under nitrogen. The reaction mixture was stirred for 1 h. During this time a precipitate of triethylamine hydrochloride formed which was removed by filtration and washed with DCM (3 x 10 cm^3). The combined filtrate and washings were washed with saturated NaCl solution (3 x 20 cm^3) and saturated ammonium chloride solution (2 x 30 cm^3). The organic layer was then dried (Na_2SO_4) and concentrated under reduced pressure to yield the *title compound* (**17**) as a colourless oil (5.65 g, 63%). The compound was to be used without further purification as decomposition occured during column chromatography; υ_{max} (liquid film) 1741 (CO_2Et), 1647 (C=N) cm^{-1};

δ_H ($CDCl_3$) 1.3 (3H, t, J=7.1 Hz, $CO_2CH_2C\underline{H}_3$), 4.2 (2H, q, J=7.1 Hz, $CO_2C\underline{H}_2CH_3$), 4.4 (2H, s, $NC\underline{H}_2CO_2$), 5.3 (1H, s, Ar-C<u>H</u>=N), 7.74 - 7.88 (5H, m, H-aromatics); δ_C ($CDCl_3$) 13.9 ($CO_2CH_2\underline{C}H_3$), 60.8 ($CO_2\underline{C}H_2CH_3$), 61.8 (N-$\underline{C}H_2$ CO_2), 128.2, 129.4, 134.1, 135.4 (C-aromatic), 165.1 (Ar-CH-N), 169.8 ($\underline{C}O_2CH_2CH_3$). m/z (C.I.) 192 $(M+1)^+$ (100%), 162 (M+1 - $C_2H_5)^+$ (25%), 118 (M+1 - CO_2Et) (25%).

Ethyl N-(diphenylmethylene)glycinate (**18**).- Benzophenone imine (5.0g, 27.6 mmol) and an equimolar amount of finely ground glycine ethyl ester hydrochloride (3.85g, 27.6 mmol) were stirred in dry DCM (100cm^3) in the presence of anhydrous magnesium sulphate (3g), at room temperature for 24 hours, with a calcium chloride drying tube. The reaction mixture was then filtered through a Celite pad to remove ammonium chloride and the solvent removed under reduced pressure. The residue was taken up in ether (100 cm^3), filtered and washed with distilled water (100 cm^3). The organic portion was then dried ($MgSO_4$), filtered and the solvent removed under reduced pressure. The solid was washed with a small amount of cold hexane and dried under high vacuum to yield a white crystalline solid (6.01g, 82%).; υ_{max} (nujol mull) 1964 (C=C=C), 1730 (CO_2Et), 1660 (C=N) cm^{-1}; δ_H ($CDCl_3$) 1.25 (3H, t, J=7.1 Hz, $CO_2CH_2C\underline{H}_3$), 4.2 (2H, q, J=7.3, 7.0 Hz, $CO_2C\underline{H}_2CH_3$), 4.2 (2H, s, $NC\underline{H}_2CO_2$), 7.74 - 7.88 (10H, m, H-aromatics) ppm; δ_C ($CDCl_3$) 14.1 ($CO_2CH_2\underline{C}H_3$), 55.6 ($CO_2\underline{C}H_2CH_3$), 60.7 (N-$\underline{C}H_2$ CO_2), 128.2, 129.4, 134.1, 135.4 (C-aromatic), 170.5 (Ar-C-Ar), 171.1 ($\underline{C}O_2CH_2CH_3$) ppm; m/z (C.I.$^+$) 268 $(M+1)^+$ (90%), 194 (100%), 91 (90%).

5-(t-Butyldiphenylsilyloxy)penta-2,3-diene-1-diethyl phosphate (**20**).- Freshly distilled pyridine (200mg, 2.53mmol) was added dropwise to a stirred solution of (**14**) (306mg, 0.905 mmol) in dry DCM (5cm^3) at 0°C, under nitrogen atmosphere, followed by diethyl chlorophosphate (218mg, 1.267 mmol). The reaction mixture was then allowed to warm to room temperature and stirred for 16 hours. The reaction mixture was then taken up in ether (25 cm^3) and washed with 2N HCl (30 cm^3), saturated aqueous $NaHCO_3$ solution (30 cm^3), and distilled water (30 cm^3).

The organic portion was then dried ($MgSO_4$), filtered, and the solvent removed under reduced pressure to yield (**20**) as a colourless oil (333mg, 77%). υ_{max} (liquid film) 1969 (C=C=C), 1277 (P=O), 1050-1020 (P-O-CH_2), 1090 and 820 (SiOC) cm^{-1}; δ_H ($CDCl_3$) 1.04 (9H,s,t-Bu), 1.32 (6H, t, J=7.0 Hz, 2 x $CO_2CH_2\underline{C}H_3$), 4.10 (4H, p, J=7.15, 7.5 Hz, 2 x $CO_2\underline{C}H_2CH_3$), 4.26 (2H, dd, J=3.0, 8.6 Hz, CH_2OSi), 4.48 (2H, ddd, J=2.6, 4.2 Hz, CH_2OP), 5.40 (2H, m, HC=C=CH), 7.37-7.69 (10H, m, H-aromatic); δ_C ($CDCl_3$) 16.1 ($OCH_2\underline{C}H_3$), 19.1 ($\underline{C}(CH_3)_3$), 26.7 ($C(\underline{C}H_3)_3$), 61.4 (CH_2OSi), 63.7 ($O\underline{C}H_2CH_3$), 63.8 (CH_2OP), 89.4 (HC=C=$\underline{C}$H-CH_2OP), 93.7 (HC=C=$\underline{C}$H-CH_2OSi), 127.6, 129.6, 133.4, 135.5 (C-aromatic), 204.5 (C=$\underline{C}$=C); m/z (F.A.B.) 475 $(M+1)^+$ (32%), 417 (80%), 219 (80%), 135 (100%).

5-(t-Butyldiphenylsilyloxy)pent-2,3-diene-1-ethyl-N-(diphenylmethylene)glycinate (**21**).- [note: all solvents were degassed] Diisopropylamine (33mg, 0.32 mmol) in dry THF (2 cm^3) was treated with 2.5N n-BuLi in hexanes (0.130 cm^3, 0.32 mmol) at -78°C, under nitrogen atmosphere, over a period of 5 minutes. (**18**) (79mg, 0.2947 mmol) dissolved in dry THF (2 cm^3) was added to the reaction mixture and the solution stirred at -78°C for 1 hour, and then allowed to warm to room temperature. A solution of $Pd_2(dba)_3$ (11mg, 0.012 mmol) and triphenyl phosphine (19mg, 0.07 mmol) in dry THF (3 cm^3) was added via a cannular, followed by the phosphate (**20**) (140mg, 0.3 mmol). The reaction mixture was then stirred at room temperature for 5 hours. The reaction mixture was then hydrolysed with saturated aqueous NH_4Cl solution (20 cm^3) and the product extracted with ether (3 x 30 cm^3). The combined organic phases were washed with distilled water (2 x 25 cm^3), dried ($MgSO_4$), filtered and the solvent removed under reduced pressure to yield a crude yellow oil (24mg). The product which was purified by column chromatography [silica gel, petrol-EtOAc (4:1)] to yield the *title compound* (**21**) (20mg, 16%) as a yellow oil; υ_{max} (liquid film) 1964 (C=C=C), 1730 (CO_2Et), 1660 (C=N), 1090 and 820 (SiOC) cm^{-1}; δ_H ($CDCl_3$) 0.9 (3H, t, J=7.33 Hz, $CO_2CH_2\underline{C}H_3$), 1.0 (9H, s, t-Bu), 2.6 (2H, dt, HC=C=CH-$C\underline{H}_2$-CH), 4.15 (3H, m, $OC\underline{H}_2CH_3$ and HC=C=CH-CH_2-$C\underline{H}$), 4.33 (2H, t, J=6.65 Hz, $C\underline{H}_2OSi$), 5.05 (1H, m, HC=C=$C\underline{H}$-CH_2-CH), 5.15 (1H, m, HC=C=$C\underline{H}$-CH_2-OSi), 7.24 - 7.92 (20H, m, H-aromatics) ppm; δ_C ($CDCl_3$) 14.2 ($CO_2CH_2\underline{C}H_3$), 19.1 ($\underline{C}(CH_3)_3$), 26.7 ($C(\underline{C}H_3)_3$),

60.9 (CH_2OSi), 62.4 ($O\underline{C}H_2CH_3$), 65.4 ($HC{=}C{=}CH\text{-}CH_2\text{-}\underline{C}H$), 65.5 ($HC{=}C{=}CH\text{-}\underline{C}H_2\text{-}CH$), 88.3 ($HC{=}C{=}\underline{C}H\text{-}CH_2\text{-}OSi$), 91.5 ($HC{=}C{=}\underline{C}H\text{-}CH_2\text{-}CH$),), 170.8 (Ar-C-Ar), 171.5 ($\underline{C}O_2CH_2CH_3$), 204.2 ($C{=}\underline{C}{=}C$) ppm; m/z (FAB) 563 $(M+1)^+$ (3%), 489 (100%), 323 (25%), 310 (10%).

Trans-1,4-bis [ethyl-N-(diphenylmethylene) glycinate)]-2-butene (**23**).-
Diisopropylamine (1.04 g,10.3 mmol) in dry THF (5cm^3) at -78°C, under nitrogen, was treated dropwise with 2.5N n-butyllithium in hexanes (4.1 cm^3, 10.3 mmol) over a period of 10 minutes. A solution of ethyl-N-(diphenylmethylene)glycinate (**18**) (2.5 g, 9.35 mmol) in dry THF (5 cm^3) was added to the solution in one go.
The resulting solution was stirred at -78°C for one hour. The solution was then allowed to warm to RT and stirred for a further 30 minutes before being cooled back to -78°C. A solution of trans-1,4-dibromo-2-butene (1.0 g, 4.67 mmol) and *tetrakis* (triphenylphosphine) palladium (0.30 g,0.256 mmol) in dry THF (5 cm^3) was added and the reaction mixture allowed to warm to RT overnight. The resulting solution was hydrolysed with distilled water (30 cm^3) and extracted with EtOAc (3 x 20 cm^3). The organic layers were combined, dried (Na_2SO_4), filtered and concentrated under reduced pressure to afford the crude product as an orange oil, which was purified by column chromatography [silica gel, petrol-EtOAc (9:1)] to yield the *title compound* (**23**) as a yellow oil (1.47 g, 60%); υ_{max} (liquid film) 1737 (CO-O), 1623 (C=N) cm^{-1}; δ_H ($CDCl_3$) 1.20 (6H, t, J=7.32 Hz, 2 x $OCH_2C\underline{H}_3$), 2.55 (4H, m, 2 x $C{=}CH\text{-}C\underline{H}_2$), 4.0 (2H, m, 2 x $N\text{-}C\underline{H}\text{-}CO_2Et$), 4.10 (4H, q, J=7.3 Hz, 2 x $OC\underline{H}_2CH_3$), 5.30 (2H, s, $\underline{H}C{=}C\underline{H}$), 7.2-7.6 (20H, m, H-aromatics) ppm; δ_C ($CDCl_3$) 14.1 (2 x $OCH_2\underline{C}H_3$), 36.8 (2 x $C{=}CH\text{-}\underline{C}H_2$), 60.7 (2 x $O\underline{C}H_2CH_3$), 65.5 (2 x $N\text{-}\underline{C}H\text{-}CO_2Et$), 127.7, 127.9, 128.3, 128.5, 128.7, 128.9, 136.4, 139.5 (C-aromatics), 170.4 (2 x Ar-C-Ar), 171.7 ($\underline{C}O_2Et$) ppm; m/z (F.A.B. accurate mass) 587 $(M+1)^+$ (100%), 193 (30%).

[6.1.0]-9,9-Dibromobicyclononane (**27a**).- A solution containing cyclooctene (0.5 cm^3, 3.85 mmol) and bromoform (1.68 cm^3, 19.23 mmol) in dry THF (10 cm^3) stirred at -78°C, under nitrogen, was treated dropwise with 1.6M n-butyllithium (2.89 cm^3, 4.62 mmol). The reaction mixture was stirred at -78°C for 30 minutes the treated with distilled water (20 cm^3) and extracted with EtOAc (3 x 20 cm^3). The organic portions were combined, dried ($MgSO_4$), filtered, and concentrated under reduced pressure to afford the crude product which was dried under high vacuum to give the *title* compound (**27**) as an orange oil (650 mg, 60%). υ_{max} (liquid film) 657 (C-Br) cm^{-1} ; δ_H ($CDCl_3$) 0.99 (4H, t, J=7.33 Hz, $C^4\underline{H_2}$ and $C^5\underline{H_2}$), 1.42 (4H, m, $C^3\underline{H_2}$ and $C^6\underline{H_2}$), 1.84 (4H, m, $C^2\underline{H_2}$ and $C^7\underline{H_2}$), 2.95 (2H, m, $C^1\underline{H}$ and $C^8\underline{H}$) ppm; δ_C ($CDCl_3$) 21.0 (C^4 and C^5), 25.3 (C^3 and C^6), 26.3 (C^2) 27.8 (C^7), 33.3 (C^1 and C^8), 35.0 (C^9Br_2) ppm.

[6.1.0]-9,9-Dibromobicyclononane (**27b**).- An aqueous 50% solution of sodium hydroxide (1.45 cm^3, 18.18 mmol) was added dropwise to a vigorously stirred solution of cyclooctene (1.18 cm^3, 9.09 mmol), bromoform (0.79 cm^3, 9.09 mmol), and benzyltriethylammonium chloride (10mg), in DCM (10 cm^3). The mixture was stirred for 3 hours at 40°C then allowed to cool to room temperature. A further 50 cm^3 of DCM was added and the organic phase washed with water (3 x 50 cm^3), dried ($MgSO_4$), filtered and concentrated under reduced pressure to give an orange oil which was dried under high vacuum to give the *title* compound (**27**) as an orange oil (1.07g, 42%). δ_H ($CDCl_3$) 1.02 (4H, t, J=7.32 Hz, $C^4\underline{H_2}$ and $C^5\underline{H_2}$), 1.46 (4H, m, $C^3\underline{H_2}$ and $C^6\underline{H_2}$), 1.90 (4H, m, $C^2\underline{H_2}$ and $C^7\underline{H_2}$), 3.01 (2H, m, $C^1\underline{H}$ and $C^8\underline{H}$) ppm; δ_C ($CDCl_3$) 21.2 (C^4 and C^5), 25.3 (C^3 and C^6), 26.3 (C^2) 28.0 (C^7), 33.0 (C^1 and C^8), 35.1 (C^9Br_2) ppm.

1,2-Cyclononadiene (**28**) - A solution containing cyclooctene (1.0 cm^3, 7.7 mmol) and carbon tetrabromide (2.5 g, 7.7 mmol) in diethyl ether (10 cm^3), under nitrogen, was cooled to -60°C and then treated dropwise with 1.0M methyllithium (7.7 cm^3, 7.7 mmol).

After stirring at -60°C for half an hour another 1.1 equivalents of 1.0 M methyllithium was added dropwise, and after another 30 minutes the reaction was allowed to warm to 0°C. The organic layer was then washed with water (3 x 20 cm^3), dried ($MgSO_4$), filtered, and concentrated under reduced pressure to give the crude *title* compound (**28**) as an oil (535 mg, 57%). υ_{max} (liquid film) 1960 (C=C=C) cm^{-1}. δ_H ($CDCl_3$) 1.4 - 2.4 (8H, m, CH_2 for C_4-C_7), 1.65 (4H, m, CH_2 for C_3 and C_8), 5.35 (2H, m, HC=C=CH) ppm. δ_C ($CDCl_3$) 23.7 (C_3 and C_8), 26.3 (C_4 and C_7), 27.2 (C_5 and C_6), 85.2 (HC=C=CH), 205.9 (HC=C=CH) ppm. B.p. Found 164-165°C. (Literature[64] = 167-169°C).

Bis-(trichloromethyl)mercury (**29**) - A solution of mercuric chloride (5.0 g, 18.4 mmol) and sodium tricloroacetate (6.8 g, 36.8 mmol) in 1,2-dimethoxyethane (30 cm^3) was refluxed for 1 hour. The solution was allowed to cool to room temperature and then poured into water (200 cm^3) and extracted with diethyl ether (3 x 100 cm^3). The combined organic extracts were washed with fresh water (2 x 100 cm^3), dried ($MgSO_4$), filtered and concentrated under reduced pressure to give 5.6g of white solid which was crystallised from chloroform resulting in the *title* compound (**29**) as white prisms (2.5g, 31%). M.p. Found= 139°C. (Literature[68] = 140-141°C).

L-Serine benzylester benzenesulphonate (**33**) - A mixture of L-serine (21.02g, 0.2 mol), 90% technical grade benzenesulphonic acid (45.0g, 0.25 mol) and benzyl alcohol (100 cm^3) in carbon tetrachloride (250 cm^3) was distilled using a Dean-Stark apparatus until no more water was collected (16 hours). The remaining solvent was removed under reduced pressure and diethyl ether (200 cm^3) was added with vigorous stirring. The solution was stored in a freezer for 24 hours and the white solid which crystallised was filtered off and washed with fresh, cold diethyl ether. The product was dried under high vacuum to give the *title compound* (**33**) as a white solid (25.94g, 40%).

δ_H (D_2O) 3.95 (2H, ddd, J=12.1, 4.2 Hz, CH_2OH), 4.2 (1H, m, $CHCO_2$), (2H, s, CH_2Ph), 7.4 -7.8 (10H, m, H-aromatics) ppm; δ_C (D_2O) 54.9 ($\underline{C}HCO_2$), 59.4 ($\underline{C}H_2OH$), 68.8 ($\underline{C}H_2Ph$), 125.5, 128.7, 129.1, 129.2, 131.8 (C-aromatics), 134.8 ((Ar)$\underline{C}$-CH_2CO_2), 142.5 ((Ar)$\underline{C}$-SO_3), 168.4 ($\underline{C}O_2CH_2$) ppm. mp 96-97 °C (Lit.[42] 97-98 °C).

L-N-t-Butoxycarbonylserine benzylester **(34)** - Di-tert-butylpyrocarbonate (16.24g, 74.41 mmol) was added to an ice cooled solution of **(33)** (25.0g, 74.41 mmol) in dry THF (200 cm^3) and 1M aqueous sodium hydroxide (75 cm^3). The resulting solution was stirred at room temperature for 16 hours. The solution was then concentrated under reduced pressure, cooled in an ice bath and covered with ethyl acetate (100 cm^3) and acidified with 10% w/v aqueous potassium hydrogen sulphate to pH 2-3. The two layers were separated and the aqueous washed with fresh ethyl acetate (2 x 100 cm^3). The combined organic fractions were washed with water (3 x 100 cm^3), dried ($MgSO_4$), filtered and concentrated under reduced pressure to give a colourless oil which solidified on drying under high vacuum to give the *title compound* **(34)** as a white solid (21.14g, 96%). δ_H ($CDCl_3$) 1.44 (9H, s, $OC(CH_3)_3$), 2.05 (1H, br s, OH), 3.95 (2H, ddd, J=7.15, 7.15 Hz, CH_2OH), 4.40 (1H, br s, $NCHCO_2$), 5.20 (2H, s, CH_2Ph), 5.51 (1H, br s, NH), 7.35 (5H, s, aromatics) ppm. δ_C ($CDCl_3$) 28.5 ($C(\underline{C}H_3)_3$), 56.0 ($\underline{C}HCO_2$), 60.6 ($\underline{C}H_2OH$), 67.6 ($\underline{C}H_2Ph$), 80.5 ($\underline{C}(CH_3)_3$), 128.3, 128.6, 128.8 (C-aromatics), 135.4 ((Ar)$\underline{C}$-CH_2CO_2), 156.5 ($NH\underline{C}O_2$), 170.9 ($\underline{C}O_2CH_2Ph$) ppm. mp 70-71 °C (Lit.[42] 70-71 °C). m/z (FAB) 296 $(M+1)^+$ (8%), 240 (M+1 - $H_2C{=}C(CH_3)_2)^+$ (15%), 196 (49%).

(S)-Benzyl-2-[(tert-butoxycarbonyl)amino]-3-p-toluenesulphonylpropionate **(35)** - Tosyl chloride (7.1g, 37.16 mmol) was added to a stirred solution of **(34)** (10g, 33.78 mmol) in dry pyridine (50 cm^3) under nitrogen at -10°C. The resulting solution was stirred for 24 hours at <0°C. The solution was then poured into ice/water (1:1, 200 cm^3) and stirred until a white solid crystallised out of solution.

The solid was filtered off and washed with water (3 x 50 cm^3) and the washed with a small amount of cold ethanol, then dried under high vacuum. The crude product was recrystallised from ethanol to give the *title compound* **(35)** as a white solid (10.44g, 68.5%). δ_H ($CDCl_3$) 1.40 (9H, s, $OC(CH_3)_3$), 2.43 (3H, s, $pSO_2C_6H_4C\underline{H}_3$), (2H, ddd, J=2.93, 19.42 Hz, CH_2OSO_2), 4.53 (1H, bd, J=8.24 Hz, $NCHCO_2$), 5.12 (2H, dd, J=12.27, 13.01 Hz, CH_2Ph), 5.35 (1H, bd, J=8.25 Hz, NH), 7.30 (7H, m, $CH_2C_6\underline{H}_5$ and tosyl meta protons), 7.71 (2H, d, J=8.24 Hz, tosyl ortho protons) ppm. δ_C ($CDCl_3$) 21.6 ($SO_2C_6H_4\underline{C}H_3$), 28.2, ($C(\underline{C}H_3)_3$), 53.0 ($N\underline{C}HCO_2$), 67.8 ($CH_2Ph$), 69.5 ($CH_2OSO_2$), 80.4 ($\underline{C}(CH_3)_3$), 127.9, 128.2, 128.5, 128.6, 129.9 (C-aromatics), 132.3 ((Ar)-$\underline{C}$-CH_3), 134.7 ((Ar)-$\underline{C}$-CH_2CO_2), 145.1 ((Ar)-$\underline{C}$-SO_2), 154.9 ($NH\underline{C}O_2$), 168.4 ($\underline{C}O_2CH_2Ph$) ppm. m/z (FAB) 450 $(M+1)^+$ (20%), 394 $(M+1 - H_2C{=}C(CH_3)_2)^+$ (100%), 350 (30%).. mp 95-97 °C (Lit. [42] 95-96 °C).

(R)-Benzyl-2-[(tert-butoxycarbonyl)amino]-3-iodopropionate **(36)** - A solution of sodium iodide (2.0g, 13.3 mmol) in dry acetone (20 cm^3) was added dropwise to a stirred solution of **(35)** (4.0g, 8.9 mmol) in dry acetone (20 cm^3) under nitrogen in the absence of light. The resulting yellow solution was stirred at room temperature, in the dark, for 24 hours. The reaction mixture was then filtered and concentrated under reduced pressure. The residue was then dissolved in chloroform (100 cm^3), washed sequentially with water (2 x 50 cm^3), sodium thiosulphate (50 cm^3), and again with water (3 x 50 cm^3), then dried ($MgSO_4$), filtered and concentrated under reduced pressure to give a pale yellow oil. The oil was dried under high vacuum then dissolved in a minimum of hot ethanol and stored in the freezer overnight. A white solid crystallised out of solution and was filtered off and washed with a small amount amount of cold ethanol. After drying under high vacuum the *title compound* **(36)** was isolated as a white solid (1.96g, 54%). The product requires storage in the dark under a nitrogen atmosphere at ambient temperature. mp 79-80 °C (Lit.[42] 79-80 °C). δ_H ($CDCl_3$) 1.45 (9H, s, $C(CH_3)_3$), (2H, m, CH_2I), 4.58 (1H, m, $NCHCO_2$), 5.20 (2H, dd, J=3.3, 12.2 Hz, CH_2Ph), 5.40 (1H, bd, J= 7.5 Hz, NH), 7.37 (5H, s, aromatics) ppm.

δ_C ($CDCl_3$) 8.0 (CH_2I), 28.4 ($C(CH_3)_3$), 53.8 ($N\underline{C}HCO_2$), 68.2 ($\underline{C}H_2Ph$), 80.7 ($\underline{C}(CH_3)_3$), 128.4, 128.7, 128.8 (CH-aromatics), 135.0 (C-aromatics),, 152.9 ($N\underline{C}O_2$), 169.7 ($\underline{C}O_2CH_2Ph$) ppm. m/z (FAB) 406 $(M+1)^+$ (5%), 350 $(M+1 - H_2C=C(CH_3)_2)^+$ (60%), 306 (40%).

(±) - 3- Butyn-2-oxy-p-toluensulphonte **(41)** - Tosyl chloride (15.0g, 78.6 mmol) was added to a stirred solution of (±)-3-butyn-2-ol (5.0g, 71.4 mmol) in dry pyridine (50 cm^3) under nitrogen at -10°C. The solution was for 2 hours at -10°C and then stored in the freezer for 24 hours. The solution was then poured in ice / water (1:1, 200 cm^3) and stirred for 20 minutes. The aqueous was then extracted with ethyl acetate (3 x 100 cm^3) and the combined organic fractions washed with 2M HCl (3 x 50 cm^3), $NaHCO_3$ (50 cm^3), and water (50 cm^3). The organic portions were then dried ($MgSO_4$), filtered and concentrated under reduced pressure to give an orange oil which solidified on storage in a freezer. The crude solid product was crystallised from ethanol to give the *title compound* **(41)** as a white solid (15.06g, 88%).
δ_H ($CDCl_3$) 1.58 (3H, d, $C\underline{H}_3CH$), 2.43 (1H, d, C≡CH), 2.50 (3H, s, Ar-CH_3), 5.16 (1H, dq, $C\underline{H}$-C≡CH), 7.33 (2H, d, tosyl meta protons), 7.82 (2H, d, tosyl ortho protons) ppm. δ_C ($CDCl_3$) 21.8 (Ar-$\underline{C}H_3$), 22.7 ($\underline{C}H_3CH$), 67.5 ($\underline{C}H$-C≡CH), 75.7 (CH-C≡$\underline{C}$H), 80.0 (CH-$\underline{C}$≡CH), 128.2, 129.9 (CH-aromatics), 133.9 ((Ar)$\underline{C}CH_3$) 145.1 ((Ar)$\underline{C}SO_2$) ppm. m/z (C.I.) 224 $(M+1)^+$ (4%), 167 (100%), 41 (20%).

Benzyl 2-(S)-[(tert-butoxycarbonyl)amino]-hepta-4,5-dienoate **(42)** - A suspension of zinc powder (1.3g, 20.1 mmol) in dry THF (10 cm^3) and 1,2-dibromoethane (0.4g, 0.2 cm^3, 4.7 mmol) was heated under nitrogen to 60°C and stirred at this temperature for 5 minutes. After cooling to 35°C, trimethylsilylchloride (0.03 cm^3, 0.2 mmol) was added and the reaction mixture stirred vigorously for 30 minutes at 35°C. A solution of **(36)** (1.81g, 4.46 mmol) in dry THF (5 cm^3) was added dropwise and the reaction stirred for 45 minutes.

The solution was then cooled to 0°C and a solution of **(41)** (1.0g, 4.46 mmol) and tetrakis(triphenylphosphine) palladium (0) (0.30g, 0.268mmol) in dry THF (5 cm^3) was added. The reaction was stirred at -10°C for 16 hours. The reaction mixture was then treated with ethyl acetate (30 cm^3) and filtered through celite. The organic portion was then washed with 0.1 M HCl (50 cm^3) and distilled water (3 x 75 cm^3). The organic portion was then dried (Na_2SO_4), filtered and concentrated under reduced pressure to give a brown oil, the analytical data on which did not correspond to the desired product **(42)**.

Benzyl 2-(S)-[(tert-butoxycarbonyl)amino]-hepta-4,5-dienoate **(42)** - A suspension of zinc powder (1.3g, 20.1 mmol) in dry THF (10 cm^3) and 1,2-dibromoethane (0.4g, 0.2 cm^3, 4.7 mmol) was heated under nitrogen to 60°C and stirred at this temperature for 5 minutes. After cooling to 35°C, trimethylsilylchloride (0.03 cm^3, 0.2 mmol) was added and the reaction mixture stirred vigorously for 30 minutes at 35°C. A solution of **(36)** (1.81g, 4.46 mmol) in dry THF (5 cm^3) was added dropwise and the reaction stirred for 45 minutes. The solution was then cooled to -10°C and a solution of copper (I) cyanide (0.30g, 3.4 mmol) and lithium chloride (0.30g, mmol) in dry THF (2 cm^3) was added. The reaction was stirred at 0°C for 10 minutes then cooled to -10°C before the addition of a solution of **(41)** (1.0g, 4.46 mmol) in dry THF (2 cm^3). The reaction was stirred at -10°C for 16 hours. The reaction mixture was then treated with ethyl acetate (30 cm^3) and filtered through celite. The organic portion was then washed with 0.1 M HCl (50 cm^3) and distilled water (3 x 75 cm^3). The organic portion was then dried (Na_2SO_4), filtered and concentrated under reduced pressure to give the *title compound* **(42)** as a yellow oil (1.27g, 86%). δ_H ($CDCl_3$) 1.45 (9H, s, $C(C\underline{H}_3)_3$), 1.67 (3H, dd, J= 7, 3.5 Hz, $C\underline{H}_3CH$) 2.45 (2H, m, $C\underline{H}_2CH$), 4.45 (1H, m, $CH_2C\underline{H}$), 4.90 (1H, m, $CH_3C\underline{H}$=C=C), 5.05-5.25 (3H, m, $CH_2C\underline{H}$=C=C and $C\underline{H}_2Ph$), 7.38 (5H, s, aromatics) ppm. δ_C ($CDCl_3$) 14.4 ($\underline{C}H_3CH$=C=C), 28.5 ($C(\underline{C}H_3)_3$), 32.2 ($\underline{C}H_2CH$=C=C), 53.3 ($N\underline{C}HCO_2$), 67.2 ($\underline{C}H_2Ph$), 80.0 ($\underline{C}(CH_3)_3$), 84.6 (C=C=$\underline{C}HCH_2$), 86.6 (C=C=$\underline{C}HCH_3$), 128.2, 128.4, 128.6 (CH-aromatics), 135.6 ((Ar)-$\underline{C}CH_2$), 156.0 ($NH\underline{C}O_2$), 172.1 ($\underline{C}O_2CH_2Ph$), 207.2 (C=$\underline{C}$=C) ppm. m/z (FAB) 332 $(M+1)^+$ (20%), 276 $(M+1 - C_4H_8)^+$ (100%), 232 (60%).

(±)-Allylglycine benzylester benzenesulphonate **(45)** - A mixture of (±)-allylglycine (10.0g, 87 mmol), technical grade benzenesulphonic acid (17.2g, 108.7 mmol) and benzyl alcohol (50 cm^3) in carbon tetrachloride (200 cm^3) was refluxed using a modified Dean-Stark apparatus until no more water was collected (16 hours). The reaction mixture was then concentrated under reduced pressure and the resulting oil treated with diethyl ether (200 cm^3) and stirred vigorously. The solution was then stored at <0°C for 24 hours and a white solid crystallised and was filtered off. The solid was washed with fresh, cold diethyl ether and dried under high vacuum to give the *title compound* **(45)** as a white solid (12.4g, 40%).
δ_H ($CDCl_3$) 2.55 (2H, m, $C\underline{H}_2CHC{=}CH_2$), 4.25 (1H, m, $NHC\underline{H}CO_2$), 5.00 (4H, m, $C\underline{H}_2{=}CH$ and $C\underline{H}_2Ph$), 5.55 (1H, m, $CH_2{=}C\underline{H}$), 7.2-7.4 (8H, m, $CH_2C_6\underline{H}_5$ and $PhSO_3^-$ meta and para protons), 7.88 (2H, d, J=7.5 Hz, $PhSO_3^-$ ortho protons), 8.1-8.4 (1H, bs, NH) ppm. δ_C ($CDCl_3$) 34.5 ($\underline{C}H_2CH{=}CH_2$), 52.9 ($N\underline{C}HCO_2$), 68.0 ($\underline{C}H_2Ph$), 121.3 ($CH_2CH{=}\underline{C}H_2$), 128.2, 128.6, 128.7, 129.9 (CH-aromatics), 130.4 ($CH_2\underline{C}H{=}CH_2$), 134.9 ((Ar)$\underline{C}CH_2$), 144.4 ((Ar)$\underline{C}SO_3^-$), 168.9 ($\underline{C}O_2CH_2Ph$) ppm.

(±)-N-t-Butoxycarbonylallylglycine benzylester **(46)** - Di-tert-butylpyrocarbonate (7.25g, 33.2 mmol) was added to an ice cooled solution of **(45)** (12.0g, 33.2 mmol) in dry THF (80 cm^3) and aqueous 1M sodium hydroxide (35 cm^3). The resulting solution was stirred at room temperature for 16 hours and then concentrated under reduced pressure, cooled in an ice bath, treated with ethyl acetate (100 cm^3) and adjusted to pH 3 with 10% aqueous potassium hydrogen sulphate. The two layers were separated and the aqueous phase was washed with fresh ethyl acetate (2 x 100 cm^3). The combined organic layers were washed with water (3 x 50 cm^3), dried (Na_2SO_4), filtered and concentrated under reduced pressure to give the *title compound* **(46)** as a colourless oil (9.43g, 93%). δ_H ($CDCl_3$) 1.42 (9H, s, ($C(C\underline{H}_3)_3$), 2.55 (2H, m, $C\underline{H}_2CH{=}CH_2$), 4.45 (1H, m, $NHC\underline{H}CO_2$), 5.20 (4H, m, $C\underline{H}_2{=}CH$ and $C\underline{H}_2Ph$), 5.65 (1H, m, $CH_2{=}C\underline{H}$), 7.35 (5H, s, $CH_2C_6\underline{H}_5$) ppm. δ_C ($CDCl_3$) 28.5 ($C(\underline{C}H_3)_3$), 36.9 ($\underline{C}H_2CH{=}CH_2$), 53.1 ($N\underline{C}HCO_2$), 67.2 ($\underline{C}H_2Ph$), 80.1 ($\underline{C}(CH_3)_3$), 119.4 ($CH_2CH{=}\underline{C}H_2$), 128.5,128.6, 128.7, (CH-aromatics), 132.4 ($CH_2\underline{C}H{=}CH_2$), 135.5 ((Ar)$\underline{C}CH_2$), 155.4 ($NH\underline{C}O_2$), 172.1 ($\underline{C}O_2CH_2Ph$) ppm. m/z (FAB) 306 $(M+1)^+$ (8%), 250 (100%), 206 (28%).

(±)-N-t-Butoxycarbonylaspartic acid-β-semialdehyde benzylester **(43)** - A solution of **(46)** (5.0g, 16.3 mmol) in dry DCM (300 cm^3) was flushed with ozone for two hours and the reaction followed by tlc (20% EtOAc/petrol). When all the starting material had reacted the flask was flushed with nitrogen to remove any excess ozone. The ozonide was decomposed by treating the solution with triethylamine (3.3g, 32.7 mmol) at -78°C over 20 minutes, and stirring the resulting solution for a further 5 hours at room temperature. The solution was concentrated under reduced pressure, then treated with ethyl acetate (100 cm^3) to precipitate triethylamine-N-oxide. The suspension was filtered and the filtrate washed with water (3 x 100 cm^3), dried (Na_2SO_4), filtered and concentrated under reduced pressure to give the crude aldehyde as an oil. The crude product was purified by column chromatography [silica gel, petrol-EtOAc (4:1)] to yield the *title compound* **(43)** as a colourless oil (3.64g, 73%). δ_H ($CDCl_3$) 1.45 (9H, s, $C(C\underline{H}_3)_3$), 3.05 (2H, m, $C\underline{H}_2CHO$), 4.65 (1H, m, $NHC\underline{H}CO_2$), 5.15 (4H, s, $C\underline{H}_2Ph$), 5.40 (1H, bd, J=8.0 Hz, NH), 7.35 (5H, m, $CH_2C_6\underline{H}_5$), 9.70 (1H, s, CHO) ppm. δ_C ($CDCl_3$) 28.4 ($C(\underline{C}H_3)_3$), 46.1 ($\underline{C}H_2CHO$), 48.9 ($N\underline{C}HCO_2$), 67.8 ($\underline{C}H_2Ph$), 77.7 ($\underline{C}(CH_3)_3$), 128.6,128.7, 128.8, (CH-aromatics), 135.3 ((Ar)$\underline{C}CH_2$), 155.7 ($NH\underline{C}O_2$), 171.1 ($\underline{C}O_2CH_2Ph$), 199.6 (CHO) ppm. m/z (FAB) 308 $(M+1)^+$ (10%), 252 (80%), 206 (55%).

(±)-Allylglycine-t-butyl ester hydrochloride **(47)** - 2-Methylpropene gas (400 cm^3) was bubbled through a stirred solution of (±)-allylglycine (5.0g, 43.48 mmol) in DCM (300 cm^3) at -78°C. Once all the gas had been added to the reaction mixture concentrated sulphuric acid (5 cm^3) was added dropwise over 20 minutes, and the solution stirred at -78°C for 30 minutes. The solution was allowed to warm to room temperature overnight then adjusted to pH 8 with saturated aqueous sodium bicarbonate solution. The two layers were separated and the organic layer washed with saturated brine solution (2 x 50 cm^3), dried ($MgSO_4$), filtered and concentrated under reduced pressure. The resulting oil was taken up in diethyl ether and stirred vigorously while concentrated hydrochloric acid was added dropwise until pH 2.

The solution was stored in a freezer overnight and a white solid crystallised out of solution which was filtered off, washed with fresh diethyl ether and dried under high vacuum to give the *title compound* **(47)** as a white solid (7.54g, 84%). δ_H ($CDCl_3$) 1.49 (9H, s, $C(C\underline{H}_3)_3$), 2.81 (2H, m, $CH_2{=}CHC\underline{H}_2$), 4.06 (1H, m, $NC\underline{H}CO_2$), 5.28 (2H, m, $C\underline{H}_2{=}CHCH_2$), 5.83 (1H, m, $CH_2{=}C\underline{H}CH_2$), 8.73 (3H, bs, NH_3) ppm. δ_C ($CDCl_3$) 27.9 ($C(\underline{C}H_3)_3$), 34.0 ($CH_2{=}CH\underline{C}H_2$), 52.9 ($N\underline{C}HCO_2$), 84.0 ($\underline{C}(CH_3)_3$), 121.1 ($\underline{C}H_2{=}CHCH_2$), 130.1 ($CH_2{=}\underline{C}HCH_2$), 167.3 ($NCH\underline{C}O_2$) ppm.

(±)-N-t-Butoxycarbonylallylglycine-t-butyl ester **(48)** - Di-tert-butylpyrocarbonate (7.91g, 36.2 mmol) was added to an ice cooled solution of **(47)** (7.5g, 36.2 mmol) in dry THF (100 cm^3) and aqueous 1M sodium hydroxide (35 cm^3). The resulting solution was stirred at room temperature for 16 hours and then concentrated under reduced pressure, cooled in an ice bath, treated with ethyl acetate (100 cm^3) and adjusted to pH 3 with 10% aqueous potassium hydrogen sulphate. The two layers were separated and the aqueous phase was washed with fresh ethyl acetate (2 x 100 cm^3). The combined organic layers were washed with water (3 x 50 cm^3), dried (Na_2SO_4), filtered and concentrated under reduced pressure to give the *title compound* **(48)** as a colourless oil (8.2g, 95%). δ_H ($CDCl_3$) 1.45 (9H, s, $NHCO_2C(C\underline{H}_3)_3$), 1.46 (9H, s, $CHCO_2C(C\underline{H}_3)_3$), 2.49 (2H, m, $CH_2{=}CHC\underline{H}_2$), 4.25 (1H, m, $NC\underline{H}CO_2$), 5.13 (3H, m, $C\underline{H}_2{=}CHCH_2$ and NH), 5.72 (1H, m, $CH_2{=}C\underline{H}CH_2$) ppm. δ_C ($CDCl_3$) 27.9 ($CHCO_2C(\underline{C}H_3)_3$), 28.2 ($NHCO_2C(\underline{C}H_3)_3$), 36.9 ($CH_2{=}CH\underline{C}H_2$), 53.2 ($NC\underline{H}CO_2$), 79.6 ($CHCO_2\underline{C}(CH_3)_3$), 81.8 ($NHCO_2\underline{C}(CH_3)_3$), 118.6 ($\underline{C}H_2{=}CHCH_2$), 132.4 ($CH_2{=}\underline{C}HCH_2$), 155.2 ($NH\underline{C}O_2$), 170.8 ($CH\underline{C}O_2$) ppm. m/z (FAB) 272 $(M+1)^+$ (15%), 216 (100%), 172 (65%).

(±)-N-t-Butoxycarbonylaspartic acid-β-semialdehyde-t-butyl ester **(49)** - A solution of **(48)** (4.2g, 17.6 mmol) in dry DCM (300 cm^3) was flushed with ozone for five hours and the reaction followed by tlc (20% EtOAc/petrol). When all the starting material had reacted the flask was flushed with nitrogen to remove any excess ozone.

The ozonide was decomposed by treating the solution with triethylamine (3.8g, 35.14 mmol) at -78°C over 20 minutes, and stirring the resulting solution at room temperature overnight. The solution was concentrated under reduced pressure, then treated with ethyl acetate (200 cm^3) to precipitate triethylamine-N-oxide. The suspension was filtered and the filtrate washed with water (3 x 100 cm^3), dried (Na_2SO_4), filtered and concentrated under reduced pressure to give the crude aldehyde as an oil. The crude product was purified by column chromatography [silica gel, petrol-EtOAc (4:1)] to yield the *title compound* **(49)** as a colourless oil (1.8g, 43%). δ_H ($CDCl_3$) 1.44 (9H, s, $NHCO_2C(C\underline{H}_3)_3$), 1.45 (9H, s, $CHCO_2C(C\underline{H}_3)_3$), 2.99 (2H, dq, CH_2CHO), 4.48 (1H, m, $NC\underline{H}CO_2$), 5.40 (1H, m, J=7.69, NH), 9.74 (1H, s, CHO) ppm. δ_C ($CDCl_3$) 27.6 ($CHCO_2C(\underline{C}H_3)_3$), 27.7 ($NHCO_2C(\underline{C}H_3)_3$), 46.3 ($N\underline{C}HCO_2$), 49.2 ($\underline{C}H_2CHO$), 79.9 ($CHCO_2\underline{C}(CH_3)_3$), 82.6 ($NHCO_2\underline{C}(CH_3)_3$), 155.3 ($NH\underline{C}O_2$), 171.0 ($CH\underline{C}O_2$), 199.3 ($\underline{C}HO$) ppm. m/z (FAB) 274 $(M+1)^+$ (5%), 218 (75%), 174 (60%).

Tert-butyl 2-(N-tertbutoxycarbonylamino)-4-hydroxy-6-(trimethylsilyl)hex-5-ynoate - A solution of **(49)** (500mg, 2.1 mmol) in dry THF (10 cm^3), under a nitrogen atmosphere, was cooled to -20°C then treated dropwise with a 0.5M solution of lithium (trimethylsilyl)acetylide in THF (4.2 cm^3, 2.1 mmol). After 2 hours the reaction was allowed to warm to room temperature and treated with a solution of saturated aqueous ammonium chloride (25 cm^3). The solution was then extracted with dichloromethane (3 x 50 cm^3). The combined organic extracts were dried ($MgSO_4$), filtered and concentrated under reduced pressure to give an orange oil (546mg), the analytical data on which did not correspond to the desired product.

Dimethyldioxirane **(53)** - To a solution of $NaHCO_3$ (47g, 0.559 mol) in water (200 cm^3) and acetone (150 cm^3) was added potassium monoperoxysulphate (100g, 0.833 mol) in 20g portions at -78°C with vigorous stirring.

5 minutes after the last addition of potassium monoperoxysulphate the reaction was allowed to warm to room temperature and a moderate vacuum (80-100 torr) was applied to distil off the DMDO / acetone 5% solution, which was collected in a receiving flask cooled to -78°C. The product was dried (K_2CO_3) and stored in the freezer (-20°C) over molecular sieves (4Å).

Tert-butyl 2-(N-tertbutyloxycarbonyl amino)pent-4-enoate epoxide **(54a)** - To a solution of **(48)** (0.22g, 0.82 mmol) in acetone (10 cm^3), cooled to -5°C, was added a solution of **(53)** (10 cm^3). The solution was stirred for 1 hour and then concentrated under reduced pressure. The crude product was dissolved in EtOAc (10 cm^3), dried ($MgSO_4$), filtered and concentrated under reduced pressure to give the *title compound* **(54a)** as a reddish oil (0.2g, 84%).δ_H ($CDCl_3$) 1.45 (9H, s, $NHCO_2C(C\underline{H}_3)_3$), 1.48 (9H, s, $CHCO_2C(C\underline{H}_3)_3$), 1.94 (2H, t, J=6 Hz, $C\underline{H}_2CHN$),2.49 (1H, m, $C\underline{H}_aH_bOCH$), 2.78 (1H, t, J=5 Hz, $CH_aH_bOC\underline{H}$), 3.03 (1H, m, $CH_a\underline{H}_bOCH$), 4.34 (1H, m, $NC\underline{H}CO_2$), 5.35 (1H, m, J=7.8, NH), ppm. δ_C ($CDCl_3$) 27.8 ($CHCO_2C(\underline{C}H_3)_3$), 28.2 $NHCO_2C(\underline{C}H_3)_3$), 35.6 ($C\underline{H}_2OCH$), 35.8 ($C\underline{H}_2CHN$), 46.7 ($N\underline{C}HCO_2$), 52.3 ($CH_2O\underline{C}H$), 79.7 ($CHCO_2\underline{C}(CH_3)_3$), 82.2 ($NHCO_2\underline{C}(CH_3)_3$), 155.2 ($NH\underline{C}O_2$), 170.9 ($CH\underline{C}O_2$) ppm. m/z 288 $(M+1)^+$ (15%), 272 (55%), 232 (100%), 188 (40%).

Tert-butyl 2-(N-tertbutyloxycarbonyl amino)pent-4-enoate epoxide **(54b)** - *m*-Chloroperbenzoic acid (0.26g, 1.48 mmol) was added to an ice cooled solution of **(48)** (0.20g, 0.74 mmol) in dry DCM (10 cm^3) under nitrogen atmosphere. The reaction mixture was stirred at 0-5°C for 14 hours then treated with an aqueous solution of 1M $NaHCO_3$ (20 cm^3), and the solution stirred for 20 minutes.
The two layers were separated and the aqueous washed with fresh DCM (2 x 50 cm^3). The combined DCM washes were washed with distilled water (3 x 50 cm^3), dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography [silica gel, petrol-EtOAc (4:1)] to yield the *title compound* **(54b)** as a colourless oil (0.116g, 55%).δ_H ($CDCl_3$)

1.45 (9H, s, $NHCO_2C(C\underline{H}_3)_3$), 1.46 (9H, s, $CHCO_2C(C\underline{H}_3)_3$), 2.01 (2H, t, J=6 Hz, $C\underline{H}_2CHN$),2.45 (1H, m, $CH_a\underline{H}_bOCH$), 2.75 (1H, t, J=5 Hz, $CH_a\underline{H}_bOC\underline{H}$), 2.97 (1H, m, $CH_a\underline{H}_bOCH$), 4.30 (1H, m, $NC\underline{H}CO_2$), 5.38 (1H, m, J=7.8, NH), ppm. δ_C ($CDCl_3$) 27.7 ($CHCO_2C(\underline{C}H_3)_3$), 28.2 ($NHCO_2C(\underline{C}H_3)_3$), 35.7 ($C\underline{H}_2OCH$), 35.9 ($C\underline{H}_2CHN$), 46.6 ($N\underline{C}HCO_2$), 52.2 ($CH_2O\underline{C}H$), 79.8 ($CHCO_2\underline{C}(CH_3)_3$), 82.3 ($NHCO_2\underline{C}(CH_3)_3$), 155.3 ($NH\underline{C}O_2$), 171.0 ($CH\underline{C}O_2$) ppm. m/z 288 $(M+1)^+$ (15%), 272 (55%), 232 (100%), 188 (40%).

Tert-butyl 2-(N-tertbutoxycarbonylamino)-4-hydroxy-6-(trimethylsilyl)hex-5-ynoate - Cerium chloride heptahydrate (0.410g, 1.1 mmol) was finely ground and placed in a two necked round bottom flask, which was placed in an oil bath and heated *in vacuo* to 135-140°C at 0.5 mmHg for 2 hours. Nitrogen was then added to the flask which was then cooled in an ice bath and dry THF (5 cm^3) added. After stirring in an ultrasonic bath at room temperature for 1 hour the resulting slurry was cooled to -78°C and treated dropwise with a 0.5M solution of lithium (trimethylsilyl)acetylide in THF (1.5 cm^3, 0.75 mmol), and the solution stirred for 1 hour. A solution of **(49)** (0.20g, 0.75 mmol) in THF (3 cm^3) was added dropwise and the solution stirred at -78°C for 18 hours. The reaction mixture was then treated with a saturated aqueous solution of ammonium chloride (20 cm^3), followed by ethyl acetate (40 cm^3). The solution was stirred for 10 minutes, the two layers were then separated, and the aqueous extracted with fresh EtOAc (3 x 40 cm^3). The combined organic layers were dried (Na_2CO_3), filtered and concentrated under reduced pressure to give a brown oil, the analytical data on which did not correspond to the desired product.

Tert-butyl 2-(N-tertbutoxycarbonylamino)-4-hydroxy-6-phenylhex-5-ynoate **(57)** - Cerium chloride heptahydrate (0.20g, 0.55 mmol) was finely ground and placed in a two necked round bottom flask, which was placed in an oil bath and heated *in vacuo* to 135-140°C at 0.5 mmHg for 2 hours. Nitrogen was then added to the flask which was then cooled in an ice bath and dry THF (2 cm^3) added.

After stirring in an ultrasonic bath at room temperature for 1 hour the resulting slurry was cooled to -78°C and treated dropwise with a 1M solution of lithium phenylacetylide (0.55 cm^3, 0.55 mmol), and the solution stirred for 1 hour. A solution of **(49)** (0.10g, 0.37 mmol) in THF (2 cm^3) was added dropwise and the solution stirred at -78°C for 18 hours. The reaction mixture was then treated with a saturated aqueous solution of ammonium chloride (10 cm^3), followed by ethyl acetate (20 cm^3). The solution was stirred for 10 minutes, the two layers were then separated, and the aqueous extracted with fresh EtOAc (3 x 20 cm^3). The combined organic layers were dried (Na_2CO_3), filtered and concentrated under reduced pressure. This gave a brown oil which was purified by column chromatography [silica gel, petrol-EtOAc (3:1)] to yield the *title compound* **(57)** as a yellow oil (0.08g, 60%). δ_H ($CDCl_3$) 1.46 (9H, s, $NHCO_2C(C\underline{H}_3)_3$), 1.48 (9H, s, $CHCO_2C(C\underline{H}_3)_3$), 1.7-2.4 (2H, m, $C\underline{H}_2CHN$), 3.68 (1H, m $CH_2C\underline{H}OH$), 4.46 (1H, m, $NC\underline{H}CO_2$), 5.46 (1H, m, J=7.6, NH), 7.28-7.44 (5H, m, aromatics) ppm. δ_C ($CDCl_3$) 27.9 ($CHCO_2C(\underline{C}H_3)_3$), 28.2 ($NHCO_2C(\underline{C}H_3)_3$), 40.3 ($\underline{C}H_2CHOH$), 59.6 ($CH_2\underline{C}HOH$), 80.3 ($CHCO_2\underline{C}(CH_3)_3$), 80.7 ($C\equiv\underline{C}CHOH$), 82.6 ($NHCO_2\underline{C}(CH_3)_3$), 88.9 ($Ph\underline{C}\equiv C$), 122.6 (aromatics($\underline{C}$)-C≡C), 128.2, 131.7, 131.8 (aromatics($\underline{C}H$), 155.8 ($NH\underline{C}O_2$), 169.9 ($CH\underline{C}O_2$) ppm.

Tert-butyl 2-(N-tertbutoxycarbonylamino)-4-hydroxy-4-thiophen-2-ylbutanoate **(61)**- A 0.25M solution of lithium-2-thienylcyanocuprate in THF (8.25 cm^3, 2.06 mmol), at -78°C under a nitrogen atmosphere, was treated dropwise with an 11% suspension of sodium acetylide in xylene (0.085g, 1.9 ml, 2.06 mmol) and the solution stirred at -78°C for 1 hour. A solution of **(49)** (0.500g, 2.06 mmol) in THF (2 ml) was added in one portion and the reaction allowed to warm to room temperature over 2 hours. The THF was removed under reduced pressure and the resulting oil taken up in EtOAc (20 cm^3), then treated with a saturated aqueous solution of ammonium chloride (15 cm^3) and stirred for 10 minutes at room temperature. The two layers were separated, and the aqueous wahed with fresh EtOAc (3 x 20 cm^3). The combined EtOAc layers were washed with distilled water (2 x 50 cm^3), dried (Na_2SO_4), filtered and concentrated under reduced pressure to give a brown oil.

This was purified by column chromatography [silica gel, petrol-EtOAc (2:1)] to yield the *title compound* **(61)** as an oil (0.40g, 61%). δ_H ($CDCl_3$) 1.44 (9H, s, $NHCO_2C(C\underline{H}_3)_3$), 1.45 (9H, s, $CO_2C(C\underline{H}_3)_3$), 2.71-2.97 (2H, dq, J=4.8, 12.1 Hz, $CHOHC\underline{H}_2$), 4.30 (1H, t, J=6.7 Hz, $C\underline{H}OH$), 4.45 (1H, m, $NC\underline{H}CO_2$), 5.45 (1H, bd, J=8.25 Hz, NH), 6.99-7.74 (3H, m, Thienyl CH) ppm. δ_C ($CDCl_3$) 27.8 ($CO_2C(\underline{C}H_3)_3$), 28.3 ($NHCO_2C(\underline{C}H_3)_3$), 50.4 ($N\underline{C}HCO_2$), 60.8 ($CHOH\underline{C}H_2$), 65.5 ($\underline{C}HOHCH_2$), 79.8 ($CO_2\underline{C}(CH_3)_3$), 82.2 ($NHCO_2\underline{C}(CH_3)_3$), 123.7, 124.3, 127.0 (Thienyl-$\underline{C}$H), 130.9 (Thienyl-$\underline{C}$), 155.4 ($NH\underline{C}O_2C(CH_3)_3$), 170.0 ($\underline{C}O_2C(CH_3)_3$) ppm.

Tert-butyl 2-(N-tertbutoxycarbonylamino)-4-hydroxy-pentanoate **(66)** - Copper (II) cyanide (0.74g, 0.82 mmol) in THF (1 cm^3) was treated dropwise with a 1.0M solution of methyllithium in THF (2equiv, 1.65 cm^3, 1.65 mmol) at -78°C, under nitrogen, and the solution stirred for 30 minutes. The reaction mixture was allowed to warm to room temperature and treated dropwise with tributyl(ethynyl)tin (0.24 cm^3, 0.82 mmol) and the solution stirred at room temperature for 1 hour. The solution was cooled again to -78°C and treated dropwise with a solution of **(49)** (0.200g, 0.823 mmol) in THF (2 cm^3) and the solution allowed to warm to room temperature overnight. The THF was removed under reduced pressure, then saturated aqueous solution of ammonium chloride (10 cm^3) and EtOAc (20 cm^3) were added, and the solution stirred for 10 minutes. The two layers were separated and the aqueous washed with fresh EtOAc (3 x 30 cm^3). The combined EtOAc layers were washed with distilled water (2 x 100 cm^3), dried (Na_2SO_4), filtered and concentrated under reduced pressure to give a yellow oil. The crude product was purified by column chromatography [silica gel, petrol-EtOAc (3:1)] to yield the *title compound* **(66)** as a colourless oil (0.190g, 89%).δ_H ($CDCl_3$) 1.45 (9H, s, $NHCO_2C(C\underline{H}_3)_3$), 1.46 (9H, s, $CO_2C(C\underline{H}_3)_3$), 1.64 (3H, m, $C\underline{H}_3CHOH$), 2.75-3.05 (2H, dq, J=5.1, 12.4 Hz, $CHOHC\underline{H}_2$), 4.15 (1H, m, $C\underline{H}OH$), 4.48 (1H, m, $NC\underline{H}CO_2$), 5.45 (1H, bd, J=8.7 Hz, NH), ppm. δ_C ($CDCl_3$) 26.8 ($\underline{C}H_3CHOH$), 27.8 ($CO_2C(\underline{C}H_3)_3$), 27.9 ($NHCO_2C(\underline{C}H_3)_3$), 50.5 ($N\underline{C}HCO_2$), 60.8 ($CHOH\underline{C}H_2$), ($NHCO_2\underline{C}(CH_3)_3$), 155.0 ($NH\underline{C}O_2C(CH_3)_3$), 170.0 ($\underline{C}O_2C(CH_3)_3$) ppm.

Benzyl 2-(N-tertbutyloxycarbonylamino)-3-triethoxyphononylpropionate **(69)** - A solution of **(36)** (1.0g, 2.46 mmol) and triethylphosphite (0.41g, 2.46 mmol) in dry THF (20 cm^3) was stirred at reflux for 16 hours. The reaction mixture was then concentrated under reduced pressure and the resulting oil purified by column chromatography [silica gel, MeOH-DCM (1:20)] to yield the *title compound* **(69)** as a white solid (0.65g, 65%). δ_H ($CDCl_3$) 1.35 (6H, t, J=7.02 Hz, 2 x $POCH_2C\underline{H}_3$), 1.44 (9H, s, $C(CH_3)_3$), 3.55 (2H, m, CH_2P), 4.09 (4H, dq, J=7.02 Hz, 2 x $POC\underline{H}_2CH_3$), 4.55 (1H, m, $NCHCO_2$), 5.21 (2H, s, CH_2Ph), 5.43 (1H, bd, J= 7.63 Hz, NH), 7.37 (5H, s, aromatics) ppm. δ_C ($CDCl_3$) 7.6 (CH_2P), 15.8 ($POCH_2\underline{C}H_3$), 16.2 ($PO\underline{C}H_2CH_3$), 28.1 ($C(\underline{C}H_3)_3$), 53.2 ($N\underline{C}HCO_2$), 67.7 ($\underline{C}H_2Ph$), 80.3 ($\underline{C}(CH_3)_3$), 128.4, 128.6, 129.7 (CH-aromatics), 134.7 (C-aromatics), 154.7 ($N\underline{C}O_2$), 169.8 ($\underline{C}O_2CH_2Ph$) ppm. m/z (FAB) 416 $(M+1)^+$ (6%), 325 $(M+1 - CH_2Ph)^+$ (20%), 279 $(M+1 - PO(OEt)_2)^+$ (25%), 280 $(M+1 - CO_2CH_2Ph)^+$ (100%), 91 (100%).

Propynal **(70)** - A solution of chromium trioxide (30.0g, 0.3 mol) in concentrated sulphuric acid (20 cm^3) and water (60 cm^3) was added dropwise over 1 hour to a solution of propynol (18.0g, 18.7 cm^3, 0.3 mol) in MEK (50 cm^3). The temperature was maintained between 20-25°C using an ice/water bath. After addition was complete the reaction mixture was stirred for 4 hours and then diluted with water (50 cm^3). The organic layer was separated and the aqueous washed with diethyl ether (2 x 50 cm^3). The combined organic extracts were dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The residual liquid was distilled at atmospheric pressure (b.p. 54-57°C) to give three batches of the *title compound* **(70)** all contaminated with MEK, (total yield=15.6g, 90%). δ_H ($CDCl_3$) 3.71 (1H, s, $\underline{H}C\equiv C$), 9.22 (1H, s, $C\underline{H}O$) ppm. δ_C ($CDCl_3$) 65.5 ($H\underline{C}\equiv C$), 83.7 ($HC\equiv\underline{C}$), 209.1 ($\underline{C}HO$) ppm.

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