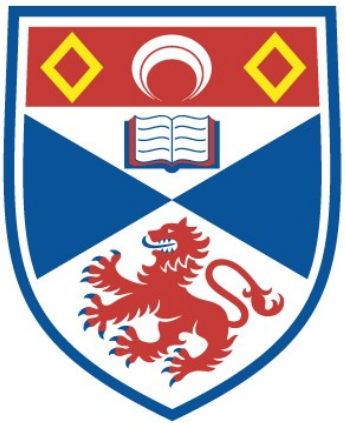
# PEPTIDE SECONDARY STRUCTURE MIMETICS: THE ALPHA-HELIX AND BETA-TURN

Arwel Lewis

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



1998

# PEPTIDE SECONDARY STRUCTURE MIMETICS:

# THE α-HELIX AND β -TURN

a thesis presented by

**Arwel Lewis**

to the

UNIVERSITY OF ST. ANDREWS

in application for

THE DEGREE OF DOCTOR OF PHILOSOPHY

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I was admitted to the Faculty of Science of the University of St. Andrews as a research student in October 1994 and as a candidate for the degree of Ph.D. in October 1995. The research of which this is a record was carried out in the University of St. Andrews between 1994 and 1998.

Signed ..... Date 6 May 1998

I hereby certify that the candidate has fulfilled the conditions of the Resolution and Regulations appropriate to the degree of Ph.D.

Signature of Supervisor

Date ..... 6 May '98

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Finally, I am grateful to the Wellcome Trust for the generous Prize Studentship which funded this Ph.D.

# ABSTRACT

This thesis describes research undertaken in peptidomimetic chemistry, concerned with stabilisation of peptide secondary structure.

The first section describes the successful synthesis of two triazepinediones for potential application as *cis* prolyl mimetics and/or β-turn mimetics. The first triazepinedione **64**, carrying an *N*-phenyl substituent, was subjected to reductive conditions in an unsuccessful attempt to generate a *cis* prolyl peptide. The second triazepinedione **72** was designed as a Gly-*cis* Pro-Phe peptidomimetic. Its synthesis proceeded in 8 steps, *via* a chiral α-hydrazino acid. Triazepinedione **72** was extended from both the *N* and *C* termini, demonstrating that it could be incorporated into a peptide.

The second section describes the design and attempted synthesis of an *N*-terminal template for stabilisation of α-helical structure in an attached peptide. Preliminary efforts to produce a Pro-Pro-Ala macrocycle **91** and a Pro-Pro-Pro ether macrocycle **98** were unsuccessful. Attempts to synthesise a Pro-Pro-Pro thioether macrocycle **112** resulted in formation of a cyclic dimer **122**. The formation of this dimer was proposed to be due to excessive steric hindrance in the transition state to cyclisation. Attempts to stabilise the desired folded transition state by α-methylation of proline residues or hydrogen bonding did not allow synthesis of any of the desired monomeric cyclic material.

A monomeric cyclic compound was finally obtained by increasing the flexibility of the linear precursor, affording a Pro-(2*R*)-Ala-Pro thioether macrocycle **151**. X-Ray crystallography and 2D NMR studies established that this macrocycle lacked the required arrangement of carbonyl groups conducive to initiation of an α-helix. Further NMR studies of macrocycle-peptide conjugates suggested that the macrocycle exerted a conformational influence only over the first residue of the attached peptide. The carbonyl groups of the macrocycle were found to adopt a significant degree of α-helical geometry upon attachment of a *C*-terminal cationic trialkylammonium group.

# ABBREVIATIONS

| | |
|---|---|
| Ac | acetyl |
| Alloc | allyloxycarbonyl |
| Ar | aromatic group |
| BOC | *tert*-butoxycarbonyl |
| BOP-Cl | *N*,*N*-bis(2-oxo-3-oxazolidinyl)phosphinic chloride |
| CD | circular dichroism |
| CI | chemical ionisation |
| DBU | 1,8-diazabicyclo[5.4.0]undec-7-ene |
| def. | deformation |
| DIAD | di-*iso*propyl azodicarboxylate |
| DIEA | *N*,*N*-di-*iso*propylethylamine |
| DMAP | 4-(*N*,*N*-dimethylamino)pyridine |
| DMF | *N*,*N*-dimethylformamide |
| DMSO | dimethyl sulfoxide |
| DPPA | diphenylphosphoryl azide |
| EDCI | 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride |
| EI | electron impact ionisation |
| ES | electrospray |
| Et | ethyl |
| FAB | fast atom bombardment |
| FKBP | FK506 binding protein |
| FMDV | foot and mouth disease virus |
| Fmoc | 9-fluorenylmethoxycarbonyl |
| h | hours |
| HATU | *N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-yl-methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide |

| | |
|---|---|
| HBTU | *O*-(1*H*-benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate |
| HRMS | high-resolution mass spectroscopy |
| IBCF | *iso*butyl chloroformate |
| IR | infra-red |
| IUPAC | International Union of Pure and Applied Chemistry |
| *J* | coupling constant |
| kcal $mol^{-1}$ | kilocalories per mole |
| kJ $mol^{-1}$ | kilojoules per mole |
| LDA | lithium di*iso*propylamide |
| Me | methyl |
| min | minutes |
| NMM | *N*-methylmorpholine |
| NMR | Nuclear Magnetic Resonance |
| NOE | Nuclear Overhaüser Effect |
| NOESY | NOE correlation spectroscopy |
| Ph | phenyl |
| PP | polyproline |
| PPIase | peptidyl-prolyl *cis-trans* isomerase |
| ppm | parts per million |
| pyBOP | (1*H*-benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate |
| pyBroP | bromotripyrrolidinophosphonium hexafluorophosphate |
| RNA | ribonucleic acid |
| ROESY | rotating frame NOE correlation spectroscopy |
| rt | room temperature |
| TASP | Template Assembled Synthetic Protein |
| TBTU | *O*-(1*H*-benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium tetrafluoroborate |

| | |
|---|---|
| TFA | trifluoroacetic acid |
| TFE | 2,2,2-trifluoroethanol |
| THF | tetrahydrofuran |
| Tic | 1,2,3,4-tetrahydro*iso*quinoline-3-carboxylate |
| TMS | tetramethylsilane |
| UV | ultra-violet |
| Z | benzyloxycarbonyl |

# AMINO ACID ABBREVIATIONS

| Three-Letter Code | One-Letter Code | Amino Acid |
|---|---|---|
| Aib | U | α-amino*iso*butyric acid |
| Ala | A | alanine |
| Arg | R | arginine |
| Asn | N | asparagine |
| Asp | D | aspartic acid |
| Cys | C | cysteine |
| Gln | Q | glutamine |
| Glu | E | glutamic acid |
| Gly | G | glycine |
| His | H | histidine |
| Hyp | – | hydroxyproline |
| Ile | I | isoleucine |
| Leu | L | leucine |
| Lys | K | lysine |
| Phe | F | phenylalanine |
| Pro | P | proline |
| $Pro^{Me}$ | – | α-methylproline |
| Ser | S | serine |
| Thr | T | threonine |
| Trp | W | tryptophan |
| Tyr | Y | tyrosine |
| Val | V | valine |
| Xaa | – | any amino acid |
| Yaa | – | any amino acid |

# CONTENTS

# 1. INTRODUCTION

## 1.1 Biologically Active Peptides

Peptides are essential to almost every biochemical process, regulating diverse functions in the cardiovascular, gastrointestinal, immunological, and central nervous systems. These crucially important molecules span a wide range of sizes from those with fewer than ten constituent amino acids up to proteins containing hundreds of residues. The function of these compounds varies as much as their size and structural complexity. Proteins may serve structural roles or act as enzymes to catalyse a myriad of complex chemical transformations.

This report will focus on structural aspects of peptides, especially those containing fewer than 50 residues. These compounds generally serve roles as chemical messengers, eliciting a physiological response in a cell by interacting with a membrane-bound receptor, thus acting as hormones and neurotransmitters in the regulation of a host of essential processes.

Important examples include the opioid peptides (endorphins, enkephalins and dynorphins) which possess analgesic properties; [1, 2] angiotensin II **1**, which helps regulate blood pressure; [3] bradykinin **2**, a vasodilator and bronchioconstrictor; [4] oxytocin **3**, responsible for uterine contraction; [5] and somatostatin **4** which controls the release of growth factor from the hypothalamus, amongst other effects (Fig. 1.1). [1, 3, 5] Whilst hardly critical for human function, the dipeptide sweetener Aspartame™ **5** certainly possesses significant economic importance.

Peptides bind to their receptors in a particular conformation, the so-called *bioactive conformation*, even though they possess great conformatonal flexibility in solution. As such, the three-dimensional structure adopted by a peptide upon binding is crucial to its function. A description of the various structure types available to peptides and a discussion of the factors which determine the energetically accessible conformations adopted now follows.

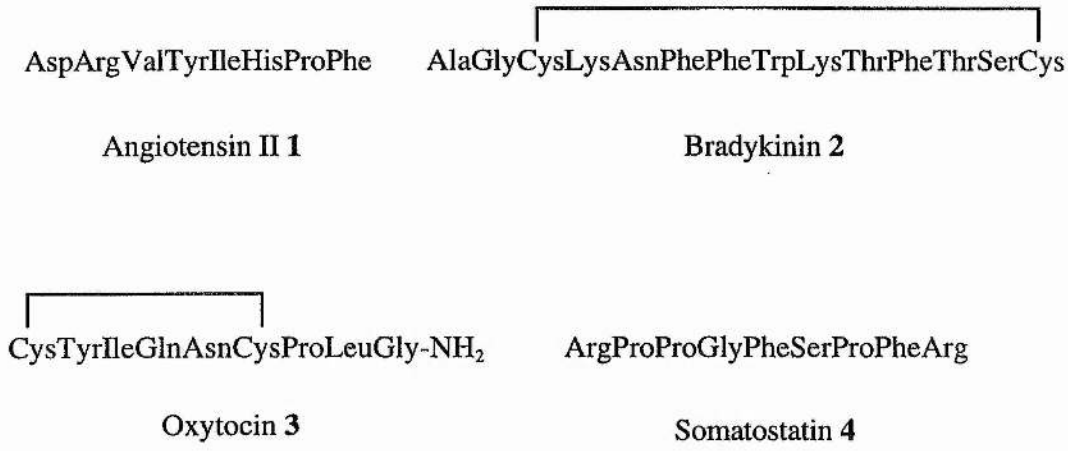



**Figure 1.1:** *Some biologically important peptides*

## 1.2 Peptide Structure

### 1.2.1 General Features

The description of peptide and protein structure is usually split into four groups, each reflecting a different level of complexity:

- Primary Structure, the sequence of amino acids;
- Secondary Structure, the folding of this sequence into basic motifs (*e.g.* α-helix, β-sheet, β-turn);
- Tertiary Structure, how these motifs pack together;
- Quaternary Structure, how large areas of tertiary structure interact between separate sub-units of a protein.

The interactions between individual residues, peptide chains or whole units of structure which govern the structures adopted may be covalent (disulfide bridges),

electrostatic (hydrogen bonds, salt bridges) or hydrophobic (clustering of non-polar groups) in nature.

In the case of the shorter peptides, only primary and secondary structures are important, the latter being especially critical in interactions with receptors. Each of the types of interaction listed above may be involved to a greater or lesser extent in determining stable conformations.

Secondary structure of peptides and proteins is described by a set of *torsion angles* for each residue within the chain (Fig. 1.2).

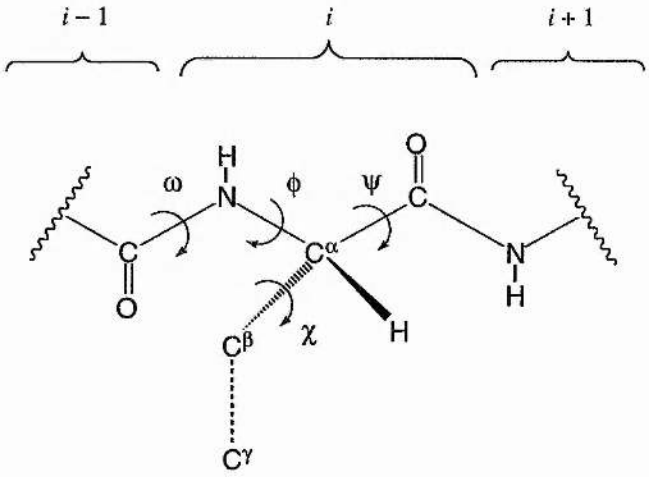



**Figure 1.2:** *Dihedral angles used to describe peptide conformation*

The important torsion angles are:

Phi ($\phi$), around the N–$C^{\alpha}$ bond;

Psi ($\psi$), around the $C^{\alpha}$–CO bond;

Omega ($\omega$), around the CO–N bond;

Chi ($\chi$), around the $C^{\alpha}$–$C^{\beta}$ bond.

Knowledge of each of these angles for the whole peptide describes its conformation; certain values for the residues within a sequence defines the peptide's structure as a β-turn, β-sheet or an α-helix. [6-8]

The angles $\phi$, $\psi$ and $\chi$ may cover a wide range, but $\omega$ can only adopt values close to 0° (*cis*) or 180° (*trans*), due to the partial double bond character inherent in the amide bond.

The two most important parameters $\phi$ and $\psi$ can be plotted on a *Ramachandran Plot.* [7-9] This is a useful graphical representation of the low-energy $\phi$ and $\psi$ values allowed for residues in a peptide, as depicted in Fig. 1.3. Less than half the area of the plot is accessible for the naturally-occurring L-series of amino acids, with each separate area corresponding to a certain structure type. The bottom left quadrant contains right-handed α-helices ($\alpha_R$), the top left quadrant β-structures, and the top right quadrant left-handed α-helices ($\alpha_L$).

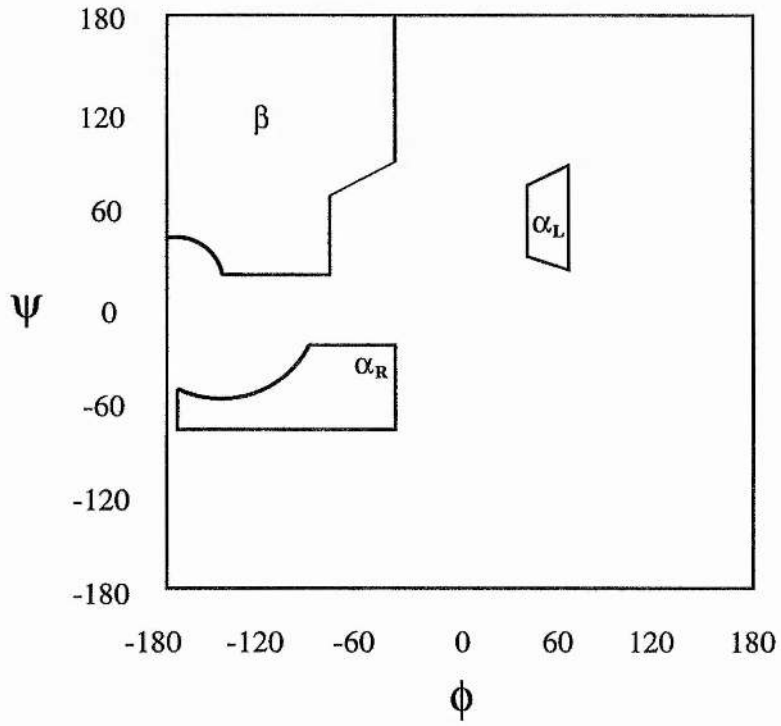



**Figure 1.3** *Ramachandran plot showing low-energy dihedral angles*

These β-structures (turns and sheets) and α-helices account for the majority of conformations adopted by peptides and protein chains. The β-turn and α-helix in particular are the central areas of study in this thesis, and a detailed description follows.

### 1.2.2 The β-Turn

A β-turn (reverse turn or hairpin bend) is a sequence of the peptide chain where it folds back on itself (Fig. 1.4).

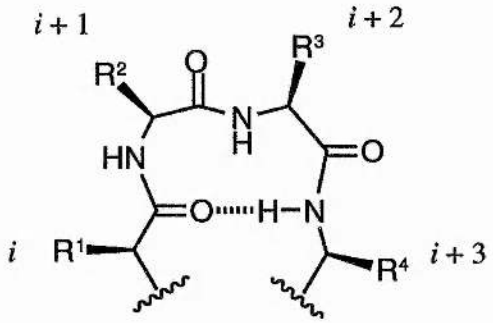



**Figure 1.4:** *General structure of a β-turn*

β-Turns contain four residues which each possess a different set of (ϕ, ψ) values, and a distance between the $C^{\alpha}$ atoms of the first residue *i* and the fourth residue (*i* + 3) of < 7Å. [8, 10, 11] Most β-turns possess a stabilising hydrogen bond between the carbonyl group of residue *i* and the NH of residue (*i* + 3), which closes a ten-membered ring. Model studies show that the ten-membered ring formed by the hydrogen bond is enthalpically favoured, with a near optimal hydrogen bond geometry, but entropically disfavoured, since chain flexibility is reduced. [12, 13]

β-Turns are classified into several groups according to the (ϕ, ψ) values for the middle two residues (*i* + 1) and (*i* + 2). The type VI turn is a special case which incorporates a *cis* peptide bond between residues (*i* + 1) and (*i* + 2), residue (*i* + 2) being proline (Fig. 1.5). [11, 14, 15]

Proline's unique cyclic nature renders it common within β-turns, at positions (*i* + 1) (*trans* Pro) or (*i* + 2) (*cis* Pro). [15-21] A β-turn is especially favoured in model peptides containing proline where the preceding residue has the D configuration. Glycine is also prevalent; due to its lack of α-substitution, it is more flexible and is able to adopt a wider range of dihedral angles, thus avoiding unfavourable

interactions which might disfavour the turn. [8, 21, 22] The [(*i* + 1), (*i* + 2)] = (Pro, Gly) sequence is very common; half of all proline residues followed by glycine are involved in a β-turn. [23]

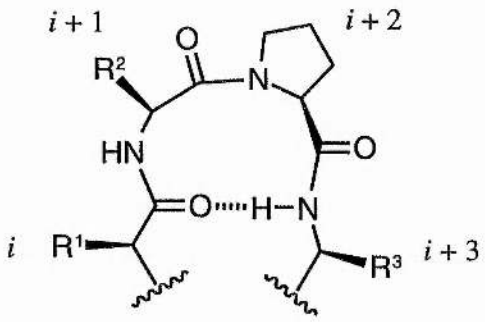



**Figure 1.5:** *A type VI β-turn*

Reverse turns are often found at the surfaces of proteins, and are important for linking together sections of α-helix and β-sheet, especially in joining adjacent strands of an anti-parallel β-sheet. [8] They have been implicated in initiation of protein folding [22, 24] since they are integral to β-sheet structure and can help "start off" a helix. [25] Many biologically active peptides including bradykinin **2**, [26, 27] somatostatin **4**, [28] and tuftsin [18] have been proposed to adopt a β-turn conformation in the receptor-bound form, especially when proline is a constituent residue.

A rather less common type of reverse turn exists which is closely related to the β-turn. This is the γ-turn, which comprises three residues rather than four, usually with a hydrogen bond between the CO of residue *i* and the NH of residue (*i* + 2), forming a seven-membered ring. [29] Model studies in isolation from other stabilising forces suggest this form to have lower enthalpic stability than the β-turn, [30] but γ-turns have been postulated to be present in the bioactive conformations of several peptides. [15, 31, 32]

### 1.2.3 The β-Sheet

β-Sheets involve interactions between two or more extended peptide chains which may be aligned in a parallel or anti-parallel manner relative to one another. An anti-parallel β-sheet is shown in Fig. 1.6.

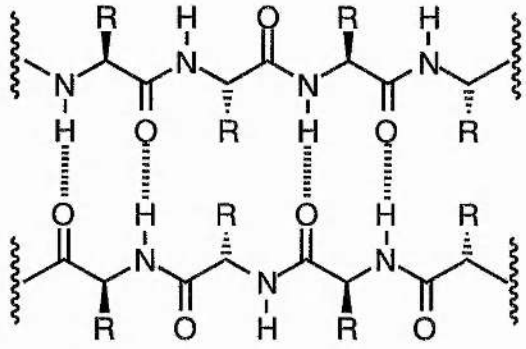


**Figure 1.6:** *Two strands of an anti-parallel β-sheet*

Each strand is "pleated", with α-carbons successively a little above and a little below the plane of the sheet. The strands may be directly connected *via* a β-turn (anti-parallel β-sheet) or further apart in a protein sequence (parallel β-sheet). [33] This is a repetitive structure type, with ($\phi$, $\psi$) values identical for each residue in the sheet. For the parallel β-sheet, ($\phi$, $\psi$) = (−119°, +133°), and for the anti-parallel β-sheet, ($\phi$, $\psi$) = (−139°, +135°). [8]

Hydrogen bonds between each β-strand stabilise these structures, and other longer-range hydrophobic and electrostatic forces are also important, which may explain why β-sheets are very rarely found in shorter peptides.

### 1.2.4 The α-Helix

#### 1.2.4.1 Introduction

The α-helix (Fig. 1.7) was first proposed as a stable structure for a peptide chain in 1951 by Linus Pauling. [34] Experimental proof of its existence followed shortly afterwards, and this structural motif is now recognised as being ubiquitous in proteins, comprising over 40% of their structure.

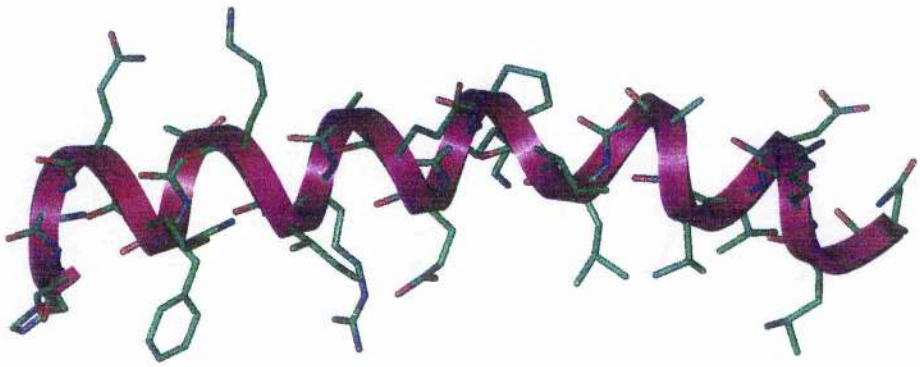


**Figure 1.7:** *A section of α-helix incorporating a proline residue*

Like the β-sheet, the α-helix is a repetitive structure, with each residue adopting identical ($\phi$, $\psi$) values of (–57°, –47°). These negative values correspond to a right-handed helix; the mirror-image left-handed helix, with ($\phi$, $\psi$) = (+57°, +47°) is of higher energy for the naturally occurring L-amino acids and is seldom observed.

The α-helix is stabilised by hydrogen bonds between the carbonyl group of residue $i$ and the NH group of residue ($i$ + 4). In the usual nomenclature for description of polypeptide helices this is a $3.6_{13}$ helix, 3.6 being the number of residues per turn and 13 the number of atoms in the ring created by the ($i$ + 4) → $i$ hydrogen bond. [7, 8] In aqueous media, the actual enthalpic gain imparted by these backbone hydrogen bonds is disputed; little, if any, advantage exists over a fully

solvated, extended peptide, and there is an unfavourable loss of entropy upon restraining the conformation using a hydrogen bond. However, within the hydrophobic shell of the helix, no solvent is present and in this situation hydrogen bonds certainly have a stabilising influence, by occupying polar amide groups which do not suit this non-polar environment.

The regular repeating structure of the α-helix aligns all of the carbonyl groups of each peptide unit. Consequently, the helix as a whole possesses a significant dipole. This is a destabilising factor, but is offset by the enthalpic gain from hydrogen bonding and other stabilising factors. The positive end of the dipole is at the *N*-terminus of the helix, and the negative end at the *C*-terminus. This may be important in binding charged ligands (*e.g.* negatively charged phosphate groups at the *N*-terminus). [35]

### 1.2.4.2 Preferred Residues Within an α-Helix

Certain amino acid residues play particular stabilising or destabilising roles depending upon their position within the helix. This position can be roughly described as internal (residues midway through the peptide sequence) or terminal (residues near the ends of the helix); residues which are favoured at the termini may be disfavoured internally, and *vice versa*.

Favoured internal residues often have hydrophobic side-chains. These side-chains can cluster together preventing access of water which may disrupt hydrogen bonding in the helix backbone. These hydrophobic interactions are thought to be extremely important in determining helix stability. [36-39] Alanine is particularly suitable; it can provide some hydrophobic stabilisation but lacks a γ-carbon, so less entropy is lost when its side-chain packs together with others. [40-42] The least favoured residues internally are proline (see Section 1.3.1.2, p18) and glycine. Glycine cannot contribute to hydrophobic interactions, and also loses more entropy when its dihedral angles are fixed to α-helical values, since it is more flexible. [36, 43]

Propensity for positioning at one of the helix termini is a quite distinct parameter. Preference for these positions depends primarily on the fact that the last 4 NH groups at the *N*-terminus and the last 4 CO groups at the *C*-terminus lack hydrogen bond partners within the backbone. Therefore, residues near the termini are preferred if their side-chains are able to supply hydrogen bond partners for unpaired main-chain NH and CO groups. [44]

Residues with high *N*-cap propensities (*i.e.* those which often appear in the last *N*-terminal turn) tend to possess small polar side-chains (*e.g.* Asn, Ser, Thr) which can provide hydrogen bonds to unsatisfied main chain NH groups. [42, 44-48] For the same reason, these residues tend to be rather less common deeper within a helix, since their hydrogen bonding capabilities may disrupt essential backbone hydrogen bonds.

Residues with negatively charged side-chains (Asp, Glu) are also favoured at the *N*-terminus, since they can interact with the positive end of the helix macrodipole, as well as providing a hydrogen bond to an *N*-terminal amide proton which lacks a hydrogen bond partner. [42, 44-51]

Glycine is common at the *N*-terminus because of its small size; lacking a side-chain, it does not impede the access of solvent which can also form hydrogen bonds with unsatisfied main-chain donors. [42, 44-50]

Proline is another special case. Whilst highly disfavoured further into a helix, it fits very well into the first *N*-terminal turn because its torsion angle $\phi$ is fixed to an $\alpha$-helical value ($\phi = -60$ °), and it requires no hydrogen bonding partner since it is devoid of an NH group (see Section 1.3.1.2, p18). [52]

Although there is again a bias towards small, polar residues in the last *C*-terminal turn, the most significant preference is for glycine as the penultimate *C*-terminal residue in $\alpha$-helices within proteins. $\alpha$-Helices often end at the *C*-terminus with short sections of $3_{10}$ helix involving $(i + 3) \rightarrow i$ hydrogen bonds (see Section 1.2.5, p15). These sections are often followed by a residue in the *left-handed* $\alpha$ -helical conformation ($\alpha_L$) (of lowest energy for glycine and asparagine), which forms a

"π-turn", with an $(i + 5) \rightarrow i$ hydrogen bond. This so-called *Schellman Motif* forces termination of the α-helix. [8, 37, 45, 53, 54]

Charged residues are also commonly found near *C*-termini of helices. At this position, the side-chains carry positive charges (Lys, Arg, His) which interact favourably with the negative end of the helix macrodipole. [47, 51, 55] This stabilising effect was demonstrated in a model study by Gellman *et al.* [56] Schneider and DeGrado showed that *C*-capping auxiliaries supplying a cationic group and hydrogen bond donors for carbonyl groups at the helix *C*-terminus imparted a significant helix-stabilising influence. [57]

### 1.2.4.3 Helix-Stabilising Pairs and Groups of Residues

In addition to the propensities of individual residues for various positions in a helix, certain pairs or groups of residues in defined positions relative to one another impart stability, a consequence of favourable side-chain → side-chain interactions. These residues are usually placed three or four positions apart in the helix. This brings the interacting side-chains close to one another on the same face of the helix (Fig. 1.8).

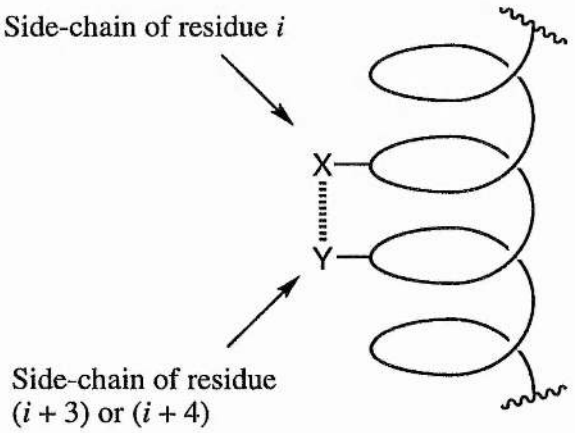



**Figure 1.8:** *Stabilising interactions between side-chains in an α-helix*

These interactions may be hydrophobic or electrostatic in nature. Various non-polar side-chains can pack together well [*e.g.* $(i, i + 4)$ = (Tyr, Leu)]. The stabilisation imparted depends on the loss of entropy involved, and also on tertiary interactions with other parts of the protein. [38, 50]

Salt bridges between oppositely charged side-chains [*e.g.* $(i, i + 4)$ = (Glu, Lys)] are often found in stable helices, [50, 58, 59] as are side-chain → side-chain hydrogen bonds [*e.g.* $(i, i + 4)$ = (Phe, His) [60] or (Gln, Asp), [61] the former utilising the aromatic ring as the hydrogen bond acceptor from the NH of the histidine side-chain].

Groups of residues of similar polarity are often found on one face of a helix, enabling non-polar side-chains to cluster on the hydrophobic face (often buried in the protein interior), and polar side-chains to cluster on the hydrophilic face (often interacting with solvent) (Fig. 1.9). Such helices are termed amphiphilic or amphipathic helices. [62]

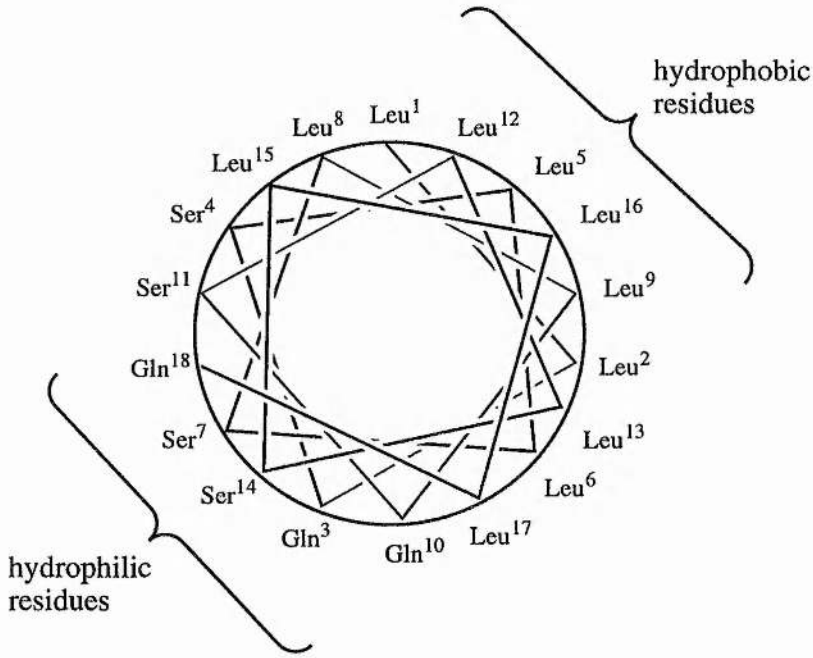



**Figure 1.9:** *Helical wheel representation of a designed amphiphilic α-helix*

Peptides designed to be amphiphilic have been shown to be α-helical in the presence of micelles, [63, 64] because the hydrophobic face present in the helical

conformation can be shielded from the solvent upon interaction with the micelle. This stabilisation of structure is thought to be analogous to the binding of peptide hormones to membrane-bound receptors. [65, 66]

### 1.2.4.4 Calculation of Helix Stability

An algorithm for the calculation of helix stability relative to the random coil state was first suggested by Zimm and Bragg in 1959 [67] and by Lifson and Roig shortly afterwards. [68] Using the Zimm-Bragg parameters, helix stability is related to an initiation constant sigma ($\sigma$), which reflects the probability of aligning the first three residues in an $\alpha$-helical conformation. Without stabilising hydrogen bonds, this is a highly disfavoured process, due to repulsive interactions between the aligned dipoles and the loss of entropy, [39] so $\sigma$ generally takes very low values of $<10^{-3}$, where a value of 1 would indicate that the first three residues were fixed in an $\alpha$-helical conformation. The other important Zimm-Bragg parameter is the propagation constant *s*, which reflects the likelihood of an amino acid residue adopting $\alpha$-helical ($\phi$, $\psi$) values when added to the end of a pre-existing helix. There is a different *s* value for each amino acid, and the value changes depending on the position within the helix (since some residues are more likely to be found at the helix termini, for example). Values for *s* of >1 indicate helix-*making* amino acids (*e.g.* Ala), values <1 indicate helix-*breaking* residues (*e.g.* Pro).

Values of *s* have been determined experimentally, [62, 69] theoretically [39] and by analysis of protein crystal structure databases. [37, 49] Together, these Zimm-Bragg parameters $\sigma$ and *s* give a useful quantitative indication of the helical propensities of each amino acid, which is important in understanding protein folding and structure.

### 1.2.4.5 Prediction of Peptide and Protein Secondary Structure

The secondary structure of a peptide or protein is a function of the primary structure, but accurate prediction of the secondary structure of a given sequence is still difficult. A number of reasonably reliable methods are available, the most widely used being the rules developed by Chou and Fasman. [70] These rules classify each amino acid as favouring either α or β structures, as determined from analysis of protein crystal structure databases and *s* values for each amino acid. An algorithm based on the number of each type in the sequence determines whether the peptide is likely to form an α-helix or β-structure in a protein-like environment. This method gives a 60–70% success rate for prediction of the correct structure type, and is a useful guide in design of peptides where particular secondary structures are required, though it is intrinsically unable to take into account important longer range interactions which also have a crucial effect on the assumed structure.

### 1.2.4.6 α-Helices in Short Peptides

In general, the α-helix is not a stable element of structure in short peptides in aqueous solution, and peptides of fewer than about 20 residues usually exist as random coils. This is due to the competition for backbone hydrogen bonds by solvent, the low value of the initiation constant σ, and the lack of specific helix-stabilising interactions.

However, there are several examples of short peptides which do show α-helical structure in aqueous solution. The first of these to be studied in depth were the S-peptide and C-peptide, which are both part of an α-helical segment from the enzyme ribonuclease A. [55, 71, 72] Baldwin *et al.* later showed these structures to be stabilised by charge-dipole interactions [51] and salt bridges. [59] Bombolitin I, a 17-residue peptide isolated from bumble-bee venom, is predominantly α-helical in the presence of micelles, [63] and molitin, a 22-residue gastrointestinal peptide hormone, is stabilised as an α-helix by charge-dipole interactions and salt bridges. [29] A variety of other peptide hormones also show α-helical structure during interactions with receptors. These

peptides rely on amphiphilicity for their structural stability when associated with non-polar surfaces. [65, 66]

In fact, virtually all short peptides which show observable helical structure in aqueous solution owe this feature to stabilisation contributed by amphiphilicity, charge-dipole interactions, salt bridges or involvement of helix-fortifying residues. [73] These factors have been used in the *de novo* design of helical peptides, *e.g.* the 17-residue peptide of Marqusee and Baldwin which contains three (Glu $\rightarrow$ Lys) salt bridges, charged groups of appropriate sign at both termini, and a high proportion of helix-stabilising alanine residues; [58] and the 18-residue peptide of Zhou *et al.* which combines charge-dipole effects, salt bridges, high alanine content and amphiphilicity to generate helicity. [62]

Although it is possible to synthesise helices by use of these stabilising forces, rigorous rules must be followed and there is consequently little opportunity for structural variation when using the proteinogenic amino acids.

### 1.2.5 Other Helix Types

The right-handed α-helix is the most stable helical form for oligomers of L-amino acids, but other forms do exist. A π-helix is possible, incorporating $(i + 5) \rightarrow i$ hydrogen bonds, but this requires distortion of backbone dihedral angles. [66] Polymers of proline form helices which are not stabilised by hydrogen bonds. These are of two types; the PP I form contains all *cis* amide bonds whilst the PP II has all *trans* amide bonds. These helix types are still accessible when some of the residues are not proline, and may contribute to the bound form of some peptide ligands. The structural protein collagen adopts a PP II conformation in each of its three constituent strands. [74-76]

Oligomers of β-amino acids [$H_2NCH(R)CH_2CO_2H$] show stable $3_{14}$-helical structure in solution, which may switch to other helical forms under certain conditions. [77-79]

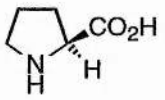
The only helix besides the α form which is of appreciable significance in peptide and protein structure is the $3_{10}$ helix, which has $(\phi, \psi) = (-60°, -30°)$, and an $(i + 3) \rightarrow i$ hydrogen bonding pattern, equivalent to an infinite series of type III β-turns. This is closely related to the α-helix, and only small alterations are required to interconvert the two forms. [73, 80-83] The 9:1 ratio of α:$3_{10}$ helix observed in protein structures in addition to theoretical calculations shows that the α-helix is the more stable of the two forms for proteinogenic amino acids. [40] In mammalian systems, the $3_{10}$-helix is only found in short stretches, [84] particularly at the *C*-termini of α-helices. [8]

## 1.3 Structurally Important Amino Acids

### 1.3.1 Proline

#### 1.3.1.1 Introduction

Proline **6** is unique amongst proteinogenic amino acids because its amino group is secondary, a consequence of the side-chain cyclising back on to the nitrogen atom to create a pyrrolidine ring. This feature has direct consequences on the conformations available to this residue. The torsion angle $\phi$ is fixed to values close to $-60°$ (corresponding to an $\alpha$-helix), and the angle $\psi$ is restricted and can only assume values in two fairly narrow ranges around $-40°$ and $+140°$. These values of $\psi$ correspond to $\alpha$-helical and $\beta$-structure respectively, [23, 76, 85] and are fairly equally populated throughout peptide structure, depending on the nature of adjacent residues.




**6**

The pyrrolidine ring itself can exist in one of two conformations. These both have the $C^{\delta}$, N, $C^{\alpha}$ and $C^{\beta}$ atoms nearly co-planar, with the $C^{\gamma}$ atom displaced either to the same side as the carbonyl group ($C^{\gamma}$-endo) or the opposite side ($C^{\gamma}$-exo) (Fig. 1.10).


$C^{\gamma}$ *endo* $C^{\gamma}$ *exo*

**Figure 1.10:** *Pyrrolidine ring pucker in proline residues*

Theoretical calculations suggest that the $C^{\gamma}$-exo form is slightly more stable than the $C^{\gamma}$-endo form, by about 1 kJ $mol^{-1}$. [86]

### 1.3.1.2 Structural Role of Proline

The cyclic, *N*-alkylated nature of proline has profound effects on the types of secondary structures it can adopt. In peptides, its lack of an amide proton means that it cannot participate as a hydrogen bond donor in repetitive structural motifs. This fact, along with the unfavourable steric interactions associated with the pyrrolidine ring, and the constraint of the $\phi$ angle to $\alpha$-helical values, indicates that proline is highly disfavoured in $\beta$-sheets. [23, 64]

Its role in $\alpha$-helical structure is more complex. It is certainly significantly disfavoured in the middle of a helical sequence, because it disrupts the hydrogen bonding within the backbone, and the pyrrolidine ring does not fit well into a helical structure, [23, 45, 52, 76] although it *can* be accommodated with a 20–30° bend in the direction of the helix axis, as demonstrated by the helix in Fig. 1.7. [43, 64, 87]

However, proline is actually *favoured* in the first *N*-terminal turn of a helix, and 89% of all proline residues in $\alpha$-helices are found here. [23] In this position, its lack of an NH group is advantageous, since it would otherwise lack a hydrogen bonding partner. It also prevents propagation of the helix in the *N*-terminal direction. The $\phi$ angle is already fixed to an ideal $\alpha$-helical value, so proline loses less freedom of movement when constrained in the first turn of the helix. [23, 42, 45, 52] The carbonyl group of the residue directly preceding proline is more basic than for any other residue, since it is involved in a tertiary amide bond, and therefore forms stronger hydrogen bonds to stabilise the first turn of the helix. [88]

The nature of the tertiary amide bond linking proline to the preceding residue has the additional consequence of imparting a relatively high polarity to these regions in peptides. Whilst also being another factor acting against burial within a helix, this results in proline residues often taking up positions at the surfaces of proteins, in loops and turns. [32] Proline's propensity to be involved in hydrogen bonding schemes and its constrained nature also lend themselves to these elements of secondary structure. [64, 89] In particular, there are many examples of peptides containing proline which adopt $\beta$ or $\gamma$-turn structures. [11, 15-20, 82, 85, 90]

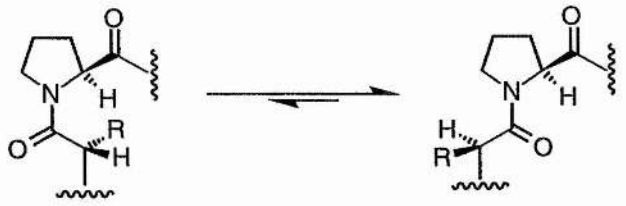
### 1.3.1.3 Effect of Proline on Neighbouring Residues

The presence of a proline residue also constrains the conformations available to the adjacent residues within the peptide, especially that directly preceding it in the sequence. Steric clashes are possible between the pyrrolidine ring and the *N* and $C^{\beta}$-atoms of the preceding residue, if the preceding residue attempts to adopt an α-helical conformation. Consequently, its torsion angles are more likely to take up values corresponding to β-structure, again disfavouring formation of an α-helix in the vicinity of the proline residue. [16, 76]

The α-helical geometry of the residue prior to proline is almost always found when proline is also in the α-form and both are part of an α-helix with hydrogen bond stabilisation rendering the otherwise inaccessible structure feasible. [23]

### 1.3.1.4 *cis-trans* Isomerisation of Prolyl Peptides

The amino acids in peptides are usually linked *via* secondary amide bonds. These are resonance stabilised and therefore have partial double bond character. Consequently, *cis* and *trans* isomers are possible. These interconvert with an energy barrier of ~90 kJ $mol^{-1}$, which corresponds to the resonance stabilisation of the amide bond. [86] The *cis* form is destabilised by steric interactions, and the *trans* form is more stable by ~11 kJ $mol^{-1}$. Therefore, less than 0.1% of secondary amide bonds are found in the *cis* form; [23, 86] these tend to occur in cyclic peptides where the *trans* form is disfavoured, [91-93] or where there are other external forces influencing the equilibrium. [63]




*cis* Xaa-Pro *trans* Xaa-Pro

**Figure 1.11: Cis-trans *isomerisation of Xaa-Pro peptides***

The amide bond directly preceding proline is tertiary in nature, which leads to differences in the *cis-trans* equilibrium process (Fig. 1.11). The *trans* form is now also destabilised by steric interactions involving the pyrrolidine ring, so the energy difference between the two configurations drops to only 1–6 kJ $mol^{-1}$. [86, 94, 95] As a result, this amide bond has a much increased frequency (10–40%) of being found in the *cis* form. [23] The rate of isomerisation between the two forms is also increased, because the tertiary amide bond is longer (1.36Å *versus* 1.33Å for a secondary amide bond); the energy barrier for interconversion drops to 75–80 kJ $mol^{-1}$. [23, 86, 96, 97]

#### 1.3.1.5 Factors Affecting Thermodynamics and Kinetics of *cis-trans* Isomerisation

The rate of isomerisation and the position of equilibrium for the process is dependent on several factors:

- a bulky side-chain on the residue preceding proline slows the process, [23] and may also affect the ratio. [86] If the residue following proline carries an aromatic group on its side-chain, the amount of *cis* isomer is increased due to a stabilisation of the *cis* form and/or a destabilisation of the *trans* form. [98]

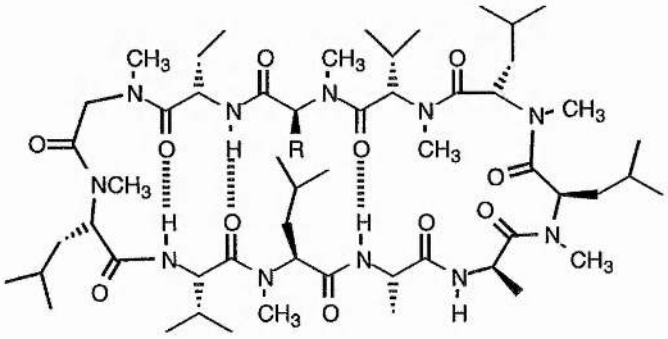
- if substituents are incorporated into the pyrrolidine ring, the ratio is affected. α-Methylproline peptides show no *cis* isomer at all, [99] whilst δ-methylproline shows comparable amounts of *cis* and *trans* isomers. [100, 101] Electron-withdrawing substituents increase the rate of isomerisation by promoting pyramidalisation of the nitrogen atom, which reduces the double bond character of the amide bond. [100, 102]

- the solvent has some effect on the rate, which is slower in aqueous solution than in organic solution, since water interacts more strongly with the more

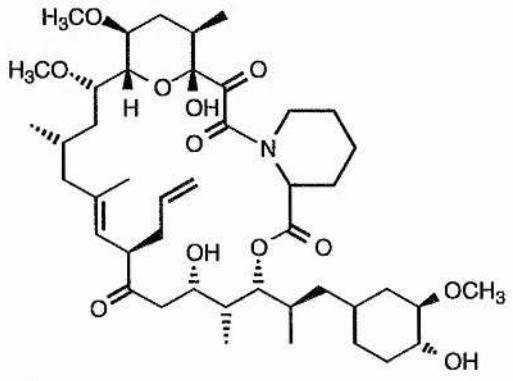
polar planar ground state forms, thereby increasing the activation energy. [97, 103-105] Rich *et al.* showed that addition of lithium chloride to trifluoroethanol solutions of peptides containing tertiary amide bonds greatly increases the amount of *cis* isomer to ~70%. [106]

### 1.3.1.6 Peptidyl-Prolyl *cis-trans* Isomerases

The rate of isomerisation of the tertiary amide bond preceding proline is increased *in vivo* by a group of enzymes known as peptidyl-proline *cis-trans* isomerases (PPIases), the first enzymes found to be specialised for catalysis of conformational changes. [107] These enzymes exist because the uncatalysed rate is not fast enough to meet the biological demands of protein folding. [89, 104, 107-109]



Cyclosporin A **7** R=



FK506 **8**

The PPIases are also known as immunophilins, [107, 110] since they are binding proteins for immunosuppressant drugs. The immunophilin cyclophilin binds the immunosuppressant cyclosporin A **7** whilst FKBP binds FK506 **8**. [107, 111] Therefore, PPIases have sparked considerable interest as the targets for potential therapeutic agents.

### 1.3.1.7 Mechanism of *cis-trans* Isomerisation

The non-enzymatic process has been the subject of several mechanistic studies, one of the most important conclusions being that the activation barrier is almost entirely enthalpic, with no significant entropy of activation. [103, 111] This suggests a unimolecular reaction with no involvement of solvent, presumably proceeding *via* a transition state with a twisted amide bond (Fig. 1.12). [111, 112]

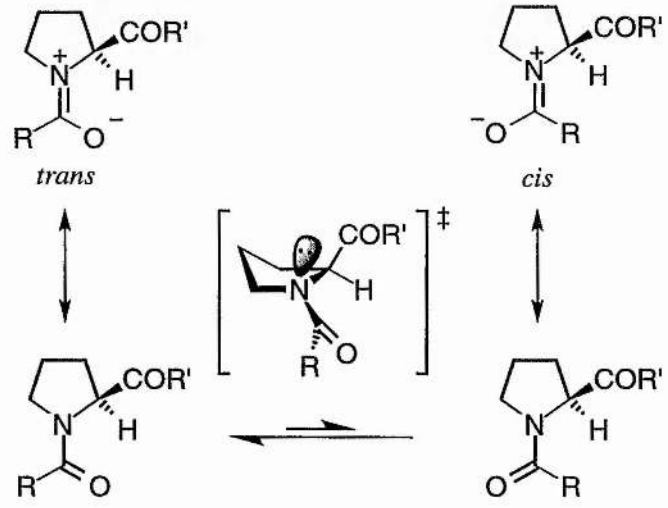



**Figure 1.12:** *Mechanism for prolyl isomerisation* via *a transition state with a twisted amide bond*

This theory is supported by the fact that the rate is increased by organic solvents, which can interact more favourably with the transition state than with the more polar ground states. [97]

The mechanism changes at the extremes of pH. In alkaline solutions, a tetrahedral adduct may be formed by attack of hydroxide at the amide carbonyl, removing double bond character and increasing the rate of rotation about the amide bond. In acidic solution, the rate also increases, due to *N*-protonation which again destroys resonance stabilisation. [103, 111]

Exactly how the PPIases lower the activation barrier is still not certain. The transition state was originally proposed to be a tetrahedral adduct formed with thiol groups in the enzyme active site, but further research showed that no such groups were present. [103] It now seems more likely to be catalysis by distortion, where the less polar transition state structures are stabilised in a non-polar pocket in the enzyme active site. This effect may be enhanced by hydrogen bonding to the prolyl nitrogen atom which further enhances its $sp^3$ character. [103, 111, 113] This effect has been demonstrated in a model peptidomimetic study. [114] The immunosuppressant drugs cyclosporin A **7** and FK506 **8** are thought to act by incorporating substructures which mimic a twisted amide bond, thus acting as transition state analogues. [110]

#### 1.3.1.8 Dynamic Role of Proline

In addition to its importance in structural aspects of peptides and proteins, the *cis-trans* isomerisation process which accompanies prolyl peptides is thought to have a role in dynamic events. Foremost amongst these is the part it plays in protein folding, being the rate-limiting step in the folding pathways of many proteins. [16, 43, 86, 109, 115-117] In addition to its presence in structures which might initiate folding (beginning of α-helix, β-turns), this dynamic role underlines the central role that proline plays in the mechanisms of protein folding.

Even though it is a fairly polar residue, proline is found fairly frequently in transmembrane helices. This may be because it serves an active role; isomerisation between the *cis* and *trans* forms can exert a transient "pull" on the local protein segment, thus acting a switch. This could be involved in the function of membrane proteins, perhaps by opening and closing transport channels. [23, 32, 64, 86, 87, 118]

### 1.3.1.9 Biologically Active Peptides Containing Proline

Many biologically active peptides contain one or more proline residues, including corticotropin, [119] angiotensin II **1**, [120] bradykinin **2** [26, 85, 98] and oxytocin **3**. [5] Its prevalence may be due to the constraints that its structure imposes upon these hormones and neurotransmitters. Being more constrained, the peptides can bind more effectively to their receptors, [76] and are less likely to adopt peptidase-bound conformations, resulting in increased stability and bioavailability. Peptidases frequently will not cleave *cis* peptide bonds, another advantage conferred by the presence of proline. [89]

The most abundant protein in vertebrates, collagen, is also rich in proline and hydroxyproline residues which reinforce its stable structure. [121, 122]

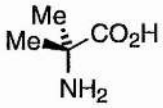
Proline is not the only *N*-alkyl amino acid found in biological systems. *N*-Methyl residues are prevalent in microbial peptides such as the fungal metabolite cyclosporin A **7**. These have similar structural effects to those of proline, although thay lack the constraint on $\phi$, so are somewhat more flexible. [123] *Cis-trans* isomerisation also occurs in peptides containing *N*-methylated residues. [97, 123, 124]

### 1.3.2 $C^{\alpha}$-Disubstituted Amino Acids

The other common modification of amino acids besides *N*-alkylation is $C^{\alpha}$-alkylation. This severely limits the conformational freedom of the residue, compressing the internal angle so that folded structures such as helices and turns are highly favoured over extended structures. [81, 125]

By far the most widespread of these amino acids is α-amino*iso*butyric acid (Aib, **9**). The dihedral angles for this residue are fixed to helical values, but its lack of chirality means that both right and left-handed helices are of equal energy for Aib oligomers. [73, 126, 127] These form fully developed $3_{10}$-helices at the octamer level, and show high helicity even with only 4-7 residues in aqueous solution. [73, 81, 83, 125, 126, 128-132] When present in peptides containing L-amino acid residues, the right handed helical forms are promoted. [131] α-Helices are preferred in

these cases for peptides containing 9 or more residues, and for those with < 50% Aib content. Aib also favours type III β-turns (which are equivalent to a four-residue section of $3_{10}$ helix). [26, 133]




**9**

Aib is commonly found in the pentaibol family of naturally occurring antibiotics which are produced by microbial sources. These alter the permeability of biological membranes by forming channels for ion transport. [73, 81] The presence of these structure-stabilising residues helps to direct folding of these molecules without other longer range forces, and may also be important in reduction of the entropic cost of receptor binding.

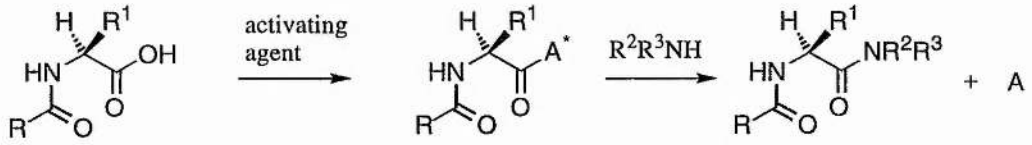
Other $C^{\alpha}$-disubstituted amino acids have come under study. Being chiral, (2*S*)-α-methylvaline was found to favour right-handed helical forms. [81, 129] α-Methylglutamic acid, α-methylaspartic acid and α-methylproline were all found to promote β-turns in short sequences. [127, 134]

## 1.4 Synthesis of Peptides Containing Hindered Residues

### 1.4.1 Difficulties Encountered in Coupling Hindered Residues

The synthesis of biologically active peptides and pseudopeptides such as cyclosporins [135, 136] and didemnins [137, 138] is an important area of research in the development of potential therapeutic agents. As discussed, many of these compounds contain one or more unusual residues which are *N*-methylated, $C^{\alpha}$-disubstituted and/or β-branched. These classes of amino acid share a trait with the proteinogenic amino acid proline; they are significantly more sterically hindered than other proteinogenic amino acids. Consequently, formation of amide bonds involving such residues can be problematic. [139, 140]

In these reactions, amide bonds are synthesised from an *N*-protected segment with a free carboxyl group, and a *C*-protected segment with a free amino group. The free acid is converted to an active species, usually by action of a *peptide coupling reagent.* This active species is used in the acylation of the amine, forming the amide bond with the release of a good leaving group (Scheme 1.1).




**Scheme 1.1:** *General mechanism of formation of an amide bond*

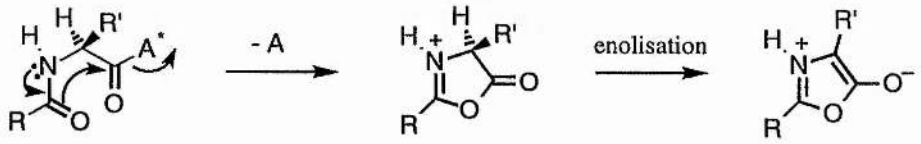
During the acylation of hindered residues, the presence of more than one substituent on the *N*, $C^{\alpha}$ or $C^{\beta}$ atoms appreciably diminishes the reaction rate. Whilst this in itself may only cause the minor inconvenience of a prolonged reaction, in some cases side reactions and racemisation processes which would otherwise be too slow to cause problems become competitive.

The major concerns associated with these hindered residues include:

- *Racemisation*. The loss of chiral integrity at the α-carbon of *N*-protected residues involved in amide bond forming reactions generally occurs by one of two mechanisms, *enolisation* or *via* an *oxazolonium salt.*

  Enolisation is not usually a problem, since most *N*-protected residues possess an amide proton which is more acidic than the α-proton and will be removed first. However, for *N*-alkyl residues there is no amide proton and therefore enolisation is more likely.

  The oxazolonium salt **10** is produced by a side-reaction from the activated species (Scheme 1.2). This species can then undergo racemisation by enolisation (giving the mesoionic oxazolone **11**) or tautomerisation. [139, 140]



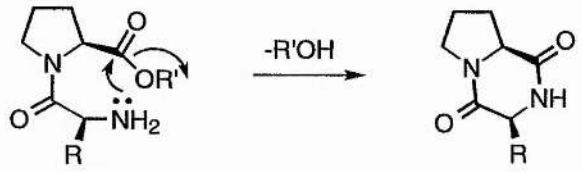

**10** **11**

activated species — oxazolonium salt — pseudoaromatic mesoionic oxazolone

**Scheme 1.2:** *Formation of oxazolone species during amide bond formation*

Urethane protection is an effective way to reduce the degree of racemisation because urethanes are less readily converted to oxazolonium salts. This is a result of the reduced double bond character of the urethane bond, which results in reduced nucleophilicity of the carbonyl oxygen. [96, 139, 140] Fortunately, proline suffers from few of these undesirable side-effects, because the addition of another contiguous ring on to the pyrrolidine ring generates cyclic strain. Therefore, formation of an oxazolonium salt is only possible under highly forcing conditions (*e.g.* on treatment with perchloric acid). [139, 140] Consequently, proline is resistant towards racemisation.

- *Dioxopiperazine Formation.* This is a potentially serious problem when coupling a *C*-protected dipeptide which has an *N*-alkyl residue at the *C*-terminal position, as shown in Scheme 1.3.




**12**

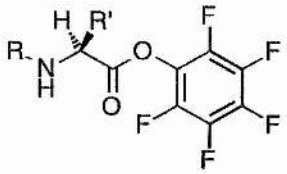
**Scheme 1.3:** *Dioxopiperazine formation from a dipeptide ester*

The greatly increased likelihood of a *cis* amide bond in such peptides facilitates cyclisation to form a six-membered dioxopiperazine ring **12**, which possesses two *cis* amide bonds. The rate of this process may not be fast enough to compete with intermolecular amide bond formation, but if the *N*-terminal residue is also *N*-alkylated or if the activated acid is hindered it can become an important consideration. [139, 140]

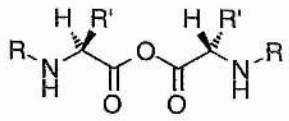
### 1.4.2 Peptide Coupling Reagents

Many of these undesirable processes would diminish in importance if a particularly effective acylating agent could be produced. With this goal in mind, a wide range of new reagents has appeared in the last 10-15 years specifically for reactions involving hindered residues. These are usually tested using the problematic residues Pro, *N*-methyl Ala, Aib and *N*-methyl Aib. [139, 140 141]

The older methods such as formation of mixed anhydrides *via* action of alkyl chloroformates, [139, 142] or use of carbodiimides [141] are often ineffective. Rather more successful as the acylating agent are pentafluorophenyl esters **13**, [139, 142] symmetrical anhydrides **14**, and pivalic mixed anhydrides **15**. [139, 141]



**13**



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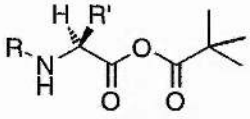
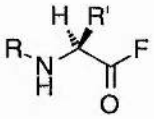


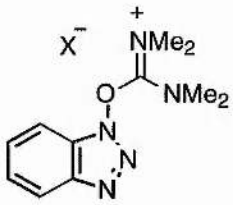
**15**



**16**

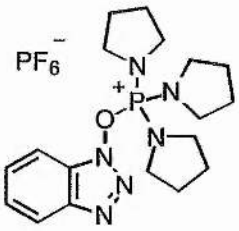
Recently, Carpino has developed the use of acid fluorides **16** as highly active, stable acylating species. [143] They are able to perform acylations of the extremely hindered *N*-methyl Aib residue with reasonable efficiency, [143, 144] and appear to be superior to the less stable acid chlorides which are also used occasionally. [145-147]

Perhaps the most widely used families of reagents used in modern peptide synthesis are the uronium and phosphonium salts which include HBTU **17**, TBTU **18** and pyBOP **19**. [148, 149]



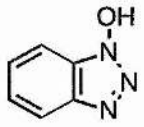
X = $PF_6$ **17**

X = $BF_4$ **18**

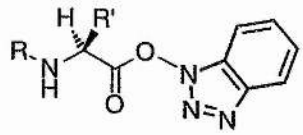


**19**

These reagents initially react with the carboxyl group to produce an active oxyuronium or oxyphosphonium species.

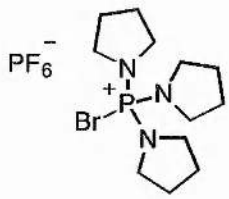


**20**



**21**

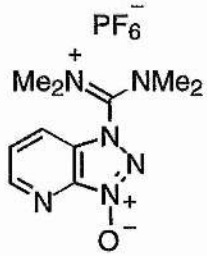
Hydroxybenzotriazole **20** or its anion is also present in the solution when these reagents are used, and this is thought to react with the oxyuronium or oxyphosphonium salt to give an oxybenzotriazolyl ester **21** which is the actual acylating agent for the amine. [141, 149-151] Unfortunately, this is fairly stable and often lacks sufficient reactivity in difficult couplings. Noting this deleterious effect, Coste *et al.* introduced analogues which do not incorporate a hydroxybenzotriazole group, such as pyBroP **22**. [149]



**22**

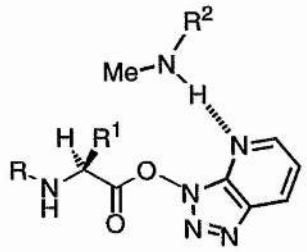
These reagents avoid formation of the benzotriazolyl ester, and as a consequence, pyBroP is preferable to pyBOP for difficult couplings. [136, 151]

The latest advance in this reagent family was the development of HATU **23**, incorporating an azabenzotriazole group, by Carpino *et al.* in 1993. [152]

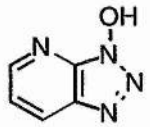


**23**

This gives increased reaction yields and a decrease in racemisation, even for very hindered couplings. [134, 136, 140, 153, 154] Its efficacy was suggested to be due to a favourable complex **24** between the azabenzotriazolyl ester and the incoming nucleophile, which brings it into closer proximity to the reaction centre.



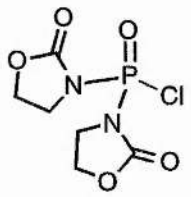
**24**



**25**

Such active esters can also be produced by using 7-azabenzotriazole **25** in conjunction with other coupling reagents such as carbodiimides. [136]

Finally, mention should be made of BOP-Cl **26**, a phosphinic chloride reagent first used for the formation of peptide bonds in 1985 by Rich *et al.* [139, 155, 156] This activates the carboxyl component by formation of a mixed phosphorus anhydride.



**26**

This reagent is best used with secondary amines, since it has a tendency to react with primary amines in preference to forming the anhydride. BOP-Cl is effective in sterically congested couplings and was used in a synthesis of cyclosporin A **7**, [135, 157] though it does not tolerate bulky protecting groups or β-branched residues particularly well. [140]

## 1.5 Peptidomimetics

### 1.5.1 Introduction

The wide range of effects exerted by peptides suggests potential applications in the therapy of many disorders. This has proved to be possible in several cases, the best known of which is the administration of insulin, a two-strand 51 residue peptide, for the treatment of diabetes.

Unfortunately, the medical application of peptides as drugs has a number of limitations:

- they cannot usually be administered orally, since they are rapidly degraded in the stomach and gut and are poorly absorbed into the bloodstream, a consequence of their relatively high molecular mass and the lack of membrane transport systems;

- they are *conformationally flexible*. Their ability to adopt many different conformations in solution means that they are capable of binding to several different receptors, which can result in undesirable side-effects;

- the *structure-activity relationship* is not known in most cases. That is, the precise 3D conformation of the peptide which binds to the receptor remains a mystery. In some cases, this has been determined by NMR or X-Ray studies of ligand-receptor complexes, but these experiments are difficult and lengthy processes. Far more progress has been made in NMR studies of unbound peptides, [18, 85, 98, 119] although any stable structure found in solution does not necessarily reflect the bound conformation.

An enormous research effort has gone into finding ways of overcoming these problems. [6, 158] Some approaches use the peptides themselves, but in a "masked"

form which veils their true nature and increases bioavailability. For example, Bodor and Buchwald [159] have synthesised a series of derivatives of neurotransmitter peptides such as enkephalins which can be transported across the blood-brain barrier into the brain, a process highly disfavoured for the peptides themselves. This was achieved by disguising the peptides by protection with large alkyl groups, which rendered them lipophilic enough to cross the membrane. Once there, endogenous brain enzymes are able to cleave the protecting groups to release the active neurotransmitter or hormone.

Whilst "smuggling" peptides into a target organ in this manner is a promising area of study, most efforts to overcome the innate problems of peptides as drugs focus on the design and synthesis of *peptidomimetics*, non-peptide molecules designed to simulate the biological action of endogeneous peptides. [1, 4] Peptidomimetics should possess biological activity equal to or greater than that of the mimicked peptide, whilst lacking the aforementioned disadvantages inherent in the use of peptides as drugs.

This is universally achieved by designing *conformationally constrained* analogues which simulate the receptor bound conformation as shown in Fig. 1.13.

If the bioactive conformation is correctly mimicked, this approach offers several merits:

- *increased receptor affinity*, since the bound conformation is fixed in the mimetic and entropic losses are greatly reduced;

- *increased stability to peptidases*, which may be unable to bind this fixed conformation;

- *minimal side-effects*, since other receptors which trigger undesirable responses are unable to bind the mimetic. [1, 4]

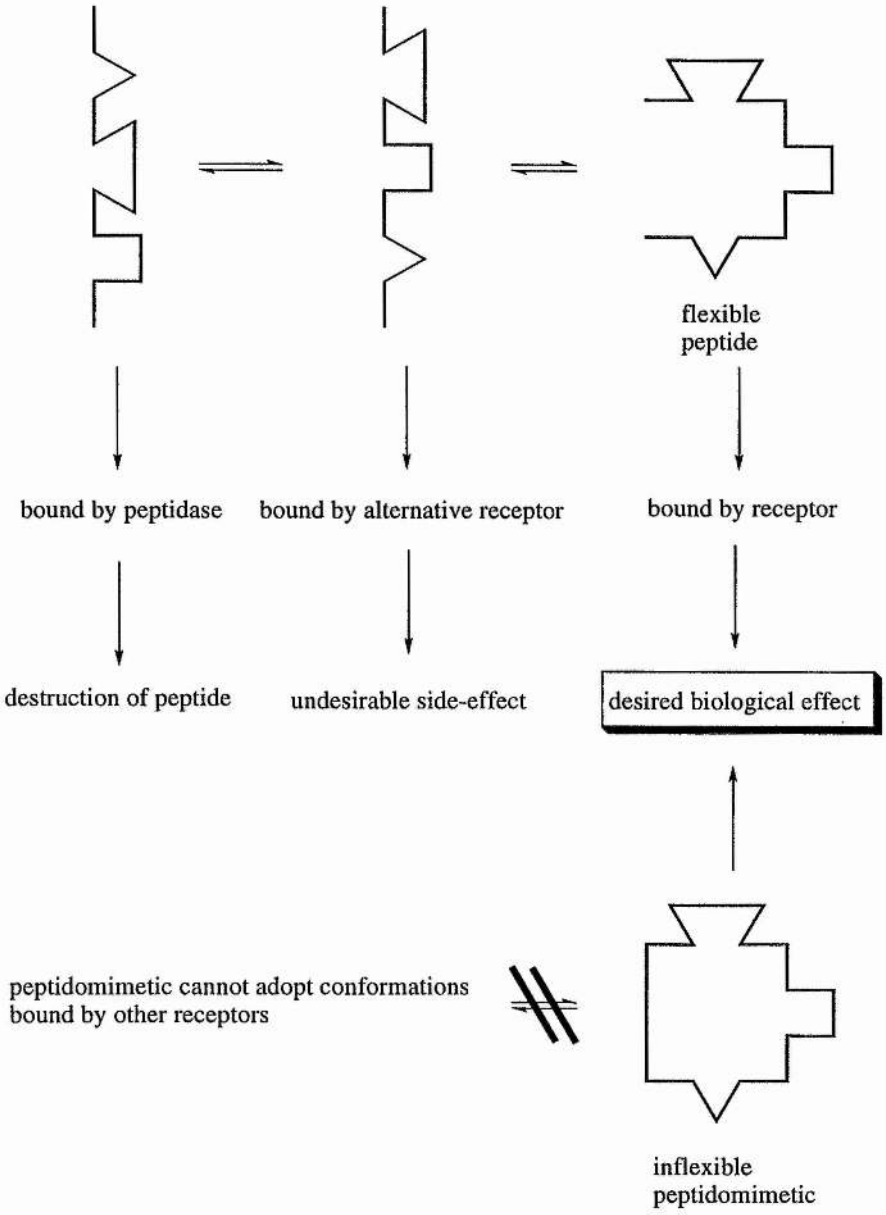
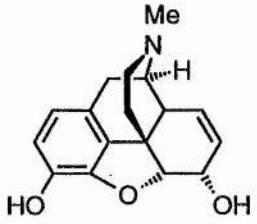



**Figure 1.13:** *Comparison of the biological fates of a peptide and a constrained peptidomimetic*

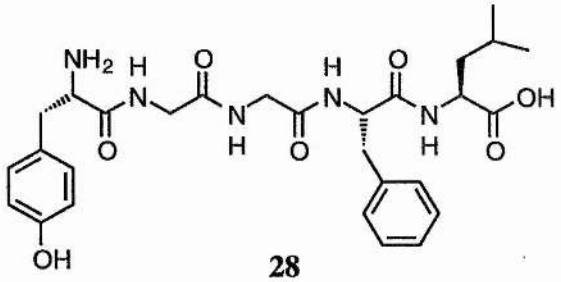
Such a peptidomimetic needs to supply the essential pharmacophoric elements from the primary sequence of the peptide in the correct three-dimensional positions. [160] Knowledge of the biologically active conformation of the parent peptide would be a great advantage for this design, but is not usually known. Therefore,

peptidomimetic design is often based on the *predicted* stable structure of the endogeneous peptide, which may be calculated from the Chou-Fasman rules. [70]

Peptidomimetics cover a wide range of structural types, varying from those which differ from the parent compounds only by incorporation of a few unusual amino acids, to those which bear no readily apparent resemblance to the peptide whatsoever. One such example is morphine **27**, which is a completely non-peptidic mimetic of endogenous opioids such as Leu-enkephalin **28**. [1]



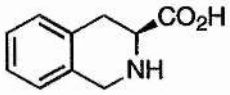

**27**




**28**

### 1.5.2 Constraints at the Amino Acid Level

Incorporation of more highly constrained analogues of naturally occurring amino acids into peptides can exert a significant influence over the conformation adopted, perhaps stabilising a certain element of secondary structure. This has already been discussed in the area of naturally occurring $C^{\alpha}$ disubstituted and *N*-methylated residues (see Section 1.3.2, p24). Other changes are possible; substitution of D for L residues favours reverse turns, and increases stability towards peptidases. Incorporation of any of the above residue types alters the conformation of the *backbone*; other amino acids have been designed for control of the *side-chain* conformations, or *topology* of the peptide. [4] Such control has been exerted by Hruby *et al.* using cyclic amino acids such as 1,2,3,4-tetrahydroisoquinoline-3-carboxylate (Tic, **29**), an analogue of phenylalanine with a constrained side-chain, [6] or amino acids with β-substitution and/or substitution on aromatic rings. [161-165]

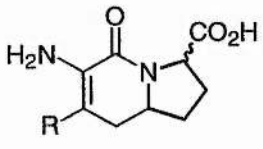


**29**

These substitutions severely restrict available values of the side-chain dihedral angles $\chi_1$ and $\chi_2$. Certain side-chain topologies may be present in the bound states of bioactive peptides, and this has been demonstrated by the incorporation of such residues into analogues of endogenous opioids which have high affinity and selectivity. [4, 6, 165, 166]

### 1.5.3 Dipeptide Analogues

The conformation of a peptide may be constrained at a slightly higher level by bridging neighbouring residues to control the structure of dipeptide units within the chain. This is often done by creating dipeptidomimetics containing lactam or piperazinone structures which in some cases can be substituted with appropriate side-chains to control the topology of the dipeptide sequence. Examples include the 6,5-fused bicyclic lactam **30**, [167] the Leu-Ala dipeptidomimetic **31**, [168] the diazepinone **32**, [169, 170] and the piperazinone **33**; [1] there are dozens of others, predominantly based on fused 5,6 or 5,7 bicyclic skeletons. [1, 4, 171-174]



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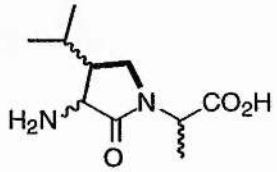

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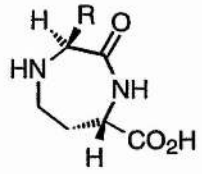

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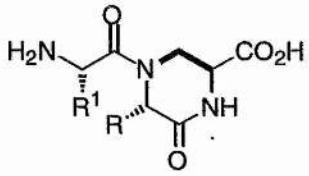
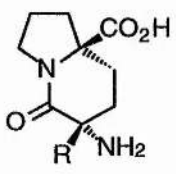


**33**

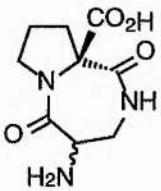
More global conformational constraint can be introduced in a similar manner by linking residues further apart in sequence *via* a variety of methods including disulfide bridges and larger lactam rings. [4]

### 1.5.4 Mimetics of *Cis* and *Trans* Peptide Bonds

*Cis* amide bonds may be present in the bioactive conformations of prolyl peptides, often in type VI β-turns. In order to test such theories, *cis*-prolyl peptidomimetics are useful. Some of the aforementioned dipeptidomimetics incorporate this feature (*e.g.* mimetics **32** and **33**); [169, 172] others are specifically designed to do so. [175, 176]




**34**

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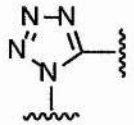


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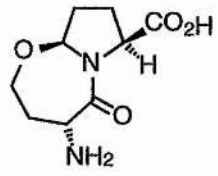


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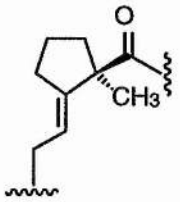
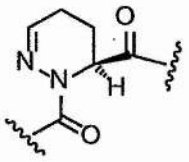



**38**




**39**

Connection of the α-carbons of proline and the preceding residue with an ethylene bridge gives *cis*-prolyl peptidomimetic **34**. [27, 28, 177] The Phe-*cis*-Pro mimetic (R=$CH_2Ph$) was incorporated into somatostatin **4** to produce a high-affinity ligand, which is suggestive of a possible β-turn conformation in the bound form of this peptide. [28] Mimetic **35** connects the same two α-carbons using a lactam bridge. [178]

Marshall *et al.* [179, 180] have designed tetrazoles **36** which are produced directly from the amide bond and mimic its *cis* form. Other groups have produced mimetics of

*trans* prolyl amide bonds, such as the 5,7-fused bicycle **37** or the *trans* alkene **38**. [171, 181]

Piperazic acid derivative **39** was shown to have an increased bias towards the *trans* form compared to proline. [182]

### 1.5.5 Modification of the Peptide Backbone

Altering the amide unit in some way usually produces a mimetic which has improved stability towards degradation, since it is no longer a substrate for peptidases. However, these compounds show no significant conformational constraint compared with the parent peptide. [1, 183] In many cases, completely new secondary structures may be formed which are inaccessible to the parent peptide. The types of modification possible include:

- Isosteric bond replacements; swapping the CONH unit for CH=CH, CF=CH, $CH_2CH$ or $CH_2S$ produces more stable analogues, but they are also more flexible; [183, 184]

- Peptoids; these are oligomers of *N*-substituted glycines (Fig. 1.14). They sample more conformational space than peptides themselves due to the lack of hydrogen bonding and chirality, but show improved stability; [185, 186]

- Retro-Inverso Isomers; these possess a reversed sequence of amino acids and a change in the chirality of each residue from L to D (Fig. 1.14). This maintains side-chain topology but increases resistance to peptidases. In some cases, such isomers have shown biological activity, [187] but in general their poor affinity suggests that the backbone sequence is also important in binding. [183]

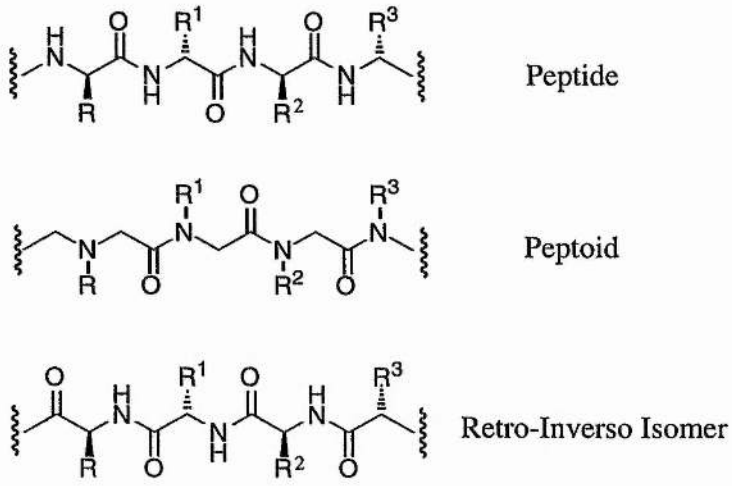



**Figure 1.14:** *Modification of the peptide backbone*

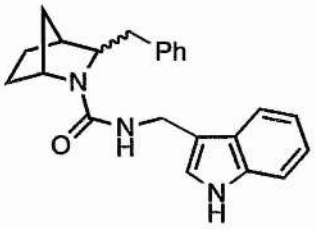
### 1.5.6 Secondary Structure Mimetics

The majority of secondary structures adopted by peptides and proteins involve the β-turn, β-sheet and α-helix. Consequently, much effort has gone into the design of systems to simulate these motifs. These are known as *secondary structure mimetics*, and find uses not only in the design of bioactive compounds but also in studies of protein folding.

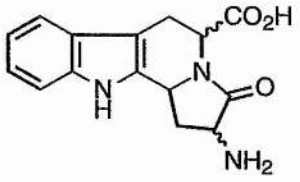
#### 1.5.6.1 β-Turn Mimetics

The β-turn is a localised structural element and as such is much the easiest of the three main motifs to mimic. As discussed, β-turns can be stabilised simply by judicious use of turn-stabilising residues such as proline or $C^{\alpha}$-disubstituted amino acids. [127, 134, 188] The majority of other mimetics involve cyclic scaffolds which simulate the 10-membered hydrogen bonded ring usually present in a β-turn. Ideally, these structures should also carry the relevant side-chains of the β-turn in the native peptide, [1, 173, 174] possess a $C^{\alpha}_{i} - C^{\alpha}_{(i+3)}$ interatomic distance of < 7 Å, and cause a reversal in the direction of the peptide chain. A host of compounds have been synthesised to meet one or more of these conditions. Representative examples include the aforementioned type VI β-turn mimetic **34**; [27, 177] the 2,3-disubstituted

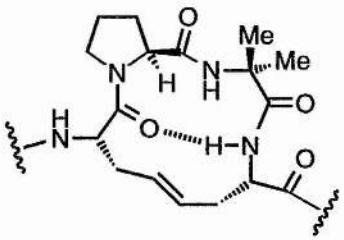
azanorbornane **40**; [189] indolizoindole derivatives **41**; [190] the 14-membered macrocycle **42**; [191] and the 9-membered scaffolds **43**. [192, 193]



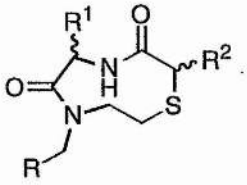
**40**



**41**



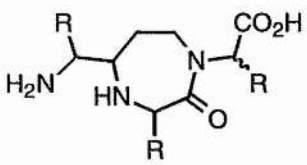
**42**



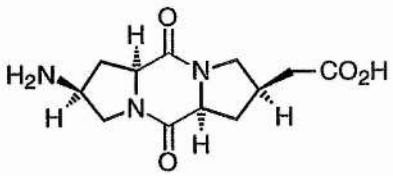
**43**

In some cases these have been incorporated into biologically active peptides to give analogues possessing reasonable affinity, but in most cases their application is unproven. The frequent incorporation of bulky artificial frameworks and insufficiently precise side-chain topologies may result in peptides incorporating these mimetics lacking activity upon application to biological systems.

Compounds designed to mimic other turn structures such as the γ-turn mimetic **44**, [194] or the Ω-loop template **45** [195] have also been suggested. [174, 186]



**44**



**45**

### 1.5.6.2 Stabilisation of β-Sheet Structure

Mimicry of larger, repeating structures is a more challenging task than that of localised motifs. The approaches towards β-sheet stabilisation involve molecular scaffolds which provide attachment points for two or more peptide strands such that intramolecular hydrogen bonding is favoured (Fig. 1.15). [196]

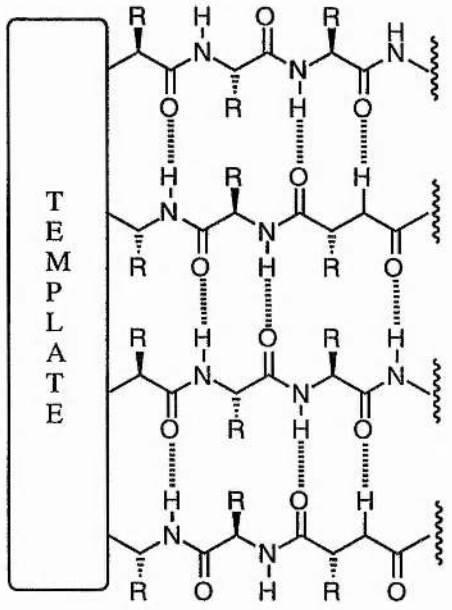
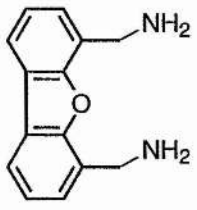
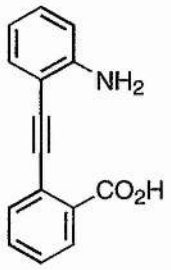



**Figure 1.15:** *Anti-parallel β-sheet nucleated from a template*

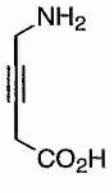
Examples of these *nucleating turn mimics* include the benzofuran scaffold **46** of Skar *et al.* [197], the diphenylacetylene **47** of Kemp *et al.* [198, 199] and the acetylene derivative **48** of Hartzoulakis *et al.* [200]




**46**

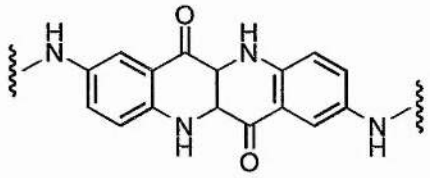



**47**



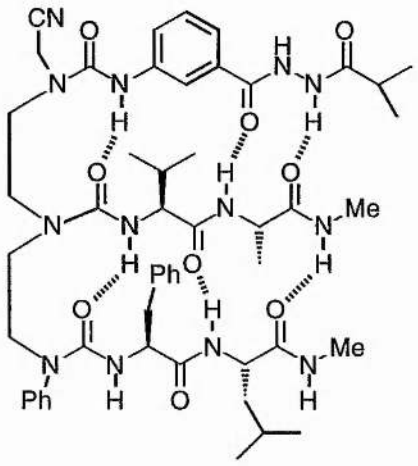

**48**

In some cases mimics of the β-strands themselves are used to initiate formation of sheet structure, by acting as *conformational templates* to propagate a conformation from an ordered region (the template) to a disordered region (the peptide). Kemp *et al.* used diacylaminoepindolidiones **49** to simulate a β-strand; in conjunction with known β-turn favouring Pro-D-Ala sequences, these mimics stabilise the β-sheet by hydrogen bonding to adjacent β-strands. [201]



**49**

Nowick *et al.* used a hydrazine β-strand template (upper chain in system **50**) attached to a turn mimic to stabilise a β-sheet structure. [202]



**50**

### 1.5.6.3 Stabilisation of α-Helical Structure: Solvent Effects

The addition of 2,2,2-trifluoroethanol (TFE) or other alcohols to aqueous solutions of peptides is known to promote helix formation. This is due to the reduced dielectric constant which approximates to that of the protein interior; [203] its less polar nature compared to water decreases the strength of peptide-solvent hydrogen bonding

found in random coil states, thus favouring the intramolecular hydrogen bonding of the helix. [72, 203-205]

The helicity induced correlates well with the helicity predicted by the Chou-Fasman rules; [203] TFE does not stabilise helical structure in peptides which have little or no helix propensity. Its relatively hydrophobic nature is disruptive of tertiary structure, which may explain why it has no stabilising effect on β-sheets which depend on longer-range hydrophobic interactions rather more than do α-helices. [196, 205]

### 1.5.6.4 Stabilisation of α-Helical Structure: Side-Chain Constraints

Naturally occurring peptide and protein helices are often stabilised by favourable interactions between the overlapping side-chains of residues three or four apart in sequence, such as hydrogen bonds, hydrophobic interactions or salt bridges. This side-chain proximity has been used to similar effect by several groups who have found ways to force interactions between side-chains, thus enforcing α-helical conformation in the peptide, in an analogous way to the helix-stabilising interactions found between these side-chains in natural peptides (see Fig. 1.8, p11).

Various types of conformational constraint have been used. Co-ordination of the $i$ and $(i + 4)$ side-chains to metal ions has been accomplished using acid or amine and thiol groups. [206, 207] Similarly, aspartic acid residues at positions $i$ and $(i + 4)$ co-ordinate a positively charged guanidinium receptor, enforcing the conformation as an α-helix. [208]

Other methods use covalent linkages; [$i$ to $(i + 7)$] disulfide bonds, [209] [$i$ to $(i + 4)$] [210] or [$i$ to $(i + 7)$] [211] lactam bridges and [$i$ to $(i + 7)$] alkyl bridges [212] have all been shown to enforce α-helical conformations in peptides. In one case, the $i$ and $(i + 11)$ side-chains were connected to a porphyrin macrocycle, and the structure of the peptide between the attachment points was found to be an α-helix. [213]

### 1.5.6.5 Stabilisation of α-Helical Structure: Helix Templates

All the methods discussed to this point have been shown to stabilise α-helical structure to a greater or lesser extent. However, they all involve perturbation of the system by introduction of alien residues, organic solvents or "forcing" cyclisation strategies. In the study of protein folding processes and synthesis of analogues of bioactive peptides, such alterations may prove too drastic.

A preferable method is to bias the conformation towards an α-helix without changing its primary structure. This is possible by use of templates which initiate structure in attached peptides. [196] This concept operates on two levels. Mutter's template-assembled synthetic protein (TASP) approach uses macrocyclic templates for attachment of three or four peptide chains (Fig. 1.16). These fold into α-helices because of favourable inter-helical tertiary interactions which are present. [214]

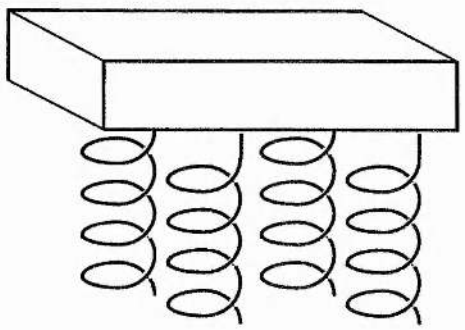


**Figure 1.16:** *Template assembled synthetic protein approach to helix stabilisation*

Other groups use different templates, such as porphyrins or transition metals, [196] but they serve the same purpose of bringing the peptide chains into close proximity to allow tertiary interactions.

This kind of template allows study of the importance of inter-helix forces in stabilisation of these structures. They do not allow for study of the influence of residues within an isolated helix without longer range forces, nor for synthesis of potential bioactive analogues of endogenous peptides. This can only be done if an α-

helix is stabilised in an isolated peptide chain. Without the tertiary interactions present in the TASP approach, the template itself must impart some stabilising influence upon the structure of the peptide (unlike the TASP templates, which are designed merely to bring the strands close together). These are further examples of conformational templates. In the case of the α-helix, the template must provide a set of carbonyl groups which are rigidly aligned in an α-helical conformation (Fig. 1.17). [196, 201]

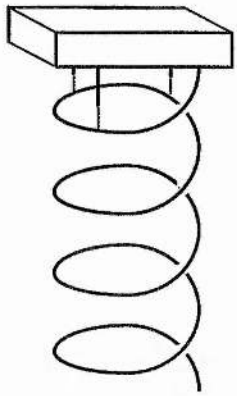
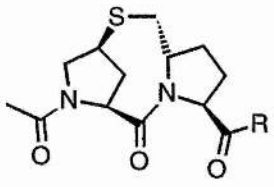


**Figure 1.17:** *Stabilisation of an α-helix* via *an* N-*terminal template*

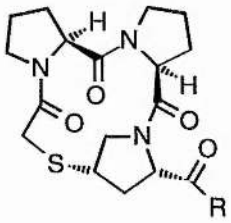
An attached peptide can hydrogen bond to these carbonyl groups *via* its amide groups; if it does so, it will itself adopt an α-helical conformation. Such a template operates by a massive increase in the value of the initiation constant σ from ~$10^{-3}$ to ~1, by reduction of the entropic loss inherent in helix initiation. Design and synthesis of such a template with the correct structural features is far from trivial, but some success has been achieved by several groups.

Kemp and co-workers have led the work in this area, developing templates based on proline-containing macrocycles. [201, 205, 215-228] Most of this work has focussed on template **51**.

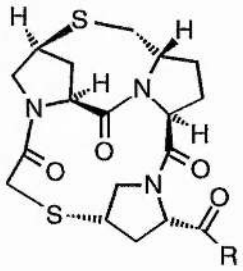


**51**

Ideally, the three carbonyl groups present in this system should be aligned, and thus able to generate α-helical structure in an attached peptide. In the event, the macrocycle shows a degree of flexibility, interconverting between three conformations, only one of which is able to nucleate a helix. [224] The amount of template present in this nucleating conformation (detectable by NMR spectroscopy) correlates with the degree of helicity of the attached peptide, and as such is a useful "reporter" on the conformation of the peptide. This has proved useful for quantification of amino acid *s* values, [226, 229] and for study of the helix-stabilising effects of side-chains [228] and TFE. [205] However, the lack of rigidity significantly decreases the efficiency of the template as an α-helix initiator (σ estimated at ~0.15 in water, as compared to 1 for an exactly α-helical array of carbonyl groups). [226] In an attempt to overcome this limitation, Kemp has also studied the triproline analogues **52** and **53**.



**52**



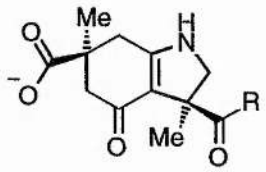
**53**

Macrocycle **52** can supply three aligned carbonyl groups only if all three amide bonds within the ring possess the *trans* configuration (*ttt*). In solution, the macrocycle was found to exist as two conformers, *cct* and *ctt*, and did not have useful helix-

initiating properties. [219, 220] Introduction of a methylenethio bridge to rule out the *cis* configuration of the second amide bond gave macrocycle **53**. However, the desired *ttt* conformer is still not present, the macrocycle favouring the *ctt* form. Preliminary results suggested that this template can generate a $3_{10}$-helix which may convert to an α-helix further along the peptide sequence, [221, 222] but these results require more investigation. Whilst it apparently possesses greater initiating power than Kemp's previous two templates **51** and **52**, template **53** again lacks the required number of carbonyl groups for α-helix induction, and involves a laborious, inefficient 26-step synthesis. [221, 222]

Besides the Kemp group, three others have suggested α-helix templates based on different cyclic structures.

Bartlett *et al.* [227] recently reported the hexahydroindol-4-one **54** as a so-called "aglet", to prevent *N*-terminal fraying of helices.




**54**

This template requires the carboxyl group on the six-membered ring to be deprotonated in order to function, presumably using a charge-dipole interaction to stabilise the helix. The peptides must also be linked *via* a lactate residue to the other carboxylic acid. If these criteria are met, this template is able to generate an α-helix, as demonstrated by CD and NMR studies. The actual degree of helicity induced in the peptide is unclear; standard methods suggest around 30% helicity, but these methods were thought inappropriate for the short peptide sequence involved (six residues). Accounting for this factor produced an estimate of 50-75% helicity. Whilst this template clearly does have a stabilising influence over α-helical conformations, the extent of this stabilisation is unclear, since the peptide appended to the template already possesses high helical propensity (consisting largely of alanine residues, and

initiating properties. [219, 220] Introduction of a thiamethylene bridge to rule out the *cis* configuration of the second amide bond gave macrocycle **53**. However, the desired *ttt* conformer is still not present, the macrocycle favouring the *ctt* form. Preliminary results suggested that this template can generate a $3_{10}$-helix which may convert to an α-helix further along the peptide sequence, [221, 222] but these results require more investigation. Whilst it apparently possesses greater initiating power than Kemp's previous two templates **51** and **52**, template **53** again lacks the required number of carbonyl groups for α-helix induction, and involves a laborious, inefficient 26-step synthesis. [221, 222]

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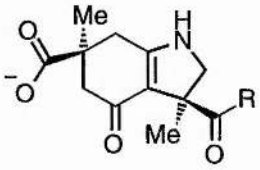


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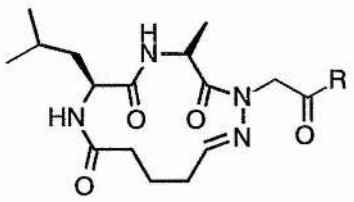
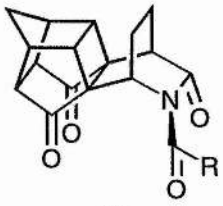
incorporating a salt bridge). How much additional stabilisation does such a peptide need to form a helix? Studies using peptides with a lower helical propensity will help to determine the extent of the conformational influence exerted by the template.

Arrhenius and Satterthwait have also proposed an α-helix template in the form of the 13-membered macrocycle **55**. [230] However, this compound's templating effect was only demonstrated in TFE, and the helix appeared to fray quite quickly moving away from the template.



**55**



**56**

Finally, Müller *et al.* suggested several lactam derivatives as helix initiators, of which the cage compound **56** is representative. [230] Peptide conjugates of these compounds show improved helicity compared to the untemplated peptides, but once again the experiments are performed in the presence of TFE and the peptides incorporate additional stabilising features.

Such templates have proven influence over peptide conformation. However, the energetic gain imparted is debatable, since in all cases other helix-favouring factors are present in the form of solvents or sets of residues with high helix propensities, so the energy required to convert these peptides from the random coil state to the helical state may be small. As such, the generality of their use is called into doubt; will the templates suggested to date only be applicable in certain favourable situations? The template approach has potential, and there is much scope for optimisation of these existing structures and design of new templates with substantial, proven influence over peptide structure.

## 1.6 Protein Processing in Foot and Mouth Disease Virus

Peptidomimetics may assist in the elucidation of enzyme mechanism. One potential example is in polyprotein processing of the foot-and-mouth disease virus (FMDV).

Foot-and-mouth disease is an extremely contagious, acute viral condition of cloven-footed animals characterised by fever and formation of painful vesicles in the mouth and on the feet. [231] Although a vaccine is available, it is expensive and a slaughter policy is often employed instead. In the case of farm livestock such as cattle, the disease prevents weight gain in beef animals and greatly reduces milk yields in dairy cows, resulting in substantial economic losses. Other significant expenditure goes into diagnosis of the condition, quarantine procedures, disposal of carcasses and compensation to affected farmers. The UK epidemic of 1967-68 cost an estimated £150 million.

The foot and mouth disease virus belongs to the picornavirus family of viruses. This family possesses four sub-classes, or genera, of viruses. These are the enteroviruses (which include the virus responsible for polio), the rhinoviruses, the cardioviruses, and the apthoviruses; it is this last genus to which FMDV belongs.

Picornaviruses are based on a single strand of positive sense ribonucleic acid (RNA) consisting of some 8400 base pairs, surrounded by an icosahedral capsid. This capsid has twenty faces, each consisting of sixty copies of each of four capsid proteins. [232]

In order to replicate, the viral capsid must be discarded and the RNA strand passed into a host cell. Upon infection of the cell, the virus is able to suppress cellular protein synthesis, enabling more efficient production of viral proteins. Acting as messenger RNA (mRNA) on cellular ribosomes, the viral nucleic acid strand undergoes translation, resulting in the production of viral proteins. These include RNA polymerase, a replicative enzyme which catalyses synthesis of new RNA strands, using the genome of the viral RNA strand as a template. Translation from

viral RNA strands also produces the various proteins required to synthesise the capsids of new virus particles. Together, these proteins produce new copies of the original virus particle which can then leave the cell. This procedure is very efficient, and can result in the formation of up to 100,000 new virus particles from a single host cell in only 4 hours.

The initial product of this replicative process is a large polyprotein. This must be processed into the smaller encapsidative and replicative proteins *via* a series of cleavage reactions (Fig. 1.18). These are normally carried out by proteases within the polyprotein itself (*e.g.* L, 3C). [232]

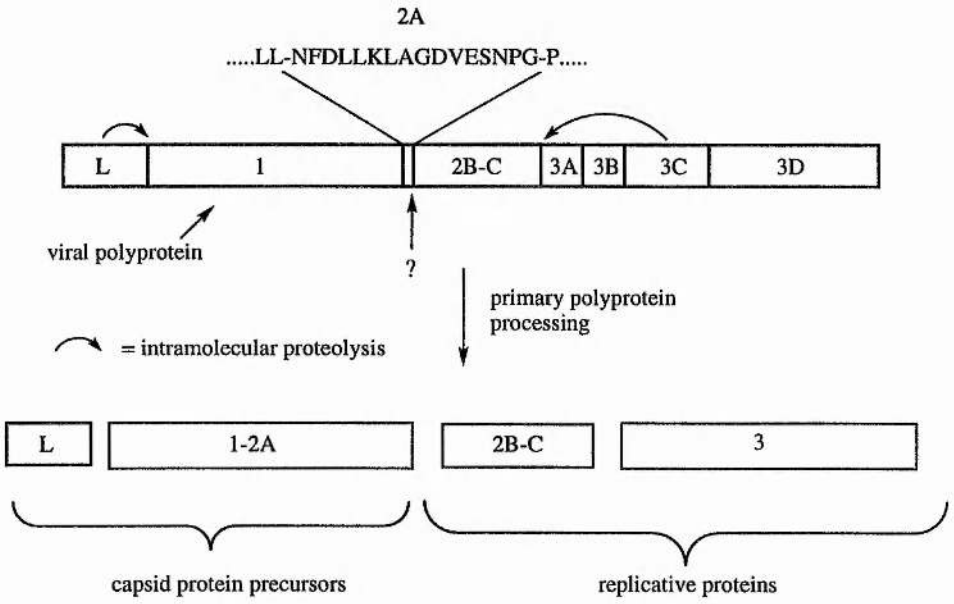



**Figure 1.18** *Polyprotein processing of the foot and mouth disease virus*

The most interesting aspect of FMDV viral polyprotein processing is the cleavage catalysed by the 2A region. In cardioviruses, this region is 143 residues long; in FMDV it contains only 16 amino-acids. Molecular biology studies have ruled out involvement of other regions of the polyprotein in this cleavage *via* creation of a new plasmid pCAT2AGUS which enabled synthesis of a polyprotein in which a sequence spanning the 2A region of the FMDV polyprotein was inserted between two unrelated

proteins CAT and GUS. This resulted in the production of the cleavage products CAT2A and GUS, showing that the process does not require the presence of any other part of the FMDV polyprotein. [233, 234]

Synthetic analogues of 2A show no ability to perform such cleavage reactions in aqueous solution. Initially, this was thought to be due to the lack of a defined secondary structure in this medium, as demonstrated by NMR and CD spectroscopic studies. [235] It is now believed that the cleavage is *not* a proteolytic event akin to those catalysed by the L and 3C proteases, but instead occurs when the 2A region is still attached to RNA on the ribosome; *i.e.* the cleavage takes place at an *ester* bond and the 2A region is an *esterase* (Fig. 1.19). This conclusion was reached after observation that greater amounts of the CAT2A cleavage product were formed than the GUS product. This argues against proteolysis which would produce equal amounts of both products. This difference in the quantities of protein produced may serve a function in the reproductive process, since the virus requires more copies of encapsidative proteins (the region upstream of 2A) than of replicative proteins (the region downstream of 2A).

It is clear that the 2A region itself is involved in catalysing the reaction. This requires a particular reactive structure for the 2A region. Modelling studies by computational methods and structure prediction using the Chou-Fasman rules both strongly suggest the presence of an α-helix throughout the *N*-terminal region of the protein segment.

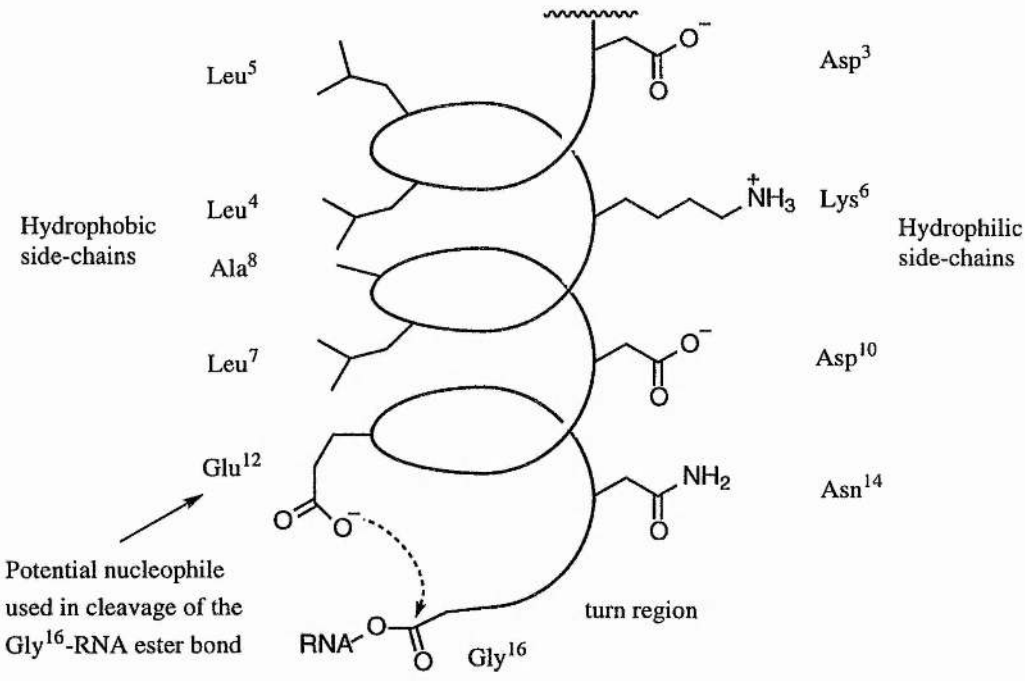



**Figure 1.19:** *Proposed α-helical conformation of the 2A region showing its amphiphilic nature and a possible mechanism of cleavage of the Gly16-RNA bond*

This α-helical structure is stabilised by a series of [$i \rightarrow (i + 3)$] or [$i \rightarrow (i + 4)$] side-chain interactions: a salt-bridge ($Asp^3 \rightarrow Lys^6$); hydrogen bonds ($Lys^6 \rightarrow Asp^{10}$ and $Asp^{10} \rightarrow Asn^{14}$) and a set of hydrophobic interactions. The helix is also amphiphilic in nature, with hydrophilic residues grouped on one face and hydrophobic residues on the other. The importance of these residues is supported by mutation studies; changes to residues which are proposed to stabilise the helical structure are not tolerated and the cleavage process fails to occur. Structural studies of synthetic 2A peptides in water/TFE mixtures also indicate a small degree of helicity. [235]

Besides the α-helical region, cleavage also requires correct positioning of the scissile $Gly^{16}$-RNA ester bond underneath $Glu^{12}$ which might act as a nucleophile or as part of a general base mechanism in the cleavage reaction. This would be possible if a type VI (*cis* Pro) β-turn exists in the $Ser^{13}$-$Asn^{14}$-$Pro^{15}$-$Gly^{16}$ sequence. If a helix-

turn motif is present, the $Gly^{16}$-RNA bond scission could occur in preference to nucleophilic attack of the next amino acid (the first of the 2B region) at the $Gly^{16}$ carbonyl; *i.e.* ester bond cleavage takes place instead of continued peptide synthesis. Significantly, the next residue is proline, the least effective nucleophile of the proteinogenic amino acids. The slow rate of Gly-Pro amide bond formation might allow the $Glu^{12}$-catalysed ester bond cleavage to become competitive.

Molecular biology experiments are still in progress to elucidate further aspects of the 2A-mediated process. However, these studies will not be able to supply information on crucial structural aspects. Fortunately, the extraordinary small size of the 2A region renders it amenable to study by chemical methods.

It has already been demonstrated that isolated 2A peptides lack stable secondary structure in aqueous solution. We intend to produce analogues in which the proposed structure is stabilised using peptidomimetics, whilst maintaining maximum integrity of the primary structure of the 2A sequence. This is possible by the application of a type VI β-turn mimetic which is closely related structurally to the β-turn found in the putative bioactive structure, and by use of an *N*-terminal α-helix template which allows the proposed helical structure of the 2A peptide to be strengthened without perturbation of its primary structure. Such mimetics, which have minimal effect on the primary structure of the sequence but a major stabilising effect on the secondary structure, will enable reliable conclusions to be drawn on the mechanism of action of this tiny enzyme. Our intention is to append RNA analogues to the end of a structure-stabilised 2A analogue to study the extent of ester bond cleavage in these systems, thus establishing if our proposed bioactive structure is accurate. If this proves to be the case, major advances can be made in the elucidation of the mode of action of this fascinating self-cleaving peptide.

This goal, along with the variety of other applications available in other areas of peptide chemistry, inspired us to begin work on the synthesis of peptidomimetics for the stabilisation of β-turns and α-helices.

# RESULTS AND DISCUSSION

# 2 Triazepinediones as *cis*-Prolyl Peptidomimetics

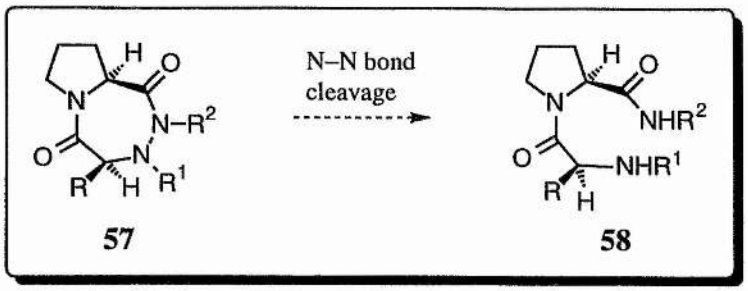
## 2.1 Introduction

The novel fused triazepinedione system **57** is a potential mimetic of *cis*-prolyl peptides. [176, 236]

**57**

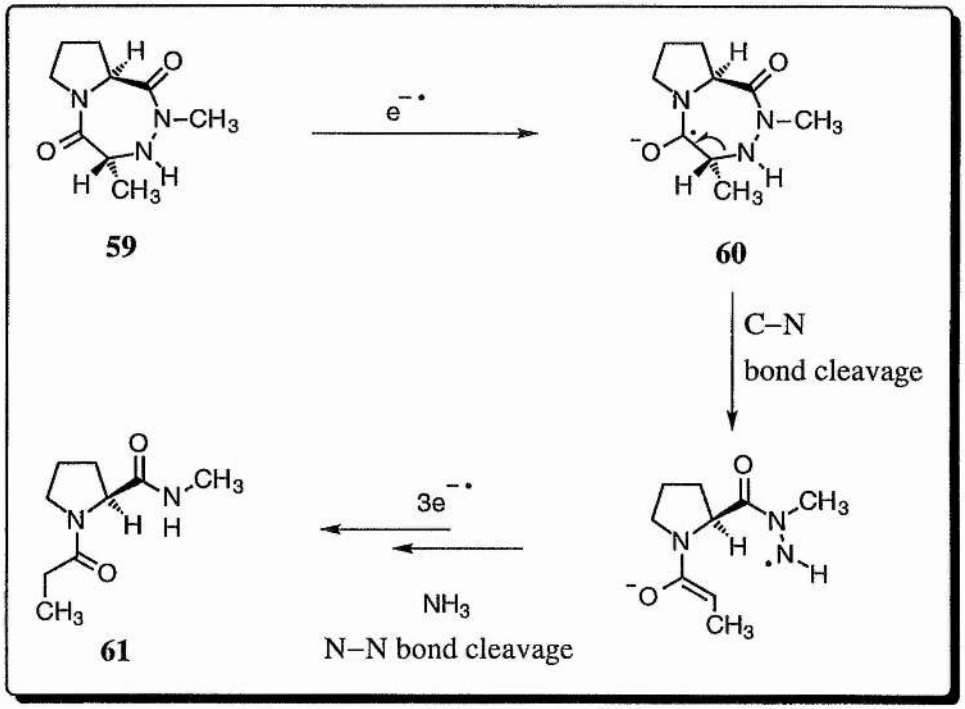
The fusion of the seven-membered triazepine-1,5-dione ring on to the proline ring fixes the amide bond preceding proline in the *cis* form. Incorporation of this mimetic into a peptide may lead to stabilisation of a type VI β-turn, which requires a *cis*-proline at position $(i + 2)$ and a distance between the $C^{\alpha}$ atoms of residues $i$ and $(i + 3)$ of < 7 Å. The molecular constraint imposed by the N–N bond in system **57** ensures that both of these conditions are met for peptides containing this mimetic.

The initial objective in synthesising such compounds was to investigate their possible conversion to *cis*-prolyl peptides **58**, useful for studying the *cis* to *trans* isomerisation process. This conversion could occur by scission of the N–N bond which is relatively weak (dissociation energy 37 kcal $mol^{-1}$) (Scheme 2.1). The most common methods for this process are catalytic hydrogenolysis [237, 238] and dissolving metal reductions. [239, 240]




**Scheme 2.1:** *Potential synthesis of a* cis*-prolyl peptide from a triazepinedione*

Unfortunately, synthesis of *cis*-prolyl peptides using this method by Lenman *et al.* did not prove possible. In all cases, the compound was either resistant to reduction (catalytic hydrogenolysis) or both N–N and C–N bond cleavage occurred (dissolving metal reductions). For example, the dimethyl analogue **59** gave (2*S*)-propionylproline methylamide **61** upon treatment with sodium in liquid ammonia (Scheme 2.2).




**Scheme 2.2:** *C–N and N–N bond cleavage during dissolving metal reduction of triazepinedione* ***59***

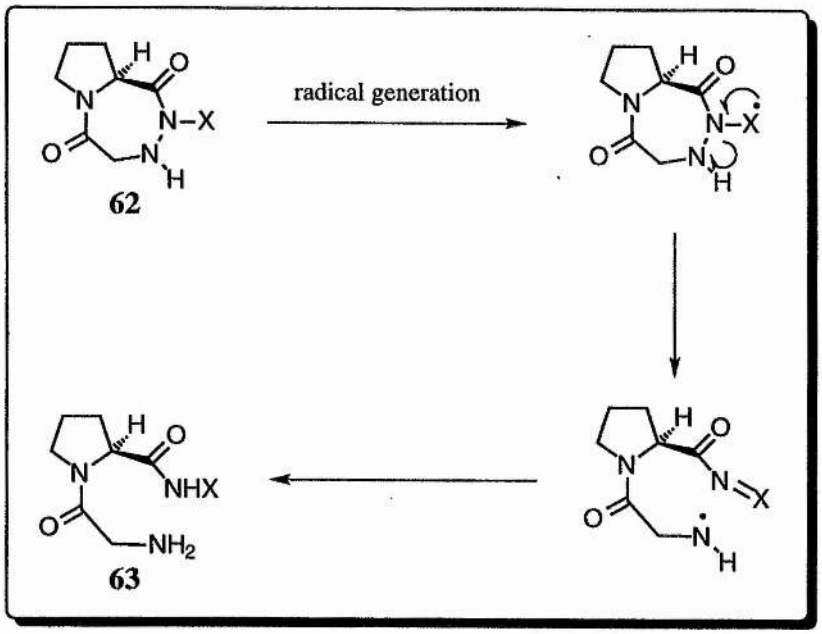
This reactivity can be explained if the carbonyl group α to the cleaved C–N bond accepts an electron to give a radical anion **60**. The C–N bond can then undergo homolytic cleavage, leading eventually to the undesired "double reduction" product **61**.

The aim of this work was twofold:

- synthesise a triazepinedione with the potential to stabilise a radical α to the N–N bond, allowing this bond to cleave prior to the C–N bond to produce a *cis*-prolyl peptide **58**;

- show that the triazepinedione system **57** can be incorporated into a peptide chain for use as a potential *cis*-prolyl peptidomimetic and/or a type VI β-turn mimetic.

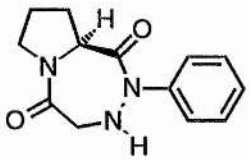
Previous studies on these fused triazepinediones by Lenman *et al.*[176, 236] showed that either, or both, of the hydrazide nitrogen atoms could be substituted with a methyl group. We hoped to be able to use a similar methodology to synthesise a derivative **62** with an electron acceptor group X as substituent $R^2$, enabling stabilisation of a radical α to the N–N bond which could allow this bond to be selectively cleaved under reductive conditions to give the desired *cis*-prolyl peptide **63** (Scheme 2.3).




**Scheme 2.3:** *Generation of a radical α to the N–N bond*

## 2.2 Synthesis of an *N*-Phenyl Triazepinedione Peptidomimetic

Our first target was the fused triazepinedione **64** (X = Ph in structure **62**).

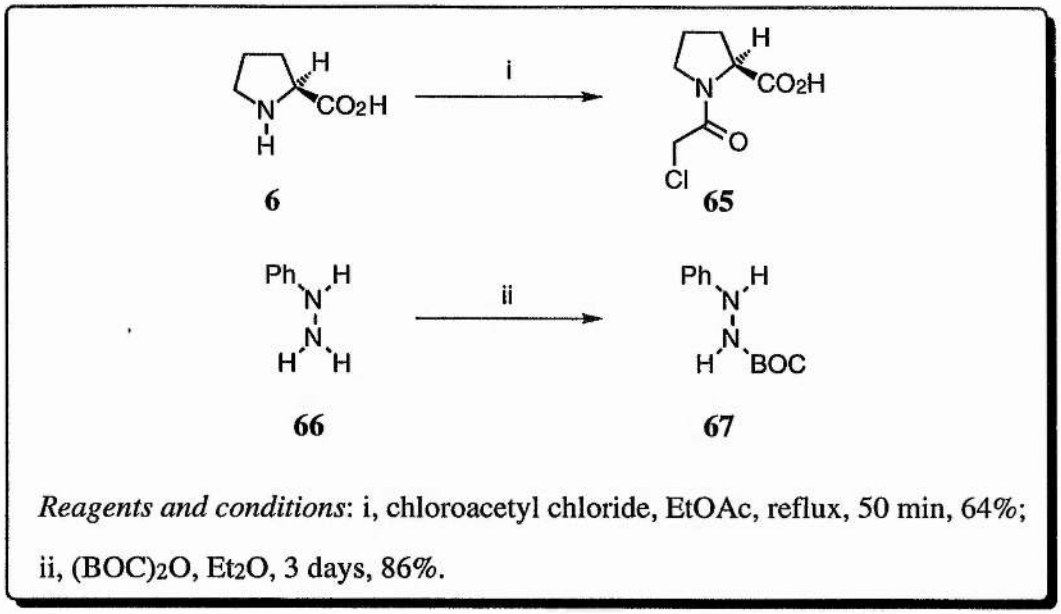


**64**

The amide substituent on the phenyl ring can potentially *destabilise* an anion α to it but *stabilise* a radical, the required situation for cleavage of the N–N bond.

Construction of the triazepinedione **64** was undertaken in a similar manner to that used by Lenman *et al.* for the *N*-methyl analogue. [176, 236]

Direct acylation of proline **6** with chloroacetyl chloride using the method of Ronwin [241] gave (2*S*)-chloroacetylproline **65** in good yield (Scheme 2.4).




*Reagents and conditions*: i, chloroacetyl chloride, EtOAc, reflux, 50 min, 64%; ii, $(BOC)_2O$, $Et_2O$, 3 days, 86%.

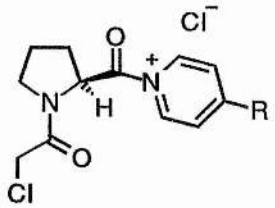
**Scheme 2.4:** *Synthesis of precursors to triazepinedione* ***64***

The two remaining nitrogen atoms of the triazepinedione and its phenyl substituent were derived from phenylhydrazine **66**. In order to achieve the correct regiochemistry in the product, we required formation of an amide bond between the acid group of proline derivative **65** and $N^{\alpha}$ (the nitrogen atom bearing the phenyl substituent). Direct coupling of the two species **65** and **66** would be unlikely to achieve this aim, since *N*-arylhydrazines tend to acylate on $N^{\beta}$ (the unsubstituted nitrogen). [242] This is a consequence of the low nucleophilicity of $N^{\alpha}$ caused by the steric influence of the phenyl ring and the delocalising effect it has on the lone pair of $N^{\alpha}$. This contrasts with acylation of alkylhydrazines which tends to occur predominantly on the substituted nitrogen. [243]

The superiority of $N^{\beta}$ as a nucleophile was masked by treating hydrazine **66** with $(BOC)_2O$ to give the hydrazide **67**, thus drastically reducing the nucleophilicity of $N^{\beta}$, allowing $N^{\alpha}$ to be acylated without competition.

Formation of the required amide bond between species **65** and **67** proved to be relatively difficult, a consequence of the aforementioned poor nucleophilicity of the secondary amino group of hydrazide **67**. The use of standard methods for the

formation of amide bonds in peptide synthesis such as the carbodiimide EDCI or the uronium salt TBTU failed to promote any coupling between the two segments. In the event, a fairly crude method proved to be the most effective. This was the methodology of Matsuda *et al.* [146, 147] which involves activation of the acid component as an acyl chloride or acyl pyridinium salt **68**, by action of thionyl chloride in the presence of pyridine and DMAP.

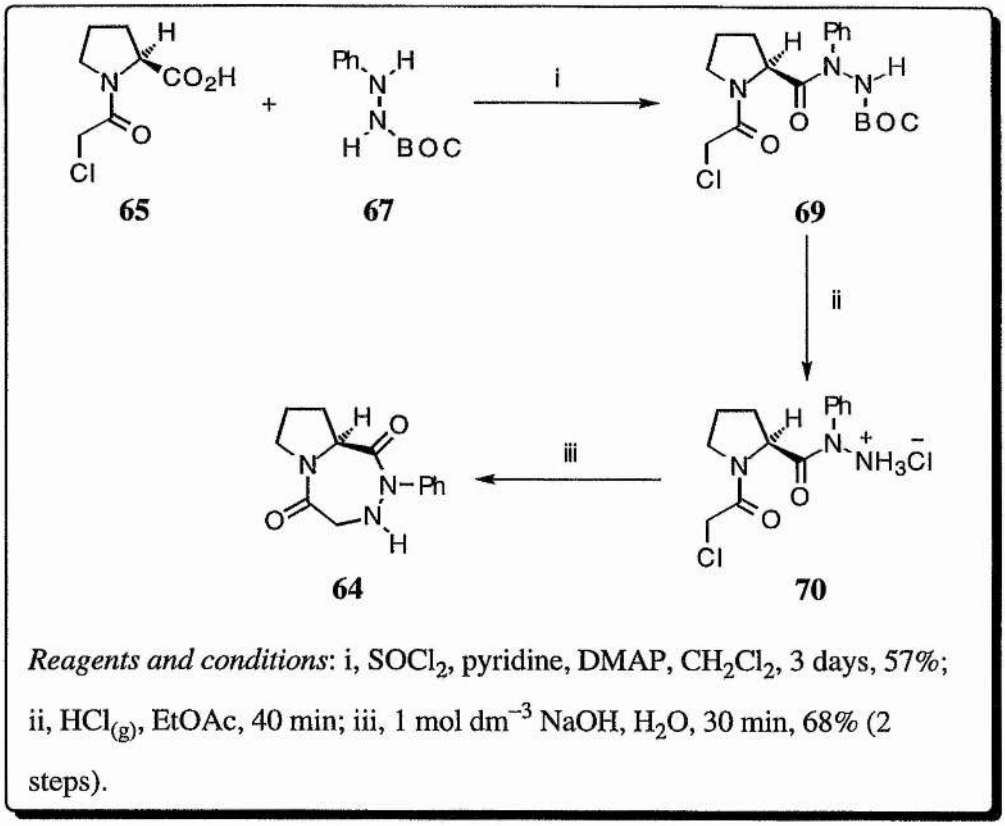



**68**

These pyridinium salts possess high reactivity and are relatively unhindered. These properties render them ideal for acylation of unreactive and hindered nucleophiles such as hydrazide **67**. Coupling of the two components using this method gave a 57% yield of the desired hydrazide **69** (Scheme 2.5).

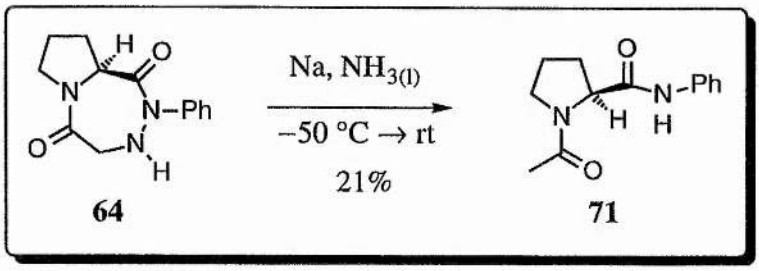
Removal of the BOC protecting group using dry HCl gas gave hydrochloride salt **70**. Upon neutralisation with aqueous sodium hydroxide solution, cyclisation occurred *via* $S_N2$ displacement of chloride by the liberated primary amino group to give the fused bicyclic compound **64**.

Whilst demonstrating that these mimetics can be elaborated with more complex substituents, we required to establish whether or not the dissolving metal conditions applied to triazepinedione **64** would give rise to the desired reduction product; *i.e.* does the phenyl ring allow creation of a radical $\alpha$ to the N–N bond which can give rise to its fission?




**Scheme 2.5:** *Synthesis of triazepinedione* ***64***

Therefore, triazepinedione **64** was treated with sodium in liquid ammonia. Unfortunately, the only product which could be obtained from this reduction process was that formed by cleavage of both N–N and C–N bonds, as before, to give (2*S*)-*N*-acetylprolinanilide **71** (Scheme 2.6).




**Scheme 2.6:** *Reductive cleavage of triazepinedione* ***64***

It appears that the carbonyl group α to the C–N bond is still a better electron acceptor than the substituent α to the N–N bond. Even though a radical anion could be generated in the phenyl ring, an electron is most likely to add to the carbonyl group first, leading to rapid fission of the C–N bond. Subsequent N–N bond cleavage leads to the undesired anilide. These dissolving metal conditions allow a radical to be generated at *any* position in the fused triazepinedione where it is relatively stable. If we can design a system where the radical is created exclusively α to the N–N bond, then cleavage of the C–N bond would not be possible. Work is currently under way with the aim of generating such a radical using a variety of methods.

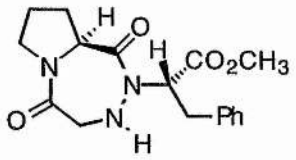
Although it appears that these mimetics may not lead to a synthetic route to elaborate *cis*-prolyl peptides, it may soon be possible to create short model systems containing a *cis* amide bond for study of the *cis* to *trans* isomerisation process.

### 2.3 Extension of *cis* Xaa-Pro Mimetics at the *N*- and *C*- Termini

Fused bicyclic compounds of type **57** may serve as effective mimetics of *cis* Xaa-Pro peptides. A primary requirement for a useful peptidomimetic is that it can be incorporated into a peptide sequence. For these triazepinediones, we must therefore show that extension is possible from both $N^{\alpha}$ and $N^{\beta}$.

Previous work in the group has showed that extension from $N^{\beta}$ (equivalent to the *N*-terminus of an open chain peptide) was straightforward, using standard mixed anhydride coupling methodology to add on further amino acid residues. [236] Extension from $N^{\alpha}$ is significantly more difficult, requiring synthesis of more complex substituted hydrazines for incorporation into the previously described synthetic route.

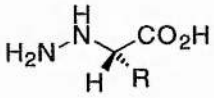
We chose to attempt the synthesis of the triazepinedione **72**, a potential mimetic of H-Gly-*cis*-Pro-Phe-OMe.



72

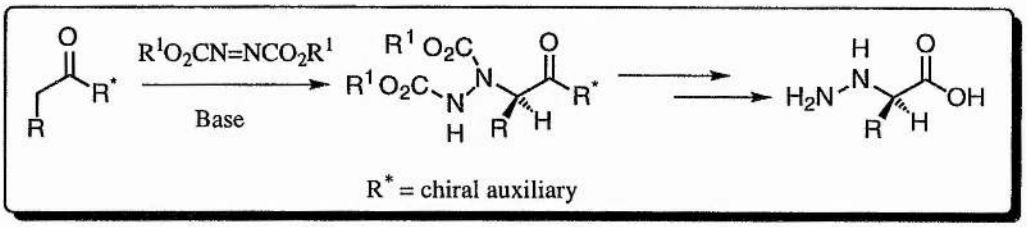
### 2.3.1 Synthesis of Protected (2*S*)-*N*-Aminophenylalanine

Chiral α-hydrazino acids **73** have received relatively little attention in the study of peptidomimetics due to difficulties in their synthesis and coupling reactions.



73

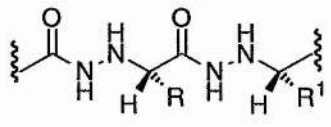
Few methods exist for the synthesis of these compounds. Some groups have utilised stereoselective aminations of chiral enolates using azodicarboxylates (Scheme 2.7). [244-246]



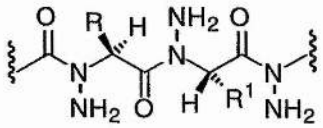

**Scheme 2.7:** *Synthesis of a chiral α-hydrazino acid* via *asymmetric amination*

Whilst these methods can in principle supply any desired α-hydrazino acid, they all involve the manipulation of expensive chiral auxiliaries, potentially difficult low temperature reactions to produce the new stereogenic centre, and several synthetic steps. Less elegant methods also exist based on *N*-homologisation of amino acids. [247, 248] These avoid the generation of a new chiral centre, since the desired stereocentre is already present in a starting material obtained from the chiral pool.

α-Hydrazino acids find uses in the construction of hydrazino peptides **74** or *N*-amino peptides **75** for peptidomimetic studies. [183, 249-252]



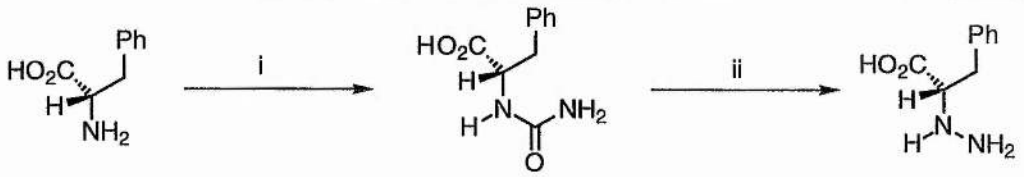
**74**



**75**

We employed the two-step *N*-homologisation procedure of Viret *et al.* [247] for the synthesis of (2*S*)-*N*-aminophenylalanine **78**.

Accordingly, (2*S*)-phenylalanine **76** was converted to the corresponding hydantoic acid **77** upon treatment with a basic aqueous potassium cyanate solution (Scheme 2.8).



**76** **77** **78**

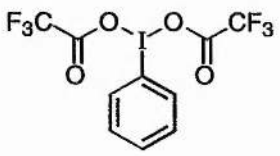
*Reagents and conditions*: i, KOCN, $H_2O$, 60 °C, 4 h, 86%; ii, KOCl, KOH, $H_2O$, 0 → 70–80 °C, 90 min, 43%.

**Scheme 2.8:** *Synthesis of α-hydrazino acid 78*

Conversion of the hydantoic acid **77** to the required α-hydrazino acid **78** was carried out using a Shestakov rearrangement, a transformation which converts a urea into a hydrazine. This is related to the better-known Hofmann rearrangement which transforms an amide into an amine. [253] Action of a basic potassium hypochlorite solution on the hydantoic acid **77** resulted in formation of the hydrazine **78**, in an optimised yield of 43%. Whilst these conditions enabled synthesis of the desired

compound, the relatively low yield and poor reproducibility prompted a study of alternative reagents which might improve the process.

The Hofmann rearrangement can also be brought about by the action of [*I,I*-bis(trifluoroacetoxy)iodo]benzene **79**, a hypervalent iodine reagent which has proved useful for amides derived from peptides and acylamino acids. [254, 255]

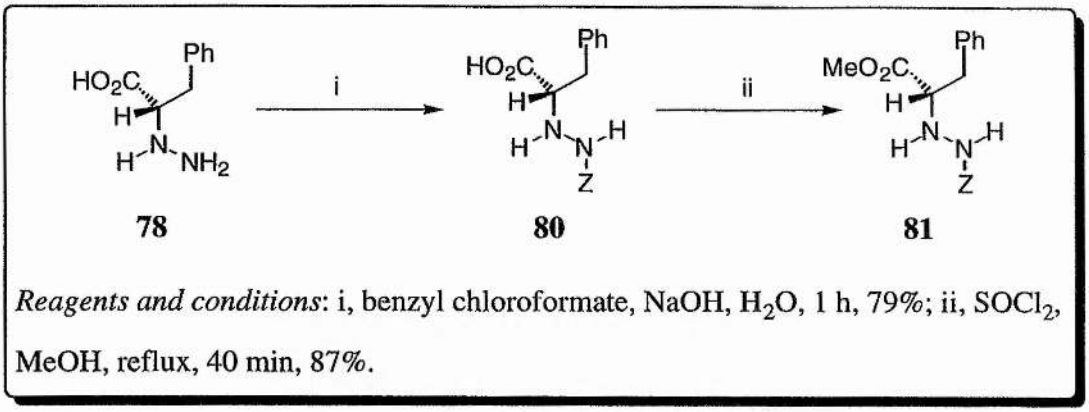


**79**

However, action of reagent **79** on hydantoic acid **77** failed to give any of the hydrazino acid **78**. It was apparent that the synthesis of the α-hydrazino acid was unlikely to be significantly improved without substantial further work, so no further efforts were made to increase the yield.

### 2.3.2 Synthesis of a Gly-*cis*-Pro-Phe Triazepinedione Peptidomimetic

We proposed to use the α-hydrazino acid in place of the simpler hydrazines used in previous syntheses of less complex triazepinediones. As for phenylhydrazine, this required selective protection of the unsubstituted amino group $N^{\beta}$. However, in this case the hydrazine is $N^{\alpha}$-*alkylated* rather than arylated, which might result in a different and undesirable regiochemistry of protection. Alkylhydrazines generally alkylate on the more nucleophilic substituted nitrogen $N^{\alpha}$, [242] except in cases where the substituent imparts excessive steric hindrance. Fortunately, α-hydrazino acids possess sterically demanding substitution which significantly lowers the nucleophilicity of $N^{\alpha}$, [250] so Z-protection of the primary amino group of hydrazino acid **78** proceeded in good yield with no detectable acylation of $N^{\alpha}$, to give hydrazide **80** (Scheme 2.9).



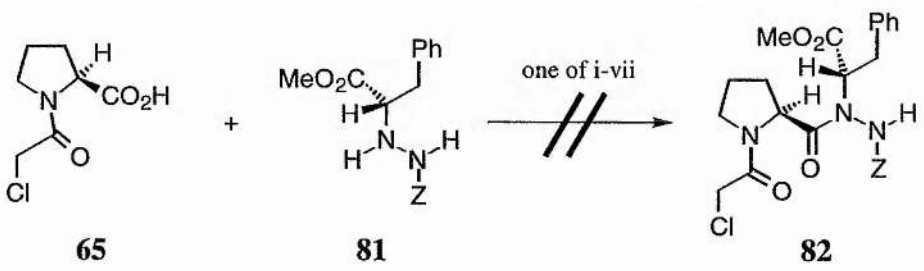

**78** **80** **81**

*Reagents and conditions*: i, benzyl chloroformate, NaOH, $H_2O$, 1 h, 79%; ii, $SOCl_2$, MeOH, reflux, 40 min, 87%.

**Scheme 2.9:** *Synthesis of protected α-hydrazino acid 81*

As the next step is a peptide coupling reaction, the acid group of hydrazide **80** also needed protection; this was done by the formation of a methyl ester using thionyl chloride in methanol in 87% yield, giving hydrazide **81**.

Considering the difficulties encountered in the attempted coupling of BOC-protected phenylhydrazine **67**, we predicted similar problems with the subsequent synthetic step in this sequence, the formation of an amide bond between $N^{\alpha}$ of hydrazide **81** and the acid group of proline derivative **65** to give trisubstituted hydrazide **82** (Scheme 2.10).

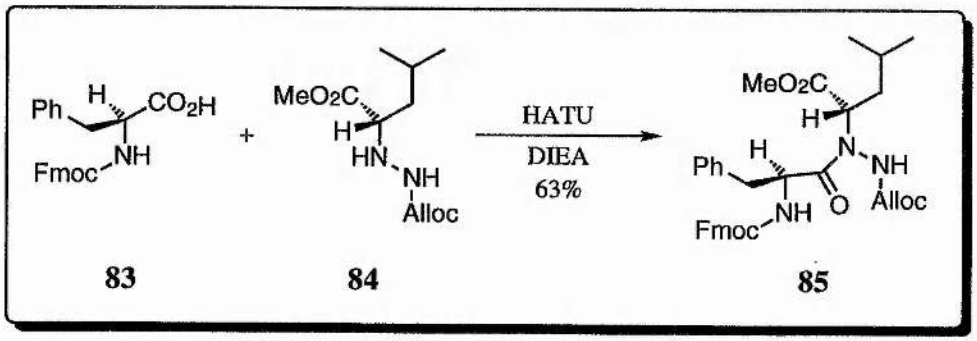



**65** **81** **82**

*Reagents*: i, IBCF; ii, EDCI; iii, pyBOP; iv, TBTU; v, DPPA; vi, pyBroP; vii, BOP-Cl.

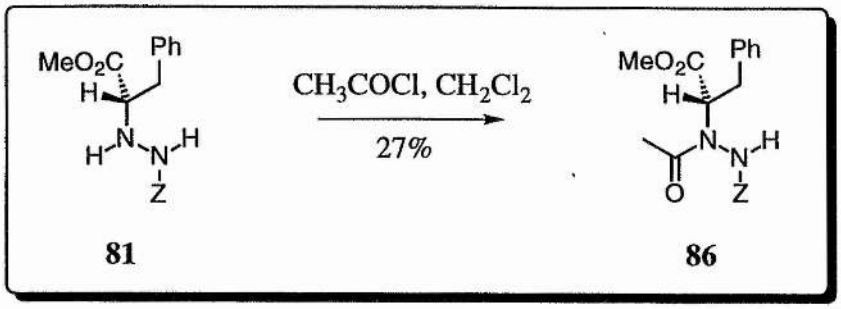
**Scheme 2.10:** *Failed attempts to couple acid 65 and hydrazide 81*

A greater range of coupling methods were employed here, covering every major family of reagents available. *Iso*butyl chloroformate, EDCI, TBTU, DPPA and pyBOP all met with failure. Disturbingly, even BOP-Cl and pyBroP, usually so effective for difficult couplings, were unsuccessful in this case. Problems in coupling on to $N^{\alpha}$ of hindered hydrazides have been encountered before. Lecoq *et al.* [250] used strong activation *via* symmetrical anhydrides or acid chlorides, but yields for these processes were not quoted. Nugiel *et al.* [256] required an acid chloride to acylate $N^{\alpha}$ of their hydrazide in only 38% yield. Finally, Kim *et al.* [251] utilised a double coupling procedure using the extremely powerful HATU reagent, in order to achieve a reasonably efficient reaction between Fmoc-Phe-OH **83** and protected (2*S*)-*N*-aminoleucine **84** to give hydrazide **85** (Scheme 2.11).




**Scheme 2.11:** *Difficult coupling reaction of a* $N^{\beta}$*-protected α-hydrazino acid*

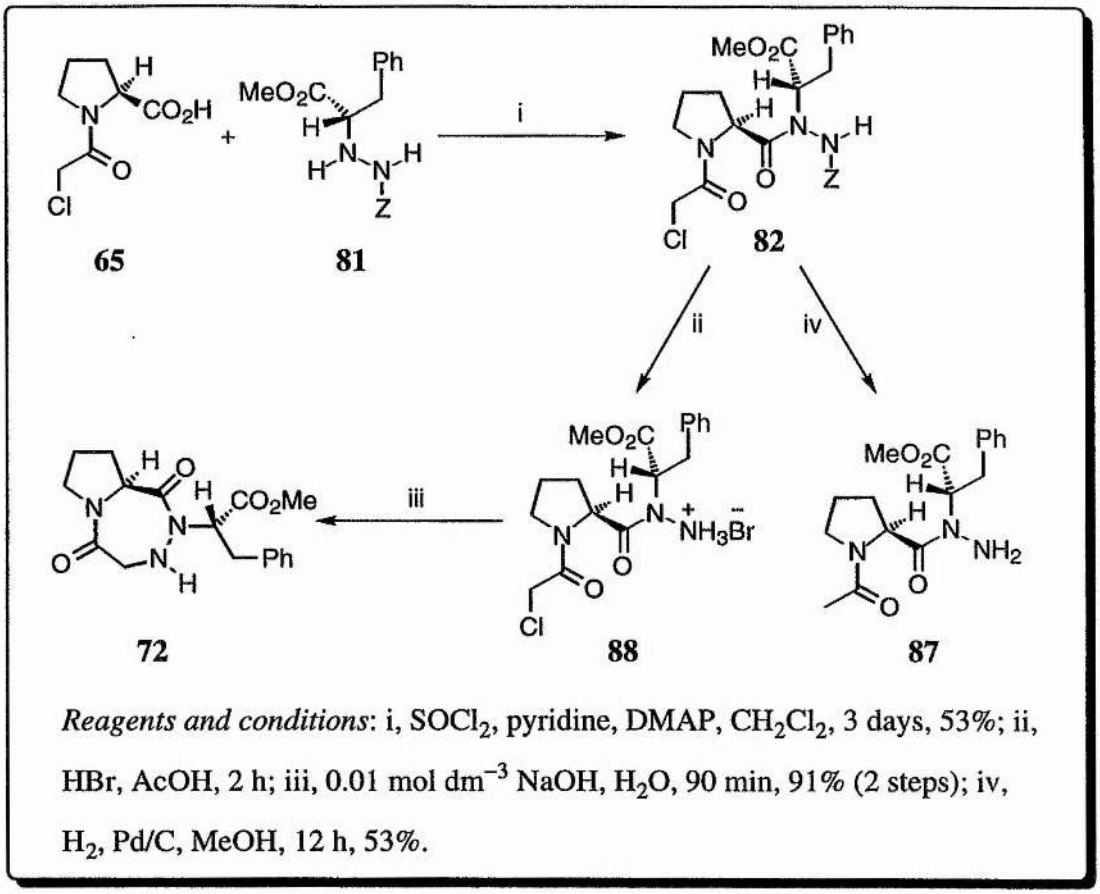
In the event, an acid chloride method proved to be effective in our synthesis. The potential of this methodology was shown by a test reaction between hydrazide **81** and acetyl chloride (Scheme 2.12).




**Scheme 2.12:** *Test acylation of hydrazide **81***

This succeeded in acetylation of $N^{\alpha}$, giving the $N^{\alpha}$-acetyl derivative **86**, albeit in low yield. The success of this procedure indicated that the thionyl chloride/pyridine/DMAP methodology of Matsuda *et al.* [146, 147] which worked well for the phenylhydrazine derivative **67**, might also be applicable here. Fortunately, this was indeed the case and coupling between acid **65** and hydrazide **81** was achieved in yields of 40–53%, giving compound **82** (Scheme 2.13).

The standard method for removal of the Z protecting group is catalytic hydrogenolysis, but application of this technique to compound **82** resulted in reduction of the primary alkyl chloride as well, leading to *N*-acetyl compound **87**. Acidolysis is an alternative method, and treatment of hydrazide **82** with a solution of HBr in acetic acid successfully cleaved the protecting group to give the hydrobromide salt **88**. Neutralisation of this with aqueous sodium hydroxide solution liberated the primary amino group which underwent cyclisation to give the fused triazepinedione **72**, in 8% overall yield for the 8 steps from proline, phenylalanine and chloroacetyl chloride.




**Scheme 2.13:** *Synthesis of triazepinedione 72*

### 2.3.3 Further Studies of Triazepinedione 72

Compound **72** showed two sets of peaks in its $^1H$ and $^{13}C$ NMR spectra in $C^2HCl_3$. This effect was also observed in some of the other triazepinediones in this series, [236] and is probably due to conformational isomerisation of the triazepine ring. This seven-membered ring may be in equilibrium between two sets of conformations, a chair family and a boat family (Fig. 2.1). Each of these families can undergo pseudorotations to more stable twist forms, in an analogous manner to cycloheptane. [257] The two conformations will be discernable in NMR spectra if the interconversion is slow on the NMR timescale.

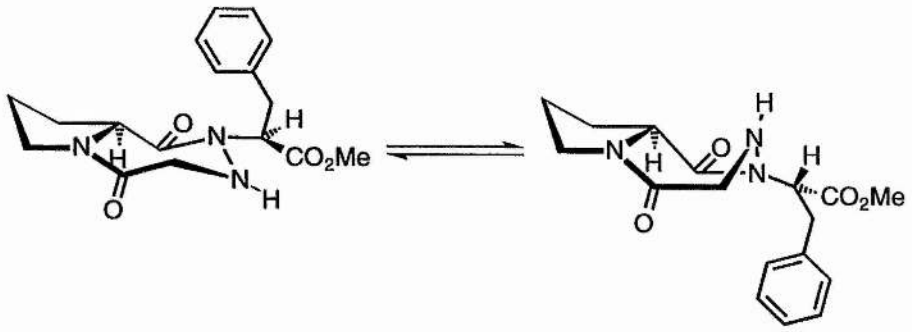


**Figure 2.1:** *Possible chair-boat conformational isomerisation of triazepinedione 72*

Hydrazines may also show stereochemical isomerisation *via* nitrogen inversion. This process occurs at pyramidal nitrogen atoms, and consequently will not take place at the amide nitrogen of this hydrazide, which has a roughly planar configuration. However, this process is not usually observed at room temperature by NMR spectroscopy, making the chair-boat flip explanation more likely.

Another interesting feature of the $^1$H NMR spectrum of compound **72** is a substantial upfield shift of the triazepinedione methylene protons in one of the two conformations. These diastereotopic protons appear as two doublets at δ 2.61 and 3.09, well upfield of the shifts of the corresponding protons of the other conformation, which both resonate at around δ 3.8.

This effect may be due to a shielding interaction, between the phenyl ring and the methylene protons, which only exists in one of the conformations. This shielding phenomenon has been observed in peptides containing the Gly-*cis*-Pro-Phe sequence. [98, 258] In these systems, the *cis* configuration of the Gly-Pro amide bond is stabilised, probably by an attractive interaction between the phenyl ring and the Gly carbonyl group which is not possible in the *trans* form (Fig. 2.2).

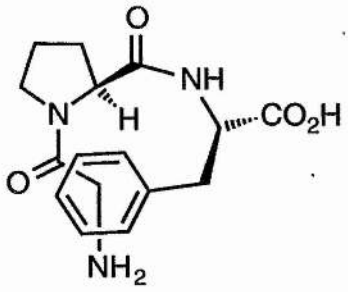
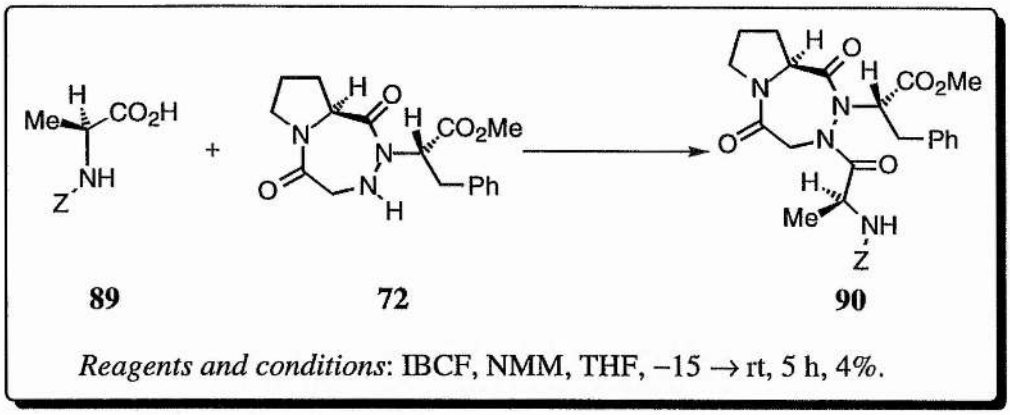



**Figure 2.2:** *Attractive interaction between phenyl ring and glycine residue in a Gly-*cis*-Pro-Phe tripeptide*

This brings the aromatic ring into close proximity with the glycine methylene protons, resulting in an upfield shift of their signals in the $^1$H NMR spectrum.

A similar effect was observed in studies of dioxopiperazines by Kopple *et al.* [122] and Vicar *et al.* [259] These cyclic dipeptides have amide bonds fixed in the *cis* configuration, and resonances of protons in the piperazine ring show upfield shifts of over 1 ppm when an aromatic substituent is present which is capable of folding over the ring.

Our main objective here was to determine if this mimetic could be incorporated into a peptide chain. Extension from $N^{\alpha}$ (the "*C* -terminus") appears to be fairly straightforward, and all that remained was to couple another residue on to the secondary amino group of the triazepinedione **72**. Yet again, this nucleophile is very hindered, and both BOP-Cl and HATU failed to promote coupling to Z-alanine **89**. A final effort using *iso*butyl chloroformate succeeded in production of the desired peptide **90**, albeit in very low yield (Scheme 2.14).




**Scheme 2.14:** N-*Terminal extension of triazepinedione* **72**

These fused triazepinediones differ from the mimicked peptide by only 2 hydrogen atoms and an N–N bond. However, they still restrict the Xaa-Pro amide bond to the *cis* conformation, allow retention of all side-chains, and possess a $C^{\alpha}_{i} \rightarrow C^{\alpha}_{(i+3)}$ interatomic distance of < 7 Å. These properties suggest that they may function as effective *cis*-prolyl mimetics and/or β-turn mimetics. The synthetic route is fairly straightforward and should prove general for any Xaa-Pro-Yaa sequence, which can be incorporated into a longer peptide.

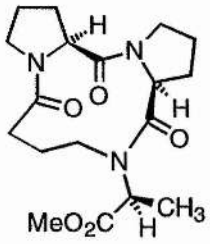
# 3 *N*-Terminal α-Helix Templates

## 3.1 Attempted Synthesis of a Pro-Pro-Ala Macrocycle

We began our studies towards a macrocyclic *N*-terminal α-helix template by consideration of the necessary features required of such a compound and how these features could be incorporated into our design:

- it must provide three correctly oriented carbonyl groups;
- it should be conformationally rigid;
- it should lack hydrogen bond donors of its own to preclude other hydrogen bonded conformations;
- the synthesis should be reasonably concise.

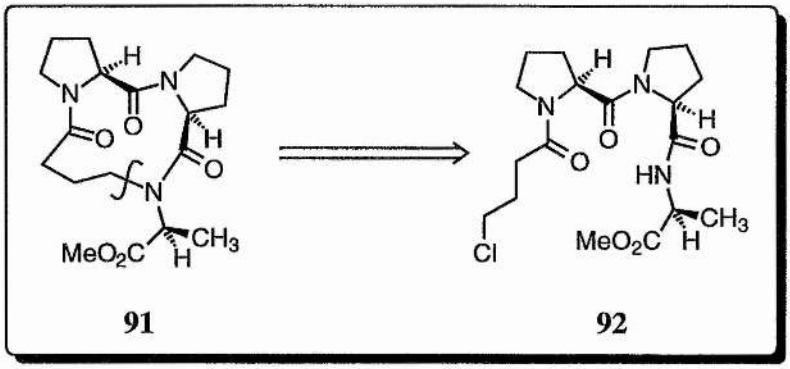
With its restricted dihedral angles and *N*-alkylated nature, proline meets the first three criteria more effectively than any other proteinogenic amino acid. [52] If the macrocycle could be constructed largely from proline residues, this would also simplify the synthesis. Accordingly, our first template design was the 11-membered macrocycle **91**, which consists of a Pro-Pro-Ala tripeptide sequence cyclised *via* a butyryl bridge between the $Pro^1$ and $Ala^3$ nitrogen atoms. This bridge further restricts conformational flexibility and rules out non-productive extended structures, thereby decreasing the entropic losses inherent in forming an α-helical conformation.



**91**

In order to emulate an α-helical pitch of carbonyl groups, each of the three amide bonds within the ring must be in the *trans* configuration; molecular modelling suggested this to be an energetically accessible situation. The tertiary nature of each amide bond implies that *cis* isomers might be present, which would lead to ineffective templates.

The disconnection of the macrocycle (Scheme 3.1) suggested a similar strategy to that used in the synthesis of the previously described triazepinedione mimetics.

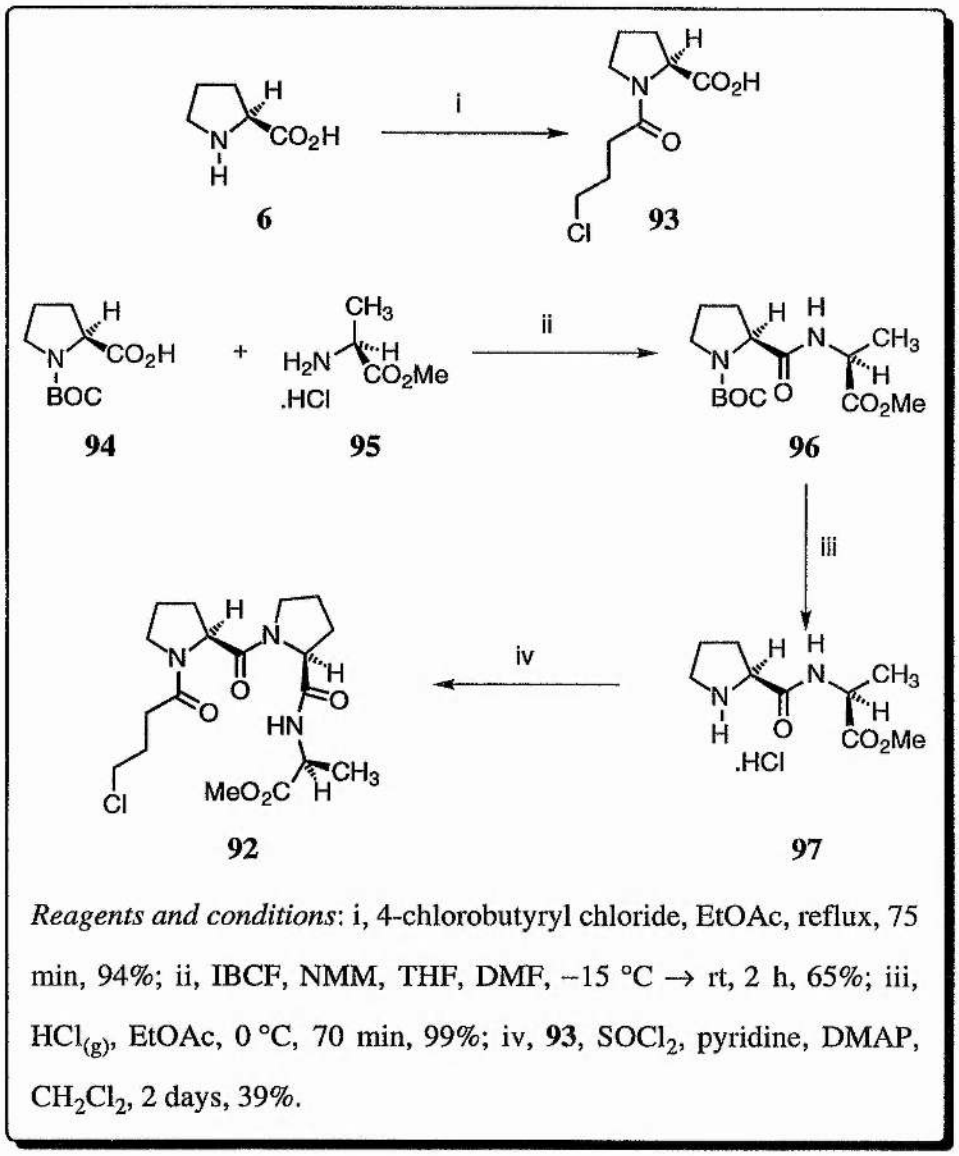


**91** **92**

**Scheme 3.1:** *Possible disconnection of macrocycle* ***91***

Cyclisation could occur by $S_N2$ attack of a nitrogen nucleophile at an alkyl chloride. Synthesis of tripeptide **92** was achieved in five steps from commercially available starting materials. Proline **6** was directly acylated using 4-chlorobutyryl chloride to afford the *N*-acyl derivative **93**. (2*S*)-*N*-BOC-proline **94** was coupled to

(2*S*)-alanine methyl ester **95** using mixed anhydride methodology to give dipeptide **96** in moderate yield (Scheme 3.2).




*Reagents and conditions*: i, 4-chlorobutyryl chloride, EtOAc, reflux, 75 min, 94%; ii, IBCF, NMM, THF, DMF, −15 °C → rt, 2 h, 65%; iii, $HCl_{(g)}$, EtOAc, 0 °C, 70 min, 99%; iv, **93**, $SOCl_2$, pyridine, DMAP, $CH_2Cl_2$, 2 days, 39%.

**Scheme 3.2:** *Synthesis of linear precursor 92*

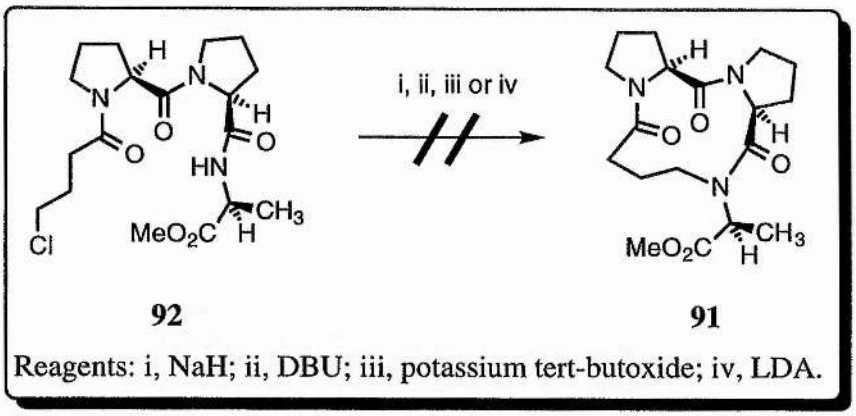
Removal of the *N*-terminal protecting group with HCl afforded amine hydrochloride **97** in quantitative yield. The subsequent reaction of this amine with (2*S*)-*N*-(4-chlorobutyryl)proline **93** involved amide bond formation between two proline residues. This is one of the most hindered couplings possible when using the

proteinogenic amino acids. Standard mixed anhydride methodology gave no detectable product; therefore the more powerful coupling reagents TBTU and pyBroP were utilised, but to no avail. These failures suggested the application of the thionyl chloride/pyridine methodology, which had already proven its utility for similar hindered systems. This produced a 25-39% yield of the desired tripeptide **92**.

Compound **92** displayed three conformations in its $^{1}H$ and $^{13}C$ NMR spectra, in a ratio of approximately 2:1:1. A similar peptide studied by Venkatachalapathi and Balaram showed comparable conformational isomerisation by NMR spectroscopy. [82] One or more of these conformations may incorporate a *cis* amide bond, preceding one or both of the proline residues. Hydrogen bonding between the $Ala^{3}$ NH and one of the carbonyl groups may also be a factor in stabilising certain conformations. Such hydrogen bonding could form a 10-membered ring (β-turn/$3_{10}$-helical turn) or 7-membered ring (γ-turn) (Fig. 3.1).


**Figure 3.1:** *Potential conformations available to peptide* ***92***

Cyclisation of compound **92** requires deprotonation of the Ala NH, since the amide nitrogen itself lacks sufficient reactivity. Analogous amide alkylations have been achieved using a variety of bases. [260-263] We attempted this intramolecular amide alkylation using DBU, sodium hydride, potassium *tert*-butoxide and LDA, but in no case could any of the desired macrocycle **91** be obtained (Scheme 3.3).




**Scheme 3.3:** *Attempted synthesis of macrocycle **91***

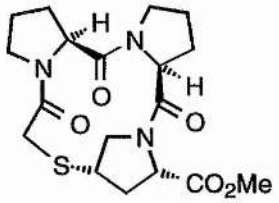
This disappointing result must stem from the hindered nature of the nucleophile, and the conformations adopted by the acyclic compound. Any potentially useful conformation-directing effects of hydrogen bonding present in the linear precursor will be destroyed upon deprotonation of the amide group; without this stabilising factor, the tripeptide is liable to adopt a more extended conformation which precludes close proximity of the reactive centres, thus disfavouring the cyclisation. Efforts to perform the difficult *N*-alkylation earlier in the synthesis in an intermolecular manner also proved fruitless.

Considering the significant problems we encountered in the synthesis of this macrocycle, we decided to refocus our efforts on a redesign of Kemp's template **52** (see p. 46). As it stands, this macrocycle fails to induce α-helicity, but we believed that prudent alterations to the structure and synthetic route might lead to an optimal α-helical template.

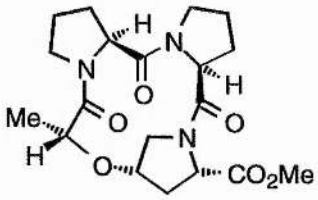
### 3.2 Attempted Synthesis of a Pro-Pro-Pro Ether Macrocycle

The Kemp macrocycle **52** adopts the unproductive *ctt* and *cct* conformations in solution. [219, 220] Molecular modelling indicates that the presence of the *ttt* form is the primary requirement for correct alignment of the carbonyl groups. We chose to

promote this conformer by use of a simple methyl substitution at the methylenethio bridge, which should destabilise the *cis* form of the first amide bond, thus disfavouring the undesired *ctt* and *cct* forms. In order to simplify the synthesis we also chose to avoid incorporation of the sulfur atom. These two elements of redesign led to our target, macrocyclic ether **98**.

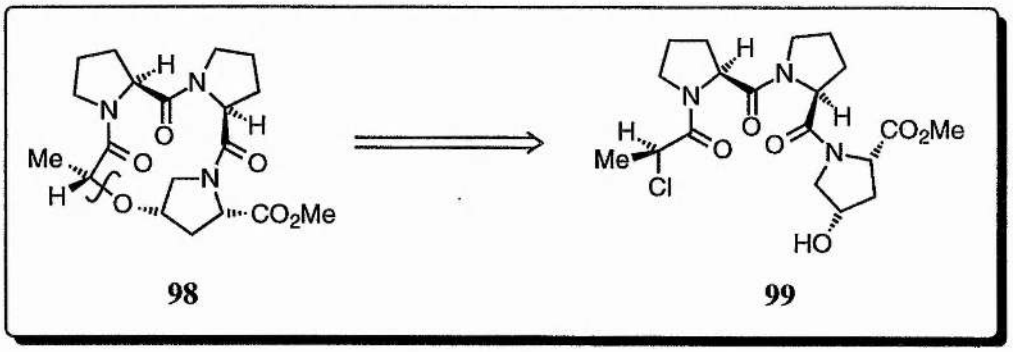



**52**




**98**

These structural alterations required a different synthetic route, since Kemp's methodology does not allow for substitution at the intended position. [219] Instead of ring closure *via* amide bond formation, we proposed a similar disconnection to our previous peptidomimetic syntheses (Scheme 3.4). This suggests cyclisation *via* $S_N2$ displacement of chloride by an alcohol **99**.



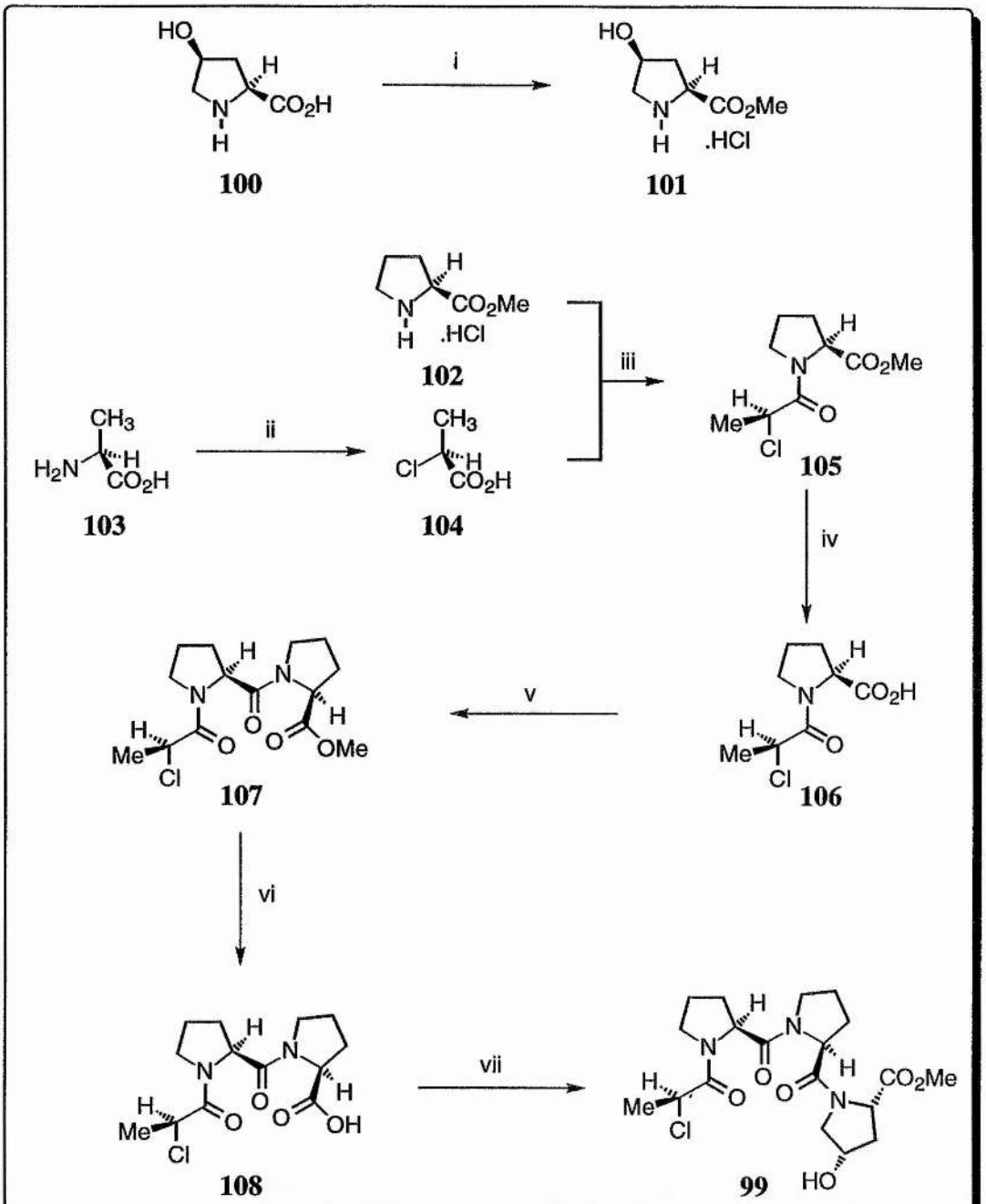

**Scheme 3.4:** *Disconnection of macrocycle **98***

The synthesis of linear precursor **99** proceeded in eight steps from the amino acids (2*S*)-proline **6**, *cis*-4-hydroxyproline **100** and (2*S*)-alanine **103** (Scheme 3.5).

(2*S*)-Alanine **103** was converted to (2*S*)-2-chloropropionic acid **104** *via* diazotisation-chlorination. Condensation of this acid with (2*S*)-proline methyl ester **102** using standard mixed anhydride methodology gave only 35-50% of the desired dipeptide **105**, a conversion efficiency not improved by the use of the more specialised reagents BOP-Cl or PyBOP. Despite its somewhat hindered nature, the amine component of this coupling should not prevent any real difficulties, so the problem must lie in the activation and reaction of the α-chloro acid. The electron-withdrawing nature of the chloro substituent may render the active species rather unstable. In the case of the *iso*butyl chloroformate mediated reaction, significant amounts of a urethane **109** were formed. This arises from attack of the amine on unreacted chloroformate or at the carbonate carbonyl group of the mixed anhydride **110**. The α-chloro substituent must mediate this undesirable change in acid reactivity.

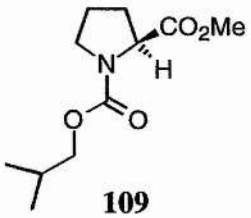
The methyl ester **105** was subsequently converted to the corresponding acid **106** by alkaline hydrolysis without disturbing the chloro-substituted chiral centre; bromo-substituted analogues suffer racemisation during this process. [236] Acid **106** was coupled to (2*S*)-proline methyl ester **102** using the acid chloride method to afford the tripeptide **107** in 33% yield. Saponification gave acid **108** in almost quantitative yield.

The final residue to be attached to this sequence was *cis*-4-hydroxyproline, in the form of its methyl ester **101**. Coupling of this with acid **108** gave a moderate 42% yield of the triprolyl peptide alcohol **99**.

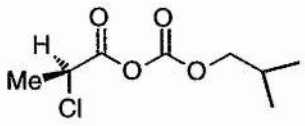



*Reagents and conditions*: i, $SOCl_2$, MeOH, reflux, 90 min, 100%; ii, $NaNO_2$, HCl, KCl, $H_2O$, 0–5 °C, 2 h, 73%; iii, IBCF, NMM, THF, DMF, −15 °C → rt, 18 h; iv, 1.0 mol $dm^{-3}$ NaOH, MeOH, $H_2O$, 2 h, 36% (2 steps); v, **102**, $SOCl_2$, pyridine, DMAP, $CH_2Cl_2$, 4 days, 33%; vi, 1.0 mol $dm^{-3}$ NaOH, MeOH, $H_2O$, 90 min, 98%; vii, **101**, $SOCl_2$, pyridine, DMAP, $CH_2Cl_2$, 2 days, 42%.

**Scheme 3.5:** *Synthesis of linear precursor **99***




**109**




**110**

The proposed cyclisation of compound **99** is a variant of the Williamson ether synthesis, which involves reaction of an alkoxide with an alkyl halide. [264] The weaker amine bases pyridine and DBU failed to promote cyclisation even upon use of large excesses and prolonged reaction times. A similar lack of reactivity was found with stronger bases such as sodium methoxide, potassium *tert*-butoxide, sodium hydride and potassium hexamethyldisilazide (KHMDS) in a variety of solvents either at ambient or elevated temperatures.

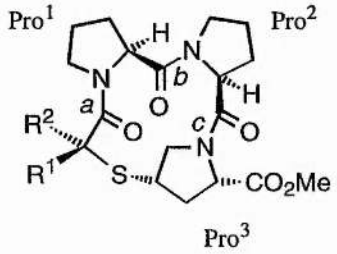
Compound **99** seems to suffer from similar drawbacks to amide **92** with regard to this cyclisation process. The nucleophilic and electrophilic sites are both at less reactive secondary centres. The carbon-oxygen bonds present in the proposed macrocycle **98** are shorter than the carbon-sulfur bonds found in Kemp's macrocycle **52**; this could result in excessive strain in the molecule, precluding its formation. The linear precursor **99** may avoid the folded conformations which are required to bring the reacting centres together, instead favouring extended structures such as the polyproline II helix often found in oligoprolines, [75, 265] which minimise unfavourable steric interactions between the pyrrolidine rings.

The problems of low nucleophile reactivity and potential cyclic strain may be overcome by switching to a thioether macrocycle akin to that of Kemp. Thiolates are significantly better nucleophiles than alkoxides, and the longer carbon-sulfur bonds could help to relax cyclic strain.

### 3.3 Studies of Pro-Pro-Pro Thioether Macrocycles

#### 3.3.1 Molecular Modelling Studies

The substitution of sulfur for oxygen provides the new target molecules **111** and **112**:




Extensive molecular modelling studies were carried out on this macrocycle, assessing the isomers with both *S* (**111**)and *R* (**112**) stereochemistry at the new chiral centre compared to Kemp's unsubstituted version **52**.

The results are summarised in Tables 3.1 and 3.2, where the fifteen computed lowest energy conformations for each macrocycle are shown. *Cis-trans* isomerisation at each amide bond generates 8 possible configurational isomers. Only the *ttt* form is capable of α-helix initaition. Each of these 8 "parent" conformations of defined ω values possesses a set of substates which differ in the ϕ and ψ dihedral angles for each proline residue and by flexibility in the thioether bridge.

The relative conformational energies of Kemp's macrocycle **52** indicate that six isomeric forms exist which are of greater stability than the most likely α-helix-initiating *ttt* substate ***U***. All but one of these contain a *cis* amide bond at position *a*, next to our proposed site of substitution. The modelling study correctly predicts the structure of this compound, which was experimentally determined by Kemp *et al.* to assume the *ctt* and *cct* forms. [219]

**Table 3.1:** *Molecular mechanics energy calculations (in kcal mol$^{-1}$) for* ***52*** *and* ***111***

| **52** | | | **111** | | |
|---|---|---|---|---|---|
| **Code** | **State** | **Energy** | **Code** | **State** | **Energy** |
| *G* | *ctt* | 71.73 | *G* | *ctt* | 93.02 |
| *B* | *cct* | 71.92 | *AE* | *ctt* | 95.20 |
| *AE* | *ctt* | 73.44 | *AG* | *ctt* | 95.73 |
| *R* | *ctt* | 74.40 | *R* | *ctt* | 96.09 |
| *K* | *ctt* | 74.64 | *AB* | *ctt* | 99.14 |
| *M* | *ttt* | 75.95 | *A* | *cct* | 100.21 |
| ***U**** | *ttt* | 76.24 | ***U**** | *ttt* | 101.34 |
| ***S**** | *ttt* | 77.49 | *D* | *ctt* | 101.49 |
| *AA* | *tct* | 77.60 | *I* | *ttt* | 101.75 |
| *AB* | *ctt* | 77.78 | *M* | *ttt* | 101.78 |
| ***O**** | *ttt* | 77.97 | *Q* | *tct* | 102.53 |
| *I* | *ttt* | 78.23 | ***O**** | *ttt* | 103.21 |
| *N* | *cct* | 78.96 | *AA* | *tct* | 103.34 |
| *A* | *cct* | 78.98 | ***S**** | *ttt* | 103.65 |
| *Y* | *tcc* | 79.46 | *P* | *tct* | 103.67 |

*Conformational energies were calculated using the AMBER all-atoms molecular mechanics force-field* [266] *and the Insight II program.*[267] *Energies are relative to an arbitrary minimum and are given in kcal mol$^{-1}$.* "State" *describes the configurations of the three amide bonds a, b, and c in the 12-membered ring. Conformers with the correct set of dihedral angles for α-helix initiation are highlighted in bold and marked with an asterisk.*

**Table 3.2:** *Molecular mechanics energy calculations (in kcal mol$^{-1}$) for* ***112***

| **112** | | |
|---|---|---|
| **Code** | **State** | **Energy** |
| *A* | *cct* | 94.00 |
| *M* | *ttt* | 96.99 |
| *AA* | *tct* | 98.83 |
| ***S**** | *ttt* | 99.01 |
| *I* | *ttt* | 99.95 |
| ***U**** | *ttt* | 100.30 |
| ***O**** | *ttt* | 100.93 |
| *Q* | *tct* | 101.14 |
| *F* | *ttt* | 101.47 |
| *N* | *cct* | 101.54 |
| *G* | *ctt* | 101.87 |
| *Y* | *tcc* | 102.11 |
| *Z* | *ttt* | 103.17 |
| *P* | *tct* | 103.77 |
| *AG* | *ctt* | 104.42 |

Surprisingly, methyl substitution at the methylenethio bridge with *S* stereochemistry in compound **111** seems to have a detrimental effect on the relative stability of α-helix initiating conformers. Isomers with amide bond *a* in the *cis* form are still of significantly lower energy. However, *R* stereochemistry appears to have a pronounced destabilising effect on the *cis* form. In general, isomers of the *R* form **112** with amide bond *a cis* are now of significantly higher energy. This has the desired effect of increasing the *relative* stability of the α-helix-initiating *ttt* substates ***S***, ***U*** and ***O***. It should be noted that not all *ttt* states are capable of α-helix initiation. However, if another *ttt* state is present, the energy required to alter the conformation to an

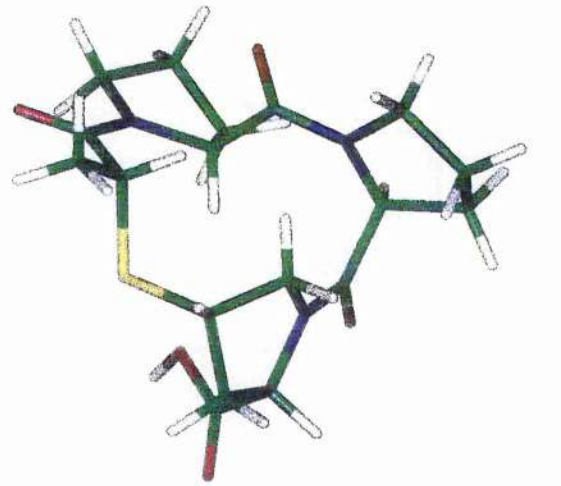
initiating state should be relatively small, involving changes to $\phi$ and $\psi$ values rather than the large changes in $\omega$ required for *cis-trans* isomerisation.

It is apparent from comparison of the sets of conformational energies for each compound that macrocycle **112**, with *R* stereochemistry, is the most likely to afford $\alpha$-helix-initiating properties, and this became our target. Three of the lowest energy modelled conformations are shown in Fig. 3.2. Conformer ***A*** is predicted to be the most stable of all, with a *cct* arrangement of amide bonds. Conformer ***M*** is the lowest energy *ttt* substate, possessing no net dipole and lacking $\alpha$-helix-initiating capability. Conformer ***S*** is the most stable of the potentially $\alpha$-helix-initiating *ttt* substates with a correctly aligned set of carbonyl groups and an associated dipole.

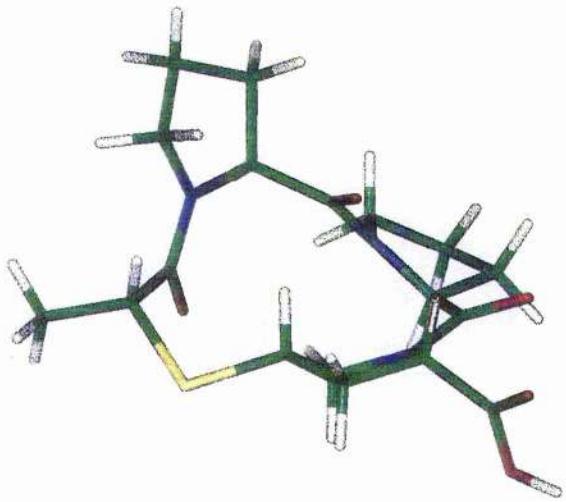
### 3.3.2 Synthetic Studies

Synthesis of macrocycle **112** required elaboration of the route for incorporation of the sulfur atom in place of oxygen. This could be done quite easily based on a similar strategy to the preceding synthesis. In this instance, *trans*-4-hydroxyproline methyl ester **114** was required, and this was prepared from the amino acid **113** by action of thionyl chloride in methanol in almost quantitative yield. The amine **114** was coupled to acid **108**, as for the *cis* analogue **101**, to give the triprolyl peptide alcohol **115** (Scheme 3.6).

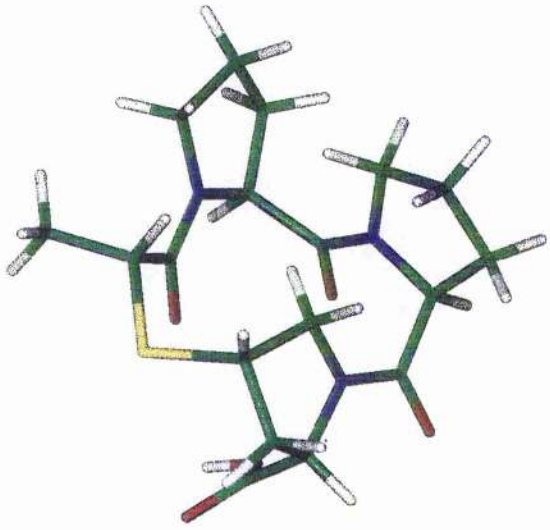
Our first attempt to incorporate sulfur began with tosylation of the secondary alcohol, which resulted in a disappointingly inefficient transformation to the desired material **116**. The presence of two leaving groups in species **115** suggested an ambitious one-step conversion to the desired macrocycle **112**, *via* a double displacement of chloride and tosylate using sulfide ($S^{2-}$) as the nucleophile. This method has been used in the preparation of symmetrical sulfides, giving almost quantitative yields even when using a secondary alkyl chloride as the electrophile. [268] In our hands, none of the desired macrocycle **112** could be obtained; $^{1}H$ and $^{13}C$ NMR spectra of the reaction products suggested that polymerisation had occurred, despite the high dilution conditions employed.



*A*



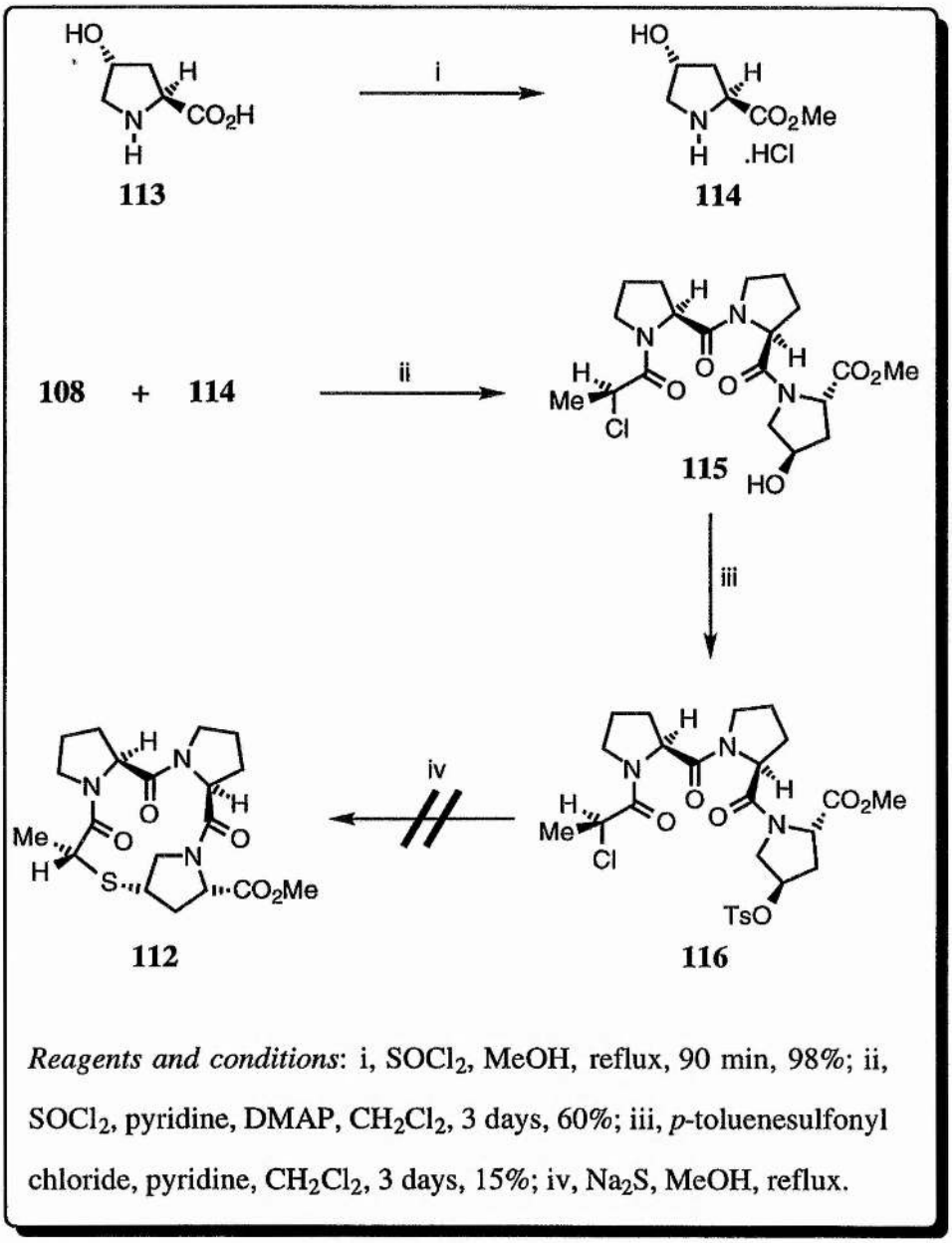
*M*



*S*

**Figure 3.2:** *Low energy modelled conformations of macrocycle* ***112***

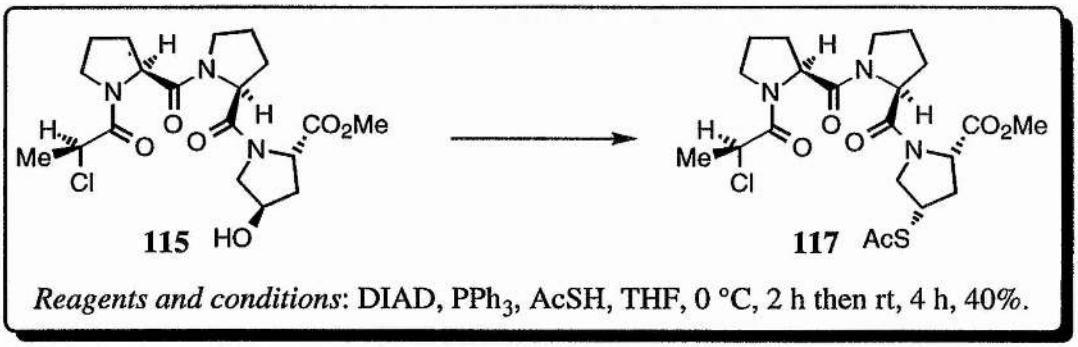
In view of the inefficient production of the tosylate and failure to achieve cyclisation, we decided first to convert the alcohol into a protected form of thiol which could then be unmasked in a controlled manner to undergo reaction with the alkyl chloride.




*Reagents and conditions*: i, $SOCl_2$, MeOH, reflux, 90 min, 98%; ii, $SOCl_2$, pyridine, DMAP, $CH_2Cl_2$, 3 days, 60%; iii, *p*-toluenesulfonyl chloride, pyridine, $CH_2Cl_2$, 3 days, 15%; iv, $Na_2S$, MeOH, reflux.

**Scheme 3.6:** *Attempted synthesis of macrocycle 112*

Conversion of alcohols to thiolethers or thiolesters with the required inversion of stereochemistry is well-established. [269-271] We chose to use the Mitsunobu-type transformation of Volante, [272] which was expected to convert the alcohol **115** directly into the corresponding thiolester of correct stereochemistry.



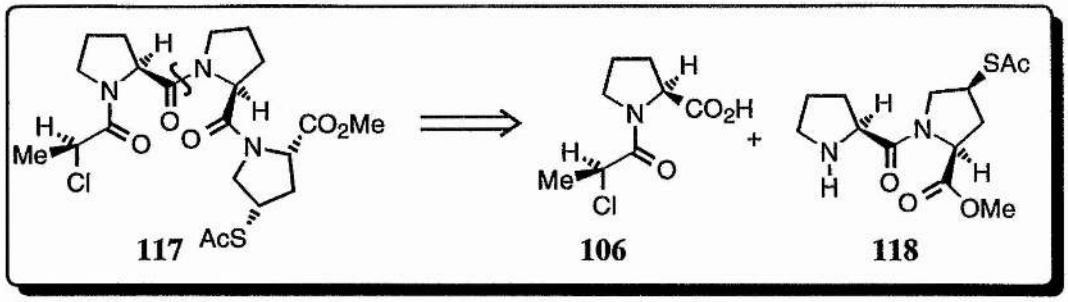

*Reagents and conditions*: DIAD, $PPh_3$, AcSH, THF, 0 °C, 2 h then rt, 4 h, 40%.

**Scheme 3.7:** *Synthesis of thiolester **117***

Treatment of alcohol **115** with diethyl azodicarboxylate (DEAD) or di*iso*propyl azodicarboxylate (DIAD) and triphenylphosphine in the presence of thiolacetic acid afforded 30-40% of the desired thiolacetate **117** (Scheme 3.7).

This yield was not considered sufficient at this late stage in the reaction sequence. We decided to adapt the synthesis so that the Mitsunobu reaction could be performed at an earlier point.

Accordingly, the original linear synthesis was superseded by a more convergent approach. Retrosynthetic analysis suggested amine **118** and acid **106** as intermediates (Scheme 3.8).



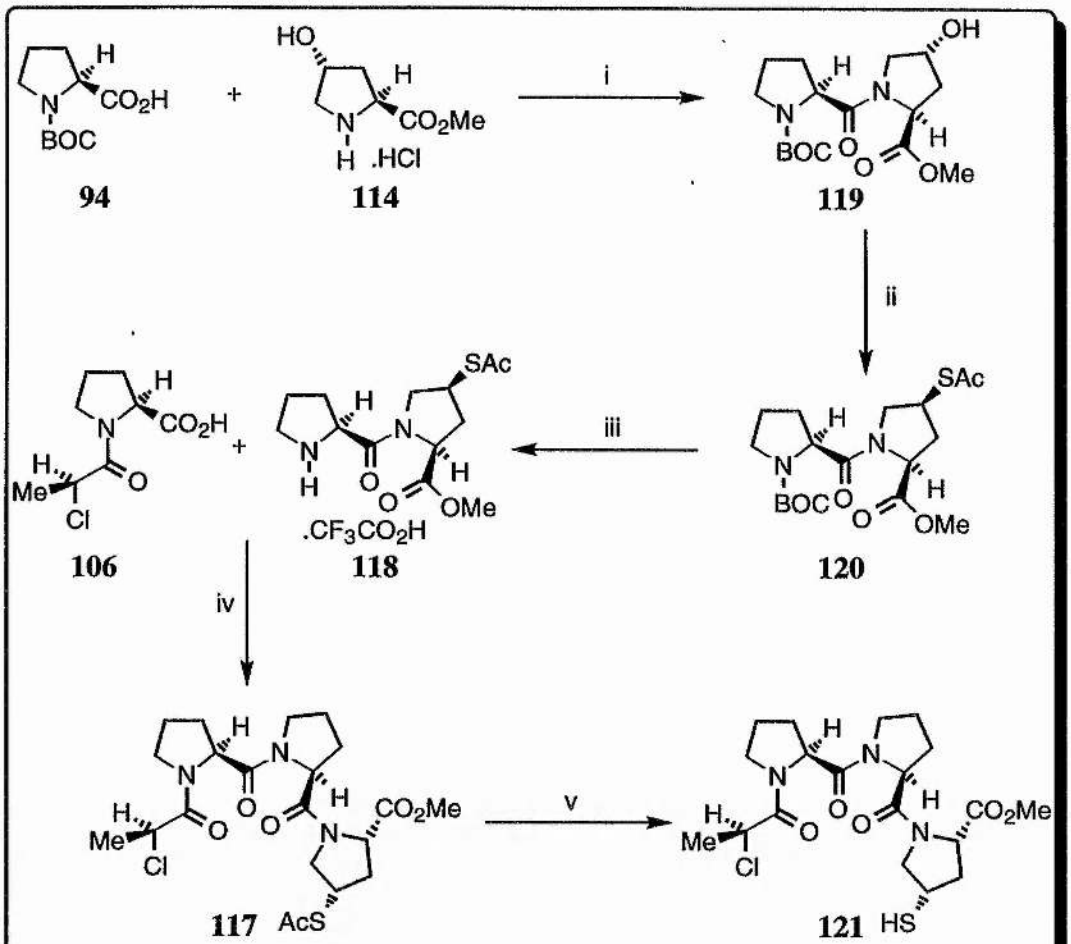

**Scheme 3.8:** *Disconnection of thiolester 117*

Acid **106** was already available from the previous synthetic route, so we needed only to devise a synthesis of the amine fragment **118**. In the event, this was possible in only three steps from intermediates in the previous synthesis (Scheme 3.9).

(2*S*)-*tert*-Butoxycarbonylproline **94** and *trans*-4-hydroxyproline methyl ester **114** were coupled using BOP-Cl in 62% yield to give the dipeptide **119**. BOP-Cl proved to be the reagent of choice for coupling reactions involving proline residues.

Following this, the alcohol was converted to the thiolester **120** of opposite stereochemistry using Volante's procedure. [272] This transformation and subsequent acid-catalysed BOC group removal afforded 69% of the desired amine trifluoroacetate salt **118**.

Using BOP-Cl, the acid **106** and amine **118** were coupled in very good yield to give the triprolyl thiolester **117**. The yield of target compound **117** from acid **106** was thus increased four-fold in comparison with the previous less convergent route.




*Reagents and conditions*: i, BOP-Cl, DIEA, $CH_2Cl_2$, 0 °C → rt, 6 days, 62%; ii, DIAD, $PPh_3$, AcSH, THF, 0 °C, 2 h then rt, 15 h; iii, TFA, $CH_2Cl_2$, 0 °C, 2 h, 69% (2 steps); iv, BOP-Cl, DIEA, $CH_2Cl_2$, 0–5 °C, 2 h then rt, 8 days, 90%; v, 0.05 mol $dm^{-3}$ KOH, MeOH, $H_2O$, 4 h, 95%.

**Scheme 3.9:** *Synthesis of linear precursor **121***

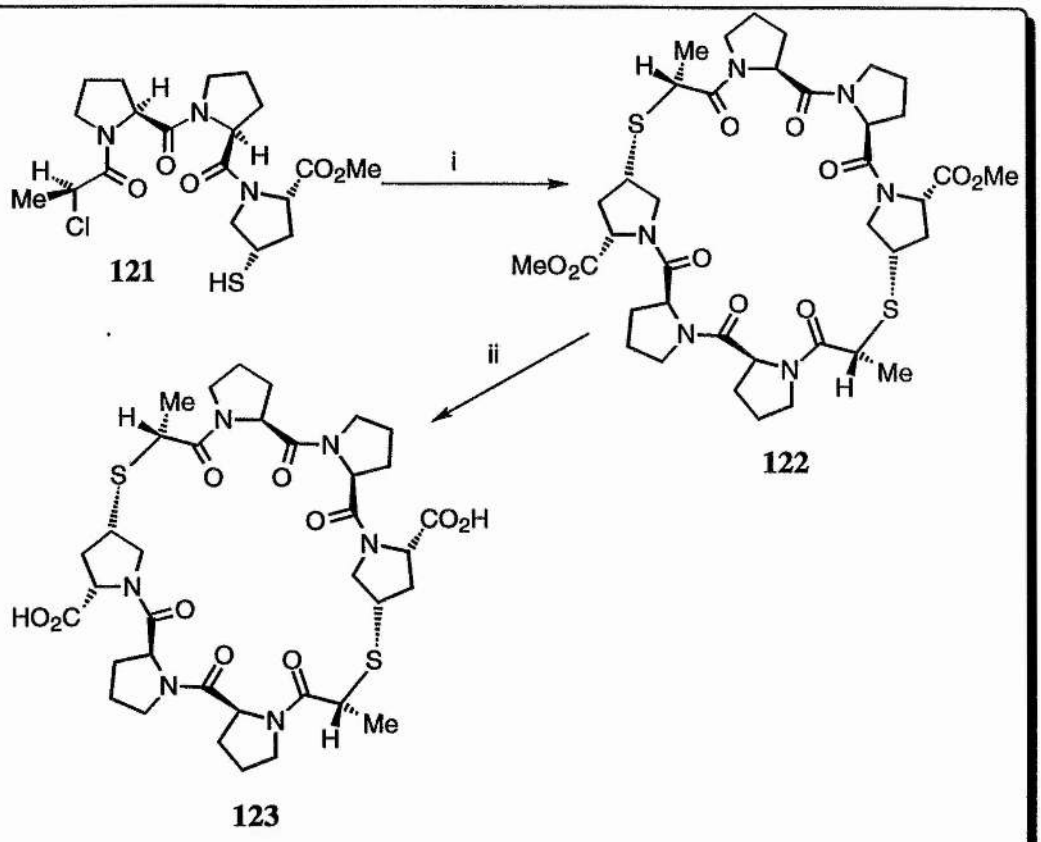
Cyclisation required the thiol to be unmasked. This was achieved in 95% yield by very mild alkaline hydrolysis of thiolester **117** to afford thiol **121** without affecting the methyl ester. A thiolate anion is generated during this process, but this underwent neither intramolecular nor intermolecular reaction with the alkyl chloride. Solutions of thiol **121** in various solvents were therefore subjected to a variety of other bases in

an attempt to achieve cyclisation. Use of DBU, sodium methoxide or potassium *tert*-butoxide failed to produce any of the desired product. Only when solutions of thiol **121** were heated in the presence of sodium hydride were significant changes seen in the NMR spectra of the crude product, indicating that some reaction had occurred. Column chromatography of the crude material allowed isolation of a compound in 15–25% yield, the NMR spectra of which corresponded to those expected for the macrocycle **112**.

In the $^{1}H$ NMR spectrum, a striking 0.3 ppm upfield shift of the doublet attributed to the methyl group at the stereogenic centre was highly indicative of a change of substituent at that position. The $^{13}C$ NMR spectrum also showed two upfield shifts, of the methyl group carbon (by ~3 ppm) and the stereogenic carbon atom (by >10 ppm). These changes would be expected for the decreased electronegativity of the substituent upon exchanging chloride for sulfur. The single set of resonances in the $^{13}C$ NMR spectrum ruled out acyclic oligomers and polymers. The product lacked the S–H stretch in the infra-red found for the starting material, supporting the NMR evidence that a sulfide rather than a thiol was now present.

These results gave a strong indication that the desired macrocycle **112** had formed. However, mass spectra of the compound failed to show the desired molecular ion ($M^+ = 409$). Instead, a peak at mass 819 was observed, indicating that the reaction product was actually the cyclic dimer **122** containing a 24-membered ring (Scheme 3.10).

The dimeric product **122** was indistinguishable from monomeric compound **112** by any of the other analytical techniques employed because it possesses $C_2$ symmetry. Conformational analysis of the product in its acid form **123** by high-field 2D NMR experiments gave similarly ambiguous results. The set of ROESY cross-peaks observed is equally possible for both the monomer **112** (in the ***M*** conformation) and the dimer **122**, with all amide bonds in the *trans* configuration.




*Reagents and conditions*: i, NaH, THF, reflux, 2 h, 22%; ii, 1.0 mol $dm^{-3}$ NaOH, MeOH, $H_2O$, 2 h, 65%.

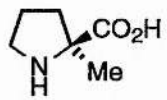
**Scheme 3.10:** *Cyclodimerisation upon attempted synthesis of macrocycle **112***

This disappointing result implies that the steric strain inherent in the folded conformations of thiol **121** which are required for intramolecular reaction prohibited cyclisation. Instead, only intermolecular thiol alkylation was possible. This produced an acyclic dimer which had sufficient flexibility to allow the reacting centres to approach one another without energetically prohibitive steric interactions between the pyrrolidine rings.

Formation of a macrocyclic template akin to **112** requires stabilisation of folded forms of thiol **121** which allow close intramolecular approach of the nucleophile and electrophile.

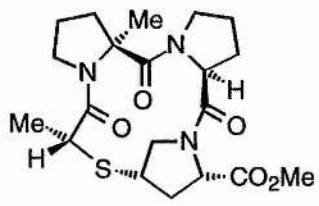
### 3.4 Studies of a Pro$^{Me}$-Pro-Pro Thioether Macrocycle

α-Alkyl amino acids such as Aib are known to favour folded conformations, [81, 132, 133] and to promote cyclisation reactions *via* the *gem*-dimethyl effect. [273] If such an α-alkyl residue could be incorporated into the thiol **121**, it could appreciably increase the time that such a molecule spends in folded conformations conducive to monomeric cyclisation. The minimum change which could be envisaged is the substitution of α-methylproline residue **124** for one of the proline residues in macrocycle **112**.




**124**

α-Methylproline (Pro$^{Me}$) has received little attention to date. Those studies which have appeared indicate that the amide bond preceding an α-methylproline residue is exclusively *trans*.[99, 105] Likewise, the *cis* configuration of the following amide bond is also destabilised. Substitution of Pro for Pro$^{Me}$ at position ($i$ + 1) in tetrapeptide sequences by Robinson and co-workers was found to restrict the conformational space available to the peptide so that it occupied turn-like conformations for longer periods of time, [15, 134] exactly the type of effect we hoped to utilise. The positioning of the Pro$^{Me}$ residue in Robinson's sequences corresponds to Pro$^{1}$ in the triprolyl peptide **112**. Incorporation at this point affords new target **125.**




**125**

Molecular mechanics simulations of the *ttt* substates of this molecule suggested a further advantage. The helix-initiating ***S*** substate is now predicted to be of *lower* energy than the non-productive ***M*** substate, in contrast to the original thioether macrocycle **112** (Table 3.3).

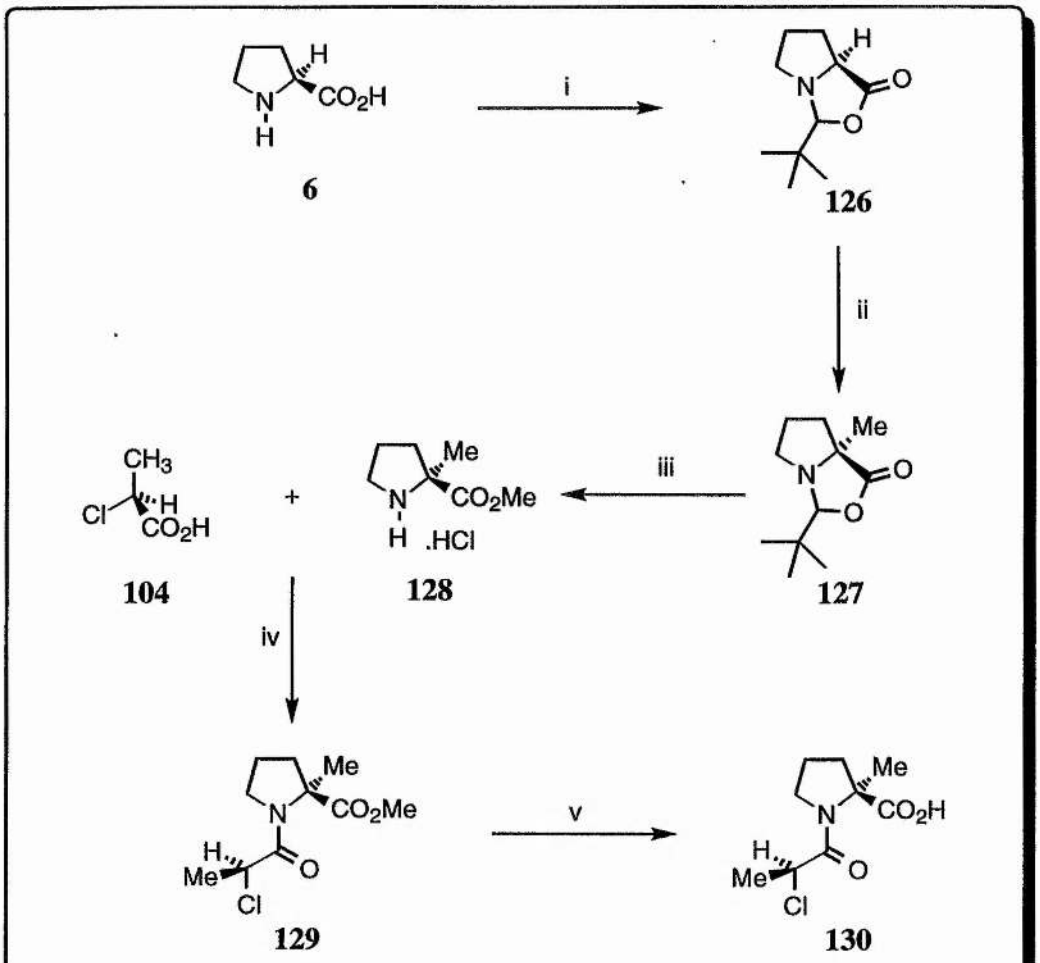
**Table 3.3:** *Energy differences (in kcal mol$^{-1}$) between non-helical (**M**) and helical (**S**)* ttt *conformations of macrocycle **112** and analogue **125** containing an α-methylproline residue*

| Compound | *M* | *S* | Difference |
|---|---|---|---|
| **112** | 96.99 | 99.01 | +2.02 |
| **125** | 118.95 | 117.39 | −1.56 |

We envisaged using a very similar synthetic route towards macrocycle **125** to that used in the previous synthesis. First, we required a convenient synthesis of α-methylproline.

α-Alkyl amino acids can be made in a variety of ways, [274] but we chose Seebach's methodology for the synthesis of α-substituted proline derivatives as the most convenient (Scheme 3.11). [275]

(2*S*)-Proline **6** was condensed with pivalaldehyde to give bicyclooctanone **126** in 96% yield. Deprotonation α to the carbonyl group using LDA at −78 °C followed by addition of methyl iodide gave 57% of the α-methyl derivative **127**. The *tert*-butyl group directs the electrophile by controlling the conformation of the bicyclic enolate so that the alkylation must occur from the same side as deprotonation. Since the 1,3-induction results in complete retention of configuration, this procedure is termed a "self-reproduction of chirality". [275] No trace of the (2*R*) diastereoisomer could be detected in the NMR spectra of compound **127**.




*Reagents and conditions*: i, pivalaldehyde, pentane, reflux, Dean-Stark, 7 days; ii, LDA, THF, −78 °C, 1 h; then MeI, −78 → −30 °C, 2 h, 55% (2 steps); iii, $SOCl_2$, MeOH, reflux, 2 h, 58%; iv, BOP-Cl, DIEA, $CH_2Cl_2$, 0–5 °C, 4 h then rt, 15 h; v, NaOH, MeOH, $H_2O$, 2 days, 43% (2 steps).

**Scheme 3.11:** *Synthesis of α-methylproline derivative* ***130***

The bicyclic compound **127** was cleaved by action of thionyl chloride in methanol to give (2*S*)-2-methylproline methyl ester hydrochloride **128** in reasonable yield.

Coupling of the methyl ester **128** with (2*S*)-2-chloropropionic acid **104** using *iso*butyl chloroformate or pyBroP gave yields of only 31% and 34% respectively of the dipeptide **129**. BOP-Cl proved more effective; a slight excess of the activated acid

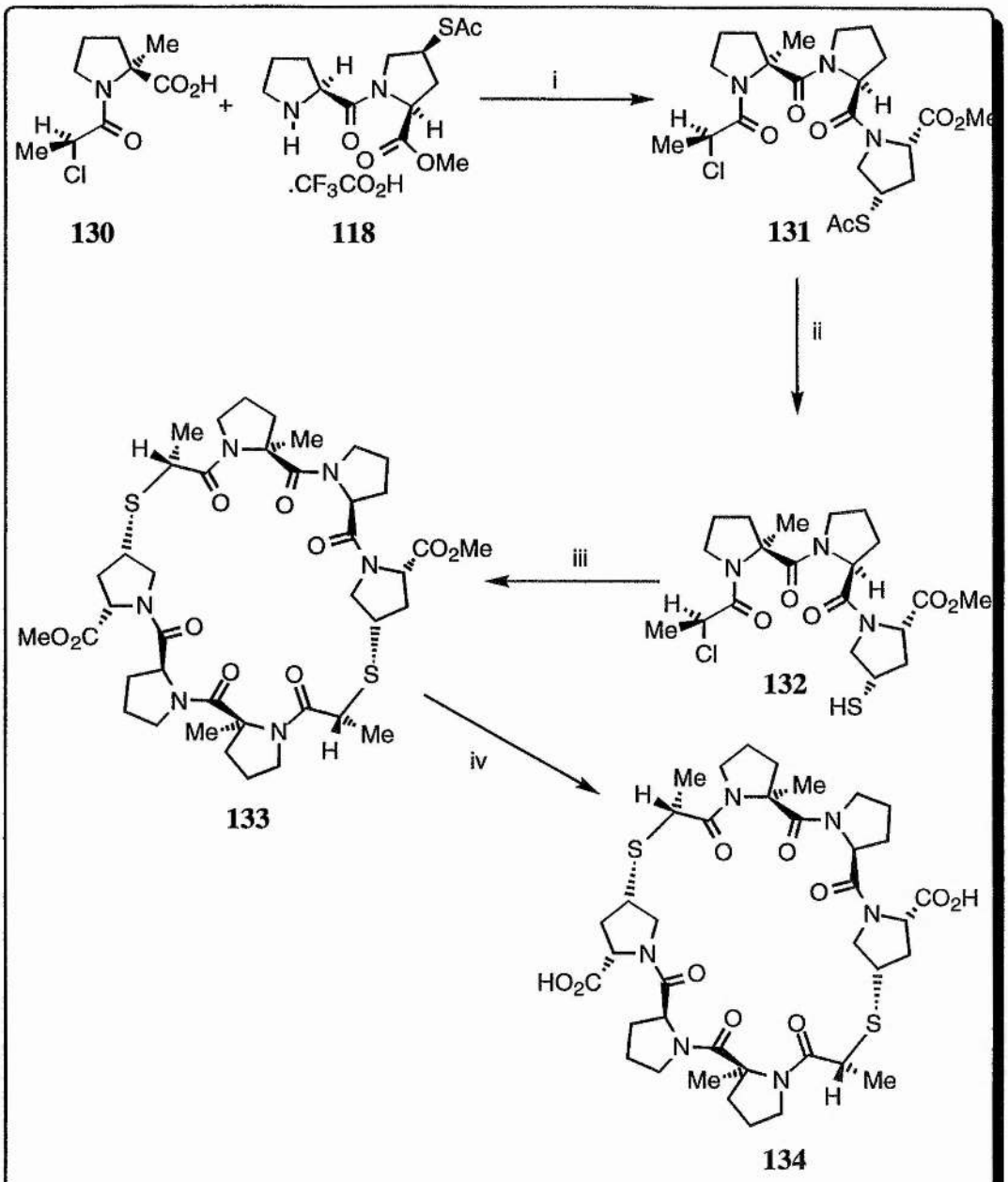
afforded a moderate 44% yield of the desired product. Although a three-fold excess increased the yield to 70%, this resulted in intolerable racemisation at the chloro-substituted chiral centre. Further optimisation established that a two-fold excess of activated acid gave a satisfactory 62% yield with no significant racemisation.

Alkaline hydrolysis of the methyl ester **129** to the acid **130** was sluggish, a consequence of the adjacent quaternary centre. With care, complete cleavage took place over 1-2 days. Increases in either alkali concentration or reaction time led to various degrees of substitution of chloride for hydroxide.

The next synthetic step required coupling of the acid **130** to the amine thiolester **118**, an intermediate in the previous synthesis. The powerful coupling reagent HATU afforded only 21% of the desired peptide **131,** whilst BOP-Cl was more slightly more efficient, giving 31% yield. The slow acylation rate, related to the significant associated steric hindrance of both species, may have rendered dioxopiperazine formation from the amine fragment **118** competitive. This compound is particularly prone to this cyclisation process due to the presence of proline and hence its higher propensity to contain a *cis* amide bond. Such a side-reaction may be responsible for the low yield in this coupling reaction.

Nevertheless, useful quantities of the thiolester **131** could be obtained. This was converted to the thiol **132** using mild alkaline hydrolysis (Scheme 3.12).

In this case, caesium carbonate proved slightly more effective than sodium hydride for the subsequent cyclisation, affording 30% of a compound suggested by NMR to be cyclic in nature. Once again, mass spectral analysis confirmed formation of a cyclic dimer **133**. It appears that incorporation of a $Pro^{Me}$ residue failed to promote sufficiently the required folded forms of the acyclic precursor. However, NMR spectra of the cyclic dimer **133** do highlight the influence that the two $Pro^{Me}$ residues have on the conformation of the macrocycle.




*Reagents and conditions*: i, BOP-Cl, DIEA, $CH_2Cl_2$, 0–5 °C, 5 h then rt, 15 h; ii, 0.05 mol $dm^{-3}$ KOH, MeOH, $H_2O$, 4 h, 29% (2 steps); iii, $Cs_2CO_3$, DMF, 80–90 °C, 2.5 h, 30%; iv, 1.0 mol $dm^{-3}$ NaOH, MeOH, $H_2O$, 2 h, 78%.

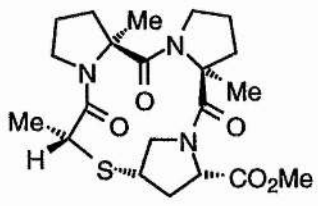
**Scheme 3.12:** *Synthesis of cyclic dimers **133** and **134***

Cyclic dimer **122** containing only ordinary proline residues showed predominantly one conformational state, with others accounting for <5% of the total in both chloroform and water. In contrast, the bis-($Pro^{Me}$) macrocycle **133** displayed solvent-dependent conformational isomerisation. In chloroform, three sets of peaks were observed in both $^{1}H$ and $^{13}C$ NMR spectra, in relative abundance 3:2:2. This may be due to the presence of three distinct $C_2$-symmetric conformations which interconvert slowly on the NMR timescale, or alternatively one $C_2$-symmetric conformation and one which lacks an axis of symmetry. In aqueous solution, only one set of NMR resonances was observed, suggesting that the interconversion between the various conformations was too rapid to be discernable in this medium.

The acid form of the dimer **134** showed isomerisation in aqueous solution, the NMR spectra showing three sets of peaks in relative abundance 81:13:6. High-field 2D NMR experiments confirmed that the most populated conformer was the same as that found for macrocycle **123**, with six *trans* amide bonds.

### 3.5 Attempted Synthesis of a $Pro^{Me}$-$Pro^{Me}$-Pro Thioether Macrocycle

Further promotion of folded forms of the linear precursor was clearly necessary to enable cyclisation to take place. Extension of the principles applied in design of the $Pro^{Me}$ macrocycle **123** leads to the new target **135** which incorporates *two* $Pro^{Me}$ residues.




**135**

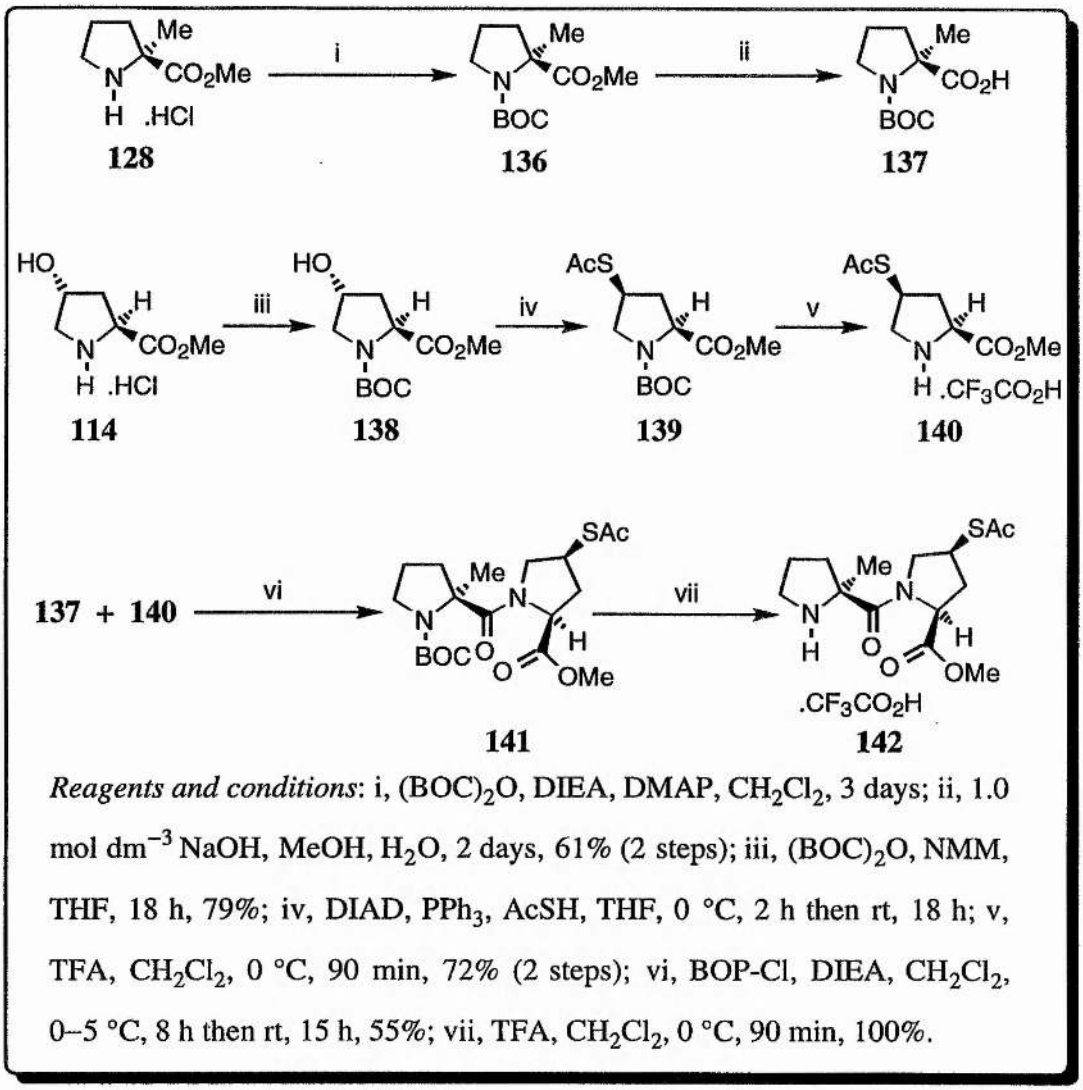
Molecular mechanics simulations of compound **135** predicted a further advantage: an increased preference for the potential helix-initiating ***S ttt*** substate over the ***M*** substate (Table 3.4).

**Table 3.4:** *Energy differences (in kcal mol$^{-1}$) between non-helical (**M**) and helical (**S**)* ttt *conformations of macrocycle **112** and analogues **125** and **135** containing one and two α-methylproline residues respectively*

| Compound | *M* | *S* | Difference |
|---|---|---|---|
| **112** | 96.99 | 99.01 | +2.02 |
| **125** | 118.95 | 117.39 | −1.56 |
| **135** | 178.43 | 175.34 | −3.09 |

A second Pro$^{Me}$ residue would also have an additional destabilising effect on the *cis* configuration of the second and third amide bonds. Hence, if the 12-membered ring could be formed, a helix-initiating conformation is even more likely for this macrocycle. Unfortunately, the presence of two Pro$^{Me}$ residues was certain to pose serious synthetic problems.

This attempted synthesis of macrocycle **135** required a modification to the amine fragment **118**. Accordingly, (2*S*)-2-methylproline methyl ester hydrochloride **128** was converted to the corresponding BOC derivative **136** upon action of $(BOC)_2O$ with DMAP catalysis and a prolonged reaction time. Alkaline hydrolysis then gave the free acid **137** in a satisfactory 61% yield over the two steps (Scheme 3.13).

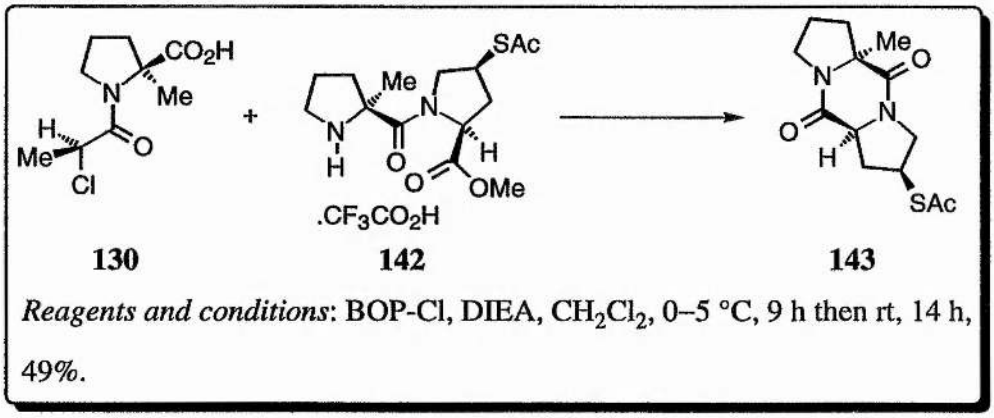



*Reagents and conditions*: i, $(BOC)_2O$, DIEA, DMAP, $CH_2Cl_2$, 3 days; ii, 1.0 mol $dm^{-3}$ NaOH, MeOH, $H_2O$, 2 days, 61% (2 steps); iii, $(BOC)_2O$, NMM, THF, 18 h, 79%; iv, DIAD, $PPh_3$, AcSH, THF, 0 °C, 2 h then rt, 18 h; v, TFA, $CH_2Cl_2$, 0 °C, 90 min, 72% (2 steps); vi, BOP-Cl, DIEA, $CH_2Cl_2$, 0–5 °C, 8 h then rt, 15 h, 55%; vii, TFA, $CH_2Cl_2$, 0 °C, 90 min, 100%.

**Scheme 3.13:** *Synthesis of Pro$^{Me}$ thiolester dipeptide 142*

We chose to incorporate the thiolacetate group prior to the coupling of the two proline residues in order to avoid manipulation of the precious Pro$^{Me}$-Pro dipeptide. Accordingly, (2*S*)-4-*trans*-hydroxyproline methyl ester hydrochloride **114** was converted to its *N*-BOC derivative **138** which was in turn converted to the thiolester **139** using the Mitsunobu-type inversion. Trifluoroacidolysis of the amino protecting group afforded the amine trifluoroacetate salt **140**. The acid **137** and amine **140** were coupled using BOP-Cl to give 55% of the Pro$^{Me}$-Pro dipeptide **141**. Removal of the *N*-terminal protecting group gave amine trifluoroacetate salt **142**.

The subsequent coupling reaction between two $Pro^{Me}$ residues proved to be most difficult yet, due to both fragments **130** and **142** possessing extreme steric hindrance. Although BOP-Cl had proved to be clearly superior to any other reagent in such hindered reactions, in this case it failed to produce any of the desired product. Instead, 49% of the dioxopiperazine **143** was isolated (Scheme 3.14). The very slow rate of *intermolecular* acylation rendered *intramolecular* acylation the faster process, despite the presence of a $Pro^{Me}$ residue which disfavoured the required *cis* amide bond configuration of amine **142**.

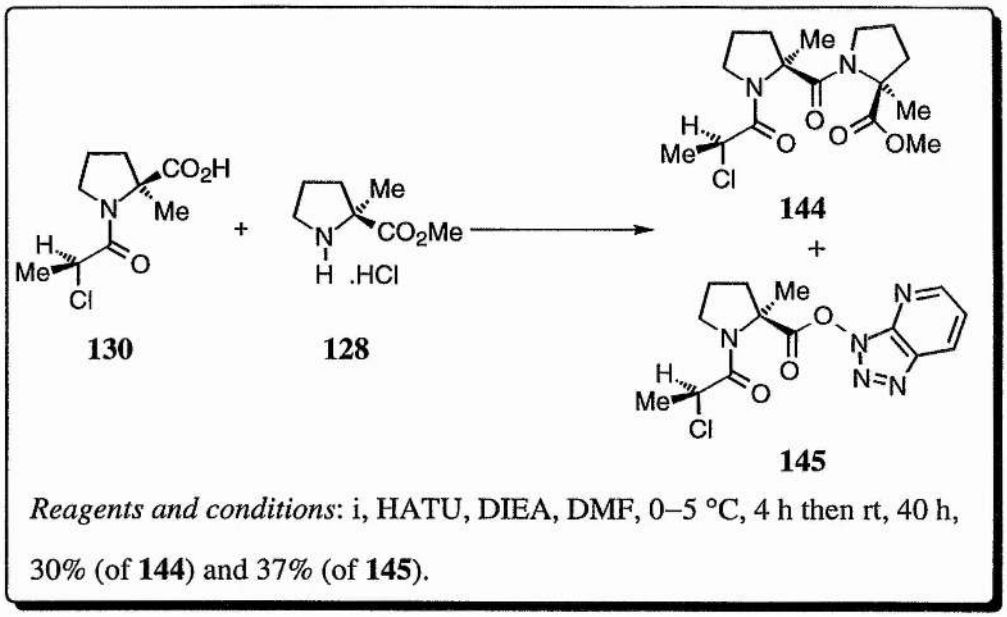



**130** **142** **143**

*Reagents and conditions*: BOP-Cl, DIEA, $CH_2Cl_2$, 0–5 °C, 9 h then rt, 14 h, 49%.

**Scheme 3.14:** *Dioxopiperazine formation upon attempted coupling of $Pro^{Me}$ residues*

In order to overcome the problem of dioxopiperazine formation, we decided to pursue a linear synthetic route. We attempted to mediate amide bond formation between acid **130** and amine **128** using HATU (Scheme 3.15). Gratifyingly, this was partially successful, affording 21–30% of $Pro^{Me}$-$Pro^{Me}$ peptide **144**. A second compound could also be isolated from the reaction mixture, and this appeared to be the azabenzotriazolyl ester **145**. This acylating agent survived an aqueous work-up and silica column chromatography, demonstrating the extraordinary low reactivity that the α-methyl group imparts on the adjacent carbonyl group.




*Reagents and conditions*: i, HATU, DIEA, DMF, 0–5 °C, 4 h then rt, 40 h, 30% (of **144**) and 37% (of **145**).

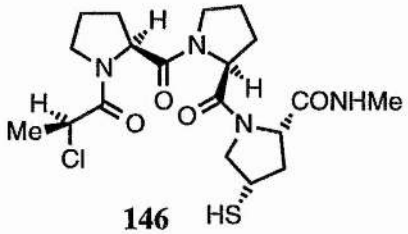
**Scheme 3.15:** *Partially successful coupling of two Pro$^{Me}$ residues*

Although it was possible to form the exceptionally difficult Pro$^{Me}$-Pro$^{Me}$ sequence, the yields to this point were low, and we did not envisage being able to produce useful amounts of more advanced intermediates. This view was supported by an attempt to hydrolyse the methyl ester of compound **144**. This usually facile reaction did not go to completion even after a week in strongly alkaline conditions. Therefore, we decided to abandon synthesis of the bis-(Pro$^{Me}$) macrocycle, and considered other ways of stabilising the required folded transition state of the linear precursor.

### 3.6 Effect of Hydrogen Bonding on the Conformations of Linear Precursors

We have established that steric effects alone are insufficient to promote the necessary folding of the triprolyl sequence. In nature, folded structures in peptides and proteins are often stabilised by hydrogen bonding. Can we use this phenomenon to favour the cyclisation reaction?

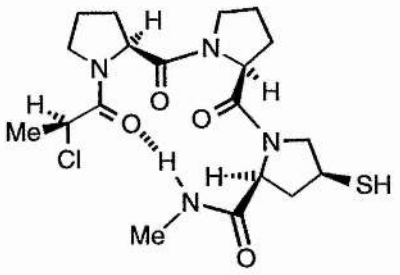
Conversion of the methyl ester of the original linear precursor to a methylamide gives triprolyl peptide **146**.



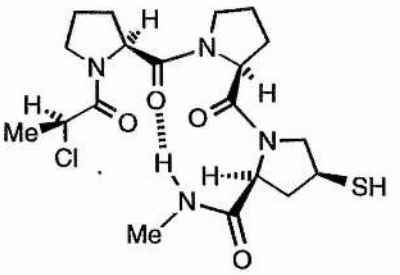

**146**

The potential now exists for formation of intramolecular hydrogen bonds which could stabilise folded states approximating to the desired cyclic structure, thus assisting cyclisation. Possible hydrogen bonding schemes involve the terminal methyl amide in $i \rightarrow (i-5)$ (α-helical turn), $i \rightarrow (i-4)$ ($3_{10}$-helical/β-turn) or $i \rightarrow (i-3)$ (γ-turn) interactions with preceding carbonyl groups (Fig. 3.3). Model studies on other peptide sequences have shown that the β-turn seems to be the most stable of the three in isolation. [13, 56]

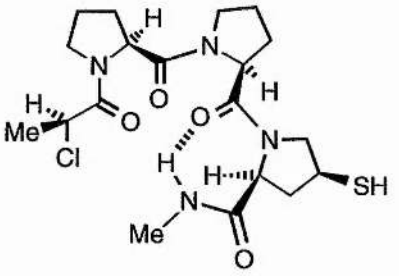
These conformations may allow favourable geometry for intramolecular reaction, particularly if the α-helical turn makes a prominent contribution to the secondary structure of the linear precursor. Hydrogen bonding has found uses in favouring cyclisations in the past, for example in Wenger's synthesis of cyclosporin A **7**. [276]



α-helical turn



β-turn

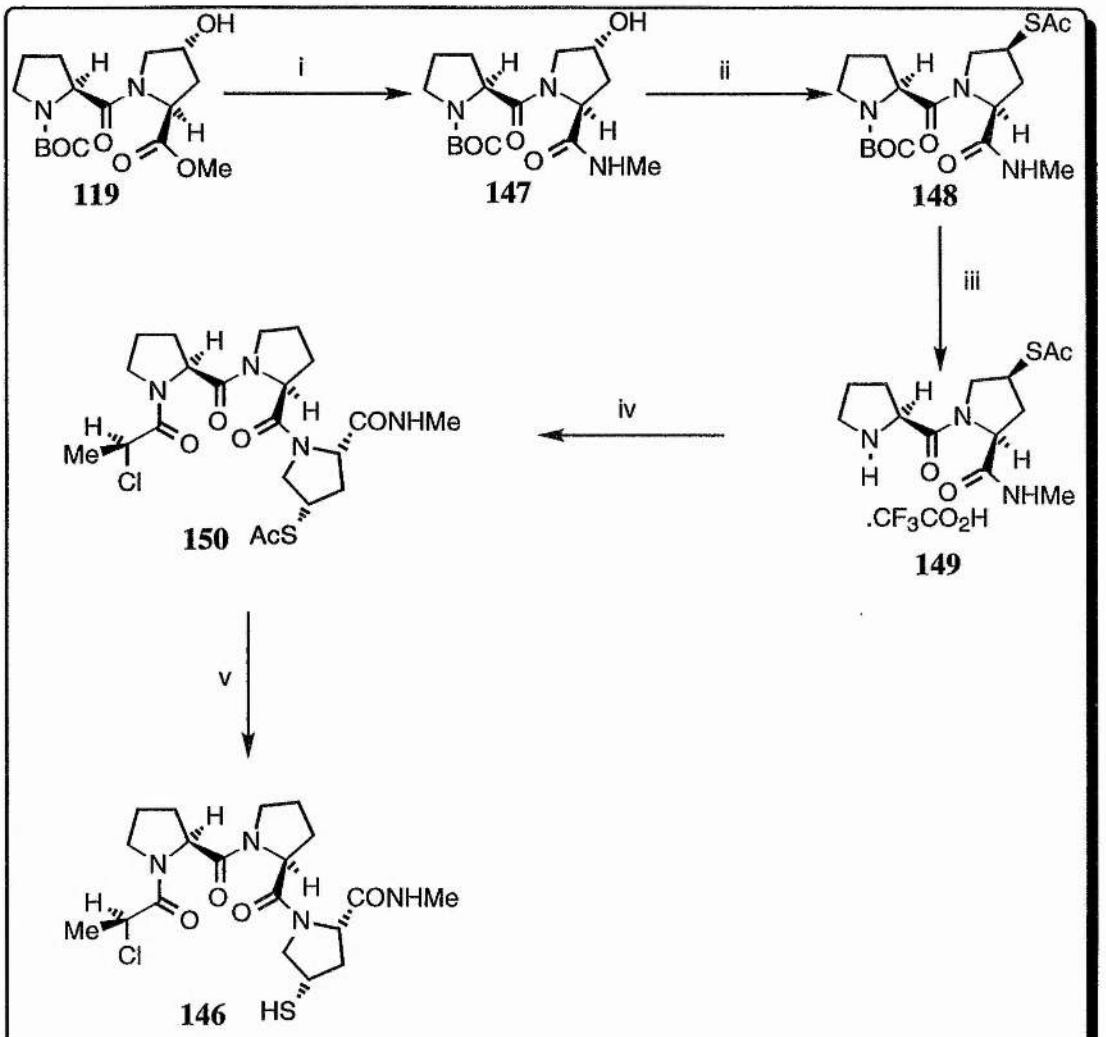


γ-turn

**Figure 3.3:** *Possible hydrogen bonded conformations of linear precursor* ***146***

Hydrogen bonding of this sort provides an enthalpic bias towards certain folded conformations, but the actual geometry adopted depends on a fine balance between this favourable effect and other forces which act against compact structures, such as loss of entropy upon formation of hydrogen bonded rings, torsional strain, and repulsive dipolar interactions. [12, 13, 16] It remained to be seen whether the enthalpic gain of a hydrogen bond would be enough to overcome the repulsive steric interactions which prohibited cyclisation of earlier linear precursors. Hydrogen bonding interactions in Pro-Pro-Ala tripeptides have been demonstrated to promote folded forms of this sequence. [82] We hoped that this might also be possible in the similar Pro-Pro-Pro sequence.

The synthetic route to the linear precursor **146** used a very similar strategy to the previous synthesis (Scheme 3.16). To begin with, methyl ester **119** was converted to the corresponding methyl amide **147** in quantitative yield.

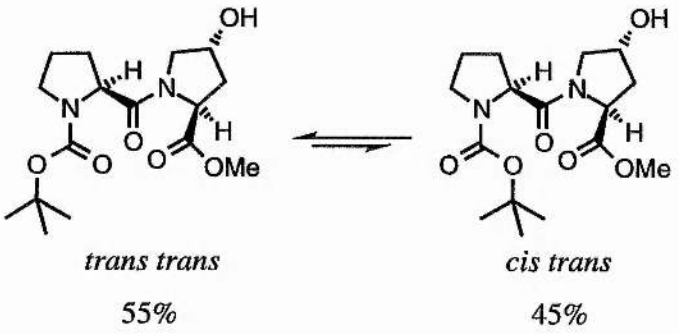



*Reagents and conditions*: i, $NH_2CH_3$, MeOH, 1 day, 100%; ii, DIAD, $PPh_3$, AcSH, THF, 0 °C, 2.5 h then rt, 15 h, 50%; iii, TFA, $CH_2Cl_2$, 0 °C, 2.5 h; iv, **105**, BOP-Cl, DIEA, $CH_2Cl_2$, 0–5 °C, 2 h then rt, 2 days, 69% (2 steps); v, 0.05 mol $dm^{-3}$ KOH, MeOH, $H_2O$, 4 h, 66%.

**Scheme 3.16:** *Synthesis of linear precursor* ***146***

The methyl ester **119** shows two conformations in single-dimensional NMR spectra in $C^2HCl_3$. Examination of the proline $C^\beta$ and $C^\gamma$ $^{13}C$ chemical shifts allows for designation of *cis* and *trans* isomers about the preceding urethane or amide bond.

In ester **119**, the conformations present are thus identified as *trans trans* (*tt*) and *cis trans* (*ct*), in 55% and 45% abundance respectively (Fig. 3.4).




*trans trans* 55%

*cis trans* 45%

**Figure 3.4:** *Conformations adopted by methyl ester **119***

*Cis* and *trans* isomers of urethane bonds are of very similar energy due to the similar size of the carbonyl oxygen and the alkoxy substituent. The slight preference for the *trans* configuration may be due to an O–H·····O=C 10-membered ring hydrogen bond which can form between the $Pro^2$ γ-hydroxy substituent and the urethane carbonyl oxygen. No trace of the *cis* configuration about the Pro-Pro amide bond could be found, indicating that it is of significantly higher energy than the *trans* form in the absence of external stabilising forces.

In contrast, the methyl amide **147** displays *three* conformational isomers. Since the only change is incorporation of a hydrogen bond donor, the additional conformation must be one stabilised by a hydrogen bond.

The NMR spectra reveal that the *tc*, *tt*, and *ct* configurations are present, in relative abundance 14:62:24 (Fig. 3.5).

The *tc* state is not found in the methyl ester, and must therefore be stabilised by an N–H·····O=C hydrogen bond involving the methyl amide NH and the urethane carbonyl oxygen. The presence of a hydrogen bond is further indicated by a 0.4 ppm upfield shift of the amide proton resonance relative to the non-hydrogen bonded form; infra-red spectra in dichloromethane solution also show two N–H stretch bands at

3448 and 3342 $cm^{-1}$, which correspond to non-hydrogen bonded and intramolecularly hydrogen bonded N–H groups respectively.

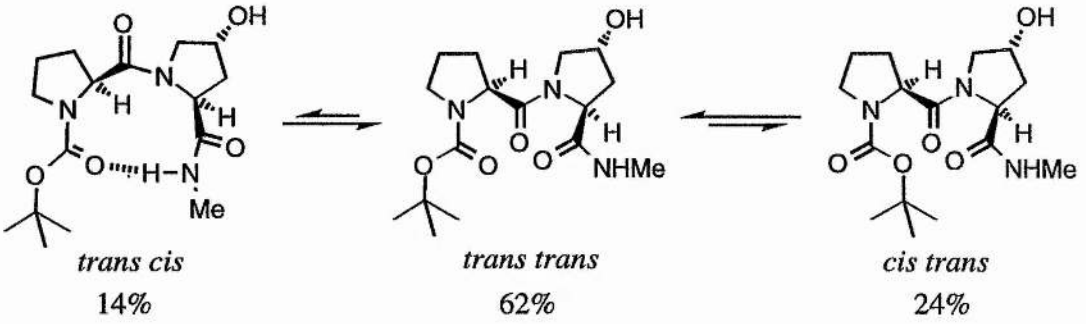



**Figure 3.5:** *Conformations adopted by methylamide **147***

Alcohol **147** was converted to thiolester **148**, and subsequent deprotection of the amino terminus gave amine trifluoroacetate salt **149** which showed only one conformation (Scheme 3.16). There is now only one isomerisable bond, and the removal of the urethane hydrogen bond acceptor site destroys any stabilising influence over the *cis* configuration, so only the *trans* form is now stable. Amine trifluoroacetate salt **149** was subsequently coupled to acid **105** to afford the thiolester **150** in 69% yield. This thiolester shows two conformations, corresponding to *ttt* (45%) and *ttc* (55%), due to re-establishment of *cis-trans* isomerisation about the $Pro^2$-$Pro^3$ amide bond (Fig. 3.6).

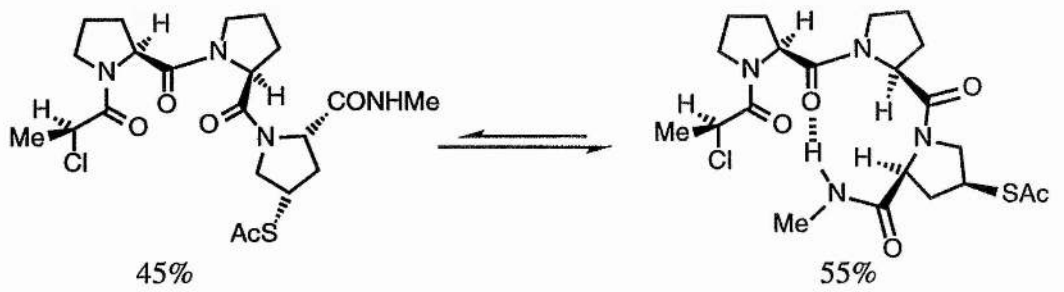



**Figure 3.6:** *Conformations adopted by thiolester **150***

Conversion of thiolester **150** to the free thiol **146** does not change the conformational equilibrium adopted in $C^2HCl_3$. Spectra in $^2H_2O$ show only the *ttt* state because the intramolecular hydrogen bond found in the *ttc* state is replaced by intermolecular solvent-peptide hydrogen bonds.

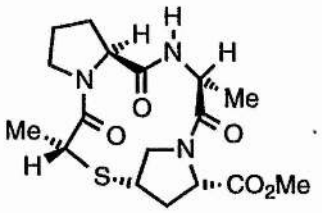
It appears that addition of a hydrogen bonding group at the *C*-terminus will not be able to assist the thiol to adopt the required conformations involving close approach of the electrophile and nucleophile. A hydrogen bond *is* present in about half the molecules in chloroform solution and to some degree is able to overcome repulsive interactions which otherwise prohibit the folded form. However, the stabilised conformation is one in which the thiol group is held *away* from the intended site of reaction, and as such is of no use in assisting the cyclisation process.

## 3.7 Studies of a Pro-(2*R*)-Ala-Pro Thioether Macrocycle

### 3.7.1 Design

Steric and hydrogen bonding effects both failed to promote the required folds of linear precursors to our desired macrocycles. This is largely due to the excessive non-bonded interactions between the three pyrrolidine rings which greatly inhibit the backbone flexibility. Rather than trying to overcome such unfavourable steric effects in the transition state, a better method may be to increase the flexibility of the backbone. [277] This can be done by substituting $Pro^2$ by a less sterically demanding residue, thus removing undesirable interactions between $Pro^2$ and the two adjacent Pro residues which preclude folded conformations. Of course, this substitution will also increase the conformational space available to the macrocycle.

Initially we chose to exchange $Pro^2$ for Ala, a helix-favouring residue which will significantly increase the conformational space available to the sequence. We decided on a (2*R*)-Ala residue because the Pro-(2*R*)-Ala sequence is well-established as an effective β-turn inducer, [133, 252, 277-279] and should therefore favour the elusive conformations wherein the reactive centres are proximal.



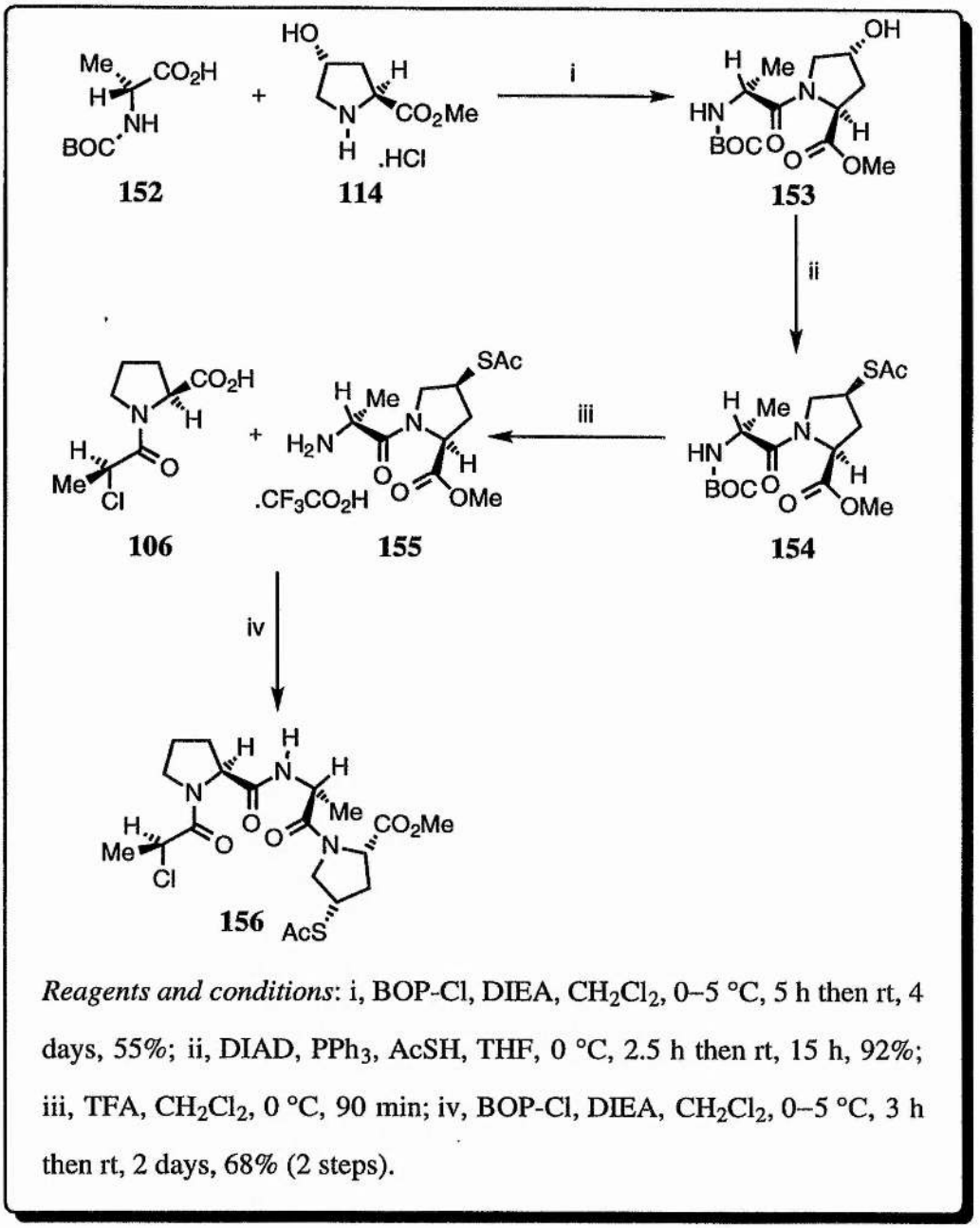
**151**

Molecular mechanics modelling of the proposed template **151** has not yet been completed because of the large increase in accessible conformations resulting upon substitution of $Pro^2$ for Ala. Although *cis* configurations of secondary amides have been found in cyclic peptides, [91, 93] we can predict that *ttt* conformations of the macrocycle will be of relatively low energy because the secondary $Pro^1$-$Ala^2$ amide bond will have a significant bias towards the *trans* form.

### 3.7.2 Synthetic Studies

The synthesis followed the familiar pattern (Scheme 3.17). BOC-(2*R*)-Ala-OH **152** was coupled with (2*S*)-*trans*-4-hydroxyproline methyl ester **114** using BOP-Cl to give dipeptide **153** in 55% yield. Mitsunobu inversion gave the thiolacetate **154**, and removal of the BOC group gave amine trifluoroacetate salt **155** cleanly in 92% yield over the 2 steps.

These compounds all contain small amounts (10–20%) of a conformation with a *cis* $Ala^2$-$Pro^3$ amide bond, which was never present in Pro-Pro sequences without external stabilisation. Peptide bond formation between amine trifluoroacetate **155** and the ubiquitous acid **105** was mediated by BOP-Cl to give 68% of the Pro-(2*R*)-Ala-Pro peptide **156**.

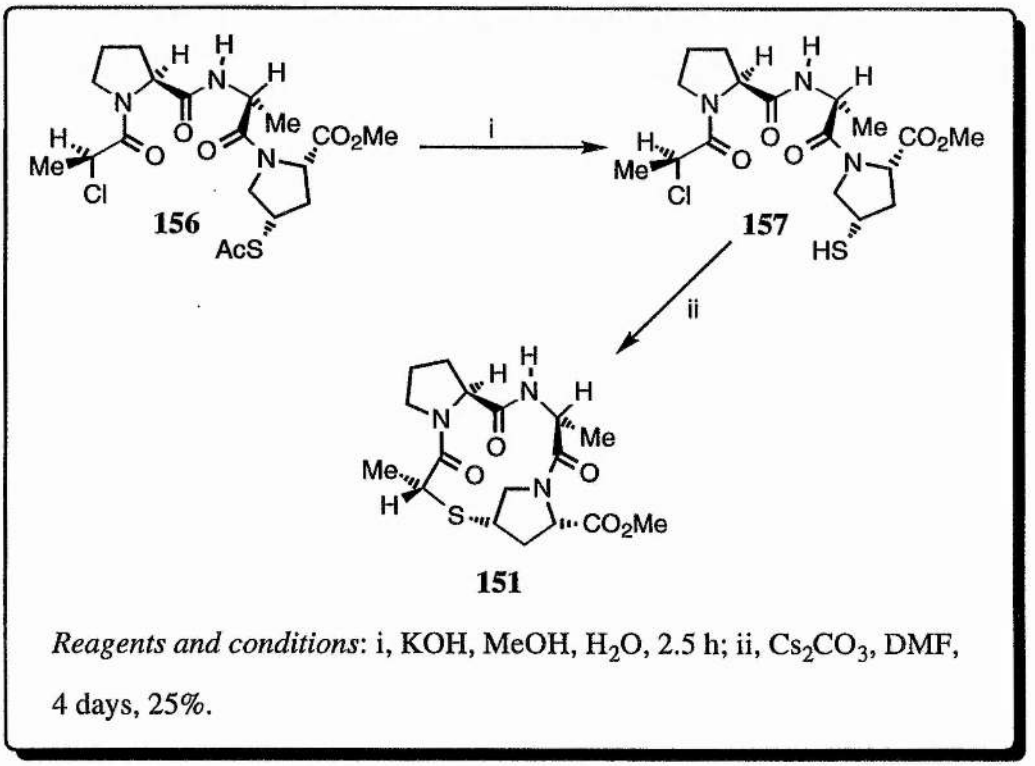



*Reagents and conditions*: i, BOP-Cl, DIEA, $CH_2Cl_2$, 0–5 °C, 5 h then rt, 4 days, 55%; ii, DIAD, $PPh_3$, AcSH, THF, 0 °C, 2.5 h then rt, 15 h, 92%; iii, TFA, $CH_2Cl_2$, 0 °C, 90 min; iv, BOP-Cl, DIEA, $CH_2Cl_2$, 0–5 °C, 3 h then rt, 2 days, 68% (2 steps).

**Scheme 3.17:** *Synthesis of linear precursor* ***156***

Surprisingly, mild alkaline hydrolysis of the thiolester **156** to the free thiol **157** did not give a pure product. It appeared that some other process had occurred in addition to cleavage of the thiolester. Apparently, some thiolate alkylation had already taken place. The impure material **157** was taken straight on and subjected to

cyclisation conditions using caesium carbonate in DMF under high dilution conditions at room temperature over 4 days (Scheme 3.18). Column chromatography of the crude product again enabled isolation of a compound displaying NMR spectra corresponding to those expected of the 12-membered macrocycle **151**. Gratifyingly, mass spectroscopy revealed this to be the desired *monomeric* cyclic compound **151** ($[M + H]^+$ = 384). Some cyclic dimer was also formed, but was easily separated by chromatography. This methodology allowed a 25% conversion of thiolacetate **156** to the macrocyclic compound **151**. Optimisation of the process established that compound **151** could be produced directly from the thiolacetate *via* action of a dilute solution of potassium hydroxide in methanol–water at 65 °C for 2.5 h in an excellent 69% yield. [280]




*Reagents and conditions*: i, KOH, MeOH, $H_2O$, 2.5 h; ii, $Cs_2CO_3$, DMF, 4 days, 25%.

**Scheme 3.18:** *Synthesis of macrocycle **151***

### 3.7.3 Conformational Studies

Macrocycle **151** proved conducive to analysis by X-ray diffraction of a crystal grown as a hemihydrate from ethyl acetate–hexane. The solid-state structure of the compound is shown below (Fig. 3.9).

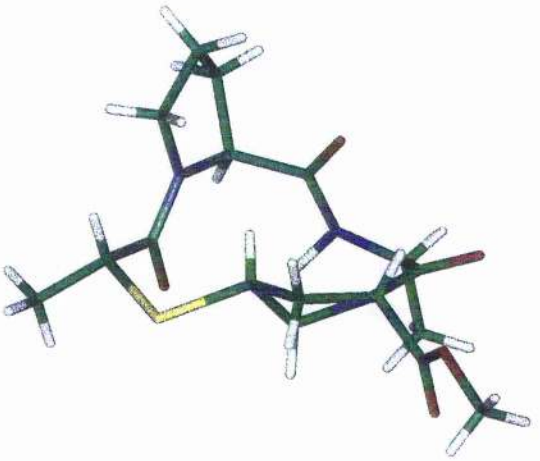
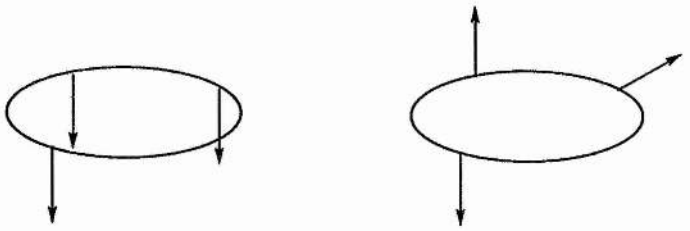


**Figure 3.9:** *Crystal structure of macrocycle **151***

All amide bonds take the *trans* configuration, fulfilling our first objective. However, the *ttt* substate adopted is incapable of helix initiation, with the carbonyl groups lacking the required alignment. In fact, the molecule lacks any significant dipole. Although the carbonyl group in the mercaptopropionyl bridge possesses an approximately α-helical geometry, the $Pro^1$ carbonyl has roughly opposite alignment, and the $Ala^2$ carbonyl is splayed out from the desired position, as the cartoon depiction in Fig. 3.10 demonstrates.



ideal α-helical geometry of carbonyl groups

geometry of carbonyl groups experimentally observed for macrocycle **151**

**Figure 3.10:** *Ideal and actual carbonyl alignments of macrocycle **151***

In $^2H_6$-DMSO solution, the macrocycle **151** displays only one conformation, which corresponds almost exactly to that found in the solid state. Any other conformation must be present in less than ~10% abundance, or NOESY crosspeaks derived from this second conformation would be observable. The diagnostic NOE's which indicate the "up" alignment of the Pro[1] carbonyl group all involve the Ala[2] amide proton. This shows interactions with the Pro[1] αCH and both of the Pro[3] $\delta CH_2$ protons (Fig. 3.11). The absence of NOE's to either Pro[1] $\beta CH_2$ proton argues against any significant population of the α-helical conformation with the Pro[1] carbonyl "down".

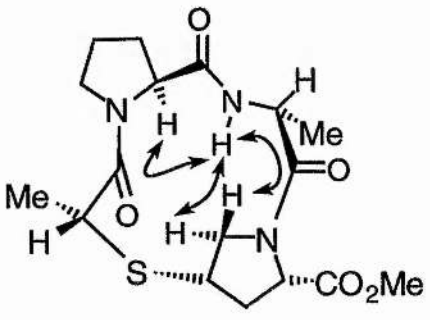



**Figure 3.11:** *NOE's observed for macrcocycle **151** in $^2H_6$-DMSO solution*

### 3.8 Evaluation of Macrocycle 151 as an α-Helix Initiator

Is the template **151** capable of initiating helical structure in an attached peptide? At first glance, the arrangement of carbonyl groups does not appear favourable. The $Pro^1$ carbonyl has exactly the opposite alignment to that required, and the $Ala^2$ carbonyl is also appreciably displaced from the position expected for an α-helix. However, the mercaptopropionyl carbonyl is in approximately the correct position to accept a hydrogen bond from the first amide group of an attached peptide and thus induce an α-helical geometry in at least the first appended residue. If it does so, the next NH group along the attached peptide chain will be in the correct position to hydrogen bond to the $Pro^1$ carbonyl *if the carbonyl group flips alignment* (Fig. 3.12). A similar effect may then occur for the $Ala^2$ carbonyl group which requires less movement to form a hydrogen bond with the next amide group of the growing helix.

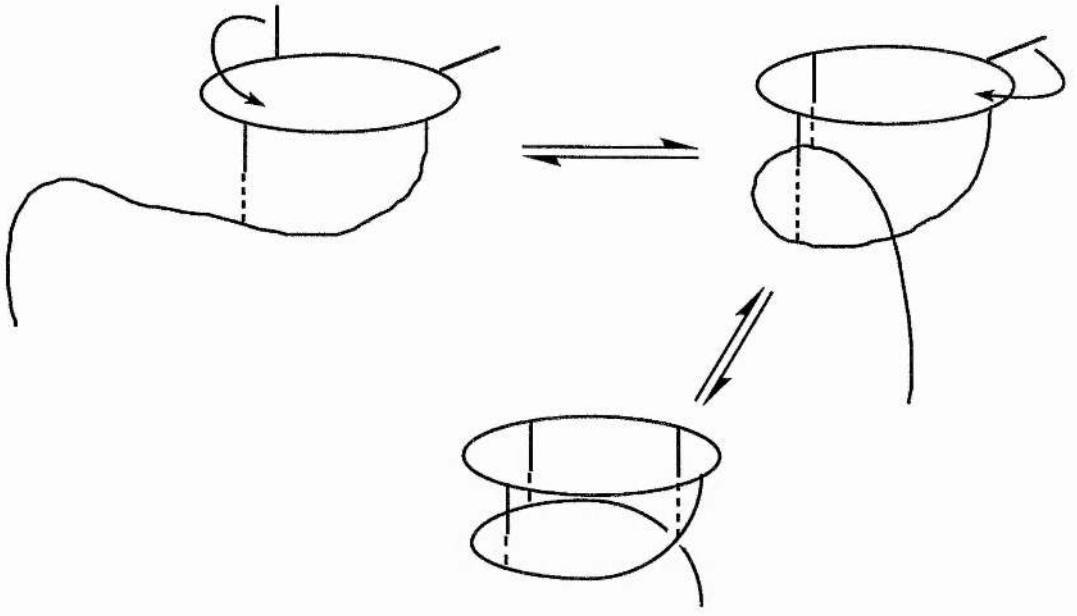


**Figure 3.12:** *Potential complementary conformational changes in macrocycle and attached peptide*

In this way, changes in conformation of both template and peptide would be complementary, in an analogous way to the changes in both receptor *and* ligand

### 3.8 Evaluation of Macrocycle 151 as an α-Helix Initiator

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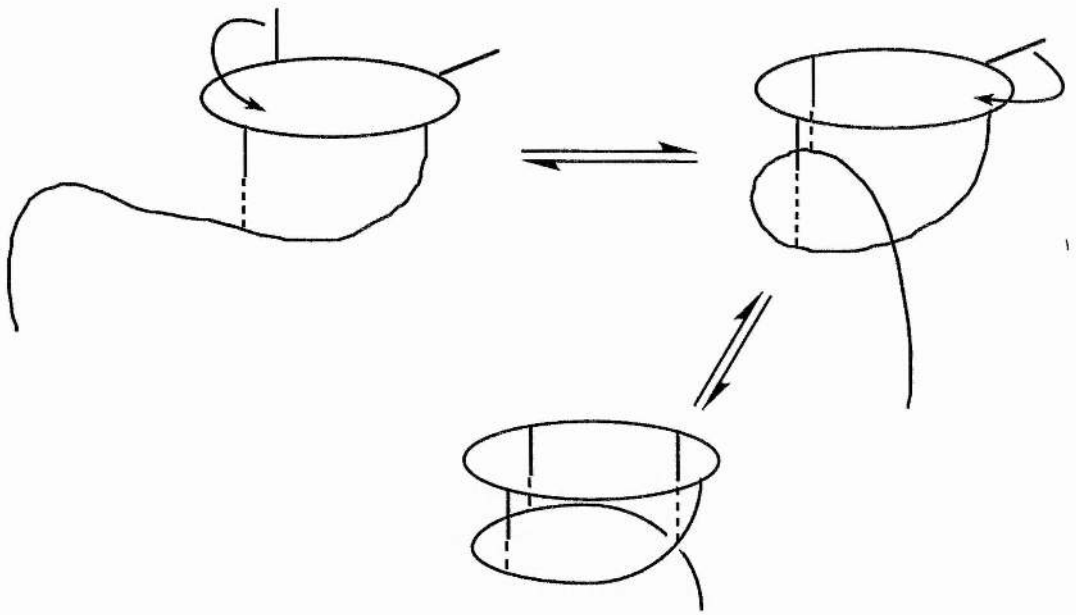


**Figure 3.12:** *Potential complementary conformational changes in macrocycle and attached peptide*

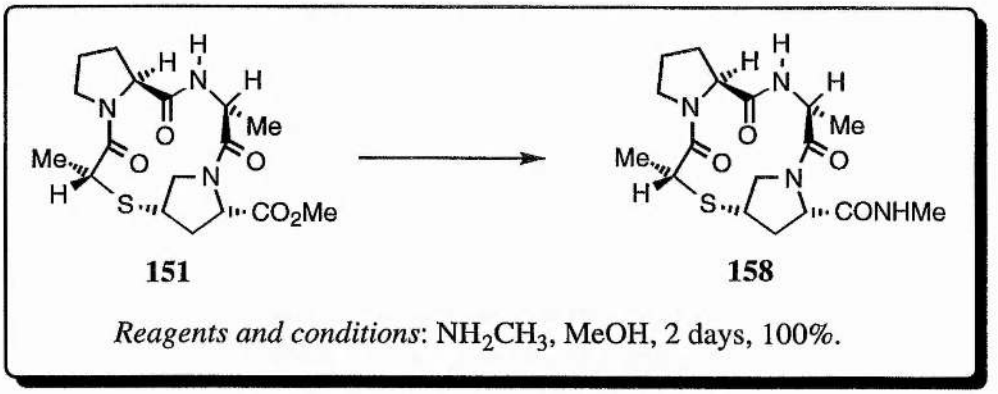
In this way, changes in conformation of both template and peptide would be complementary, in an analogous way to the changes in both receptor *and* ligand

conformation upon binding of bioactive peptides. Like Kemp's first template **51** (p. 46), NMR-observable changes in the macrocycle conformation might thus be used as a "reporter" on the conformation of the peptide, since the population of the α-helix-initiating conformation of the macrocycle would be directly proportional to the degree of α-helicity of the attached peptide. [224]

This "conformational co-operativity" is favoured by the formation of hydrogen bonds, but disfavoured on entropic grounds and by repulsive dipolar interactions. It was also unclear if the large change in the $Pro^1$ ψ dihedral angle required to bring its carbonyl group into the correct alignment possessed a prohibitively large activation energy due to hindered rotation within the cyclic structure.

This study of the feasibility of α-helix induction by template **151** requires synthesis of derivatives with appended peptide chains. The hydrogen bond donors of the attached peptide may be able to trigger conformational changes in the template and therefore the peptide itself.

One hydrogen bond donor was introduced upon conversion of the methyl ester **151** to the methylamide **158** (Scheme 3.19).




**151** **158**

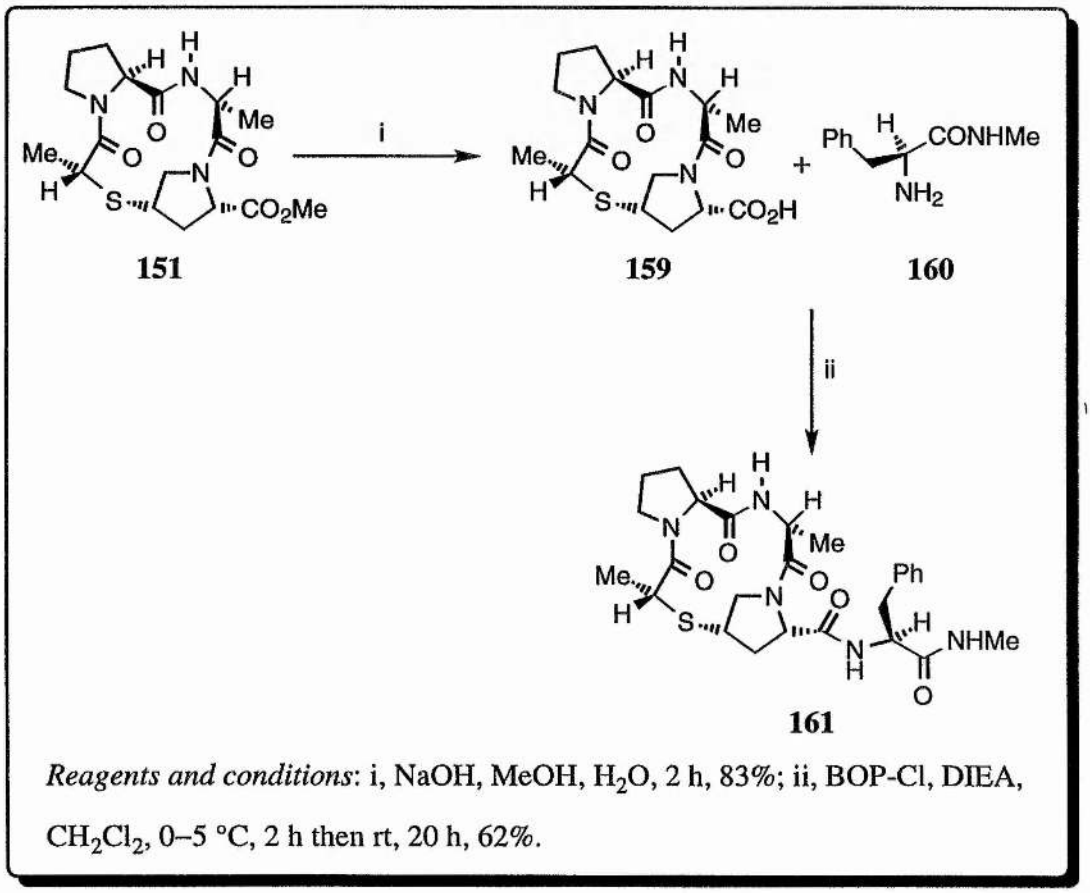
*Reagents and conditions*: $NH_2CH_3$, MeOH, 2 days, 100%.

**Scheme 3.19:** *Synthesis of macrocycle methylamide **158***

Conformational analysis of compound **158** in $H_2O$–$^2H_2O$ (9:1) indicated that there was no change in the conformation of the macrocycle upon addition of the hydrogen bond donor. The $Pro^1$ carbonyl group does not flip round to take part in a

$3_{10}$-helical hydrogen bond with the methylamide NH. Experiments to establish whether there is an α-helical hydrogen bond from this NH to the mercaptopropionyl carbonyl were inconclusive; weak NOE's show that the methylamide group may spend some time folded underneath the macrocycle, but the presence of a hydrogen bond could not be firmly established. In aqueous solution, the solvent may sequester the hydrogen bond donor so that the time it spends taking part in an α-helical hydrogen bond is greatly reduced.

Attachment of more than one hydrogen bond donor site required hydrolysis of the methyl ester **151** to the free acid **159**. The acid was coupled to (2*S*)-phenylalanine methyl amide **160** to give the template-peptide conjugate **161** (Scheme 3.20).




**151** **159** **160** **161**

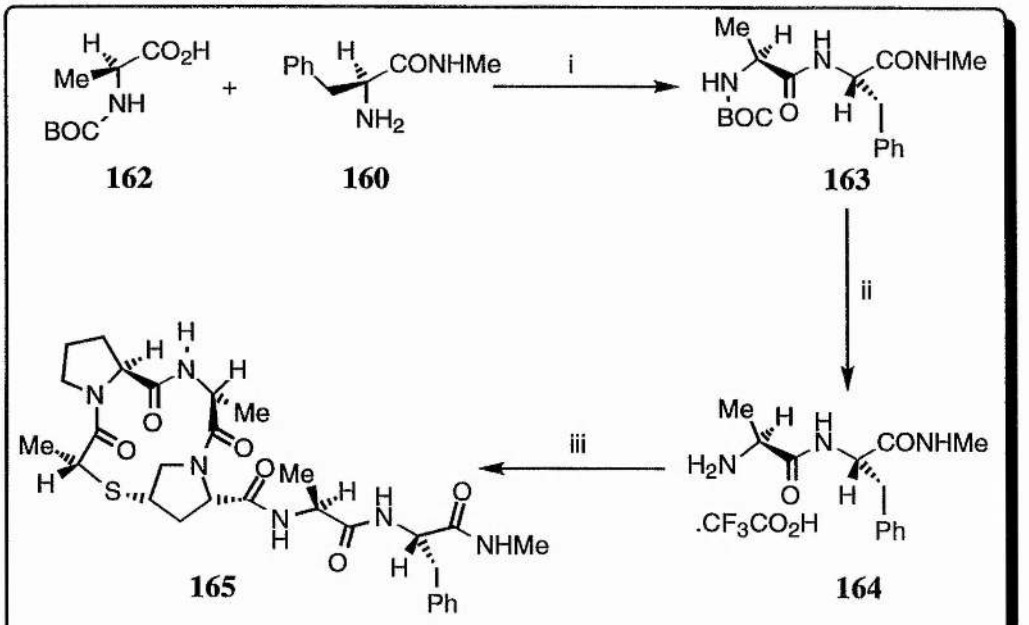
*Reagents and conditions*: i, NaOH, MeOH, $H_2O$, 2 h, 83%; ii, BOP-Cl, DIEA, $CH_2Cl_2$, 0–5 °C, 2 h then rt, 20 h, 62%.

**Scheme 3.20:** *Synthesis of macrocycle-peptide conjugate **161***

In $C^2HCl_3$ solution, the macrocycle in compound **161** shows no change in conformational preference relative to that in the methyl ester **151**. However, several NOE effects suggest some interaction between the lower face of the macrocycle and the attached peptide. This implies that the phenylalanine residue spends an appreciable amount of time folded underneath the macrocycle in a position conducive to the presence of a hydrogen bond between the phenylalanine amide proton and the mercaptopropionyl carbonyl. Such a hydrogen bond necessitates an α-helical position for the phenylalanine residue. This model is supported by a 0.2 ppm upfield shift of the mercaptopropionyl methyl protons, consistent with a ring current effect caused by the close proximity of the phenyl ring. There is also a 0.8 ppm downfield shift of the phenylalanyl amide proton relative to its position in a peptide known to lack intramolecular hydrogen bonding.

Although this folded conformation may be populated to some extent, other evidence suggests that there is still significant mobility associated with the attached peptide. Some NOE's are present which are not possible for the folded conformation, and the $^3J_{\alpha N}$ value for the phenylalanine residue is 8.0 Hz, which implies conformational averaging and not a fixed α-helical geometry.

In order to provide hydrogen bond donors for each acceptor carbonyl in the macrocycle, one further derivative was required. The peptide component was synthesised by mixed anhydride coupling of BOC-(2*S*)-Ala-OH **162** and (2*S*)-phenylalanine methylamide **160** to afford dipeptide **163**. TFA mediated removal of the BOC group yielded the trifluoroacetate salt **164**. This was coupled to the macrocycle acid **159** to give the desired conjugate **165** (Scheme 3.21).




*Reagents and conditions*: i, IBCF, NMM, THF, −15 °C → rt, 2 h, 67%; ii, TFA, $CH_2Cl_2$, 0 °C, 2 h; iii, **159**, BOP-Cl, DIEA, $CH_2Cl_2$, 0–5 °C, 2 h then rt, 18 h, 33% (2 steps).

**Scheme 3.21:** *Synthesis of macrocycle-peptide conjugate **165***

NMR studies in $C^2HCl_3$ solution supply no evidence to suggest that the attached hydrogen bond donors are altering the conformation of the macrocycle. However, there is fairly convincing evidence for the presence of an intramolecular hydrogen bond between the NH of the appended alanine residue and the thiapropionyl carbonyl. There is a relatively strong set of NOE's similar to those found for compound **161**, indicative of folding of the peptide under the macrocycle. The $^3J_{\alpha N}$ value for the attached alanine residue is 3.9 Hz, which corresponds exactly with the value expected for an α-helical geometry, and the high-field position of the NH resonance is indicative of involvement in a hydrogen bond. These results suggest that the macrocycle may be inducing α-helical character in the first attached amino acid residue, but not in any of the subsequent residues. The more convincing data for this

conjugate compared with derivative **161** may reflect the higher intrinsic α-helical propensity of alanine in comparison with phenylalanine. [37, 42]

### 3.9 Effect of a *C*-terminal Cation on the Conformation of Macrocycle 151

The enthalpic benefit of hydrogen bonding between the appended peptide and the template in the "helical" conformation appears unable to counterbalance the repulsive dipolar forces and entropic losses associated with this conformation.

However, hydrogen bonds are not the only stabilising force acting upon peptide and protein α-helices in nature. Another common feature of these α-helices is the presence of an associated charge of the appropriate sign near one of the helix termini, which interacts favourably with the helix macrodipole. [46, 51] The desired alignment of carbonyl groups in our macrocycle is disfavoured by repulsive dipole-dipole interactions. This repulsive force could be replaced by a favourable effect if a negatively charged group could be attached over the *N*-terminal face of the macrocycle (as found in Bartlett's template **54**, p 47 [227]) or if a positively charged group could be attached under the *C*-terminal face, as shown in Fig. 3.13. The latter effect was demonstrated by Gellman *et al.* who appended a tetraalkylammonium group at the *C*-terminus of a short depsipeptide with resultant induction of essentially complete α-helical structure. [56]

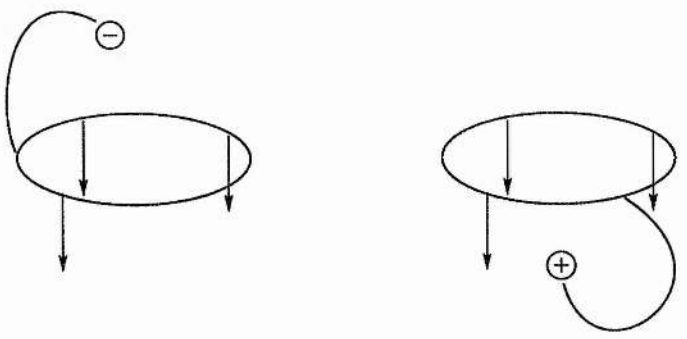
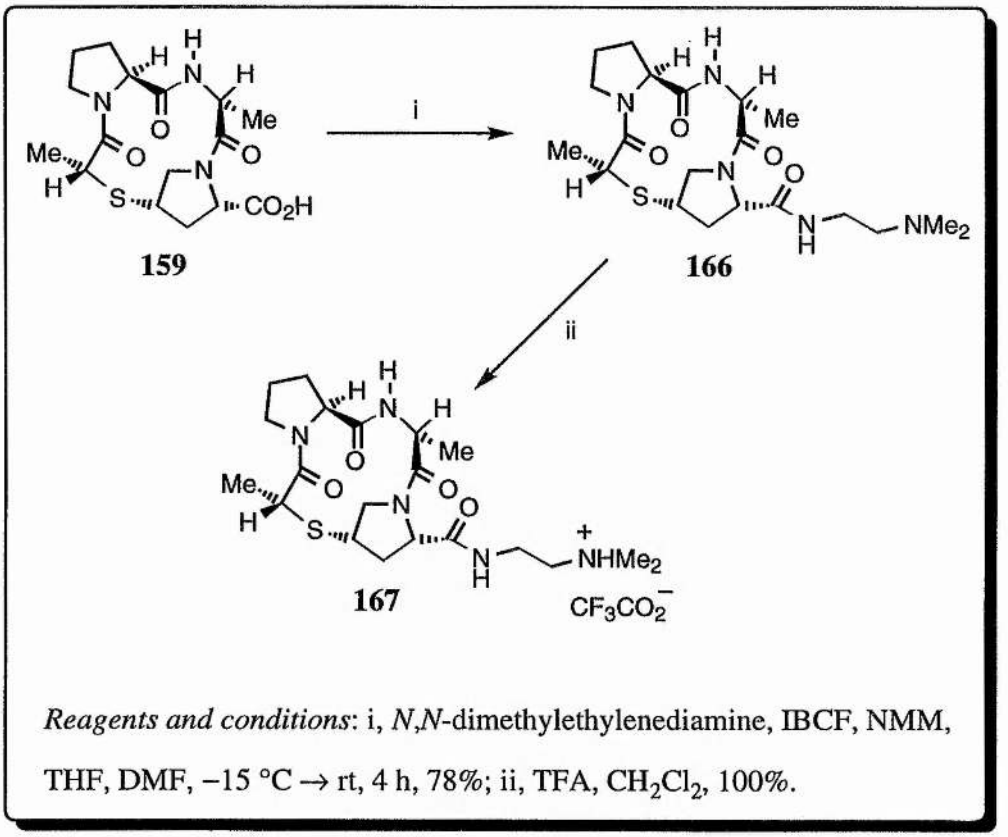


**Figure 3.13:** *Charge-template models for control of macrocycle conformation*

Introduction of an anionic group on the *N*-terminal face is synthetically challenging and cannot be accomplished from macrocycle **159**. Therefore, we chose to attach a cationic group at the *C*-terminus *via* the carboxyl group of macrocycle **159**. This proved to be straightforward, with mixed anhydride coupling of acid **159** to *N,N*-dimethylethylenediamine affording macrocycle **166** with a pendant tertiary amine (Scheme 3.22).




**Scheme 3.22:** *Synthesis of macrocycle-cation conjugate **167***

The amine **166** was converted to its trifluoroacetate salt **167** to produce the positively charged group intended to interact with the set of carbonyl groups in the macrocycle; two hydrogen bond donors are also present which may assist in stabilisation of α-helical macrocycle conformations.

In organic solution, the macrocycle-cation conjugate **167** behaves in a different manner to any of the previous systems. In this case, the macrocycle equilibriates between *two* conformations. One set of resonances was observed at room temperature, but the $^1H$ NMR resonances of both conformations could be frozen out at $-80$ °C in $C^2H_2Cl_2$. The rapid rate of interconversion between the two conformations resulted in significant complications even at this low temperature, but sufficient NOE constraints were obtained to allow simulated annealing and generation of reliable structures for both conformations.

The major conformation, accounting for ~60% of the population, was found to be similar to those observed in previous cases, although the $Ala^2$ carbonyl appears to have been pulled down from its previous position, presumably to interact with the appended positive charge (Fig. 3.14).

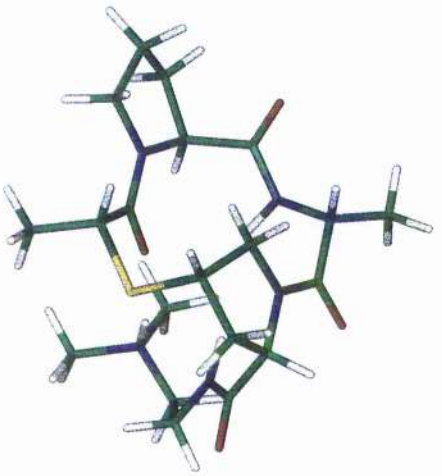


**Figure 3.14:** *Major conformation of macrocycle-cation conjugate **167***

In contrast, the minor conformation, accounting for ~40% of the population possesses an α-helical arrangement of carbonyl groups (Fig. 3.15). This conclusion is based on a set of NOE's involving the $Ala^2$ amide proton and protons on the upper face of $Pro^1$ which are only possible for a conformation with the $Pro^1$ carbonyl correctly aligned in an α-helical geometry.

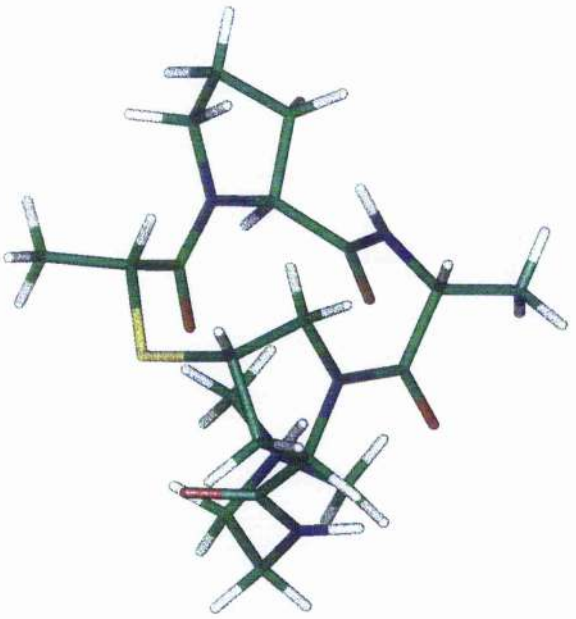


**Figure 3.15:** *Minor conformation of macrocycle-cation conjugate* ***167***

Dynamic modelling of the minor conformation using a dielectric constant of 4 (approximating to a non-polar environment) showed no evidence of hydrogen bonding between the macrocycle and the pendant chain. Instead, the trialkylammonium cation was positioned directly underneath the centre of the macrocycle, allowing maximum charge-dipole interaction with the aligned carbonyl groups. When the dielectric constant was increased to simulate an aqueous environment, the trialkylammonium group was found to drift away from the position directly beneath the macrocycle.

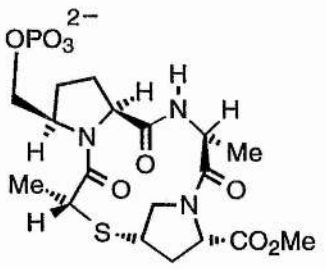
This striking change in macrocycle conformation shows that the generation of potential α-helix initiating templates is possible by judicious use of charge-dipole interactions. Modelling studies suggest that these initiating forms are stabilised solely by the charged group effect without contribution from hydrogen bonding.

### 3.10 Conclusions and Future Work

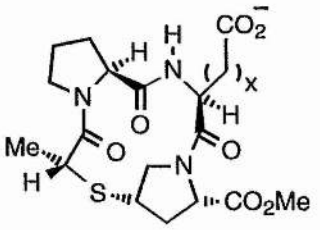
Under the influence of a *C*-terminal cationic group, the Pro-(2*R*)-Ala-Pro template has been demonstrated to adopt a significant population of the desired α-helical conformation in organic solution. Further derivatives of macrocycle **151** are under study, where additional amino acid residues are incorporated between the template and the pendant cation to establish the effect of the charge-template system on the conformational preference of an attached peptide. This may also allow us to determine if the NMR signature of the template in the α-helical state can act as a "reporter" on the degree of helicity adopted by the attached peptide. Experiments will be designed to calculate the dependence of the peptide conformation on the charge alone *i.e.* is it possible that any helical character induced in a peptide is caused solely by the charge-dipole interaction without the template exerting any significant stabilising influence? The solvent dependence of the conformational equilibrium exhibited by these systems will also be assessed, since the template must ideally be able to function in aqueous media which may lessen the beneficial charge-dipole interactions and disrupt intramolecular hydrogen bonding.

A more useful template would carry an anionic group at its "*N*-terminus", allowing for general use in conjunction with any peptide of interest without the need for synthesis of peptide derivatives with pendant amine groups. The anion-stabilised model also allows for the maximum possible ion-dipole effect to be exerted upon the template; this favourable influence is likely to be significantly diminished in the cation-stabilised model if longer peptides are inserted between the macrocycle and the pendant ammonium ion.

The first design **168** incorporates a side-chain carrying a phosphate group on $C^{\delta}$ of $Pro^{1}$. This side-chain can fold over the *N*-terminus of the helix to allow the doubly-charged anionic group to be positioned directly over the axis of the helix barrel where it can exert the maximum influence.



**168**



**169** x = 1
**170** x = 2

The alternative designs **169** and **170** are synthetically more accessible and could be made using the well-established methodology by substituting Ala[2] for a protected form of aspartic acid (macrocycle **169**) or glutamic acid (macrocycle **170**). This template follows nature's lead; Pro is the most common residue at the *N*-terminal position of α-helices, whilst Asp and Glu are the most common residues at the next *C*-terminal position. Although the negative charge attached to these templates is less likely to be positioned directly over the helix, they can also favour the "down" alignment of the Pro[1] carbonyl by forming a hydrogen bond from the carboxyl side-chain to the Ala[2] NH.

The optimised template can then be applied to a variety of peptide conjugates under various conditions to determine whether the template is an efficient "reporter" system (showing quantifiable spectral changes as a function of peptide helicity) and/or an efficient α-helix initiator (calculation of the helix initiation constant σ for the template).

Further into the future, an α-helix template might be applied to study of the mechanism of the FMDV 2A peptide. The template could be attached to the *N*-terminus of 2A analogues, resulting in stabilisation of α-helical structure. If the proposed bioactive structure involving a functional helical segment is correct, such stabilisation should allow the cleavage reaction to take place, allowing study of the cleavage mechanism catalysed by this protein fragment.

# 4. EXPERIMENTAL

Melting points were taken on an Electrothermal melting point apparatus and are uncorrected.

Optical rotations were measured at 22 °C on an Optical Activity AA-1000 polarimeter using a 10 cm path length cell.

Elemental microanalyses were performed in the departmental microanalytical laboratory.

IR spectra were recorded on a Perkin-Elmer 1710 f.t. IR spectrometer. The samples were prepared as Nujol mulls, thin films between sodium chloride discs or solutions in the specified solvent. The frequencies ($\upsilon$) of absorption maxima are given in wavenumbers ($cm^{-1}$) relative to a polystyrene standard.

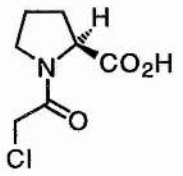
NMR spectra were recorded on a Brüker AM-300 ($^1H$, 300 MHz; $^{13}C$, 74.76 MHz), Varian 300 ($^1H$, 300 MHz; $^{13}C$, 75.44 MHz) or Varian gemini 200 ($^1H$, 200 MHz; $^{13}C$, 50.31 MHz) spectrometers. Chemical shifts are described in parts per million downfield from $SiMe_4$ and are reported consecutively as position ($\delta_H$ or $\delta_C$), relative integral, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; dd, doublet of doublets; m, multiplet and br, broad), coupling constant (*J*/Hz) and assignment (numbering according to the IUPAC nomenclature for the compound). $^1H$-NMR spectra were referenced internally on $^2HOH$ ($\delta$ 4.68), $CHCl_3$ ($\delta$ 7.27) or DMSO ($\delta$ 2.47). $^{13}C$-NMR spectra were referenced on $CH_3OH$ ($\delta$ 49.3), $C^2HCl_3$ ($\delta$ 77.5), or DMSO ($\delta$ 39.7). Carbon and proton resonances of amino acids in NMR spectra are assigned as $\alpha$, $\beta$, $\gamma$ and $\delta$ according to normal convention. Where more than one conformational isomer is present due to the presence of a tertiary amide bond, these are assigned as *c* (*cis*) or *t* (*trans*), according to the isomeric state of the amide bond. If the isomeric states of the amide bonds are not known, the conformations are assigned as *A*, *B*, *C etc.*

Mass spectra and accurate mass measurements were recorded on a VG 70-250 SE, a Kratos MS-50 or by the SERC service at Swansea using a VG AZB-E. Fast atom bombardment spectra were recorded using glycerol as a matrix. Major fragments were given as percentages of the base peak intensity (100%).

All experiments were performed at room temperature (20–25 °C) unless otherwise stated. Flash chromatography was performed according to the method of Still *et al.* [281] using Sorbsil C 60 (40-60 mm mesh) silica gel. Analytical thin layer chromatography was carried out on 0.25 mm precoated silica gel plates (Macherey-Nagel SIL g/UV254) and compounds were visualised using UV fluorescence, iodine vapour or ninhydrin.

The solvents used were either distilled or of analar quality. Light petroleum ether refers to that portion boiling between 40 and 60 °C and ether refers to diethyl ether. Solvents were dried according to literature procedures. Ethanol and methanol were dried using magnesium turnings. DMF, $CH_2Cl_2$ and pyridine were distilled over $CaH_2$. THF and ether were dried over sodium/ benzophenone and distilled under nitrogen. Thionyl chloride was distilled over sulfur and the initial fractions were always discarded. *N*-Methylmorpholine was distilled over ninhydrin. All other reagents were used without further purification.

**(2*S*)-*N*-(Chloroacetyl)proline 65**

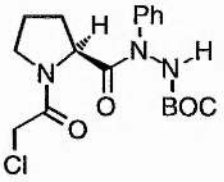



A stirred suspension of (2*S*)-proline **6** (7.5 g, 65.1 mmol) in ethyl acetate (150 $cm^3$) was treated dropwise with chloroacetyl chloride (8.0 $cm^3$, 99.7 mmol), and heated at reflux for 50 min. Upon cooling, colourless crystals were formed which were filtered off and washed with cold ethyl acetate to afford acid **65** (7.9 g, 64%), mp 106–108 °C (lit., [241] 112 °C) (Found: C, 43.85; H, 5.15; N, 7.2. Calc. for $C_7H_{10}ClNO_3$: C, 43.9; H, 5.25; N, 7.3%); $[\alpha]_D$ −110.6 (*c* 0.9 in $H_2O$) [lit., [241] − 114 (*c* 2 in $H_2O$)]; $\upsilon_{max}$(Nujol)/$cm^{-1}$ 2900 (OH), 1722 (acid CO), 1620 (amide CO) and 690 (C–Cl); $\delta_H$(200 MHz; $C^2H_3O^2H$) 1.90–2.13 (3 H, m, $\frac{1}{2}\beta CH_2$ and $\gamma CH_2$), 2.18–2.35 (1 H, m, $\frac{1}{2}\beta CH_2$), 3.51–3.74 (2 H, m, $\delta CH_2$), 4.28 (2 H, AB quartet, *J* 13.6, $CH_2Cl$), 4.41–4.48 (*t*, 1 H, m, αCH) and 4.62–4.69 (*c*, 1 H, m, αCH); $\delta_C$(74.76 MHz; $C^2H_3O^2H$) 23.49 (*c*, $\gamma CH_2$), 25.99 (*t*, $\gamma CH_2$), 30.51 (*t*, $\beta CH_2$), 32.37 (*c*, $\beta CH_2$), 43.12 (*c*, $CH_2Cl$), 43.23 (*t*, $CH_2Cl$), 48.46 (*t*, $\delta CH_2$), 48.73 (*c*, $\delta CH_2$) 60.98 (αCH), 167.76 (*t*, amide CO), 168.17 (*c*, amide CO), 174.87 (*c*, acid CO) and 175.34 (*t*, acid CO); *m/z* (EI) 193 and 191 (2 and 6%, chlorine isotopes, $M^+$), 148 and 146 (26 and 52, chlorine isotopes, $[M - CO_2H]^+$), 112 (36, $[M - Cl - CO_2H + H]^+$) and 70 (100, $C_4H_8N^+$).

***N*-(*tert*-Butoxycarbonyl)-*N'*-phenylhydrazine 67**

Di-*tert*-butyl dicarbonate (11.1 g, 50.8 mmol) was added to a solution of phenylhydrazine **66** (5.0 $cm^3$, 30.8 mmol) in ether (125 $cm^3$) and the mixture stirred at room temperature for 3 days. The solvent was removed under reduced pressure and the residual solid washed with light petrol, aqueous HCl (0.5 mol $dm^{-3}$) and water to afford hydrazide **67** (9.1 g, 86%); a small portion was recrystallised from light petrol–ethyl acetate to afford colourless needles, mp 88–90 °C (lit., [282] 91–93 °C); (Found: C, 63.55; H, 7.75; N, 13.65. Calc. for $C_{11}H_{16}N_2O_2$: C, 63.45; H, 7.75; N, 13.45%); $\upsilon_{max}$(Nujol)/$cm^{-1}$ 3354 (amine NH), 3278 (amide NH) and 1700 (CO); $\delta_H$(200 MHz; $C^2HCl_3$) 1.48 [9 H, s, $C(CH_3)_3$], 5.90 (1 H, s, amine NH), 6.64 (1 H, s, urethane NH), 6.80 (2 H, d, *J* 7.8, Ar–H *ortho*), 6.89 (1 H, t, *J* 7.8, Ar–H *para*) and 7.23 (2 H, t, *J* 7.8, Ar–H *meta*); $\delta_C$(74.76 MHz; $C^2HCl_3$) 28.15 [$C(CH_3)_3$], 81.03 [$C(CH_3)_3$)], 112.88 (Ar–CH *ortho*), 120.56 (Ar–CH *para*), 129.00 (Ar–CH *meta*), 148.33 (Ar–C quaternary) and 156.25 (urethane CO); *m/z* (EI) 208 (9%, $M^+$), 152 (81, [M – $C_4H_9$ + H]$^+$), 108 (57, [M – $CO_2C_4H_9$ + H]$^+$), 92 (44, $C_6H_5NH^+$), 77 (52, $C_6H_5^+$), 65 (31, $C_5H_5^+$) and 57 (100, $C_4H_9^+$).

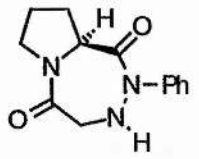
***N*-(*tert*-Butoxycarbonyl)-*N'*-[(2*S*)-*N*-(chloroacetyl)prolyl]-*N'*-phenylhydrazine 69**




Thionyl chloride (1.00 $cm^3$, 13.9 mmol) was added dropwise to a stirred solution of (2*S*)-*N*-(chloroacetyl)proline **65** (2.20 g, 11.5 mmol) and pyridine (1.27 $cm^3$, 15.8 mmol) in dichloromethane (50 $cm^3$). After 10 min, a solution of hydrazide **67** (2.00 g, 9.6 mmol) and DMAP (2.60 g, 21.4 mmol) in dichloromethane (50 $cm^3$) was added in one portion. The dark brown solution was stirred for 3–4 days, then washed

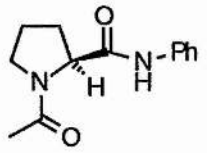
with aqueous HCl (0.5 mol $dm^{-3}$, 2 × 70 $cm^3$), aqueous sodium hydrogen carbonate solution (5%, 2 × 70 $cm^3$) and brine (100 $cm^3$). The organic phase was dried ($MgSO_4$), and the solvent removed under reduced pressure to yield a brown oil which was purified by column chromatography using light petrol–ethyl acetate (3:2) as the eluent to afford hydrazide **69** as a tan oil (2.09 g, 57%); $R_f$ 0.17; (Found: C, 56.05; H, 6.2; N, 10.75. $C_{18}H_{24}ClN_3O_4$ requires C, 56.6; H, 6.35; N, 11.0%); (HRMS: found $[M - OC_4H_9]^+$, 308.0808. $C_{14}H_{15}{}^{35}ClN_3O_3$ requires 308.0802); $[\alpha]_D$ −21.5 (*c* 0.4 in MeOH); $\upsilon_{max}$(thin film)/$cm^{-1}$ 1733 (urethane CO), 1694 (amide CO), 1654 (amide CO) and 1162 (C–O); $\delta_H$(200 MHz; $C^2HCl_3$) (2 conformations, *A* and *B*) 1.28 [9 H, s, $C(CH_3)_3$], 1.66–2.02 (4 H, m, $\beta CH_2$ and $\gamma CH_2$), 3.28–3.53 (2 H, m, $\delta CH_2$), 3.90–3.99 (2 H, m, $CH_2Cl$), 4.30 (*A*, 1 H, m, αCH), 5.05 (*B*, 1 H, m, αCH), 6.96–7.42 (5 H, m, Ar–H), 8.95 (*A*, 1 H, br. s, NH) and 9.25 (*B*, 1 H, br. s, NH); $\delta_C$(74.76 MHz; $C^2HCl_3$) 25.28 (*A*, $\gamma CH_2$), 25.42 (*B*, $\gamma CH_2$), 28.60 [$C(CH_3)_3$], 28.95 (*B*, $\beta CH_2$), 29.20 (*A*, $\beta CH_2$), 42.50 ($CH_2Cl$), 47.86 ($\delta CH_2$), 58.17 (*B*, αCH), 58.39 (*A*, αCH), 81.86 [$C(CH_3)_3$], 125.15 and 126.95 (Ar–CH *ortho*), 128.37 and 128.87 (Ar–CH *para*), 129.11 and 129.95 (Ar–CH *meta*), 141.56 and 142.14 (Ar–C quaternary), 154.97 (*B*, urethane CO), 155.96 (*A*, urethane CO), 165.39 (*B*, $COCH_2Cl$), 165.83 (*A*, $COCH_2Cl$) and 173.47 (*C*ONPh); *m/z* (EI) 382 (2%, $[M + H]^+$), 310 and 308 (2 and 6, chlorine isotopes, $[M - OC_4H_9]^+$), 283 and 281 (4 and 14, chlorine isotopes, $[M - CO_2 - C_4H_8]^+$), 176 and 174 (32 and 55, chlorine isotopes, $C_7H_9NO_2Cl^+$), 148 and 146 (46 and 100, chlorine isotopes, $C_6H_9NOCl^+$), 70 (77, $C_4H_8N^+$) and 57 (57, $C_4H_9^+$).

**(9a*S*)-2-Phenyl-2,3,4,5,7,8,9,9a-octahydro-1*H*-pyrrolo-[2,1-*d*][1,2,5]triazepine-1,5-dione 64**



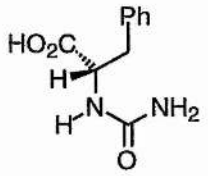

Dry HCl gas was bubbled through a solution of dipeptide **69** (0.84 g, 2.2 mmol) in ethyl acetate (25 $cm^3$) for 40 min. The solvent was removed under reduced pressure to give amine hydrochloride salt **70**, which was redissolved in aqueous HCl (0.5 mol $dm^{-3}$, 25 $cm^3$). This solution was washed with ether (30 $cm^3$), and basified with aqueous sodium hydroxide solution (1.0 mol $dm^{-3}$, 35 $cm^3$). The solution was allowed to stand for 30 min and then extracted with ethyl acetate (3 × 50 $cm^3$). The organic phase was washed with water (50 $cm^3$), dried ($MgSO_4$), and the solvent removed under reduced pressure to yield the triazepinedione **64** as an orange oil (0.36 g, 68%); (HRMS: found $M^+$, 245.1170. $C_{13}H_{15}N_3O_2$ requires 245.1164); $[\alpha]_D$ −27.9 (*c* 1.0 in MeOH); $\upsilon_{max}$(thin film)/$cm^{-1}$ 3336 (NH) and 1650 (CO); $\delta_H$(300 MHz; $C^2HCl_3$) 1.90–2.04 (3 H, m, $\frac{1}{2}\beta CH_2$ and $\gamma CH_2$), 2.30–2.45 (1 H, m, $\frac{1}{2}\beta CH_2$), 3.48–3.64 (2 H, m, $\delta CH_2$), 4.17–4.35 (2 H, m, $CH_2NH$), 5.57 (1 H, m, αCH) and 7.19–7.52 (5 H, m, Ar–H); $\delta_C$(74.76 MHz; $C^2HCl_3$) 25.55 ($\gamma CH_2$), 29.84 ($\beta CH_2$), 42.65 ($CH_2NH$), 48.04 ($\delta CH_2$), 58.17 (αCH), 127.99 (Ar–CH *ortho*), 128.93 (Ar–CH *para*), 130.08 (Ar–CH *meta*), 142.35 (Ar–C quaternary), 165.28 ($COCH_2N$) and 171.15 (*C*ONPh); *m/z* (EI) 245 (15%, $M^+$), 217 (33, $[M - CO]^+$), 110 (40, $C_6H_8NO^+$), 93 (57, $PhNH_2^+$), 77 (42, $Ph^+$) and 70 (100, $C_4H_8N^+$).

**(2*S*)-*N*-Acetylprolinanilide 71**



Sodium metal (70 mg, 3.0 mmol) was added to a solution of triazepinedione **64** (0.20 g, 0.82 mmol) in liquid ammonia (10 $cm^3$) under nitrogen at –50°C, such that a dark blue colour persisted for 10 min. The solution was allowed to warm up to room temperature, after which methanol (10 $cm^3$) and aqueous ammonium chloride solution (0.4 mol $dm^{-3}$, 10 $cm^3$) were added. The mixture was stirred overnight to allow evaporation of ammonia, and the remaining solvent removed under reduced pressure. The residue was purified by column chromatography using ethyl acetate as the eluent to yield (2*S*)-*N*-acetylprolinanilide **71** as pale yellow crystals (40 mg, 21%), mp 168–170 °C; $R_f$ 0.23; (HRMS: found $M^+$, 232.1212. $C_{13}H_{16}N_2O_2$ requires 232.1203); $[\alpha]_D$ –66.7 (*c* 0.5 in MeOH); $\upsilon_{max}$(Nujol)/$cm^{-1}$ 3270 (NH), 3132 (NH), 1695 (secondary amide CO) and 1628 (tertiary amide CO); $\delta_H$(300 MHz; $C^2HCl_3$) 1.80–2.19 (3 H, m, $\frac{1}{2}\beta CH_2$ and $\gamma CH_2$), 2.14 (3 H, s, $COCH_3$), 2.54–2.61 (1 H, m, $\frac{1}{2}\beta CH_2$), 3.39–3.60 (2 H, m, $\delta CH_2$), 4.76 (1 H, d, *J* 7.2, αCH), 7.04 (1 H, t, *J* 7.5, Ar–H *para*), 7.26 (2 H, t, *J* 7.5, Ar–H *ortho*), 7.51 (2 H, d, *J* 7.8, Ar–H *meta*) and 9.68 (1 H, s, NH); $\delta_C$(74.76 MHz; $C^2HCl_3$) 22.88 ($COCH_3$), 25.45 ($\gamma CH_2$), 27.05 ($\beta CH_2$) 48.90 ($\delta CH_2$), 60.88 (αCH), 120.20 (Ar–CH *ortho*), 124.36 (Ar–CH *para*), 129.34 (Ar–CH *meta*), 138.90 (Ar–C quaternary), 169.48 (acetyl CO) and 172.33 (anilide CO); *m/z* (EI) 232 (32%, $M^+$), 140 (23, [M – NHPh]$^+$), 112 (76, [M – CONHPh]$^+$), 70 (100, $C_4H_8N^+$) and 43 (35, $COCH_3^+$).

**(2*S*)-2-Benzylhydantoic acid 77**



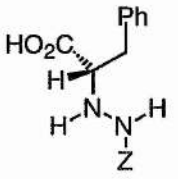

Potassium cyanate (22.9 g, 0.28 mol) was added to a stirred suspension of (2*S*)-phenylalanine **76** (5.0 g, 30.3 mmol) in water (80 $cm^3$). The resulting mixture was heated at 60 °C for 4 h. The solution was cooled to 0 °C and carefully acidified with concentrated HCl (50 $cm^3$). The precipitate thus formed was filtered off, washed with cold water and dried under vacuum to yield (2*S*)-2-benzylhydantoic acid **77** as a white solid (5.4 g, 86%), mp 189–190 °C (lit., [247] 189 °C); $[\alpha]_D$ +38.5 (*c* 1.0 in MeOH) [lit., [247] +45.0 (*c* 1.0 in EtOH)]; $\upsilon_{max}$(Nujol)/$cm^{-1}$ 3450 (NH), 3297 (NH), 2926 (OH), 1693 (acid CO) and 1659 (urea CO); $\delta_H$(200 MHz; $^2H_6$–DMSO) 2.85 (1 H, dd, $J_1$ 13.7, $J_2$ 7.9, $\frac{1}{2}\beta CH_2$), 3.01 (1 H, dd, $J_1$ 13.9, $J_2$ 5.1, $\frac{1}{2}\beta CH_2$), 4.35 (1 H, m, αCH), 5.70 (2 H, s, $NH_2$), 6.23 (1 H, d, *J* 8.0, NH) and 7.19–7.45 (5 H, m, Ar–H); $\delta_C$(50.31 MHz; $^2H_6$–DMSO) 37.79 ($\beta CH_2$), 53.92 (αCH), 126.65 (Ar–CH *para*), 128.41 (Ar–CH *ortho*), 129.47 (Ar–CH *meta*), 137.70 (Ar–C quaternary), 158.53 (urea CO) and 174.17 (acid CO); *m/z* (EI) 208 (6%, $M^+$), 148 (86, $[M - NHCONH_2 + H]^+$), 120 (41, $C_8H_{10}N^+$), 91 (100, $C_7H_7^+$), 74 (61, $C_2H_4NO_2^+$) and 65 (28, $C_5H_5^+$).

**(2*S*)-*N*-Aminophenylalanine 78**

A solution of (2*S*)-2-benzylhydantoic acid **77** (8.00 g, 38.4 mmol) in aqueous potassium hydroxide solution (2.5 mol $dm^{-3}$, 56 $cm^3$) at 0 °C was treated with aqueous potassium hypochlorite solution (2.1 mol $dm^{-3}$, 25 $cm^3$). The solution was heated at 70–80 °C for 1.5 h, after which toluene (100 $cm^3$), hydrazine hydrate (2.4 $cm^3$) and concentrated HCl (32 $cm^3$) were added. The mixture was cooled to room temperature, the phases separated, and the solvent removed from the aqueous

phase under reduced pressure. The residual salts were extracted with hot ethanol (3 × 20 $cm^3$) and the extracts brought to pH 6.3 by addition of diethylamine, resulting in formation of a white solid which was filtered off, washed with ethanol and dried under vacuum to yield (2*S*)-*N*-aminophenylalanine **78** (2.99 g, 43%), mp 199–201 °C (lit., [283] 197–200 °C); (Found: C, 58.2; H, 7.1; N, 15.05. Calc. for $C_9H_{12}N_2O_2 \cdot \frac{1}{4}H_2O$: C, 58.5; H, 6.8; N, 15.15%); (HRMS: found $M^+$, 180.0904. Calc. for $C_9H_{12}N_2O_2$: 180.0899); $[\alpha]_D$ −10.0 (*c* 1.2 in 5 mol $dm^{-3}$ HCl) [lit., [247] −15.8 (*c* 0.5 in 6 mol $dm^{-3}$ HCl)]; $\upsilon_{max}$(Nujol)/$cm^{-1}$ 2900 (OH), 1735 (CO) and 1377 (C–O); $\delta_H$(200 MHz; $^2H_2O$) 2.96–3.16 (2 H, m, $\beta CH_2$), 3.67 (1 H, t, *J* 6.2, αCH) and 7.16–7.42 (5 H, m, Ar–H); $\delta_C$(74.76 MHz; $^2H_2O$) 33.49 ($\beta CH_2$), 63.46 (αCH), 125.54 (Ar–CH *para*), 127.03 (Ar–CH *ortho*), 127.36 (Ar–CH *meta*), 133.67 (Ar–C quaternary) and 172.76 (acid CO); *m/z* (EI) 180 (5%, $M^+$), 91 (100, $C_7H_7^+$), 89 (100, $[M - C_7H_7]^+$), 77 (23, $Ph^+$), 71 {91, $[COCH(CH_2)NH + H]^+$} 65 (35, $C_5H_5^+$) and 43 (46, $[CH_2CHNH + H]^+$).

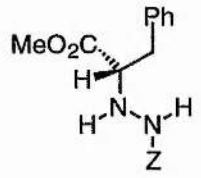
**(2*S*)-*N*-(Benzyloxycarbonylamino)phenylalanine 80**




A solution of (2*S*)-*N*-aminophenylalanine **78** (1.50 g, 8.3 mmol) in aqueous sodium hydroxide solution (0.2 mol $dm^{-3}$, 40 $cm^3$) at 0 °C was treated with benzyl chloroformate (1.42 g, 8.4 mmol) and the mixture stirred vigorously whilst warming to room temperature over 1 h. Additional sodium hydroxide solution (1.0 mol $dm^{-3}$, 10 $cm^3$) was then added to redissolve the white precipitate. The solution was washed with ether (2 × 30 $cm^3$) and the aqueous phase acidified with aqueous HCl (0.5 mol $dm^{-3}$) to pH 3. The white solid thus formed was filtered off, washed with cold water and dried under vacuum to afford (2*S*)-

*N*-(benzyloxycarbonylamino)phenylalanine **80** (2.06 g, 79%), mp 163–165 °C; (Found: C, 64.7; H, 5.75; N, 8.85. $C_{17}H_{18}N_2O_4$ requires C, 64.95; H, 5.75; N, 8.9%); $[\alpha]_D$ –3.9 (*c* 0.5 in MeOH); $\upsilon_{max}$(Nujol)/cm$^{-1}$ 3365 (NH), 3287 (NH), 2900 (OH), 1749 (acid CO), 1710 (amide CO) and 1273 (C–O); $\delta_H$(200 MHz; $C^2H_3O^2H$) 2.96–3.01 (2 H, m, $\beta CH_2$), 3.85 (1 H, m, $\alpha CH$), 5.10 (2 H, s, $OCH_2Ph$) and 7.21–7.34 (10 H, m, Ar–H); $\delta_C$(74.76 MHz; $C^2H_3O^2H$) 38.07 ($\beta CH_2$), 65.92 ($OCH_2Ph$), 68.12 ($\alpha CH$), 127.99, 129.23, 129.40, 129.78, 130.67 (Ar–CH), 138.26, 138.71 (Ar–C quaternary), 159.51 (urethane CO) and 176.13 (acid CO); *m/z* (EI) 314 (13%, $M^+$), 223 (42, $[M - CH_2Ph]^+$), 179 (22, $[M - CO_2CH_2Ph]^+$), 91 (100, $C_7H_7^+$), 77 (22, $Ph^+$) and 65 (34, $C_5H_5^+$).

**Methyl (2*S*)-*N*-(benzyloxycarbonylamino)phenylalaninate 81**




Thionyl chloride (0.62 cm$^3$, 8.5 mmol) was added dropwise to methanol (15 cm$^3$) at 0 °C. Hydrazide **80** (1.80 g, 5.73 mmol) was added and the resulting solution heated under reflux for 40 min, cooled to room temperature, and the solvent and excess thionyl chloride removed under reduced pressure to yield a pale yellow oil. The crude oil was redissolved in ethyl acetate (80 cm$^3$) and the solution washed with aqueous sodium hydrogen carbonate solution (5%, 2 × 40 cm$^3$) and water (2 × 20 cm$^3$). The organic phase was dried ($MgSO_4$) and the solvent removed under reduced pressure to yield a pale yellow solid (1.63 g, 87%). A small portion of this was recrystallised from ethyl acetate–light petrol to afford the methyl ester **81** as colourless crystals, mp 62–64 °C; (Found: C, 65.55; H, 6.0; N, 8.45. $C_{18}H_{20}N_2O_4$ requires C, 65.85; H, 6.15; N, 8.55%); (HRMS: found $M^+$, 328.1414. $C_{18}H_{20}N_2O_4$ requires 328.1423); $[\alpha]_D$ +10.9 (*c* 0.2 in MeOH); $\upsilon_{max}$(Nujol)/cm$^{-1}$ 1735 (CO), 1261 and 1080 (C–O); $\delta_H$(200

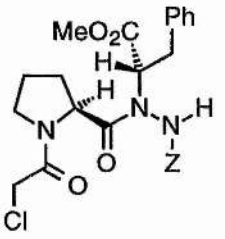
MHz; $C^2HCl_3$) 2.95 (1 H, dd, $J_1$ 14.0, $J_2$ 8.0, $\frac{1}{2}\beta CH_2$), 3.11 (1 H, dd, $J_1$ 14.0, $J_2$ 5.4, $\frac{1}{2}\beta CH_2$), 3.70 (3 H, s, $CO_2CH_3$), 4.00 (1 H, t, αCH), 4.20 (1 H, br. s, amine NH), 5.09 (2 H, s, $OCH_2Ph$), 6.52 (1 H, br. s, urethane NH) and 7.22–7.31 (10 H, m, Ar–H); $\delta_C$(50.31 MHz; $C^2HCl_3$) 37.34 ($\beta CH_2$), 52.48 ($CO_2CH_3$), 64.43 (αCH), 67.50 ($OCH_2Ph$), 127.45, 128.66, 128.71, 128.99, 129.06 and 129.61 (Ar–CH), 136.55 and 136.83 (Ar–C quaternary), 157.42 (urethane CO) and 173.57 (ester CO); *m/z* (EI) 328 (5%, $M^+$), 237 (57, $[M - C H_2Ph]^+$), 193 (35, $[M - CO_2CH_2Ph]^+$), 162 (26, $PhCH_2CCO_2CH_3^+$), 105 (17, $[CHCH_2Ph + H]^+$), 91 (100, $C_7H_7^+$) and 65 (22, $C_5H_5^+$).

**Methyl (2*S*)-*N*-(benzyloxycarbonylamino)-*N*-acetylphenylalaninate 86**


A solution of hydrazide **81** (100 mg, 0.3 mmol) in dichloromethane (15 $cm^3$) was treated with acetyl chloride (70 mg, 0.9 mmol) and the mixture stirred for 4 days. The solution was washed with aqueous HCl (0.5 mol $dm^{-3}$, 2 × 10 $cm^3$), aqueous sodium hydrogen carbonate solution (5%, 2 × 10 $cm^3$) and water (2 × 10 $cm^3$). The organic phase was dried ($MgSO_4$) and the solvent removed under reduced pressure to yield a light yellow solid, which was washed with methanol and dried under reduced pressure to yield compound **86** as a white solid (30 mg, 27%), mp 130–132 °C; (Found: C, 65.05; H, 5.95; N, 7.55. $C_{20}H_{22}N_2O_5$ requires C, 64.85; H, 6.0; N, 7.55%); $[\alpha]_D$ −112.5 (*c* 0.2 in MeOH); $\upsilon_{max}(CH_2Cl_2)/cm^{-1}$ 3388 (NH), 3054 (CH), 1751 (ester CO), 1685 (amide CO) and 1272 (C–O); $\delta_H$(200 MHz; $C^2HCl_3$) 2.08 (3 H, s, $COCH_3$), 3.09–3.27 (2 H, m, $\beta CH_2$), 3.62 (3 H, s, $CO_2CH_3$), 4.68 (1 H, s, NH), 5.19 (2 H, s, $OCH_2Ph$), 5.51 (1 H, m, αCH) and 7.14–7.65 (10 H, m, Ar–H); $\delta_C$(50.31 MHz; $C^2HCl_3$) 21.16 ($COCH_3$), 35.17 ($\beta CH_2$), 52.82 ($CO_2CH_3$), 59.33 (αCH), 68.43

($OCH_2Ph$), 127.28, 128.78, 128.90, 129.09, 129.28 and 129.49 (Ar–CH), 135.84 and 136.93 (Ar–C quaternary), 155.57 (urethane CO), 171.70 (amide CO) and 172.90 (ester CO); *m/z* (EI) 371 (1%, $[M + H]^+$), 313 (38, $[M - C_4H_9]^+$), 237 (39, $[M - COCH_3 - CH_2Ph]^+$), 193 (22, $[M - COCH_3 - NHCO_2CH_2Ph + H]^+$), 91 (100, $C_7H_7^+$) and 43 (32, $COCH_3^+$).

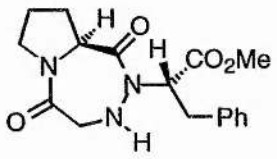
**Methyl (2*S*)-*N*-(benzyloxycarbonylamino)-*N*-[(2*S*)-*N*-(chloroacetyl)prolyl] phenylalaninate 82**




A solution of (2*S*)-*N*-(chloroacetyl)proline **65** (0.53 g, 2.8 mmol) and pyridine (0.31 $cm^3$, 3.9 mmol) in dichloromethane (10 $cm^3$) was treated dropwise with thionyl chloride (0.26 $cm^3$, 3.6 mmol). After 10 min, a solution of hydrazide **81** (0.60 g, 1.8 mmol) and DMAP (0.49 g, 4.0 mmol) in dichloromethane (10 $cm^3$) was added in one portion. After 3–4 days the dark brown solution was washed with aqueous HCl (0.5 mol $dm^{-3}$, 2 × 20 $cm^3$), aqueous sodium hydrogen carbonate solution (5%, 2 × 20 $cm^3$) and brine (30 $cm^3$). The organic phase was dried ($MgSO_4$), and the solvent removed under reduced pressure to yield a brown oil. The crude oil was purified by column chromatography using light petrol–ethyl acetate (3:2) as the eluent to afford unchanged starting material **81** (0.10 g, 17%) and hydrazide **82** as a light oil (0.49 g, 53%); $R_f$ 0.10; (HRMS: found $M^+$, 501.1675. $C_{25}H_{28}{}^{35}ClN_3O_6$ requires 501.1667); $[\alpha]_D$ –66.8 (*c* 1.1 in MeOH); $\upsilon_{max}$(thin film)/$cm^{-1}$ 3241 (NH), 1744 (ester CO), 1690 (urethane CO) and 1645 (amide CO); $\delta_H$(200 MHz; $C^2HCl_3$) 1.92–2.06 [3 H, m, $\frac{1}{2}\beta CH_2$(Pro) and $\gamma CH_2$(Pro)], 2.11–2.18 [1 H, m, $\frac{1}{2}\beta CH_2$(Pro)], 3.00–3.21 [2 H, m, $\beta CH_2$(Phe)], 3.55–3.65 [2 H, m, $\delta CH_2$(Pro)], 3.70 (3 H, s, $CO_2CH_3$), 3.93–4.08 (2 H, dd, $J_1$ 13.2, $J_2$ 4.0, $CH_2Cl$), 4.61 [*t*, 1 H, m, $\alpha$CH(Pro)], 4.73 [*c*, 1 H, m,

αCH(Pro)], 5.10–5.25 [3 H, m, *c*, αCH(Phe) and $OCH_2Ph$], 5.50 [*t*, 1 H, m, αCH(Phe)], 7.25–7.39 (10 H, m, Ar–H) and 8.10 (1 H, br. s, NH); $\delta_C$(74.76 MHz; $C^2HCl_3$) 22.40 [*c*, $\gamma CH_2$(Pro)], 25.38 [*t*, $\gamma CH_2$(Pro)], 29.11 [*t*, $\beta CH_2$(Pro)], 31.00 [*c*, $\beta CH_2$(Pro)], 34.79 [$\beta CH_2$(Phe)], 42.09 (*c*, $CH_2Cl$), 42.16 (*t*, $CH_2Cl$), 47.74 [$\delta CH_2$(Pro)], 52.51 (*c*, $CO_2CH_3$), 53.02 (*t*, $CO_2CH_3$), 60.42 [αCH(Pro) and αCH(Phe)], 68.21 (*c*, $OCH_2Ph$), 68.80 (*t*, $OCH_2Ph$), 127.10–129.63 (Ar–CH), 136.15 and 137.09 (Ar–C quaternary), 155.94 (urethane CO), 165.62 ($COCH_2Cl$), 169.93 (amide CO) and 174.37 (ester CO); *m/z* (EI) 504 and 502 (3 and 9%, chlorine isotopes, $[M + H]^+$), 328 (31, $[M - ClCH_2CONC_4H_7CO + H]^+$), 237 (21, $[M - ClCH_2CONC_4H_7CO - C_7H_7 + H]^+$), 176 and 174 (29 and 57, chlorine isotopes, $ClCH_2CONC_4H_7CO^+$), 148 and 146 (43 and 100, chlorine isotopes, $ClCH_2CONC_4H_7^+$), 91 (71, $C_7H_7^+$) and 70 (74, $[C_4H_7N + H]^+$).

**(9a*S*)-2-[(1*S*)-1-Methoxycarbonyl-2-phenylethyl]-2,3,4,5,7,8,9,9a-octahydro-1*H*-pyrrolo-[2,1-*d*][1,2,5]triazepine-1, 5-dione 72**



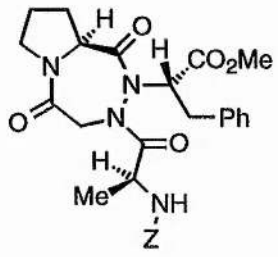

Peptide **82** (0.10 g, 0.2 mmol) was treated with 30% HBr in acetic acid (6 $cm^3$). The solution was stirred for 2 h and then poured on to ether (100 $cm^3$), resulting in formation of a flocculent white precipitate which decomposed to a brown oil if exposed to air. The solvents were carefully decanted and the suspension diluted with ether, shaken, and the solvent again decanted. The remaining solvents were removed under reduced pressure and the residue redissolved in aqueous sodium hydroxide solution (0.01 mol $dm^{-3}$, 20 $cm^3$) to give neutral pH. The solution was allowed to stand for 90 min, and extracted with ethyl acetate (3 × 20 $cm^3$). The combined organic extracts were washed with water (50 $cm^3$) and dried ($MgSO_4$). The solvent was removed under reduced pressure to yield a clear oil (60 mg, 91%); a small

portion was crystallised from ethyl acetate–light petrol to give the triazepinedione **72** as pale yellow crystals, mp 114–116 °C; (HRMS: found $M^+$, 331.1523. $C_{17}H_{21}N_3O_4$ requires 331.1532); $[\alpha]_D$ −78.3 (*c* 0.8 in MeOH); $\upsilon_{max}$(thin film)/cm$^{-1}$ 3353 (NH), 1740 (ester CO), 1672 (amide CO), 1170 (C–O) and 1093 (C–N).

NMR spectra of compound **72** show two conformations. These are defined as follows for each of the three spin systems: *A* and *B* (pyrrolidine ring); *C* and *D* ($CH_2$ in triazepinedione ring) and *E* and *F* (phenylalanine).

$\delta_H$(300 MHz; $C^2HCl_3$) 1.75–2.15 [3 H, m, $\frac{1}{2}\beta CH_2$(Pro) and $\gamma CH_2$(Pro)], 2.18 [*A*, 1 H, m, $\frac{1}{2}\beta CH_2$(Pro)], 2.32 [*B*, 1 H, m, $\frac{1}{2}\beta CH_2$(Pro)], 2.61 (*C*, 1 H, d, *J* 11.3, $\frac{1}{2}COCH_2N$), 2.99–3.50 [2 H, m, $\beta CH_2$(Phe)], 3.09 (*C*, 1 H, d, *J* 11.3, $\frac{1}{2}COCH_2N$), 3.50–3.82 [2 H, m, $\delta CH_2$(Pro)], 3.67 and 3.76 (3 H, s, $CO_2CH_3$), 3.78–3.91 (*D*, 2 H, m, $COCH_2N$), 5.12 [*B*, 1 H, dd, $J_1$ 8.9, $J_2$ 3.0, $\alpha$CH(Pro)], 5.29 [*A*, 1 H, dd, $J_1$ 8.9, $J_2$ 3.0, $\alpha$CH(Pro)], 5.45 [*F*, 1 H, dd, $J_1$ 9.2, $J_2$ 6.3, $\alpha$CH(Phe)], 5.65 [*E*, 1 H, dd, $J_1$ 12.3, $J_2$ 4.8, $\alpha$CH(Phe)] and 7.19–7.33 (5 H, m, Ar–H); $\delta_C$(300 MHz; $C^2HCl_3$) 22.36 [*B*, $\gamma CH_2$(Pro)], 24.66 [*A*, $\gamma CH_2$(Pro)], 27.29 (*C*, $COCH_2N$), 27.42 (*D*, $COCH_2N$), 29.00 [*A*, $\beta CH_2$(Pro)], 31.52 [*B*, $\beta CH_2$(Pro)], 33.59 [*E*, $\beta CH_2$(Phe)], 34.10 [*F*, $\beta CH_2$(Phe)], 47.80 [*B*, $\delta CH_2$(Pro)], 48.12 [*A*, $\delta CH_2$(Pro)], 52.41 and 52.77 ($CO_2CH_3$), 57.57 [*A*, $\alpha$CH(Pro)], 57.96 [*F*, $\alpha$CH(Phe)], 58.13 [*B*, $\alpha$CH(Pro)], 58.69 [*E*, $\alpha$CH(Phe)], 127.27 and 127.81 (Ar–CH *para*), 128.39 and 128.82 (Ar–CH *ortho*), 129.05 and 129.36 (Ar–CH *meta*), 135.90 and 136.42 (Ar–C quaternary), 165.14 and 165.25 ($COCH_2N$), 171.42 and 172.05 (amide CO) and 174.71 (ester CO); *m/z* (EI) 331 (53%, $M^+$), 212 (63, $[M - COCH_2Ph]^+$), 168 (38, $[M - PhCH_2CHCO_2CH_3]^+$), 125 (39, $NC_4H_7CONCH_2^+$), 112 (51, $NC_4H_8CON^+$), 91 (48, $C_7H_7^+$) and 70 (100, $C_4H_8N^+$).

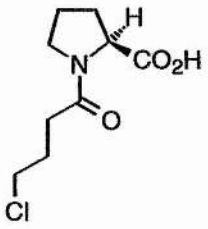
**(9a*S*)-2-[(1*S*)-1-Methoxycarbonyl-2-phenylethyl]-3-[(2*S*)-*N*-benzyloxycarbonylalanyl]-2,3,4,5,7,8,9,9a-octahydro-1*H*-pyrrolo-[2,1-*d*][1,2,5]triazepine-1,5-dione 90**




A solution of (2*S*)-*N*-(benzyloxycarbonyl)alanine **89** (76 mg, 0.34 mmol) and *N*-methylmorpholine (40 $mm^3$, 0.36 mmol) in dry THF (3 $cm^3$) was cooled to −15 °C, and *iso*butyl chloroformate (50 $mm^3$, 0.32 mmol) was added with stirring. After 2 min, a solution of triazepinedione **72** (100 mg, 0.30 mmol) and *N*-methylmorpholine (40 $mm^3$, 0.36 mmol) in DMF (1 $cm^3$) was added in one portion to the stirred suspension. The reaction mixture was allowed to warm to room temperature and stirred for a further 5 h. The hydrochloride salts were filtered off and the solvent removed under reduced pressure. The residual oil was redissolved in ethyl acetate (10 $cm^3$) and the solution was washed with aqueous HCl (0.5 mol $dm^{-3}$, 2 × 5 $cm^3$), aqueous sodium hydrogen carbonate solution (5%, 2 × 5 $cm^3$) and brine (5 $cm^3$). The solution was dried ($MgSO_4$), and the solvent removed under reduced pressure to yield a pale yellow oil which was purified by column chromatography using ethyl acetate as the eluent to afford the product **90** as a clear oil (6 mg, 4%); $R_f$ 0.62; (HRMS: found $M^+$, 536.2283. $C_{28}H_{32}N_4O_7$ requires 536.2271); $\upsilon_{max}$(thin film)/$cm^{-1}$ 1742 (ester CO), 1725 (urethane CO), 1680 (secondary amide CO), 1650 (tertiary amide CO) and 1180 (C–O); $\delta_H$(300 MHz; $C^2HCl_3$) (2 conformations) 1.42 [3 H, d, *J* 7.1, βCH$_3$(Ala)], 1.79–2.27 [4 H, m, βCH$_2$(Pro) and γCH$_2$(Pro)], 3.18–3.49 [2 H, m, βCH$_2$(Phe)], 3.62–3.87 [2 H, m, δCH$_2$(Pro)], 3.71, 3.76 (3 H, s, $CO_2CH_3$), 4.00–4.08 (2 H, m, $COCH_2N$), 4.36 [1 H, m, αCH(Ala)], 5.11 (2 H, s, $OCH_2Ph$),

5.21–5.42 [2 H, m, αCH(Pro) and αCH(Phe)], 6.64 and 6.80 (1 H, m, NH) and 7.07–7.36 (10 H, m, Ar–H); $\delta_C$(74.76 MHz; $C^2HCl_3$) 18.65 [βCH$_3$(Ala)], 22.27 and 24.99 [γCH$_2$(Pro)], 29.03 and 31.61 [βCH$_2$(Pro)], 33.39 and 34.33 [βCH$_2$(Phe)], 41.79 and 42.01 (CO*C*H$_2$N), 47.67 and 47.97 [δCH$_2$(Pro)], 49.70 [αCH(Ala)], 52.68 and 52.97 (CO$_2$*C*H$_3$), 58.43 and 58.69 [αCH(Pro)], 64.10 and 64.33 [αCH(Phe)], 67.05 (O*C*H$_2$Ph), 127.04, 127.48, 128.24, 128.36, 128.70, 128.75, 128.89, 129.16 and 129.39 (Ar–CH), 136.65 and 137.76 (Ar–C quaternary), 141.71 and 143.09 (Ar–C quaternary), 155.83 (urethane CO), 164.95 (amide CO), 169.79 (amide CO) and 172.33 (ester CO); *m/z* (CI) 536 (8%, M$^+$), 190 [27, OCNCH(CO$_2$)CH$_2$Ph$^+$], 174 [30, OCNCH(CO)CH$_2$Ph$^+$], 146 (66, OCNCHCH$_2$Ph$^+$), 140 (29, CH$_3$CONC$_4$H$_7$CO$^+$), 112 (45, NC$_4$H$_8$CON$^+$), 91 (100, C$_7$H$_7^+$) and 70 (96, C$_4$H$_8$N$^+$).

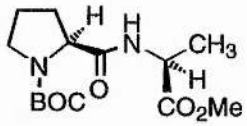
**(2*S*)-*N*-(4-Chlorobutyryl)proline 93**




A suspension of (2*S*)-proline **6** (4.0 g, 34.7 mmol) in ethyl acetate (100 cm$^3$) was treated with 4-chlorobutyryl chloride (6.0 cm$^3$, 53.5 mmol), and the mixture heated at reflux for 75 min. After cooling to room temperature, the solvent was removed under reduced pressure. The residual oil was washed with several portions of ethyl acetate–light petrol (1:1) to remove unreacted acid chloride, affording (2*S*)-*N*-(4-chlorobutyryl)proline **93** as a pale yellow oil (7.2 g, 94%); (HRMS: found M$^+$, 220.0740. C$_9$H$_{15}$$^{35}$ClNO$_3$ requires 220.0736); $[\alpha]_D$ –19.9 (*c* 1.0 in MeOH); $\upsilon_{max}$(thin film)/cm$^{-1}$ 2966 (OH), 1735 (acid CO) and 1605 (amide CO); $\delta_H$(300 MHz; $C^2HCl_3$) 1.93–2.27 (6 H, m, βC*H*$_2$, γCH$_2$ and C*H*$_2$CH$_2$Cl), 2.47–2.55 (2 H, m, βCH$_2$ and CH$_2$Cl), 3.47–3.69 (4 H, m, δCH$_2$ and CH$_2$CO), 4.50 (1 H, m, αCH) and 10.14 (1 H,

br. s, $CO_2H$); $\delta_C$(74.76 MHz; $C^2HCl_3$) 24.96 ($\gamma CH_2$), 27.82 ($CH_2CH_2Cl$), 28.92 ($\beta CH_2$), 31.33 ($CH_2Cl$), 44.83 ($CH_2CO$), 48.03 ($\delta CH_2$), 59.78 ($\alpha CH$), 173.34 (amide CO), 174.76 (acid CO); *m/z* (EI) 222 and 220 (2 and 5%, chlorine isotopes, $M^+$), 176 and 174 (13 and 25, chlorine isotopes, $[M - CO_2H - H]^+$), 157 (14, $[M - CH_2CH_2Cl]^+$), 140 (26, $[M - CO_2H - Cl]^+$), 107 and 105 [4 and 14, chlorine isotopes, $CO(CH_2)_3Cl^+$], 70 (100, $C_4H_8N^+$) and 41 (37, $C_3H_5^+$).

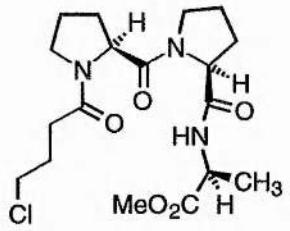
**Methyl (2*S*,2'*S*)-*N*-[*N*-(*tert*-butoxycarbonyl)prolyl]alaninate 96**




A solution of (2*S*)-*N*-(*tert*-butoxycarbonyl)proline **94** (2.5 g, 11.6 mmol) and *N*-methylmorpholine (1.3 $cm^3$, 11.6 mmol) in dry THF (20 $cm^3$) was cooled to −15 °C, and *iso*butyl chloroformate (1.55 $cm^3$, 11.4 mmol) was added with stirring. After 2 min, a solution of (2*S*)-alanine methyl ester hydrochloride **95** (1.6 g, 11.6 mmol) and *N*-methylmorpholine (1.3 $cm^3$, 11.6 mmol) in THF (10 $cm^3$) and DMF (26 $cm^3$) was added in one portion to the stirred suspension. The reaction mixture was allowed to warm to room temperature over 2 h and left standing overnight. The hydrochloride salts were filtered off and the solvent removed under reduced pressure. The residual oil was redissolved in ethyl acetate (50 $cm^3$) and the solution washed with aqueous HCl (0.5 mol $dm^{-3}$, 2 × 30 $cm^3$), aqueous sodium hydrogen carbonate solution (5%, 2 × 30 $cm^3$) and brine (30 $cm^3$). The solution was dried ($MgSO_4$), and the solvent removed under reduced pressure to afford the product as a white solid which was recrystallised from ethyl acetate–light petrol to yield the dipeptide **96** as colourless crystals (2.4 g, 65%), mp 78–79 °C (lit., [284] 79–81 °C); $[\alpha]_D$ −94.8 (*c* 1.1 in MeOH) [lit., [284] −92.3 (*c* 1.0 in MeOH)]; $\upsilon_{max}$(Nujol)/$cm^{-1}$ 3267 (NH), 1752 (ester CO), 1695 (urethane CO), 1658 (amide CO) and 1163 (C–O); $\delta_H$(200 MHz; $C^2HCl_3$) 1.33 [3 H, d, *J* 7.2, $\beta CH_3$(Ala)], 1.41 [9 H, s, $C(CH_3)_3$],

1.73–1.98 [3 H, m, $\frac{1}{2}\beta CH_2$(Pro) and $\gamma CH_2$(Pro)], 2.01–2.27 [1 H, m, $\frac{1}{2}\beta CH_2$(Pro)], 3.31–3.50 (2 H, m, $\delta CH_2$), 3.68 (3 H, s, $CO_2CH_3$), 4.23 [1 H, m, αCH(Ala)], 4.50 [1 H, m, αCH(Pro)], 6.61 and 7.34 (*c* and *t*, 1 H, br. s, NH); $\delta_C$(74.76 MHz; $C^2HCl_3$) 17.90 [$\beta CH_3$(Ala)], 23.64 [*c*, $\gamma CH_2$(Pro)], 24.34 [*t*, $\gamma CH_2$(Pro)], 28.18 [$C(CH_3)_3$ and *t*, $\beta CH_2$(Pro)], 30.88 [*c*, $\beta CH_2$(Pro)], 46.93 [$\delta CH_2$(Pro)], 47.88 [αCH(Ala)], 52.15 ($CO_2CH_3$), 59.71 and 60.59 [*c* and *t*, αCH(Pro)], 80.12 [$C(CH_3)_3$], 154.49 and 155.48 (*c* and *t*, urethane CO), 172.43 (amide CO) and 173.22 (ester CO); *m/z* (EI) 300 (2%, $M^+$), 227 (7, $[M - OC_4H_9]^+$), 199 (7, $[M - CO_2C_4H_9]^+$), 170 (31, $C_8H_{14}N_2O_2^+$), 114 (71, $C_5H_{10}N_2O^+$), 70 (100, $C_4H_8N^+$) and 57 (72, $C_4H_9^+$).

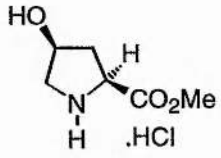
**Methyl (2*S*)-*N*-{*N*-[*N*-(4-chlorobutyryl)-(2*S*)-prolyl]-(2*S*)-prolyl}alaninate 92**




Dipeptide **96** (2.09 g, 7.0 mmol) was dissolved in ethyl acetate (75 $cm^3$) at 0 °C and dry HCl gas was bubbled through the cold solution for 70 min. The solution was allowed to stand at 0 °C for 90 min, after which time the solvent was removed under reduced pressure to yield the amine hydrochloride salt **97** as a clear oil (1.64 g, 99%). A solution of (2*S*)-*N*-(4-chlorobutyryl)proline **93** (1.55 g, 7.1 mmol) and pyridine (0.89 $cm^3$, 11.0 mmol) in dichloromethane (40 $cm^3$) was treated dropwise with thionyl chloride (0.66 $cm^3$, 9.0 mmol). After 5 min, a solution of the amine hydrochloride **97** (1.20 g, 5.1 mmol) and DMAP (1.24 g, 10.2 mmol) in dichloromethane (35 $cm^3$) was added in one portion. The solution was allowed to stir for 2 days, after which time it was washed with aqueous HCl (0.5 mol $dm^{-3}$, 2 × 50 $cm^3$), aqueous sodium hydrogen carbonate solution (5%, 2 × 50 $cm^3$) and brine (50 $cm^3$). The solution was dried ($MgSO_4$), and the solvent removed under reduced

pressure to afford a brown oil, which was purified by column chromatography using ethyl acetate–methanol (9:1) as the eluent to yield the peptide **92** as an oil (0.79 g, 39%); $R_f$ 0.20; (HRMS: found $M^+$, 401.1712. $C_{18}H_{28}{}^{35}ClN_3O_5$ requires 401.1717); $[\alpha]_D$ −84.5 (*c* 1.6 in MeOH); $\upsilon_{max}$(thin film)/cm$^{-1}$ 3329 (NH), 2955 (CH), 1739 (ester CO), 1652 (amide CO) and 1165 (C–O); $\delta_H$(300 MHz; $C^2HCl_3$) (3 conformations) 1.32, 1.38 and 1.45 [3 H, d, *J* 7.2, $\beta CH_3$], 1.89–2.19 [10 H, m, 2 × $\beta CH_2$(Pro), 2 × $\gamma CH_2$(Pro) and $CH_2CH_2Cl$], 2.45–2.49 (2 H, m, $CH_2Cl$), 3.50–3.71 [6 H, m, 2 × $\delta CH_2$(Pro) and $CH_2CO$], 3.63 (3 H, s, $CO_2CH_3$), 3.97 and 4.20–4.58 [3 H, m, 2 × $\alpha$CH(Pro) and $\alpha$CH(Ala)], 7.10, 7.55, and 7.68 (1 H, br. d, NH); $\delta_C$(74.76 MHz; $C^2HCl_3$) 16.35, 17.23 and 17.90 [$\beta CH_3$(Ala)], 22.41, 22.78, 24.22, 24.94, 25.27 and 25.43 [2 × $\gamma CH_2$(Pro)], 27.58 and 28.01 ($CH_2CH_2Cl$), 28.53 and 28.95 [2 × $\beta CH_2$(Pro)], 31.26 and 31.54 ($CH_2Cl$), 44.51 and 44.73 ($CH_2CO$), 47.15, 47.34 and 47.62 [2 × $\delta CH_2$(Pro)], 48.25 and 48.89 [$\alpha$CH(Ala)] 52.17 and 52.40 ($CO_2CH_3$), 57.91, 58.17 and 58.85 [$\alpha$CH(Pro)], 59.82, 60.45 and 61.11 [2 × $\alpha$CH(Pro)], 170.76, 171.50, 171.73 and 171.99 (amide CO), 173.18 and 173.52 (ester CO); *m/z* (EI) 403 and 401 (1 and 4%, chlorine isotopes, $M^+$), 204 and 202 (9 and 29, chlorine isotopes, $C_9H_{13}NO_2Cl^+$), 174 (51, $C_8H_{13}NOCl^+$) and 70 (100, $C_4H_8N^+$).

**Methyl (2*S*,4*S*)-4-hydroxyprolinate hydrochloride 101**



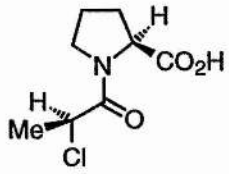

Thionyl chloride (0.35 cm$^3$, 4.1 mmol) was added dropwise to methanol (10 cm$^3$) at 0 °C. (2*S*,4*S*)-4-Hydroxyproline **100** (0.40 g, 2.3 mmol) was added and the resulting solution heated under reflux for 90 min. The solvents were removed under reduced pressure to afford the hydrochloride **101** as a white solid in quantitative yield. A small portion was recrystallised from ethyl acetate–hexane to yield colourless needles, mp 79–80 °C; (Found: C, 36.4; H, 6.75; N, 7.5. $C_6H_{11}NO_3{\cdot}HCl{\cdot}H_2O$ requires C, 36.3; H,

7.1; N, 7.05%); $[\alpha]_D$ −49.2 (*c* 0.5 in MeOH); $\upsilon_{max}$(Nujol)/cm$^{-1}$ 3327 (OH), 2921 (CH), 1732 (CO), 1252 (OH def.) and 1182 (C–O); $\delta_H$(300 MHz; $^2H_2O$) 2.27–2.39 (2 H, m, $\beta CH_2$), 3.34 (2 H, br. s, $\delta CH_2$), 3.72 (3 H, s, $CO_2CH_3$) and 4.51–4.60 (2 H, m, $\alpha$CH and $\gamma$CH); $\delta_C$(74.76 MHz; $^2H_2O$) 36.76 ($\beta CH_2$), 53.55 ($\delta CH_2$), 53.93 ($CO_2CH_3$), 58.45 ($\alpha$CH), 69.00 ($\gamma$CH) and 170.93 (ester CO); *m/z* (EI) 145 (11%, [M – HCl]$^+$), 101 (13, [M – HCl – $CH_2NH$ – $CH_3$]$^+$), 86 (100, [M – HCl – $CO_2CH_3$]$^+$), 68 (44, $C_4H_6N^+$) and 58 (16, $NHCH_2CHO^+$).

**(2*S*)-2-Chloropropionic acid 104**

A stirred suspension of (2*S*)-alanine **103** (15.0 g, 0.17 mol) in saturated potassium chloride solution (36 cm$^3$) was treated dropwise with concentrated HCl (60 cm$^3$). The mixture was cooled to 0 °C, and sodium nitrite (23.3 g, 0.34 mol) was added in portions over 75 min. After completion of addition, the brown solution was stirred at 0–5 °C for a further 1 h, then allowed to reach room temperature overnight. The solution was extracted with ether (3 × 100 cm$^3$), the organic phase dried ($MgSO_4$), and the solvent removed under reduced pressure to yield acid **104** as a pale yellow oil (13.4 g, 73%), bp 80 °C/~15 mmHg (lit., [285] 77 °C/10 mmHg); $[\alpha]_D$ −17.7 (*c* 0.8 in MeOH) (lit., [285] −18.2); $\upsilon_{max}$(thin film)/cm$^{-1}$ 3110 (OH), 1735 (CO), 1457 ($CH_3$ def.), 1210 (C–O) and 668 (C–Cl); $\delta_H$(300 MHz; $C^2HCl_3$) 1.73 (3 H, d, *J* 7.2, $\beta CH_3$), 4.45 (1 H, q, *J* 6.9, $\alpha$CH) and 10.70 (1 H, br. s, $CO_2H$); $\delta_C$(74.76 MHz; $C^2HCl_3$) 21.71 ($\beta CH_3$), 52.63 ($\alpha$CH) and 176.75 (acid CO); *m/z* (CI) 111 and 109 (3 and 11%, chlorine isotopes, [M + H]$^+$) and 57 (100, $CHCO_2^+$).

**(2*S*,2'*S*)-*N*-(2'-Chloropropionyl)proline 106**

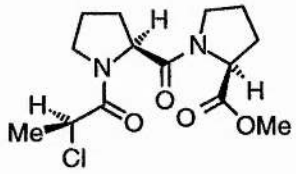



A stirred solution of (2*S*)-2-chloropropionic acid **104** (6.0 g, 55.3 mmol) and *N*-methylmorpholine (6.2 $cm^3$, 55.3 mmol) in dry THF (100 $cm^3$) at −15 °C was treated dropwise with *iso*butyl chloroformate (7.40 $cm^3$, 54.5 mmol), followed after 2 min by a solution of (2*S*)-proline methyl ester hydrochloride **102** (9.2 g, 55.5 mmol) and *N*-methylmorpholine (6.2 $cm^3$, 55.3 mmol) in DMF (30 $cm^3$). The mixture was allowed to reach room temperature and stirred for 18 h. The hydrochloride salts formed were filtered off and the solvents removed under reduced pressure to yield an orange oil, which was redissolved in dichloromethane (140 $cm^3$). The solution was washed with aqueous HCl (0.5 mol $dm^{-3}$, 2 × 100 $cm^3$), aqueous sodium hydrogen carbonate solution (5%, 2 × 100 $cm^3$) and brine (50 $cm^3$). The solvent was removed under reduced pressure to yield a pale yellow oil which was purified by gradient column chromatography using light petrol–ethyl acetate as the eluent to give the dipeptide methyl ester **104** as a pale yellow oil (4.9 g).

The above oil was dissolved in methanol (45 $cm^3$), and aqueous sodium hydroxide solution (1.0 mol $dm^{-3}$, 55 $cm^3$) was added. The mixture was stirred at room temperature for 2 h, after which time aqueous HCl (1.0 mol $dm^{-3}$, 55 $cm^3$) was added. Methanol was removed under reduced pressure, the solution further acidified to pH 1, and the solution extracted with ethyl acetate (3 × 80 $cm^3$). The combined organic extracts were dried ($MgSO_4$), and the solvent removed under reduced pressure to yield the acid **106** as a white solid (4.05 g, 36%). A small portion was recrystallised to give colourless crystals, mp 161–162 °C (lit., [286] 164–165 °C); $[\alpha]_D$ −59.2 (*c* 1.0 in MeOH) [lit., [286] −62.1 (*c* 1.0 in MeOH)]; $\upsilon_{max}$(Nujol)/$cm^{-1}$ 3000 (OH), 1739 (acid CO), 1621 (amide CO) and 652 (C–Cl); $\delta_H$(200 MHz; $C^2HCl_3$) 1.64 (3 H, d, *J* 6.6, CHC$H_3$), 1.97–2.29 (4 H, m, β$CH_2$ and γ$CH_2$), 3.60–3.89 (2 H, δ$CH_2$),

4.46–4.56 (2 H, m, αCH and C*H*$CH_3$) and 10.05 (1 H, s, $CO_2H$); $\delta_C$(50.31 MHz; $C^2HCl_3$) 20.89 (CH*C*$H_3$), 25.25 ($\gamma CH_2$), 29.25 ($\beta CH_2$), 47.69 ($\delta CH_2$), 51.53 (*C*$HCH_3$), 60.03 (αCH), 169.13 (amide CO) and 175.78 (acid CO); *m/z* (EI) 208 and 206 (4 and 15%, chlorine isotopes, $M^+$), 170 (39, $[M - HCl]^+$), 162 and 160 (22 and 60, chlorine isotopes, $[M - CO_2H - H]^+$), 126 (49, $[M - CO_2H - Cl]^+$), 114 (51, $C_5H_8NO_2^+$), 70 (100, $C_4H_8N^+$) and 57 (29, $[CH_3CHCO + H]^+$).

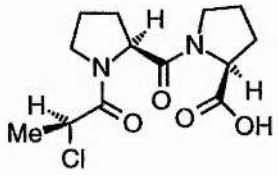
**Methyl (2*S*)-*N*-{*N*-[(2*S*)-2-chloropropionyl]-(2*S*)-prolyl}prolinate 107**




This was prepared in an identical manner to hydrazide **69**, using (2*S*,2'*S*)-*N*-(2'-chloropropionyl)proline **106** (0.70 g, 3.4 mmol) and methyl (2*S*)-prolinate hydrochloride **102** (0.51 g, 3.08 mmol) to yield a pale brown oil which was purified by gradient column chromatography using ethyl acetate–light petrol as the eluent to afford methyl ester **107** as colourless crystals (0.32 g, 33%), mp 126–128 °C; $R_f$ 0.25 (ethyl acetate); (Found: C, 53.25; H, 6.8; N, 8.6. $C_{14}H_{21}ClN_2O_4$ requires C, 53.1; H, 6.7; N, 8.85%); $[\alpha]_D$ –131.0 (*c* 0.9 in MeOH); $\upsilon_{max}$(Nujol)/$cm^{-1}$ 1735 (ester CO) and 1652 (amide CO); $\delta_H$(300 MHz; $C^2HCl_3$) 1.54 (3 H, d, *J* 6.6, CHC*H*$_3$), 1.90–1.98 [6 H, m, 2 × $\frac{1}{2}\beta CH_2$(Pro) and 2 × $\gamma CH_2$(Pro)], 2.10–2.15 [2 H, m, 2 × $\frac{1}{2}\beta CH_2$(Pro)], 3.56–3.78 [4 H, m, 2 × $\delta CH_2$(Pro)], 3.62 (3 H, s, $CO_2CH_3$), 4.44–4.48 [2 H, m, αCH(Pro) and C*H*$CH_3$] and 4.53 [1 H, dd, $J_1$ 7.8, $J_2$ 3.9, αCH(Pro)]; $\delta_C$(74.76 MHz; $C^2HCl_3$) 20.71 (CH*C*$H_3$), 24.93 and 25.13 [2 × $\gamma CH_2$(Pro)], 28.49 and 28.99 [2 × $\beta CH_2$(Pro)], 46.89 and 47.44 [2 × $\delta CH_2$(Pro)], 51.49 (*C*$HCH_3$), 52.31 ($CO_2CH_3$), 58.52 and 58.91 [2 × αCH(Pro)], 167.90 (*C*$OCHCH_3$), 170.70 (amide CO) and 173.06 (ester CO); *m/z* (EI) 318 and 316 (14 and 36%, chlorine isotopes, $M^+$), 280 (14, $[M - HCl]^+$), 225 {11, $[M - ClCH(CH_3)CO]^+$}, 190 and 188 [7 and 21, chlorine

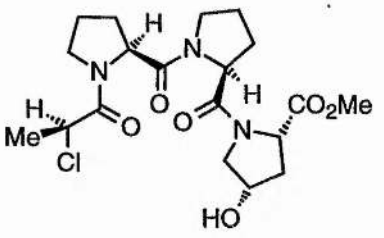
isotopes, $ClCH(CH_3)CONC_4H_7CO^+$], 162 and 160 [52 and 96, chlorine isotopes, $ClCH(CH_3)CONC_4H_7^+$], 128 (29, $NC_4H_7CO_2CH_3^+$) and 70 (100, $C_4H_8N^+$).

**(2*S*)-*N*-{*N*-[(2*S*)-2-Chloropropionyl]-(2*S*)-prolyl}proline 108**



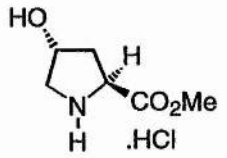

A solution of methyl ester **107** (0.30 g, 3.0 mmol) in methanol (2 $cm^3$) was treated with aqueous sodium hydroxide solution (1.0 mol $dm^{-3}$, 2 $cm^3$) and the mixture stirred for 90 min. The solution was treated with aqueous HCl (0.5 mol $dm^{-3}$, 2 $cm^3$) and the methanol removed under reduced pressure. The solution was further acidified to pH 1–2 and extracted with ethyl acetate (5 × 20 $cm^3$). The combined organic extracts were dried ($MgSO_4$), and the solvent removed under reduced pressure to yield acid **108** as a white solid (0.28 g, 98%), mp 84–86 °C; (Found: C, 50.9; H, 6.55; N, 8.65. $C_{13}H_{19}ClN_2O_4 \cdot \frac{1}{4}H_2O$ requires C, 50.8; H, 6.4; N, 9.1%); $[\alpha]_D$ −104.5 (*c* 0.9 in MeOH); $\upsilon_{max}$(Nujol)/$cm^{-1}$ 2922 (CH), 1702 (acid CO) and 1642 (amide CO); $\delta_H$(300 MHz; $C^2HCl_3$) 1.57 (3 H, d, *J* 6.6, $CHCH_3$), 1.97–2.08 [6 H, m, 2 × $\frac{1}{2}\beta CH_2$(Pro), 2 × $\gamma CH_2$(Pro)], 2.11–2.22 [2 H, m, 2 × $\frac{1}{2}\beta CH_2$(Pro)], 3.52–3.59 and 3.63–3.84 [4 H, m, 2 × $\delta CH_2$(Pro)], 4.46–4.53 [2 H, m, αCH(Pro) and $CHCH_3$], 4.59–4.63 [1 H, m, αCH(Pro)] and 9.34 (1 H, br. s, $CO_2H$); $\delta_C$(74.76 MHz; $C^2HCl_3$) 20.60 ($CHCH_3$), 25.07 [2 × $\gamma CH_2$(Pro)], 28.57 and 28.76 [2 × $\beta CH_2$(Pro)], 47.31 and 47.58 [2 × $\delta CH_2$(Pro)], 51.52 ($CHCH_3$), 58.68 and 59.31 [2 × αCH(Pro)], 168.47 ($COCHCH_3$), 171.77 (amide CO) and 174.23 (acid CO); *m/z* (EI) 304 and 302 (4 and 14%, chlorine isotopes, $M^+$), 266 (7, $[M - HCl]^+$), 260 and 258 (4 and 11, chlorine isotopes, $[M - CO_2]^+$), 162 and 160 [46 and 86, chlorine isotopes, $ClCH(CH_3)CONC_4H_7CO^+$], 128 (14, $NC_4H_7CO_2CH_3^+$) and 70 (100, $C_4H_8N^+$).

**Methyl (2*S*,4*S*)-*N*-(*N*-{*N*-[(2*S*)-2-chloropropionyl]-(2*S*)-prolyl}-(2*S*)-prolyl)-4-hydroxyprolinate 99**



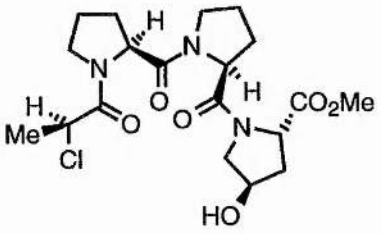

This was prepared in an identical manner to hydrazide **69**, using acid **108** (0.23 g, 0.76 mmol) and methyl (2*S*,4*S*)-4-hydroxyprolinate hydrochloride **101** (0.12 g, 0.66 mmol) to yield the peptide **99** as a brown oil (0.12 g, 42%) which defied further purification; (HRMS: found $M^+$, 429.1662. $C_{19}H_{28}{}^{35}ClN_3O_6$ requires 429.1667); $\upsilon_{max}$(thin film)/cm$^{-1}$ 3441 (OH), 2981 (CH), 1746 (ester CO), 1649 (amide CO), 1435 (CH def.) and 1066 (C–O); $\delta_H$(300 MHz; $C^2HCl_3$) 1.61 (3 H, d, *J* 6.3, $CHCH_3$), 1.91–2.38 [10 H, m, 2 × $\beta CH_2$(Pro), $\beta CH_2$(Hyp) and 2 × $\gamma CH_2$(Pro)], 3.56–3.86 [6 H, m, 2 × $\delta CH_2$(Pro) and $\delta CH_2$(Hyp)], 3.68 (3 H, s, $CO_2CH_3$), 4.47–4.69 [4 H, m, $CHCH_3$, 2 × αCH(Pro) and αCH(Hyp)] and 5.10–5.21 [1 H, m, γCH(Hyp)]; $\delta_C$(50.31 MHz; $C^2HCl_3$) 20.78 ($CHCH_3$), 25.08 and 25.17 [2 × $\gamma CH_2$(Pro)], 28.36 and 28.52 [2 × $\beta CH_2$(Pro)], 37.04 [$\beta CH_2$(Hyp)], 47.37 and 47.61 [2 × $\delta CH_2$(Pro)], 51.56 ($CHCH_3$), 52.94 ($CO_2CH_3$), 55.85 [$\delta CH_2$(Hyp)], 57.80, 58.21 and 58.77 [2 × αCH(Pro) and αCH(Hyp)], 71.41 [γCH(Hyp)], 168.08 ($COCHCH_3$), 170.56 and 171.56 (2 × amide CO) and 174.75 (ester CO); *m/z* (EI) 431 and 429 (1 and 3%, chlorine isotopes, $M^+$), 393 (6, $[M - HCl]^+$), 287 and 285 {4 and 7, chlorine isotopes, $[M - NCH_2CH(OH)CH_2CHCO_2CH_3 + H]^+$}, 259 and 257 {4 and 13, chlorine isotopes, $[M - CONCH_2CH(OH)CH_2CHCO_2CH_3 + H]^+$}, 190 and 188 (17 and 53, chlorine isotopes, $C_8H_{11}NO_2Cl^+$), 162 and 160 (34 and 87, chlorine isotopes, $C_7H_{11}NOCl^+$), 130 {69, $[NCH_2CH(OH)CH_2CHCO_2H + H]^+$}, 86 (84, $C_4H_8NO^+$) and 70 (100, $C_4H_8N^+$).

**Methyl (2*S*,4*R*)-4-hydroxyprolinate hydrochloride 114**




This was prepared in an identical manner to methyl (2*S*,4*S*)-4-hydroxyprolinate hydrochloride **101**, using (2*S*,4*R*)-4-hydroxyproline **113** (10.0 g, 76.3 mmol) to give the hydrochloride **114** as a white solid (13.5 g, 98%). A small portion was recrystallised to afford white needles, mp 162–164 °C (lit., [287] 162–164 °C); $[\alpha]_D$ –25.3 (*c* 1.0 in MeOH) [lit., [287] –24.3 (*c*1.05 in MeOH)]; $\upsilon_{max}$(Nujol)/cm$^{-1}$ 3325 (OH), 1742 (CO), 1246 (OH def.) and 1180 (C–O); $\delta_H$(200 MHz; $^2H_2O$) 2.19 (1 H, ddd, $J_1$ 14.8, $J_2$ 8.8, $J_3$ 4.2, $\frac{1}{2}\beta CH_2$), 2.40 (1 H, dd, $J_1$ 14.8, $J_2$ 8.8, $\frac{1}{2}\beta CH_2$), 3.25 (1 H, d, $J$ 12.6, $\frac{1}{2}\delta CH_2$), 3.35 (1 H, dd, $J_1$ 12.6, $J_2$ 3.6, $\frac{1}{2}\delta CH_2$), 3.68 (3 H, s, $CO_2CH_3$) and 4.48–4.58 (2 H, m, αCH and γCH); $\delta_C$(50.31 MHz; $^2H_2O$) 37.07 ($\beta CH_2$), 53.87 ($\delta CH_2$), 54.23 ($CO_2CH_3$), 58.65 (αCH), 69.85 (γCH) and 170.62 (ester CO); *m/z* (EI) 146 (11%, [M – HCl]$^+$), 86 (100, $OC_4H_8N^+$), 68 (44, $C_4H_6N^+$) and 58 (21, $NHCH_2COH^+$).

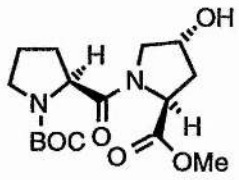
**Methyl (2*S*,4*R*)-*N*-(*N*-{*N*-[(2*S*)-2-chloropropionyl]-(2*S*)-prolyl}-(2*S*)-prolyl)-4-hydroxyprolinate 115**




This was prepared in an identical manner to hydrazide **69**, using acid **108** (1.27 g, 4.2 mmol) and methyl (2*S*,4*R*)-4-hydroxyprolinate hydrochloride **114** (0.79 g, 4.4 mmol) to afford the product as a brown oil (1.08 g, 60%) which defied further purification;

(HRMS: found $M^+$, 429.1657. $C_{19}H_{28}{}^{35}ClN_3O_6$ requires 429.1667); $\delta_H$(300 MHz; $C^2HCl_3$) 1.56 (3 H, d, *J* 6.6, $CHCH_3$), 1.90–2.42 [10 H, m, 2 × β $CH_2$(Pro), β$CH_2$(Hyp) and 2 × γ$CH_2$(Pro)], 3.54–3.82 [6 H, m, 2 × δ$CH_2$(Pro) and δ$CH_2$(Hyp)] 3.63 (3 H, s, $CO_2CH_3$) and 4.40–4.65 [5 H, m, $CHCH_3$, 2 × αCH(Pro), αCH(Hyp) and γCH(Hyp)]; $\delta_C$(74.76 MHz; $C^2HCl_3$) 20.52 ($CHCH_3$), 24.75 and 24.79 [2 × γ$CH_2$(Pro)], 28.01 and 28.32 [2 × β$CH_2$(Pro)], 37.27 [β$CH_2$(Hyp)], 47.13 and 47.37 [2 × δ$CH_2$(Pro)], 51.34 ($CHCH_3$), 52.22 ($CO_2CH_3$), 54.57 [δ$CH_2$(Hyp)], 57.63, 58.01 and 58.55 [2 × αCH(Pro) and αCH(Hyp)], 69.91 [γCH(Hyp)], 167.85 ($COCHCH_3$), 170.52 and 170.89 (2 × amide CO) and 172.91 (ester CO); *m/z* (EI) 431 and 429 (2 and 7%, chlorine isotopes, $M^+$), 287 and 285 {2 and 9, chlorine isotopes, [M – $NCH_2CH(OH)CH_2CHCO_2CH_3$ + H]$^+$}, 259 and 257 {3 and 11, chlorine isotopes, [M – $CONCH_2CH(OH)CH_2CHCO_2CH_3$ + H]$^+$}, 190 and 188 (17 and 61, chlorine isotopes, $C_8H_{11}NO_2Cl^+$), 162 and 160 (24 and 79, chlorine isotopes, $C_7H_{11}NOCl^+$) and 70 (100, $C_4H_8N^+$).

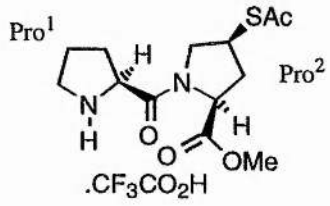
**Methyl (2*S*,4*R*)-*N*-[*N*-(*tert*-butoxycarbonyl)-(2*S*)-prolyl]-4-hydroxyprolinate 119**




A solution of (2*S*)-*N*-(*tert*-butoxycarbonyl)proline **94** (4.0 g, 18.6 mmol) and *N,N*-di-*iso*propylethylamine (9.0 $cm^3$, 52.0 mmol) in dry dichloromethane (115 $cm^3$) was treated with BOP-Cl (4.92 g, 19.3 mmol) and the resulting suspension stirred under nitrogen at 0 °C for 20 min. A suspension of methyl (2*S*,4*R*)-4-hydroxyprolinate hydrochloride **114** (2.93 g, 16.1 mmol) in dry dichloromethane (35 $cm^3$) was then added. The mixture was allowed to warm to room temperature and stirred for a further 6 days. The solution was washed with aqueous HCl (0.5 mol $dm^{-3}$, 2 × 100 $cm^3$), aqueous sodium hydrogen carbonate solution (5%, 2 × 100 $cm^3$) and brine

(100 $cm^3$), then dried ($MgSO_4$). The solvent was removed under reduced pressure, and the residual oil purified by column chromatography using ethyl acetate as the eluent to afford the dipeptide **119** as colourless crystals (3.41 g, 62%), mp 92–94 °C; $R_f$ 0.24; (Found: C, 55.95; H, 7.8; N, 8.15. $C_{16}H_{26}N_2O_6$ requires C, 56.15; H, 7.65; N, 8.2%); $[\alpha]_D$ −112.6 (*c* 0.8 in MeOH); $\upsilon_{max}(CH_2Cl_2)/cm^{-1}$ 3458 (OH), 2981 (CH) 1748 (ester CO), 1690 (urethane CO), 1662 (amide CO), 1407 (alcohol C–O) and 1165 (ester C–O); $\delta_H$(200 MHz; $C^2HCl_3$) (2 conformations, *ct* and *tt*) 1.17 [*ct*, 9 H, $C(CH_3)_3$], 1.21 [*tt*, 9 H, $C(CH_3)_3$], 1.61–2.06 [6 H, m, $\beta CH_2$(Pro), $\beta CH_2$(Hyp) and $\gamma CH_2$(Pro)], 3.14–3.89 [4 H, m, $\delta CH_2$(Pro) and $\delta CH_2$(Hyp)], 3.47 (3 H, s, $CO_2CH_3$) and 4.16–4.42 [3 H, m, αCH(Pro), αCH(Hyp) and γCH(Hyp)]; $\delta_C$(50.31 MHz; $C^2HCl_3$) 23.47 [*ct*, $\gamma CH_2$(Pro)], 23.98 [*tt*, $\gamma CH_2$(Pro)], 28.26 [*ct*, $C(CH_3)_3$], 28.38 [*tt*, $C(CH_3)_3$)], 29.00 [*tt*, $\beta CH_2$(Pro)], 29.73 [*ct*, $\beta CH_2$(Pro)], 36.95 [*tt*, $\beta CH_2$(Hyp)], 37.15 [*ct*, $\beta CH_2$(Hyp)], 46.51 [*ct*, $\delta CH_2$(Pro)], 46.86 [*tt*, $\delta CH_2$(Pro)], 52.08 ($CO_2CH_3$), 54.45 [$\delta CH_2$(Hyp)], 57.70 [αCH(Pro)], 57.93 [αCH(Hyp)], 69.68 [*ct*, γCH(Hyp)], 69.99 [*tt*, γCH(Hyp)], 79.71 [$C(CH_3)_3$], 153.96 (*ct*, urethane CO), 154.47 (*tt*, urethane CO), 171.36 (*tt*, amide CO), 171.78 (*ct*, amide CO), 172.65 (*ct*, ester CO) and 172.75 (*tt*, ester CO); *m/z* (CI) 343 (42%, $[M + H]^+$), 287 (48, $[M - C_4H_8 + H]^+$), 243 (100, $[M - CO_2 - C_4H_8 + H]^+$) and 57 (84, $[C_4H_8 + H]^+$).

**Methyl (2*S*,4*R*)-*N*-[(2*S*)-prolyl]-4-(acetylthio)prolinate trifluoroacetate 118**

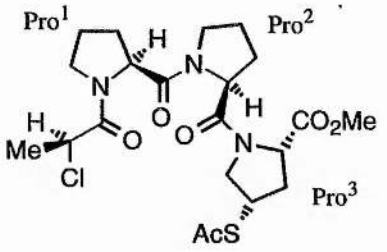



Di-*iso*propyl azodicarboxylate (0.46 g, 2.3 mmol) was added to a solution of triphenylphosphine (0.61 g, 2.3 mmol) in dry THF (15 $cm^3$) at 0 °C, and the resulting suspension stirred under nitrogen at 0 °C for 30 min. A solution of alcohol **119**

(0.69 g, 2.0 mmol) and thiolacetic acid (0.17 $cm^3$, 2.5 mmol) in dry THF (10 $cm^3$) was then added dropwise to this solution. The mixture was stirred at 0 °C for 2 h, and then at room temperature for 18 h. The solvent was removed under reduced pressure to yield a pale orange oil which was partially purified by column chromatography, using ethyl acetate–light petrol (2:1) as the eluent, to afford the thiolester **120** as an oil (1.12 g).

The above oil was redissolved in dichloromethane (20 $cm^3$), the solution cooled to 0 °C, and trifluoroacetic acid (10 $cm^3$) was added. The solution was stirred at 0 °C for 2 h, and the solvents were removed under reduced pressure. The residual oil was redissolved in water (20 $cm^3$) and the solution washed with ether (2 × 10 $cm^3$). The aqueous phase was concentrated under reduced pressure and lyophilised to yield the amine trifluoroacetate salt **118** as a clear colourless oil (0.58 g, 69%); (Found: C, 42.0; H, 5.3; N, 6.35. $C_{13}H_{20}N_2O_4S{\cdot}CF_3CO_2H{\cdot}H_2O$ requires C, 41.65; H, 5.35; N, 6.5%); (HRMS: found $[M - CF_3CO_2]^+$, 301.1222. $C_{13}H_{21}N_2O_4S$ requires 301.1222); $[\alpha]_D$ –93.8 (*c* 0.2 in MeOH); $\upsilon_{max}(CH_2Cl_2)/cm^{-1}$ 2956 (CH), 1750 (ester CO), 1696 (thiolester CO), 1663 (amide CO) and 1200 (C–O); $\delta_H$(300 MHz; $C^2HCl_3$) 1.90–2.15 [4 H, m, $\beta CH_2(Pro^1)$ and $\gamma CH_2(Pro^1)$], 2.31 (3 H, s, $SCOCH_3$), 2.44 [1 H, m, $\frac{1}{2}\beta CH_2(Pro^2)$], 2.73 [1 H, dt, $J_1$ 13.2, $J_2$ 7.8, $\frac{1}{2}\beta CH_2(Pro^2)$], 3.35 [1 H, dd, $J_1$ 10.2, $J_2$ 7.8, $\frac{1}{2}\delta CH_2(Pro^2)$], 3.41–3.58 [2 H, m, $\delta CH_2(Pro^1)$], 3.71 (3 H, s, $CO_2CH_3$), 4.00 [1 H, quintet, $J$ 7.8, $\gamma CH(Pro^2)$], 4.12 [1 H, dd, $J_1$ 10.2, $J_2$ 7.2, $\frac{1}{2}\delta CH_2(Pro^2)$] and 4.49–4.69 [2 H, m, 2 × $\alpha CH(Pro)$], 7.71 (1 H, br. s, NH) and 11.80 (1 H, br. s, $CO_2H$); $\delta_C$(50.51 MHz; $C^2HCl_3$) 24.70 [$\gamma CH_2(Pro^1)$], 29.01 [$\beta CH_2(Pro^1)$], 30.77 ($SCOCH_3$), 34.45 [$\beta CH_2(Pro^2)$], 39.87 [$\gamma CH(Pro^2$], 47.27 [$\delta CH_2(Pro^1)$], 52.56 ($CO_2CH_3$), 53.09 [$\delta CH_2(Pro^2)$], 59.05 [$\alpha CH(Pro^1)$], 60.15 [$\alpha CH(Pro^2)$], 116.46 (q, $J_{CF}$ 286, $CF_3CO_2H$), 161.93 (q, $J_{CF}$ 38, $CF_3CO_2H$), 167.95 (amide CO), 171.49 (ester CO) and 195.04 (thiolester CO); *m/z* (CI) 301 (70%, $[M - CF_3CO_2]^+$), 269 (100, $[M - CF_3CO_2 - OCH_3]^+$), 160 [30, $NC_4H_7(S)CO_2CH_3^+$] and 70 (17, $C_4H_8N^+$).

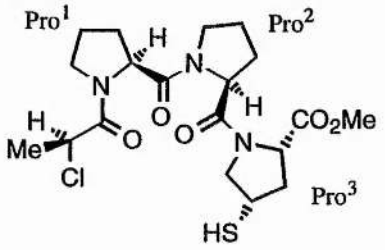
**Methyl (2*S*,4*S*)-*N*-(*N*-{*N*-[(2*S*)-2-chloropropionyl]-(2*S*)-prolyl}-(2*S*)-prolyl)-4-(acetylthio)prolinate 117**




This was prepared in an identical manner to dipeptide **119**, using (2*S*,2'*S*)-*N*-(2'-chloropropionyl)proline **106** (0.38 g, 1.85 mmol) and amine trifluoroacetate **118** (0.50 g, 1.21 mmol) to afford thiolacetate **117** as a white foam (0.53 g, 90%); (Found: C, 49.95; H, 6.0; N, 8.65. $C_{21}H_{30}ClN_3O_6S$ requires C, 49.85; H, 6.35; N, 8.3%); (HRMS: found $M^+$, 487.1552. $C_{21}H_{30}{}^{35}ClN_3O_6S$ requires 487.1544); $[\alpha]_D$ −133.1 (*c* 0.3 in MeOH); $\upsilon_{max}$(thin film)/cm$^{-1}$ 1748 (ester CO), 1695 (thiolacetate CO), 1652 (amide CO), 1435 (CH def.) and 1200 (C–O); $\delta_H$(200 MHz; $C^2HCl_3$) 1.63 (3 H, d, *J* 6.6, CHC*H*$_3$), 1.85–2.20 [9 H, m, βC H$_2$(Pro$^1$), β CH$_2$(Pro$^2$), $\frac{1}{2}$βCH$_2$ (Pro$^3$), γCH$_2$(Pro$^1$) and γCH$_2$(Pro$^2$)], 2.31 (3 H, s, SCOCH$_3$), 2.60–2.80 [1 H, m, $\frac{1}{2}$βCH$_2$(Pro$^3$)], 3.42 [1 H, dd, *J*$_1$ 10.1, *J*$_2$ 7.2, $\frac{1}{2}$δCH$_2$(Pro$^3$)], 3.57–3.84 [4 H, m, δCH$_2$(Pro$^1$) and δCH$_2$(Pro$^2$)], 3.70 (3 H, s, CO$_2$CH$_3$), 4.01–4.10 [1 H, m, γCH(Pro$^3$)], 4.30 [1 H, dd, *J*$_1$ 10.1, *J*$_2$ 7.2, $\frac{1}{2}$δCH$_2$(Pro$^3$)] and 4.48–4.71 (4 H, m, C*H*CH$_3$ and 3 × αCH(Pro)]; $\delta_C$(74.76 MHz; $C^2HCl_3$) 20.88 (CH*C*H$_3$), 25.19 and 25.26 [γCH$_2$(Pro$^1$) and γCH$_2$(Pro$^2$)], 28.39 and 28.66 [βCH$_2$(Pro$^1$) and βCH$_2$(Pro$^2$)], 30.87 (SCO*C*H$_3$), 35.13 [βCH$_2$(Pro$^3$)], 40.38 [γCH$_2$(Pro$^3$)], 47.35 and 47.71 [δCH$_2$(Pro$^1$) and δCH$_2$(Pro$^2$)], 51.68 (*C*HCH$_3$), 52.57 [δCH$_2$(Pro$^3$)], 52.76 (CO$_2$*C*H$_3$), 58.29, 58.54 and 58.84 [3 × αCH(Pro)], 168.16 (*C*OCHCH$_3$), 170.71 and 171.14 (2 × amide CO), 172.36 (ester CO) and 194.84 (thiolester CO); *m/z* (EI) 489 and 487 (7 and 16%, chlorine isotopes, $M^+$), 451 (7, [M − HCl]$^+$), 327 {14, [M − ClCH(CH$_3$)CONC$_4$H$_7$]$^+$}, 287 and 285 (6 and 21, chlorine isotopes, [M − CH$_3$COSC$_4$H$_6$NCO$_2$CH$_3$]$^+$), 190 and 188 [32 and 100, chlorine isotopes,

$ClCH(CH_3)CONC_4H_7CO^+$], 162 and 160 [26 and 77, chlorine isotopes, $ClCH(CH_3)CONC_4H_7^+$] and 70 (53, $C_4H_8N^+$).

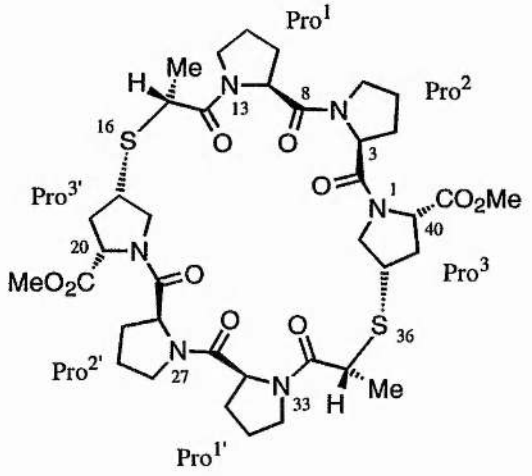
**Methyl (2*S*,4*S*)-*N*-(*N*-{*N*-[(2*S*)-2-chloropropionyl]-(2*S*)-prolyl}-(2*S*)-prolyl)-4-mercaptoprolinate 121**




Thiolacetate **117** (0.15 g, 0.31 mmol) was dissolved in methanol (10 $cm^3$) and the solution treated with aqueous potassium hydroxide solution (0.05 mol $dm^{-3}$, 10 $cm^3$). The mixture was stirred for 4 h, after which time aqueous HCl (0.05 mol $dm^{-3}$, 10 $cm^3$) was added and the methanol removed under reduced pressure. The solution was further acidified to pH 1–2 and extracted with ethyl acetate (5 × 30 $cm^3$). The combined organic extracts were dried ($MgSO_4$) and the solvent removed under reduced pressure to yield the thiol **121** as a clear colourless oil (0.13 g, 95%); (HRMS: found $[M + H]^+$, 446.1506. $C_{19}H_{28}{}^{35}ClN_3O_5S$ requires 446.1516); $[\alpha]_D$ −135.5 (*c* 0.4 in MeOH); $\upsilon_{max}$(thin film)/$cm^{-1}$ 2954 (CH), 2539 (SH), 1744 (ester CO), 1654 (amide CO), 1434 (CH def.) and 1199 (C–O); $\delta_H$(200 MHz; $C^2HCl_3$) 1.60 (3 H, d, *J* 6.6, $CHCH_3$), 1.80–2.20 [9 H, m, $\beta CH_2(Pro^1)$, $\beta CH_2(Pro^2)$, $\frac{1}{2}\beta CH_2(Pro^3)$, $\gamma CH_2(Pro^1)$ and $\gamma CH_2(Pro^2)$], 2.64–2.71 [1 H, m, $\frac{1}{2}\beta CH_2(Pro^3)$], 3.32–3.45 [2 H, m, $\gamma CH(Pro^3)$ and $\frac{1}{2}\delta CH_2(Pro^3)$], 3.58–3.86 [4 H, m, $\delta CH_2(Pro^1)$ and $\delta CH_2(Pro^2)$], 3.69 (3 H, s, $CO_2CH_3$), 4.27 [1 H, dd, $J_1$ 8.4, $J_2$ 5.7, $\frac{1}{2}\delta CH_2(Pro^3)$], 4.49 [2 H, m, $CHCH_3$ and αCH(Pro)] and 4.67 [2 H, m, 2 × αCH(Pro)]; $\delta_C$(74.76 MHz; $C^2HCl_3$) 20.61 ($CHCH_3$), 24.97 and 25.04 [$\gamma CH_2(Pro^1)$ and $\gamma CH_2(Pro^2)$], 28.27 and 28.43 [$\beta CH_2(Pro^1)$ and $\beta CH_2(Pro^2)$], 35.82 [$\beta CH_2(Pro^3)$], 39.26 [$\gamma CH_2(Pro^3)$], 47.22 and 47.50 [$\delta CH_2(Pro^1)$ and $\delta CH_2(Pro^2)$], 51.43 ($CHCH_3$), 52.53 ($CO_2CH_3$), 56.20

[$\delta CH_2(Pro^3)$], 58.10, 58.63 and 58.74 [3 × αCH(Pro)], 167.96 (*C*OCHCH$_3$), 170.67 and 170.83 (2 × amide CO) and 172.25 (ester CO); *m/z* (CI) 448 and 446 (21 and 60%, chlorine isotopes, $[M + H]^+$), 412 (100, $[M - Cl + H]^+$), 380 (32, $[M - Cl - OCH_3 + H]^+$), 227 {49, $[M - ClCH(CH_3)CONC_4H_7 - CO_2CH_3 + H]^+$}, 154 (31, $[CH_3CHCONC_4H_7CO + H]^+$), 126 (26, $OCNC_4H_8CO^+$) and 70 (24, $C_4H_8N^+$).

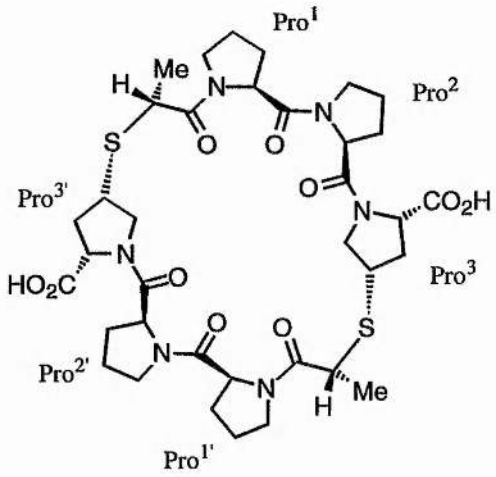
**Dimethyl (3*S*,9*S*,15*R*,17*S*,20*S*,23*S*,29*S*,35*R*,37*S*,40*S*)-15,35-dimethyl-2,8,14,22,28,34-hexaoxo-16,36-dithia-1,7,13,21,27,33-hexaazaheptacyclo-[24.2.2.1.1.1.1.0$^{3,7}$.0$^{9,13}$.0$^{23,27}$.0$^{29,33}$]tetracontane-20,40-dicarboxylate 122**




Sodium hydride (60% dispersion in mineral oil, 11 mg, 0.29 mmol) was added to a stirred solution of thiol **121** (105 mg, 0.24 mmol) in dry THF (40 cm$^3$) and the mixture heated at reflux under nitrogen for 2 h. The suspension was cooled to room temperature, and the solvent removed under reduced pressure. The residual oil was purified by gradient column chromatography using dichloromethane–methanol as the eluent to yield the cyclic product **122** as a pale oil (21 mg, 22%); $R_f$ 0.65 (dichloromethane–methanol, 9:1); (HRMS: found $[\frac{1}{2}M + H]^+$, 410.1742. $C_{19}H_{27}N_3O_5S$ requires 410.1750); $[\alpha]_D$ –64.1 (*c* 0.3 in MeOH); $\upsilon_{max}$(thin film)/cm$^{-1}$ 1742 (ester CO), 1634 (amide CO) and 1200 (C–O); $\delta_H$(300 MHz; $C^2HCl_3$) 1.35 (6 H, d, *J* 6.6, 2 × CHC*H*$_3$), 1.85–2.35 [18 H, m, $\beta CH_2(Pro^{1,1'})$, $\beta CH_2(Pro^{2,2'})$,

$\frac{1}{2}\beta CH_2(Pro^{3,3'})$, $\gamma CH_2(Pro^{1,1'})$ and $\gamma CH_2(Pro^{2,2'})$], 2.76 [2 H, dt, $J_1$ 13.2, $J_2$ 8.4, $\frac{1}{2}\beta CH_2(Pro^{3,3'})$], 3.25 [2 H, dd, $J_1$ 10.2, $J_2$ 7.5, $\frac{1}{2}\delta CH_2(Pro^{3,3'})$], 3.45–3.92 [10 H, m, $\gamma CH(Pro^{3,3'})$, $\delta CH_2(Pro^{1,1'})$ and $\delta CH_2(Pro^{2,2'})$], 3.69 (6 H, s, 2 × $CO_2CH_3$), 4.23 [2 H, dd, $J_1$ 10.2, $J_2$ 7.9, $\frac{1}{2}\delta CH_2(Pro^{3,3'})$] and 4.62–4.71 [8 H, m, 2 × C*H*$CH_3$ and 6 × $\alpha CH(Pro)$]; $\delta_C$(74.76 MHz; $C^2HCl_3$) 17.56 (2 × $CHCH_3$), 25.11 and 25.54 [$\gamma CH_2(Pro^{1,1'})$ and $\gamma CH_2(Pro^{2,2'})$], 28.20 and 28.58 [$\beta CH_2(Pro^{1,1'})$ and $\beta CH_2(Pro^{2,2'})$], 38.42 [$\beta CH_2(Pro^{3,3'})$], 40.45 [$\gamma CH_2(Pro^{3,3'})$], 40.90 (2 × *C*$HCH_3$), 47.35 and 48.36 [$\delta CH_2(Pro^{1,1'})$ and $\delta CH_2(Pro^{2,2'})$], 52.39 (2 × $CO_2CH_3$), 52.68 [$\delta CH_2(Pro^{3,3'})$], 58.58, 58.82 and 59.06 [6 × $\alpha CH(Pro)$], 170.63 and 170.78 (4 × amide CO), 172.08 (2 × *C*$OCHCH_3$) and 172.67 (2 × ester CO); *m/z* ($ES^+$) 857 (8%, $[M + K]^+$), 841 (57, $[M + Na]^+$), 819 (16, $M^+$) and 364 (96, $[\frac{1}{2}M - 2CH_3 - OCH_3]^+$).

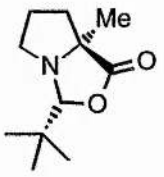
**(3*S*,9*S*,15*R*,17*S*,20*S*,23*S*,29*S*,35*R*,37*S*,40*S*)-15,35-Dimethyl-2,8,14,22,28,34-hexaoxo-16,36-dithia-1,7,13,21,27,33-hexaazaheptacyclo-[24.2.2.1.1.1.1.$0^{3,7}$.$0^{9,13}$.$0^{23,27}$.$0^{29,33}$]tetracontane-20,40-dicarboxylic acid 123**




A solution of methyl ester **122** (11 mg, 27 μmol) in methanol (0.2 $cm^3$) was treated with aqueous sodium hydroxide solution (1.0 mol $dm^{-3}$, 0.2 $cm^3$) and the solution stirred for 2 h. The solvents were removed and the residue redissolved in water (5 $cm^3$). The aqueous solution was acidified to pH 1 with aqueous HCl (1 mol $dm^{-3}$) and

washed with dichloromethane (2 × 4 $cm^3$). The solvent was removed from the aqueous phase and the residual solids extracted several times with hot dichloromethane. The combined extracts were filtered and the solvent removed under reduced pressure to afford the acid **123** as a colourless glass (7 mg, 65%); $[\alpha]_D$ –33.9 (*c* 0.4 in MeOH); $\upsilon_{max}(CH_2Cl_2)/cm^{-1}$ 2978 (CH), 1754 (acid CO), 1642 (amide CO) and 1437 (CH def.); $\delta_H$(300 MHz; $^2H_2O$) 1.32 (6 H, d, *J* 7.2, 2 × $CHCH_3$), 1.86–2.01 [4 H, m, $\frac{1}{2}\beta CH_2(Pro^{1,1'})$ and $\frac{1}{2}\beta CH_2(Pro^{2,2'})$], 2.00–2.13 [10 H, m, $\frac{1}{2}\beta CH_2(Pro^{3,3'})$, $\gamma CH_2(Pro^{1,1'})$ and $\gamma CH_2(Pro^{2,2'})$] 2.31–2.44 [4 H, m, $\frac{1}{2}\beta CH_2(Pro^{1,1'})$ and $\frac{1}{2}\beta CH_2(Pro^{2,2'})$], 3.02 [2 H, dt, $J_1$ 12.6, $J_2$ 7.8, $\frac{1}{2}\beta CH_2(Pro^{3,3'})$], 3.35 [2 H, m, $\frac{1}{2}\delta CH_2(Pro^{3,3'})$], 3.60 [2 H, m, $\gamma CH(Pro^{3,3'})$], 3.62–3.91 [8 H, m, $\delta CH_2(Pro^{1,1'})$ and $\delta CH_2(Pro^{2,2'})$], 4.17 [2 H, dd, $J_1$ 9.0, $J_2$ 6.0, $\frac{1}{2}\delta CH_2(Pro^{3,3'})$], 4.27 (2 H, q, *J* 7.2, 2 × $CHCH_3$), 4.51 [2 H, t, *J* 7.8, $\alpha CH(Pro^{3,3'})$] and 4.71–4.78 [4 H, m, $\alpha CH(Pro^{1,1'})$ and $\alpha CH(Pro^{2,2'})$]; $\delta_C$(75.44 MHz; $^2H_2O$) 16.13 (2 × $CHCH_3$), 24.58 and 24.78 [$\gamma CH_2(Pro^{1,1'})$ and $\gamma CH_2(Pro^{2,2'})$], 27.71 and 28.25 [$\beta CH_2(Pro^{1,1'})$ and $\beta CH_2(Pro^{2,2'})$], 37.96 [$\beta CH_2(Pro^{3,3'})$], 40.30 [2 × $CHCH_3$ and $\gamma CH(Pro^{3,3'})$], 47.83 and 48.94 [$\delta CH_2(Pro^{1,1'})$ and $\delta CH_2(Pro^{2,2'})$], 51.34 [$\delta CH_2(Pro^{3,3'})$], 58.73 and 59.51 [6 × αCH(Pro)], 171.56 and 171.97 (4 × amide CO), 174.13 (2 × $COCHCH_3$) and 175.27 (2 × acid CO).

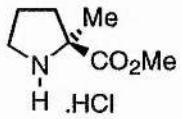
**(2*R*,5*S*)-2-*tert*-Butyl-5-methyl-1-aza-3-oxabicyclo[3.3.0]octan-4-one 127**




Pivalaldehyde (25 $cm^3$, 0.23 mol) was added to a suspension of (2*S*)-proline **6** (14.0 g, 0.12 mol) in pentane (400 $cm^3$) and the mixture heated under reflux, using a Dean-Stark apparatus, for 7 days. During this time three further 25 $cm^3$ portions of pivalaldehyde were added at 2 day intervals. The solvent and excess pivalaldehyde

were removed under reduced pressure to afford the bicyclooctanone **126** as a pale orange-brown oil (21.5 g). A portion of this oil (6.3 g, 34.4 mmol) was redissolved in dry THF (140 $cm^3$) and the solution cooled under nitrogen to −78 °C. A solution of lithium di-*iso*propylamide in hexanes–THF (1 mol $dm^{-3}$, 36 $cm^3$) was added and the mixture stirred for 1 h at −78 °C. Methyl iodide (2.4 $cm^3$, 38.6 mmol) was then added dropwise and the suspension allowed to warm to −30 °C over 2 h. The suspension was poured into a mixture of water (100 $cm^3$) and dichloromethane (250 $cm^3$). The organic phase was separated, dried ($MgSO_4$) and the solvents removed under reduced pressure to give a yellow-orange solid. The crude solid was redissolved in dichloromethane and the solution was filtered. The filtrate was concentrated under reduced pressure to yield a brown semi-solid mass; Kugelrohr distillation of this residue under reduced pressure afforded bicyclooctanone **127** as a clear colourless oil (3.9 g, 55%), bp 110 °C/~0.05 mmHg (lit., [275] 85 °C/0.05mmHg); $[\alpha]_D$ −28.7 (*c* 1.05 in $CHCl_3$) [lit., [275] −29.8 (*c* 0.7 in $CHCl_3$)]; $\upsilon_{max}$(thin film)/$cm^{-1}$ 2973 (CH), 1784 (CO) and 1192 (C–O); $\delta_H$(200 MHz; $C^2HCl_3$) 0.90 [9 H, s, $C(CH_3)_3$], 1.37 (3 H, s, $\beta CH_3$), 1.64–1.86 (3 H, m, $\frac{1}{2}\beta CH_2$ and $\gamma CH_2$), 2.12–2.19 (1 H, m, $\frac{1}{2}\beta CH_2$), 2.76–2.87 (1 H, m, $\frac{1}{2}\delta CH_2$), 3.05–3.18 (1 H, m, $\frac{1}{2}\delta CH_2$) and 4.25 (1 H, s, OCHN); $\delta_C$(75.44 MHz; $C^2HCl_3$) 24.54 [$C(CH_3)$], 25.84 and 25.96 ($\beta CH_3$ and $\gamma CH_2$), 36.86 [$C(CH_3)_3$)] 39.14 ($\beta CH_2$), 58.34 ($\delta CH_2$), 69.30 ($\alpha$C), 106.36 (NCO) and 179.76 (CO); *m/z* (CI) 198 (82%, $[M + H]^+$), 140 (6, $[M - C_4H_9]^+$), 130 (100, $[M - C_5H_7 + H]^+$) and 84 (14, $NC_4H_7CH_3^+$).

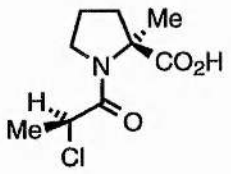
**Methyl (2*S*)-2-methylprolinate hydrochloride 128**




An ice-cooled solution of bicyclooctanone **127** (6.6 g, 33.5 mmol) in dry methanol (75 $cm^3$) was treated dropwise with thionyl chloride (4.0 $cm^3$, 54.6 mmol). The

solution was heated under reflux for 2 h, then cooled and the solvents removed under reduced pressure to yield a dark brown solid. The crude solid was recrystallised from ethyl acetate–methanol to afford the amine hydrochloride **128** as colourless crystals (3.5 g, 58%), mp 106–108 °C; (Found: C, 46.5; H, 8.15; N, 7.7. $C_7H_{14}ClNO_2$ requires C, 46.8; H, 7.85; N, 7.8%); $[\alpha]_D$ –31.6 (*c* 1.3 in MeOH); $\upsilon_{max}(CH_2Cl_2)/cm^{-1}$ 2995 (CH) and 1747 (CO); $\delta_H$(200 MHz; $^2H_2O$) 1.68 (3 H, s, $\beta CH_3$), 1.99–2.21 (3 H, m, $\frac{1}{2}\beta CH_2$ and $\gamma CH_2$), 2.36–2.46 (1 H, m, $\frac{1}{2}\beta CH_2$), 3.41–3.51 (2 H, m, $\delta CH_2$) and 3.84 (3 H, s, $CO_2CH_3$); $\delta_C$(75.44 MHz; $C^2H_3O^2H$) 21.86 ($\beta CH_3$), 23.97 ($\gamma CH_2$), 36.75 ($\beta CH_2$), 46.75 ($\delta CH_2$), 54.68 ($CO_2CH_3$), 70.50 ($\alpha C$) and 173.17 (ester CO); *m/z* (CI) 144 (100%, $[M - Cl]^+$) and 84 (26, $[M - HCl - CO_2CH_3]^+$).

**(2*S*,2'*S*)-*N*-(2'-Chloropropionyl)-2-methylproline 130**

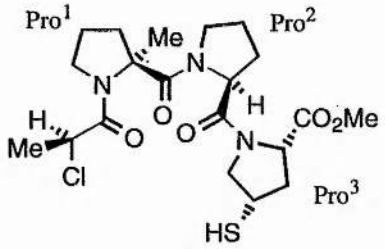



A solution of (2*S*)-2-chloropropionic acid **104** (1.21 g, 11.1 mmol), methyl (2*S*)-2-methylprolinate hydrochloride **128** (1.00 g, 5.5 mmol) and *N*,*N*-di-*iso*propylethylamine (3.3 $cm^3$, 18.9 mmol) in dry dichloromethane (100 $cm^3$) was treated with BOP-Cl (2.98 g, 11.7 mmol) and the resulting suspension stirred under nitrogen at 0–5 °C for 4 h. The mixture was allowed to warm to room temperature and stirred for a further 15 h. The solution was washed with aqueous HCl (0.5 mol $dm^{-3}$, 2 × 50 $cm^3$), aqueous sodium hydrogen carbonate solution (5%, 2 × 50 $cm^3$) and brine (50 $cm^3$), then dried ($MgSO_4$). The solvent was removed under reduced pressure to yield the dipeptide **129** as a colourless oil (0.78 g).

The above oil was redissolved in methanol (7 $cm^3$), treated with aqueous sodium hydroxide solution (1.0 mol $dm^{-3}$, 9 $cm^3$), then stirred for 2 days. Following addition of aqueous HCl (1.0 mol $dm^{-3}$, 9 $cm^3$), the solution was concentrated to ~15 $cm^3$

under reduced pressure and then extracted with ethyl acetate (3 × 25 $cm^3$). The combined organic extracts were dried ($MgSO_4$) and the solvent removed under reduced pressure to afford the product as a tan solid (0.53 g, 43%); a small portion was recrystallised to yield the acid **130** as colourless crystals, mp 136–137 °C; (Found: C, 49.5; H, 6.8; N, 6.45. $C_9H_{14}ClNO_3$ requires C, 49.2; H, 6.4; N, 6.4%); $[\alpha]_D$ −3.9 (*c* 0.3 in MeOH); $\upsilon_{max}(CH_2Cl_2)/cm^{-1}$ 3000 (OH), 2989 (CH), 1758 (ester CO), 1674 (amide CO) and 1260 (C–O); $\delta_H$(300 MHz; $C^2HCl_3$) 1.53 (3 H, s, $\beta CH_3$), 1.57 (3 H, d, *J* 6.6, $CHCH_3$), 1.85–2.06 (3 H, m, $\frac{1}{2}\beta CH_2$ and $\gamma CH_2$), 2.21–2.30 (1 H, m, $\frac{1}{2}\beta CH_2$), 3.57–3.69 (1 H, m, $\frac{1}{2}\delta CH_2$), 3.78–3.85 (1 H, m, $\frac{1}{2}\delta CH_2$), 4.42 (1 H, q, *J* 6.6, $CHCH_3$) and 7.13 (1 H, br. s, $CO_2H$); $\delta_C$(75.44 MHz; $C^2HCl_3$) 20.49 ($\beta CH_3$), 20.90 ($CHCH_3$), 24.26 ($\gamma CH_2$), 38.66 ($\beta CH_2$), 48.46 ($\delta CH_2$), 51.80 ($CHCH_3$), 67.00 ($\alpha C$), 168.33 ($COCHCH_3$) and 177.61 (acid CO); *m/z* (EI) 222 and 220 (1 and 4%, chlorine isotopes, $M^+$), 176 and 174 (32 and 56, chlorine isotopes, $[M - CO_2H + H]^+$), 140 (45, $[M - CO_2H - HCl + H]^+$), 128 {21, $[M - ClCH(CH_3)CO]^+$} and 84 (100, $C_5H_{10}N^+$).

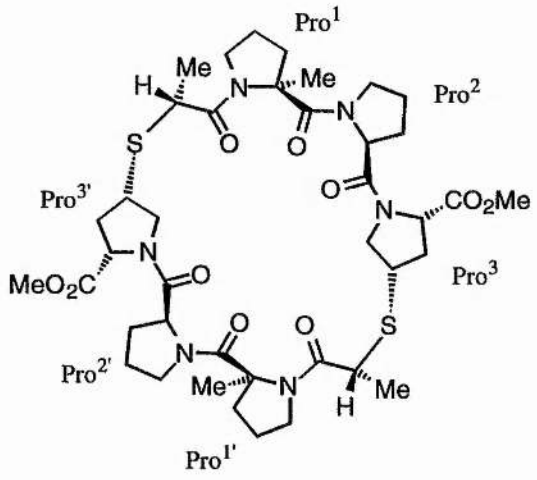
**Methyl (2*S*,4*S*)-*N*-(*N*-{*N*-[(2*S*)-2-chloropropionyl]-(2*S*)-2-methylprolyl}-(2*S*)-prolyl)-4-mercaptoprolinate 132**




A solution of (2*S*,2'*S*)-*N*-(2'-chloropropionyl)-2-methylproline **130** (0.20 g, 0.91 mmol) and *N*,*N*-di-*iso*propylethylamine (0.50 $cm^3$, 2.87 mmol) in dry dichloromethane (15 $cm^3$) was treated with BOP-Cl (0.25 g, 0.98 mmol) and the resulting suspension stirred under nitrogen at 0 °C for 20 min. A solution of amine trifluoroacetate **118** (0.28 g, 0.83 mmol) in dry dichloromethane (5 $cm^3$) was then

added. The mixture was stirred at 0–5 °C for 5 h, then at room temperature for 15 h. The solution was washed with aqueous HCl (0.5 mol $dm^{-3}$, 2 × 5 $cm^3$), aqueous sodium hydrogen carbonate solution (5%, 2 × 5 $cm^3$) and brine (100 $cm^3$) and dried ($MgSO_4$). The solvent was removed under reduced pressure, and the residual oil was purified by column chromatography using ethyl acetate–methanol (19:1) as the eluent, to yield the thiolacetate **131** as a colourless foam (0.13 g). The crude foam was redissolved in methanol (9 $cm^3$) and the solution treated with aqueous potassium hydroxide solution (0.05 mol $dm^{-3}$, 9 $cm^3$). The solution was stirred for 4 h, after which time aqueous HCl (0.05 mol $dm^{-3}$, 10 $cm^3$) was added. The methanol was removed under reduced pressure, then the solution was further acidified to pH 1 and extracted with ethyl acetate (3 × 25 $cm^3$). The combined organic extracts were dried ($MgSO_4$) and the solvent removed under reduced pressure to afford the thiol **132** as a colourless oil (0.11 g, 29%); (HRMS: found $[M + H]^+$, 460.1682. $C_{20}H_{31}{}^{35}ClN_3O_5S$ requires 460.1673); $[\alpha]_D$ −108.4 (*c* 1.1 in MeOH); $\upsilon_{max}(CH_2Cl_2)/cm^{-1}$ 2546 (SH), 1747 (ester CO), 1662 (amide CO), 1423 (CH def.) and 1200 (C–O); $\delta_H$(300 MHz; $C^2HCl_3$) 1.57 (3 H, d, *J* 6.6, CHC$H_3$), 1.78 [3 H, s, $\beta CH_3(Pro^1)$], 1.80–2.10 [8 H, m, \F(1,2)$\beta CH_2(Pro^1)$, $\beta CH_2(Pro^2)$, \F(1,2)$\beta CH_2(Pro^3)$, $\gamma CH_2(Pro^1)$ and $\gamma CH_2(Pro^2)$], 2.35 [1 H, m, $\frac{1}{2}\beta CH_2(Pro^2)$], 2.63 [1 H, dt, $J_1$ 13.3, $J_2$ 8.0, $\frac{1}{2}\beta CH_2(Pro^3)$], 3.30–3.41 [2 H, m, $\gamma CH(Pro^3)$ and $\frac{1}{2}\delta CH_2(Pro^3)$], 3.52–3.80 [4 H, m, $\delta CH_2(Pro^1)$ and $\delta CH_2(Pro^2)$], 3.62 (3 H, s, $CO_2CH_3$), 4.20 [1 H, dd, $J_1$ 10.0, $J_2$ 7.6, $\frac{1}{2}\delta CH_2(Pro^3)$], 4.42 [2 H, m, C$H$CH$_3$ and $\alpha CH(Pro)$] and 4.71 [1 H, m, $\alpha CH(Pro)$]; $\delta_C$(75.44 MHz; $C^2HCl_3$) 20.62 (CH*C*H$_3$), 23.52 and 23.60 [$\beta CH_3(Pro^1)$ and $\gamma CH_2(Pro^2)$], 25.55 [$\gamma CH_2(Pro^1)$], 27.26 [$\beta CH_2(Pro^2)$], 36.00 [$\beta CH_2(Pro^3)$], 38.23 [$\beta CH_2(Pro^1)$], 39.36 [$\gamma CH_2(Pro^3)$], 48.36 [$\delta CH_2(Pro^2)$], 48.69 [$\delta CH_2(Pro^1)$], 52.22 (*C*HCH$_3$), 52.65 ($CO_2CH_3$), 56.32 [$\delta CH_2(Pro^3)$], 58.75 [$\alpha CH(Pro^2)$], 60.51 [$\alpha CH(Pro^3)$], 68.58 [$\alpha C(Pro^1)$], 167.90 (*C*OCHCH$_3$), 171.09 and 171.71 (2 × amide CO) and 172.58 (ester CO); *m/z* (CI) 462 and 460 (33 and 100%, chlorine isotopes, $[M + H]^+$), 426 (13, $[M - HCl + H]^+$), 392 (13, $[M - Cl - SH + H]^+$), 301 and 299 {3 and 7, chlorine isotopes, $[M - NC_4H_6(SH)CO_2CH_3]^+$)}.

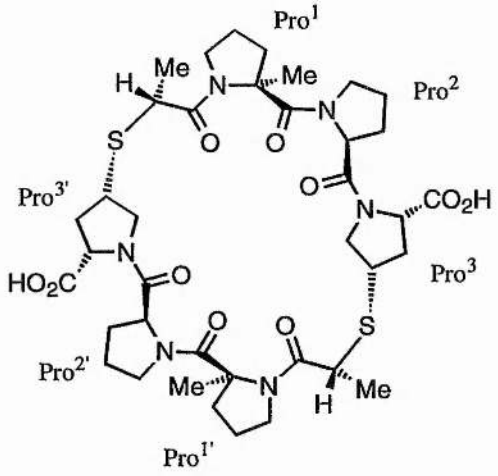
**Dimethyl (3*S*,9*S*,15*R*,17*S*,20*S*,23*S*,29*S*,35*R*,37*S*,40*S*)-9,15,29,35-tetramethyl-2,8,14,22,28,34-hexaoxo-16,36-dithia-1,7,13,21,27,33-hexaazaheptacyclo [24.2.2.1.1.1.1.$0^{3,7}$.$0^{9,13}$.$0^{23,27}$.$0^{29,33}$]tetracontane-20,40-dicarboxylate 133**




Caesium carbonate (210 mg, 0.65 mmol) was added to a solution of thiol **132** (150 mg, 0.33 mmol) in DMF (80 $cm^3$) and the mixture strirred with heating at 80–90 °C for 2.5 h. The suspension was cooled and the solvent removed under reduced pressure. The residual orange-brown oil was purified by column chromatography using dichloromethane–methanol (19:1) as the eluent to afford the macrocycle **133** as a white foam (41 mg, 30%); $R_f$ 0.43; (Found: C, 54.4; H, 7.1; N, 9.5. $C_{40}H_{58}N_6O_{10}S_2{\cdot}2H_2O$ requires: C, 54.4; H, 7.0; N, 9.8%); $[\alpha]_D$ −58.4 (*c* 0.1 in MeOH); $\upsilon_{max}(CH_2Cl_2)/cm^{-1}$ 2983 (CH), 1747 (ester CO), 1658 (amide CO), 1626 (amide CO) and 1200 (C–O); $\delta_H$(300 MHz; $^2H_2O$) 1.31 (6 H, d, *J* 6.9, 2 × $CHCH_3$), 1.80 [6 H, s, $\beta CH_3(Pro^{1,1'})$], 1.93–2.21 [18 H, m, $\beta CH_2(Pro^{1,1'})$, $\beta CH_2(Pro^{2,2'})$, $\frac{1}{2}\beta CH_2(Pro^{3,3'})$, $\gamma CH_2(Pro^{1,1'})$ and $\gamma CH_2(Pro^{2,2'})$], 2.85–2.94 [2 H, m, $\frac{1}{2}\beta CH_2(Pro^{1,1'})$], 3.44 [2 H, t, *J* 9.4, $\frac{1}{2}\delta CH_2(Pro^{3,3'})$], 3.58–3.69 [4 H, m, $\frac{1}{2}\delta CH_2(Pro^{1,1'})$ and $\gamma CH(Pro^{3,3'})$], 3.76 (6 H, s, 2 × $CO_2CH_3$), 3.76–3.85 [6 H, m, $\frac{1}{2}\delta CH_2(Pro^{1,1'})$ and $\delta CH_2(Pro^{2,2'})$], 4.11–4.22 [4 H, m, 2 × $CHCH_3$ and $\frac{1}{2}\delta CH_2(Pro^{3,3'})$], 4.56 [2 H, t, *J* 7.3, $\alpha CH(Pro^{3,3'})$] and 4.70–4.79 [4 H, m, $\alpha CH(Pro^{1,1'})$ and $\alpha CH(Pro^{2,2'})$]; $\delta_C$(75.44 MHz; $^2H_2O$) 16.41 (2 × $CHCH_3$), 23.01

and 23.24 [$\beta CH_3(Pro^{1,1'})$ and $\gamma CH_2(Pro^{1,1'})$], 25.20 [$\gamma CH_2(Pro^{2,2'})$], 26.66 [$\beta CH_2(Pro^{2,2'})$], 37.55 [$\beta CH_2(Pro^{3,3'})$], 38.00 [$\beta CH_2(Pro^{1,1'})$], 40.87 [$\gamma CH_2(Pro^{3,3'})$], 41.58 (2 × $CHCH_3$), 49.20 and 49.41 [$\delta CH_2(Pro^{1,1'})$ and $\delta CH_2(Pro^{2,2'})$], 52.08 (2 × $CO_2CH_3$), 53.17 [$\delta CH_2(Pro^{3,3'})$], 59.38 [$\alpha CH(Pro^{3,3'})$], 61.03 [$\alpha CH(Pro^{2,2'})$], 69.31 [$\alpha CH(Pro^{1,1'})$], 172.55, 173.15 and 173.52 (6 × amide CO) and 174.45 (2 × ester CO); *m/z* (CI) 847 (8%, $[M + H]^+$), 452 {72, $[CONC_4H_6(CH_3)COCH(CH_3)SC_4H_6(CO_2CH_3)NCOC_4H_7NCO + H]^+$}, 354 {80, $[CONC_4H_6(CH_3)COCH(CH_3)SC_4H_6(CO_2CH_3)]^+$} and 145 {100, $[NC_4H_6(S)CO_2H]^+$}.

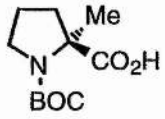
**(3*S*,9*S*,15*R*,17*S*,20*S*,23*S*,29*S*,35*R*,37*S*,40*S*)-9,15,29,35-Tetramethyl-2,8,14,22,28,34-hexaoxo-16,36-dithia-1,7,13,21,27,33-hexaazaheptacyclo [24.2.2.1.1.1.1.$0^{3,7}$.$0^{9,13}$.$0^{23,27}$.$0^{29,33}$]tetracontane-20,40-dicarboxylic acid 134**




This was prepared in an identical manner to acid **123**, using methyl ester **133** (20 mg, 4.7 μmol) to give the acid **134** as a clear colourless oil (15 mg, 78%); (HRMS: found $[M - 2H + 2Na]^+$, 863.3084. $C_{38}H_{53}N_6O_{10}S_2Na_2$ requires 863.3060); $[\alpha]_D$ −45.8 (*c* 0.1 in MeOH); $\upsilon_{max}(CH_2Cl_2)/cm^{-1}$ 2971 (CH), 1749 (acid CO), 1647 (amide CO) and 1456 (CH def.); $\delta_H$(500 MHz; $^2H_2O$) major conformation: 1.30 (6 H, d, *J* 7.2, 2 × $CHCH_3$), 1.79 [6 H, s, $\beta CH_3(Pro^{1,1'})$], 1.86–2.00 [4 H, m, $\frac{1}{2}\beta CH_2(Pro^{1,1'})$ and

$\frac{1}{2}\gamma CH_2(Pro^{2,2'})$], 2.00–2.15 [8 H, m, $\frac{1}{2}\beta CH_2$ (Pro$^{3,3'}$), $\gamma CH_2(Pro^{2,2'})$ and $\frac{1}{2}\gamma CH_2(Pro^{1,1'})$], 2.15–2.26 [4 H, m, $\frac{1}{2}\beta CH_2(Pro^{1,1'})$ and $\frac{1}{2}\beta CH_2(Pro^{2,2'})$], 2.88–2.97 [2 H, m, $\frac{1}{2}\beta CH_2(Pro^{3,3'})$], 3.37–3.42 [2 H, m, $\frac{1}{2}\delta CH_2(Pro^{3,3'})$], 3.49–3.68 [4 H, m, $\frac{1}{2}\delta CH_2(Pro^{3,3'})$ and $\gamma CH(Pro^{3,3'})$], 3.72–3.86 [6 H, m, $\delta CH_2(Pro^{1,1'})$ and $\frac{1}{2}\delta CH_2(Pro^{2,2'})$], 4.10–4.15 [2 H, m, $\frac{1}{2}\delta CH_2(Pro^{3,3'})$], 4.18 (2 H, q, *J* 7.2, 2 × C*H*CH$_3$), 4.49 [2 H, t, *J* 7.8, $\alpha CH(Pro^{3,3'})$] and 4.70–4.75 [2 H, m, $\alpha CH(Pro^{2,2'})$]; minor conformation: 1.42 [6 H, s, $\beta CH_3(Pro^{1,1'})$], 1.48 (6 H, d, *J* 7.2, 2 × C H C*H*$_3$), 1.68–1.73 [2 H, m, $\frac{1}{2}\gamma CH_2(Pro^{2,2'})$], 1.83–2.03 [6 H, m, $\gamma CH_2(Pro^{1,1'})$ and $\frac{1}{2}\gamma CH_2(Pro^{2,2'})$], 2.04–2.15 [4 H, m, $\frac{1}{2}\beta CH_2$ (Pro$^{1,1'}$) and $\frac{1}{2}\beta CH_2(Pro^{2,2'})$], 2.15–2.33 [6 H, m, $\frac{1}{2}\beta CH_2(Pro^{1,1'})$, $\frac{1}{2}\beta CH_2(Pro^{2,2'})$ and $\frac{1}{2}\beta CH_2(Pro^{3,3'})$], 3.02–3.08 [2 H, m, $\frac{1}{2}\beta CH_2(Pro^{3,3'})$], 3.49–3.56 [2 H, m, $\frac{1}{2}\delta CH_2(Pro^{3,3'})$], 3.37–3.42 [4 H, m, $\delta CH_2(Pro^{2,2'})$], 3.49–3.68 [2 H, m, $\delta CH_2(Pro^{1,1'})$], 3.99–4.06 [2 H, m, $\gamma CH(Pro^{3,3'})$], 4.10–4.22 [4 H, m, 2 × C*H*CH$_3$ and $\frac{1}{2}\delta CH_2(Pro^{3,3'})$], 4.48 [2 H, t, *J* 7.8, $\alpha CH(Pro^{3,3'})$] and 4.92 [2 H, d, *J* 7.8, $\alpha CH(Pro^{1,1'})$]; $\delta_C$(75.44 MHz; $^2H_2O$) 16.77 and 19.57 (2 × CH*C*H$_3$), 20.10 and 22.15 [$\beta CH_3(Pro^{1,1'})$], 23.04, 23.28 and 25.23 [$\gamma CH_2(Pro^{1,1'})$ and $\gamma CH_2(Pro^{2,2'})$], 26.80 and 30.84 [$\beta CH_2(Pro^{1,1'})$], 34.96 and 38.10 [$\beta CH_2(Pro^{3,3'})$], 38.86 and 39.86 [$\beta CH_2(Pro^{1,1'})$], 40.41 and 41.30 [$\gamma CH(Pro^{3,3'})$], 43.61 and 43.95 (2 × *C*HCH$_3$), 47.62, 49.03, 49.30 and 49.63 [$\delta CH_2(Pro^{1,1'})$ and $\delta CH_2(Pro^{2,2'})$], 51.70 and 52.04 [$\delta CH_2(Pro^{3,3'})$], 59.31, 61.22, 62.25 and 63.10 [$\alpha CH(Pro^{2,2'})$ and $\alpha CH(Pro^{3,3'})$], 68.32 and 69.41 [$\alpha C(Pro^{1,1'})$], 171.40, 171.57, 172.78, 173.15 and 173.95 (6 × amide CO), 178.31 and 179.18 (2 × acid CO); *m/z* (FAB) 885 (53%, [M – 2H + 3Na]$^+$), 863 (100, [M – H + 2Na]$^+$) and 817 (18, [M – H]$^+$).

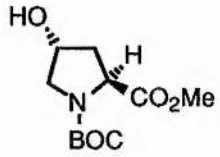
**(2*S*)-*N*-(*tert*-Butoxycarbonyl)-2-methylproline 137**




A suspension of (2*S*)-2-methylproline methyl ester hydrochloride **128** (1.00 g, 5.6 mmol) in dry dichloromethane (30 $cm^3$) at 0 °C was treated with *N*,*N*-di-*iso*propylethylamine (2.40 $cm^3$, 13.8 mmol), DMAP (0.20 g, 1.9 mmol) and di-*tert*-butyldicarbonate (1.85 g, 8.4 mmol). The mixture was allowed to reach room temperature and stirred for 3 days. The solution was washed with aqueous HCl (0.5 mol $dm^{-3}$, 2 × 20 $cm^3$), aqueous sodium hydrogen carbonate solution (5%, 2 × 20 $cm^3$) and brine (20 $cm^3$). The organic phase was dried ($MgSO_4$), and the solvent was removed under reduced pressure to yield the methyl ester **136** as a brown oil (1.24 g). The crude oil was redissolved in methanol (15 $cm^3$) and aqueous sodium hydroxide solution (1.0 mol $dm^{-3}$, 25 $cm^3$) was added. The solution was stirred for 2 days, concentrated to ~20 $cm^3$ and acidified to pH 2 with aqueous HCl (0.5 mol $dm^{-3}$). The solution was extracted with ethyl acetate (3 × 25 $cm^3$) and the combined organic extracts dried ($MgSO_4$). The solvent was removed under reduced pressure to afford the acid **137** as pale yellow crystals (0.78 g, 61%), mp 129–130 °C [lit.,[288] 129–132 °C]; (Found: C, 57.75; H, 8.4; N, 6.15. Calc. for $C_{11}H_{19}NO_4$: C, 57.6; H, 8.35; N, 6.1%); $[\alpha]_D$ –50.6 (*c* 1.4 in $CHCl_3$) [lit., [288] –41.4 (*c* 1.45 in $CHCl_3$)]; $\upsilon_{max}(CH_2Cl_2)/cm^{-1}$ 2982 (CH), 2664 (OH), 1749 (acid CO), 1692 (urethane CO) and 1165 (C–O); $\delta_H$(200 MHz; $C^2HCl_3$) 1.40 [*t*, 9 H, s, $C(CH_3)_3$], 1.44 [*c*, 9 H, s, $C(CH_3)_3$], 1.50 (*t*, 3 H, s, $\beta CH_3$), 1.57 (*c*, 3 H, s, $\beta CH_3$), 1.84–2.00 (3 H, m, $\frac{1}{2}\beta CH_2$ and $\gamma CH_2$), 2.18–2.43 (1 H, m, $\frac{1}{2}\beta CH_2$), 3.42–3.60 (2 H, m, $\delta CH_2$) and 10.05 (1 H, s, $CO_2H$); $\delta_C$(75.44 MHz; $C^2HCl_3$) 22.45 (*c*, $\beta CH_3$), 23.09 (*t*, $\beta CH_3$), 23.14 (*t*, $\gamma CH_2$), 23.39 (*c*, $\gamma CH_2$), 28.61 [*t*, $C(CH_3)_3$], 28.75 [*c*, $C(CH_3)_3$], 39.35 (*c*, $\beta CH_2$), 40.67 (*t*, $\beta CH_2$), 48.14 (*t*, $\delta CH_2$), 48.63 (*c*, $\delta CH_2$), 65.17 (*t*, αC), 66.00 (*c*, αC), 81.06 [$C(CH_3)_3$], 154.26 (*t*, urethane CO), 155.50 (*c*, urethane CO), 179.34 (*c*, acid CO) and

181.11 (*t*, acid CO); *m/z* (EI) 229 (0.5%, $M^+$), 184 (27, $[M - CO_2H]^+$), 128 (100, $[M - CO_2H - C_4H_8]^+$), 84 (78, $NC_4H_7CH_3^+$) and 57 (79, $C_4H_9^+$).

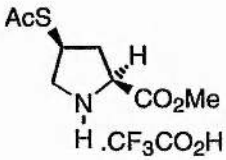
**Methyl (2*S*,4*R*)-*N*-(*tert*-butoxycarbonyl)-4-hydroxyprolinate 138**




A stirred suspension of methyl (2*S*,4*R*)-4-hydroxyprolinate hydrochloride **114** (7.0 g, 38.5 mmol) in dry THF (90 $cm^3$) was treated with *N*-methylmorpholine (8.8 $cm^3$, 78.7 mmol) and the mixture stirred for 1 h. Di-*tert*-butyl dicarbonate (9.3 g, 42.7 mmol) was added and the mixture stirred for a further 18 h. The hydrochloride salts were filtered off and the solvent removed from the filtrate under reduced pressure to give a brown oil which was redissolved in ethyl acetate (100 $cm^3$). The solution was washed with aqueous HCl (0.5 mol $dm^{-3}$, 2 × 70 $cm^3$), aqueous sodium hydrogen carbonate solution (5%, 2 × 70 $cm^3$) and brine (70 $cm^3$), then dried ($MgSO_4$). The solution was then concentrated under reduced pressure to yield compound **138** as a clear colourless oil (7.5 g, 79%); (Found: C, 53.6; H, 8.05; N, 5.8. $C_{11}H_{19}NO_5$ requires C, 53.85; H, 7.8; N, 5.75%); (HRMS: found $[M + H]^+$, 246.1341. $C_{11}H_{20}NO_5$ requires 246.1334); $[\alpha]_D$ −60.2 (*c* 1.0 in MeOH) [lit., [287] −51.0 (*c* 1.0 in MeOH)]; $\upsilon_{max}$(thin film)/$cm^{-1}$ 3443 (OH), 1750 (ester CO), 1683 (urethane CO) and 1160 (C–O); $\delta_H$(200 MHz; $C^2HCl_3$) 1.36 [*t*, 9 H, s, $C(CH_3)_3$], 1.41 [*c*, 9 H, s, $C(CH_3)_3$], 1.98–2.07 (1 H, m, $\frac{1}{2}\beta CH_2$), 2.22–2.32 (1 H, m, $\frac{1}{2}\beta CH_2$), 3.19–3.61 (2 H, m, $\delta CH_2$), 3.69 (3 H, s, $CO_2CH_3$) and 4.31–4.43 (2 H, m, αCH and γCH); $\delta_C$(75.4 MHz; $C^2HCl_3$) 28.21 [*t*, $C(CH_3)_3$], 28.35 [*c*, $C(CH_3)$], 38.36 (*c*, $\beta CH_2$), 39.12 (*t*, $\beta CH_2$), 52.03 (*t*, $CO_2CH_3$), 52.18 (*c*, $CO_2CH_3$), 55.60 (*t*, $\delta CH_2$), 55.73 (*c*, $\delta CH_2$), 58.09 (*c*, αCH), 58.17 (*t*, αCH), 68.96 (*t*, γCH), 69.66 (*c*, γCH), 80.20 [*c*, $C(CH_3)_3$], 80.33 [*t*, $C(CH_3)$], 154.13 (*t*, urethane CO), 154.75 (*c*, urethane CO), 173.68 (*c*, ester CO) and 173.92 (*t*, ester CO); *m/z* (EI) 246 (6%, $[M + H]^+$), 186 (60, [M –

$CO_2CH_3]^+$), 144 (54, $[M - CO_2C_4H_9]^+$), 130 (71, $[M - CO_2CH_3 - C_4H_9]^+$), 86 (88, $[M - CO_2CH_3 - CO_2C_4H_9]^+$) and 57 (100, $C_4H_9^+$).

**Methyl (2*S*,4*S*)-4-(acetylthio)prolinate trifluoroacetate 140**

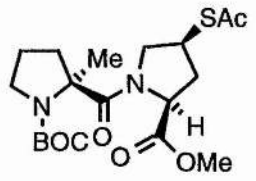



Di-*iso*propyl azodicarboxylate (3.75 g, 19.0 mmol) was added to an ice-cooled solution of triphenylphosphine (4.97 g, 19.0 mmol) in dry THF (70 $cm^3$) and the resulting suspension stirred at 0 °C for 30 min. A solution of alcohol **138** (4.04 g, 16.5 mmol) and thiolacetic acid (0.48 $cm^3$, 20.4 mmol) in dry THF (70 $cm^3$) was then added dropwise to this solution. The mixture was stirred with ice-cooling for 2 h, and at room temperature for 18 h. The solvent was removed under reduced pressure to yield a pale orange oil which was partially purified by column chromatography using light petrol–ethyl acetate (6:1) as the eluent to yield the thiolacetate **139** in an impure state as a colourless oil (4.05 g).

The above oil (1.1 g) was redissolved in dichloromethane (20 $cm^3$), the solution ice-cooled, and trifluoroacetic acid (7 $cm^3$) added. The solution was stirred with cooling for 90 min, and the solvents were removed under reduced pressure. The residual oil was redissolved in water (10 $cm^3$) and the solution washed with ether (2 × 7 $cm^3$). The aqueous phase was concentrated under reduced pressure and lyophilised to give the amine trifluoroacetate **140** as a clear colourless oil (1.03 g, 72%); (HRMS: found $[M - CF_3CO_2]^+$, 204.0691. $C_8H_{13}{}^{35}ClNO_3S$ requires 204.0694); $[\alpha]_D$ –25.9 (*c* 1.0 in MeOH); $\upsilon_{max}(CH_2Cl_2)/cm^{-1}$ 2996 (CH), 1751 (ester CO), 1698 (thiolacetate CO) and 1199 (C–O); $\delta_H$(300 MHz; $C^2HCl_3$) 2.19 (1 H, dt, $J_1$ 13.9, $J_2$ 7.8, $\frac{1}{2}\beta CH_2$), 2.32 (3 H, s, $SCOCH_3$), 2.85 (1 H, dt, $J_1$ 13.9, $J_2$ 7.8, $\frac{1}{2}\beta CH_2$), 3.37 (1 H, dd, $J_1$ 12.2, $J_2$ 7.7, $\frac{1}{2}\delta CH_2$), 3.81 (3 H, s, $CO_2CH_3$), 3.86 (1 H, dd, $J_1$ 12.2, $J_2$ 7.7, $\frac{1}{2}\delta CH_2$), 4.08 (1 H, quintet, $J$ 7.7, $\gamma$CH), 4.53 (1 H, t, $J$ 8.1, $\alpha$CH) and 8.43 (2 H, br. s, $NH_2$); $\delta_C$(50.51

MHz; $C^2HCl_3$) 30.50 ($SCOCH_3$), 34.25 ($\beta CH_2$), 38.97 ($\gamma CH_2$), 50.87 ($CO_2CH_3$), 53.84 ($\delta CH_2$), 59.06 ($\alpha CH$), 116.40 (q, $J_{CF}$ 290.8, $CF_3CO_2H$), 161.91 (q, $J_{CF}$ 36.5, $CF_3CO_2H$), 169.06 (ester CO) and 194.85 (thiolester CO); *m/z* (CI) 204 (100%, $[M - CF_3CO_2]^+$), 162 (26, $[M - CF_3CO_2 - COCH_3 + H]^+$) and 146 (19, $[M - CF_3CO_2 - CO_2CH_3 + H]^+$).

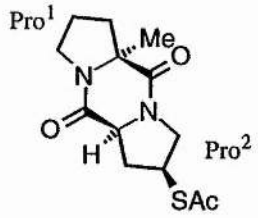
**Methyl (2*S*,4*S*)-*N*-[*N*-(*tert*-butoxycarbonyl)-(2*S*)-2-methylprolyl]-4-(acetylthio)prolinate 141**




This was prepared in an identical manner to dipeptide **119**, using acid **137** (0.15 g, 0.65 mmol) and amine trifluoroacetate **140** (0.23 g, 0.73 mmol) to yield a yellow oil which was purified by column chromatography using ethyl acetate–light petrol (1:1) as the eluent to afford the thiolacetate **141** as colourless crystals (0.21 g, 55%); $R_f$ 0.64 (ethyl acetate); mp 112–114 °C; (Found: C, 55.05; H, 7.3; N, 6.75. $C_{19}H_{30}N_2O_6$ requires: C, 55.1; H, 7.55; N, 6.75%); $[\alpha]_D$ −109.8 (*c* 1.2 in MeOH); $\upsilon_{max}(CH_2Cl_2)/cm^{-1}$ 2980 (CH), 1752 (ester CO), 1694 (urethane CO), 1640 (amide CO) and 1174 (C–O); $\delta_H$(300 MHz; $C^2HCl_3$) 1.43 [9 H, s, $C(CH_3)_3$], 1.49 [3 H, s, $\beta CH_3(Pro^1)$], 1.72–2.07 [5 H, m, $\beta CH_2(Pro^1)$, $\frac{1}{2}\beta CH_2(Pro^2)$ and $\gamma CH_2(Pro^1)$], 2.29 (3 H, s, $SCOCH_3$), 2.51 [1 H, dt, $J_1$ 13.7, $J_2$ 7.4, $\frac{1}{2}\beta CH_2(Pro^2)$], 3.15 [1 H, t, *J* 10.3, $\frac{1}{2}\delta CH_2(Pro^2)$], 3.31 [1 H, m, $\frac{1}{2}\delta CH_2(Pro^1)$], 3.50 [1 H, m, $\frac{1}{2}\delta CH_2(Pro^1)$], 3.69 (3 H, s, $CO_2CH_3$), 3.80 [1 H, m, $\gamma CH(Pro^2)$], 4.04 [1 H, m, $\frac{1}{2}\delta CH_2(Pro^2)$] and 4.39 [1 H, t, *J* 8.1, $\alpha CH(Pro^2)$]; $\delta_C$(75.44 MHz; $C^2HCl_3$) 22.46 [$\beta CH_3(Pro^1)$], 23.57 [$\gamma CH_2(Pro^1)$], 28.16 [*tt*, $C(CH_3)_3$], 28.69 [*ct*, $C(CH_3)_3$], 30.84 ($SCOCH_3$), 33.72 [$\beta CH_2(Pro^2)$], 39.79 and 40.21 [$\beta CH_2(Pro^1)$ and $\gamma CH(Pro^2)$], 47.61 [$\delta CH_2(Pro^1)$], 52.28 ($CO_2CH_3$), 52.51 [$\delta CH_2(Pro^2)$], 60.70 [$\alpha CH(Pro^2)$], 66.01 [$\alpha C(Pro^1)$], 81.25 [$C(CH_3)_3$], 154.06

(urethane CO), 172.57 (amide CO), 173.62 (ester CO) and 195.51 (thiolester CO); *m/z* (EI) 415 (13%, $M^+$), 341 (14, $[M - SCOCH_3 + H]^+$), 315 (17, $[M - CO_2C_4H_9 + H]^+$), 184 [80, $C_4H_9OC(O)NC_4H_6CH_3^+$], 128 (100, $CH_3C_4H_7NCO_2^+$), 84 (65, $NC_4H_7CH_3^+$) and 57 (53, $C_4H_9^+$).

**(2*S*,5a*S*,10a*S*)-2-(Acetylthio)-5a-methyl-5,10-dioxoperhydrodipyrrolo [1,2-*a*;1,2-*d*]pyrazine 143**

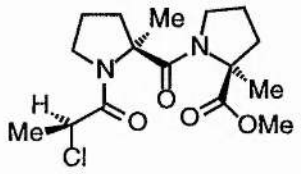


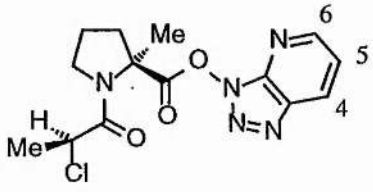

A solution of thiolester **141** (0.15 g, 0.36 mmol) in dichloromethane (3 $cm^3$) at 0 °C was treated with trifluoroacetic acid (1 $cm^3$). The mixture was stirred for a further 90 min, then the solvents were removed under reduced pressure to supply the amine thiolacetate **142** as a colourless oil.

A solution of (2*S*,2'*S*)-*N*-(2'-chloropropionyl)-2-methylproline **130** (0.16 g, 0.73 mmol) and *N,N*-di-*iso*propylethylamine (0.22 $cm^3$, 1.26 mmol) in dry dichloromethane (6 $cm^3$) was treated with BOP-Cl (0.19 g, 0.75 mmol) and the resulting suspension stirred at 0 °C for 35 min. A solution of amine trifluoroacetate **142** (0.17 g, 0.36 mmol) in dry dichloromethane (2 $cm^3$) was then added. The mixture was stirred at 0–5 °C for 9 h, then at room temperature for 14 h. The solution was diluted with dichloromethane to ~20 $cm^3$ and washed with aqueous HCl (0.5 mol $dm^{-3}$, 2 × 5 $cm^3$), aqueous sodium hydrogen carbonate solution (5%, 2 × 5 $cm^3$) and brine (5 $cm^3$) then dried ($MgSO_4$). The solvent was removed under reduced pressure, and the residual oil purified by column chromatography using ethyl acetate as the eluent to afford the dioxopiperazine **143** as a white solid (50 mg, 49%); $R_f$ 0.25 (ethyl acetate); (Found: C, 51.55; H, 6.55; N, 9.2. $C_{13}H_{18}N_2O_3S{\cdot}H_2O$ requires: C, 52.0; H, 6.7; N, 9.35%); $\upsilon_{max}(CH_2Cl_2)/cm^{-1}$ 2982 (CH), 1695 (thiolester CO), 1664

(amide CO) and 1428 (CH def.); $\delta_H$(300 MHz; $C^2HCl_3$) 1.41 [3 H, s, $\beta CH_3(Pro^1)$], 1.93–2.29 [5 H, m, $\beta CH_2(Pro^1)$, $\frac{1}{2}\beta CH_2(Pro^2)$ and $\gamma CH_2(Pro^1)$], 2.30 (3 H, s, $SCOCH_3$), 2.67 [1 H, dt, $J_1$ 12.9, $J_2$ 6.9, $\frac{1}{2}\beta CH_2(Pro^2)$], 3.42 [1 H, dd, $J_1$ 12.0, $J_2$ 8.1, $\frac{1}{2}\delta CH_2(Pro^2)$], 3.48–3.58 [2 H, m, $\delta CH_2(Pro^1)$], 3.84–3.89 [1 H, m, $\gamma CH(Pro^2)$], 3.99 [1 H, dd, $J_1$ 10.2, $J_2$ 6.6, $\frac{1}{2}\delta CH_2(Pro^2)$] and 4.30 [1 H, dd, $J_1$ 9.3, $J_2$ 7.2, $\alpha CH(Pro^2)$]; $\delta_C$(75.44 MHz; $C^2HCl_3$) 21.56 [$\beta CH_3(Pro^1)$], 23.20 [$\gamma CH_2(Pro^1)$], 30.84 ($SCOCH_3$), 34.34 [$\beta CH_2(Pro^2)$], 35.57 [$\beta CH_2(Pro^1)$], 38.33 [$\gamma CH(Pro^2)$], 45.50 [$\delta CH_2(Pro^1)$], 51.22 [$\delta CH_2(Pro^2)$], 59.61 [$\alpha CH(Pro^2)$], 66.59 [$\alpha C(Pro^1)$], 165.60 and 169.89 (2 × amide CO) and 195.44 (thiolacetate CO); *m/z* (CI) 283 (100%, $[M + H]^+$), 241 (8, $[M - COCH_3 + 2H]^+$) and 206 (9, $[M - COCH_3 - H_2S + H]^+$).

**Methyl (2*S*)-*N*-[*N*-(2-chloropropionyl)-(2*S*)-2-methylprolyl]-2-methylprolinate 144 and (2*S*,2'*S*)-*N*-(2'-chloropropionionyl)proline 7-azabenzotriazolyl ester 145**



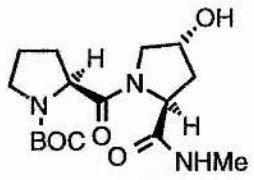

A solution of (2*S*,2'*S*)-*N*-(2'-chloropropionyl)-2-methylproline **130** (68 mg, 0.31 mmol), methyl (2*S*)-2-methylprolinate hydrochloride **128** (37 mg, 0.21 mmol) and *N*-*N*-di-*iso*propylethylamine (110 $mm^3$, 0.63 mmol) in dry DMF (1 $cm^3$) under nitrogen at 0 °C was treated with HATU (120 mg, 0.32 mmol) and the mixture stirred at 0 °C for 4 h, then at room temperature for 40 h. The solution was diluted to 30 $cm^3$ with ethyl acetate and washed with aqueous HCl (0.5 mol $dm^{-3}$, 2 × 10 $cm^3$), aqueous sodium hydrogen carbonate solution (5%, 2 × 10 $cm^3$) and brine (10 $cm^3$). The organic phase was dried ($MgSO_4$), and the solvent was removed under reduced pressure. The residual oil was purified by column chromatography using ethyl acetate–light petrol (3:2) as the eluent, enabling isolation of two products, the peptide

**144** as a white solid (21 mg, 30%) and the 7-azabenzotriazolyl ester **145** as a colourless gum (38 mg, 37%).

Data for **144**: mp 124–126 °C; $R_f$ 0.45 (ethyl acetate); (HRMS: found $[M + H]^+$, 345.1586. $C_{16}H_{25}{}^{35}ClN_2O_4$ requires 345.1581); $[\alpha]_D$ −42.5 (*c* 0.8 in MeOH); $\upsilon_{max}(CH_2Cl_2)/cm^{-1}$ 2985 (CH), 1737 (ester CO), 1666 (amide CO), 1636 (amide CO), 1402 (CH def.) and 1193 (C–O); $\delta_H$(300 MHz; $C^2HCl_3$) (2 conformations, *A* and *B*) 1.51 (*A*, 3 H, d, *J* 6.6, $CHCH_3$), 1.53 (3 H, s, $\beta CH_3$), 1.61 (*B*, 3 H, d, *J* 6.6, $CHCH_3$), 1.64 (3 H, s, $\beta CH_3$), 1.75–2.21 (8 H, m, 2 × $\beta CH_2$ and 2 × $\gamma CH_2$), 3.38–3.49 (1 H, m, $\frac{1}{2}\delta CH_2$), 3.60–3.68 (2 H, m, $\delta CH_2$), 3.70 (*B*, 3 H, s, $CO_2CH_3$), 3.74 (*A*, 3 H, s, $CO_2CH_3$), 3.87–3.95 (1 H, m, $\frac{1}{2}\delta CH_2$), 4.35 (*A*, 1 H, q, *J* 6.6, $CHCH_3$) and 4.45 (*B*, 1 H, q, *J* 6.6, $CHCH_3$); $\delta_C$(75.44 MHz; $C^2HCl_3$) 20.68, 21.79 and 22.70 ($CHCH_3$ and 2 × $\beta CH_3$), 23.67 and 25.28 (2 × $\gamma CH_2$), 37.39 and 37.69 (2 × $\beta CH_2$), 47.89 and 49.06 (2 × $\delta CH_2$), 52.18 ($CHCH_3$), 52.82 ($CO_2CH_3$), 68.46 (2 × αC), 167.55 ($COCHCH_3$), 170.83 (amide CO) and 175.29 (ester CO); *m/z* (CI) 347 and 345 (23 and 70%, chlorine isotopes, $[M + H]^+$), 315 and 313 (33 and 97, chlorine isotopes, $[M - 2CH_3 + H]^+$), 204 and 202 [32 and 100, chlorine isotopes, $ClCH(CH_3)CONC_4H_6(CH_3)CO^+$], 176 and 174 [26 and 82, chlorine isotopes, $ClCH(CH_3)CONC_4H_6CH_3^+$].

Data for **145**: (Found: C, 47.45; H, 5.1; N, 19.55. $C_{14}H_{16}ClN_5O_3 \cdot H_2O$ requires C, 47.25; H, 5.1; N, 19.7%); $\upsilon_{max}(CH_2Cl_2)/cm^{-1}$ 2987 (CH), 1750 (ester CO) and 1661 (amide CO); $\delta_H$(300 MHz; $C^2HCl_3$) 1.73 (3 H, d, *J* 6.6, $CHCH_3$), 1.81 (3 H, s, $\beta CH_3$), 2.20–2.35 (3 H, m, $\frac{1}{2}\beta CH_2$ and $\gamma CH_2$), 2.71–2.81 (1 H, m, $\frac{1}{2}\beta CH_2$), 3.75–3.83 (1 H, m, $\frac{1}{2}\delta CH_2$), 3.95–4.03 (1 H, m, $\frac{1}{2}\delta CH_2$), 4.52 (1 H, q, *J* 6.6, $CHCH_3$), 7.40 (1 H, dd, $J_1$ 6.5, $J_2$ 4.4, Ar–CH 5), 8.39 (1 H, dd, $J_1$ 8.3, $J_2$ 1.4, Ar–CH 6) and 8.71 (1 H, dd, $J_1$ 4.4, $J_2$ 1.4, Ar–CH 4); $\delta_C$(75.44 MHz; $C^2HCl_3$) 20.64 ($CHCH_3$), 21.34 ($\beta CH_3$), 24.86 ($\gamma CH_2$), 38.80 ($\beta CH_2$), 48.15 ($\delta CH_2$), 51.60 ($CHCH_3$), 66.00 (αC), 121.32 (Ar–C 5), 129.90 (Ar–C 4), 135.51 and 141.18 (2 × quaternary Ar–C), 152.39 (Ar–C 6), 168.14 (amide CO) and 170.02 (ester CO).

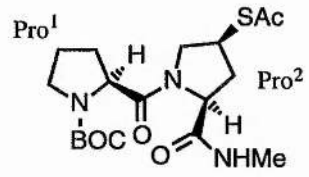
**(2*S*,4*R*)-*N*-[*N*-(*tert*-Butoxycarbonyl)-(2*S*)-prolyl]-4-hydroxyproline methylamide 147**




Methyl ester **119** (0.63 g, 1.84 mmol) was dissolved in a saturated solution of methylamine in methanol (35 $cm^3$) at 0 °C. The flask was stoppered, allowed to warm to room temperature, and left standing for 1 day. The flask was cooled to 0 °C and the stopper removed. Excess methylamine was driven off by bubbling nitrogen through the solution for 30 min. The solvent was removed under reduced pressure to yield the methylamide **147** as a white foam (0.63 g, 100%), mp 187–188 °C (Found: C, 56.6; H, 8.25; N, 12.45. $C_{16}H_{27}N_3O_5$ requires C, 56.3; H, 7.95; N, 12.3%); $[\alpha]_D$ –99.4 (*c* 1.1 in MeOH); $\upsilon_{max}(CH_2Cl_2)/cm^{-1}$ 3448 (NH), 3342 (NH), 2981 (CH), 1677 (CO) and 1164 (C–O); $\delta_H$(200 MHz; $C^2HCl_3$) (3 conformations, *ct*, *tt* and *tc*) 1.20 [*ct*, 9 H, $C(CH_3)_3$], 1.24 [*tt* and *tc*, 9 H, $C(CH_3)_3$], 1.63–1.91 [4 H, m, $\frac{1}{2}\beta CH_2$(Pro), $\frac{1}{2}\beta CH_2$(Hyp) and $\gamma CH_2$(Pro)], 1.95–2.09 [1 H, m, $\frac{1}{2}\beta CH_2$(Hyp)], 2.54 (3 H, d, *J* 4.4 NHC$H_3$), 3.20–3.62 [4 H, m, $\delta CH_2$(Pro) and $\delta CH_2$(Hyp)], 4.22–4.43 [3 H, m, αCH(Pro), αCH(Hyp) and γCH(Hyp)], 7.36 (*tt* and *ct*, 1 H, q, *J* 4.8, N*H*$CH_3$) and 7.76 (*tc*, 1 H, q, *J* 4.8, N*H*$CH_3$); $\delta_C$(50.31 MHz; $C^2HCl_3$) 23.75 [*ct*, $\gamma CH_2$(Pro)], 24.43 [*tt*, $\gamma CH_2$(Pro)], 24.76 [*tc*, $\gamma CH_2$(Pro)], 26.40 (*tt*, $NHCH_3$), 26.47 (*ct*, $NHCH_3$), 28.31 [*tc*, $C(CH_3)_3$], 28.36 [*ct*, $C(CH_3)_3$], 28.57 [*tt*, $C(CH_3)_3$], 29.34 [*tt*, $\beta CH_2$(Pro)], 29.52 [*tc*, $\beta CH_2$(Pro)], 30.24 [*ct*, $\beta CH_2$(Pro)], 36.64 [*tt* and *ct*, $\beta CH_2$(Hyp)], 37.50 [*tc*, $\beta CH_2$(Hyp)], 46.79 [*ct*, $\delta CH_2$(Pro)], 47.17 [*tt*, $\delta CH_2$(Pro)], 47.29 [*tc*, $\delta CH_2$(Pro)], 54.26 [*tc*, $\delta CH_2$(Hyp)], 54.57 [*ct*, $\delta CH_2$(Hyp)], 55.33 [*tt*, $\delta CH_2$(Hyp)], 57.82 (*tc*, αCH), 58.31 (*ct*, αCH), 58.60 (*tt*, αCH), 59.26 (*tt*, αCH), 60.00 (*ct*, αCH), 67.87 [*tc*, γCH(Hyp)], 69.97 [*ct*, γCH(Hyp)], 70.56 [*tt*, γCH(Hyp)], 79.98 [*ct*, $C(CH_3)_3$], 80.26 [*tt* and *tc*, $C(CH_3)_3$], 154.00 (*ct*, urethane CO), 155.02 (*tt* and *tc*, urethane CO),

172.04 (*tt*, amide CO), 172.10 (*ct*, amide CO), 172.40 (*ct*, amide CO), 172.71 (*tt*, amide CO) and 172.92 (*tc*, amide CO); *m/z* (CI) 342 (98%, $[M + H]^+$), 286 (100, $[M - C_4H_8 + H]^+$) and 242 (83, $[M - CO_2C_4H_8 + H]^+$).

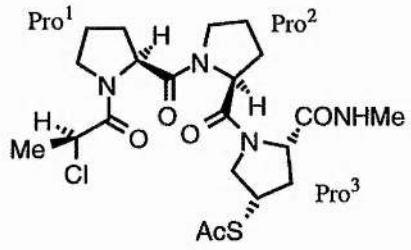
**(2*S*,4*S*)-*N*-[*N*-(*tert*-Butoxycarbonyl)-(2*S*)-prolyl]-4-(acetylthio)proline methylamide 148**




Di-*iso*propyl azodicarboxylate (0.33 g, 1.6 mmol) was added to a solution of triphenylphosphine (0.44 g, 1.7 mmol) in THF (5 $cm^3$) at 0 °C. The resulting suspension was stirred at 0 °C for 30 min, after which a solution of alcohol **147** (0.50 g, 1.5 mmol) and thiolacetic acid (0.13 $cm^3$, 1.8 mmol) in THF (5 $cm^3$) was added dropwise. The mixture was stirred at 0 °C for 2.5 h, then at room temperature overnight. The solvent was removed under reduced pressure and the residual oil purified using column chromatography with light petrol–ethyl acetate (2:1) as the eluent to afford the thiolacetate **148** as a clear colourless oil (0.29 g, 50%); $R_f$ 0.10 (ethyl acetate); (HRMS: found $[M + H]^+$, 400.1898. $C_{18}H_{30}N_3O_5S$ requires 400.1906); $\upsilon_{max}(CH_2Cl_2)/cm^{-1}$ 3346 (NH), 2980 (CH), 1688 (urethane and thiolester CO), 1666 (amide CO) and 1166 (C–O); $\delta_H$(300 MHz; $C^2HCl_3$) (3 conformations, *ct*, *tt* and *tc*) 1.31 [*ct*, 9 H, s, $C(CH_3)_3$], 1.34 [*tt* and *tc*, 9 H, s, $C(CH_3)_3$], 1.71–2.52 [6 H, m, $\beta CH_2(Pro^1)$, $\beta CH_2(Pro^2)$ and $\gamma CH_2(Pro^1)$], 2.20 (*tc*, 3H, s, $SCOCH_3$), 2.22 (*tt*, 3 H, s, $SCOCH_3$), 2.24 (*ct*, 3 H, s, $SCOCH_3$), 2.65 (*tt* and *ct*, 3 H, d, *J* 4.7, $NHCH_3$), 2.71 (*tc*, 3 H, *J* 4.7, $NHCH_3$), 3.18–3.46 [3 H, m, $\delta CH_2(Pro^1)$ and $\frac{1}{2}\delta CH_2(Pro^2)$], 3.81–4.17 [2 H, m, $\gamma CH(Pro^2)$ and $\frac{1}{2}\delta CH_2(Pro^2)$], 4.21–4.53 [2 H, m, 2 × $\alpha CH$(Pro)], 7.10 (1 H, *tt* and *ct*, q, *J* 4.4, $NHCH_3$) and 8.02 (1 H, *tc*, q, *J* 4.4, $NHCH_3$); $\delta_C$(75.44 MHz; $C^2HCl_3$) 23.75, 24.50 and 24.80 [$\gamma CH_2(Pro^1)$], 26.45 and 26.65 ($NHCH_3$),

28.57 and 29.31 [C($CH_3$)$_3$], 29.55, 30.62 and 30.65 [$\beta CH_2$(Pro$^1$)], 32.37 and 32.86 (SCO$CH_3$), 37.57 [*tt* and *ct*, $\beta CH_2$(Pro$^2$)], 38.84 [*tc*, $\beta CH_2$(Pro$^2$)], 40.09 [*tc*, $\gamma$CH(Pro$^2$)], 40.28 [*tt* and *ct*, $\gamma$CH(Pro$^2$)], 46.79, 46.96 and 42.28 [$\delta CH_2$(Pro$^1$)], 53.37 and 53.56 [$\delta CH_2$(Pro$^2$)], 58.11, 58.17, 59.23, 59.67, 60.02 and 60.48 [2 × $\alpha$CH(Pro)], 79.87, 80.13 and 80.40 [$C(CH_3)_3$], 153.99, 154.96 and 155.13 (urethane CO), 171.00, 171.50, 171.74, 172.12, 172.16 and 173.12 (2 × amide CO), 195.27, 195.77 and 196.03 (thiolester CO); *m/z* (CI) 400 (82%, [M + H]$^+$), 344 (63, [M − $C_4H_8$ + H]$^+$), 300 (100, [M − C $O_2C_4H_8$ + H]$^+$), 170 (21, $C_4H_9OCONC_4H_7^+$), 114 (21, [$NC_4H_8CONH$ + H]$^+$) and 70 (22, $C_4H_8N^+$).

**(2*S*,4*S*)-*N*-(*N*-{*N*-[(2*S*)-2-Chloropropionyl]-(2*S*)-prolyl}-(2*S*)-prolyl)-4-(acetylthio)proline methylamide 150**

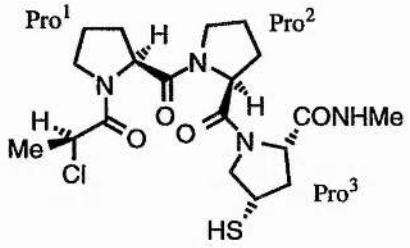



A solution of thiolester **148** (0.38 g, 0.75 mmol) in dichloromethane (7 cm$^3$) at 0 °C was treated with trifluoroacetic acid (3.5 cm$^3$) and the solution stirred at 0 °C for 2.5 h. The solvents were removed under reduced pressure to yield the trifluoroacetate salt **149** as a clear colourless oil.

A solution of acid **106** (0.23 g, 1.1 mmol) and di-*iso*propylethylamine (0.50 cm$^3$, 2.9 mmol) in dichloromethane (15 cm$^3$) was treated with BOP-Cl (0.29 g, 1.1 mmol) at 0 °C under nitrogen. After 20 min, a solution of amine trifluoroacetate **149** in dichloromethane (5 cm$^3$) was added and the mixture was stirred at 0–5 °C for 2 h, then at room temperature for 2 days. The solution was diluted to ~40 cm$^3$ with dichloromethane, then washed with aqueous HCl (0.5 mol dm$^{-3}$, 2 × 25 cm$^3$), aqueous sodium hydrogen carbonate solution (5%, 2 × 25 cm$^3$) and brine (25 cm$^3$).

The organic phase was dried ($MgSO_4$), and the solvent removed under reduced pressure to afford the thiolester **150** as a clear colourless oil (0.31 g, 69%); (HRMS: found [M + H]$^+$, 487.1788. $C_{21}H_{32}{}^{35}ClN_4O_5S$ requires 487.1782); $\upsilon_{max}(CH_2Cl_2)/cm^{-1}$ 3331 (NH), 1690 (thiolester and secondary amide CO), 1662 (tertiary amide CO) and 1433 (CH def.); $\delta_H$(300 MHz; $C^2HCl_3$) (2 conformations, *ttt* and *ttc*) 1,51 and 1.53 (3 H, d, *J* 6.3, $CHCH_3$), 1.72–2.18 [9 H, m, $\beta CH_2(Pro^1)$, $\beta CH_2(Pro^2)$, $\frac{1}{2}\beta CH_2(Pro^3)$, $\gamma CH_2(Pro^1)$ and $\gamma CH_2(Pro^2)$], 2.19 and 2.21 (3 H, s, $SCOCH_3$), 2.33–2.45 [1 H, m, $\frac{1}{2}\beta CH_2(Pro^3)$], 2.63 and 2.65 (3 H, d, *J* 5.5, $NHCH_3$), 3.25–3.35 [1 H, m, $\frac{1}{2}\delta CH_2(Pro^3)$], 3.46–3.60, 3.61–3.81 [4 H, m, $\delta CH_2(Pro^1)$ and $\delta CH_2(Pro^2)$], 3.89–3.97 [*ttc*, 1 H, m, $\gamma CH(Pro^3)$], 4.05 [*ttt*, 1 H, dd, $J_1$ 7.2, $J_2$ 13.2, $\gamma CH(Pro^3)$], 4.21–4.51 and 4.52–4.62 [4 H, m, 3 × αCH(Pro) and $\frac{1}{2}\delta CH_2(Pro^3)$], 6.81 (*ttt*, $NHCH_3$) and 8.11 (*ttc*, $NHCH_3$); $\delta_C$(75.44 MHz; $C^2HCl_3$) 20.62 and 20.77 ($CHCH_3$), 24.96, 25.11 and 25.43 [$\gamma CH_2(Pro^1)$ and $\gamma CH_2(Pro^2)$], 26.48 and 26.65 ($NHCH_3$), 28.05, 28.46 and 28.51 [$\beta CH_2(Pro^1)$ and $\beta CH_2(Pro^2)$], 30.62 and 30.66 ($SCOCH_3$), 33.15 [*ttt*, $\beta CH_2(Pro^3)$], 37.25 [*ttc*, $\beta CH_2(Pro^3)$], 38.84 [*ttc*, $\gamma CH(Pro^3)$], 40.10 [*ttt*, $\gamma CH(Pro^3)$], 47.19, 47.43, 47.46 and 47.55 [$\delta CH_2(Pro^1)$ and $\delta CH_2(Pro^2)$], 51.31 and 51.44 ($CHCH_3$), 53.44 and 53.52 [$\delta CH_2(Pro^3)$], 58.26, 58.52, 58.74, 58.87, 59.78 and 60.46 [3 × αCH(Pro)], 167.84 and 167.91 ($COCHCH_3$), 170.58, 170.95, 171.60 and 171.91 (3 × amide CO), 195.27 and 195.58 (thiolester CO); *m/z* (CI) 489 and 487 (12 and 29%, chlorine isotopes, [M + H]$^+$), 396 {77, [M – $ClCH(CH_3)CO$ + H]$^+$}, 354 {11, [M – $ClCH(CH_3)CO$ – $CH_3CO$ + 2H]$^+$}, 322 {11, [M – $ClCH(CH_3)CO$ – $CH_3CO$ – S + 2H]$^+$} and 279 (100, $[CH_3CHCONC_4H_7CONC_4H_6CONHCH_3]^+$).

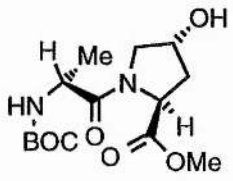
**(2*S*,4*S*)-*N*-(*N*-{*N*-[(2*S*)-2-Chloropropionyl]-(2*S*)-prolyl}-(2*S*)-prolyl)-4-mercaptoproline methylamide 146**




This was prepared in an identical manner to thiol **121**, using thiolester **150** (0.25, 0.51 mmol) to afford thiol **146** as a clear oil (0.15 g, 66%); (HRMS: found $[M + H]^+$, 445.1676. $C_{19}H_{30}{}^{35}ClN_4O_4S$ requires 445.1676); $[\alpha]_D$ −103.7 (*c* 0.6 in MeOH); $\upsilon_{max}(CH_2Cl_2)/cm^{-1}$ 3323 (NH), 2985 (CH), 1657 (CO) and 1435 (CH def.); $\delta_H$(300 MHz; $C^2HCl_3$) (2 conformations, *ttt* and *ttc*) 1.63 (*ttc*, 3 H, d, *J* 6.6, $CHCH_3$), 1.65 (*ttt*, 3 H, d, *J* 6.6, $CHCH_3$), 1.80 (1 H, d, *J* 5.8, SH), 1.91–2.32 [9 H, m, $\beta CH_2(Pro^1)$, $\beta CH_2(Pro^2)$, $\gamma CH_2(Pro^1)$, $\gamma CH_2(Pro^2)$ and *ttt*, $\frac{1}{2}\beta CH_2(Pro^3)$], 2.47–2.57 [1 H, m, $\frac{1}{2}\beta CH_2(Pro^3)$], 2.67–2.77 [*ttc*, 1 H, m, $\frac{1}{2}\beta CH_2(Pro^3)$], 2.74 (*ttc*, 3 H, d, *J* 4.7, $NHCH_3$), 2.79 (*ttt*, 3 H, d, *J* 4.7, $NHCH_3$), 3.32–3.41 [2 H, m, $\frac{1}{2}\delta CH_2(Pro^3)$ and *ttc*, $\gamma CH(Pro^3)$], 3.43–3.51 [*ttc*, 1 H, $\gamma CH(Pro^3)$], 3.59–3.70 [2 H, m, $\frac{1}{2}\delta CH_2(Pro^1)$ and $\frac{1}{2}\delta CH_2(Pro^2)$], 3.77–3.94 [2 H, m, $\frac{1}{2}\delta CH_2(Pro^1)$ and $\frac{1}{2}\delta CH_2(Pro^2)$], 4.16 [*ttc*, 1 H, dd, $J_1$ 12.7, $J_2$ 6.7, $\frac{1}{2}\delta CH_2(Pro^3)$], 4.25–4.35 [2 H, m, *ttt*, $\frac{1}{2}\delta CH_2(Pro^3)$ and *ttc*, $\alpha CH(Pro^3)$], 4.38–4.57 [2 H, m, $CHCH_3$ and *ttt*, $\alpha CH(Pro^3)$], 4.64–4.72 [2 H, m, $\alpha CH(Pro^1)$ and $\alpha CH(Pro^2)$], 6.50 (*ttt*, 1 H, d, *J* 4.1, $NHCH_3$) and 8.13 (*ttc*, $NHCH_3$); $\delta_C$(75.44 MHz; $C^2HCl_3$) 20.75 and 20.91 ($CHCH_3$), 25.12, 25.33, 25.40 and 25.61 [$\gamma CH_2(Pro^1)$ and $\gamma CH_2(Pro^2)$], 26.79 ($NHCH_3$), 28.28, 28.64, 28.71 and 28.80 [$\beta CH_2(Pro^1)$ and $\beta CH_2(Pro^2)$], 34.26 [*ttt*, $\beta CH_2(Pro^3)$], 35.94 [*ttc*, $\beta CH_2(Pro^3)$], 38.49 [*ttc*, $\gamma CH(Pro^3)$], 41.52 [*ttt*, $\gamma CH(Pro^3)$], 47.55 and 47.69 [$\delta CH_2(Pro^1)$ and $\delta CH_2(Pro^2)$], 51.48 (*ttt*, $CHCH_3$), 51.59 (*ttc*, $CHCH_3$), 56.21 [*ttc*, $\delta CH_2(Pro^3)$], 57.26 [*ttt*, $\delta CH_2(Pro^3)$], 58.55, 58.81, 59.02, 59.11, 60.60 and 60.73 [3 × $\alpha CH(Pro)$], 168.33 ($COCHCH_3$), 171.11, 171.54, 171.70 and 172.17 (3 × amide CO); $\delta_H$(300 MHz; $^2H_2O$) 1.45 (3 H, d, *J* 6.6,

CHC$H_3$), 1.61–1.98 [9 H, m, βCH$_2$(Pro$^1$), βCH$_2$(Pro$^2$), $\frac{1}{2}$βCH$_2$(Pro$^3$), γCH$_2$(Pro$^1$) and γCH$_2$(Pro$^2$)], 2.17–2.30 [1 H, m, $\frac{1}{2}$βCH$_2$(Pro$^3$)], 2.60 (3 H, s, NHC$H_3$), 3.30–3.41 [2 H, m, γCH(Pro$^3$) and $\frac{1}{2}$δCH$_2$(Pro$^3$)], 3.47–3.57, 3.64–3.78 [4 H, m, δCH$_2$(Pro$^1$) and δCH$_2$(Pro$^2$)], 4.16–4.25 [2 H, m, αCH(Pro) and $\frac{1}{2}$δCH$_2$(Pro$^3$)], 4.57–4.63 [2 H, m, 2 × αCH(Pro)] and 4.74 (1 H, q, *J* 6.6, C*H*CH$_3$); $\delta_C$(75.44 MHz; $^2H_2O$) 19.86 (CH*C*H$_3$), 24.35, 24.43 [γCH$_2$(Pro$^1$) and γCH$_2$(Pro$^2$)], 25.73 (NHCH$_3$), 27.95, 28.05 [βCH$_2$(Pro$^1$) and βCH$_2$(Pro$^2$)], 34.40 [βCH$_2$(Pro$^3$)], 39.09 [γCH(Pro$^3$)], 47.54, 47.92 [δCH$_2$(Pro$^1$) and δCH$_2$(Pro$^2$)], 51.49 (*C*HCH$_3$), 56.05 [δCH$_2$(Pro$^3$)], 58.59, 59.05, 60.78 [3 × αCH(Pro)], 170.43 (*C*OCHCH$_3$), 171.70, 172.00 and 173.67 (3 × amide CO); *m/z* (CI) 447 and 445 (8 and 18%, chlorine isotopes, [M + H]$^+$), 278 (82, CH$_2$CHCONC$_4$H$_7$CONC$_4$H$_6$CONHCH$_3^+$), 234 (17, CHCONC$_4$H$_7$CONC$_4$H$_6$CO$^+$), 208 and 206 {33 and 100, chlorine isotopes, [ClCH(CH$_3$)CONC$_4$H$_7$CO$_2$H + H]$^+$}.

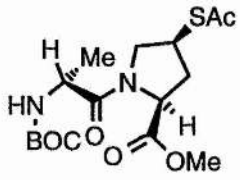
**Methyl (2*S*,4*R*)-*N*-[*N*-(*tert*-butoxycarbonyl)-(2*R*)-alanyl]-4-hydroxyprolinate 153**




This was prepared in an identical manner to alcohol **119**, using (2*R*)-(*tert*-butoxycarbonyl)alanine **152** (8.00 g, 42.3 mmol) to afford the dipeptide **153** as a white foam (6.34 g, 55%); (HRMS: found [M + H]$^+$, 317.1721. $C_{14}H_{25}N_2O_6$ requires 317.1713); $[\alpha]_D$ −16.3 (*c* 1.0 in MeOH); $\upsilon_{max}$(CH$_2$Cl$_2$)/cm$^{-1}$ 3425 (NH), 2981 (CH), 1747 (ester CO), 1708 (urethane CO), 1653 (tertiary amide CO) and 1171 (C–O); $\delta_H$(300 MHz; C$^2$HCl$_3$) 1.31 [3 H, d, *J* 6.9, βCH$_3$(Ala)], 1.42 [9 H, s, C(CH$_3$)$_3$], 2.00–2.11 [1 H, m, $\frac{1}{2}$βCH$_2$(Hyp)], 2.25–2.37 [1 H, m, $\frac{1}{2}$βCH$_2$(Hyp)], 3.56 [1 H, d, *J* 11.0, $\frac{1}{2}$δCH$_2$(Hyp)], 3.73 (3 H, s, CO$_2$CH$_3$), 3.83 [1 H, dd, $J_1$ 11.0, $J_2$ 4.4, $\frac{1}{2}$δCH$_2$(Hyp)], 4.38–4.63 [3 H, m, αCH(Hyp), αCH(Ala) and γCH(Hyp)] and 5.47 (1 H, d, *J* 8.0, N*H*CH$_3$); $\delta_C$(50.31 MHz; C$^2$HCl$_3$) 18.70 [βCH$_3$(Ala)], 28.64

[C($CH_3$)$_3$], 37.65 [βCH$_2$(Hyp)], 48.22 [αCH(Ala)], 52.65 ($CO_2CH_3$), 55.23 [δCH$_2$(Hyp)], 58.30 [αCH(Hyp)], 68.43 [γCH(Hyp)], 80.02 [$C$(CH$_3$)$_3$], 155.46 (urethane CO), 172.19 (amide CO) and 173.03 (ester CO); *m/z* (CI) 317 (40%, [M + H]$^+$), 261 (100, [M – $C_4H_8$ + H]$^+$), 217 (28, [M – $CO_2C_4H_8$ + H]$^+$) and 57 (10, $C_4H_9^+$).

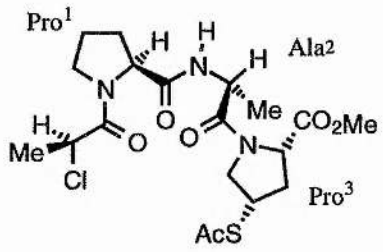
**Methyl (2*S*,4*S*)-*N*-[*N*-(*tert*-butoxycarbonyl)-(2*R*)-alanyl]-4-(acetylthio)prolinate 154**




Di-*iso*propyl azodicarboxylate (7.30 g, 34.3 mmol) was added to a solution of triphenylphosphine (9.7 g, 37.0 mmol) in THF (100 cm$^3$) at 0 °C. The resulting suspension was stirred for 30 min, after which time a solution of alcohol **153** (6.10 g, 19.0 mmol) and thiolacetic acid (2.75 cm$^3$, 37.3 mmol) in THF (50 cm$^3$) was added dropwise. The mixture was stirred at 0 °C for 2.5 h, then at room temperature overnight after which the solvent was removed under reduced pressure. Excess triphenylphosphine and its oxide were removed by crystallisation (from light petrol–ethyl acetate) and filtration. The remaining oil was purified with column chromatography using light petrol–ethyl acetate as the eluent to afford the thiolacetate **154** as a clear colourless oil (6.64 g, 92%); $R_f$ 0.49 (ethyl acetate); (HRMS: found [M + H]$^+$, 375.1593. $C_{16}H_{27}N_2O_6S$ requires 375.1590); $[\alpha]_D$ –24.1 (*c* 0.2 in MeOH); $\upsilon_{max}(CH_2Cl_2)$/cm$^{-1}$ 3428 (NH), 2982 (CH), 1749 (ester CO), 1703 (urethane and thiolester CO), 1655 (tertiary amide CO) and 1171 (C–O); $\delta_H$(300 MHz; C$^2$HCl$_3$) (2 conformations, *tt* and *tc*) 1.26 [3 H, d, *J* 6.6, βCH$_3$(Ala)], 1.36 [*tc*, 9 H, s, C(CH$_3$)$_3$], 1.38 [*tt*, 9 H, s, C(CH$_3$)$_3$], 1.84–1.95 [1 H, m, $\frac{1}{2}$βCH$_2$(Pro)], 2.26 (*tc*, 3 H, SCOCH$_3$), 2.30 (*tt*, 3 H, SCOCH$_3$), 2.60–2.72 [*tt*, 1 H, m, $\frac{1}{2}$βCH$_2$(Pro)], 2.72–2.84 [*tc*, 1 H, m,

$\frac{1}{2}\beta CH_2$(Pro)], 3.36 [*tc*, 1 H, dd, $J_1$ 12.9, $J_2$ 4.7, $\frac{1}{2}\delta CH_2$(Pro)], 3.52 [*tt*, 1 H, dd, $J_1$ 8.8, $J_2$ 7.0, $\frac{1}{2}\delta CH_2$(Pro)], 3.69 (*tt*, 3 H, s, $CO_2CH_3$), 3.74 (*tc*, 3 H, s, $CO_2CH_3$), 3.92–4.19 [2 H, m, γCH(Pro) and $\frac{1}{2}\delta CH_2$(Pro)], 4.35–4.44 [2 H, m, αCH(Ala) and *tc*, αCH(Pro)], 5.00–5.14 [*tt*, 1 H, m, αCH(Pro)] and 5.33 (1 H, d, *J* 8.2, N*H*$CH_3$); $\delta_C$(75.44 MHz; $C^2HCl_3$) 18.27 [*tc*, $\beta CH_3$(Ala)], 18.86 [*tt*, $\beta CH_3$(Ala)], 28.60 [C(*C*$H_3$)$_3$]; 30.79 (SCO*C*$H_3$), 34.83 [*tt*, $\beta CH_2$(Pro)], 37.67 [*tc*, $\beta CH_2$(Pro)], 39.04 [*tc*, γCH(Pro)], 39.83 [*tt*, γCH(Pro)], 47.55 [*tc*, αCH(Ala)], 48.15 [*tt*, αCH(Ala)], 52.38, 52.70, 52.76 and 53.03 [$\delta CH_2$(Pro) and $CO_2CH_3$], 58.77 [αCH(Pro)], 79.94 [*tt*, *C*(CH$_3$)$_3$], 80.18 [*tc*, *C*(CH$_3$)$_3$], 155.42 (*tt*, urethane CO), 155.82 (*tc*, urethane CO), 171.74 and 172.03 (*tt*, amide and ester CO), 172.75 and 173.32 (*tc*, amide and ester CO) and 195.09 (thiolester CO); *m/z* (CI) 375 (41%, $[M + H]^+$), 319 (100, $[M - C_4H_8 + H]^+$) and 275 (31, $[M - CO_2C_4H_8 + H]^+$).

**Methyl (2*S*,4*S*)-*N*-(*N*-{*N*-[(2*S*)-2-chloropropionyl]-(2*S*)-prolyl}-(2*R*)-alanyl)-4-(acetylthio)prolinate 156**

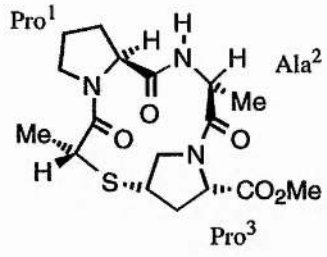



A solution of thiolester **154** (3.25 g, 8.7 mmol) in dichloromethane (30 cm$^3$) at 0 °C was treated with trifluoroacetic acid (15 cm$^3$) and the solution stirred at 0 °C for 90 min. The solvents were removed under reduced pressure to give the trifluoroacetate salt **155** as a yellow oil.

A solution of acid **106** (2.37 g, 11.5 mmol) and *N*,*N*-di-*iso*propylethylamine (6.0 cm$^3$, 34.5 mmol) in dichloromethane (120 cm$^3$) was treated with BOP-Cl (3.10 g, 12.2 mmol) at 0 °C. After 45 min, a solution of amine trifluoroacetate **155** in dichloromethane (40 cm$^3$) was added and the mixture was stirred at 0–5 °C for 3 h,

then at room temperature for 2 days. The solution was washed with aqueous HCl (0.5 mol $dm^{-3}$, $2 \times 50\ cm^3$), aqueous sodium hydrogen carbonate solution (5%, $2 \times 50\ cm^3$) and brine ($80\ cm^3$). The organic phase was dried ($MgSO_4$), and the solvent was removed under reduced pressure. The residual oil was purified using column chromatography with ethyl acetate as the eluent to afford the thiolester **156** as a thick yellow oil (2.73 g, 68%), $R_f$ 0.28; (Found: C, 47.85; H, 6.3; N, 8.65. $C_{19}H_{28}ClN_3O_6S{\cdot}H_2O$ requires: C, 47.55; H, 6.3; N, 8.75%); (HRMS: Found $(M + H)^+$, 462.1457. $C_{19}H_{29}{}^{35}ClN_3O_6S$ requires 462.1466); $[\alpha]_D$ −31.5 (*c* 0.9 in MeOH); $\upsilon_{max}(CH_2Cl_2)/cm^{-1}$ 3404, 3310 (NH), 2955 (CH), 1748 (ester CO), 1693 (secondary amide and thiolester CO) and 1658 (tertiary amide CO); $\delta_H$(300 MHz; $C^2HCl_3$) (2 conformations, *ttt* and *ttc*) 1.28 [3 H, d, *J* 6.6, $\beta CH_3(Ala^2)$], 1.63 (*ttc*, 3 H, d, *J* 6.3, $CHCH_3$), 1.64 (*ttt*, 3 H, d, *J* 6.6, $CHCH_3$), 1.85–2.26 [5 H, m, $\beta CH_2(Pro^1)$, $\frac{1}{2}\beta CH_2(Pro^3)$ and $\gamma CH(Pro^1)$], 2.27 (*ttc*, 3 H, s, $SCOCH_3$), 2.31 (*ttt*, 3 H, s, $SCOCH_3$), 2.60–2.72 [*ttt*, 1 H, m, $\frac{1}{2}\beta CH_2(Pro^3)$], 2.74–3.08 [*ttc*, 1 H, m, $\frac{1}{2}\beta CH_2(Pro^3)$], 3.36 [*ttc*, 1 H, dd, $J_1$ 12.9, $J_2$ 4.7, $\frac{1}{2}\delta CH_2(Pro^3)$], 3.48–3.70 [3 H, m, $\delta CH_2(Pro^1)$ and *ttt*, $\frac{1}{2}\delta CH_2(Pro^3)$], 3.68 (*ttt*, 3 H, s, $CO_2CH_3$), 3.74 (*ttc*, 3 H, s, $CO_2CH_3$), 3.92–4.16 [2 H, m, $\gamma CH(Pro^3)$ and $\frac{1}{2}\delta CH_2(Pro^3)$], 4.37–4.57 [3 H, m, $CHCH_3$, $\alpha CH(Pro^1)$ and $\alpha CH(Pro^3)$], 4.63 [1 H, quintet, *J* 6.9, $\alpha CH(Ala)$] and 7.15 (1 H, m, NH); $\delta_C$(75.44 MHz; $C^2HCl_3$) 17.82 [*ttc*, $\beta CH_3(Ala^2)$], 18.04 [*ttt*, $\beta CH_3(Ala^2)$], 20.98 ($CHCH_3$), 25.06 [*ttt*, $\gamma CH_2(Pro^1)$], 25.34 [*ttc*, $\gamma CH_2(Pro^1)$], 28.44 [$\beta CH_2(Pro^1)$], 30.85 ($SCOCH_3$), 34.86 [*ttt*, $\beta CH_2(Pro^3)$], 37.56 [*ttc*, $\beta CH_2(Pro^3)$], 39.08 [*ttc*, $\gamma CH(Pro^3)$], 39.87 [*ttt*, $\gamma CH(Pro^3)$], 46.78, 47.05, 47.40 and 47.60 [$\alpha CH(Ala^2)$ and $\delta CH_2(Pro^1)$], 51.30 (*ttc*, $CHCH_3$), 51.69 (*ttt*, $CHCH_3$), 52.33, 52.46, 52.71 and 53.05 [$\delta CH_2(Pro^3)$ and $CO_2CH_3$], 58.75 [$\alpha CH(Pro^3)$], 60.12 [*ttc*, $\alpha CH(Pro^1)$], 60.74 [*ttt*, $\alpha CH(Pro^1)$], 169.10 ($COCHCH_3$), 170.83, 171.03 (2 × amide CO), 171.99 (*ttt*, ester CO), 172.88 (*ttc*, ester CO) and 195.13 (thiolester CO); *m/z* (CI) 464 and 462 (21 and 48%, chlorine isotopes, $[M + H]^+$), 371 {26, $[M - ClCH(CH_3)CO + H]^+$}, 208 and 206 {56 and 100, chlorine isotopes, $[ClCH(CH_3)CONC_4H_7CO_2H]^+$} and 170 (48, $[CH_2CHCONC_4H_7CO_2H + H]^+$).

**Methyl (1*S*,3*R*,9*S*,12*R*,15*S*)-3,12-Dimethyl-4,10,13-trioxo-2-thia-5,11,14-triazatricyclo[12.2.1.0$^{5,9}$]heptadecane-15-carboxylate 151**

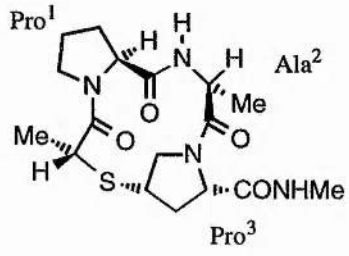



Thiolester **156** (0.34 g, 0.74 mmol) was dissolved in a mixture of 0.05 mol dm$^{-3}$ aqueous potassium hydroxide solution (25 cm$^3$) and methanol (25 cm$^3$). After stirring for 2.5 h, aqueous HCl (0.5 mol dm$^{-3}$, 2.5 cm$^3$) was added and the methanol was removed under reduced pressure. The solution was then further acidified to pH 1 and extracted with ethyl acetate (6 × 40 cm$^3$). The combined organic extracts were dried ($MgSO_4$) and the solvent removed under reduced pressure to afford thiol **150** in an impure state as a colourless oil (0.22 g).

The above oil was redissolved in DMF (150 cm$^3$) and the solution treated with caesium carbonate (0.34 g, 1.04 mmol). The suspension was stirred under nitrogen for 4 days after which it was filtered through Celite, and the solvent removed under reduced pressure to give a brown oil. The crude oil was purified by column chromatography using dichloromethane–methanol (24:1) as the eluent to afford macrocycle **151** as colourless crystals (70 mg, 25%), mp 202–204 °C; (Found: C, 51.9; H, 6.45; N, 10.55. $C_{17}H_{25}N_3O_5S\cdot\frac{1}{2}H_2O$ requires C, 52.05; H, 6.7; N, 10.7%); $[\alpha]_D$ −149.9 (*c* 0.5 in MeOH); $\upsilon_{max}(CH_2Cl_2)$/cm$^{-1}$ 3308 (NH), 2953 (CH), 1763 (ester CO), 1682 (secondary amide CO), 1663 (tertiary amide CO) and 1450 (CH def.); $\delta_H$(500 MHz; $^2H_6$-DMSO) 1.27 [3 H, d, *J* 6.5, CHC$H_3$], 1.28 [3 H, d, *J* 6.6, βCH$_3$(Ala$^2$)], 1.57–1.62 [1 H, m, $\frac{1}{2}$βCH$_2$(Pro$^3$)], 1.80–1.88 [1 H, m, $\frac{1}{2}$βCH$_2$(Pro$^3$)], 1.96–2.00 [1 H, m, $\frac{1}{2}$γCH$_2$(Pro$^1$)], 2.10–2.14 [1 H, m, $\frac{1}{2}$βCH$_2$(Pro$^1$)], 2.20–2.28 [1 H, m, $\frac{1}{2}$γCH$_2$(Pro$^1$)], 2.48–2.55 [1 H, m, $\frac{1}{2}$γCH$_2$(Pro$^1$)], 2.90 [1 H, dd, $J_1$ 11.8, $J_2$ 6.8, $\frac{1}{2}$δCH$_2$(Pro$^3$)], 3.12–3.17 [1 H, m, γCH(Pro$^3$)], 3.61–3.67 [2 H, m, δCH$_2$(Pro$^1$)], 3.65

(3 H, s, $CO_2CH_3$), 4.09 (1 H, q, *J* 6.9, $CHCH_3$), 4.09–4.16 [1 H, m, αCH(Ala$^2$)], 4.31 [1 H, dd, $J_1$ 11.7, $J_2$ 4.8, $\frac{1}{2}$δCH$_2$(Pro$^3$)], 4.41[ 1 H, dd, $J_1$ 9.3, $J_2$ 5.4, αCH(Pro$^3$)], 4.88 [1 H, d, *J* 7.2, αCH(Pro$^1$)] and 8.17 (1 H, d, *J* 4.3, NH); $\delta_C$(125.73 MHz; $^2H_6$-DMSO) 16.45 [βCH$_3$(Ala$^2$)], 16.73 ($CHCH_3$), 24.41 [γCH$_2$(Pro$^1$)], 25.62 [βCH$_2$(Pro$^1$)], 33.56 [βCH$_2$(Pro$^3$)], 40.26 ($CHCH_3$), 41.56 [γCH(Pro$^3$)], 46.38 [δCH$_2$(Pro$^1$)], 50.85 [δCH$_2$(Pro$^3$)], 51.08 [αCH(Ala$^2$)], 51.79 ($CO_2CH_3$), 57.12 [αCH(Pro$^3$)], 57.70 [αCH(Pro$^1$)], 169.26 [CO(Ala$^2$)], 170.17 [CO(Pro$^1$)], 171.05 [CO(Pro$^3$)] and 171.48 (ester CO); *m/z* (FAB$^+$) 406 (5%, [M + Na]$^+$), 384 (64, [M + H]$^+$) and 128 {84, [$NC_4H_6$(S)CO]$^+$}.

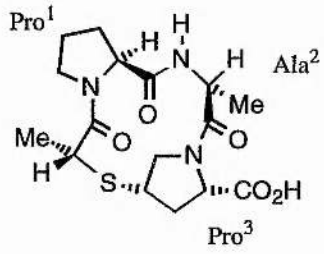
**_N_-15-Methyl-(1*S*,3*R*,9*S*,12*R*,15*S*)-3,12-Dimethyl-4,10,13-trioxo-2-thia-5,11,14-triazatricyclo[12.2.1.0$^{5,9}$]heptadecane-15-carboxamide 158**




Methyl ester **151** (42 mg, 0.11 mmol) was dissolved in a saturated solution of methylamine in methanol (5 cm$^3$) at 0 °C. The flask was stoppered, allowed to warm to room temperature, and left standing for 2 days. The flask was cooled to 0 °C, and the stopper removed. The excess methylamine was driven off by bubbling nitrogen through the solution for 30 min. The solvent was removed under reduced pressure to afford methylamide **158** as white crystals in quantitative yield, mp 280 °C (decomp.); (Found: C, 53.85; H, 6.8; N, 14.5. $C_{17}H_{26}N_4O_4S$ requires C, 53.4; H, 6.85; N, 14.65%); $[\alpha]_D$ –46.3 (*c* 0.6 in MeOH); $\upsilon_{max}(CH_2Cl_2)$/cm$^{-1}$ 3348 (NH), 2940 (CH), 1671 (secondary amide CO), 1621 (tertiary amide CO) and 1532 (NH def.); $\delta_H$(300 MHz; $C^2HCl_3$) 1.34 [3 H, d, *J* 6.6, $CHCH_3$], 1.37 [3 H, d, *J* 7.1, βCH$_3$(Ala$^2$)], 1.88–2.01 [2 H, m, $\frac{1}{2}$βCH$_2$(Pro$^3$) and $\frac{1}{2}$γCH$_2$(Pro$^1$)], 2.02–2.16 [2 H, m, $\frac{1}{2}$βCH$_2$(Pro$^1$)

and $\frac{1}{2}\gamma CH_2(Pro^1)$], 2.37–2.53 [2 H, m, $\frac{1}{2}\beta CH_2(Pro^1)$ and $\frac{1}{2}\beta CH_2(Pro^3)$], 2.82 (3 H, d, *J* 4.9, NHC$H_3$), 3.22 [1 H, d, *J* 11.0, $\frac{1}{2}\delta CH_2(Pro^3)$], 3.49–3.58 [1 H, m, $\gamma CH(Pro^3)$], 3.61–3.72 [3 H, m, $\delta CH_2(Pro^1)$ and $\frac{1}{2}\delta CH_2(Pro^3)$], 3.81 (1 H, q, *J* 7.1, C*H*$CH_3$), 4.38–4.51 [2 H, m, $\alpha CH(Ala^2)$ and $\alpha CH(Pro^3)$], 4.83 [1 H, d, *J* 7.1, $\alpha CH(Pro^1)$], 7.51 [1 H, d, *J* 6.9, $NH(Ala^2)$] and 7.64 (1 H, m, N*H*$CH_3$); $\delta_C$(75.44 MHz; $C^2HCl_3$) 17.10 and 17.15 [CHC$H_3$ and $\beta CH_3(Ala^2)$], 25.55 [$\beta CH_2(Pro^1)$ and $\gamma CH_2(Pro^1)$], 26.71 ($NHCH_3$), 34.70 [$\beta CH_2(Pro^3)$], 43.08 and 43.15 [*C*H$CH_3$ and $\gamma CH(Pro^3)$], 47.49 [$\delta CH_2(Pro^1)$], 49.43 [$\alpha CH(Ala^2)$], 51.37 [$\delta CH_2(Pro^3)$], 59.73 [$\alpha CH(Pro^3)$], 61.36 [$\alpha CH(Pro^1)$], 169.66 [$CO(Pro^1)$], 172.19 [$CO(Pro^3)$], 172.69 [$CO(Ala^2)$] and 174.63 (*C*O$CHCH_3$); *m/z* (CI) 383 (100%, $[M + H]^+$) and 351 (23, $[M - S + H]^+$).

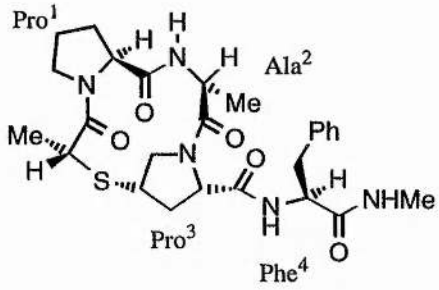
**(1*S*,3*R*,9*S*,12*R*,15*S*)-3,12-Dimethyl-4,10,13-trioxo-2-thia-5,11,14-triazatricyclo[12.2.1.0$^{5,9}$]heptadecane-15-carboxylic acid 159**




A solution of methyl ester **151** (0.30 g, 0.79 mmol) in methanol (3 $cm^3$) was treated with aqueous sodium hydroxide solution (1.0 mol $dm^{-3}$, 3.5 $cm^3$), and the mixture allowed to stir for 2 h. After addition of aqueous HCl (1.0 mol $dm^{-3}$, 5 $cm^3$), the methanol was removed under reduced pressure. The solution was acidified with aqueous HCl, saturated with NaCl, and exhaustively extracted with ethyl acetate (9 × 20 $cm^3$). The combined organic extracts were dried ($MgSO_4$), and the solvent removed under reduced pressure to afford the macrocycle acid **159** as colourless crystals (0.24 g, 83%), mp 225–226 °C (decomp.); (Found: C, 52.15; H, 6.5; N, 11.0. $C_{16}H_{23}N_3O_5S$ requires C, 52.0; H, 6.3; N, 11.35%); $[\alpha]_D$ −134.4 (*c* 0.2 in MeOH); $\upsilon_{max}(CH_2Cl_2)/cm^{-1}$ 3317 (NH), 1751 (ester CO), 1686 (secondary amide CO) and

1672 (tertiary amide CO); $\delta_H$(300 MHz; $C^2HCl_3$) 1.32 [6 H, m, $CHCH_3$ and $\beta CH_3(Ala^2)$], 1.90–2.10 [3 H, m, $\frac{1}{2}\beta CH_2(Pro^1)$, $\frac{1}{2}\beta CH_2(Pro^3)$ and $\frac{1}{2}\gamma CH_2(Pro^1)$], 2.38–2.43 [3 H, m, $\frac{1}{2}\beta CH_2(Pro^1)$, $\frac{1}{2}\beta CH_2(Pro^3)$ and $\frac{1}{2}\gamma CH_2(Pro^1)$], 3.11 [1 H, d, *J* 11.8, $\frac{1}{2}\delta CH_2(Pro^3)$], 3.50–3.66 [3 H, m, $\gamma CH(Pro^3)$ and $\delta CH_2(Pro^1)$], 3.83–3.91 [2 H, m, $CHCH_3$ and $\frac{1}{2}\delta CH_2(Pro^3)$], 4.46–4.50 [1 H, m, $\alpha CH(Ala^2)$], 4.54–4.58 [1 H, m, $\alpha CH(Pro^3)$], 4.81 [1 H, d, *J* 7.7, $\alpha CH(Pro^1)$], 7.63 (1 H, d, *J* 6.6, NH) and 9.77 (1 H, br. s, $CO_2H$); $\delta_C$(75.44 MHz; $C^2HCl_3$) 16.94 and 17.00 [$CHCH_3$ and $\beta CH_3(Ala^2)$], 25.37 and 25.73 [$\beta CH_2(Pro^1)$ and $\gamma CH_2(Pro^1)$], 34.25 [$\beta CH_2(Pro^3)$], 42.62 and 42.75 [$CHCH_3$ and $\gamma CH(Pro^3)$], 47.48 [$\delta CH_2(Pro^1)$], 49.47 [$\alpha CH(Ala^2)$], 51.54 [$\delta CH_2(Pro^3)$], 59.82 and 60.13 [$\alpha CH(Pro^1)$ and $\alpha CH(Pro^3)$], 169.98 [$CO(Pro^1)$], 172.56, 173.02 and 174.63 [$COCHCH_3$, $CO(Ala^2)$ and $CO(Pro^3)$]; *m/z* (CI) 370 (77%, $[M + H]^+$), 326 (14, $[M - CO_2 + H]^+$), 257 (59, $[M - NC_4H_7CO_2 + H]^+$) and 170 (100, $SCHCONC_4H_7CO^+$).

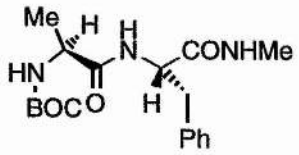
**(2*S*)-*N*-{(1*S*,3*R*,9*S*,12*R*,15*S*)-3,12-Dimethyl-4,10,13-trioxo-2-thia-5,11,14-triazatricyclo[12.2.1.0$^{5,9}$]heptadecane-15-carbonyl}phenylalanine methylamide 161**




A solution of acid **159** (54 mg, 0.15 mmol) and *N,N*-di-*iso*propylethylamine (80 µl, 0.46 mmol) in dry dichloromethane (2 $cm^3$) was treated with BOP-Cl (40 mg, 0.16 mmol) and the resulting suspension stirred under nitrogen at 0 °C for 1 h. A solution of (2*S*)-phenylalanine methylamide **160** (30 mg, 0.17 mmol) in dry dichloromethane (1 $cm^3$) was then added. The mixture was stirred at 0–5 °C for 2 h, then allowed to

warm to room temperature and stirred for a further 20 h. The solution was diluted to ~15 $cm^3$ with dichloromethane, washed with aqueous HCl (0.5 mol $dm^{-3}$, 2 × 5 $cm^3$), aqueous sodium hydrogen carbonate solution (5%, 2 × 5 $cm^3$) and brine (5 $cm^3$), then dried ($MgSO_4$). The solvent was removed under reduced pressure to afford the peptide **161** as a white solid (48 mg, 62%). A small portion of this was recrystallised from ethyl acetate to afford colourless needles, mp 258–259 °C; (Found: C, 58.95; H, 6.65; N, 13.05. $C_{26}H_{35}N_5O_5S$ requires C, 58.95; H, 6.65; N, 13.2%); $[\alpha]_D$ −62.7 (*c* 0.15 in MeOH); $\upsilon_{max}(CH_2Cl_2)/cm^{-1}$ 3364 (NH), 2937 (CH), 1679 (secondary amide CO), 1642 (tertiary amide CO) and 1531 (NH def.); $\delta_H$(500 MHz; $C^2HCl_3$) 1.14 [3 H, d, *J* 7.2, CHC$H_3$], 1.31 [3 H, d, *J* 6.6, βCH$_3$(Ala$^2$)], 1.84–1.96 [2 H, m, $\frac{1}{2}$βCH$_2$(Pro$^1$) and $\frac{1}{2}$βCH$_2$(Pro$^3$)], 2.04–2.11 [1 H, m, $\frac{1}{2}$γCH$_2$(Pro$^1$)], 2.37–2.54 [3 H, m, $\frac{1}{2}$βCH$_2$(Pro$^1$), $\frac{1}{2}$βCH$_2$(Pro$^3$) and $\frac{1}{2}$γCH$_2$(Pro$^1$)], 2.81 (3 H, d, *J* 4.7, NHC$H_3$), 3.12 [1 H, dd, $J_1$ 15.9, $J_2$ 10.0, $\frac{1}{2}$βCH$_2$(Phe$^4$)], 3.24 [1 H, d, *J* 11.9, $\frac{1}{2}$δCH$_2$(Pro$^3$)], 3.48 (1 H, q, *J* 7.9, C$H$CH$_3$), 3.58–3.67 [5 H, m, $\frac{1}{2}$βCH$_2$(Phe$^4$), γCH(Pro$^3$), δCH$_2$(Pro$^1$) and $\frac{1}{2}$δCH$_2$(Pro$^3$)], 4.45–4.49 [2 H, m, αCH(Ala$^2$) and αCH(Pro$^3$)], 4.84 [1 H, dd, $J_1$ 7.9, $J_2$ 1.5, αCH(Pro$^1$)], 5.02 [1 H, m, αCH(Phe$^4$)], 6.90 (1 H, q, *J* 4.3, N$H$CH$_3$), 7.15 (1 H, t, *J* 7.1, Ar–H *para*), 7.23–7.28 (4 H, m, Ar–H *ortho* and *meta*), 7.60 [1 H, d, *J* 7.2, NH(Ala$^2$)] and 7.86 [1 H, d, *J* 8.0, NH(Phe$^4$)]; $\delta_C$(75.44 MHz; $C^2HCl_3$) 17.01 (CH$C$H$_3$), 17.46 [βCH$_3$(Ala$^2$)], 25.52 [βCH$_2$(Pro$^1$)], 25.71 [γCH$_2$(Pro$^1$)], 26.98 (NHCH$_3$), 34.48 [βCH$_2$(Pro$^3$)], 36.59 [βCH$_2$(Phe$^4$)], 43.34 [γCH(Pro$^3$)], 43.61 [$C$HCH$_3$], 47.49 [δCH$_2$(Pro$^1$)], 48.85 [αCH(Ala$^2$)], 51.19 [δCH$_2$(Pro$^3$)], 53.60 [αCH(Phe$^4$)], 59.98 [αCH(Pro$^1$)], 62.36 [αCH(Pro$^3$)], 126.59 (Ar–CH *para*), 128.70 (Ar–CH *meta*), 129.11 (Ar–CH *ortho*), 138.78 (Ar–C quaternary), 169.65 [CO(Pro$^1$)], 172.07 [CO(Pro$^3$)], 172.76 [CO(Phe$^4$)], 173.00 [CO(Ala$^2$)] and 174.47 ($C$OCHCH$_3$); *m/z* (CI) 530 (22%, [M + H]$^+$), 243 {100, [$NC_4H_6CONHCH(CH_2Ph)CO]^+$} and 201 {37, [$SCH(CH_3)CONC_4H_7CONH + H]^+$}.

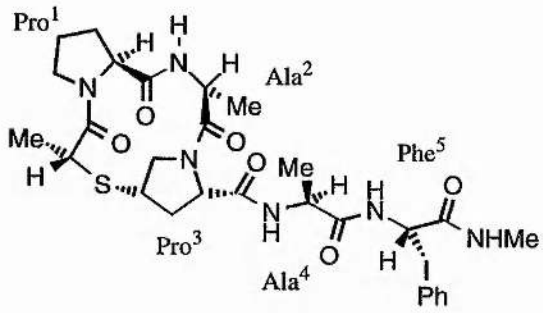
**(2*S*)-*N*-[(*tert*-Butoxycarbonyl)-(2*S*)-alanyl]phenylalanine methylamide 163**




A stirred solution of (2*S*)-(*tert*-butoxycarbonyl)alanine **162** (0.64 g, 3.4 mmol) and *N*-methylmorpholine (0.38 $cm^3$, 3.4 mmol) in dry THF (15 $cm^3$) at −15 °C was treated dropwise with *iso*butyl chloroformate (0.46 $cm^3$, 3.4 mmol), followed after 2 min by a solution of (2*S*)-phenylalanine methylamide **160** (0.60 g, 3.4 mmol) and *N*-methylmorpholine (0.38 $cm^3$, 3.4 mmol) in dry THF (5 $cm^3$).

The suspension was diluted with additional THF to allow efficient stirring, allowed to reach room temperature, then stirred for 2 h. The hydrochloride salts formed were filtered off and the solvents removed under reduced pressure to yield a solid which was redissolved in ethyl acetate (50 $cm^3$). This solution was washed with aqueous HCl (0.5 mol $dm^{-3}$, 2 × 30 $cm^3$), aqueous sodium hydrogen carbonate solution (5%, 2 × 30 $cm^3$), and brine (30 $cm^3$). The solvent was removed under reduced pressure to yield the dipeptide methylamide **163** as a white solid (0.78 g, 67%). A small portion of this was recrystallised from ethyl acetate to afford colourless needles, mp 157–158 °C (lit., [101] 157–158 °C); $[\alpha]_D$ −38.7 (*c* 1.1 in MeOH) [lit., [101] −37.7 (*c* 1.0 in MeOH]; $\upsilon_{max}(CH_2Cl_2)/cm^{-1}$ 3411 (NH), 2981 (CH), 1701 (urethane CO), 1670 (amide CO) and 1505 (NH def.); $\delta_H$(200 MHz; $C^2HCl_3$) 1.27 [3 H, d, *J* 7.0, βCH$_3$(Ala)], 1.37 [9 H, s, C(CH$_3$)$_3$], 2.70 (3 H, d, *J* 4.8, NHC*H*$_3$), 3.01–3.25 [2 H, m, βCH$_2$(Phe)], 4.05 [1 H, m, αCH(Ala)], 4.66 [1 H, m, αCH(Phe)], 5.10 (1 H, m, NH), 6.74 (1 H, m, NH) and 7.14–7.32 (5 H, m, Ar–H); $\delta_C$(50.31 MHz; $C^2HCl_3$) 18.57 [βCH$_3$(Ala)], 26.82 (NHCH$_3$), 28.69 [C(*C*H$_3$)$_3$], 38.36 [βCH$_2$(Phe)], 51.56 [αCH(Ala)], 54.42 [αCH(Phe)], 80.93 [*C*(CH$_3$)$_3$], 127.39 (Ar–CH *para*), 129.04 (Ar–CH *meta*), 129.70 (Ar–CH *ortho*), 137.11 (Ar–C quaternary), 156.10 (urethane CO), 171.70 and 173.15 (2 × amide CO); *m/z* (CI) 351 (100%, $[M + H]^+$), 294 (93, $[M - C_4H_8]^+$) and 250 (12, $[M - CO_2C_4H_8]^+$).

**(2*S*)-*N*-(*N*-{(1*S*,3*R*,9*S*,12*R*,15*S*)-3,12-Dimethyl-4,10,13-trioxo-2-thia-5,11,14-triazatricyclo[12.2.1.0$^{5,9}$]heptadecane-15-carbonyl}-(2*S*)-phenylalanyl)alanine methylamide 165**

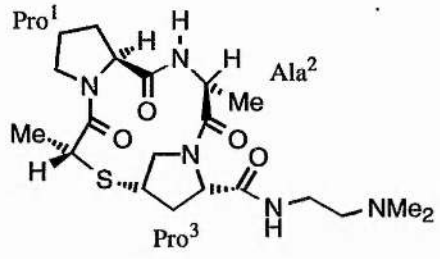



A solution of methylamide **163** (0.50 g, 1.4 mmol) in dichloromethane (15 cm$^3$) at 0 °C was treated with trifluoroacetic acid (8 cm$^3$) and the solution stirred for 2 h. The solvents were removed under reduced pressure to yield the trifluoroacetate salt **164** as a white solid.

A solution of acid **159** (80 mg, 0.22 mmol) and *N*,*N*-di-*iso*propylethylamine (0.12 cm$^3$, 0.69 mmol) in dichloromethane (5 cm$^3$) was treated with BOP-Cl (60 mg, 0.24 mmol) at 0 °C. After 50 min, amine trifluoroacetate **164** (90 mg, 0.25 mmol) was added and the mixture was stirred at 0–5 °C for 2 h, then at room temperature for 18 h. The solution was diluted to ~15 cm$^3$ with dichloromethane, then washed with aqueous HCl (0.5 mol dm$^{-3}$, 2 × 6 cm$^3$), aqueous sodium hydrogen carbonate solution (5%, 2 × 6 cm$^3$) and brine (6 cm$^3$). The organic phase was dried ($MgSO_4$), and the solvent removed under reduced pressure. The crude residue was purified by column chromatography using dichloromethane–methanol (20:1) as the eluent to afford the peptide **165** as a white solid (43 mg, 33%), mp 129–131 °C; (HRMS: Found (M + H)$^+$, 601.2798. $C_{29}H_{41}N_6O_6S$ requires 601.2808); $[\alpha]_D$ −25.7 (*c* 0.3 in MeOH); $\upsilon_{max}(CH_2Cl_2)$/cm$^{-1}$ 3338 (NH), 2935 (CH), 1680 (secondary amide CO), 1662 (tertiary amide CO) and 1536 (NH def.); $\delta_H$(300 MHz; $C^2HCl_3$) 1.16 [3 H, d, *J* 7.4, $\beta CH_3$(Ala$^4$)], 1.27 [3 H, d, *J* 6.6, $\beta CH_3$(Ala$^2$)], 1.30 (3 H, d, *J* 7.4, $CHCH_3$), 1.89–1.98 [2 H, m, $\frac{1}{2}\beta CH_2$(Pro$^1$) and $\frac{1}{2}\beta CH_2$(Pro$^3$)], 2.02–2.09 [1 H, m, $\frac{1}{2}\gamma CH_2$(Pro$^1$)],

2.33–2.40 [1 H, m, $\frac{1}{2}\gamma CH_2(Pro^1)$], 2.43–2.55 [2 H, m, $\frac{1}{2}\beta CH_2(Pro^1)$ and $\frac{1}{2}\beta CH_2(Pro^3)$], 2.74 (3 H, d, *J* 4.7, NHC$H_3$), 2.86 [1 H, dd, $J_1$ 14.4, $J_2$ 11.7, $\frac{1}{2}\beta CH_2(Phe^5)$], 3.22 [1 H, d, *J* 11.8, $\frac{1}{2}\delta CH_2(Pro^3)$], 3.44–3.69 [4 H, m, $\frac{1}{2}\beta CH_2(Phe^5)$, $\delta CH_2(Pro^1)$ and $\frac{1}{2}\delta CH_2(Pro^3)$], 3.74–3.85 [2 H, m, C*H*$CH_3$ and $\gamma CH(Pro^3)$], 4.15–4.19 [1 H, m, $\alpha CH(Ala^4)$], 4.37 [1 H, d, *J* 10.4, $\alpha CH(Pro^3)$], 4.41–4.46 [1 H, m, $\alpha CH(Ala^2)$], 4.60–4.68 [1 H, m, $\alpha CH(Phe^5)$], 4.75 [1 H, d, *J* 6.6, $\alpha CH(Pro^1)$], 6.87 (1 H, q, *J* 4.7, N*H*$CH_3$), 7.06 [1 H, d, *J* 9.1, $NH(Phe^5)$], 7.11–7.22 (5 H, m, Ar–H), 7.58 [1 H, d, *J* 7.1, $NH(Ala^2)$] and 8.33 [1 H, d, *J* 8.0, $NH(Ala^4)$]; $\delta_C$(125.8 MHz; $C^2HCl_3$) 16.31 [$\beta CH_3(Ala^4)$], 16.79 (CH*C*$H_3$), 17.03 [$\beta CH_3(Ala^2)$], 25.16 [$\beta CH_2(Pro^1)$], 25.34 [$\gamma CH_2(Pro^1)$], 26.47 (NHC$H_3$), 34.39 [$\beta CH_2(Pro^3)$], 37.31 [$\beta CH_2(Phe^5)$], 43.30 [$\gamma CH(Pro^3)$], 43.57 [*C*H$CH_3$], 47.31 [$\delta CH_2(Pro^1)$], 48.24 [$\alpha CH(Ala^2)$], 50.74 [$\delta CH_2(Pro^3)$], 51.01 [$\alpha CH(Ala^4)$], 54.24 [$\alpha CH(Phe^5)$], 59.88 [$\alpha CH(Pro^1)$], 62.11 [$\alpha CH(Pro^3)$], 126.32 (Ar–CH *para*), 128.22 (Ar–CH *meta*), 129.14 (Ar–CH *ortho*), 138.68 (Ar–C quaternary), 169.20 [$CO(Pro^1)$], 171.90, 172.18, 172.48 and 172.60 [$CO(Ala^2)$, $CO(Pro^3)$, $CO(Ala^4)$ and $CO(Phe^5)$], and 174.24 (*C*O$CHCH_3$); *m/z* (CI) 601 (100%, $[M + H]^+$), 569 (31, $[M - NH_2CH_3]^+$), 439 (10, $[M - CH_3NHCOCHCH_2Ph + H]^+$) and 372 {100, $[CONC_4H_6CONHCH(CH_3)CONHCH(CH_2Ph)CONHCH_3]^+$}.

***N*-{(1*S*,3*R*,9*S*,12*R*,15*S*)-3,12-Dimethyl-4,10,13-trioxo-2-thia-5,11,14-triazatricyclo [12.2.1.0$^{5,9}$]heptadecane-15-carbonyl}-*N'*-(dimethyl)ethylenediamine 166**




A stirred solution of acid **159** (0.15 g, 0.41 mmol) and *N*-methylmorpholine (0.10 cm$^3$, 0.91 mmol) in dry THF (3 cm$^3$) and DMF (1 cm$^3$) under nitrogen at −15 °C was treated dropwise with *iso*butyl chloroformate (55 μl, 0.42 mmol), followed after 10 min. by a solution of *N*,*N*-dimethylethylenediamine (60 μl, 0.55 mmol) in dry THF (1 cm$^3$). The solution was allowed to reach room temperature and stirred for 4 h. The solvents were removed under reduced pressure, and the residual oil redissolved in ethyl acetate (20 cm$^3$). The solution was washed with aqueous sodium hydrogen carbonate solution (5%, 2 × 5 cm$^3$), and brine (5 cm$^3$) then dried ($MgSO_4$). The solvent was removed under reduced pressure to afford the amine **166** as colourless crystals (0.14 g, 78%), mp 174–176 °C; (Found: C, 53.95; H, 7.6; N, 15.25. $C_{20}H_{33}N_5O_4S{\cdot}\frac{1}{2}H_2O$ requires C, 53.55; H, 7.65; N, 15.6%); $[\alpha]_D$–48.0 (*c* 0.85 in MeOH); $\upsilon_{max}(CH_2Cl_2)/cm^{-1}$ 3337 (NH), 2983 (CH), 1668 (secondary amide CO), 1634 (tertiary amide CO), 1531 (NH def.) and 1269 (C–N); $\delta_H$(300 MHz; $C^2HCl_3$) 1.23 [3 H, d, *J* 6.9, CHC*H*$_3$], 1.29 [3 H, d, *J* 7.1, βCH$_3$(Ala$^2$)], 1.81–2.04 [3 H, m, $\frac{1}{2}$βCH$_2$(Pro$^1$), $\frac{1}{2}$βCH$_2$(Pro$^3$) and $\frac{1}{2}$γCH$_2$(Pro$^1$)], 2.18 [6 H, s, N(CH$_3$)$_2$], 2.31–2.43 [4 H, m, C*H*$_2$N(CH$_3$)$_2$, $\frac{1}{2}$βCH$_2$(Pro$^1$) and $\frac{1}{2}$γCH$_2$(Pro$^1$)], 2.51–2.60 [1 H, m, $\frac{1}{2}$βCH$_2$(Pro$^3$)], 3.15–3.22 [2 H, m, γCH(Pro$^3$) and $\frac{1}{2}$δCH$_2$(Pro$^3$)], 3.35–3.62 [5 H, m, δCH$_2$(Pro$^1$), $\frac{1}{2}$δCH$_2$(Pro$^3$) and C*H*$_2$NHCO], 3.71 (1 H, q, *J* 7.1, C*H*CH$_3$), 4.37–4.43 [2 H, m, αCH(Ala$^2$) and αCH(Pro$^3$)], 4.76 [1 H, d, *J* 8.2, αCH(Pro$^1$)], 7.70 (1 H, t, *J* 7.7, N*H*CH$_2$) and 7.90 [1 H, d, *J* 8.0, NH(Ala$^2$)]; $\delta_C$(75.44 MHz; $C^2HCl_3$) 16.93 and 17.10 [CHC*H*$_3$ and β CH$_3$(Ala$^2$)], 25.47 [βCH$_2$(Pro$^1$) and γCH$_2$(Pro$^1$)], 34.52

[βCH$_2$(Pro$^3$)], 37.63 (*C*H$_2$NHCO), 43.02 and 43.10 [*C*HCH$_3$ and γCH(Pro$^3$)], 45.49 [N(CH$_3$)$_2$], 46.97 [δCH$_2$(Pro$^1$)], 48.79 [αCH(Ala$^2$)], 51.16 [δCH$_2$(Pro$^3$)], 57.96 [*C*H$_2$N(CH$_3$)$_2$], 59.59 [αCH(Pro$^1$)], 61.41 [αCH(Pro$^3$)], 169.53 [CO(Pro$^1$)], 171.92 and 172.30 [CO(Ala$^2$) and CO(Pro$^3$)] and 173.87 (*C*OCHCH$_3$); *m/z* (CI) 440 (100%, [M + H]$^+$), 408 (23, [M – 2CH$_3$ + H]$^+$), 369 {19, [M – CHCH$_2$N(CH$_3$)$_2$ + H]$^+$} and 241 {82, [CH$_3$CHCONC$_4$H$_7$CONHCH$_2$CH$_2$N(CH$_3$)$_2$ + H]$^+$}.

A solution of amine **166** in dichloromethane was treated with a slight excess of trifluoroacetic acid, followed by removal of the solvent under reduced pressure to afford the amine trifluoroacetate **167** as a colourless oil.

$\delta_H$(500 MHz; C$^2$HCl$_3$) 1.36 [6 H, d, *J* 7.1, CHC*H*$_3$ and βCH$_3$(Ala$^2$)], 2.01–2.08 [2 H, m, ½βCH$_2$(Pro$^3$) and ½γCH$_2$(Pro$^1$)], 2.11–2.18 (1 H, m, ½βCH$_2$(Pro$^1$)], 2.28–2.33 [1 H, m, ½βCH$_2$(Pro$^1$)], 2.39–2.45 [1H, m, ½γCH$_2$(Pro$^1$)], 2.45–2.51 [1 H, m, ½βCH$_2$(Pro$^3$)], 2.98 (3 H, s, NCH$_3$), 3.02 (3 H, s, NCH$_3$), 3.25–3.35 [1 H, m, ½C*H*$_2$N(CH$_3$)$_2$], 3.45–3.57 [4 H, m, ½C*H*$_2$NHCO, ½C*H*$_2$N(CH$_3$)$_2$, ½δCH$_2$(Pro$^1$) and ½δCH$_2$(Pro$^3$)], 3.68 [1 H, dd, $J_1$ 12.5, $J_2$ 4.5, ½δCH$_2$(Pro$^3$)], 3.82 (1 H, q, *J* 7.3, C*H*CH$_3$), 3.89–3.98 [3 H, m, ½C*H*$_2$NHCO, γCH(Pro$^3$) and ½δCH$_2$(Pro$^1$)], 4.23–4.33 [1 H, m, αCH(Ala$^2$)], 4.46 [1 H, d, *J* 10.5, αCH(Pro$^3$)], 4.55–4.62 [1 H, m, αCH(Pro$^1$)], 8.24 [1 H, d, *J* 6.8, NH(Ala$^2$)], 8.79 (1 H, br. s, N*H*CH$_2$) and 9.16 [1 H, br. s, N*H*(CH$_3$)$_2$]; $\delta_C$(125.8 MHz; C$^2$HCl$_3$) 16.10 and 17.07 [CH*C*H$_3$ and βCH$_3$(Ala$^2$)], 25.93 [γCH$_2$(Pro$^1$)], 26.84 [βCH$_2$(Pro$^1$)], 34.39 [βCH$_2$(Pro$^3$)], 35.00 (*C*H$_2$NHCO), 43.16 [γCH(Pro$^3$)], 43.82 (*C*HCH$_3$), 44.02 [NCH$_3$], 45.38 [NCH$_3$], 47.24 [δCH$_2$(Pro$^1$)], 49.00 [αCH(Ala$^2$)], 51.81 [δCH$_2$(Pro$^3$)], 57.92 [*C*H$_2$N(CH$_3$)$_2$], 62.37 [αCH(Pro$^1$) and αCH(Pro$^3$)], 116.12 (q, $J_{CF}$ 289, *C*F$_3$CO$_2$), 160.88 (q, $J_{CF}$ 38.5, CF$_3$*C*O$_2$), 173.43, 174.06 and 174.32 (4 × amide CO).

# APPENDIX

## CRYSTALLOGRAPHIC DATA FOR MACROCYCLE 151

$C_{17}H_{26}N_3O_{5.5}S$, M = 392.47, orthorhombic, space group $C222_1$ (#20), $a$ = 9.371(10), $b$ = 16.744(9), $c$ = 25.705(7) Å, V = 4033(4) Å$^3$, Z = 8, $D_{calc}$ = 1.293 g/cm$^3$, T = 293 K. 1511 unique reflections were collected on a Rigaku AFC7S, diffracting with graphite monochromated Mo-K$\alpha$ radiation ($\lambda$ = 0.71069 Å) of which 1020 [$I > 3\sigma(I)$] were used for refinement. Convergence at R(F) = 4.9%, $R_W$(F) = 4.6% for 240 variable parameters. The structure was solved and expanded using Fourier techniques and was refined using teXsan. [289]

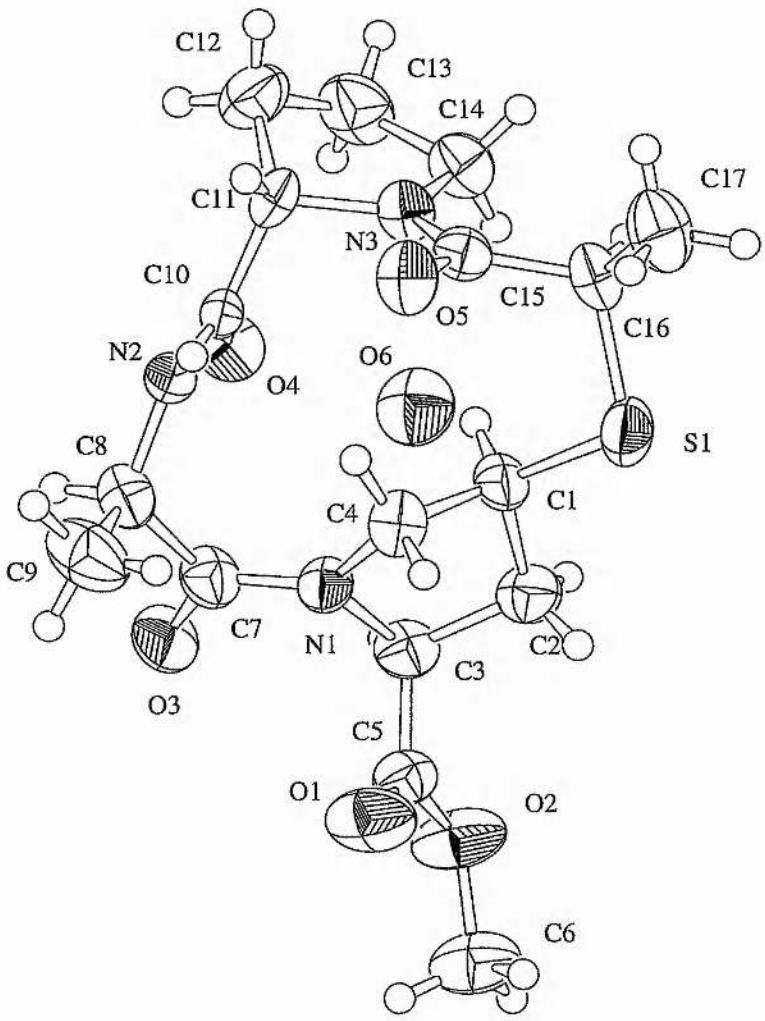


**Table A 1.1 Bond Lengths (Å) for Macrocycle 151**

| atom | atom | distance | atom | atom | distance |
|---|---|---|---|---|---|
| S(1) | C(1) | 1.82(1) | S(1) | C(16) | 1.82(1) |
| O(1) | C(5) | 1.17(1) | O(2) | C(5) | 1.34(1) |
| O(2) | C(6) | 1.45(1) | O(3) | C(7) | 1.21(1) |
| O(4) | C(10) | 1.24(1) | O(5) | C(15) | 1.25(1) |
| N(1) | C(3) | 1.47(1) | N(1) | C(4) | 1.49(1) |
| N(1) | C(7) | 1.33(1) | N(2) | C(8) | 1.46(1) |
| N(2) | C(10) | 1.32(1) | N(3) | C(11) | 1.48(1) |
| N(3) | C(14) | 1.45(1) | N(3) | C(15) | 1.34(1) |
| C(1) | C(2) | 1.53(1) | C(1) | C(4) | 1.54(1) |
| C(2) | C(3) | 1.54(1) | C(3) | C(5) | 1.51(1) |
| C(7) | C(8) | 1.55(1) | C(8) | C(9) | 1.50(1) |
| C(10) | C(11) | 1.52(1) | C(11) | C(12) | 1.51(1) |
| C(12) | C(13) | 1.52(2) | C(13) | C(14) | 1.54(1) |
| C(15) | C(16) | 1.52(1) | C(16) | C(17) | 1.56(1) |
| | | | | | |
| N(2) | H(25) | 1.10 | C(1) | H(1) | 0.95 |
| C(2) | H(2) | 0.95 | C(2) | H(3) | 0.95 |
| C(3) | H(4) | 0.95 | C(4) | H(5) | 0.95 |
| C(4) | H(6) | 0.95 | C(6) | H(7) | 0.95 |
| C(6) | H(8) | 0.95 | C(6) | H(9) | 0.95 |
| C(8) | H(10) | 0.95 | C(9) | H(11) | 0.95 |
| C(9) | H(12) | 0.95 | C(9) | H(13) | 0.95 |
| C(11) | H(14) | 0.95 | C(12) | H(15) | 0.95 |
| C(12) | H(16) | 0.95 | C(13) | H(17) | 0.95 |
| C(13) | H(18) | 0.95 | C(14) | H(19) | 0.95 |

| | | | | | |
|---|---|---|---|---|---|
| C(14) | H(20) | 0.95 | C(16) | H(21) | 0.95 |
| C(17) | H(22) | 0.95 | C(17) | H(23) | 0.95 |
| C(17) | H(24) | 0.95 | | | |

**Table A 1.2 Bond Angles (°) for Macrocycle 151**

| atom | atom | atom | angle | atom | atom | atom | angle |
|---|---|---|---|---|---|---|---|
| C(1) | S(1) | C(16) | 101.5(5) | C(5) | O(2) | C(6) | 116.2(8) |
| C(3) | N(1) | C(4) | 108.6(7) | C(3) | N(1) | C(7) | 119.9(9) |
| C(4) | N(1) | C(7) | 130.9(9) | C(8) | N(2) | C(10) | 124.0(8) |
| C(11) | N(3) | C(14) | 111.9(8) | C(11) | N(3) | C(15) | 121.6(8) |
| C(14) | N(3) | C(15) | 126.3(9) | S(1) | C(1) | C(2) | 112.9(7) |
| S(1) | C(1) | C(4) | 112.3(7) | C(2) | C(1) | C(4) | 100.6(8) |
| C(1) | C(2) | C(3) | 104.4(8) | N(1) | C(3) | C(2) | 104.2(8) |
| N(1) | C(3) | C(5) | 110.1(9) | C(2) | C(3) | C(5) | 112.1(9) |
| N(1) | C(4) | C(1) | 99.5(7) | O(1) | C(5) | O(2) | 123.3(9) |
| O(1) | C(5) | C(3) | 127(1) | O(2) | C(5) | C(3) | 109.4(10) |
| O(3) | C(7) | N(1) | 121(1) | O(3) | C(7) | C(8) | 118.2(10) |
| N(1) | C(7) | C(8) | 120.1(9) | N(2) | C(8) | C(7) | 115.5(8) |
| N(2) | C(2) | C(9) | 112.9(9) | C(7) | C(8) | C(9) | 110.7(9) |
| O(4) | C(10) | N(2) | 122.1(10) | O(4) | C(10) | C(11) | 121.4(9) |
| N(2) | C(10) | C(11) | 116.5(9) | N(3) | C(11) | C(10) | 107.4(8) |
| N(3) | C(11) | C(12) | 104.0(9) | C(10) | C(11) | C(12) | 115.5(9) |
| C(11) | C(12) | C(13) | 102.6(9) | C(12) | C(13) | C(14) | 107.0(9) |
| N(3) | C(14) | C(13) | 103.4(9) | O(5) | C(15) | N(3) | 121.4(9) |
| O(5) | C(15) | C(16) | 121.2(9) | N(3) | C(15) | C(16) | 117.3(9) |
| S(1) | C(16) | C(15) | 10.7(7) | S(1) | C(16) | C(17) | 7.1(8) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| C(15) | C(16) | C(17) | 109.2(8) | | | | |
| C(8) | N(2) | H(25) | 115.1 | C(10) | N(2) | H(25) | 116.9 |
| S(1) | C(1) | H(1) | 110.2 | C(2) | C(1) | H(1) | 110.1 |
| C(4) | C(1) | H(1) | 110.4 | C(1) | C(2) | H(2) | 110.7 |
| C(1) | C(2) | H(3) | 110.8 | C(3) | C(2) | H(2) | 110.6 |
| C(3) | C(2) | H(3) | 110.7 | H(2) | C(2) | H(3) | 109.5 |
| N(1) | C(3) | H(4) | 110.1 | C(2) | C(3) | H(4) | 110.1 |
| C(5) | C(3) | H(4) | 110.2 | N(1) | C(4) | H(5) | 111.9 |
| N(1) | C(4) | H(6) | 111.8 | C(1) | C(4) | H(5) | 111.9 |
| C(1) | C(4) | H(6) | 111.7 | H(5) | C(4) | H(6) | 109.7 |
| O(2) | C(6) | H(7) | 109.5 | O(2) | C(6) | H(8) | 109.5 |
| O(2) | C(6) | H(9) | 109.5 | H(7) | C(6) | H(8) | 109.5 |
| H(7) | C(6) | H(9) | 109.4 | H(8) | C(6) | H(9) | 109.4 |
| N(2) | C(8) | H(10) | 105.5 | C(7) | C(8) | H(10) | 105.7 |
| C(9) | C(8) | H(10) | 105.5 | C(8) | C(9) | H(11) | 109.4 |
| C(8) | C(9) | H(12) | 109.3 | C(8) | C(9) | H(13) | 109.3 |
| H(11) | C(9) | H(12) | 109.7 | H(11) | C(9) | H(13) | 109.6 |
| H(12) | C(9) | H(13) | 109.5 | N(3) | C(11) | H(14) | 109.7 |
| C(10) | C(11) | H(14) | 109.9 | C(12) | C(11) | H(14) | 110.1 |
| C(11) | C(12) | H(15) | 111.1 | C(11) | C(12) | H(16) | 111.2 |
| C(13) | C(12) | H(15) | 111.2 | C(13) | C(12) | H(16) | 111.3 |
| H(15) | C(12) | H(16) | 109.4 | C(12) | C(13) | H(17) | 110.1 |
| C(12) | C(13) | H(18) | 110.0 | C(14) | C(13) | H(17) | 110.0 |
| C(14) | C(13) | H(18) | 110.1 | H(17) | C(13) | H(18) | 109.7 |
| N(3) | C(14) | H(19) | 110.0 | N(3) | C(14) | H(20) | 111.0 |
| C(13) | C(14) | H(19) | 110.9 | C(13) | C(14) | H(20) | 110.9 |
| H(19) | C(14) | H(20) | 109.6 | S(1) | C(16) | H(21) | 110.1 |
| C(15) | C(16) | H(21) | 109.7 | C(17) | C(16) | H(21) | 110.0 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| C(16) | C(17) | H(22) | 109.6 | C(16) | C(17) | H(23) | 109.3 |
| C(16) | C(17) | H(24) | 109.3 | H(22) | C(17) | H(23) | 109.8 |
| H(22) | C(17) | H(24) | 109.5 | H(23) | C(17) | H(24) | 109.4 |

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