SELF-ASSEMBLY STUDIES WITH HETEROCYCLIC AMINO ACIDS

Anna Bertram

A Thesis Submitted to the University of Nottingham for the degree of Doctor of Philosophy

October 2000

To Mum, Dad, Rich and James

DECLARATION

I declare that the substance of this Thesis has not been submitted, nor is concurrently being submitted in candidature for any other degree. I also declare that the work embodied in this Thesis is the result of my own investigations. If the work of other investigators has been used, then this has been fully acknowledged in the text.

A Bertram

Anna Bertram

G. Pattenden

Prof. G. Pattenden

ACKNOWLEDGEMENTS

I would like to express my sincere thanks to my supervisor Professor Pattenden for his help, advice and encouragement throughout the course of my PhD studies. Thanks also go to Dr Kate Jolliffe for her support and many helpful discussions, and to Dr B Lygo for helping with the molecular modelling. I would also like to thank all the technical staff at the University of Nottingham for all their help, in particular Mr D. Toplis for teaching me all I know about hplc!

I would like to acknowledge the support of AstraZeneca and Pfizer Ltd for partly covering the costs of my research, especially consumable items and spectroscopic services.

Thanks also go to the members of C25 for providing a constant source of entertainment!

Finally I would like to thank my family and James for their endless help and support, and a big thanks to my parents for their financial support.

ABSTRACT

This thesis describes investigations towards the self-assembly of heterocyclic amino acids to form cyclopeptides, including the natural products dendroamide A and nostacyclamide.

The *Introduction* highlights a variety of different natural products including the lissoclinum cyclopeptides and other oxazole and thiazole based natural products. The conformation of the lissoclinum cyclopeptides and the affect different substituents have on their conformation is explored. The ability of a variety of natural products to chelate metal ions and the evidence for metal ion chelation within the lissoclinum cyclopeptides is also discussed. The Introduction is concluded with a statement of the aims and objectives of our research.

The *Results and Discussion* section of the thesis details the development of a novel cyclooligomerisation reaction of heterocyclic amino acids. This cyclooligomerisation is applied to the self-assembly of thiazole and oxazole amino acids to form analogues of naturally occurring cyclopeptides. The protocol is then extended to the self-assembly of the natural products dendroamide A and nostacyclamide. Additionally the ability of metal ions to act as templates and promote the formation of particular products is illustrated throughout these studies. Detailed discussions are also presented within this section into the methods for thiazole and oxazole formation.

The third part of the thesis is the *Experimental* section containing full details of the preparative work completed and listing spectroscopic and analytical data on all new compounds synthesised during this study.

CONTENTS

ABBREVIATIONS

Boc	*tert*-Butoxycarbonyl
BOP	Benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate
CbZ	Carboxybenzyl
DAST	(Diethylamino)sulfur trifluoride
DBU	1, 8-Diazabicyclo[5.4.0]undec-7-ene
DCM	Dichloromethane
DIAD	Diisopropyl azodicarboxylate
DIPEA	*N, N*-Diisopropylethylamine
DME	Ethylene glycol dimethyl ether
DMF	Dimethylfomamide
DPPA	Diphenylphosphorylazide
DPPCl	Diphenylphosphinyl chloride
EDC	1-(3-dimethylaminopropyl)-3-ethylcarbodiimide
Et	Ethyl
EtOH	Ethanol
Et_3N	Triethylamine
FDPP	Pentafluorophenyl diphenylphosphinate
HMTA	Hexamethylene-tetramine
HOBt	1-Hydroxybenzotriazole
IR	Infrared
Me	Methyl
Ph	Phenyl
rt	room temperature
NMM	*N*-Methylmorpholine
NMR	Nuclear magnetic resonance

TBS	*tert*-Butyldimethylsilyl
TFA	Trifluoroacetic acid
TFAA	Trifluoroacetic anhydride
THF	Tetrahydrofuran
tlc	Thin layer chromatography
uv	ultraviolet

INTRODUCTION

1.1 Marine and Terrestrial Secondary Metabolites

The marine environment is a treasury of fascinating and brightly coloured organisms such as sea squirts, sponges, soft corals and molluscs (**Figure 1**). These organisms are a source of a great wealth of novel and unusual secondary metabolites which exhibit a unique molecular architecture. However, despite the apparent wealth of carbon in the marine environment few organisms can exploit this source photosynthetically. It is not then surprising that while marine organisms can produce these tremendously complex organic extracts, each component is present in only trace quantities. A remarkable preference for nitrogenous metabolites derived from amino acids is a characteristic feature of natural products isolated from ascidians. The isolation and structural elucidation of such compounds has only become relatively straightforward since the advent of modern separation and spectroscopic techniques.

Not only do some of these compounds possess a magnificent molecular architecture but they often exhibit a broad range of bioactivities, such as cytotoxicity, tumour promotion, anticancer, antiviral, and anti-inflamatory activities. The immense challenge associated with the total synthesis of such formidable targets, in addition to the problem of isolating pure metabolites in suitable quantities to study their biological activity have, together, long stimulated synthetic chemists. Indeed almost since the discovery of aspirin, and its application in medical practice in the 1890s, natural products have been regarded as an excellent starting point in the search for lead compounds for drug development. Arguably few discoveries of the twentieth century can claim higher notoriety than that of penicillin **1** which was introduced as a drug during World War II and saved countless lives. However, although Alexander Fleming[1] discovered penicillin in 1928 from the secretion of the mold *Pencillium notatum*, its molecular structure which contained the unique and strained β-lactam ring was not confirmed until 1949 by Dorothy Crowfoot Hodgkin[2] by X-ray crystallography.

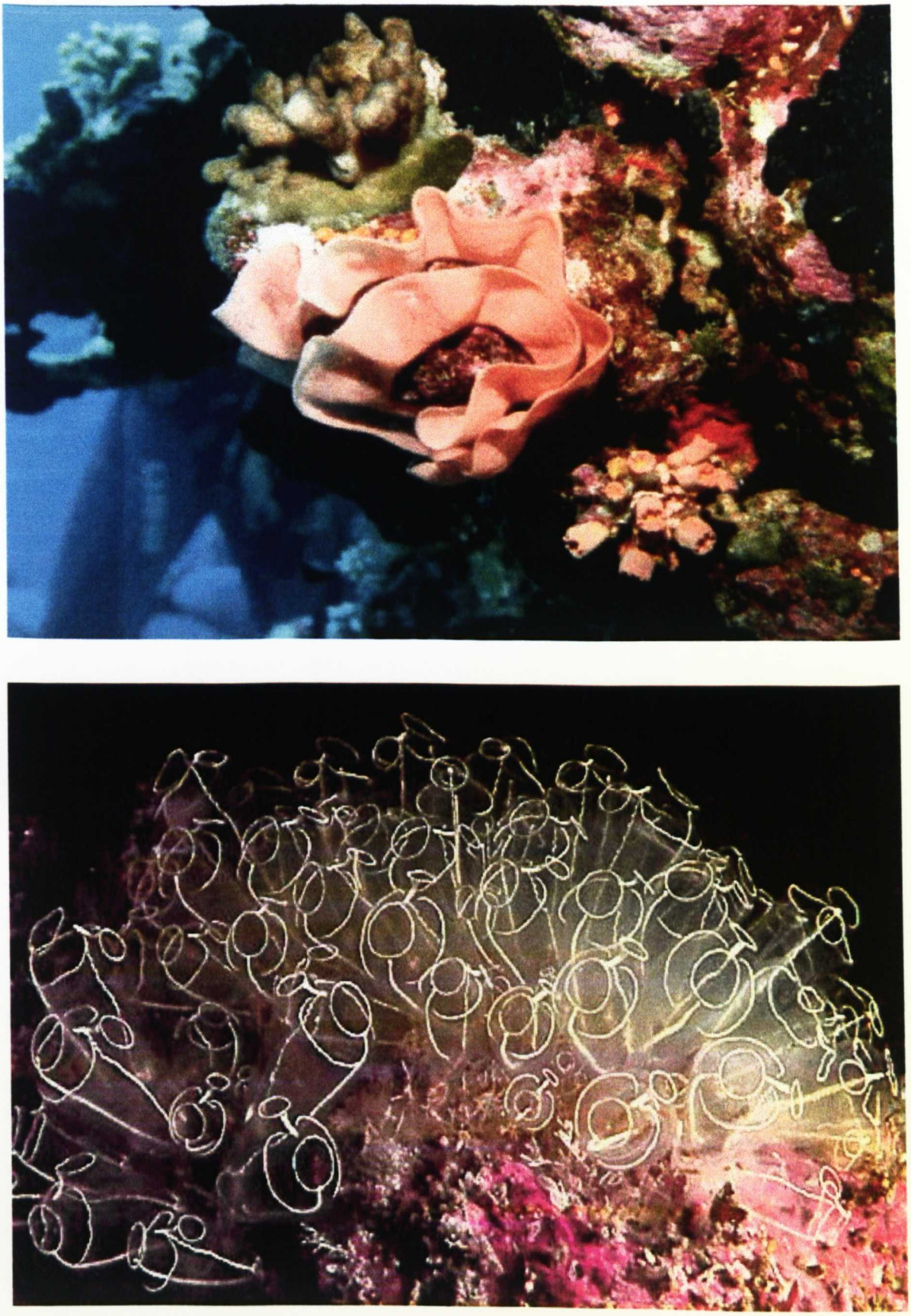
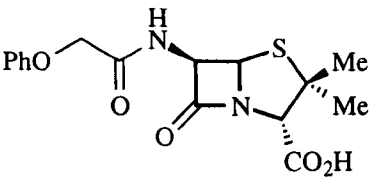
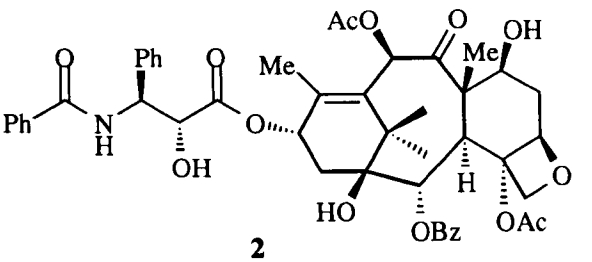


Figure 1 The marine environment displaying its rich source of fascinating organisms (top). An example of a sea squirt from which so many cyclic peptides have been isolated (bottom).

Eight years later Sheehan and Henery-Logan[3] completed the first ever total synthesis. In complete contrast to penicillin, Taxol® **2**, is a further celebrated natural product which was isolated from the pacific yew tree, *Taxus brevifolia,* and its structure reported in 1971.[4] Twenty one years later it was approved by the Food and Drug Administration for the treatment of ovarian cancer,[5] and in 1994 two simultaneous reports by Nicolaou[6] and Holton[7] described two distinctly different total syntheses.

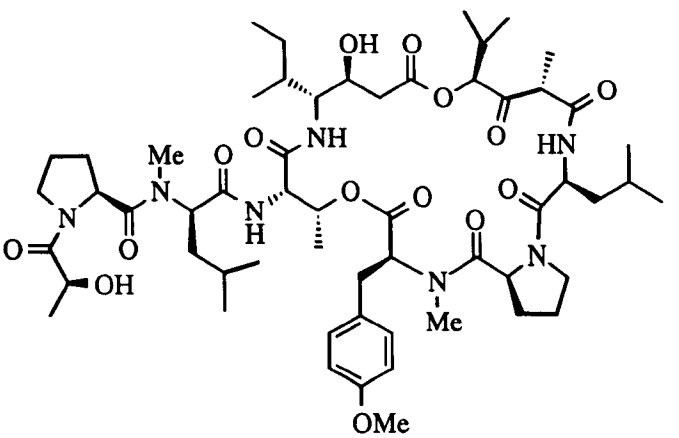


1



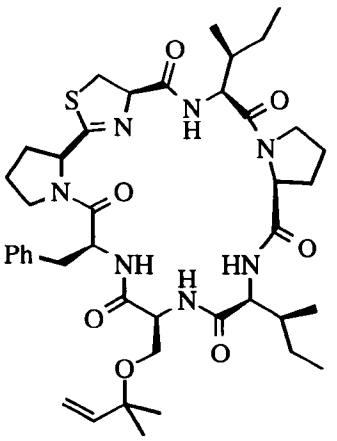
2

In the marine environment one of the major chemical structural classes isolated from ascidians are cyclopeptides. One of the most significant cyclopeptide metabolites isolated is didemnin B **3** which was isolated from the Caribbean tunicate *Tridemnum solidum* in 1981.[8] Didemnin B was the first marine natural product to enter clinical trials as a potential anticancer agent. It has also been reported to be extremely active as an immunosuppressive agent, in addition to inhibiting *herpes simplex* virus types I and II and other lethal RNA viruses. The first total synthesis of didemnin B was reported in 1987 by Rinehart *et al.*[9]

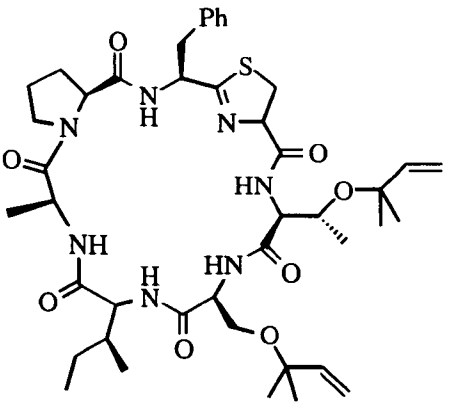


3

Mollamide **4**, which is a cyclic heptapeptide, was isolated in 1994 from the ascidian *Didemnum molle* and shows cytotoxicity against a range of cell lines. It belongs to a rare family of cyclic peptides where the structure has been modified by the inclusion of a reverse prenyl unit associated as a serine ether residue.[10] Another interesting member of this family is the doubly prenylated compound, trunkamide A **5** isolated from the ascidian *Lissoclinum patella* in 1996.[11] A total synthesis of mollamide was reported in 1999 by McKeever and Pattenden.[12] Pattenden and Wipf and their respective colleagues then simultaneously synthesised trunkamide A **5** in 2000 with a revised stereochemistry.[13]



4



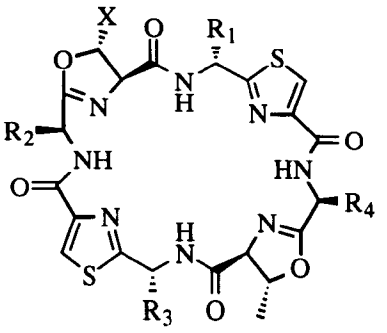
5

Didemid ascidians, especially of the *Lissoclinum* species isolated from the Great Barrier Reef, have been prolific sources of new classes of biologically active cyclopeptides. The patellamides, lissoclinamides and patellazoles have all been isolated from *Lissoclinum patella* and the bistratamides and bistratenes were isolated from *Lissoclinum bistratum.* Their complex 18 to 24 membered hexa-, hepta-, and octapeptide macrocycles are characterised by the presence of highly modified amino acid residues: thiazoles, thiazolines, oxazoles, and oxazolines. Some of these metabolites are potent cytotoxins, for example ulithiacyclamide **8** and lissoclinamide 7 (**9g**), while others such as the bistratenes cause human cells to differentiate.

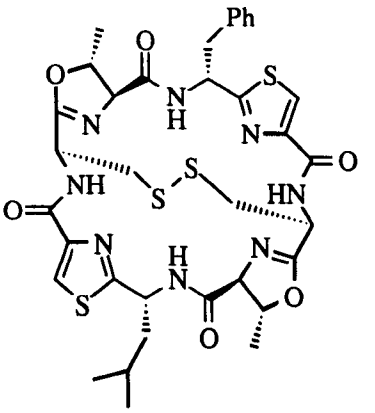
1.2 The Lissoclinum Cyclopeptides

The most widely investigated tunicates are those of the genus *Lissoclinum patella*, the metabolites of which differ with geographical location. The largest group of compounds consists of a range of cyclic hepta- and octapeptides distinguished by the presence of thiazole (or thiazoline) and oxazoline rings in the macrocyclic periphery.

The patellamides A-F (**6a-f**),[14] ulithiacyclamide **8**,[15] and the C_2-symmetric ascidiacyclamide **7**[16] make up the group of cyclic octapeptides. The similarities within the octapeptides are very marked; they all possess two oxazoline, and two thiazole moieties which are alternatively linked to form the core macrocycle. Unlike the patellamides, ulithiacyclamide **8** incorporates a disulfide bridge forming a strap across its slightly convex surface which is thought to contribute to making it the most potent cytotoxin in this group.[17]

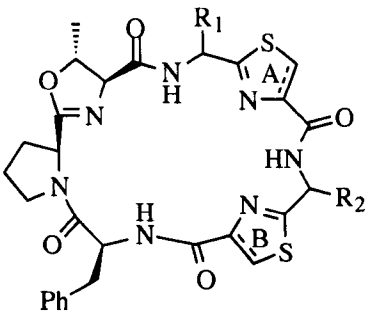


	X	R_1	R_2	R_3	R_4
6a	H	(*R*)-Val	(*S*)-Ile	(*R*)-Val	(*S*)-Ile
6b	Me	(*R*)-Ala	(*S*)-Leu	(*R*)-Phe	(*S*)-Ile
6c	Me	(*R*)-Ala	(*S*)-Val	(*R*)-Phe	(*S*)-Ile
6d	Me	(*R*)-Ala	(*S*)-Ile	(*R*)-Phe	(*S*)-Ile
6e	Me	(*R*)-Val	(*S*)-Val	(*R*)-Phe	(*S*)-Ile
6f	Me	(*R*)-Val	(*S*)-Val	(*R*)-Phe	(*R*)-Val
7	Me	(*R*)-Val	(*S*)-Ile	(*R*)-Val	(*S*)-Ile



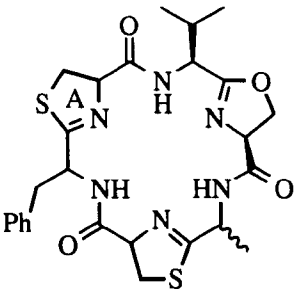
8

The heptapeptides isolated from the aplousobranch ascidian *Lissoclinum patella*[18] include lissoclinamides 1-8 (**9a-h**) and ulicyclamide **10**.[16] They conform to a common macrocycle disposition, which includes one oxazoline ring and two thiazole or thiazoline derived amino acids.

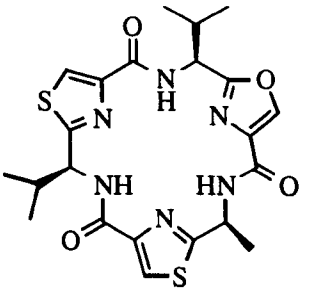


	R_1	R_2	A	B
9a	(*S*)-Val	(*R*)-Ile	thiazole	thiazole
9b	(*R*)-Ile	(*R*)-Ala	thiazoline	thiazole
9c	(*R*)-Ile	(*S*)-Ala	thiazoline	thiazole
9d	(*S*)-Val	(*R*)-Phe	thiazoline	thiazole
9e	(*S*)-Val	(*R*)-Phe	thiazole	thiazole
9f	(*R*)-Val	(*R*)-Phe	thiazoline	thiazole
9g	Val	(*R*)-Phe	thiazoline	thiazoline
9h	Val	(*R*)-Phe	thiazoline	thiazole
10	(*S*)-Ile	(*R*)-Ala	thiazole	thiazole

The bistratamides A-D (**11-13**) are cyclic hexapeptides isolated from *Lissoclinum bistratum* in Australia,[19] and the Philippines,[20] and are characterised by the presence of one oxazoline or oxazole derived amino acid and two thiazoline or thiazole derived amino acids.



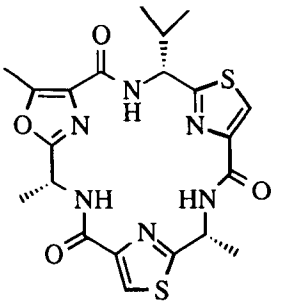
11 (A = Thiazoline)
12 (A = Thiazole)



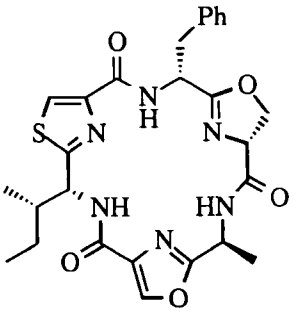
13

Another interesting hexapeptide is the C_3-symmetric cyclo-oxazoline westiellamide **17**, isolated from both an ascidian *Lissoclinum bistratum*[21] and a terrestrial blue-green algae *Westiellopsis prolifica*.[22] In addition to marine ascidians terrestrial freshwater and marine cyanobacterial species have been shown to be prolific sources of secondary metabolites including cyclic peptides and depsipeptides. More recently terrestrial blue-

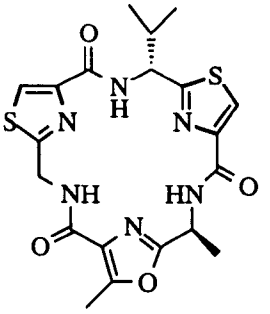
green algae have provided several 'bistratamide type' cyclic hexapeptides. Thus, dendroamides A-C *e.g.* dendroamide A **14** were isolated in 1996 from the cyanobacterium, *Stigonema dendroideum*[23] and raocyclamide A **15** and nostacyclamide **16** were obtained from the cyanobacterium *Oscillatoria raoi*[25] and *Nostoc* sp.[24] respectively.



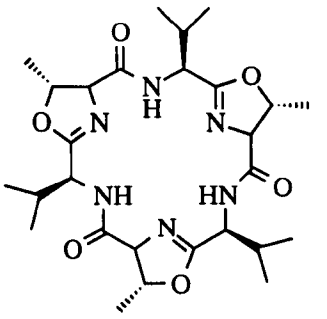
14



15



16



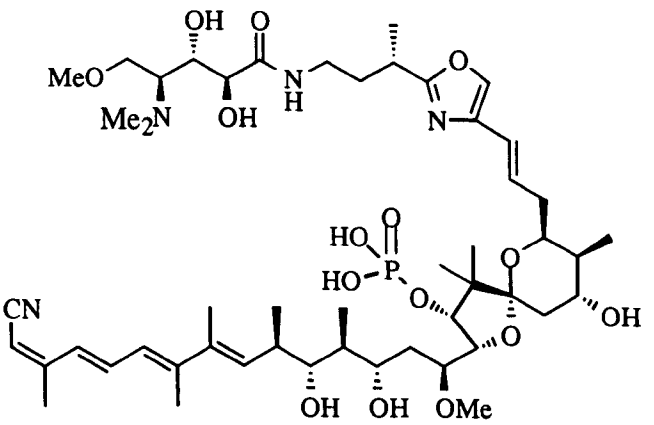
17

Interestingly all of these natural products contain at least one unnatural D-amino acid residue. Indeed all the amino acid residues in dendroamide A **14** are derived from D-amino acids! This intriguing property of the lissoclinamide cyclopeptides prompts chemists to ask why does nature incorporate these unnatural residues? Are they incorporated early on in the biosynthesis, or does epimerisation take place late on in the biosynthesis? These are some questions which will be explored later in this Thesis.

1.3 Azole Containing Natural Products

Oxazole-containing natural products often possess significant biological activities, including antifungal, cytotoxic, and tumour promoting properties. A large proportion of these secondary metabolites incorporate isolated oxazole rings, *e.g.* phenoxan **19**. More recently interest has grown in natural products containing two or three contiguously linked oxazoles. Of the vast array of oxazole containing natural products now isolated a select number have stimulated particular interest due to their unique molecular architecture and biological activity, and a few of these are highlighted below.

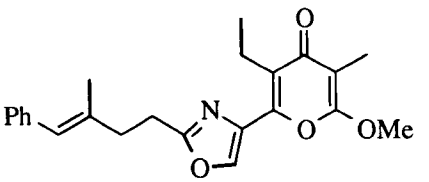
Calyculin A **18** was isolated from the marine sponge, *Discodermi calyx* in 1986.[26] Calyculin A is a novel spiro-ketal with an unprecedented skeleton containing a 2, 4-disubstituted oxazole moiety amongst a wide array of functionality which includes a phosphate and cyanotetraene. Calyculin A has generated considerable interest owing to its ability to inhibit protein phosphatases 1 and 2a, two of the four major protein-serine/threonine phosphatases. The total synthesis of calyculin A has been reported by the research groups of Evans,[27] Masamune, [28] and Smith.[29]



18

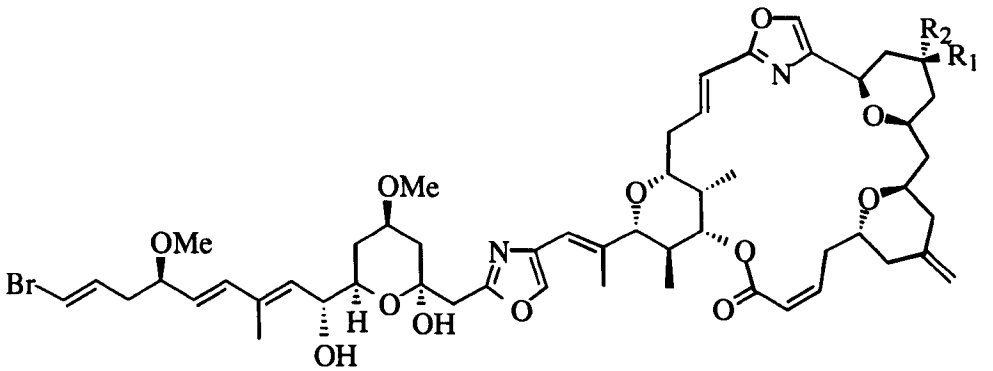
One of the simplest oxazole containing natural products is phenoxan **19**. Phenoxan was isolated in 1991 from a soil micro-organism and was shown to exhibit anti-HIV activity.[30] The structure of phenoxan is characterised by the pyran-4-one ring fused to the oxazole in the 4-position, a structural feature analogous to the oxazole-pyran sub-

unit contained within the phorboxazoles (A and B) **20** and **21**. To date there have been two published syntheses of phenoxan **19**.[31]



19

The phorboxazoles were isolated from the Indian Ocean marine sponge *Phorbas* sp. in 1995 by Molinski and Searle.[32] Their gross structure is based upon a 21-membered macrolactone ring and embodies three tetrahydropyran rings, four oxetane rings and two 2, 4-disubstituted oxazole rings. The phorboxazoles also possess exceptional biological activity, exhibiting cytotoxic, cytostatic and antifungal properties. A total synthesis of phorboxazole A was completed in 1998 by Forsyth *et al.*[33] and then by Evans *et al.* in 2000.[34]

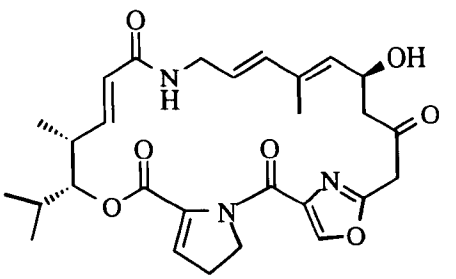


20 $R_1 = H, R_2 = OH$

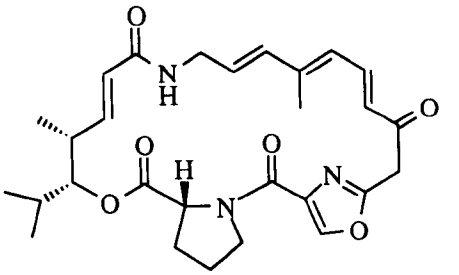
21 $R_1 = OH, R_2 = H$

One of the first families of oxazole containing natural products to be characterised were the group A virginiamycins family of antibiotics.[35] The group A virginiamycins are all based on a common 23-membered macrolactone core incorporating a variety of functionality including a 2, 4-disubstituted oxazole, a 1, 3-diene, an acrylamide unit and an amino acid residue. Both virginiamycin M_2 **22** and 14, 15-anhydropristinamycin II_B

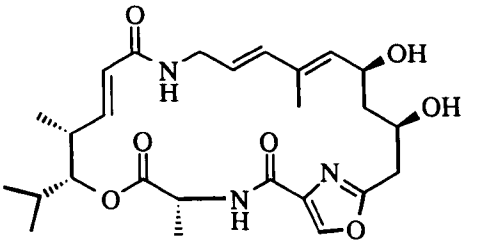
23 act as cholecystokinin antagonist for treating panic and anxiety.[36] A total synthesis of virginiamycin M_2 **22** was finally reported in 1996 by Schlessinger *et al.* some 30 years after its isolation.[37] The total synthesis of madumycin II **24**, by Meyers *et al.*,[38] and of the related antibiotic 14, 15-anhydropristinamycin II_B **23**, by Pattenden *et al.*,[39] were reported simultaneously.



22

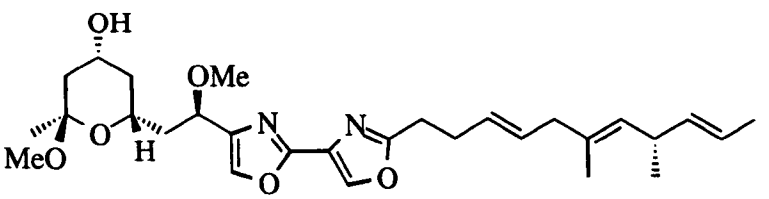


23



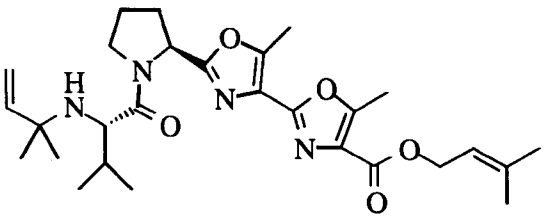
24

Natural products possessing two or three contiguously linked oxazole rings have also been isolated. Hennoxazole A **25**, a *bis*-oxazole containing natural product was isolated from the marine sponge *Polyfibrospongia* sp.[40] and is highly active against the herpes simplex virus. The diverse structure of hennoxazole A **25** incorporates a 2, 4-disubstituted *bis*-oxazole, a pyranoid glycoside and an unusual skipped triene unit. Wipf *et al.* reported the total synthesis of the unnatural enantiomer of hennoxazole A **25** in 1995,[41] and this was followed in 1999 by the total synthesis of hennoxazole A by Williams *et al.*.[42]



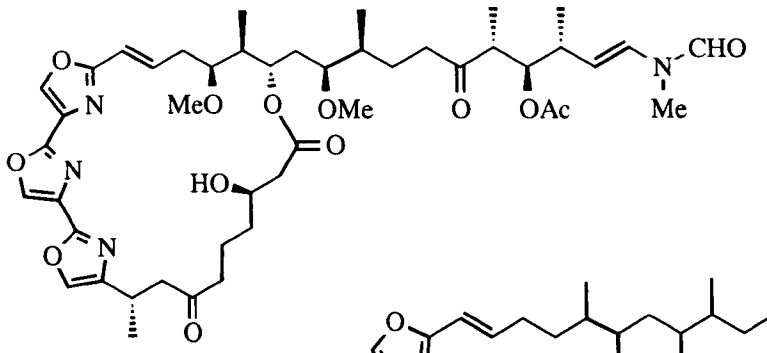
25

Muscoride A **26** is another novel *bis*-oxazole based peptidic alkaloid isolated from the freshwater cyanobacterium *Nostoc muscarum* in 1995.[43] It has weak antibacterial activity and is related structurally to hennoxazole A **25**. The *bis*-oxazole core of muscoride A **26** is formally derived from two threonine residues, which is rather remarkable since even isolated oxazoles derived from threonine are rare. The total synthesis of muscoride A **26** was reported by Wipf *et al.* in 1996,[44] and in 1998 by Pattenden *et al.*[45]

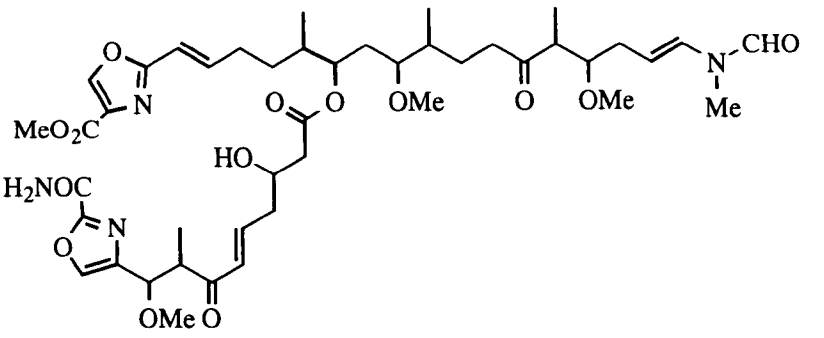


26

A family of marine metabolites known as the ulapualides which include the halichondramides,[46] the kabiramides[47] and the mycalolides[48] have structures based on three contiguously linked 2, 4-disubstituted oxazole rings. The ulapualides were first isolated from the egg masses of the nudibranch *Hexabranchus sanguineus* and some members of the family possess an interesting biological profile including antifungal, anti-leukaemic and ichthyotoxic activity.[49] A total synthesis of ulapualide A **27** was completed by Chattopadhyay and Pattenden in 1998.[50] However, the isolation of the halishigamides in which one oxazole ring of the *tris*-oxazole backbone is incomplete, *e.g.* halishigamide D **28**, has stimulated much speculation about the possible biosynthesis of these compounds. It would be quite reasonable to suggest that oxazole formation may take place late on in the biosynthesis, maybe even as the last step. This postulation paved the way for a new synthetic approach to ulapualide A in which the middle oxazole of the *tris*-oxazole backbone is formed during the last step in the synthesis.[51]

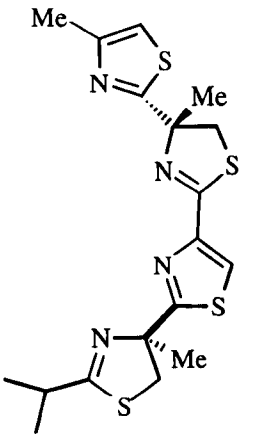


27

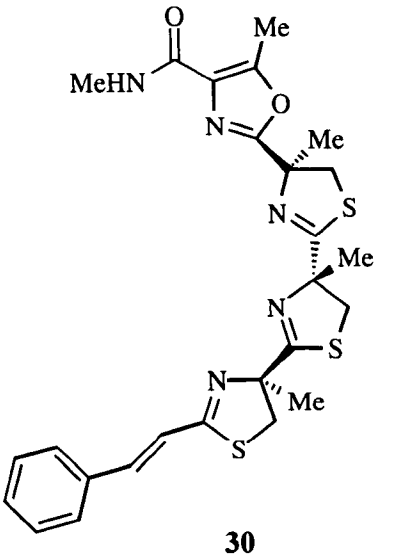


28

Similarly interesting compounds possessing contiguously linked sulfur containing azoles have also been isolated. The mirabazoles are a unique family of thiazole/thiazoline based cytotoxic alkaloids isolated from the blue-green algae *Scytonema mirabile*.[52] Their structures, including didehydromirabazole A **29** together with the related tantazoles, (*e.g.* thiangazole **30**, isolated in 1992 from the bacterium *Polyangium* sp.)[53] have aroused considerable interest amongst synthetic chemists as a result of their novel biological properties. Total syntheses of didehydromirabazole and thiangazole were completed by Pattenden *et al.* in 1994[54] and 1995[55] respectively. In addition Ehrler and Farooq[56] reported a total synthesis of thiangazole **30** in 1994 utilising Heathcock's $TiCl_4$ protocol for the cyclodehydration of a series of cysteine amides.[57] Within the same year Heathcock and Parsons reported the total synthesis of thiangazole,[58] and just a year later Wipf and Venkatraman completed a total synthesis.[59]



29



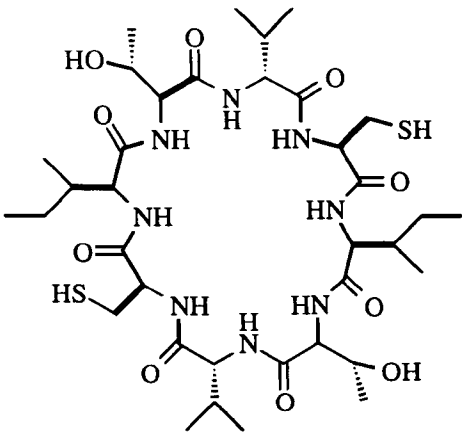
30

1.4 Conformations of Cyclopeptides

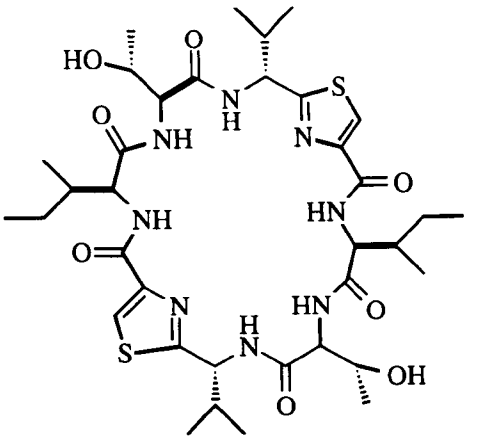
Several aspects of the diverse structure and properties of marine metabolites render them particularly interesting to synthetic and medicinal chemists alike. While their varied and intriguing architecture initially attracts the synthetic chemist, questions relating to their conformation, biological activity and possible metal ion chelation attracts the medicinal chemist. The structural variety of oxazole and thiazole containing alkaloids that have recently been isolated from both marine and terrestrial micro-organisms is astounding. The key questions are: a) what is the relationship between chemical structure and molecular conformation, and b) how is their biological activity dependant on their conformation? Cyclic peptides exhibiting potent cytotoxic activity share a common ring structure and it has long been postulated that this may be the key feature in promoting their cytotoxic activity.

The formation of the macrocycle from the linear oligomer restricts conformational freedom of the polypeptide backbone with respect to the open chain, but as the cycle increases in size additional constraints become necessary to control conformation. Nature utilises a number of devices to restrict conformational freedom in peptides, including disulfide bonds (ulithiacyclamide **8**), H-bonds, salt bridges, and metal ions, but the most common devices are molecular constraints. These constraints come in the

form of azole heterocycles namely oxazoles, oxazolines, thiazoles, and thiazolines. Indeed, a study carried out by Fairlie *et al.*[60] demonstrates the conformational control induced by the formation of azole heterocycles. These authors studied the octapeptides **31** and **32**. Comparison of NMR data for the cyclic peptide **31** containing no azole heterocycles with that for the cyclic peptide **32** in which two thiazole rings are present did indeed show a reduction in flexibility.

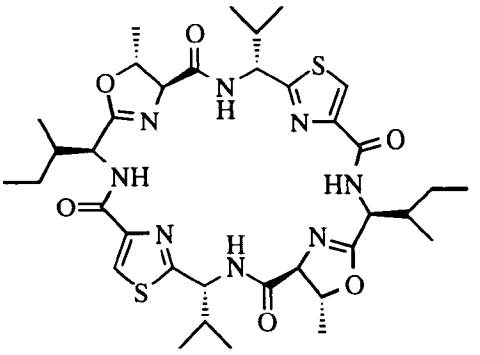


31

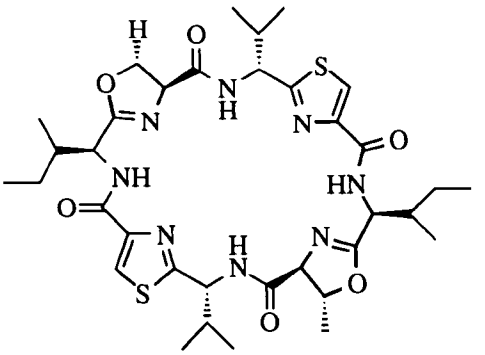


32

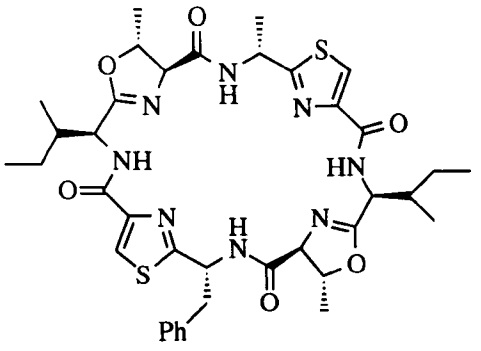
The increase in rigidity of the molecule by the introduction of the two thiazoles is thought to induce clefts and unusual shapes within the molecule which may provide sites for metal chelation and be a major factor contributing to the bioactivity of some metabolites. In an attempt to further elucidate the relationship between chemical structure, conformation and biological activity, Schmitz *et al.*[61] analysed the conformations of the cyclic hexapeptides; ascidiacyclamide **7**, patellamide A **6a**, patellamide D **6d**, and ulithiacyclamide **8**.



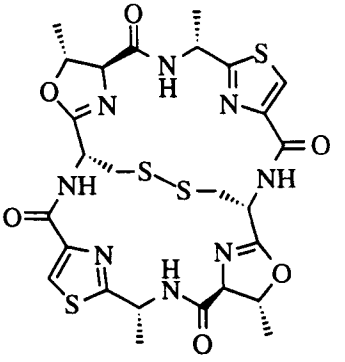
7



6a



6d



8

The molecular conformations of these cyclic hexapeptides were found to be closely related to their chemical structures, and were found to be especially dependent on the degree of asymmetry induced in the molecule by the side chains tethered to either half of the C_2-symmetric backbone. It was deduced that molecules possessing C_2-symmetry (*e.g.* ascidiacyclamide **7**, and ulithiacyclamide **8**) adopted a 'type 1' conformation (**Figure 2**). Indeed a small deviation from the C_2-symmetry as present in patellamide A **6a** at the oxazoline methyl group was not sufficient to deform the conformation from that of 'type 1'. However, the less subtle deviations from C_2-symmetry in patellamide B **6b**, C **6c**, and D **6d** resulted in a twisted, figure of eight conformation as indicated by 'type 4', stabilised by four intra-molecular hydrogen bonds (**Figure 2**).

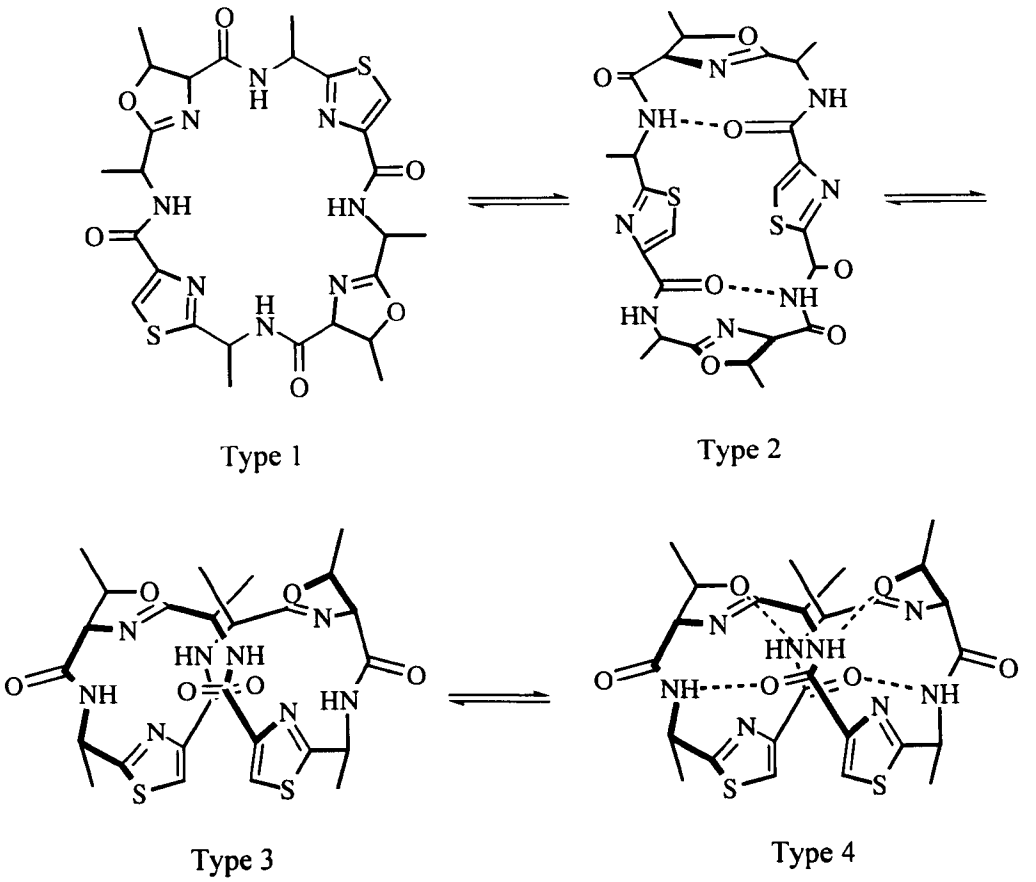


Figure 2: Molecular Conformations of Cyclic Octapeptides

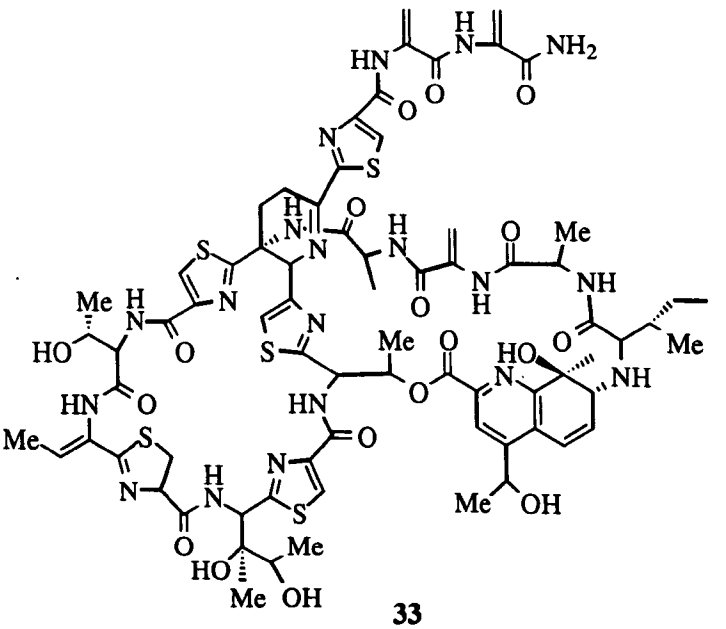
Ulithiacyclamide is locked conformationally by the disulfide linkage into a 'type 1' conformation, and as it is the most potent cytotoxic compound among the related peptides the 'type 1' conformation was proposed to exert the greatest biological activity.

1.5 Biosynthesis of Cyclopeptides

The marine environment is relatively rich in carbon, buffered by carbonate and bicarbonate to pH 8.2-8.5, but few organisms other than algae can exploit this carbon source photosynthetically. Important building blocks such as amino acids and sugars are available at only parts per billion levels, while nitrogen which is principally available as the ammonium or nitrate ion is generally the limiting nutrient. It is not then surprising that although the marine environment is rich in life forms the formidable compounds it produces are formed very slowly and in only trace amounts. It has

generally been assumed that the pathways involved in the formation of marine metabolites do not differ substantially from those that have been established for metabolites in terrestrial animals and plants. However, consideration of the variations in terrestrial and marine metabolism uncovers some striking differences. For example, halogens and isocyanide groups appear frequently as substituents in marine metabolites, yet they are rarely observed in products of terrestrial metabolism.

The biosynthesis of the thiopeptide antibiotic thiostrepton **33** has been investigated by administration of isotopically labelled precursors to cultures of *Streptomyces azureus* and *Streptomyces laurentii* and the amino acid origin of all components of the antibiotic were determined. The four thiazoles, as well as the thiazoline ring and their attached carboxy groups, each arise from the condensation of a molecule of cysteine and the carboxyl group of the adjacent amino acid.[62] However, this study does not provide any information about the order of assembly of the amino acids. This raises the question of whether thiazole/oxazole based marine cyclopeptides in particular, the lissoclinum cyclopeptides are formed by the assembly of preformed oxazole/thiazole based amino acids residues or whether the heterocyclic rings are elaborated late on in the biosynthesis following macrocyclisation to form the cyclopeptide core.



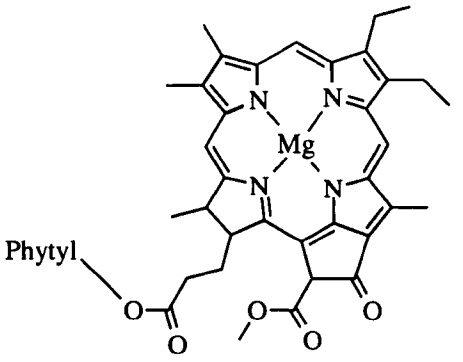
33

A large number of cyclic peptides isolated from both marine and terrestrial organisms exhibit quite an unusual, and as yet unexplainable trait where thiazole and dihydrothiazole amino acids exist in both (*L*) and (*D*) forms with the latter predominating. It is not clear whether differences such as these reflect the individuality of the producer organism or are more fundamentally important, possibly of evolutionary significance. Is this feature associated with some specific folding of a peptide chain prior to macrocycle formation? The origin of optical activity is not clear. Some reports have suggested that it may be related to the difference in catalytic activity of (*L*)- and (*D*)-amino acids which permit the selection of optically active catalysts at early stages in development. In a study, (*L*)-cysteine was oxidised with iron over a wide range of pH values (6-11.5) in the presence of complexes of (*D*)- and (*L*)-alanine. At pH 7 the catalytic activity of the (*D*)-alanine iron complex was seven times greater than the (*L*)-alanine complex.[63] Although it is only speculated that such processes could be responsible for the unusual distribution of chirality in the marine environment it raises the question yet again, do metals play a part in marine metabolism?

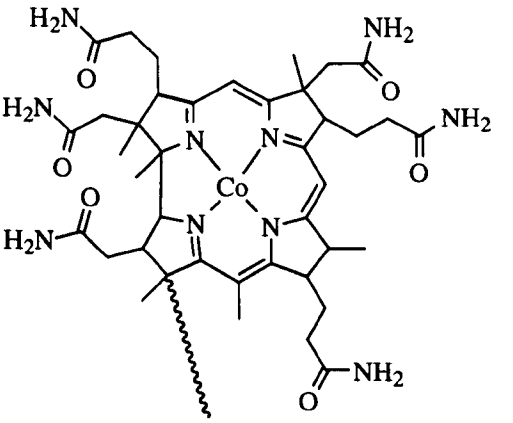
1.6 Metal Chelation of Natural Products

Metal ions are essential to the proper functioning of all living cells, and it is critical that the minute amounts of ions required be delivered to the right compartments and that they be present in the correct oxidation state and co-ordination geometry to carry out selective functions. In fact each organism could be considered as a highly tuned multi-metal, multi-ligand system capable of both controlling the amounts of each of these elements delivered and distinguishing between them. All of these requirements can be and are achieved by selective chelation. Indeed without chelation as a control any free transition metal ions would bind indiscriminately to a range of biological molecules with concomitant physiological disorders.

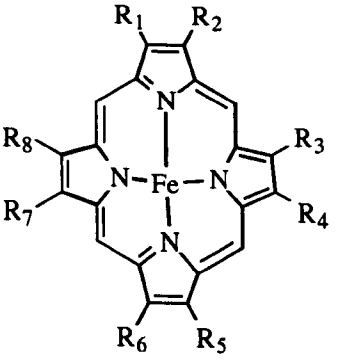
It is believed that chelation could have played a role in early protein nucleation and in the development of stereoselectivity in biological molecules. Indeed evidence that complex ligands were formed early on in the stages of biological evolution was found in the discovery of metal-porphyrin complexes in the biolithosphere. Porphyrins are widely found in nature as metal complexes; as magnesium complexes in the chlorophylls, for example chlorophyl A **34**; as cobalt complexes in the corrinoids, for example vitamin B_{12} **35**, and in the haem groups of many iron proteins. Iron-porphyrin proteins share a general structure **36** and differ from one another by the nature of the pyrrole substituents.



34



35

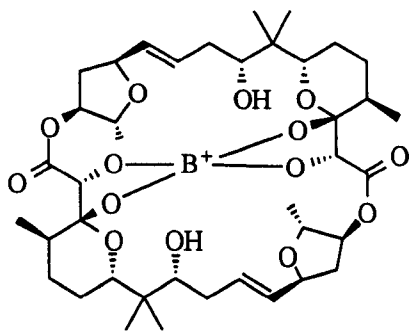


36

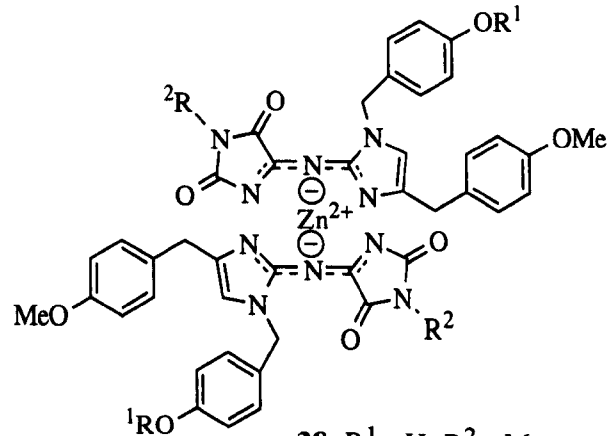
The seas and oceans contain substantial amounts of dissolved inorganic salts, common metal cations such as sodium, potassium, magnesium, and calcium are present in concentrations often in excess of 10^{-3} M, while important trace elements such as vanadium, manganese, cobalt, copper, zinc and molybdenum often reach concentrations as high as 10^{-6} M.[64] It would be quite amazing if this wealth of metal ions was

insignificant in the modes of formation and origins of the marine metabolites. Indeed the organisms that produce these metabolites concentrate metal ions, including copper to 10,000 times that found in the local marine environment. This provides further evidence that these metabolites do sequester metals. Furthermore the ability of terrestrial metabolites to act as ionophores provides a premise for the possible involvement of metal ions in marine metabolism. Can marine metabolites sequester and transport metal ions *in vivo*? Do metal ions provide a convenient template for biological assembly of the metabolites? Could metal-ligand complexation play a part in the pronounced biological activity of many of these compounds?

Many marine natural products have structural features which would make them ideal for interacting with metals, for example the presence of macrocylic cavities and polar functional groups in chelating positions with the potential for wrapping around metal ions, however, as yet few examples exist in the literature. A novel boron containing antibacterial metabolite was isolated from the cultured marine microorganism *Streptomyces griseus* in which boron is encapsulated into the macrocyclic cavity as a tetracoordinate borate **37**.[65] In addition a novel mixed polyketide-depsipeptide, jasplakinolide isolated from a marine sponge was found to form a complex with lithium.[66] More recently Schmitz *et al.* isolated two zinc complexes derived from isonamidine B and D **38** and **39**, from a pacific sponge, *Leucetta*.[67]



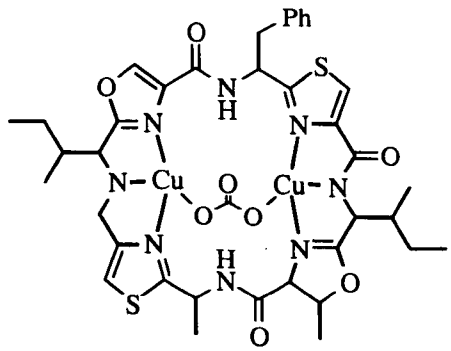
37



38 R^1= H, R^2= Me

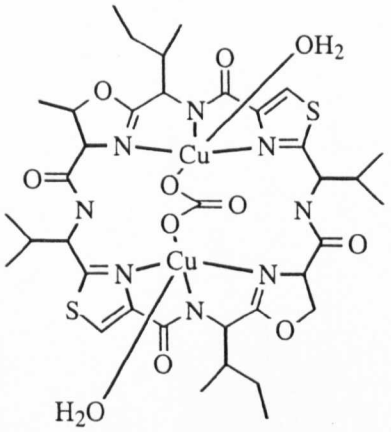
39 R^1= H, R^2=H

The lissoclinum cyclopeptides have an almost symmetrical array of oxazolines, oxazoles, thiazolines, and thiazoles which is reminiscent of ring-extended porphyrins and aza-crown ethers, but the role of these cyclopeptides as ligands in metal ion complexation and transport within their environment has not yet been fully elucidated. The cyclic octapeptides posses a 24-aza-crown-8 structure which is ideally set up for chelating a metal, but as yet only a handful of metal complexes have been isolated. Fairlie *et al.* uncovered a number of copper complexes with the cyclic octapeptide, patellamide D **6d**. Patellamide D **6d** was found to form multiple mononuclear copper(II) complexes in which there are three ligating nitrogen atoms coordinated to the copper(II) ion and the fourth binding site is occupied by a chloride ion, water or carbonate. Three binuclear copper (II) complexes, $[Cu_2(pat.H_2)]^{2+}$, $[Cu_2(pat.H_2)(OH)]^{+}$ and $[Cu_2(pat.H_2)(CO_3)]$ **40** were also isolated.[68]

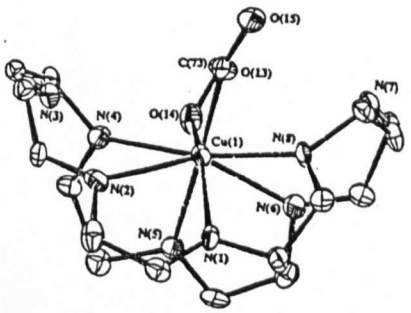


40

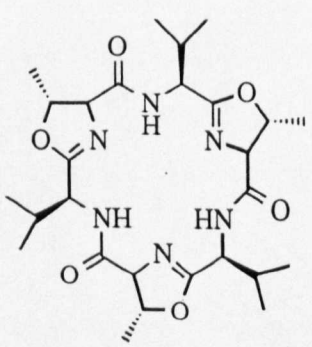
Fairlie *et al.* also isolated blue crystals of a *bis*-copper(II) complex of ascidiacyclamide **7** in which the copper ion is coordinated by three nitrogen donors, one each from an oxazoline, a thiazole, and a deprotonated amide. A water molecule and the oxygen atom of a bridging carbonate anion complete the coordination sphere **41**.[69] The formation of three complexes of ascidiacyclamide **7** with zinc have also been reported,[70] along with a novel potassium binding hydrolysis product of ascidiacyclamide.[71] In addition, work within our own group has shown the ability of the patellamides, A, B and E **6a**, **b** and **e** to bind one or two copper or zinc ions.[72]



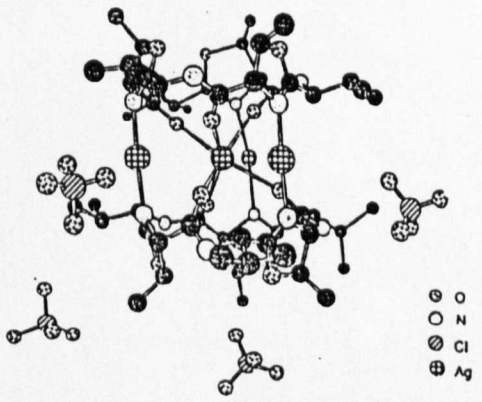
41



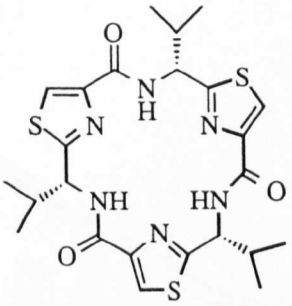
Biological activity and metal binding properties are also said to be critically dependent upon the presence of oxazoline rings which are shown to induce a boat shaped cleft ideal for encapsulating a metal. A prime example of the ability of oxazoline containing natural products to complex metals is the silver complex of the highly modified cyclic peptide, westiellamide **17**.[22] Wipf *et al.* isolated a novel $[Ag_4(\mathbf{17})_2]$ complex in which all N-H bonds and the lone pairs of the oxazoline nitrogen atoms are directed towards the interior of the 18-membered macrocycle.[73] This complexation induced drastic changes in the geometry of the macrocycle with a previously 'flat' macrocycle being turned 'inside out.' The amide carbonyls rotated from pointing out of the ring to pointing inwards and the oxazolines adopted a position orthogonal to the macrocycle. This allowed the oxazoline nitrogen atoms and the amide carbonyl oxygen atoms to complex to silver.



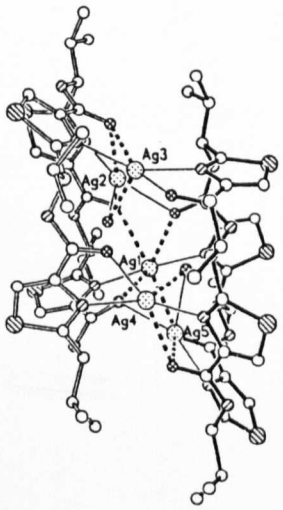
17



Recent work by Jolliffe and Pattenden using analogues of naturally occurring cyclic peptides **42** culminated in the isolation of a cyclic peptide sandwich in which five silver atoms were bound between two cyclic peptide rings.[74]



42



1.7 Aims and Objectives

Nature has delivered a plethora of structurally novel and unusual peptidic alkaloids. One group of peptidic alkaloids, the lissoclinum cyclopeptides possess macrocyclic structures accommodating thiazole and oxazole rings. Little is known about the order of the assembly of the constituent amino acids to form these metabolites. Is the macrocyclic core formed at an early stage in the biosynthesis and the heterocyclic rings elaborated later on *in vivo* to form the cyclopeptide? or are these cyclopeptides formed *via* the self-assembly of preformed oxazole/thiazole based amino acids residues?

These ring systems are clearly reminiscent of macrocyclic ligands such as aza-crown ethers and extended porphyrins. There has therefore been much speculation about the possible role of metal ions as convenient templates for the biological assembly of these cyclopeptide metabolites.

The aim of this research is to attempt to find some answers to these questions:-

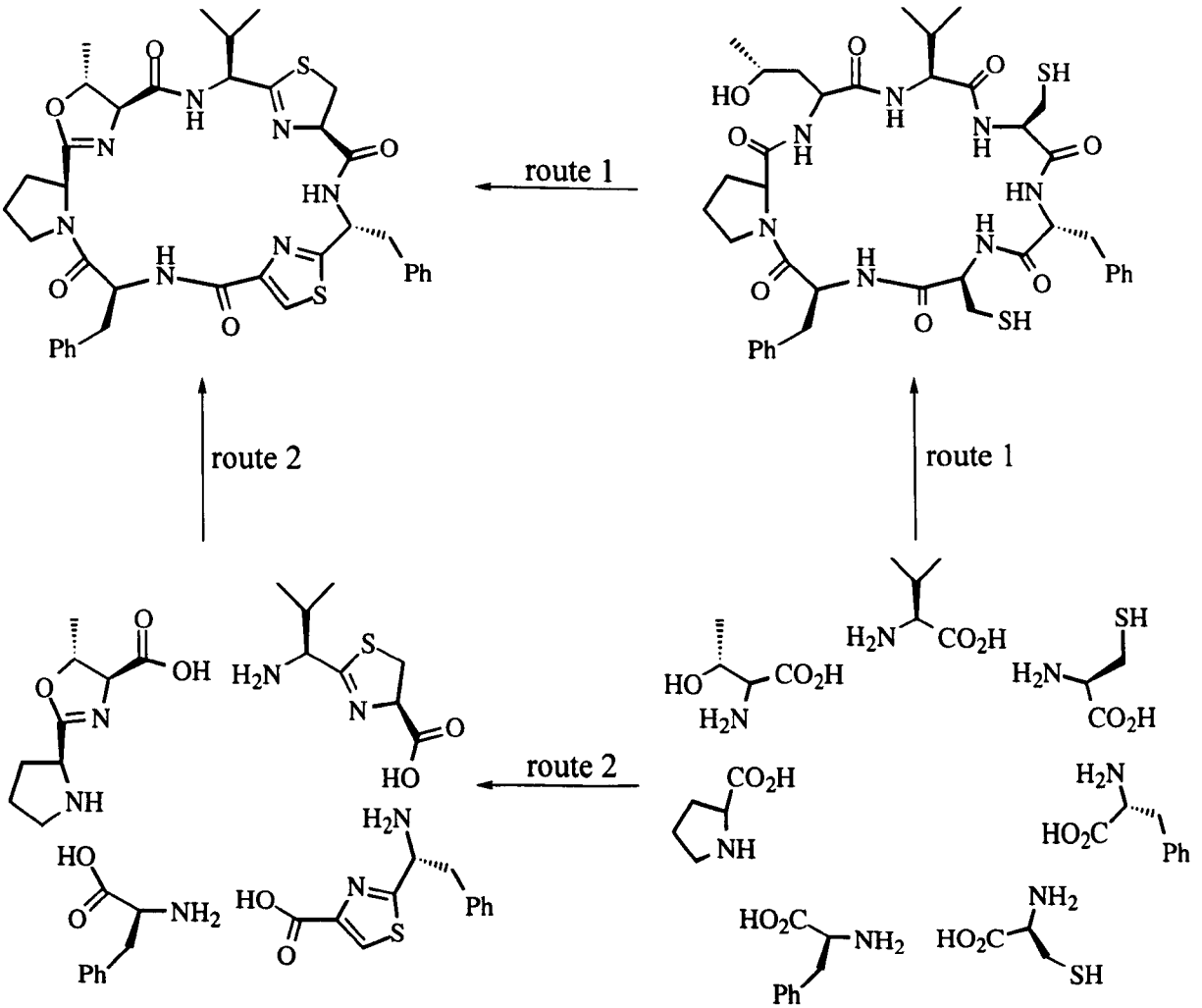
- Are the lissoclinum cyclopeptides elaborated *in vivo* by the self-assembly of preformed oxazole/thiazole based amino acid residues?

- Could metal ions be used as templates to tailor the distribution of products formed from a self-assembly reaction of azole heterocycles?

RESULTS AND DISCUSSION

2.1 The Questions Inspired by Nature

Returning to the lissoclinum cyclic peptides, when considering the biosynthesis of these types of compounds, two main pathways can be envisaged for their formation from constituent amino acids:- Firstly the macrocyclic core could be formed early on in the biosynthesis, *via* a series of peptide coupling reactions from the constituent amino acids. The natural product could then be elaborated from the cyclic core *via* a series of cyclodehydration reactions to form the heterocycles late on in the biosynthesis (route 1). Alternatively, the heterocyclic components of the cyclopeptide could be formed early on in the biosynthesis. The heterocycles could then self-assemble, and, following a series of peptide coupling reactions, form the macrocycle core, possibly as the final step in the biosynthesis (route 2) (**Scheme 1**).



Scheme 1

There is another question which must be addressed. Thus, if the organisms that produce these metabolites can concentrate metal ions to 10, 000 times that found in the local marine environment, do these ions play a role in the formation of the cyclic compounds? Could they provide a convenient template for the biological assembly of these metabolites? Perhaps metal ions are responsible for bringing together heterocyclic amino acids and holding them in the required conformation and proximity to one another to facilitate macrocylisation (**Figure 3**). This is not such an outrageous idea, since the cyclic octapeptides possess a 24-aza-crown-8 structure which is perfectly set up for chelating a metal and examples of such complexes already exist in the literature (*cf* **section 1.6**, p 19-24).

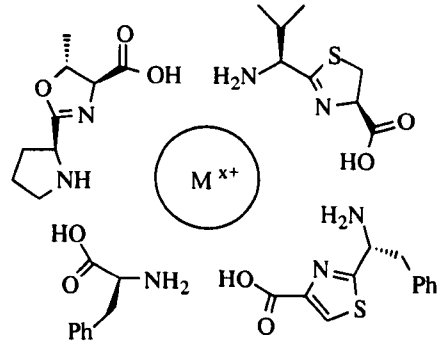


Figure 3

At the outset of this project our goal was to tackle the question of how nature constructs such heterocyclic-based cyclopeptides. To this end we decided to investigate the self-assembly of thiazole and oxazole heterocycles to form analogues of naturally occurring cyclopeptides.

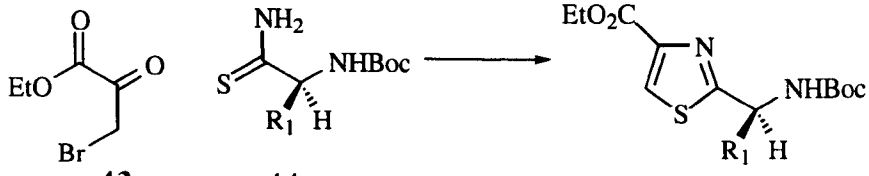
2.2 Thiazole Ring Formation

Thiazolines and thiazoles are important building blocks in pharmaceutical agents and in biologically active natural products. They are characteristic structural segments of cyclopeptide alkaloids such as lissoclinamides, patellins and bistramides, as previously described. The multitudinous synthetic stratagems employed by researchers to produce

thiazolines and thiazoles can be divided into two main classes, *i.e.* condensation reactions and cyclodehydration reactions.

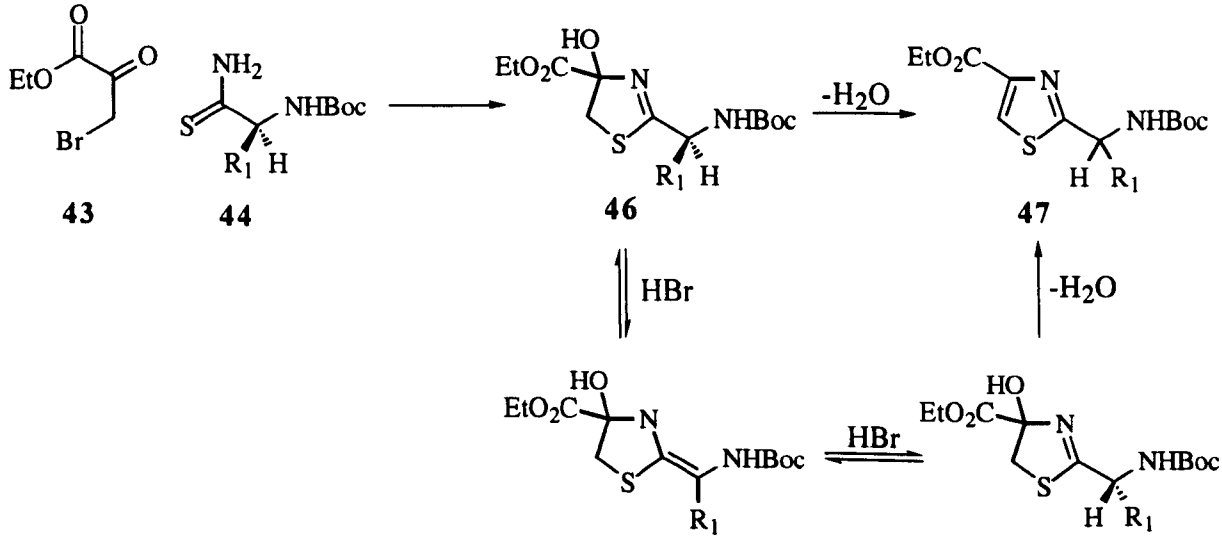
2.2.1 Condensation Reactions

Condensation reactions leading to thiazoles can themselves be divided into two sub-classes, *i.e.* [3+2] and [4+1] atom cyclisations. The Hantzsch reaction involves a [3+2] atom cyclisation between a thioamide **44**, and a α-halo-carbonyl compound **43**, and this is one of the most direct routes to thiazoles *viz* **45**.



43 **44** **45**

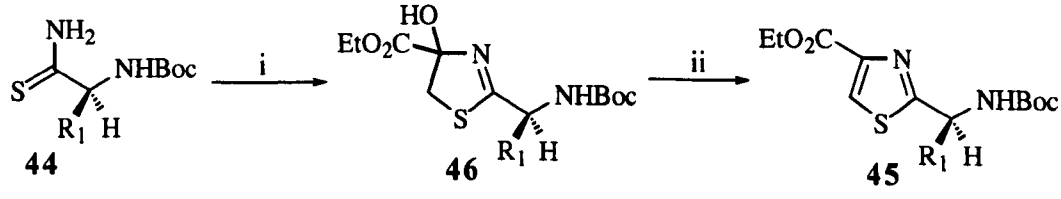
The reaction conditions reported by Pettit and Holzapfel[75] simply involve stirring the thioamide and the bromopyruvate together at room temperature in a solvent, and lead to amino acid thiazoles in excellent yields. However, these conditions do result in complete racemisation of the amino-substituted chiral centre **47**. Pettit suggested that the racemisation took place at the intermediate thiazoline **46** stage, mediated by acidic hydrobromic acid, a by-product of the reaction (**Scheme 2**).



43 **44** **46** **47**

Scheme 2

Hemichart later published a synthesis of a number of thiazoles under very similar condensation conditions *i.e.* stirring the thioamide and bromopyruvate together for two days at room temperature.[76] He claimed that the production of homochiral thiazoles was due to the improved procedure for the conversion of amides to thioamides using Lawesson's reagent,[77] but no optical rotations were published. Attempts by Schmidt *et al.* to reproduce Hemichart's work met with failure.[78] Schmidt decided to limit the exposure time of the thiazoline to the racemising effect of hydrobromic acid by adding the dehydrating agent, trifluroacetic anhydride, in order to increase the rate of conversion of the intermediate thiazoline to the thiazole. In addition, the reaction was carried out at -10 °C. The combined effect of these modifications was the production of thiazoles with enantiomeric excesses of up to 90 %. In 1986 Kelly *et al.* published work in which calcium carbonate was used to mop up the unwanted hydrobromic acid.[79] These two modifications were combined by Holzapfel *et al.* in work published between 1990 and 1992 which forms the basis of the widely utilised modified Hantzsch condensation for the formation of thiazoles.[80] Sodium hydrogen carbonate or potassium hydrogen carbonate is stirred in an aprotic solvent, such as DME, along with the thioamide **44**, and the solution is then treated with ethyl bromopyruvate. When the reaction mixture contains significant amounts of thiazoline **46**, trifluoroacetic anhydride is added to produce the thiazole **45** (**Scheme 3**).



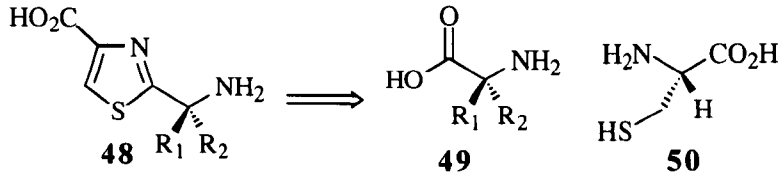
Reagents: i, EtO_2CCOCH_2Br, $KHCO_3$, DME, -10 °C; ii, $(CF_3CO)_2O$, pyridine, DME,-10°C.

Scheme 3

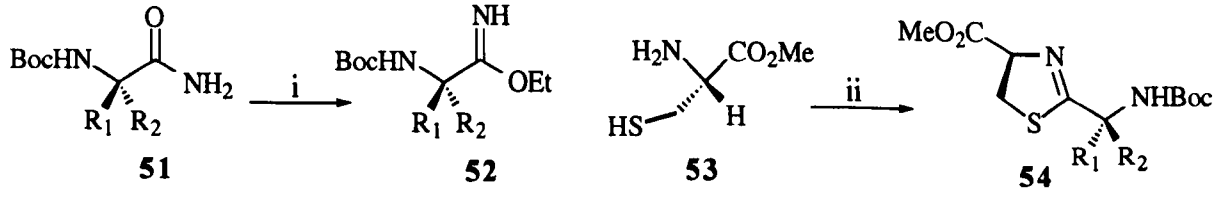
This method was successful in producing a wide range of optically pure thiazoles, however, with some amino acids a small amount of racemisation was still observed. Meyers *et al.*, in 1994, reported that by using the same conditions at -15 °C rather than

-10 °C these problems could be eliminated.[81] This subsequently allowed Meyers to complete a total synthesis of bistratamide C.[82]

Biosynthetic investigations on molecules containing the thiazole moiety, such as nosiheptide[83] and thiostrepton,[84] show that they result from a condensation reaction between the carboxyl of an amino acid **49** and cysteine **50**.



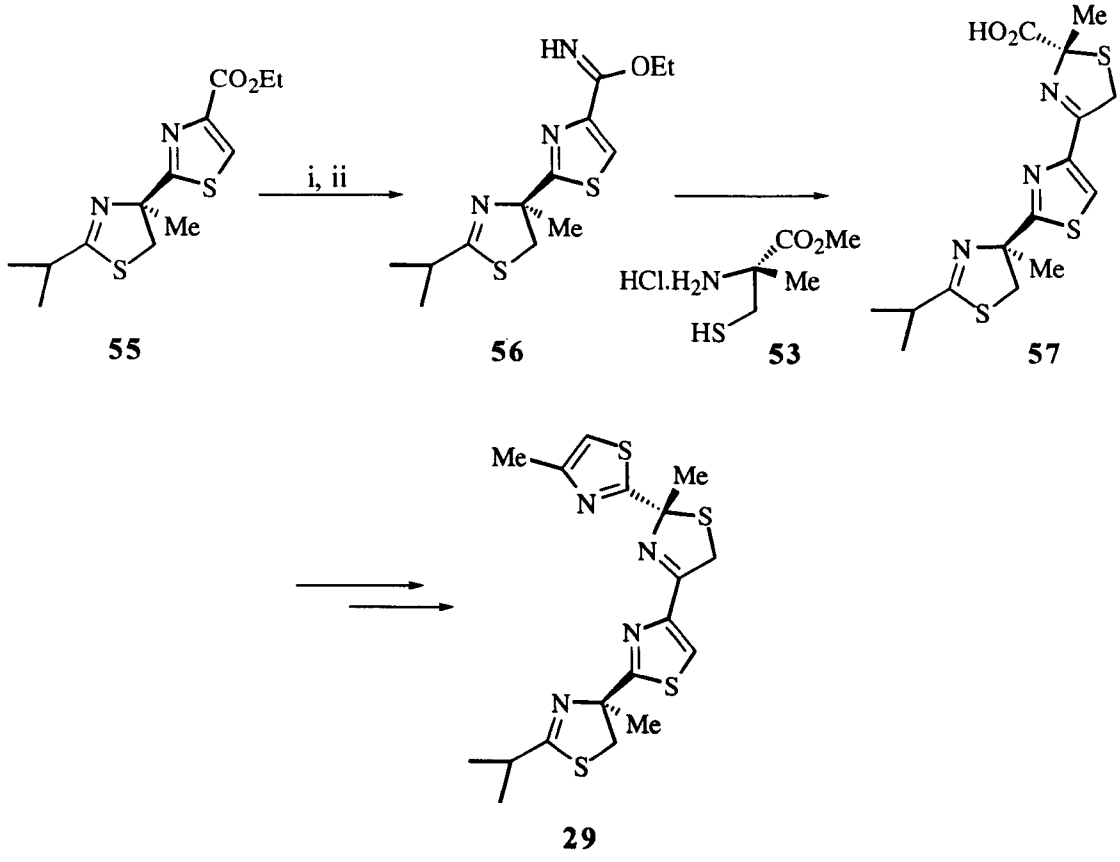
While procedures that furnish thiazoles in one pot from simple starting materials are very valuable, thiazolines occur very frequently in nature; thus routes to form thiazoles *via* thiazoline formation are equally valuable. Amino acids can be converted into imino ethers, and these can then undergo condensation with cysteine to form a thiazoline. In early experiments carried out by Hirotsu, Kaneka, and Shiba[85] thiazolines were formed, but only as racemic compounds. It wasn't for another twenty years when Pattenden and North[86] published the synthesis of a number of thiazoles *via* the imino ether route that the procedure was finally adapted to the synthesis of optically pure compounds. In this work, an imino ether **52** was formed by treating the corresponding amide **51** with triethyloxonium hexafluorophosphate. A solution of the imino ether **52** and cysteine methyl ester **53** in ethanol was then stirred at room temperature until thiazoline **54** formation was detected by tlc. The reaction mixture was then immediately worked up to avoid any racemisation of the thiazoline (**Scheme 4**).



Reagents: i, $(Et)_3OPF_6$, EtOH; ii, EtOH

Scheme 4

Pattenden and Boyce later employed an imino ether to form one of the thiazoline units in their total synthesis of didehydromirabazole **29**.[87] Thus, manipulation of the tetrapeptide **55** to the imino ether **56** and subsequent condensation with cysteine methyl ester installed the third azole heterocycle in **57**. Repetition of this sequence, followed by oxidation of the terminal thiazoline then gave didehydromirabazole **29** (**Scheme 5**).



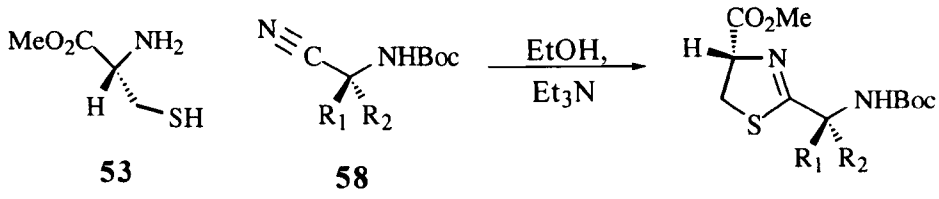
Reagents: i, EtOH / aq. NH_3; ii, $Et_3O^+PF_6^-$, DCM.

Scheme 5

There are many methods available for the oxidation of thiazolines to thiazoles in the literature, and a few of these will be highlighted later on in this Thesis.

A very similar [4+1] atom cyclisation is that involving amino nitriles **58** and cysteine methyl ester **53**. These reactions are carried out in alcoholic solutions in which an imino ether is generated *in situ* (**Scheme 6**). This method has an advantage over the usual

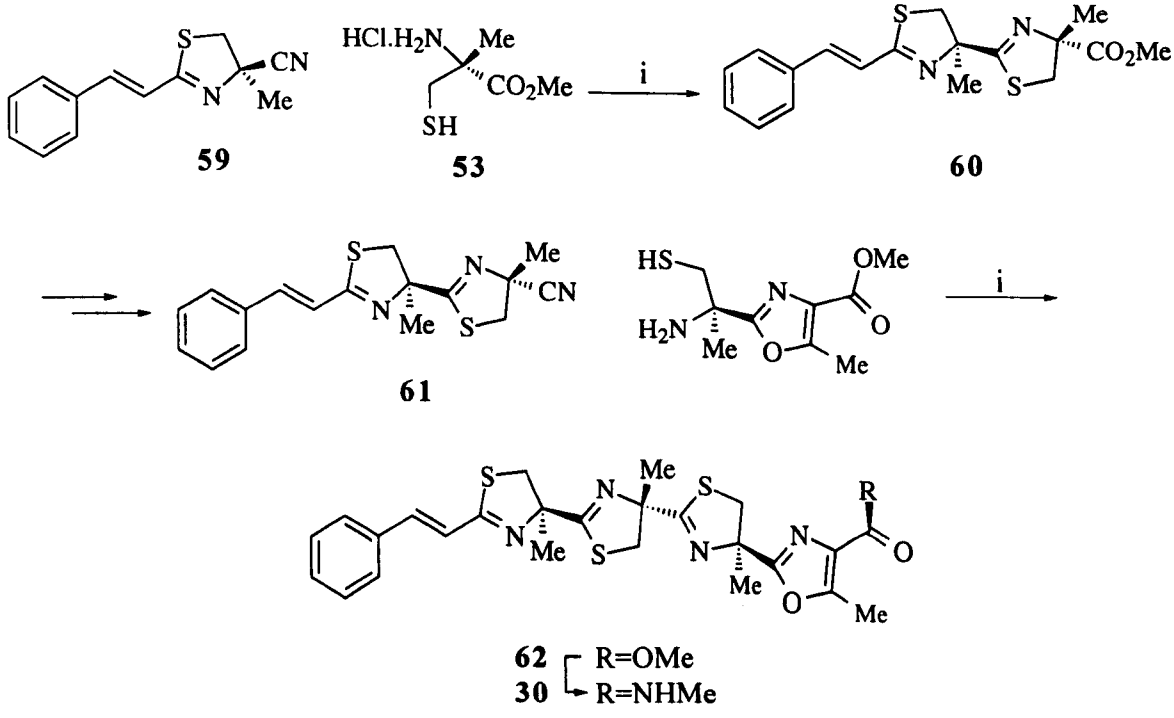
imino ether procedure as a common problem with imino ethers is their conversion into methyl esters before they have chance to react with cysteine.



53 **58**

Scheme 6

Pattenden and colleagues used these thiazoline forming conditions in the total synthesis of the *tris*-thiazoline containing metabolite, thiangazole **30**.[88] Thus condensation of the thiazoline **59** with cysteine methyl ester **53** in an alcoholic solution first yielded the *bis*-thiazoline **60**, which was then manipulated to the amino nitrile **61**. Further condensation with a cysteine residue attached to an oxazole led to the *tris*-thiazoline oxazole backbone **62** which on treatment with methylamine followed by crystallisation finally led to thiangazole **30** (**Scheme 7**).



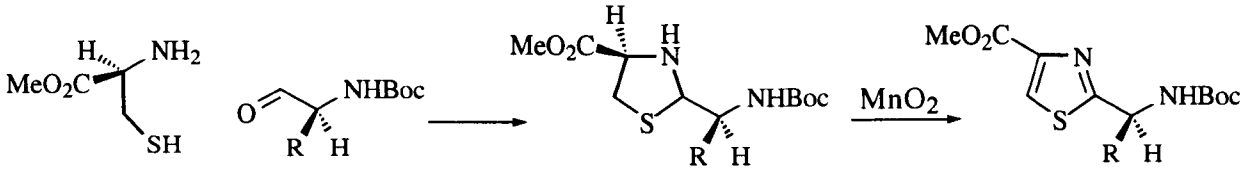
61

62 R=OMe
30 R=NHMe

Reagents: i, Et_3N, MeOH, Δ

Scheme 7

Finally, thiazolidine containing amino acids, *viz* **64**, can be obtained when cysteine methyl ester **53** is condensed with an α-amino aldehyde **63**. Oxidation of the thiazolidine **64** with manganese dioxide then furnishes the corresponding thiazole (**Scheme 8**).[89]

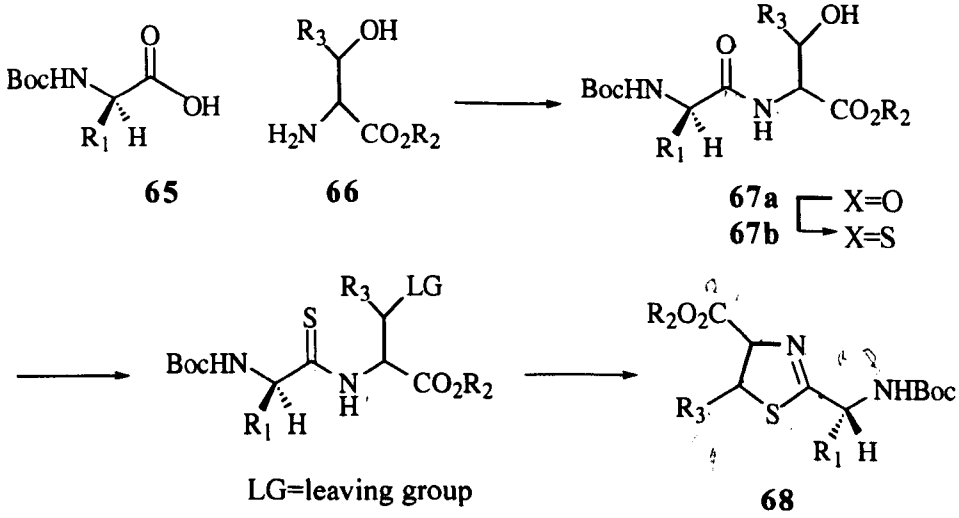


53 **63** **64**

Scheme 8

2.2.2 Cyclodehydration Reactions

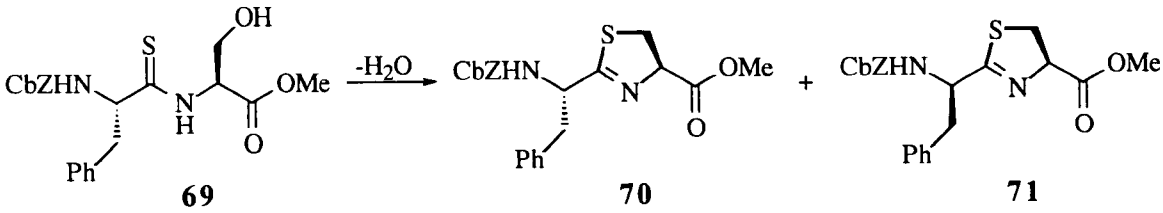
In general cyclodehydration reactions leading to thiazolines require the formation of a dipeptide **67** from one amino acid possessing the desired chiral centre **65** and another amino acid **66** containing a hydroxy moiety such as serine (R_3=H) or threonine (R_3=CH_3). The dipeptide **67a** can then be manipulated to form a thioamide **67b**, most commonly using Lawesson's reagent, and all that is then required is activation of the hydroxyl to promote a cyclodehydration to form the thiazoline **68** (**Scheme 9**).



65 **66** **67a** **67b** **68**

Scheme 9

Some of the simplest reagents available for activating alcohols include tosyl chloride and thionyl chloride. Over fifty years ago Attenburrow, Elliott, and Penny discovered that tosyl chloride in the presence of triethylamine induced cyclodehydration of dipeptides to form oxazolines.[90] Just a year later Elliott discovered the effectiveness of thionyl chloride as a cyclodehydrating agent.[91] In an attempt to pinpoint the cyclodehydration method which produced thiazolines in the highest yield and with the least amount of epimerisation, Wipf and Fritch investigated a number of alternative methods. However, they found that upon subjecting an *N*-protected thiopeptide methyl ester **69** to either of the conditions described above, a rather disappointing 40-50 % yield of racemic thiazoline **70** and **71** was obtained (entries 1 and 2, **Table 1**). Thiazolines, oxazolines and aziridines have also been formed from β-hydroxy-α-amino acids[92,93] using the Mitsunobu conditions[94] but side chain epimerisations have not been reported. Cyclodehydrations under Mitsunobu conditions occur at low temperature, and thus, when *N*-protected thiopeptide methyl ester **69** was exposed to triphenylphosphine and diisopropylazodicarboxylate, a 4 : 1 ratio of **70** : **71** was formed in an overall 80 % yield (entry 3, **Table 1**). Burgess' reagent ($MeO_2CNSO_2NEt_3$)[95] is nothing more than a fancy equivalent to thionyl chloride but has an added advantage, not only does it activate the hydroxyl but it also acts as an intramolecular base to facilitate the cyclisation process. The use of Burgess' reagent[95] to effect the cyclodehydration of thiopeptides was found to be superior to other procedures, with the thiazolines being produced in yields up to 96 %, in greater than 97 : 3 ratio of **70** : **71** (entry 4, **Table 1**).

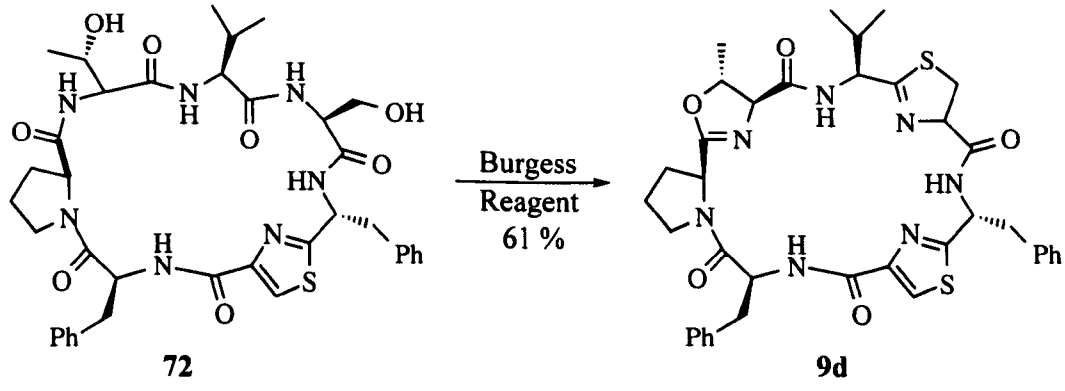


69 70 71

Entry	Method	Yield (%)	Ratio **70:71**
1	TsCl, Et_3N, DCM, 42 °C, 1h	40	1:1
2	1. $SOCl_2$, 0°C, 2h; 2. Pyridine, THF, 0 °C, 15min	49	1:1
3	Ph_3P, DIAD, DCM, -78 °C to 22 °C, 30min	80	78:22
4	Burgess' Reagent	96	>97:3

Table 1

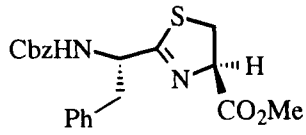
Pattenden and Boden have utilised Burgess' reagent to effect a double cyclodehydration of the cyclopeptide **72** to install both the oxazoline and thiazoline rings simultaneously as the final step in their total synthesis of lissoclinamide 4 (**9d**) (**Scheme 10**).[96]



72 9d

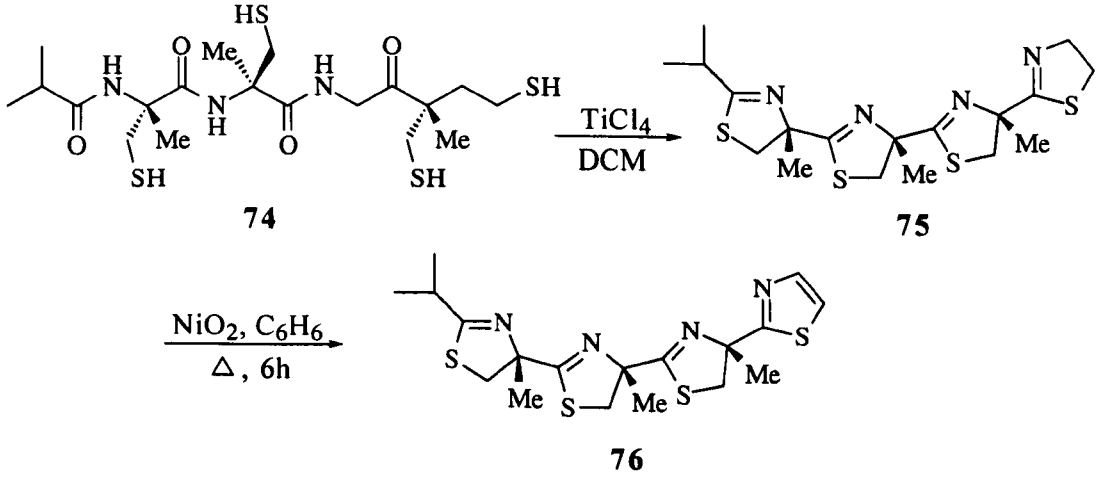
Scheme 10

Lellouche reported the use of DAST (diethylaminosulfur trifluoride) in 1995 as a powerful hydroxyl activating agent towards 1, 2-thioamido alcohols.[97] This reagent induces a fast (<1h) and stereoselective cyclisation at -78 °C to afford the corresponding 2-thiazolines. Pattenden and Rutt found that DAST was highly efficient in producing thiazolines with good retention of chirality. In particular, the phenylalanine derived thiazoline **73** was formed in >99 % ee as shown by chiral HPLC.[98]



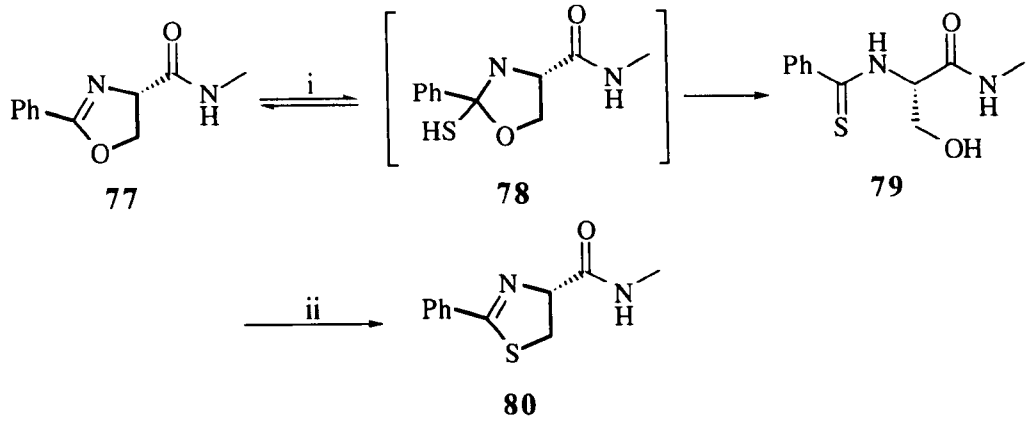
73

Heathcock and Walker employed a different strategy to activate the hydroxyl to cyclodehydration in their total synthesis of (-)-mirabazole C **76**.[57] The peptidic backbone **74** was synthesised *via* a series of peptide coupling/deprotection steps, which upon exposure to titanium tetrachloride in dichloromethane afforded the tetra-thiazoline **75** in a 63 % yield. The terminal thiazoline was oxidised using nickel peroxide in 60 % yield to give (-)-mirabazole C **76** (**Scheme 11**).[99]



Scheme 11

In total contrast to all the methods already described for the formation of thiazolines, it is possible to directly convert an oxazoline into a thiazoline.[100] The advantage of such a transformation arises from the fact that oxazolines are synthetically more accessible than the corresponding thiazolines. Thus, treatment of the oxazoline **77** with a saturated solution of hydrogen sulfide in methanol at room temperature leads to the formation of the thioamide **79**, probably *via* reversible formation of the orthoamide intermediate **78**. Subsequent cyclodehydration of **79** with Burgess' reagent then provides the thiazoline **80** in 89 % yield (**Scheme 12**).

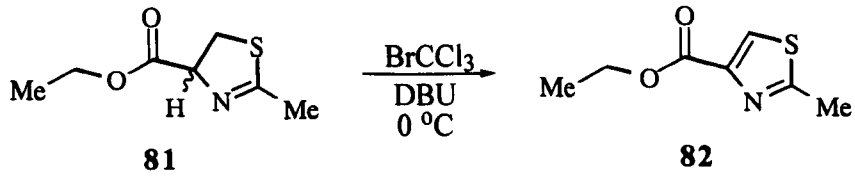


Reagents: i, Et_3N, MeOH, H_2S, 23 °C; ii, Burgess' reagent, THF, 70 °C.

Scheme 12

2.2.3 Oxidations of Thiazolines

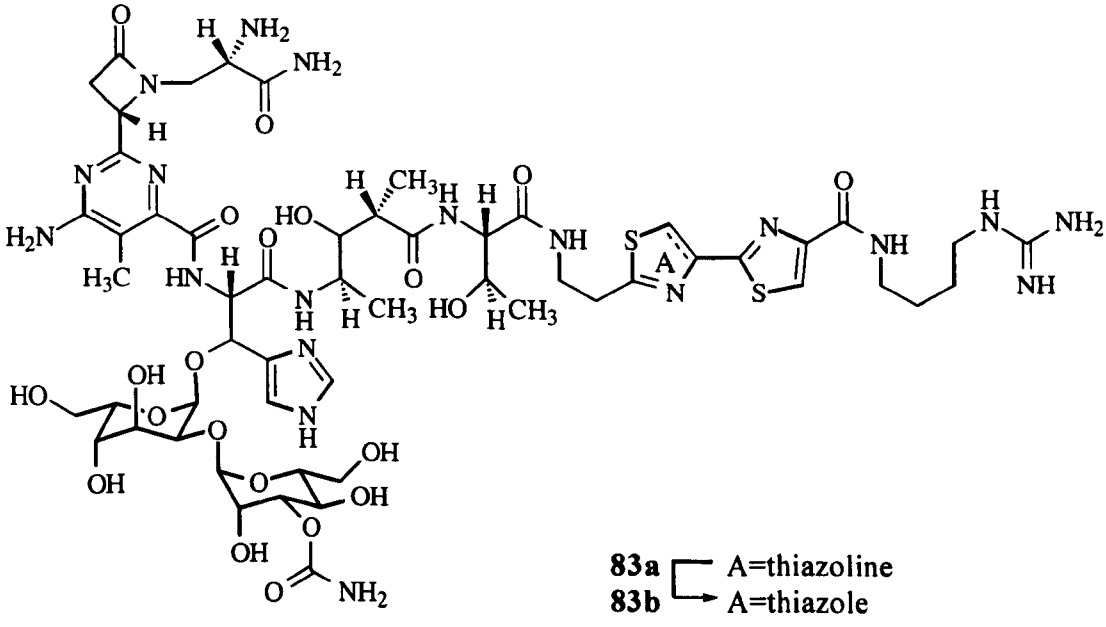
The oxidation of thiazolines to thiazoles has been developed extensively over recent years and most methods proceed either by a radical pathway or *via* an addition-elimination sequence. In order to effect these transformations in good yield an enolisable group at the 4-position in the thiazoline seems necessary. A few of the methods available for the oxidation of oxazolines involve the use of copper(I) and copper(II) salts. A modification of the Kharasch-Sosnovsky reaction,[101] by the Meyers group[102] involves treatment of thiazolines with a cocktail of copper(II) acetate, copper(I) bromide and *tert*-butyl hydroperoxide to give thiazoles in high yields. More recently Williams *et al.* have developed a mild, addition-elimination 'oxidation' method using $BrCCl_3$ and DBU. Thus, when a solution of the thiazoline **81** in dichloromethane is treated with DBU and $BrCCl_3$, at 0 °C for six hours, a 95 % yield of the thiazole **82** is obtained (**Scheme 13**).



Scheme 13

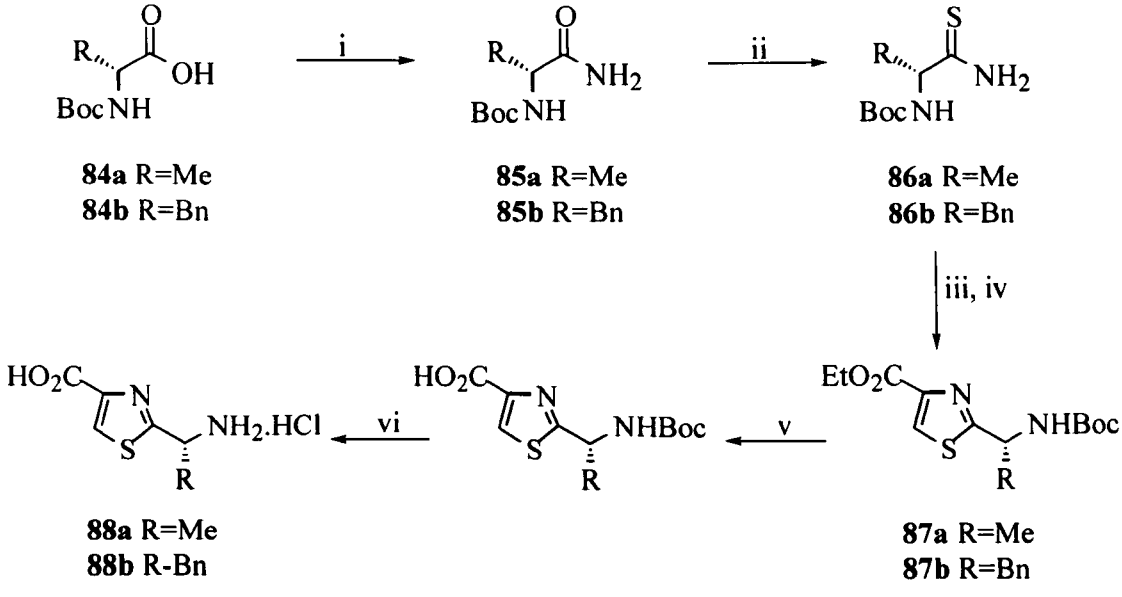
Comparisons to other oxidation techniques have shown that this procedure is cleaner and higher yielding, and as a consequence this protocol is becoming increasingly popular in organic synthesis.[103] Jung *et al.* have developed a very similar oxidation to Williams, using DBU, CCl_4 in pyridine and acetonitrile.[104]

Oxidation of thiazolines to thiazoles can also be effected under radical conditions. Meyers has shown the utility of NiO_2 in oxidising both oxazolines and thiazolines to the corresponding azole heterocycles in good yields.[99] The mild and selective nature of this oxidation is illustrated by the successful conversion of phleomycin A_2 **83a** to bleomycin A_2 **83b**.[105]



Our goal was to explore the cyclooligomerisation reactions of thiazoles with side chains of varying size, particularly those thiazoles found in natural products. We decided that a good starting point was the thiazole **88a** derived from (*D*)-alanine, and that we would employ the modified Holzapfel-Hantzsch procedure in its formation. Thus, exposure of Boc-(*D*)-alanine **84a** to ammonia gas in the presence of *N*-methylmorpholine and isobutylchloroformate first gave rise to the amide **85a**, which upon treatment with Lawesson's reagent afforded the thioamide **86a**.

Condensation of the thioamide **86a** with ethyl bromopyruvate then smoothly led to the thiazole **87a** in a pleasing 71 % yield. Subsequent saponification of the thiazole **87a** and Boc-deprotection finally gave the totally deprotected thiazole **88a** in 71 % yield over the two steps (**Scheme 14**).



84a R=Me
84b R=Bn

85a R=Me
85b R=Bn

86a R=Me
86b R=Bn

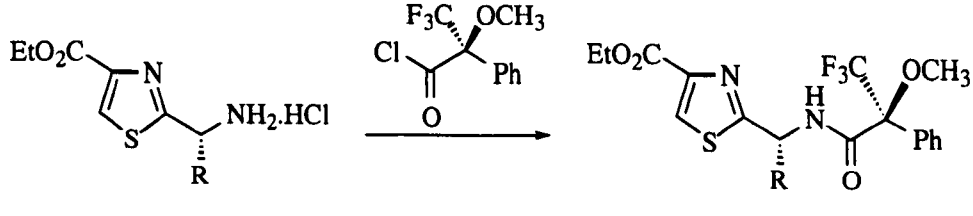
88a R=Me
88b R-Bn

87a R=Me
87b R=Bn

Reagents: i, NH_3(g), NMM, ^{i}BuOCOCl, THF, -13 °C; ii, Lawesson's reagent, THF, rt; iii, $KHCO_3$, DME, EtO_2CCOCH_2Br; iv, collidine, $(CF_3CO)_2O$, DME; v, NaOH, THF, H_2O; vi, 4M HCl, dioxane.

Scheme 14

To our delight Moshers amide formation **90a**[106] from the free amine **89a** and subsequent analysis of the ^{19}F NMR spectrum indicated that the acid **88a** had an ee ≥95 % (δ_F 71.64 ((-)-Moshers amide), δ_F 72.12 ((+)-Moshers amide) (**Scheme 15**).



89a R=Me
89b R=Bn

90a R=Me
90b R=Bn

Scheme 15

Having successfully synthesised the alanine derived thiazole **88a** we then set about the synthesis of the thiazole derived from phenylalanine *i.e.* **88b** in the same manner (**Scheme 14**). Once we had material in hand we performed a Boc-deprotection of the protected thiazole **87b** and prepared Moshers amides **90b** from the free amine **89b** (**Scheme 15**). To our surprise the synthetic phenylalanine derived thiazole had an enantiomeric excess of only 60%. We were able to improve this marginally to 70% by performing the condensation/dehydration steps at -20°C, but further attempts at -78°C lead only to the recovery of starting material. However, not wanting to loose sight of our aim, *i.e.* to attempt cyclooligomerisation reactions of these thiazoles, we decided to proceed with the 70 % enantiomerically enriched thiazole **88b**.

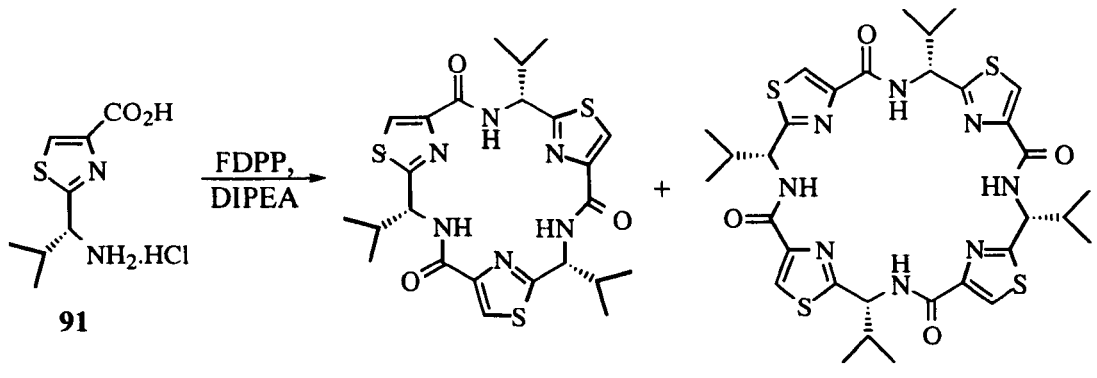
2.3 Cyclooligomerisation Reactions

Preliminary experiments were performed in our laboratories by Jeffrey Hannam, using the thiazole **91** derived from (*D*)-valine. Cyclooligomerisation reactions were attempted using a number of peptide coupling reagents (**Table 2**), of which pentafluorophenyl diphenylphosphinate (FDPP) was found to give consistently high yields (80-90 %) of cyclic products. The optimum concentration for the cyclooligomerisation of the valine thiazole was found to be in the range 10-50 mM (**Table 2**),[107] and under these conditions two major products were isolated, *i.e.* the cyclic trimer **42** and the cyclic tetramer **92**, in a 5 : 2 ratio (**Scheme 16**). The X-ray structure of **42** indicated that it is C_3-symmetric with a rigid structure in which all the amide protons are directed towards the centre of the macrocycle and with all of the valine side chains lying on the same face, adopting axial positions.

Coupling Reagent	Concentration	Yield Of Cyclic Products
DPPA[a]	0.05 M	47 %
DPPCl[b]	0.05 M	55 %
EDC[c]	0.05 M	43 %
FDPP[d]	0.5 M	60 %
FDPP	0.1 M	62 %
FDPP	0.05 M	91 %
FDPP	0.01 M	93 %
FDPP	0.005 M	60 %

a) diphenyl phosphoraziridate; b) diphenylphosphinyl chloride; c) 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; d) pentafluorophenyl diphenylphosphinate.

Table 2



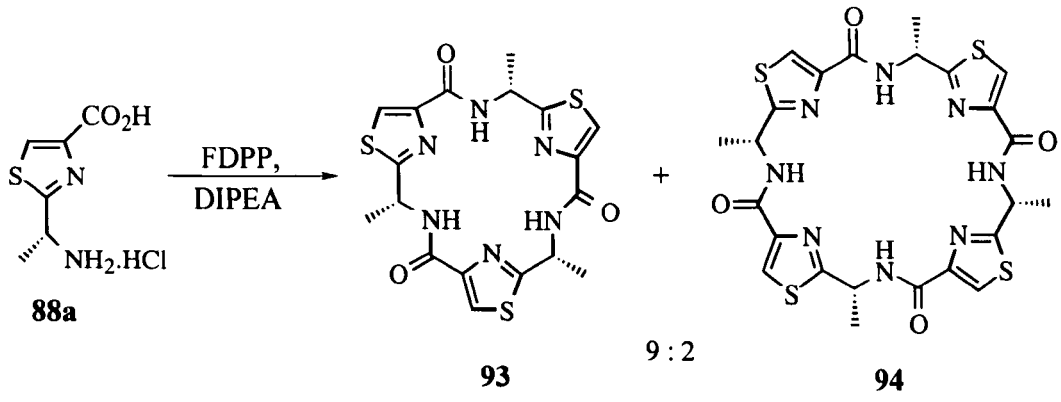
91 **42** **92**

Scheme 16

2.3.1 Cyclooligomerisations of (D)-Alanine Thiazole

The (*D*)-alanine derived thiazole **88a** was exposed to the optimum cyclooligomerisation conditions of FDPP and DIPEA in anhydrous acetonitrile, with the solution being stirred at room temperature under an atmosphere of nitrogen for 3 days. Upon work-up an overall 55 % yield of cyclic products were isolated; the slight decrease in yield can be attributed to the very poor solubility of **88a** in acetonitrile. Interestingly, the ratio of cyclic trimer **93** to cyclic tetramer **94** was found to vary compared with the valine derived thiazole, *i.e.* a 9 : 2 ratio of **93** : **94** was now obtained (**Scheme 17**).

HPLC was used to determine the ratio of cyclic products formed in all the experiments performed during this research. A refractive index detector was used to determine the ratio of cyclic products formed as the cyclopeptides all have different λ_{Max} values. A C_{18} column was used with an acetonitrile/water solvent system.

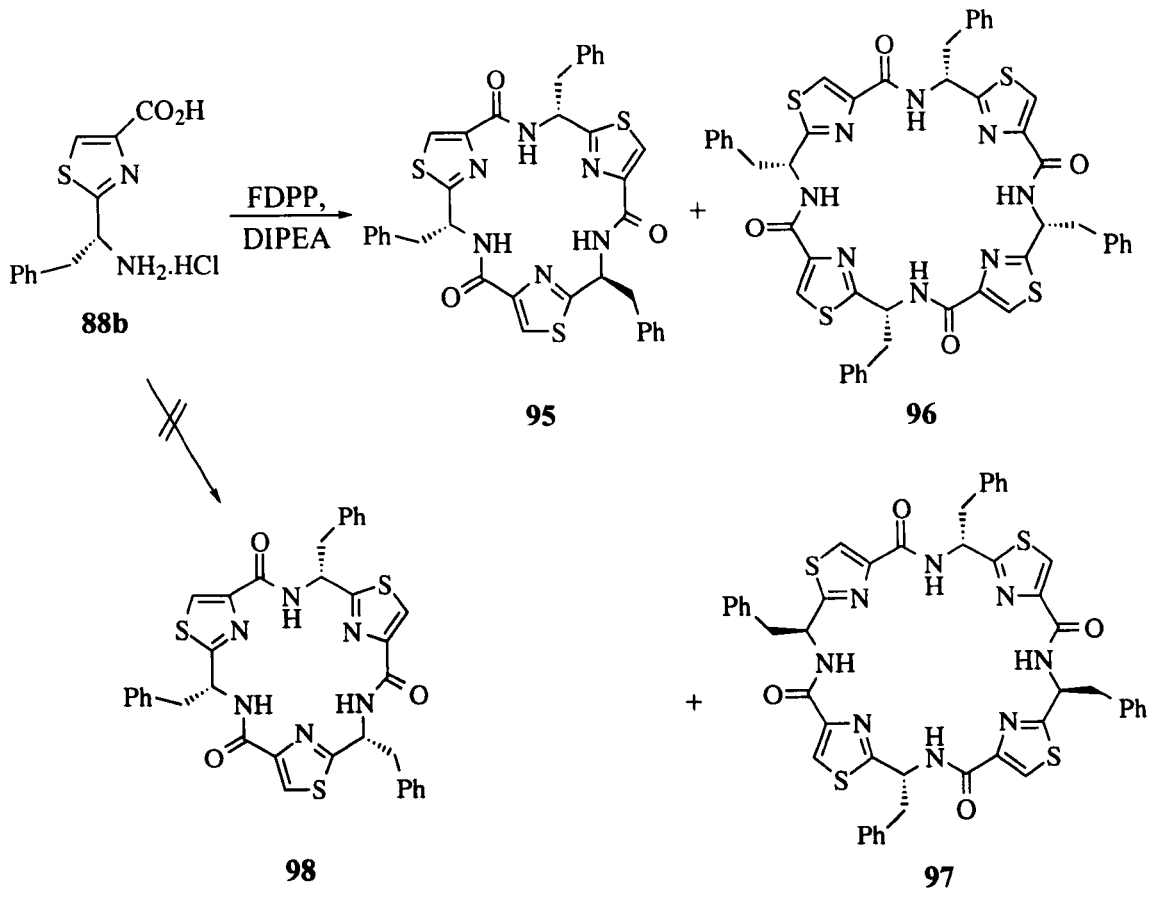


Scheme 17

The cyclic trimer **93** and cyclic tetramer **94** are C_3 and C_4 symmetric respectively as deduced from ^1H NMR spectra of the separated cyclopeptides in $CDCl_3$ at 21 °C. With the exception of the signals attributable to the amide protons, the PMR spectra were very similar. The amide protons in the trimer **93**, however, appeared 0.7 ppm further downfield from those in the tetramer **94**, suggesting that intramolecular H-bonding is probably more significant in the trimer (see experimental section pages 96-98).

2.3.2 Cyclooligomerisations of (D)-Phenylalanine Thiazole

Cyclooligomerisation reactions of the enantiomerically enriched (*D*)-phenylalanine thiazole **88b** with the coupling reagent FDPP proceeded smoothly to give an overall 63% yield of cyclic products. Interestingly, none of the expected C_3-symmetric cyclic trimer **98** was produced in any of these cyclisations. In this instance the cyclic products included a mixture of the trimer **95** and the isomeric tetramers, **96** and **97** in a 2 : 6 ratio of cyclic trimer to cyclic tetramers (**Scheme 18**)(**Appendix 4.1**).[107]



Scheme 18

The 'unsymmetrical' cyclic trimer isolated *viz* **95** contains one benzyl group on the opposite face to the other two, and most probably arises as a result of the incorporation of a small amount of the (*L*)-phenylalanine thiazole present (ee 70%) in the starting material (**Appendix 4.1**). Molecular modelling studies of trimers **95** and **98** found the trimer **95** to be energetically more favourable than trimer **98**. When the three benzyl groups lie on the same face, as in trimer **98**, the energy minimum is 264 $kJmol^{-1}$ compared with 242 $kJmol^{-1}$ for trimer **95** in which one benzyl group is on the opposite face. Indeed this is evident when visually comparing the lowest energy conformations of **95** and **98**. It is, however, interesting to note the presence of π-π interactions between two of the phenyl rings in both of these compounds (**Figure 4**).

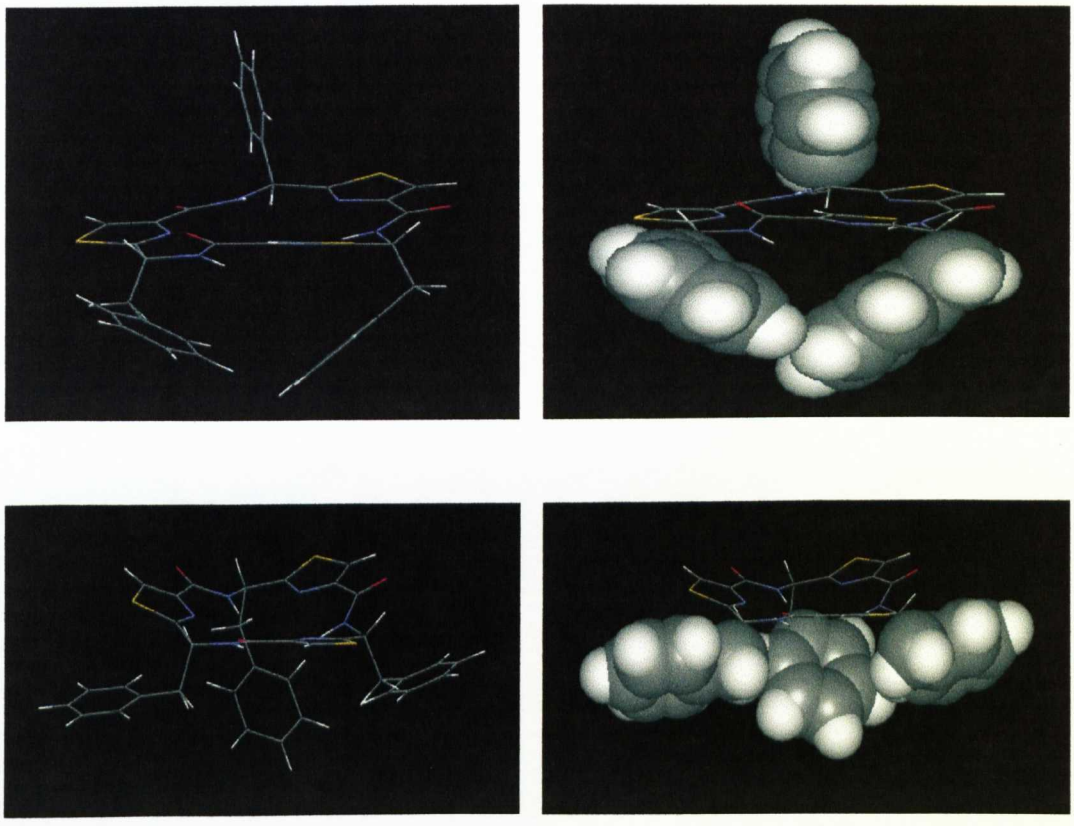
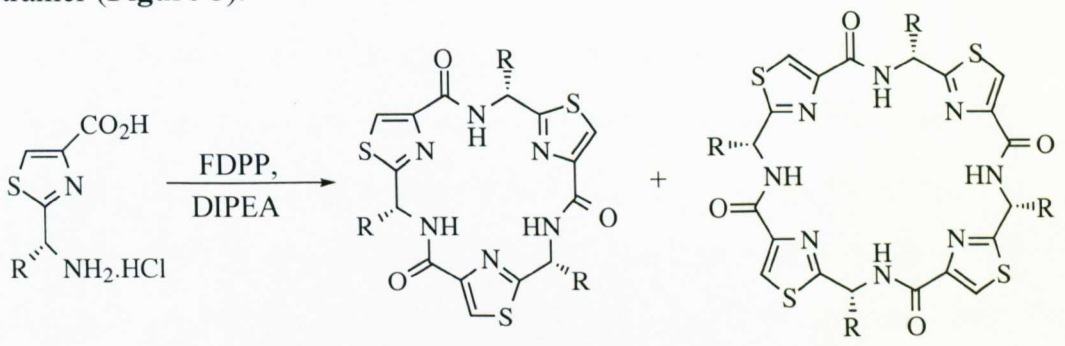


Figure 4 : Cyclic peptide **95** energy minimised (top LHS); Cyclic peptide **95** - a space filling representation of the benzyl side chains (top RHS); Cyclic peptide **98** energy minimised (bottom LHS); Cyclic peptide **98** - a space filling representation of the benzyl side chains (bottom RHS).

In addition it is very interesting to note the marked difference in the ratio of trimer to tetramer to that observed in the valine and alanine thiazole cyclooligomerisations (**Table 3**). There is an obvious trend in the ratio of cyclic products formed which is dependent upon the nature of the alkyl side chain. As the size of the alkyl side chain is decreased, the amount of cyclic trimer produced in the cyclooligomerisation process increases. In the case where R=CH_2Ph, almost three times as much cyclic tetramer is formed compared with cyclic trimer. By changing the benzyl side chain for a methyl group, the thiazole derived from alanine, sees nearly five times as much cyclic trimer than cyclic tetramer formed. Indeed molecular modelling of the trimer **93** found the compound to adopt an energy minimum of just 212 $kJmol^{-1}$, thus

confirming that an increase in the size of the alkyl side chain increases the minimised energy of the cyclic trimer and therefore disfavours its formation *cf* the tetramer (**Figure 5**).



R	Trimer : Tetramer
CH_2Ph	1 : 2.8
$CH(CH_3)_2$	3.5 : 1
CH_3	4.3 : 1

Table 3

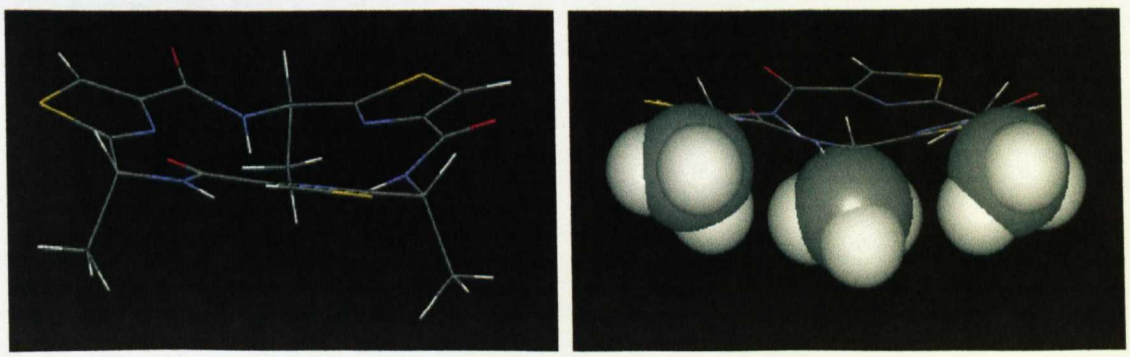
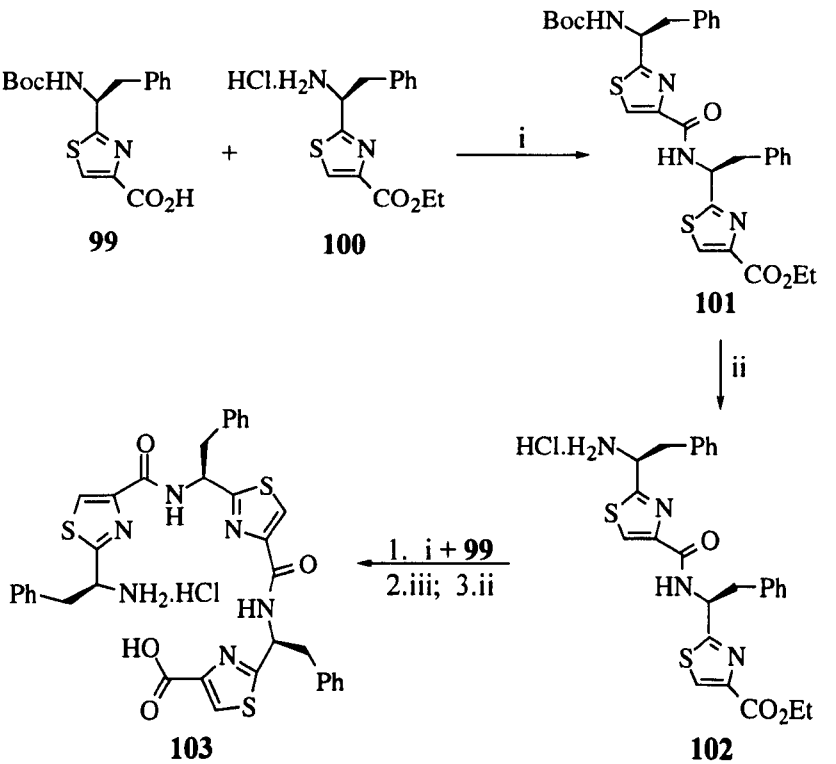


Figure 5 : Cyclic peptide **93** energy minimised (LHS); Cyclic peptide **93** - a space filling representation of the methyl side chains (RHS)

We postulated that the competition between the formation of the cyclic trimer and the cyclic tetramer may depend on the difference in rates of the two competing reactions. It is thought that during the cyclooligomerisation reactions a linear precursor is formed which then macrocyclises to form the cyclic product. However, the steric interactions between the benzyl groups in the linear trimer, prior to macrocyclisation, may disfavour the linear trimer adopting the required

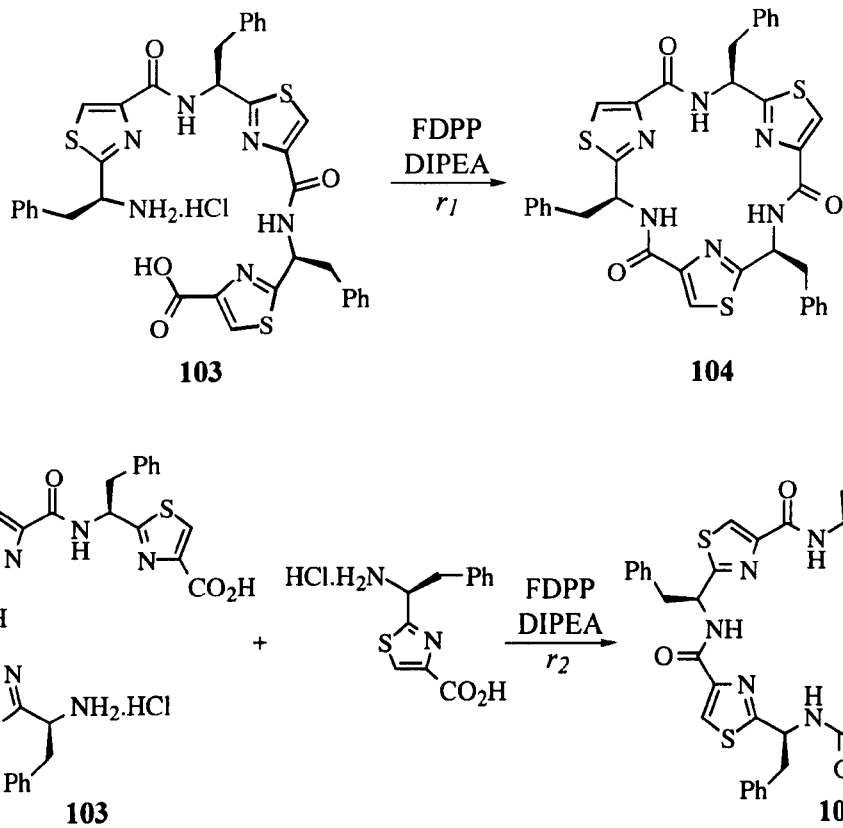
conformation to cyclise. In an attempt to investigate this further, we wanted to know whether it would be possible to synthesise the cyclic trimer in which the benzyl groups all lay on the same face *via* a linear method. Is this compound too hindered to form at all, or was the absence of this trimer in our cyclooligomerisation products simply the result of a competitive tetramerisation with a more favoured rate? We therefore decided to prepare the *L, L, L*-linear trimer **103** and examine its cyclisation to **104**. Thus, coupling of the thiazole acid **99** with the thiazole amine **100** using carbodiimide mediated conditions led smoothly to the dimer **101**. Boc-deprotection of this dimer **101** using 4M HCl in dioxane then gave the amine **102** in a 96 % yield. Further coupling of **102** under carbodiimide mediated conditions with the thiazole acid **99**, followed by saponification, and Boc-deprotection finally gave the macrocyclic precursor **103** (**Scheme 19**).



Reagents: i, EDC, NMM, ^{i}BuOCOCl, 0 °C; ii, 4M HCl, dioxane; iii, NaOH, THF H_2O,

Scheme 19

Cyclisation of **103** with FDPP did indeed lead to the formation of the C_3-symmetric trimer **104** in which all side chains lie on the same face of the molecule (**Scheme 20**). Clearly steric effects do not directly prevent the formation of this 'symmetrical' macrocycle in the cyclooligomerisation reactions. More likely, they just slow down the rate of macrocyclisation of the linear trimer sufficiently, so that the competing reaction, the formation of the linear tetramer **105** from the linear trimer, predominates (i.e. $r_2 > r_1$) (**Scheme 20**).



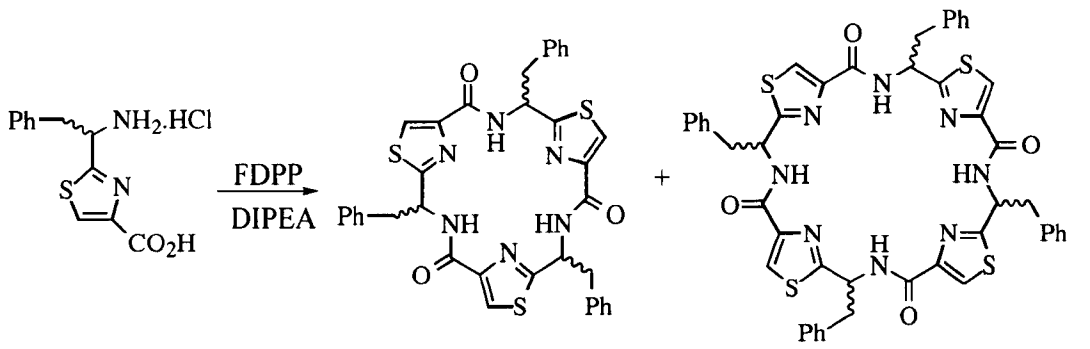
103 **104**

103 **105**

Scheme 20

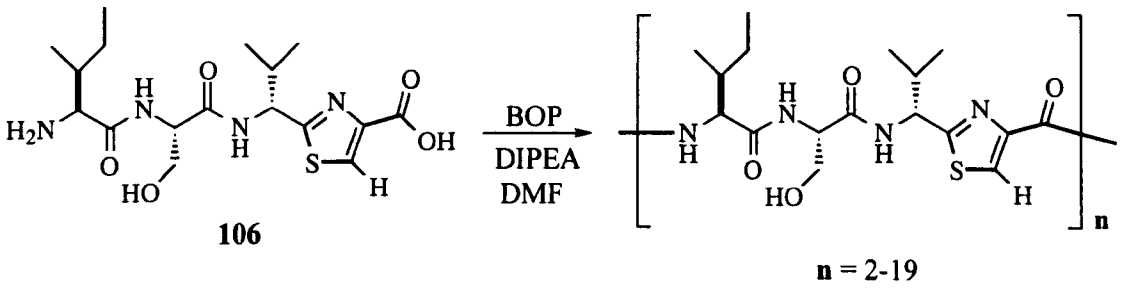
Macrocyclisation of a racemic mixture of the phenylalanine thiazole **93** was also performed (**Scheme 21**), giving a different product ratio to that obtained using enantiomerically enriched material. In this case, the product mixture contained, 'unsymmetrical' trimer **95** and a mixture of isomeric tetramers in approximately a 2 : 2.5 ratio. This increase in the formation of the cyclic trimer over cyclic tetramer when cyclisation takes place from a racemic mixture, further supports our notion

that the 'symmetrical trimer' **98** doesn't form due to steric hinderance, and thus a higher energy minimum *cf* the 'unsymmetrical trimer' **95**.



Scheme 21

At this stage in our research we had developed novel, high yielding cyclooligomerisation reactions of amino-acid based thiazoles to selectively form analogues of naturally occurring cyclopeptides in just one step from relatively accessible starting materials. Contemporaneous work by Fairlie *et al.* reported similar cyclooligomerisations but from an amino acid sequence, *i.e* **106** (**Scheme 22**).[108]



Scheme 22

Cyclooligomerisation of this amino acid was much less selective compared to our cyclooligomerisations with thiazole heterocycles. Exposure of **106** to the coupling reagent BOP with DIPEA in DMF led to the isolation of 18 cyclopeptides

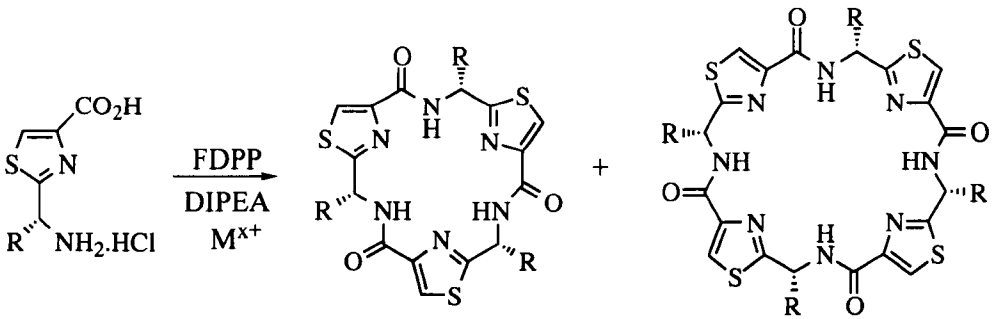
(**Scheme 22**). The presence of unmodified amino acids, not in the form of azole heterocycles, is the most likely cause of the decrease in selectivity. In light of the efficiency and selectivity of our reactions leading to just two main products, we feel we have provided some circumstantial evidence that naturally occurring cyclopeptides could be formed from preformed heterocyclic amino acids as opposed to free amino acids.

2.4 Metal Ions as Templates in Cyclooligomerisations

We now wished to challenge our cyclooligomerisation reactions even further and assert an even greater control over them. Templates have already proved useful in the promotion of cyclisation reactions and can often dramatically increase yields.[109] Most templation reactions are performed under kinetic control, with the template being responsible for bringing the two ends of a particular intermediate into close proximity, and thus promoting their reaction.[110] We were interested in using a metal ion as a template under thermodynamic control to promote formation of the product that it binds best. In this instance, using a single component mixture, templating could potentially bias the reaction between different sized cyclopeptides.

After considering the abundance of particular metal ions in the oceans and seas, we decided to attempt templation of our cyclooligomerisation reactions initially using the alkali metals, and then later with silver, cadmium and zinc. We decided to use the tetrafluoroborate and chloride salts of these metals due to their good solubility in organic solvents. Thus, a solution of the alanine derived thiazole **88a** in acetonitrile was stirred with DIPEA, and a metal salt, then, after stirring for 5 minutes the coupling reagent FDPP was added. This reaction mixture was stirred for 3 days, the reaction worked up and the residue subjected to HPLC analysis. Interestingly, we found that using lithium and cadmium as templates in the cyclooligomerisation of the alanine derived thiazole **88a** we promoted the formation

of only the cyclic trimer **93** (**Scheme 23**)(**Table 4**). Using sodium as a template also biased the reaction towards the formation of the cyclic trimer **93**, increasing the ratio from 9 : 2 without a metal to 12 : 2; potassium and silver were similarly found to bias the distribution of products, but to a lesser extent.



88a R=Me
88b R=CH_2Ph
91 R=$CH(CH_3)_2$

93 R=Me
95 R=CH_2Ph
42 R=$CH(CH_3)_2$

94 R=Me
96 R=CH_2Ph
92 R=$CH(CH_3)_2$

Scheme 23

Metal Salt	Ionis Radius /Å	Trimer:Tetramer
No Metal	–	9:2
$LiBF_4$	0.73	1:0
$CdCl_2$	0.92	1:0
$NaBF_4$	1.13	12:2
$AgBF_4$	1.14	9:2
KBF_4	1.51	10:2

Table 4

It is not possible to molecularly model metals encapsulated in the macroyclic cavities of these cyclopeptides due to the highly complex nature of the calculations required. It is, however, possible to generate a space filling representation of a lithium ion encapsulated in the macrocyclic cavity of a cyclic trimer, this illustrates just how tailor made these macrocylic cavities are for sequestering an ion (**Figure 6**). It is very difficult to postulate which properties of these metals render them better templates than others. The size of the metal ion is probably very crucial; if the metal is to template the monomer thiazoles to form, for example, a cyclic

trimer, then it must be able to chelate three monomers and hold them in favourable positions to promote cyclisation (**Figure 6**).

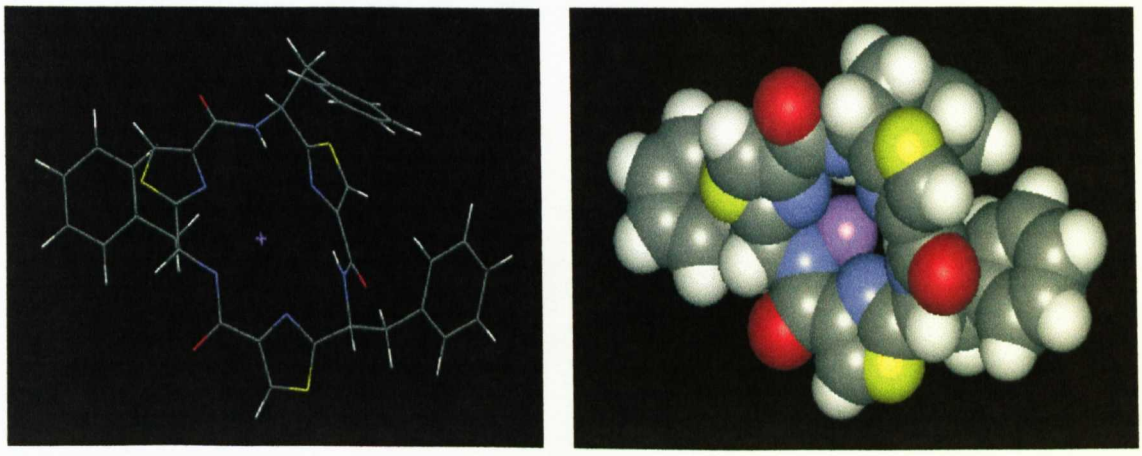


Figure 6 : Cyclic peptide **95** encapsulating a Li^+ ion (LHS); Cyclic peptide **95** encapsulating a Li^+ ion - a space filling representation (RHS).

Why does cadmium produce the same effect as lithium? What properties do these metals share? Well they have very similar ionic radii. Interestingly, if we correlate our templation results to the ionic radii of the metals (with a co-ordination number of 4) we find that as the size of the ion increases the selection for the cyclic trimer over the cyclic tetramer decreases (**Table 4**). It is very important to stress here, that although, with the exception of potassium, this relationship holds, we don't know what co-ordination number the ions adopt in these reactions (this would affect their actual ionic radius), or what affect charge density may have.

We then went on to attempt the templation of the cyclooligomerisation of the phenylalanine derived thiazole **88b** (**Table 5**). Where, once again the results displayed a similar correlation with the size of the metal ion. Moving down the first group, the affects with lithium are negligible, but sodium and potassium appear

to promote the formation of the tetramer **96**. Alternatively, we could consider sodium and potassium to be less effective templates for the formation of the cyclic trimer **95**. The strong selectivity for the phenylalanine derived thiazole **88b** to form the cyclic tetramer **96** in cyclooligomerisations without metal ions probably decreases the possibility of templates promoting the formation of the cyclic trimer **95**. This may, therefore, explain why the templation effects are not fantastically large.

Metal Salt	Ionis Radius /Å	Trimer:Tetramer
No Metal	–	1:2.8
$LiBF_4$	0.73	1:2.7
$NaBF_4$	1.13	1:3
KBF_4	1.51	1:3.5

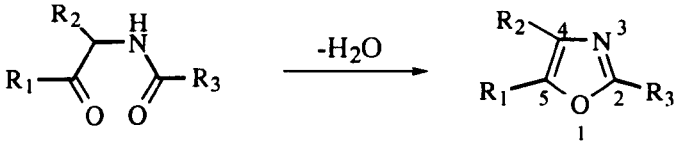
Table 5

These trends are further supported by work carried out in our laboratories by my colleagues Jolliffe and Hannam into the templation of the valine derived thiazole **91**. Lithium was found to increase the ratio of cyclic trimer **42** to cyclic tetramer **92** from 3 : 1 to 4 : 1; once more, as the metal ion was increased in size the formation of the tetramer became more significant.[74] It was during concurrent studies of the complexation behaviour of these cyclopeptides that the novel crystalline sandwich complex of **42** was isolated (**Section 1.6**). Further studies will be described into the use of metal ions as templates for natural products later on in this Thesis (**Section 2.7.2**, and **2.8.1**).

2.5 Oxazole Ring Formation

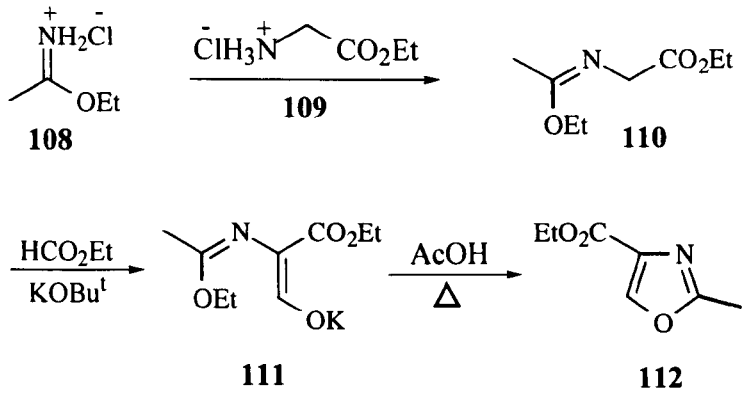
The chemistry of oxazoles began in 1876 with the synthesis of 2-methyloxazole. The development of penicillin as a drug during the World War ignited further interest in this heterocycle as it was initially thought to feature in the overall structure of penicillin. More recently, the isolation of many oxazole containing natural products, many of them peptide alkaloids has renewed the interest in oxazole chemistry, with many methods now available for their efficient construction.

The most common type of oxazole found in natural products is a 2, 4-disubstituted oxazole and the vast majority of research to date has been towards those compounds possessing a 4-carboxyl group. One of the oldest methods available for oxazole synthesis is the Robinson-Gabriel reaction.[111] This reaction involves cyclodehydration of α-acylamino ketones **107** in the presence of dehydrating agents such as PCl_5, P_2O_5, $POCl_3$, $SOCl_2$ and polyphosphoric acid.



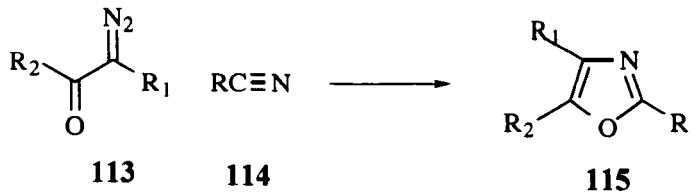
107

In 1947 Cornforth developed a synthesis of oxazoles starting from an imidate and an amino acid.[112] Thus condensation between ethyl acetimidate hydrochloride **108** and glycine ethyl ester hydrochloride **109** led to an imidate **110**. The imidate was next formylated to give **111**, which was immediately cyclised to the oxazole ester **112**, in good yields, by refluxing in acetic acid (**Scheme 24**).

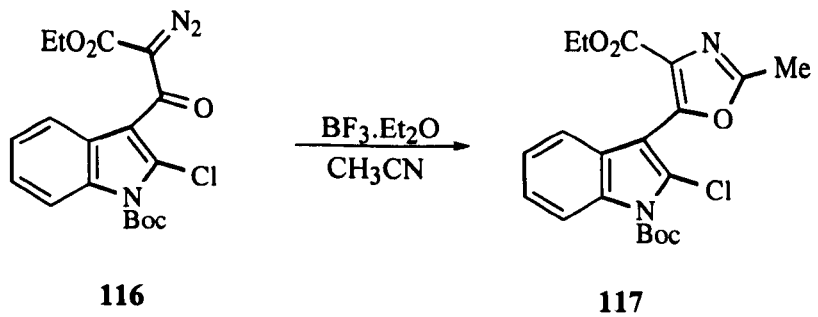


Scheme 24

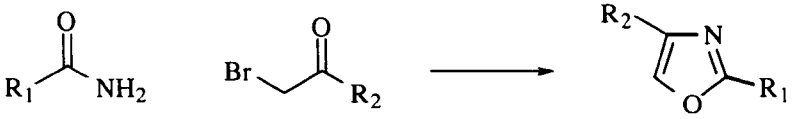
α-Diazocarbonyl compounds **113** have also been shown to participate in oxazole ring formation by undergoing reactions with nitriles **114**.[113] There are a variety of conditions which induce these reactions, ranging from metal catalysis or Lewis acid promoted reactions, to thermally or photochemically induced reactions. This method is particularly useful in providing oxazoles functionalised at the 5-position *e.g.* **115**.



Konopelski *et al.* have utilised this reaction in their studies towards the natural product, diazonamide A.[114] Thus treatment of the α-diazo-β-keto ester **116** in acetonitrile under Lewis acid conditions led to the desired 2-methyl oxazole **117** in 64 % yield.



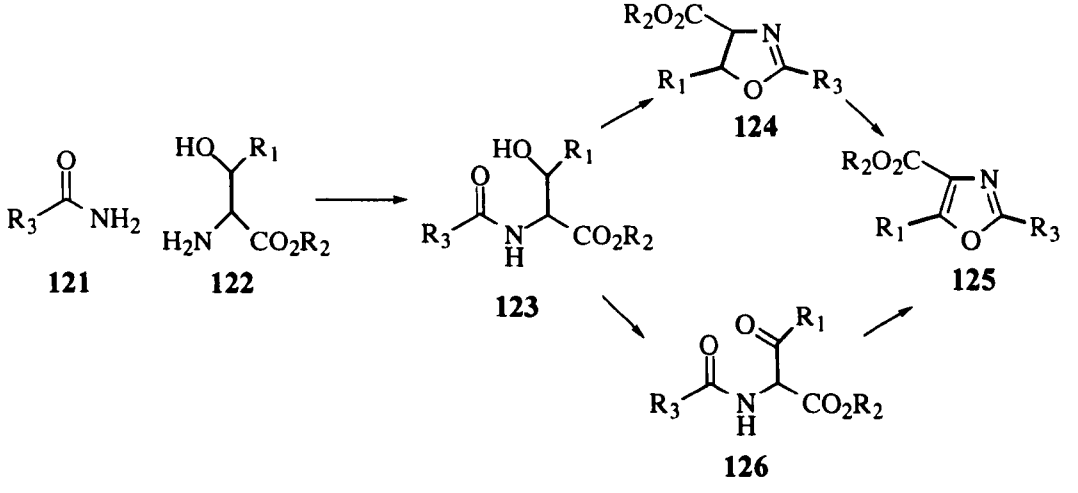
Alternatively, condensation of an α-halo-keto ester **119** with an amide **118** under the Hantzsch conditions[115] gives rise to a 2, 4-disubstituted oxazole **120** after acid mediated dehydration. The utility of this method has recently been demonstrated by Panek *et al.*, in their synthesis of the *tris*-oxazole unit of ulapualide A **27**.[116]



118 **119** **120**

2.5.1 Cyclodehydration Reactions

Probably the most widely used route towards oxazole ring formation in recent years involves the formation of a dipeptide **123**, followed either by, i) cyclisation to an oxazoline **124** and subsequent oxidation to the oxazole **125**, or ii) oxidation to the 1, 4-dicarbonyl species **126** followed by cyclodehydration to yield the oxazole **125** (**Scheme 25**).

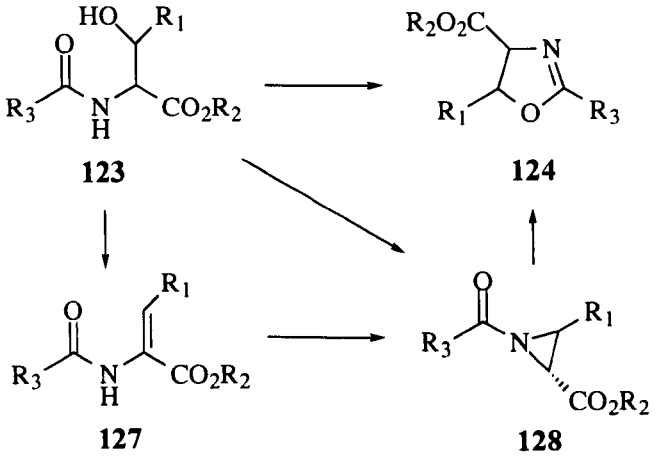


121 **122** **123** **124** **125** **126**

Scheme 25

There are, however, a number of competing reactions which may take place during the cyclodehydration of **123** to **124**. Elimination of the activated hydroxyl group in **123** may occur to produce an alkene, such as **127**. Alternatively, formation of an aziridine **128**, *via* direct attack by the amide nitrogen, is also a possibility (**Scheme 26**). A reaction which gives a multitude of products is highly undesirable.

Researchers have therefore sought to activate the alcohol in a manner that will induce the molecule to follow one of these pathways exclusively. An additional proviso is that the reaction conditions do not induce racemisation of any chiral centres.

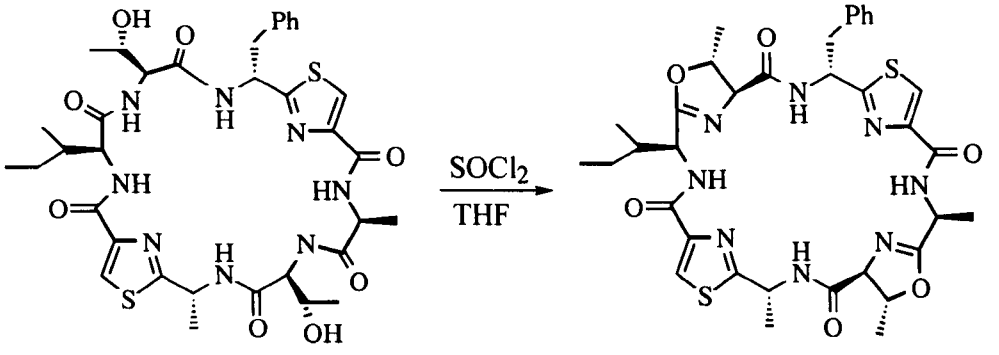


Scheme 26

A whole variety of reagents and conditions are currently available to effect these transformations. As for thiazoles, the formation of the oxazoline can be achieved in many ways from the corresponding dipeptide by activation of the hydroxyl amide prior to cyclisation. The reagents employed for the activation of dipeptides towards cyclodehydration to form oxazolines are generally identical to those used in thiazoline formation. Activation is achieved using thionyl chloride, followed by treatment with silver triflate;[117] methanesulfonyl chloride and triethylamine;[118] triphenylphospine, CCl_4 and DIPEA;[118] Mitsunobu conditions;[92, 119] phosphorus oxychloride;[120] or the more commonly encountered Burgess' reagent[95] or DAST.[97, 121]

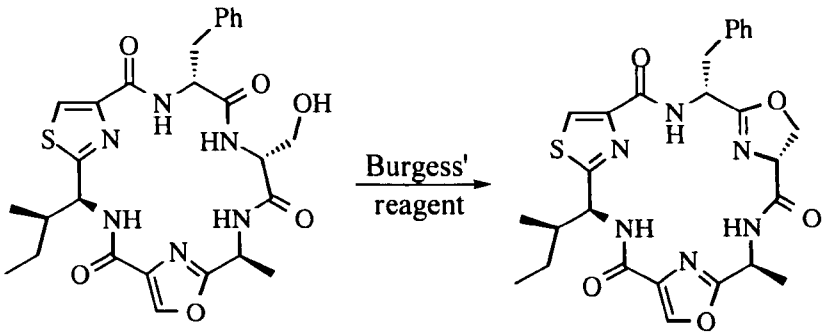
Cyclodehydration of β-hydroxy amino acid peptides to produce oxazolines was first observed by Nakajima *et al.* when their attempts to perform a β-elimination on a dipeptide of type **123** *via* an O-tosylated intermediate to afford the corresponding alkene **127** met with only oxazoline **124** formation.[122] Although the

significance of this transformation was appreciated at this time, it was not until 1985 that it was fully utilised when Shioiri *et al.* applied it to the total synthesis of ascidiacyclamide **7**[123] and later to patellamide B **6b**.[124] Thus two simultaneous cyclodehydrations induced the formation of both oxazolines in one step by treatment of the cyclic peptide **129** with thionyl chloride in THF, yielding patellamide B **6b** in 94 % yield.



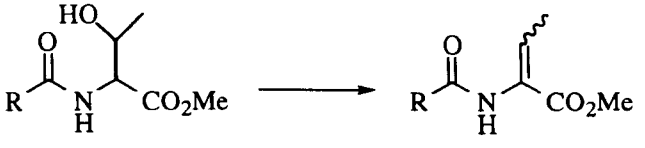
129 **6b**

Some of the yields for similar cyclisations were poor, and in these instances the addition of silver triflate was found to facilitate the reaction.[117] All the reagents listed above for the conversion of hydroxyamides into oxazolines do nothing more than activate the hydroxyl group to induce cyclodehydration. Care must, however, be taken when choosing the best reagent for a given substrate to reduce side reactions such as elimination, aziridine formation and epimerisation. Burgess reagent is widely used in the formation of oxazolines in high yields and enantiomeric purity. Pattenden and Freeman utilised Burgess' reagent to effect the cyclodehydration of **130** to form the oxazoline of raocyclamide **15** as the final step in the total synthesis.[125]



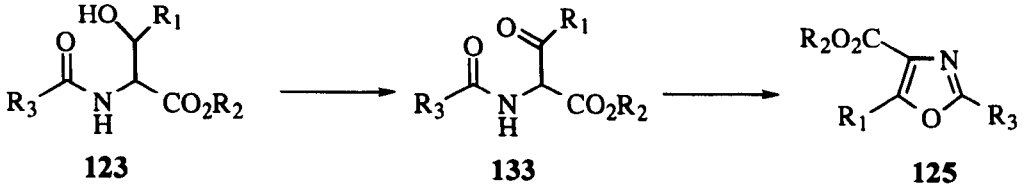
130 **15**

In situations where the hydroxyl component is derived from threonine, *e.g.* **131**, it proves difficult to effect a cyclodehydration and in many cases the main product isolated is the corresponding alkene **132**.



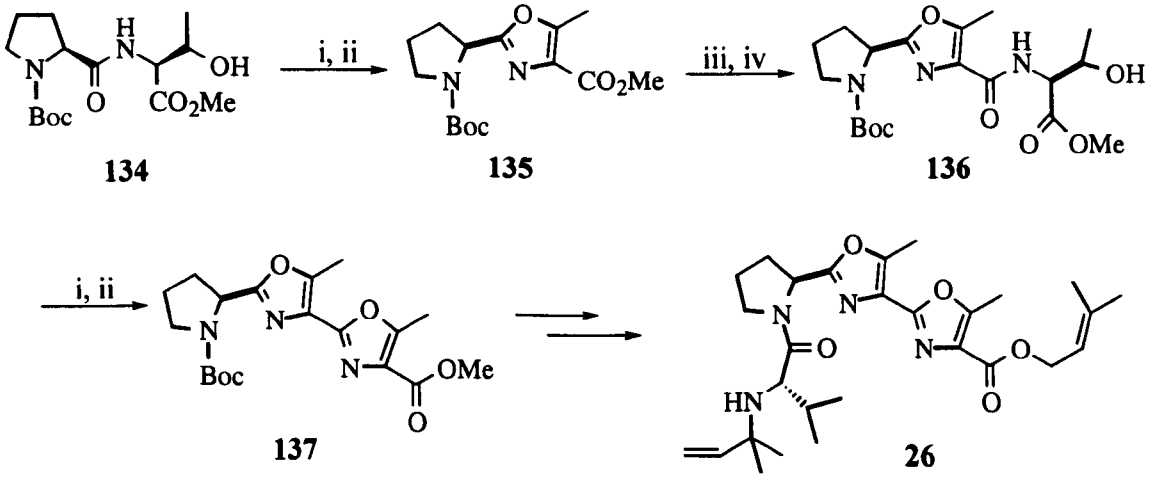
131 **132**

An alternative pathway to oxazoles from β-hydroxy amides has been developed by Wipf *et al.* and has been shown to be extremely useful in oxazole formation *en route* to natural products. Having encountered problems in the oxidation of oxazolines substituted at the 5-position, Wipf pursued a Robinson-Gabriel type cyclisation of a β-keto amide to furnish the corresponding oxazole.[126] This method avoids the problem of β-elimination to form the alkene when attempting a cyclodehydration on a dipeptide derived from threonine *e.g.* **131**, as mentioned earlier. Thus oxidation of a β-hydroxy amide **123** with Dess-Martin periodinane[127] and a subsequent mild cyclodehydration of the β-keto amide **133** with triphenylphosphine, iodine and triethylamine allowed for the rapid synthesis of highly substituted and functionalised oxazoles **125** in good yields.



123 **133** **125**

Amino aldehydes derived from serine residues were found to cyclise to the oxazole in a much less facile manner. This problem was overcome by using a bulky base, *i.e.* 2, 6-di-*tert*-butyl-4-methylpyridine in place of triethylamine, with dibromotetrachloroethane and triphenylphosphine. Under these conditions elimination did not occur spontaneously but required subsequent treatment with DBU to yield the oxazole.[128] Wipf utilised the Robinson-Gabriel oxazole synthesis to install the threonine derived *bis*-oxazole core of muscoride A **26**.[129] Thus, the dipeptide **134** was readily obtained from the condensation of Boc-(*L*)-proline with threonine methyl ester hydrochloride in the presence of isobutyl chloroformate. Oxidation and cyclodehydration of the threonine residue by side-chain oxidation with Dess Martin periodinane followed by exposure to electrophilic phosphorus reagent furnished the first oxazole **135** in 68 % yield. The oxazole **135** was next homologated by hydrolysis of the methyl ester and coupling with (*L*)-threonine to give the tripeptide **136**, which after oxidative cyclodehydration ring - closed to form the second oxazole **137** of muscoride A in 65 % yield (**Scheme 27**).



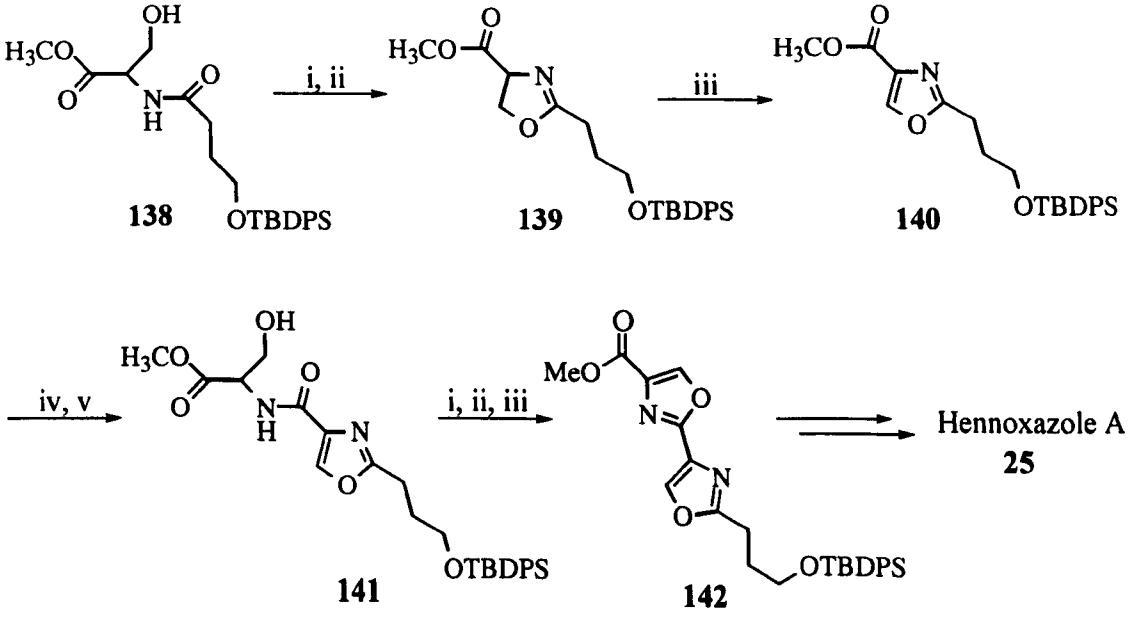
Reagents: i, Dess-Martin periodinane; ii, Ph_3P, I_2, Et_3N, DCM; iii, LiOH, H_2O, THF, 1h; iv, (*L*)-Thr-OMe, ^{i}BuOCOCl, NMM.

Scheme 27

2.5.2 Oxidations of Oxazolines

The oxidation techniques already discussed for the conversion of thiazolines into thiazoles are generally quite versatile and the majority are also applicable to the oxidation of oxazolines. A few examples of the oxidation techniques already discussed (**Section 2.2.3**) will be highlighted here towards the construction of oxazole containing natural products.

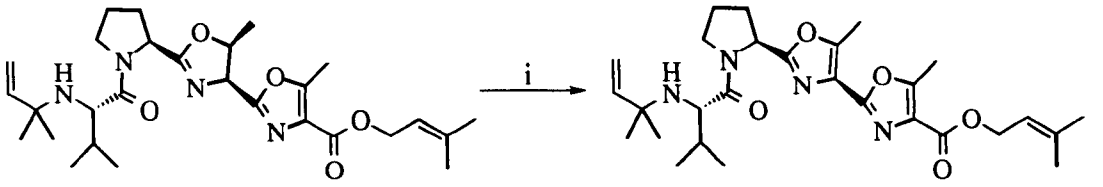
Williams employed a cyclodehydration of an amide using diethylaminosulfur trifluoride (DAST)[97] and further oxidised the oxazoline with $BrCCl_3$/DBU[103] in his total synthesis of hennoxazole A **25**.[130] Thus, cyclodehydration of **138** with DAST at -78 °C first led to the oxazoline **139**, and subsequent oxidation with $BrCCl_3$ and DBU then cleanly effected dehydration to the oxazole **140**. Saponification of the ester in **140** and condensation of the resulting acid with serine methyl ester hydrochloride led to **141**. The cyclodehydration-oxidation protocol was repeated to yield the *bis*-oxazole core **142** of hennoxazole A **25** (**Scheme 28**).



Reagents: i, DAST (1eq.), DCM, -78 °C, 1h; ii, K_2CO_3, -78 °C - 0 °C; iii, $BrCCl_3$, DBU; iv, LiOH (1eq.), THF, H_2O; v, $^tBuOCOCl$, Et_3N, (2eq.), serine methyl ester.HCl (2eq.), DCM, -20 °C.

Scheme 28

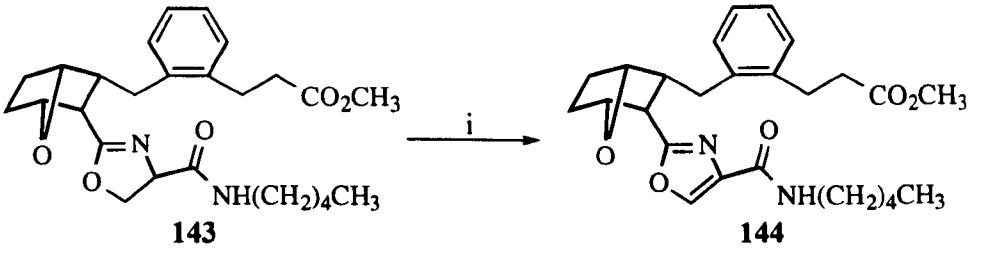
A similar oxidation to that of Williams was developed by Jung *et al.* using DBU, CCl_4 in pyridine and acetonitrile,[104] and this method was utilised by Pattenden *et al.* in their total synthesis of muscoride A (**Scheme 29**).[131]



Reagents: i, DBU, CCl_4, Pyridine, MeCN.

Scheme 29

In addition Bristol-Myers Squibb discovered a novel procedure using a mixture of copper (II) bromide and DBU to effect the oxidation of **143** to **144**.[132]

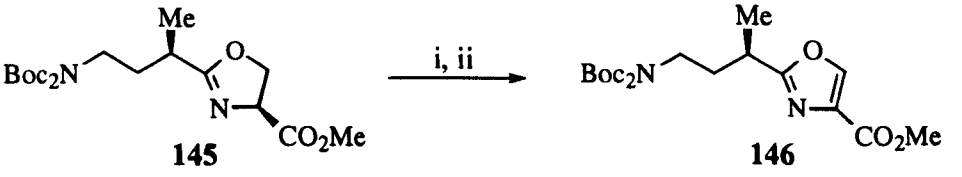


143 **144**

Reagents: i, $CuBr_2$, DBU, HMTA, DCM

Scheme 30

In contrast to these oxidations Evans *et al.* formed a 5-seleno-oxazole and performed an oxidative elimination to form an oxazole in their total synthesis of calyculin **18**.[133] Enolisation of **145** with KHMDS followed by reaction with phenylselenyl chloride and oxidative elimination of the resulting diastereomeric selenides afforded the oxazole **146** in a 57 % overall yield (**Scheme 31**).

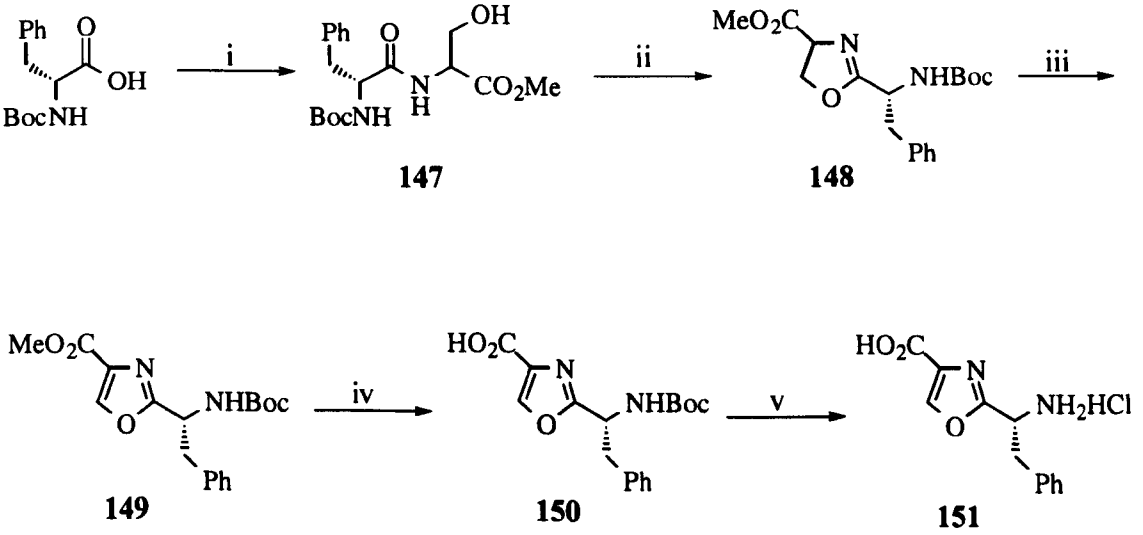


145 **146**

Reagents: i, KHMDS, THF, -78 °C, PhSeCl; ii, 30 % aq. H_2O_2, DCM, pyridine, 0°C

Scheme 31

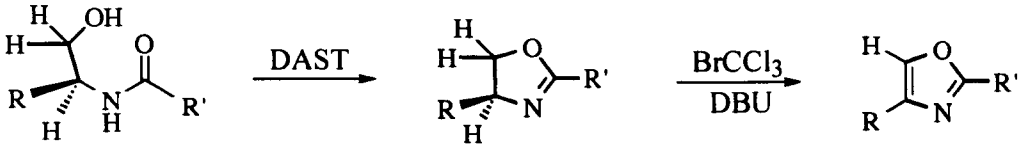
Returning to our own work we first decided to synthesise the oxazole derived from (*D*)-phenylalanine due to the high abundance of this type of substitued oxazole in natural products. This would also allow us to compare the behaviour of oxazoles with that of thiazoles. We synthesised the oxazole by cyclodehydration of the relevent dipeptide to the oxazoline, followed by oxidation to the corresponding oxazole using the mild Williams' oxidation protocol. The oxazole was produced as an enantiomerically pure compound as shown in **Scheme 32**. Thus coupling of the Boc-protected (*D*)-phenylalanine with (*D, L*)-serine methyl ester led to the dipeptide **147** in good yield. Cyclodehydration of the dipeptide **147** using DAST[97, 121] gave the oxazoline **148** in a respectable 76% yield, and oxidation with $BrCCl_3$ and DBU[103] then smoothly led to the oxazole **149**. Saponification of methyl ester **149** with lithium hydroxide afforded the acid **150**, which was subjected to Boc-deprotection with 4 M hydrochloric acid in dioxane to give the oxazole **151** in a 93 % yield (**Scheme 32**).



Reagents: i, ^{i}BuOCOCl, serine methyl ester.HCl, THF, -40°C; ii, DAST, DCM, -70°C; iii, $BrCCl_3$, DBU, 0°C; iv, LiOH, THF, H_2O; v, 4M HCl, dioxane, rt.

Scheme 32

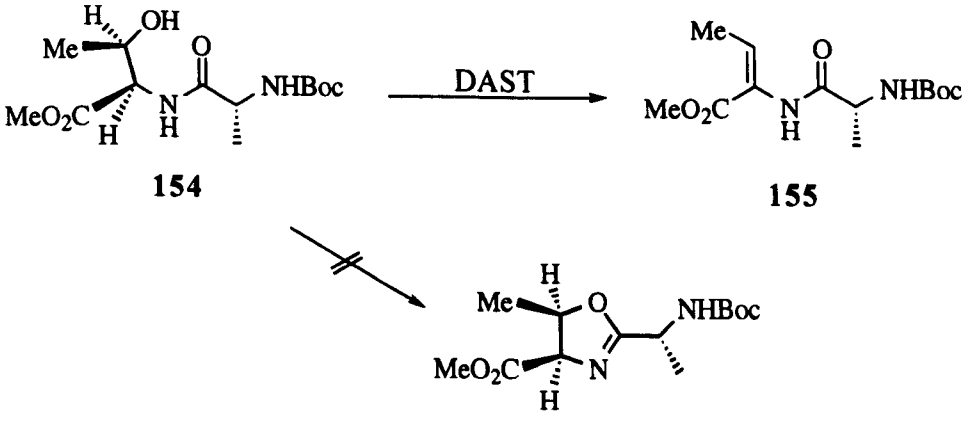
Muscoride **26**, dendroamide A **14** and nostacyclamide **16**, to name but a few, incorporate oxazoles derived from threonine. In addition Wipf *et al.* looked at cyclooligomerisation reactions of oxazolines derived from threonine within their total synthesis of westiellamide **17**.[134] We therefore decided it would be interesting to experiment with such oxazoles ourselves. However, although we had previously synthesised the oxazoles by cyclodehydration of the relevant dipeptide **152** using DAST, followed by mild dehydration using $BrCCl_3$ and DBU to furnish the oxazole **153** in good yields (~ 70 % over the two steps) (**Scheme 33**), other workers in our research group had reported a difficulty when converting dipeptides derived from threonine into oxazolines.



152 **153**

Scheme 33

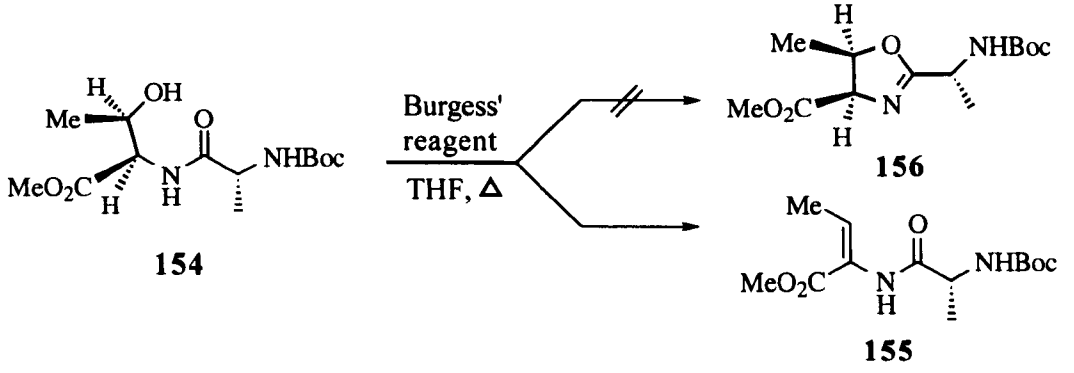
Lellouche *et al.*[135] encountered a limitation of cyclodehydrations using DAST when applied to threonine containing dipeptides, treatment of the dipeptide **154** with DAST gave rise to the corresponding *E*-dehydropeptide **155** (**Scheme 34**).



154 **155**

Scheme 34

In addition to Lellouche's attempts to cyclodehydrate threonine containing dipeptides using DAST, Wipf and Miller had reported the cyclodehydration of threonine containing dipeptides using Burgess' reagent[95, 136] and so we decided to switch tactics. Unfortunately, attempts to form the oxazoline **156** from the dipeptide **154** in reasonable yields failed, the main product obtained being the *E*-dehydropeptide **155** (**Scheme 35**). We attempted to increase the yield of oxazoline by varying reaction times from 1h to 7h, by changing the concentration and also by attempting a one-pot cyclodehydration/oxidation reaction but all attempts failed.

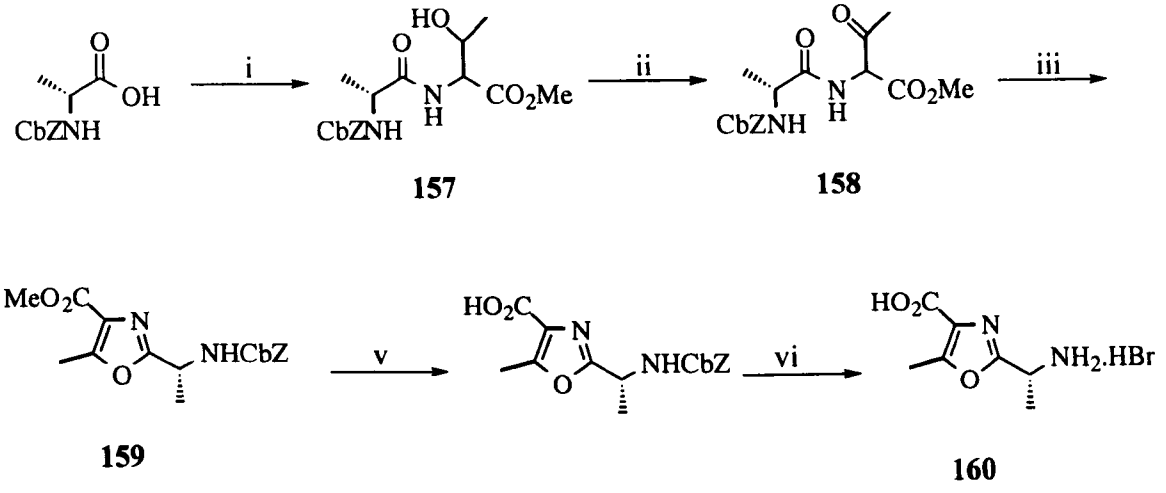


154 **156** **155**

Scheme 35

On closer examination we noticed that both Wipf and Lellouche had only used CbZ-protected amines in contrast to our Boc-protected dipeptides. Attempts within our research group to form another oxazoline had also failed when the dipeptide was Boc-protected, indeed simply swapping protecting groups for CbZ saw an increase in the yield of oxazoline formed from ~10 % to nearly 30 %. However, such a low yield would make a large scale synthesis quite laborious and as a consequence we decided to switch tactics yet again. We decided to pursue a Robinson-Gabriel type cyclisation as Wipf and Venkatraman had done in their total synthesis of muscoride A **26**.[129] Thus, coupling of CbZ-protected (*D*)-alanine with (*D, L*)-serine methyl ester hydrochloride using EDC and HOBt first gave the dipeptide **157**. Oxidation of the alcohol in **157** using Dess-Martin periodinane[127]

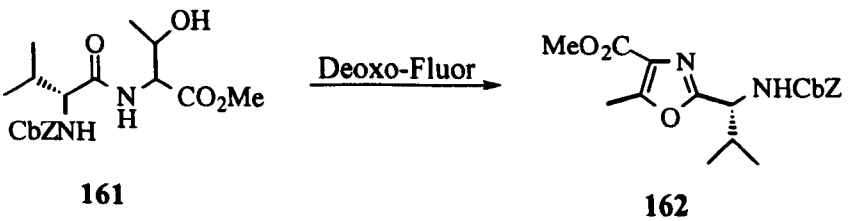
next led to the β-keto amide **158** in an 86 % yield. The β-keto amide was then exposed to Ph_3P, Et_3N and iodine at 0 °C and cyclised to the oxazole **159** in a pleasing 84 % yield. Saponification of the oxazole was carried out in ethanol using sodium hydroxide and finally the CbZ group was removed using a 33 % solution of HBr in acetic acid to leave the oxazole **160** as a brown, highly hygroscopic powder (**Scheme 36**).



Reagents: i, EDC, HOBt, NMM, THF; ii, Dess-Martin periodinane, DCM, rt; iii, Ph_3P, I_2, Et_3N, THF, -78 °C; iv, NaOH, EtOH, H_2O, rt; v, HBr, HOAc, rt.

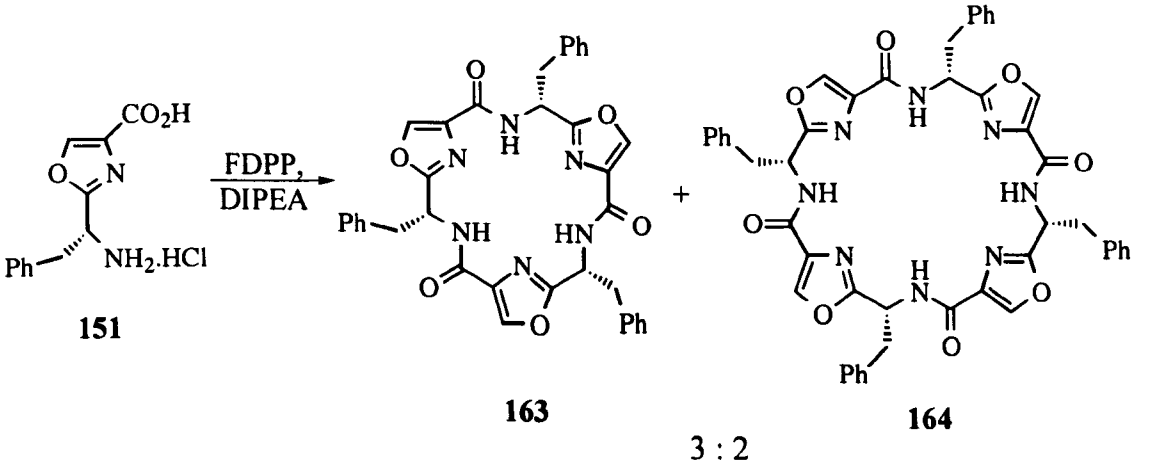
Scheme 36

It is important to note that in March 2000, after we had synthesised this oxazole, Wipf and colleagues reported the use of *bis*-(2-methoxyethyl)-aminosulfur trifluoride (Deoxo-Fluor), to effect cyclodehydrations of threonine containing dipeptides to the corresponding oxazolines. Indeed, cyclodehydration of **161** to the oxazoline **162** was achieved in a 72 % yield compared with a 27 % yield when using DAST.[137]



2.6 Cyclooligomerisations of Oxazoles

Initial attempts to cyclise the oxazole **151** derived from (*D*)-phenylalanine using the same conditions employed for the corresponding thiazoles gave a disappointing 12 % yield of cyclic trimer **163** and cyclic tetramer **164**, even following optimisation we were only able to increase this yield to 31 %. In an attempt to further optimise this reaction, we swapped the coupling reagent FDPP for DPPA and managed to increase the yield of cyclic products to 49 %. These lower yields in comparison with thiazole cyclooligomerisations probably result from the poor solubility of the oxazole in acetonitrile even after addition of base. Interestingly, the ratio of cyclic trimer **163** : cyclic tetramer **164** varied drastically from those found when performing cyclooligomerisations of the phenylalanine derived thiazoles. Unlike the phenylalanine derived thiazole the cyclic trimer **163** was now the favourable product (**Scheme 37**).

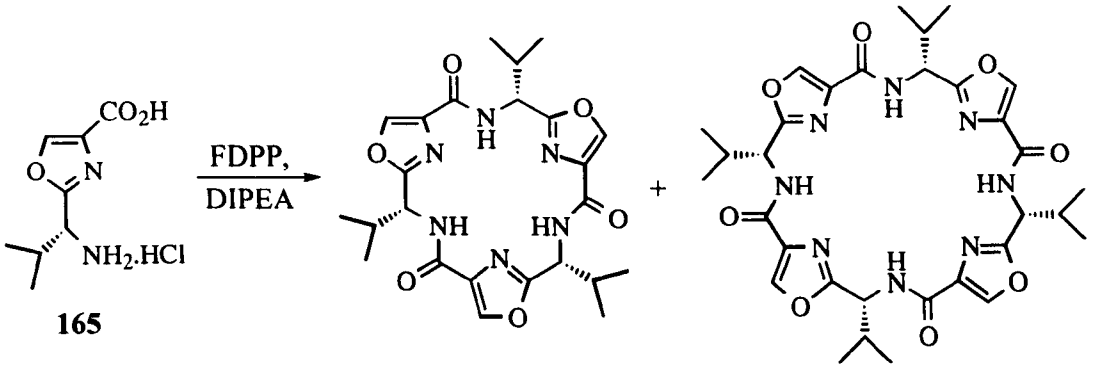


Scheme 37

The differences in product ratios of the oxazole and thiazole derivatives may be as a result of differences in their geometry, orientation of the alkyl side chains, or concentration effects as a result of the differing solubility of oxazoles and thiazoles. Molecular modelling of the cyclic trimer **163** reveals it to possess a minimum

energy conformation of just 173 kJmol^{-1} compared with a minimum energy conformation for the thiazole trimer **95** of 264 kJmol^{-1}.

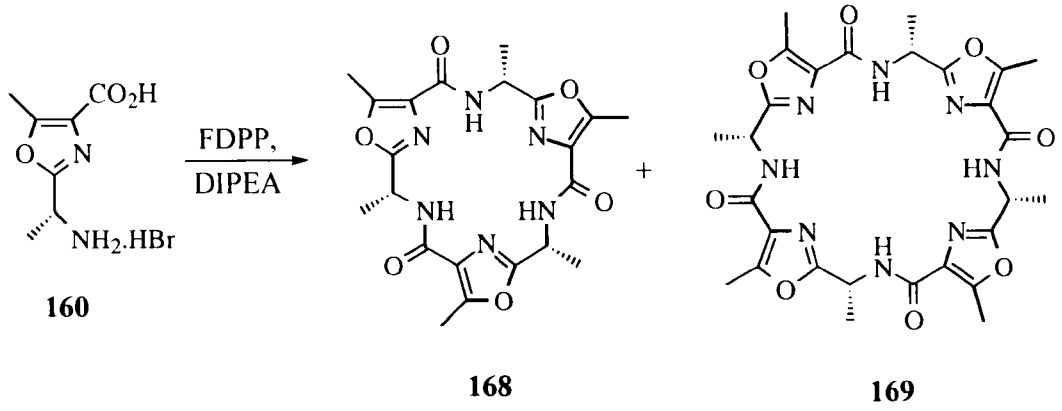
Subjection of the oxazole **165** derived from valine to our cyclooligomerisation conditions led to a very disappointing 12 % yield of cyclic products. The valine oxazole was significantly less soluble than the phenylalanine derived oxazole, thus providing further credibility for our solubility argument. The ratio of cyclic trimer **166** : cyclic tetramer **167** is drastically different to that obtained for the analogous thiazole. The cyclic trimer **166** and tetramer **167** were isolated in a 1 : 1 ratio, *cf* a 3 :1 ratio in the case of the thiazole (**Scheme 38**).



165 **166** **167**

Scheme 38

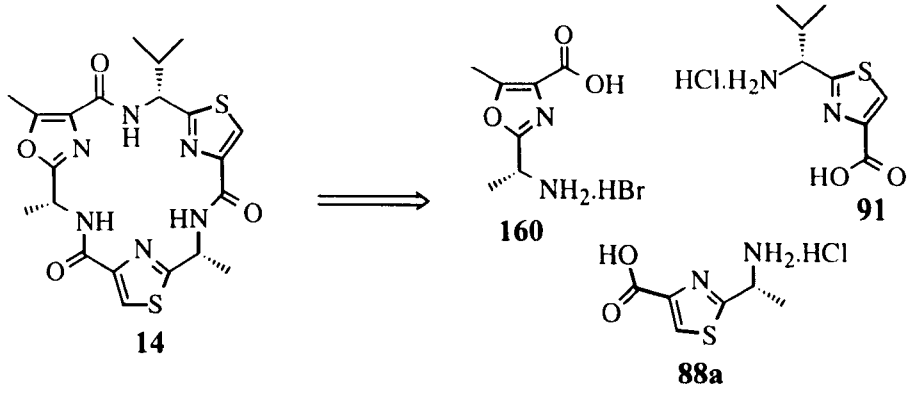
Attempts to perform a cyclooligomerisation reaction from the threonine/alanine derived oxazole **160** were equally disappointing. Exposure of **160** to the optimised coupling conditions led to only trace amounts of cyclic products. Indeed, performing the reaction on a 150 mg scale led only to the isolation of 2 mg of cyclic products comprising approximately of a 1 : 1 ratio of cyclic trimer **168** : cyclic tetramer **169** as determined by ^{1}H NMR (**Scheme 39**). The solubility of this oxazole was very poor, with the compound appearing to be present mainly as a suspension throughout the reaction. The oxazole based cyclooligomerisations now await optimisation which may just involve identifying better solvents for the reaction.



Scheme 39

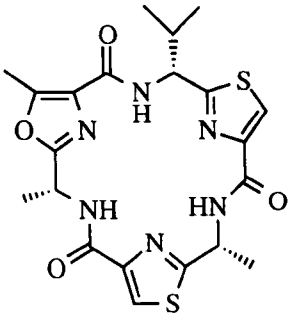
2.7 Total Synthesis of Dendroamide A

At the outset of our studies we had always been interested in applying our newly established cyclooligomerisation methodology to natural product targets. Dendroamide A **14** is a bistratamide type cyclic hexapeptide which exhibits multidrug resistance reversing activity and was isolated from the cyanobacterium *Stigonema dendroideum* in 1996.[22] Its structure is made up of three heterocyclic units, *i.e.* a thiazole derived from (*D*)-valine and cysteine **91**, a thiazole derived from (*D*)-alanine and cysteine **88a**, and an oxazole derived from (*D*)-alanine and threonine **160** .

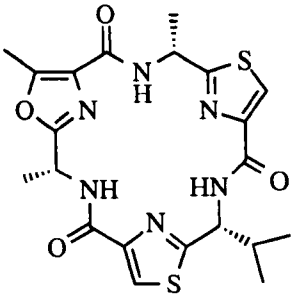


Our ultimate aim was to take a 1:1:1 mixture of these constituent heterocycles, and attempt to 'self-assemble' them to form dendroamide A **14**. This was not considered a simple task, considering that a cyclooligomerisation reaction from the three heterocycles

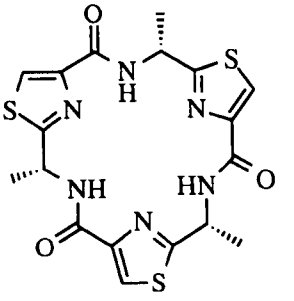
would lead to the formation of 49 possible cyclic trimers and tetramers! We did, however, have something working in our favour, that is thiazole heterocycles with smaller alkyl side chains did form cyclic trimers in preference to cyclic tetramers, but there would still be a possibility of forming eleven cyclic trimers *i.e.* **14**, **171**, **93**, **42**, **168**, **172**, **173**, **174**, **175**, **176**, and **177**. We decided the best way to move forwards was to first synthesise dendroamide A **14** totally in a linear fashion. Once the natural product was fully characterised we could then move on to our studies of the mixed cyclooligomerisation of **160**, **91**, and **88a**.



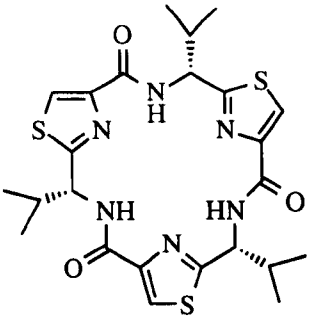
14



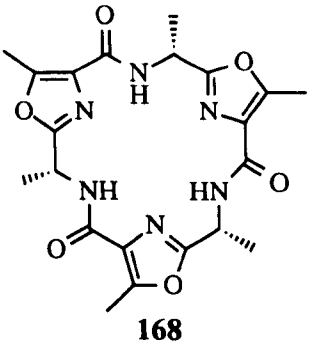
171



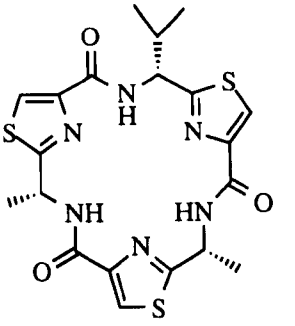
93



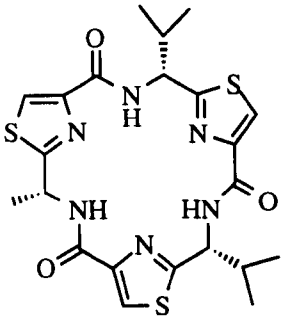
42



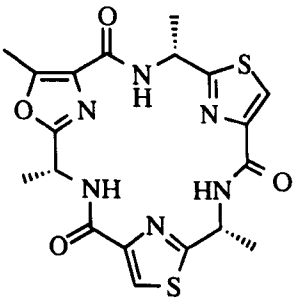
168



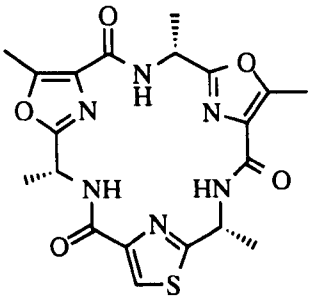
172



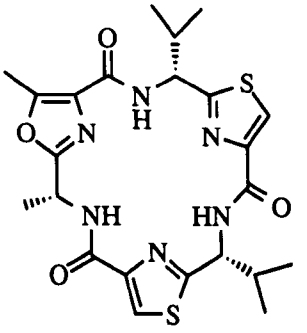
173



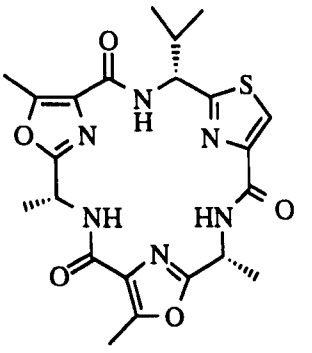
174



175

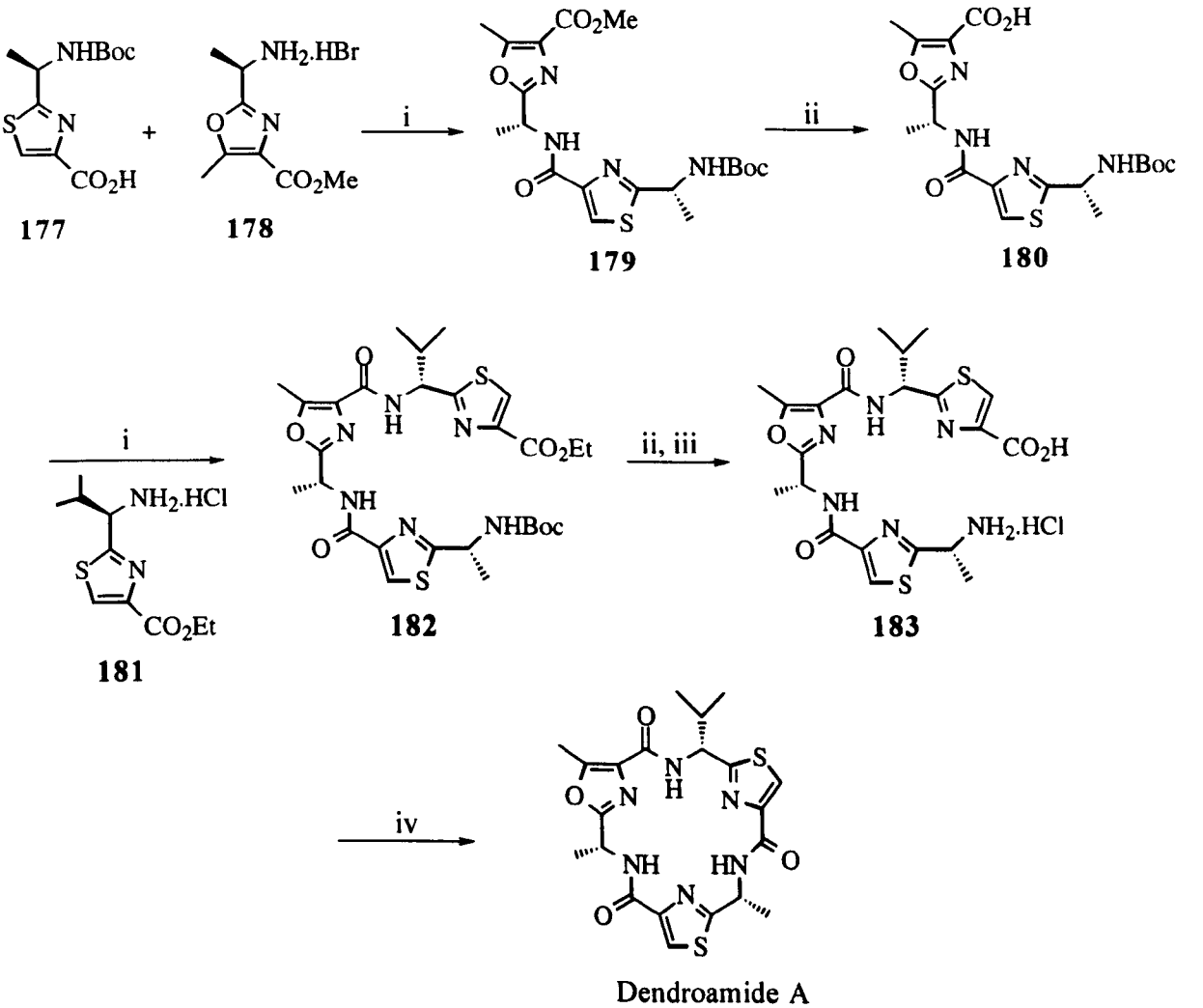


176



177

Thus coupling of the oxazole amine **178** and the thiazole acid **177** under carbodiimide mediated conditions first led to the linear tetrapeptide **179** in an 80 % yield. Saponification of this tetrapeptide **179** followed by coupling with the (*D*)-valine derived thiazole amine **181**, next gave the linear hexapeptide **182** in good overall yield. The macrocyclic precursor **183** was then obtained following saponification and Boc-deprotection of **182** in an overall 94 % yield. Macrocyclisation of **183** was achieved under our optimised coupling conditions, using a slightly higher dilution of 0.01M, to finally provide dendroamide A **14** in a pleasing 91 % yield (**Scheme 40**).



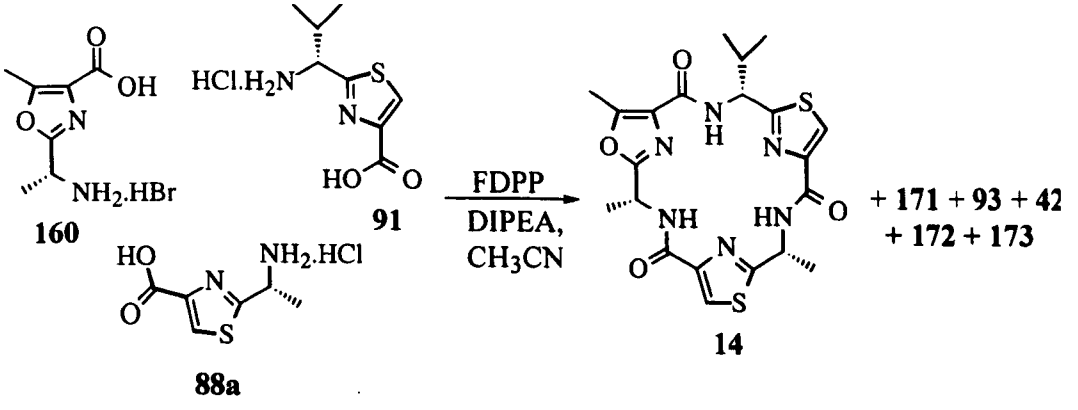
Reagents: i, EDC, HOBt, NMM, DCM, 0 °C; ii, NaOH, THF, H_2O, rt; iii, 4M HCl, dioxane, rt; iv, FDPP, DIPEA, CH_3CN, rt.

Scheme 40

The synthetic material had chiroptical and NMR spectroscopic data which were found to be identical to those reported for the natural product isolated from *S. dendroideum* (**Appendix 4.1, 4.2**).[138]

2.7.1 Cycooligomerisation Reactions towards Dendroamide A

Having obtained spectroscopic and chromatographic data for the natural dendroamide A it was now time to attempt the reaction which had provided the focus and the impetus for the whole project. A 1:1:1 mixture of the heterocyclic amino acids, **160**, **91**, and **88a** in acetonitrile was treated with DIPEA and FDPP at room temperature and the reaction was stirred for three days. Using conditions we had adapted during our earlier studies, the reaction mixture was now subjected to hplc analysis using a reverse phase C_{18} column, and an acetonitrile/water solvent system. We prepared ourselves for what could well be a very complicated hplc trace. To our delight chromatography led to the isolation of dendroamide A **14** (23 %) together with its positional isomer **171** (22 %) accompanied by only the products **93**, **42**, **172** and **173** resulting from homo-coupling of the thiazole amino acids **88a** and **91** in an overall 75 % yield (**Scheme 41**)(**Table 6**).

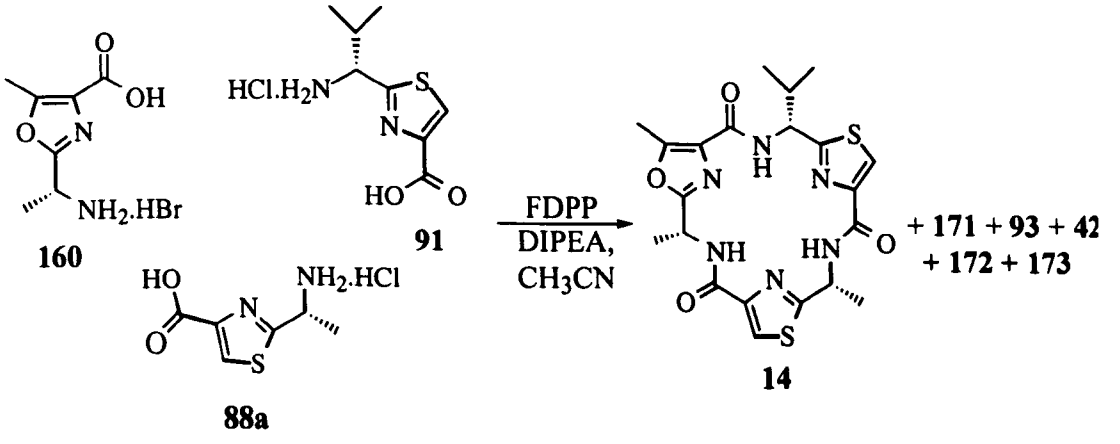


Scheme 41

Interestingly no cyclic tetramers were detected by mass spectrometry or hplc. The structures of these other cyclic compounds were determined following their independent synthesis, using a linear approach similar to that shown in **Scheme 40**, *viz* experimental pages 126 to132. In order to confirm the identity of these compounds in the hplc trace,

small amounts of the crude mixture of cyclic products obtained from the 'mixed cyclooligomerisation' were enriched in turn with each of these by-products and the natural product. These spiked samples were injected onto the hplc column, resulting in an enlargement of the peak corresponding to that product in the hplc trace of the mixture.[138]

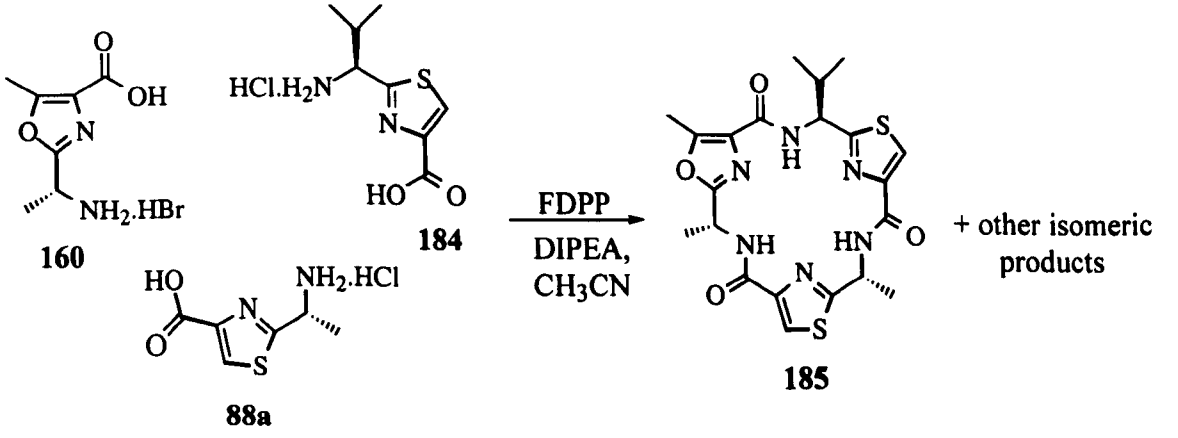
We have already shown that thiazole heterocycles undergo homo-cyclooligomeriation reactions much more readily than their oxazole counterparts (**Sections 2.3** and **2.6** respectively). Bearing this in mind we envisaged that the thiazole heterocycles would be more competitive in this mixed cyclooligomerisation than the oxazole heterocycle in forming peptide bonds. In an attempt to further tailor this 'self-assembly' reaction towards the formation of dendroamide A **14** we decided to increase the amount of oxazole in the starting mixture. By performing the reaction with a 1 : 1 : 2 ratio of **91** : **88a** : **160** it was possible to increase the composition of dendroamide A **14** to 31 % from 23 % of the cyclic products formed. Unfortunately, increasing the amount of oxazole present three-fold and four-fold, with respect to the thiazoles, failed to further increase the amount of dendroamide A **14** formed (**Table 6**).



Heterocycles	Percentage Composition of Cyclic Products						
91 : 88a : 160	**93**	**172**	Dendroamide A **14**	**171**	**168**	**173**	**42**
1:1:1	14	37	23	26			
1:1:2	16	29	31	15		4	4
1:1:3	18	23	30	21	5	2	
1:1:4	10	27	23	14	13	5	6

Table 6

As discussed already, dendroamide A **14**, unusually, contains three non-natural (*D*)-amino acid residues. In light of the results we obtained for the cyclooligomerisations of the enantiomerically enriched phenylalanine derived thiazole, in which the trimer with one side chain on the opposite face was of lower energy (and thus was the predominant product), we decided to attempt our mixed cyclooligomerisation reaction using the thiazole derived from (*L*)-valine **184** in order to ascertain whether this subtle difference affects the distribution of isomeric products. Could the results we obtain shed any light on why nature is so keen on incorporating (*D*)-amino acids? Hence, a 1:1:1 mixture of **88a**, **184**, and **160** was therefore stirred under our standard reaction conditions for three days (**Scheme 42**).



Scheme 42

Upon analysis of the crude mixture of cyclic products by hplc we found that the amount of isomeric dendromide A **185** formed was 34 % compared to the 23 % of dendroamide obtained in the analogous reaction. Molecular modelling of dendroamide A **14** and of the isomeric compound **185**, incorporating the (*L*)-valine derived thiazole showed the two compounds to adopt conformations with minimised energies of 178 $kJmol^{-1}$ and 179 $kJmol^{-1}$ respectively, suggesting that the affects of steric interactions with relatively small side chains is negligible (**Figure 9**).

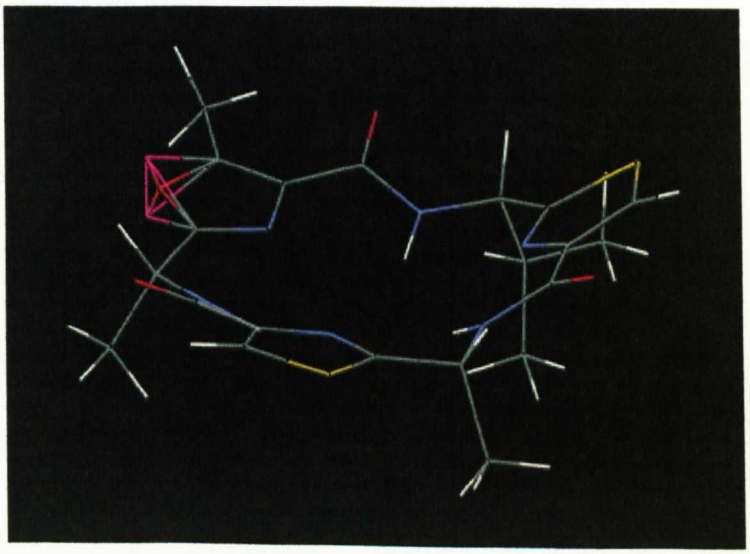
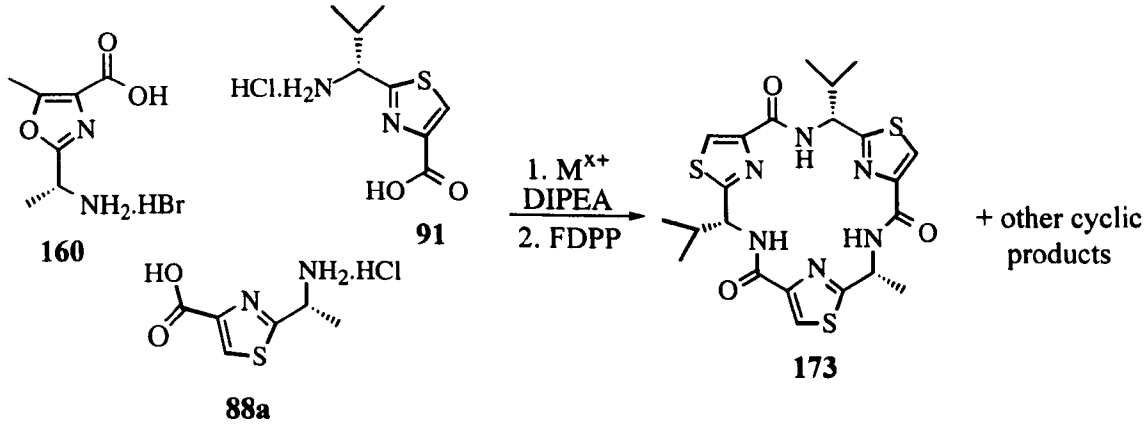


Figure 9 : Dendroamide A - energy minimised

2.7.2 Metal Templation Reactions towards Dendroamide A

Does nature use metal ions as convenient biological templates in the formation of peptidic metabolites? We have already investigated the ability of metal ions to act as templates and alter the ratio of cyclic trimer and cyclic tetramer formed from a homogenous mixture of heterocyclic amino acids (**Sections 2.3** and **2.6**). If a metal ion is used as a template in a cyclooligomerisation reaction from a heterogeneous mixture of heterocyclic amino acids, is it possible to not only increase the amount of cyclic trimer formed, *cf* cyclic tetramer, but also to bias the ratio of cyclic trimers towards a particular product?

When we reconsidered the metal templation experiments we had already carried out, we decided to increase the time the metals had to template the heterocycles before the coupling reagent was added. In previous experiments the heterocycles had been stirred in the presence of the metal salts for just five minutes before the coupling reagent was added. With hindsight this may not have been long enough for the metal to template the most favourable compound. Thus a 1:1:1 mixture of the constituent heterocycles of dendroamide A, **160**, **91**, and **88a**, was stirred in acetonitrile with DIPEA at room temperature. The appropriate metal salt was then added and the solution was stirred for six hours. We believed six hours was ample time for any equilibrium to be reached promoting the most favourable order of heterocycles to be chelated to the metal ion. FDPP was then added and the reaction was stirred for a further three days. We initially experimented with the alkali metals before moving onto the other abundant ions Cu^{2+}, Zn^{2+}, Ag^{+} and Ca^{2+} (**Scheme 43**).



Scheme 43

To our amazement the metal ions appeared to be extremely capable of altering the distribution of cyclic products. Using lithium as a template the amount of the trimer **173** was increased from 29 % without a metal to 59 % in the presence of the metal. In addition, other ions also increased the amount of **173** formed but to varying amounts. As we had previously calculated we correlated the amount of **173** formed with the ionic radii of the ions (co-ordination number = 4), and found that the trend was quite

remarkable. With the smallest ion, copper, the amount of **173** formed was approximately double the amount formed without a metal As the radius of the ions increase this affect drops off. Indeed using potassium, whose ionic radius is around twice that of copper, there is no change in the distribution of products (**Table 7**).

		Percentage Composition of Cyclic Products	
Metal Salt	Ionic Radius / Å	173	Dendroamide A
$Cu(BF_4)_2$	0.71	61	
$LiBF_4$	0.73	59	
$Zn(BF_4)_2$	0.74	59	
$NaBF_4$	1.13	48	
KBF_4	1.51	29	
No Metal	-	29	
$AgBF_4$	0.81	100	
$Ca(BF_4)_2$	1.14	23	52

Table 7

Unfortunately the yield of cyclic material obtained in these 'metal templated' reactions is considerably lower than that obtained in the analogous cyclooligomerisations in the absence of a metal. It is probable that this is a result of a decrease in the reaction rate when the amino acids are chelated to a metal ion. At present these reactions await optimisation, this could possibly be achieved by simply heating the reaction or changing the solvent or reaction time.

The macrocyclic cavity of the cyclic hexapeptides we are dealing with is reported to be in the range 5.2-5.6 Å,[24] considering the case of copper(II) (d_i = 1.42), and assuming the metal ion sits within the proximity of the macrocyclic cavity, approximately 2 Å is available around the metal ion in which intramolecular interactions with the macrocycle can take place (**Figure 10**). 2 Å is the optimum distance for intramolecular interactions

to take place. This therefore, provides further evidence that copper would be much more liable to act as a template compared with, for example, potassium. Potassium possesses twice the ionic diameter of copper, and thus would only allow approximately 1 Å space around the ion for intramolecular interactions to take place.

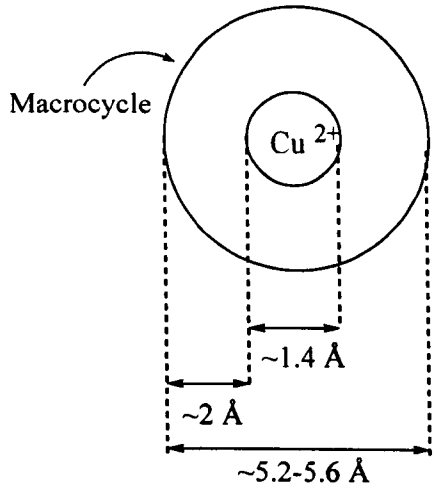


Figure 10

It is important to stress that little is known about the qualities of ions that render them good templates for certain compounds. The size of the ion is probably a major factor but, we don't know what co-ordination number the metals adopt or exactly how they interact with these heterocycles. In the case of silver and calcium the results obtained do not fit into the simplistic size correlation argument. Indeed, using silver as a template only **173** is formed. Silver is bigger than zinc and so if the size correlation was to hold we would expect around a 50 % yield of **173**.

We have found a range of metals which can be used to tailor the distribution of products towards **173**. Indeed, we can form **173** exclusively using silver as a template, but our interests were in finding a metal which would template the formation of dendroamide A **14**. When calcium was used in the self-assembly 52 % of the cyclic products consisted of dendroamide A **14**, with the amount of **173** being reduced to just 23 % (**Table 7**).

In conclusion we can deduce that silver(I) and copper(II) possess just the right geometry and electronic properties to chelate two valine derived thiazoles and an alanine derived thiazole, and thus facilitate the formation of **173**. In addition calcium(II) possesses the right properties to chelate each component of dendroamide A **14**, and thus facilitate its formation. It is not possible to generate a lowest energy conformation of a chelated cyclopeptide as the calculations required are too complex. However it is possible to insert a metal ion into the macrocyclic cavity to illustrate how well a calcium ion fits into the macrocyclic cavity of dendroamide A(**Figure 11**).

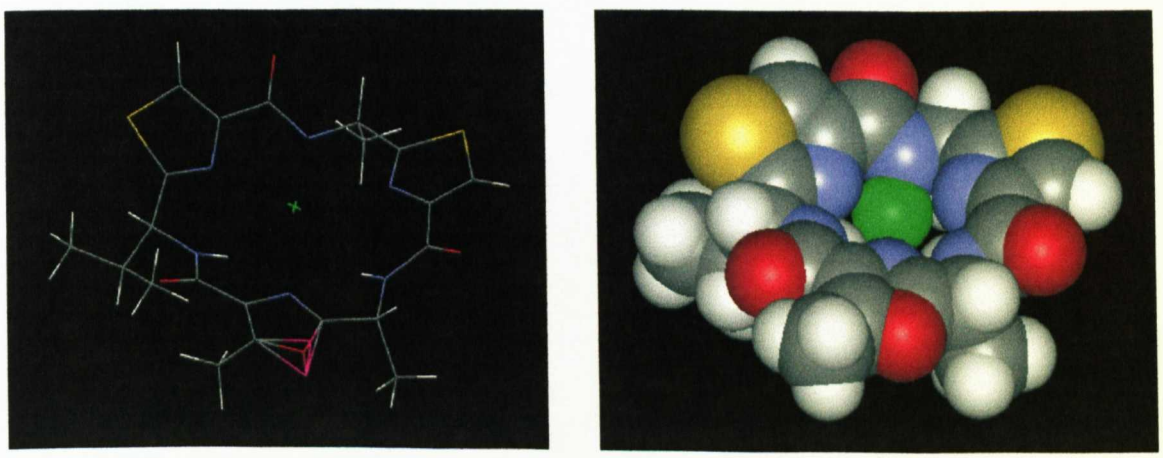


Figure 11 : Dendroamide A containing a Ca^{2+} ion (LHS); Dendroamide A containing a Ca^{2+} ion - a space filling representation (RHS)

In conclusion we have been able to tailor the mixed cyclooligomerisation reaction in which a possible forty nine cyclic trimers and tetramers could be formed into a highly selective reaction in which just one compound is produced. Furthermore, depending on the metal ion we use as a template we can form **173** exclusively or greatly increase the amount of dendroamide A **14** produced. Our next aim of our work was to attempt to apply this methodology to the self-assembly of another natural product.

2.8 Total Synthesis of Nostacyclamide

Nostacyclamide **16** is a bistratamide type cyclic hexapeptide isolated from *Nostoc* Sp.[24] Its structure consists of two thiazoles, one derived from (*D*)-valine and cysteine **91**, and the other from glycine and cysteine **185**, and the macrocycle is completed using an oxazole derived from (*L*)-alanine and threonine **186**. Moody and Bagley published a total synthesis of this cyclic hexapeptide in 1996 using their recently developed rhodium(II) catalysed N-H insertion reactions of diazoketoesters to form the oxazole.[139] We now wished to apply our novel cyclooligomerisation reactions to 'self-assemble' nostacyclamide from its constituent heterocycles (**Figure 12**).

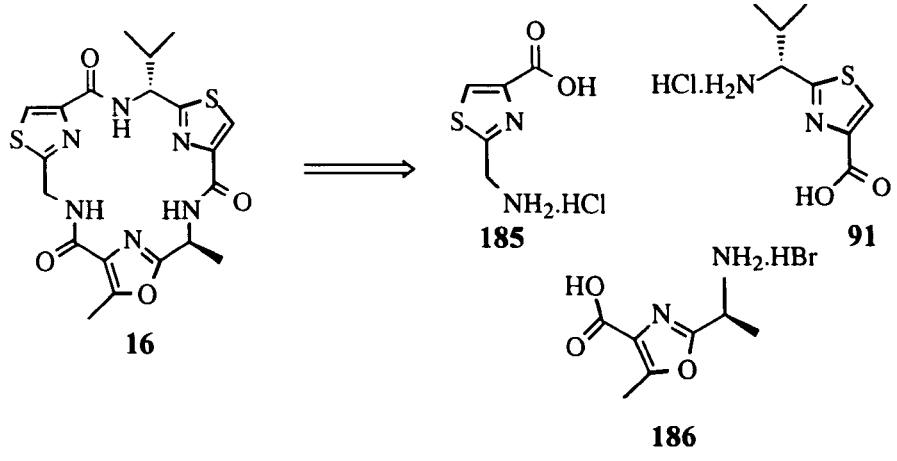
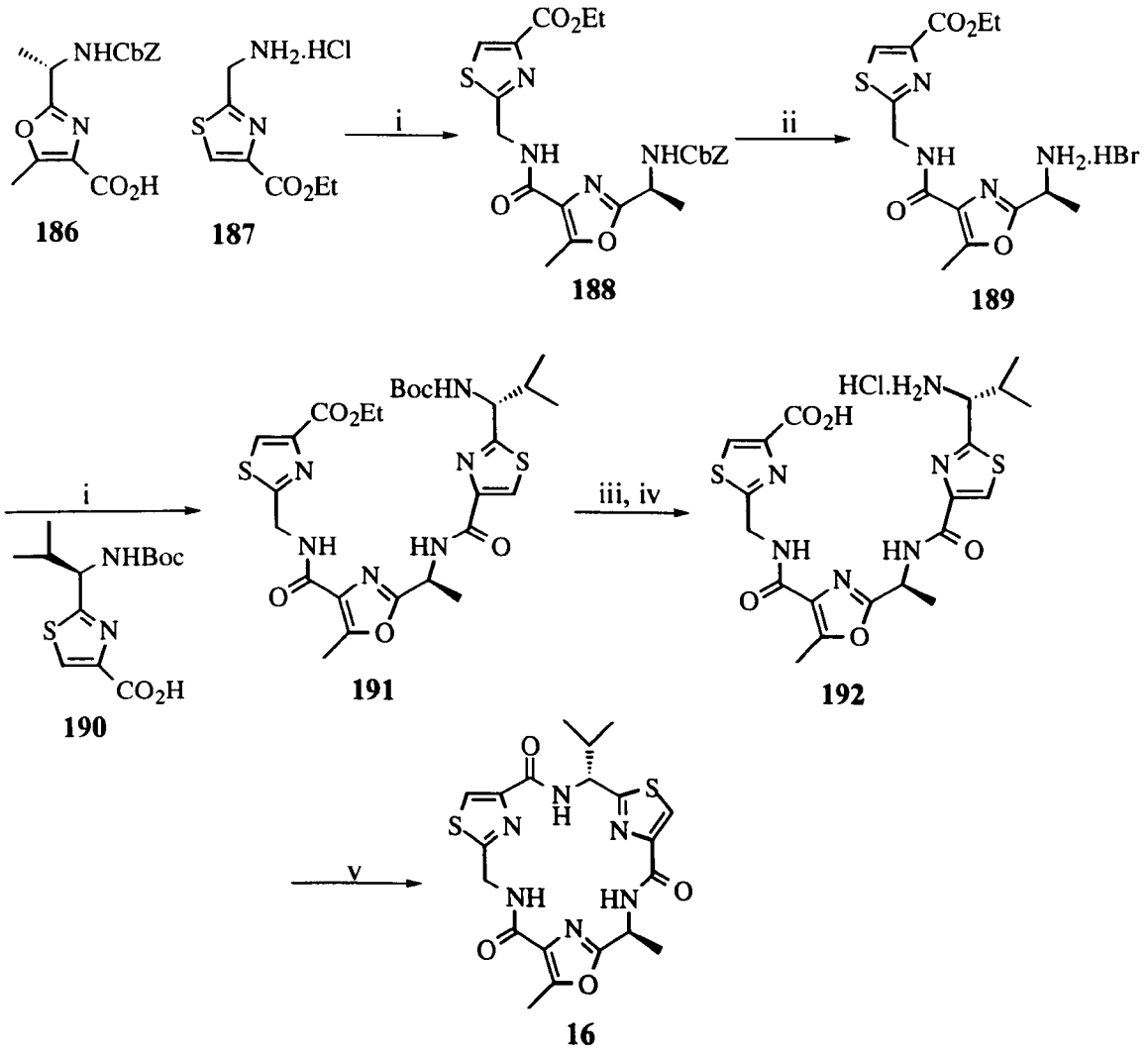


Figure 12

In a similar fashion to our approach with dendroamide A **14**, we decided to firstly synthesise nostacyclamide to aid the identification of the natural product amongst the cyclic products obtained from a mixed cyclooligomerisation. Thus, coupling of the oxazole acid **186** with the thiazole amine **187** under carbodiimide mediated conditions first led to the formation of the tetrapeptide **188** in a 60 % yield. Deprotection of the CbZ group in **188** was then achieved using a 33 % solution of HBr in acetic acid to leave the corresponding amine as its hydrobromide salt **189**. This bromide was then coupled with the thiazole acid **190** to give the linear hexapeptide **191**. Saponification and Boc-deprotection of **191** led to the macrocyclic precursor **192** in good yield. Finally exposure of **192** to FDPP, and DIPEA in anhydrous acetonitrile led to the isolation of

nostacyclamide in a 62 % yield. The synthetic material had chiroptical and spectroscopic data which were found to be identical to those reported for the natural product isolated from *Nostoc* sp. (**Scheme 44**).

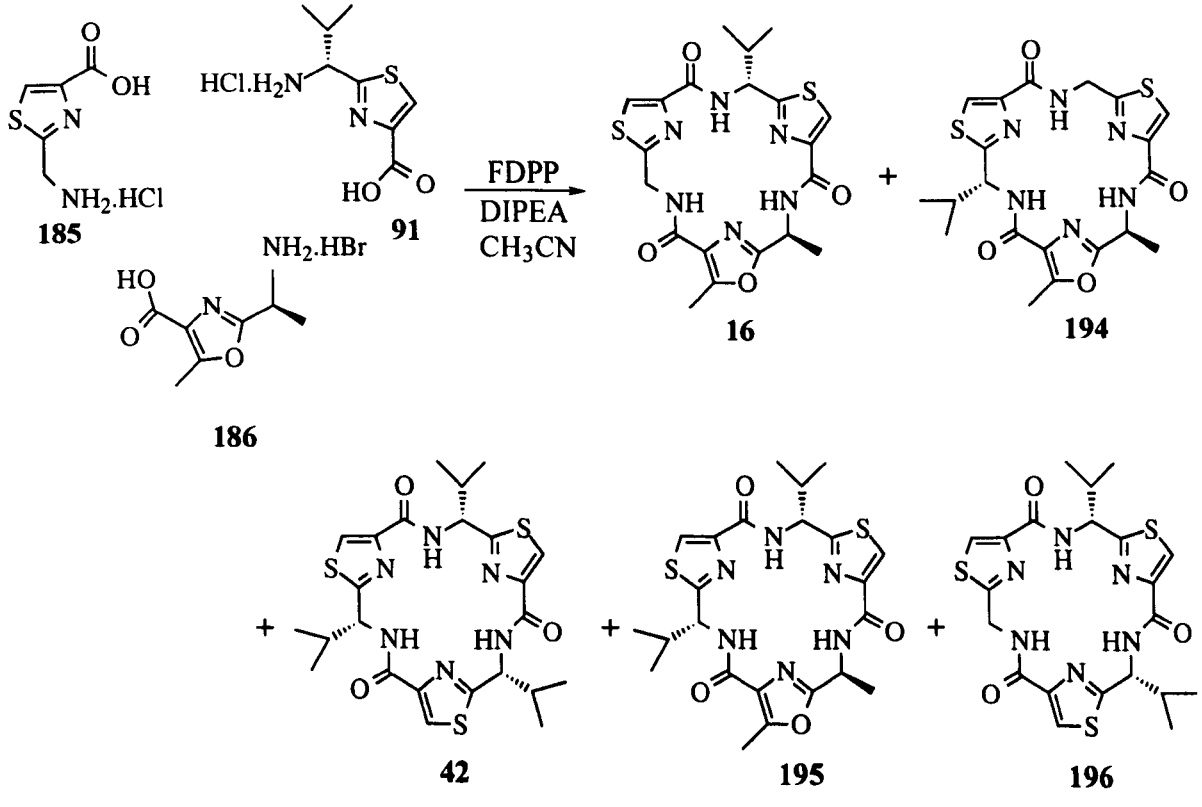


Reagents: i, EDC, HOBt, NMM, DCM, 0 °C; ii, 33 % HBr in acetic acid; iii, NaOH, THF, H_2O, rt; iv, 4M HCl, dioxane, rt; v, FDPP, DIPEA, CH_3CN.

Scheme 44

2.8.1 Cyclooligomerisation Reactions towards Nostacyclamide

A 1:1:1 mixture of **185**, **91**, and **186** was then stirred in acetonitrile with DIPEA and FDPP at room temperature for three days. Analysis of the crude mixture of cyclic products obtained, by hplc, revealed the presence of six cyclic trimers and no cyclic tetramers. The cyclic products were composed of nostacyclamide **16** (21 %), the structural isomer of nostacyclamide **194** (23 %), the cyclic trimer of the valine derived thiazole **42** (6 %), **195** (21 %), **196** (21 %), and 8 % of an unidentifiable product (**Scheme 45**). The reaction showed similar selectivity to the self-assembly we had performed towards dendroamide A **14** which was very pleasing. In a similar fashion we now wanted to attempt to metal template the formation of one of these six products, preferably nostacyclamide **16**!



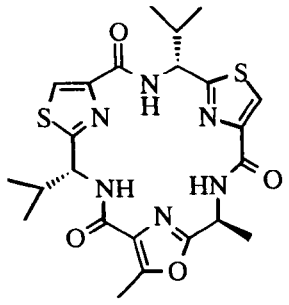
185 **91** **186** **16** **194** **42** **195** **196**

Scheme 45

2.8.2 Metal Templation Reactions towards Nostacyclamide

The metal salts which we employed in our dendroamide A **14** studies were used again to attempt templation of nostacyclamide **16**. Thus a 1 : 1 : 1 mixture of **185**, **91**, and **186** was stirred in anhydrous acetonitrile with DIPEA in the presence of a metal salt at room temperature. After stirring the mixture for six hours, FDPP was added and the mixture was again stirred for a further three days at room temperature. The crude mixture of cyclic products was analysed by reverse phase hplc using the conditions already described. The results that were obtained using these metals did not adhere to the clear cut trend as was observed for dendroamide A **14**. One group of metals promoted the formation of **195**, to varying extents, while to our delight the other group of metals promoted the formation of nostacyclamide **16** (**Scheme 45**).

Firstly, zinc, sodium, potassium and silver were found to increase the proportion of **195** formed in the cyclic products. Using $Zn(BF_4)_2$ as a template the proportion of **195** produced was increased from 21 % without a metal to 36 % composition of the total cyclic products produced. The slightly larger ion, Na^+ increased this proportion to 44 %, and using the even larger ion, K^+ the amount of **195** formed was increased to 48 % (**Table 8**). During the metal studies with dendroamide A, silver was found to be an excellent template for one particular cyclic hexapeptide, forming **173** exclusively. Returning to the nostacyclamide studies, when a 1 : 1 : 1 mixture of **185**, **41** and **186** was stirred with DIPEA and $AgBF_4$ in anhydrous acetonitrile and later with FDPP, analysis of the resulting cyclic products revealed **195** as the **only** product of the reaction (**Table 8**).



195

Metal Salt	Ionic Radius/Å	Percentage Composition of Cyclic Products		
		195	Nostacyclamide **16**	Nostacyclamide Structural Isomer **194**
no metal	-	21	21	23
$Zn(BF_4)_2$	0.74	36	25	38
$AgBF_4$	0.81	100	-	-
$NaBF_4$	1.13	44	28	28
KBF_4	1.51	48	26	26

Table 8

Cu^{2+}, Li^+ and Ca^{2+} were found to promote the formation of nostacyclamide **16**. Using copper as a template only two cyclic products were formed. The mixture comprised nostacyclamide (66 %) and the structural isomer of nostacyclamide **194** (34 %). Increasing the ionic radius of the metal ion slightly, moving to Li^+, the ion still had a significant effect on the distribution of products. The amount of nostacyclamide **16** formed was enhanced from 21 % composition to 45 % of the total cyclic products. Ca^{2+} was also found to increase the proportion of nostacyclamide **16** produced from 21 % to 48 % (**Table 9b**). In this instance, however, there was not the same correlation between the ionic radius of the template and the effectiveness of the ion as a template.

		Percentage Composition of Cyclic Products	
Metal Salt	Ionic Radius/Å	Nostacyclamide **16**	Nostacyclamide Structural Isomer **193**
$Cu(BF_4)_2$	0.71	66	34
$LiBF_4$	0.73	45	13
$Ca(BF_4)_2$	1.14	48	29
no metal	-	21	23

Table 9

The templating effect we observed for copper was really quite remarkable. The ability of the metal to selectively form a natural product, and only one other isomeric product from a possible forty nine cyclic products provides strong evidence that metal ions may indeed act as convenient biological templates in the formation of cyclopeptide metabolites. The cyclooligomerisation reactions in which metal ions are used as templates are generally slightly lower yielding than the equivalent reaction without the metal. However, in the case of copper, the total isolable yield of cyclic products was extremely low - just 11 %. It is possible that the metal binds the heterocyclic amino acids quite strongly and therefore slows down the rate of the coupling reactions. Indeed viewing a space - filling representation of a Cu^{2+} ion encapsulated in the macrocyclic cavity of nostacyclamide **16** illustrates how perfectly this ion fits (**Figure 13**).

The nature of the starting material makes it very difficult to monitor the reactions by tlc. It is not then easy to know when the reactions have gone to completion. Indeed, any unreacted monomeric heterocycles, or uncyclised linear precursor still present would simply be lost in the aqueous wash during work-up. The reduction in the yield of cyclic material obtained when metal templates are used can probably be overcome by either finding an optimum reaction time, or by heating the reaction to increase the rate of the reaction. These are investigations for the future!

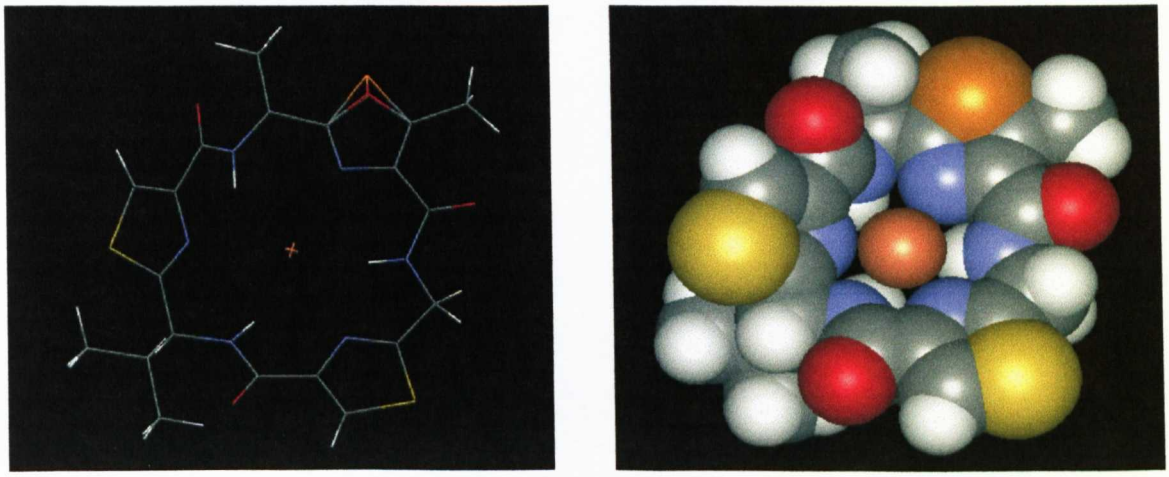


Figure 13 : Nostacyclamide containing a Cu^{2+} ion (LHS); Nostacyclamide containing a Cu^{2+} ion - a space filling representation (RHS)

2.9 Summary

We have developed a high yielding cyclooligomerisation reaction in which analogues of naturally occurring cyclopeptides are formed in just one step from thiazole and/or oxazole based amino acids. Thiazole based hexa- and octa- cyclopeptides can be formed in yields up to 80 % while the analogous reactions with oxazoles are considerably lower yielding. In the case of the thiazole cyclooligomerisations the distribution of products can be affected by altering the size of the alkyl side chain. When the reaction is performed on a thiazole with a methyl side chain *i.e.* alanine, the cyclic trimer is the predominant product. However, increasing the size of the side chain to an isopropyl group, and then to a benzyl group alters the predominant product to the cyclic tetramer.[107] Additionally metal ions can be used as templates in these reactions. In the case of the thiazole derived from (*D*)-alanine the addition of Li^{+} into the reaction results in the formation of only the corresponding cyclic trimer. As the size of metal ion is increased in radius this templating affect drops off. The use of metal ions as templates has therefore provided us with a further synthetic tool with which to manipulate the distribution of cyclic products.

We have extended these cyclooligomerisation reactions to the self-assembly of the natural products, dendroamide A **14** and nostacyclamide **16**. We successfully completed the first total synthesis of dendroamide A **14** in a linear fashion and subsequently synthesised it *via* a novel cyclooligomerisation from its constituent heterocyclic amino acids.[138] The cyclooligomerisation was highly selective, forming just four major products from a possible forty nine cyclic trimers and tetramers. Furthermore we were able to promote the formation of dendroamide A **14** to an even greater extent using Ca^{2+} as a template. In addition it was possible to form another cyclic product **173** exclusively from this cyclooligomerisation using silver as a template.

These studies were finally directed towards the synthesis of nostacyclamide **16**. A total synthesis of this natural product was completed *via* a linear method and later *via* a cyclooligomerisation reaction. The selectivities of these mixed cyclooligomerisations were similar to those obtained towards dendroamide A **14**. This time six main products were formed from a possible forty nine. Employing metal ions as templates, once again, uncovered some striking results. Using Zn^{2+}, Na^{+}, K^{+}, and Ag^{+} as templates in the self-assembly reaction towards nostacyclamide **16** we were able to tailor the reaction to form more of the cyclic product **195**. Indeed silver templated this product to perfection, producing **195** exclusively. Another group of metals, Cu^{2+}, Li^{+}, and Ca^{2+} were found to promote the formation of nostacyclamide **16**, copper being the most effective, producing a mixture of just two products composing nostacyclamide **16** (66 %), and its structural isomer **194** (34 %).

During our research we have uncovered strong circumstantial evidence which suggests that oxazole/thiazole based cyclopeptide metabolites could be produced in nature *via* a self-assembly of pre-formed oxazole/thiazole amino acids. We have also demonstrated the power of metal ions to act as templates and to promote the formation of natural products from their constituent heterocyclic amino acids, thereby giving further

evidence to the idea that metal ions are involved in the biological assembly of these compounds.

EXPERIMENTAL

General Details

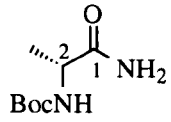
All melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Optical rotations were recorded in spectroscopic grade chloroform or ethanol on a Jasco DIP-370 polarimeter, $[\alpha]_D$ values are recorded in units of 10^{-1} deg cm^2 g^{-1}. Ultraviolet spectra were recorded on a Phillips PU 8700 spectrophotometer as solutions in spectroscopic grade ethanol, ε values are recorded in units of dm^3 mol^{-1} cm^{-1}. Infrared spectra were obtained using Perkin-Elmer 1600 series FT-IR instrument or a Nicolet Magna 550 instrument as liquid films or as dilute solutions in spectroscopic grade chloroform. Proton nmr spectra were recorded on either a Bruker DPX 360 (360 MHz), a Bruker DPX 500 (500 MHz) or a Jeol EX 270 (270 MHz) spectrometer as dilute solutions in deuterochloroform unless otherwise stated. The chemical shifts are quoted in parts per million (ppm) relative to residual chloroform (δ 7.27) or residual methanol (δ 3.35) as internal standard and the multiplicity of each signal is designated by the following abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; quin., quintet; br, broad; m, multiplet; app., apparent; obs., obscured. All coupling constants are quoted in Hertz. Carbon-13 nmr spectra were recorded on either a Bruker DPX 360 (90.5 MHz), a Bruker DPX 500 (125 MHz) or a Jeol EX 270 (67.5 MHz) spectrometer as dilute solutions in deuterochloroform unless otherwise stated. Chemical shifts are reported relative to internal chloroform (δ 77.0) or residual methanol (δ 49.0) as standard on a broad band decoupled mode, and the multiplicities determined using a DEPT sequence. Mass spectra were recorded on a VG Autospec, a MM-701CF, a VG Micromass 7070E or a Micromass LCT. Microanalytical data were obtained on a Perkin-Elmer 240B elemental analyser.

Flash chromatography was performed on Merck silica gel 60 as the stationary phase and the solvents employed were either of analytical grade or were distilled before use. All reactions were monitored by tlc using Merck silica gel 60 F_{254} precoated aluminium backed plates which were visualised with ultraviolet light and then with either acidic

ninhydrin solution, acidic alcholic vanillin solution or a basic potassium permanganate solution.

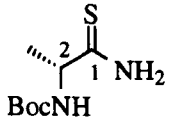
Routinely, dry organic solvents were stored under nitrogen and/or over sodium wire. Other organic solvents were dried by distillation from the following: THF and benzene (potassium benzophenone ketyl), dichloromethane (calcium hydride) and methanol (magnesium methoxide). Other organic solvents and reagents were purified by the accepted literature procedures. Dess-Martin periodinane was prepared according to the modified procedure of Ireland and Liu.[127] All organic extracts were dried with magnesium sulfate unless otherwise stated. Solvent was removed on a Büchi rotary evaporator. Where necessary, reactions requiring anhydrous conditions were performed in a flame or oven dried apparatus under nitrogen or argon atmosphere as stated.

2-(*R*)-(1-Carbamoylethyl)-carbamic acid *tert*-butyl ester (85a)



N-Methylmorpholine (3.5 ml, 31.5 mmol) and *iso*butyl chloroformate (4.1 ml, 31.5 mmol) were added to a stirred solution of Boc-(*D*)-alanine (6.0 g, 31.5 mmol) in anhydrous THF (85 ml) at -5 °C under a nitrogen atmosphere. The solution was stirred at -5 °C for 15 min, and then ammonia was bubbled through the solution for 5 min. The ice bath was removed and ammonia was bubbled through the solution for a further 20 min. The resulting white/opaque solution was stirred at room temperature for 20 min and then water (*ca* 30 ml) was added. The separated aqueous layer was extracted with dichloromethane (4 x 30 ml), the combined organic extracts were then washed with saturated sodium hydrogen carbonate solution (3 x 30 ml), and brine (3 x 30 ml), dried ($MgSO_4$) and evaporated *in vacuo* to leave the amide (5.5 g, 28.8 mmol, 92 %) as a white powder; mp. 90-93 °C (from dichloromethane) (Lit.[141] mp. 95-96 °C); υ_{max} ($CHCl_3$)/cm^{-1} 3482, 3436, 2981, 1704, 1369; δ_H (360 MHz, $CDCl_3$) 1.33 (3H, d, *J* 7.1 Hz, C*H*$_3$CH), 1.40 (9H, s, Bu^t), 4.22 (1H, br. m, C*H*CH$_3$), 5.53 (1H, br. m, N*H*Boc), 6.41 (1H, br. s, N*H*$_2$), 6.74 (1H, br. s, N*H*$_2$).

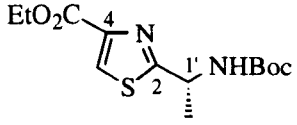
2-(*R*)-(1-Thiocarbamoylethyl)-carbamic acid *tert*-butyl ester (86a)



Lawesson's reagent (5.82 g, 14.4 mmol) was added as one portion to a stirred solution of the amide **85a** (5.46 g, 28.8 mmol) in anhydrous THF (70 ml) at room temperature under an atmosphere of nitrogen. The resulting solution was stirred at room

temperature for 14 h and concentrated *in vacuo* to leave a green oil. The residue was purified by chromatography on silica using 30 % ethyl acetate in light petroleum (40-60 °C) as eluant to give the thioamide (5 g, 24.4 mmol, 85 %) as a pale green/yellow solid; mp. 83-84 °C (from dichloromethane) (Lit.[142] mp. 85-87 °C); υ_{max} ($CHCl_3$)/cm^{-1} 3524, 3437, 2978, 1697; δ_H (360 MHz, $CDCl_3$) 1.33 (9H, s, Bu^t), 1.36-1.39 (3H, m, CH_3CH), 4.53 (1H, br., $CHCH_3$), 5.66 (1H, br., N*H*Boc); δ_C (90.5MHz, $CDCl_3$) 20.8 (q), 21.7 (d), 28.1 (q), 79.9 (s), 155.2 (s), 210.2 (s).

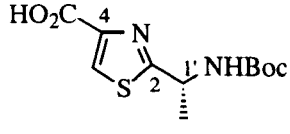
1'-(*R*)-2-(1-*tert*-Butoxycarbonylaminoethyl)-thiazole-4-carboxylic acid ethyl ester (87a)



A solution of the thioamide **86a** (1.4 g, 6.6 mmol) in DMF (9 ml) was stirred with potassium hydrogen carbonate (5.3 g, 52.6 mmol) at room temperature for 10 min. The solution was cooled to -15 °C and then ethyl bromopyruvate (2.5 ml, 19.7 mmol) was added dropwise over 5 min. The resulting yellow solution was stirred at -15 °C for 10 min and a precooled mixture of collidine (7.4 ml, 55.9 mmol) and trifluoroacetic anhydride (3.7 ml, 26.3 mmol) in dry DME (9 ml) was then added dropwise over 15 min. This solution was stirred at -15 °C for a further 15 min and then poured onto ice cold water (40 ml). The separated aqueous layer was extracted with dichloromethane (3 x 20 ml) and the combined organic extracts were then washed with 10% aqueous citric acid solution (3 x 30 ml), copper sulfate solution (3 x 30 ml), and brine (3 x 30 ml), were dried ($MgSO_4$) and evaporated *in vacuo* to leave a brown residue. This residue was purified by chromatography on silica using 30 % ethyl acetate in light petroleum (40-60 °C) as eluant to give the thiazole (1.41 g, 4.7 mmol, 71 %) as a pale yellow solid; mp. 88-90 °C (from petroleum ether/diethyl ether) (Lit.[143] mp. 89.5 °C); $[\alpha]_D^{295}$ +34.4 (c = 1.20, $CHCl_3$); υ_{max} ($CHCl_3$)/cm^{-1} 3440, 2980, 2935, 1715, 1488, 1454, 1369, 1155;

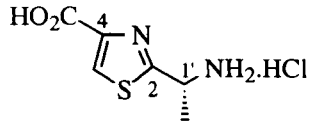
δ_H (360 MHz, $CDCl_3$) 1.28 (3H, t, *J* 7.1Hz, CH_3CH_2), 1.33 (9H, s, Bu^t), 1.51 (3H, d, *J* 7.1 Hz, CH_3CH), 4.29 (2H, q, *J* 7.1 Hz, CH_2CH_3), 4.99-5.01 (1H, m, $CHCH_3$), 5.47 (1H, d, *J* 7.2 Hz, NHBoc), 7.99 (1H, s, CHS); δ_C (90.5MHz, $CDCl_3$) 14.0 (q), 21.3 (q), 27.9 (q), 48.7 (d), 61.1 (t), 79.8 (s), 126.9 (d), 146.8 (s), 154.7 (s), 161.0 (s), 174.9 (s); *m/z* (FAB) Found: 323.1046 (MH^++Na^+, $C_{13}H_{20}O_4N_2SNa$ requires 323.1041).

1'-(*R*)-2-(1-*tert*-Butoxycarbonylaminoethyl)-thiazole-4-carboxylic acid (197)



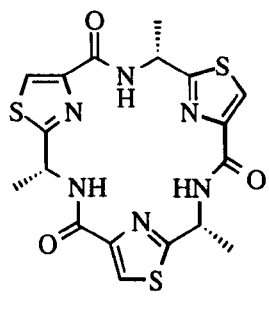
Sodium hydroxide (416 mg, 10.4 mmol) was added in one portion to a stirred solution of the thiazole **87a** (390 mg, 1.3 mmol) in a THF:H_2O (9:1) mixture (7 ml) at room temperature, and the resulting mixture was then stirred for a further 2.5 h. The separated aqueous layer was acidified to pH 4 with citric acid and then extracted with dichloromethane (3 x 30 ml). The combined organic extracts were washed with water (3 x 20 ml) and brine (3 x 20 ml), then dried ($MgSO_4$) and the solvent was removed under reduced pressure to leave the acid (291 mg, 1.07 mmol, 82 %) as a white solid; mp. 127-129 °C (from ethyl acetate/petroleum ether) (Lit.[143] mp. 128-129 °C); $[\alpha]_D^{295}$ +36.0 (c = 1.0, $CHCl_3$); Found : C, 48.3; H, 6.1; N, 9.8%; calc. for $C_{11}H_{16}O_4N_2S$: C, 48.5; H, 5.9; N, 10.3%. υ_{max} ($CHCl_3$)/cm^{-1} 3440, 2934, 2614, 1711, 1491, 1368, 1160; δ_H (360 MHz, $CDCl_3$) 1.46 (9H, s, Bu^t), 1.64 (3H, d, *J* 6.7 Hz, CH_3CH), 5.10-5.12 (1H, m, $CHCH_3$ and NHBoc), (1H, s, CHS); δ_C (90.5 MHz, $CDCl_3$) 21.5 (q), 28.3 (q), 48.7 (d), 80.4 (s), 128.5 (d), 146.4 (s), 155.0 (s), 164.2 (s).

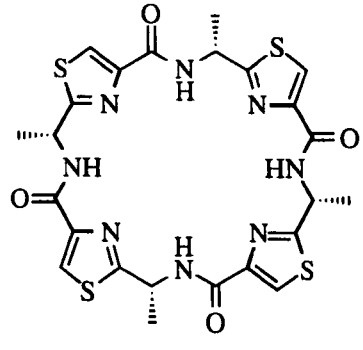
1'-(*R*)-2-(1'-Amino-ethyl)-thiazole-4-carboxylic acid (88a)



A 4M solution of hydrochloric acid in dioxane (2 ml) was added to the thiazole **197** (291 mg, 1.07 mmol) at room temperature under an atmosphere of nitrogen, and the mixture was then stirred for 2 h. The dioxane was removed *in vacuo* by azeotroping with toluene to leave the amine hydrochloride (190 mg, 0.92 mmol, 86 %) as a hygroscopic cream solid; mp. 262-264 °C (from ethanol/diethyl ether) (Lit.[144] mp. 265-267 °C); $[\alpha]_D^{293}$ +0.4 (c = 1, EtOH); Found : C, 34.1; H, 4.2; N, 13.1 %; calc. for $C_6H_9O_2N_2SCl$: C, 34.6; H, 4.4; N, 13.5 %; δ_H (360 MHz, CD_3OD) 1.79 (3H, d, *J* 6.9 Hz, CH_3CH), 4.94 (1H, app. q, *J* 6.9 Hz, $CHCH_3$), 8.52 (1H, s, CHS); δ_C (125 MHz, CD_3OD); 20.2 (q), 48-50 (d) (obscured by CD_3OD), 130.9 (d), 148.4 (s), 163.9 (s) ; *m/z* (FAB) Found: 485.0512 (MH^++Na^+, $C_{18}H_{18}O_3N_6S_3Na$ requires 485.0500).

Cyclic-*tris*-(*R*),(*R*),(*R*)-alanine-thiazole (93) and cyclic-*tetra*-(*R*),(*R*),(*R*),(*R*)-alanine-thiazole (94)

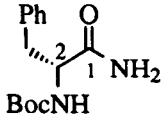




Diisopropylethylamine (0.48 ml, 2.76 mmol) was added dropwise over 5 min to a stirred solution of the thiazole **88a** (190 mg, 0.92 mmol) in anhydrous acetonitrile (18 ml) at room temperature under a nitrogen atmosphere. The solution was stirred at room

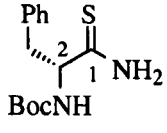
temperature for 5 min, FDPP (530 mg, 1.38 mmol) was added in one portion and the resulting solution was then stirred at room temperature for 3 days. The solvent was removed *in vacuo* to leave a yellow residue which was taken up in dichloromethane (*ca* 30 ml), washed with 2M hydrochloric acid solution (4 x 20 ml), 2M sodium hydroxide solution (3 x 20 ml), and brine (2 x 20 ml), dried ($MgSO_4$) and evaporated *in vacuo*. The white residue was purified by chromatography on silica using 70 % ethyl acetate in light petroleum (40-60 °C) to 90 % ethyl acetate in light petroleum (40-60 °C) as eluants to give, i) the *cyclic peptide* **93** (58 mg, 0.38 mmol, 41%) as a white powder; mp. 298-300 °C (from dichloromethane); $[\alpha]_D^{302}$ +48.1 (c = 1, $CHCl_3$); Found : C, 46.8; H, 4.1 %; $C_{18}H_{18}O_3N_6S_3$ requires: C, 46.8; H, 3.9 %; λ_{max} (EtOH)/nm 228 (ε/dm^3 mol^{-1}cm^{-1} 1916); υ_{max} ($CHCl_3$)/cm^{-1} 3402, 2977, 1663, 1544, 1498, 1350, 1047, 878; δ_H (360 MHz, $CDCl_3$) 1.74 (3H, d, *J* 6.8 Hz, C*H*$_3$CH), 5.64 (1H, app. quintet, *J* 6.8 Hz, C*H*CH$_3$), 8.17 (1H, s, C*H*S), 8.68 (1H, d, *J* 6.8 Hz, N*H*CO); δ_C (125 MHz, $CDCl_3$); 25.0 (q), 47.4 (d), 124.0 (d), 148.8 (s), 159.5 (s), 171.2 (s) ; *m/z* (FAB) Found: 485.0512 (MH^++Na^+, $C_{18}H_{19}O_3N_6S_3Na$ requires 485.0500), and ii) the *cyclic peptide* **94** (18 mg, 0.09 mmol, 10 %) as a white powder; mp. 268-271 °C (from dichloromethane); $[\alpha]_D^{293}$ = +17.1 (c = 0.7, $CHCl_3$); λ_{max} (EtOH)/nm 225 (ε/dm^3 mol^{-1}cm^{-1} 55593); υ_{max} ($CHCl_3$)/cm^{-1} 3632, 3402, 2928, 2856, 1715, 1665, 1543, 1462, 1297, 1016; δ_H (360 MHz, $CDCl_3$) 1.86 (3H, d, *J* 6.9 Hz, C*H*$_3$CH), 5.61 (1H, app. quintet, *J* 6.9 Hz, C*H*CH$_3$), 7.99 (1H, d, *J* 8.1 Hz, N*H*CO), 8.12 (1H, s, C*H*S); δ_C (125 MHz, $CDCl_3$) 21.2 (q), 46.0 (d), 124.8 (d), 148.2 (s), 159.9 (s), 170.8 (s); *m/z* (FAB) Found: 639.0666 (MH^+,+ Na^+, $C_{24}H_{25}O_4N_8S_4Na$ requires 639.0701).

2-(*R*)-(1-Carbomoyl-2-phenyl-ethyl)-carbamic acid *tert*-butyl ester (85b)



N-Methylmorpholine (3.2 ml, 28.8 mmol) and *iso*butyl chloroformate (3.7 ml, 28.8 mmol) were added to a solution of Boc-(*D*)-phenylalanine (8.0 g, 28.8 mmol) in anhydrous THF (7 ml) at -10 °C under an atmosphere of nitrogen. The resulting solution was stirred at -10 °C for 15 min, ammonia was then bubbled through the solution for 5 min at -10 °C, and at room temperature for a further 20 min. Water (*ca* 50 ml) was added, the aqueous layer was then separated and extracted with chloroform (4 x 30 ml). The combined organic extracts were washed with saturated sodium hydrogen carbonate solution (3 x 30 ml) and brine (3 x 30 ml), dried ($MgSO_4$) and evaporated *in vacuo* to leave the amide (7.27 g, 26.2 mmol, 91 %) as a white solid; mp. 87-89 °C (from ethyl acetate/petroleum ether) (Lit.[145] mp. 92-93 °C); δ_H (360 MHz, $CDCl_3$) 1.41 (9H, s, Bu^t), 3.07 (2H, app. d, *J* 5.8 Hz, CH_2Ph), 4.40 (1H, br. s, *J* 5.2 Hz, N*H*Boc), 5.13-5.16 (1H, br. m, C*H*CH_2), 5.71 (1H, br. s, NH_2), 5.97 (1H, br. s, NH_2), 7.22-7.33 (5H, m, Ar*H*); δ_C (90.5 MHz, $CDCl_3$) 28.2 (q), 38.4 (t), 55.3 (d), 80.1 (s), 126.9 (d), 128.6 (d), 129.3 (d), 136.5 (s), 189.1 (s).

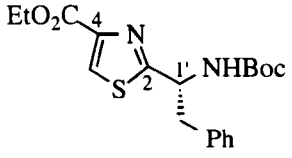
2-(*R*)-(2-Phenyl-1-thiocarbomyl-ethyl)-carbamic acid *tert*-butyl ester (86b)



Lawesson's reagent (5.3 g, 13.65 mmol) was added as one portion to a solution of the amide (7.6 g, 27.3 mmol) in anhydrous THF (13 ml) and the solution was stirred at room temperature for 15 h under an atmosphere of nitrogen. The solution was concentrated *in vacuo,* the green residue taken up in dichloromethane (40 ml), washed with 2M sodium hydroxide solution (3 x 30 ml), brine (2 x 30 ml), dried ($MgSO_4$) and evaporated *in vacuo* to leave the thioamide (7.4 g, 25.1 mmol, 92 %) as a green solid; mp. 97-99 °C (from ethyl acetate/petroleum ether) (Lit.[146] mp. 97-99 °C); δ_H (360 MHz, $CDCl_3$) 1.41 (9H, s, ^{t}Bu), 3.15-3.18 (2H, br. m, CH_2Ph), 4.62 (1H, app. quintet,

J 7.5 Hz, C*H*CH_2Ph), 7.25-7.39 (5H, m, Ar*H*); δ_C (90.5 MHz, $CDCl_3$) 28.1 (q), 60.5 (d), 71.4 (t), 80.1 (s), 126.6 (d), 128.3 (d), 129.1 (d), 155.3 (s), 208.1 (s).

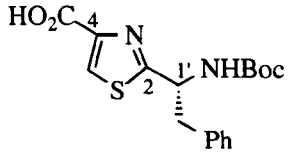
1'(*R*)-2-(1'-*tert*-Butoxycarbonylamino-2'-phenyl-ethyl)-thiazole-4-carboxylic acid ethyl ester (87b)



A solution of the thioamide **86b** (3.5 g, 12.5 mmol) in DMF (15 ml) was stirred vigorously with potassium hydrogen carbonate (10.0 g, 100 mmol) at 0 °C for 5 min under an atmosphere of nitrogen. Ethyl bromopyruvate (4.7 ml, 37.5 mmol) was added dropwise over 5 min. The resulting yellow solution was stirred at -15 °C for 5 min, and a precooled solution of collidine (14 ml, 106 mmol) and trifluoroacetic anhydride (7.1 ml, 50 mmol) in DMF (15 ml) was added dropwise over 10 min, the reaction was allowed to warm to room temperature and stirred for a further 20 min. The volatiles were removed under reduced pressure, water (40 ml) was added and the separated aqueous layer extracted with chloroform (3 x 40 ml). The combined organic extracts were washed with copper sulfate solution (3 x 30 ml), water (3 x 30 ml), and brine (2 x 30 ml), were dried ($MgSO_4$) and evaporated *in vacuo* to leave a brown residue. The residue was purified by chromatography on silica using 30% ethyl acetate in light petrol (40-60 °C) as eluant to leave the thiazole (3.9 g, 10.4 mmol, 83 %) as a cream solid; mp. 111-114 °C (from dichloromethane) (Lit.[146] mp 111 °C); $[\alpha]_D^{294}$ = +10.6 (c = 1.0, $CHCl_3$); υ_{max} ($CHCl_3$)/cm^{-1} 3432, 3125, 2982, 1715, 1489, 1455, 1393, 1369, 1300, 1154,; δ_H (360 MHz, $CDCl_3$) 1.38 (9H, br. s, Bu^t), 1.33-1.44 (3H, m, C$H_3$$CH_2O$), 3.28-3.35 (2H, m, C$H_2$Ph), 4.45 (2H, q, *J* 7.0 Hz, C$H_2$$CH_3$), 5.28 (2H, br. s, C*H*$CH_2$Ph), 5.28 (2H, br. s, N*H*Boc), 7.10-7.12 (2H, m, Ar*H*), 7.23-7.26 (3H, m, Ar*H*), 8.05 (1H, s, C*H*S); δ_C (90.5 MHz, $CDCl_3$) 14.1 (q), 28.0 (q), 41.2 (t), 53.7 (d), 61.3 (t), 80.0 (s), 126.7 (d), 127.1 (d), 128.4 (d), 129.2 (d), 136.0 (s), 146.9 (s), 154.8 (s),

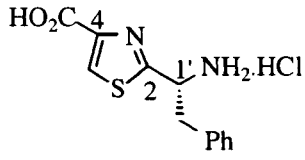
161.1 (s), 173.0 (s); *m/z* (FAB) Found: 399.1343 ($M^+ + Na^+$, $C_{19}H_{24}O_4N_2SNa$ requires 399.1354).

1'(*R*)-2-(1'-*tert*-Butoxycarbonylamino-2'-phenyl-ethyl)-thiazole-4-carboxylic acid ethyl ester (198)



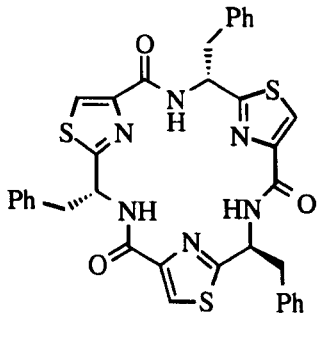
The thiazole **87b** (1.0 g, 2.7 mmol) was stirred in a THF:H_2O (9:1) mixture (14 ml) with sodium hydroxide (0.9 g, 21.3 mmol) for 12 h at room temperature. The solution was extracted with ethyl acetate (30 ml) and the separated aqueous layer was acidified to pH4 by the addition of citric acid. The acidified aqueous layer was extracted with ethyl acetate (4 x 30 ml), and these combined organic extracts were washed with brine (2 x 30 ml), dried ($MgSO_4$) and condensed *in vacuo* to leave the thiazole (670 mg, 1.9 mmol, 71 %) as a pale yellow solid; mp. 159-161 °C (from dichloromethane) (Lit.[86] describes an oil); $[\alpha]_D^{298}$ = +12.2 (c = 1.0, $CHCl_3$); υ_{max} ($CHCl_3$)/cm^{-1} 3435, 3125, 2981, 1714, 1490, 1456, 1394, 1369, 1155; δ_H (360 MHz, $CDCl_3$) 1.41 (9H, s, Bu^t), 3.33-3.39 (2H, br. m, CH_2Ph), 5.28 (1H, br., CHCH$_2$Ph), 5.29 (1H, br. s, NHBoc), 7.12-7.15 (2H, m, ArH), 7.22-7.39 (3H, m, ArH), 8.18 (1H, s, CHS); δ_C (90.5 MHz, $CDCl_3$) 28.0 (q), 41.2 (t), 53.7 (d), 80.1 (s), 126.8 (d), 128.1 (d), 128.4 (d), 128.7 (d), 146.7 (s), 154.9 (s), 163.7 (s), 173.1 (s); *m/z* (FAB) Found: 371.1021 ($MH^+ + Na^+$, $C_{17}H_{20}O_4N_2SNa$ requires 371.1040).

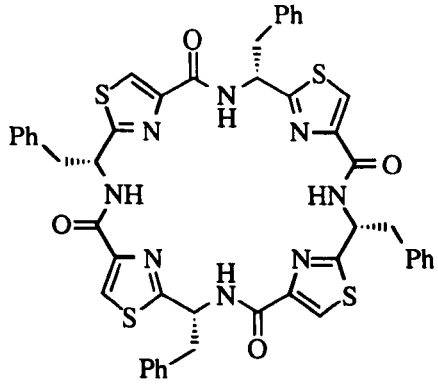
1'(*R*)-2-(1-Amino-2-phenyl-ethyl)-thiazole-4-carboxylic acid (88b)



The thiazole **198** (900 mg, 2.7 mmol) was stirred with a 4M solution of hydrochloric acid in dioxane (2 ml) at room temperature under an atmosphere of nitrogen for 2.5 h. The dioxane was removed by azeotroping with toluene to leave the thiazole (701 mg, 2.5 mmol, 91 %) as a cream/white solid; mp. 232 °C (decomp.) (Lit.[148] mp. 250-252 °C); Found; H, 4.9, N, 9.4 %; calc. for $C_{12}H_{13}O_2N_2SCl$: H, 4.6, N, 9.9 %; δ_H (360 MHz, CD_3OD) 3.34-3.39 (1H, br. m, C*H*$_2$Ph), 3.46-3.52 (1H, br. m, C*H*$_2$Ph), 5.11-5.15 (1H, br. m, C*H*CH$_2$Ph), 7.23-7.29 (2H, m, Ar*H*), 7.30-7.38 (3H, m, Ar*H*), 8.40 (1H, s, C*H*S); δ_C (90.5 MHz, CD_3OD) 54.4 (d), 68.1 (t), 128.9 (d), 130.1 (d), 130.8 (d), 135.6 (d), 148.5 (s), 163.8 (s), 165.5 (s).

Cyclic-*tris*-(*R*),(*R*),(*S*)-phenylalanine-thiazole (95) and Cyclic-*tetra*-(*R*),(*R*),(*R*),(*R*)-phenylalanine-thiazole (96).[107]

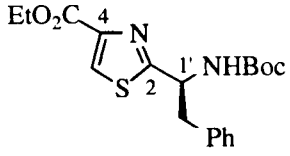




Diisopropylethylamine (0.09 ml, 0.52 mmol) and FDPP (100 mg, 0.26 mmol) were added to a suspension of the thiazole **88b** (50 mg, 0.18 mmol) in anhydrous acetonitrile (4 ml) at room temperature under an atmosphere of nitrogen, the solution was stirred

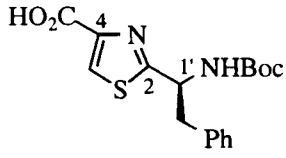
for 3 days before being evaporated to dryness *in vacuo*. The residue was taken up in dichloromethane (20 ml) and partitioned with 2M hydrochloric acid solution (15 ml) the separated organic layer was then washed with 2M hydrochloric acid solution (3 x 20 ml). The combined aqueous solutions were re-extracted with dichloromethane (2 x 20 ml) and the combined organic extracts were washed successively with 2M sodium hydroxide solution (3 x 20 ml), water (3 x 20 ml) and brine (2 x 20 ml). The organic solution was dried ($MgSO_4$) and the solvent removed under reduced pressure to leave a white residue. The *cyclic peptide* containing residue was separated by chromatography on silica using 70 % ethyl acetate in light petroleum (40-60 °C) as eluant to give, i) the cyclic trimer (4 mg, 0.017 mmol, 10 %) as a white powder; mp. 248-249 °C (from dichloromethane); $[\alpha]_D^{303}$ = -0.40 (c = 1.00, $CHCl_3$); Found : C, 62.4; H, 4.7%; $C_{36}H_{30}O_3N_6S_3$ requires; C, 62.6; H, 4.4%); λ_{max} (CH_2Cl_2)/nm 231 (ε/dm^3 mol^{-1}cm^{-1} 519003); υ_{max} ($CHCl_3$)/cm^{-1} 3400, 2927, 1667, 1550, 1492, 1464, 1360, 1306, 1140, 1061, 997, 900; δ_H (360 MHz, $CDCl_3$) 2.89 (1H, dd, *J* 9.1, 12.8 Hz, C*H*$_2$Ph), 2.90 (1H, dd, *J* 9.1, 12.8 Hz, C*H*$_2$Ph), 3.21 (1H, dd, *J* 8.4, 13.7 Hz, C*H*$_2$Ph), 3.55 (1H, dd, *J* 4.2, 12.9 Hz, C*H*$_2$Ph), 3.57 (1H, dd, *J* 4.2, 12.9 Hz, C*H*$_2$Ph), 3.74 (1H, dd, *J* 4.2, 12.9 Hz, C*H*$_2$Ph), 5.46-5.48 (1H, m, C*H*CH$_2$Ph), 5.65-5.66 (1H, m, C*H*NH), 5.86-5.87 (1H, m, C*H*NH), 7.02-7.05 (6H, m, Ar*H*), 7.18-7.31 (9H, m, Ar*H*), 8.01 (1H, s, C*H*S), 8.04 (1H, s, C*H*S), 8.14 (1H, s, C*H*S); δ_C (90.5 MHz, $CDCl_3$) 43.1 (t), 43.3 (t), 43.6 (t), 52.9 (d), 53.8 (d), 54.1 (d), 120.3 (d), 123.5 (d), 123.9 (d), 124.9 (d), 127.2 (d), 127.3 (d), 127.5 (d), 128.7 (d), 129.3 (d), 129.7 (d), 129.9 (d), 130.1 (d), 148.3 (s), 148.5 (s), 149.1 (s), 159.5 (s), 159.9 (s), 160.0 (s), 168.1 (s), 168.1 (s), 169.1(s); *m/z* (FAB) Found: 713.1450 (M^+ +Na^+, $C_{36}H_{30}O_3N_6S_3Na$ requires 713.1439), and ii) the cyclic tetramer (48 mg, 0.05 mmol, 41 %) as a white powder; mp. 250-254 °C (from dichloromethane); $[\alpha]_D^{302}$ = -5.79 (c = 1.50, $CHCl_3$); υ_{max} ($CHCl_3$)/cm^{-1} 3400, 2927, 1667, 1550, 1492, 1464, 1360, 1306, 1140, 1061, 997, 900; δ_H (360 MHz, $CDCl_3$) 3.49-3.57 (2H, m, C*H*$_2$Ph), 5.68-5.74 (1H, m, C*H*CH$_2$Ph), 7.15-7.30 (5H, m, Ar*H*), 7.95-7.98 (1H, d, *J* 8.5 Hz, N*H*CO), 7.99 (1H, s, C*H*S); δ_C (90.5 MHz, $CDCl_3$) 43.9 (t), 52.7 (d), 123.9 (d), 127.2 (d), 129.6 (d), 130.0 (d), 135.9 (s), 148.6 (s), 159.4 (s).

1'(*S*)-2-(1'-*tert*-Butoxycarbonylamino-2'-phenyl-ethyl)-thiazole-4-carboxylic acid ethyl ester (199)



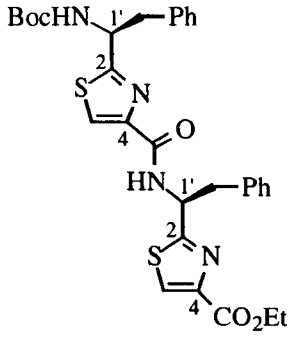
The thioamide (3.0 g, 10.2 mmol), was stirred vigorously with potassium hydrogen carbonate (8.2 g, 81.6 mmol) in DMF (12 ml) for 5 min. Ethyl bromopyruvate (3.9 ml, 30.7 mmol) was added dropwise over 5 min and the solution cooled to 0 °C. A precooled mixture of trifluoroacetic anhydride (5.8 ml, 40.8 mmol) and collidine (11.5 ml, 86.7 mmol) in DMF (12 ml) was added dropwise over 10 min, the reaction was allowed to warm to room temperature and was stirred for a further 30 min. The volatiles were removed in *vacuo*, water (*ca* 50 ml) was added and the aqueous extracted with dichloromethane (4 x 30 ml). The combined organic extracts were washed with 10% aqueous citric acid solution (3 x 30 ml), dried ($MgSO_4$) and evaporated *in vacuo* to leave a brown residue which was purified by chromatography on silica using 30 % ethyl acetate in light petroleum (40-60 °C) to leave the thiazole (2.8 g, 7.4 mmol, 73 %) as a cream solid; mp. 140-142 °C (from dichloromethane) (Lit.[146a] no mp. given); υ_{max} ($CHCl_3$)/cm^{-1} 3689, 3434, 2982, 1717, 1603, 1490; δ_H (360 MHz, $CDCl_3$) 1.31 (9H, s, Bu^t), 1.29-1.33 (3H, br. m, C*H*$_3$CH$_2$), 3.19-3.28 (1H, br. m, C*H*$_2$Ph), 3.29-3.33 (1H, br. m, C*H*$_2$Ph), 4.34 (2H, q, *J* 7.1 Hz, C*H*$_2$CH$_3$), 5.20-5.23 (1H, br. m, C*H*CH$_2$), 5.49 (1H, d, *J* 7.8 Hz, N*H*Boc), 7.04-7.19 (5H, m, Ar*H*), 7.97 (1H, s, C*H*S); δ_C (90.5 MHz, $CDCl_3$) 14.0 (q), 27.9 (q), 41.0 (t), 61.0 (t), 79.7 (s), 126.6 (d), 127.0 (d), 128.2 (d), 129.0 (d), 146.8 (s), 154.7 (s), 161.0(s), 172.9 (s).

1'(*S*)-2-(1'-*tert*-Butoxycarbonylamino-2'-phenyl-ethyl)-thiazole-4-carboxylic acid (200)



Sodium hydroxide (0.9 g, 21.3 mmol) was added to a solution of the thiazole **199** (1.0 g, 2.7 mmol) in a THF:H_2O (4:3) mixture (6 ml) at room temperature. The resulting solution was then stirred at room temperature for 13 h, and the separated aqueous extracted with dichloromethane (2 x 30 ml) to remove any impurities. The aqueous was then acidified to pH4 by the addition of citric acid and was re-extracted with dichloromethane (4 x 30 ml). These combined organic extracts were then washed with brine (3 x 30 ml), dried ($MgSO_4$) and evaporated *in vacuo* to leave the thiazole **200** (670 mg, 1.9 mmol, 71 %) as a pale yellow solid; mp. 140-142 °C (from dichloromethane) (Lit.[86] no mp. given); $[\alpha]_D^{302}$ = -8.0 (c = 1.0, $CHCl_3$); υ_{max} ($CHCl_3$)/cm^{-1} 3434, 3124, 2979, 293, 1760, 1708, 1489, 1455, 1394, 1369, 1344, 1294, 1156, 1049; δ_H (360 MHz, $CDCl_3$) 1.30 (9H, s, Bu^t), 3.01-3.03 (1H, br. m, C*H*$_2$Ph), 3.29-3.31 (1H, br. m, C*H*$_2$Ph), 4.99-5.09 (1H, br. m, C*H*CH_2Ph), 7.09-7.27 (5H, m, Ar*H*), 8.11 (1H, d, *J* 8.6 Hz, N*H*Boc), 8.16 (1H, s, C*H*S); δ_C (90.5 MHz, $CDCl_3$) 28.1 (q), 41.3 (t), 53.8 (d), 80.4 (s), 126.9 (d), 128.6 (d), 129.3 (d), 136.1 (s), 146.4 (s), 164.7 (s), 177.1 (s).

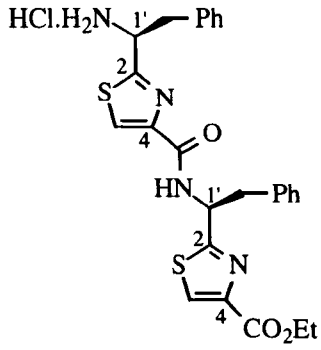
1'(*S*)-2-(1-{[2-(1-*tert*-Butoxycarbonylamino-2-phenyl-ethyl)-thiazole-4-carbonyl]-amino}-1'-(*S*)-2-phenyl-ethyl)-thiazole-4-carboxylic acid ethyl ester (101)



N-Methylmorpholine (0.21 ml, 1.9 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (364 mg, 1.9 mmol) and *N*-hydroxybenzotriazole (260 mg, 1.9 mmol) were added to a solution of the thiazole acid **200** (600 mg, 1.7 mmol) in anhydrous dichloromethane (20 ml) at 0 °C. The solution was stirred at 0 °C for 15 min, and a precooled solution of the thiazole ethyl ester hydrochloride (540 mg, 1.7 mmol) in DMF (3 ml) deprotonated with *N*-methylmorpholine (0.21 ml, 1.9 mmol) was added dropwise over 10 min. The resulting solution was stirred at 0 °C for 1 h, then at room temperature for 12 h. The solution was evaporated *in vacuo* to leave a residue which was taken up in ethyl acetate (30 ml) and washed with 10% aqueous citric acid solution (3 x 15 ml), saturated aqueous sodium bicarbonate solution (3 x 20 ml), and water (3 x 20 ml), was dried ($MgSO_4$) and evaporated *in vacuo* to leave a residue. Purification by chromatography on silica using 30 % ethyl acetate in light petroleum (40-60 °C) as eluant gave the dimer (360 mg, 0.6 mmol, 35 %) as a cream foam; $[\alpha]_D^{302}$ = -12.9 (c = 1.0, $CHCl_3$); Found : C, 61.29; H, 5.71; N, 8.82 %; $C_{31}H_{34}O_5N_4S_2$ requires: C, 61.37; H, 5.65; N, 9.24%; υ_{max} ($CHCl_3$)/cm^{-1} 3393, 3124, 2981, 2933, 1716, 1672; δ_H (360 MHz, $CDCl_3$) 1.15-1.46 (3H, m, C*H*$_3$CH$_2$), 1.31 (9H, br. s, ^{t}Bu), 2.95-3.04 (1H, m, C*H*$_2$Ph), 3.17-3.26 (1H, m, C*H*$_2$Ph), 3.42-3.44 (2H, m, C*H*$_2$Ph), 5.13-5.20 (1H, m, C*H*NHBoc), 5.65-5.71 (1H, m, C*H*CH$_2$), 5.77-5.79 (1H, m, C*H*CH$_2$), 7.01-7.26 (10H, m, Ar*H*), 7.81 (1H, s, C*H*S), 7.84 (1H, s, C*H*S), 8.23 (1H, d, *J* 7.1Hz, N*H*CO); δ_C (90.5 MHz, $CDCl_3$) 13.9 (q), 27.8 (q), 27.8 (q), 40.3 (t), 52.0 (d), 53.3

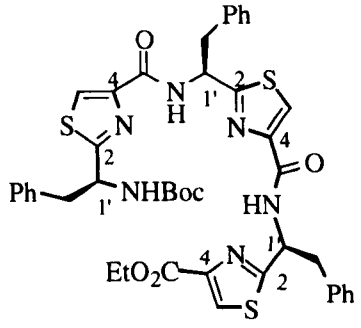
(d), 60.9 (t), 79.5 (s), 122.7 (d), 123.6 (d), 126.3 (d), 126.4 (d), 126.5 (d), 127.1 (d), 128.0 (d), 128.1 (d), 135.8 (s), 136.1 (s), 146.8 (s), 148.4 (s), 154.6 (s), 160.2 (s), 160.7 (s).

1'(*S*)-(-{[-(1-Methyl-2-phenyl-ethyl)-thiazole-4-carbonyl]-amino}1'(*S*)-2-phenyl-ethyl)-thiazole-4-carboxylic acid ethyl ester (102)



The dimer **101** (600 mg, 0.99 mmol) was stirred with a 4M solution of hydrochloric acid in dioxane (2 ml) for 1.5 h at room temperature under a nitrogen atmosphere. The dioxane was removed *in vacuo* by azeotroping with toluene to leave the amine (515 mg, 0.95 mmol, 96 %) as a yellow viscous oil; δ_H (360 MHz, CD_3OD) 1.38 (3H, t, *J* 7.0 Hz, CH_3CH_2), 3.41-3.52 (2H, m, CH_2Ph), 3.41-3.52 (2H, m, CH_2Ph), 4.38 (2H, q, *J* 7.0, Hz, CH_2CH_3), 5.24-5.27 (1H, m, $CHCH_2$), 5.77-5.81 (1H, m, $CHCH_2$), 7.19-7.41 (10H, m, Ar*H*), 8.21 (1H, s, C*H*S), 8.36 (1H, s, C*H*S); δ_C (90.5 MHz, CD_3OD) 14.6 (q), 40.8 (t), 41.3 (t), 54.3 (d), 54.4 (d), 62.5 (t), 126.5 (d), 127.3 (d), 127.8 (d), 128.6 (d), 129.4 (d), 129.7 (d), 129.8 (d), 130.4 (d), 130.5 (s), 138.1 (s), 147.0 (s), 149.4 (s), 162.1 (s), 162.3 (s).

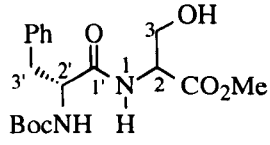
1'(*S*)-2-(1-{[2-(1-*tert*-Butoxycarbonylamino-2-phenyl-ethyl)-thiazole-4-carbonyl]-amino}-1'(*S*)-2-phenyl-ethyl)-thiazole-4-carbonyl]-amino}-1'(*S*)-2-phenyl-ethyl)-thiazole-4-carboxylic acid ethyl ester (103)



N-Methylmorpholine (0.11 ml, 1.01 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (194 mg, 1.01 mmol) and *N*-hydroxybenzotriazole (140 mg, 1.01 mmol) were added to a solution of the thiazole acid **99** (321 mg, 0.92 mmol) in anhydrous dichloromethane (20 ml) at 0 °C, the solution was stirred for 15 min and a precooled solution of the dimer amine hydrochloride **102** (500 mg, 0.92 mmol) in DMF (3 ml) deprotonated with *N*-methylmorpholine (0.11 ml, 1.01 mmol) was added dropwise over 5 min. The resulting solution was stirred at 0 °C for 1h, and at room temperature for 14 h. The solution was evaporated *in vacuo* to leave a residue which was taken up in ethyl acetate (30 ml) and washed with 10 % aqueous citric acid solution (3 x 15 ml), saturated aqueous sodium hydrogen carbonate solution (3 x 20 ml) and water (3 x 20 ml), dried ($MgSO_4$) and evaporated *in vacuo* to leave a residue. Purification by chromatography using 30 % ethyl acetate in light petroleum (40-60 °C) as eluant gave the hexapeptide (500 mg, 0.6 mmol, 65 %) as a cream foam; $[\alpha]_D^{301}$ = -28.6 (c = 1.0, $CHCl_3$); υ_{max} ($CHCl_3$)/cm^{-1} 3393, 3123, 2932, 1716, 1673; δ_H (360 MHz, $CDCl_3$) 1.16-1.35 (3H, m, C*H*$_3$CH$_2$), 1.16-1.35 (9H, m, Bu^t), 3.16-3.56 (6H, m, C*H*$_2$Ph), 4.26-4.41 (2H, m, C*H*$_2$CH$_3$), 5.21-5.24 (1H, m, N*H*Boc), 5.53-5.79 (3H, m, C*H*CH$_2$Ph), 7.04-7.21 (15H, m, Ar*H*), 7.90-7.98 (1H, m, C*H*S), 7.90-7.98 (1H, m, C*H*S), 7.90-7.98 (1H, m, C*H*S), 7.90-7.98 (1H, m, N*H*CO), 8.20 (1H, d, *J* 8.3 Hz, N*H*CO); δ_C (90.5 MHz, $CDCl_3$) 14.0 (q), 27.9 (q), 40.3 (t), 40.6 (t), 51.7 (d), 52.1 (d),

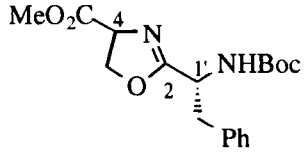
53.4 (d), 61.1 (t), 79.9 (s), 123.8 (d), 123.9 (d), 126.6 (d), 128.3 (d), 128.3 (d), 129.1 (d), 129.9 (d), 130.6 (d), 135.8 (s), 135.9 (s), 136.0 (s), 146.9 (s), 148.5 (s), 148.7 (s), 160.2 (s), 160.9 (s), 170.9 (s), 171.3 (s).

2'(*R*)-2-(2'-*tert*-Butoxycarbonylamino-3'-phenylpropionylamino)-3-hydroxypropionic acid methyl ester (147)[102, 146]



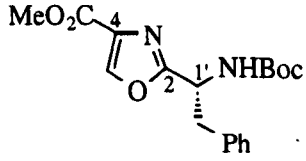
Triethylamine (1.0 ml, 7.4 mmol) was added dropwise over 2 min to a stirred solution of Boc-(*D*)-phenylalanine (1.0 g, 3.54 mmol) in THF (20 ml) at -30 °C. *Iso*butyl chloroformate (0.5 ml, 3.9 mmol) was added, the solution stirred for a further 2 min at -30 °C and (*D, L*)-serine methyl ester hydrochloride salt (0.6 g, 3.9 mmol) was added in one portion to the white suspension. The resulting solution was allowed to warm to room temperature over 15 h, the volatiles were removed *in vacuo*, and water (50 ml) was added. The solution was extracted with ethyl acetate (4 x 30 ml), the combined organic extracts were then washed with sodium bicarbonate solution (3 x 30 ml), dried ($MgSO_4$) and evaporated *in vacuo* to leave a white residue. Purification by chromatography on silica using 20 % ethyl acetate in light petroleum (40-60 °C) to 70 % ethyl acetate in light petroleum (40-60 °C) as eluants gave the dipeptide (1.3 g, 3.4 mmol, 97 %) as a sticky white foam; υ_{max} ($CHCl_3$)/cm^{-1} 3428, 2957, 2539, 2358, 1744, 1679, 1603, 1456, 1394; δ_H (360 MHz, $CDCl_3$) 1.39 (9H, s, Bu^t), 3.01-3.07 (1H, m, CH_2Ph), 3.12-3.17 (1H, br. m, CH_2Ph), 3.72 (3H, s, CH_3O), 4.02 (1H, br. s, CH_2OH), 4.46-4.48 (1H, m, C*H*NHBoc), 4.58-4.61 (1H, br. m, C*H*CO_2Me), 5.02 (1H, d, *J* 7.4 Hz, N*H*Boc), 7.10-7.31 (5H, m, ArH); δ_C (90.5 MHz, $CDCl_3$) 28.2 (q), 38.5 (t), 52.3 (q), 54.0 (d), 55.8 (d), 63.2 (t), 80.2 (s), 126.9 (d), 129.3 (d), 136.7 (s), 155.7 (s), 170.5 (s), 170.9 (s);

1'(*R*)-2-(1-*tert*-Butoxycarbonylamino-2-phenyl-ethyl)-4,5-dihydro-oxazole-4-carboxylic acid methyl ester (148)[147]



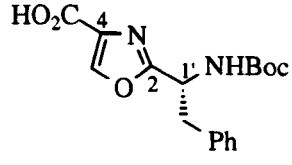
DAST (0.2 ml, 1.5 mmol) was added dropwise over 3 min to a solution of the dipeptide **147** (500 mg, 1.4 mmol) in anhydrous dichloromethane (9 ml) at -70 °C under an atmosphere of nitrogen. The resulting pale yellow solution was stirred at -70 °C for a further 2 h 40 min, aqueous ammonia (15 ml) was added in one portion and the mixture was allowed to warm to room temperature. The separated aqueous layer was extracted with dichloromethane (3 x 30 ml). The combined organic extracts were washed with water (3 x 30 ml) and brine (2 x 30 ml), dried ($MgSO_4$) and concentrated *in vacuo* to give a yellow residue which was purified by chromatography on silica using 30 % ethyl acetate in light petroleum (40-60 °C) as eluant to give the oxazoline (370 mg, 1.1 mmol, 76 %) as a pale yellow oil; υ_{max} ($CHCl_3$)/cm^{-1} 3684, 3430, 2957, 1744, 1713, 1456, 1369, 1158, 1057; δ_H (360 MHz, $CDCl_3$) 1.34 (9H, s, Bu^t), 2.93-3.11 (2H, m, CH_2Ph), 3.67 (3H, s, OCH_3), 4.31-4.37 (1H, m, C*H*CH_2Ph), 4.48 (1H, t, *J* 8.2 Hz, C*H*CO_2Me), 4.57-4.66 (2H, m, CH_2OCN), 5.21 (1H, d, *J* 8.2 Hz, N*H*Boc), 7.06-7.22 (5H, m, ArH); δ_C (90.5 MHz, $CDCl_3$) 27.8 (q), 38.4 (t), 49.5 (d), 52.3 (q), 67.3 (d), 70.8 (t), 79.2 (s), 127.2 (d), 128.2 (d), 128.9 (d), 154.5 (s), 168.9 (s), 170.6 (s);

1'(*R*)-2-(1-*tert*-Butoxycarbonylamino-2-phenyl-ethyl)-oxazole-4-carboxylic acid methyl ester (149)



Bromotrichloromethane (0.16 ml, 1.6 mmol) was added dropwise over 5 min to a stirred solution of the oxazoline **148** (500 mg, 1.4 mmol) in anhydrous dichloromethane (7 ml) at -5 °C under an atmosphere of nitrogen. The solution was stirred for 5 min at 0 °C and 1, 8-diazobicyclo[5.4.0] undec-7-ene (0.24 ml, 1.6 mmol) was added dropwise over 5 min, the resulting mixture was allowed to warm to room temperature over 1 h, and saturated ammonium chloride solution (15 ml) was added in a single portion. After stirring for 10 min the organic layer was separated and washed with water (2 x 10 ml), dried ($MgSO_4$) and evaporated *in vacuo* to leave a pale yellow solid residue. The residue was purified by chromatography on silica using 30% ethyl acetate in light petroleum (40-60 °C) as eluants to give the oxazole (370 mg, 1.07 mmol, 76 %) as a cream solid; mp. 108-110 °C (dichloromethane) (Lit.[146a] no mp. given); $[\alpha]_D^{301}$ = +9.8 (c = 1.0, $CHCl_3$); υ_{max} ($CHCl_3$)/cm^{-1} 3437, 2980, 2933, 1714, 1586, 1369, 1347, 1325, 1141, 1112, 1002; δ_H (360 MHz, $CDCl_3$) 1.36 (9H, s, Bu^t), 3.14-3.21 (2H, br. m, CH_2Ph), 3.87 (3H, s, CH_3O), 5.17-5.26 (1H, br. m, C*H*CH_2Ph), 5.28 (1H, d, *J* 8.2Hz, N*H*Boc), 7.00-7.15 (2H, m, Ar*H*), 7.17-7.23 (3H, m, Ar*H*), 8.11 (1H, s, C*H*O); δ_C (90.5 MHz, $CDCl_3$) 27.7 (q), 39.5 (t), 49.6 (d), 51.5 (q), 79.3 (s), 126.4 (d), 127.9 (d), 128.7 (d), 135.3 (s), 143.6 (d), 154.4 (s), 160.9 (s), 164.3 (s).

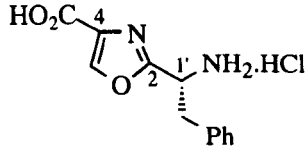
1'(*R*)-2-(*tert*-Butoxycarbonylamino-2-phenyl-ethyl)-oxazole-4-carboxylic acid (150)



Sodium hydroxide (1.1 g, 26.1 mmol) was added in one portion to a solution of the oxazole (1.1 g, 3.3 mmol) in a THF:H_2O (9:1) mixture (17 ml) at room temperature. The solution was stirred for 14 h and acidified to pH4 by the addition of citric acid, the acidified aqueous layer was then extracted with dichloromethane (3 x 40 ml). The combined organic extracts were washed with water (2 x 30 ml) and brine (2 x 30 ml),

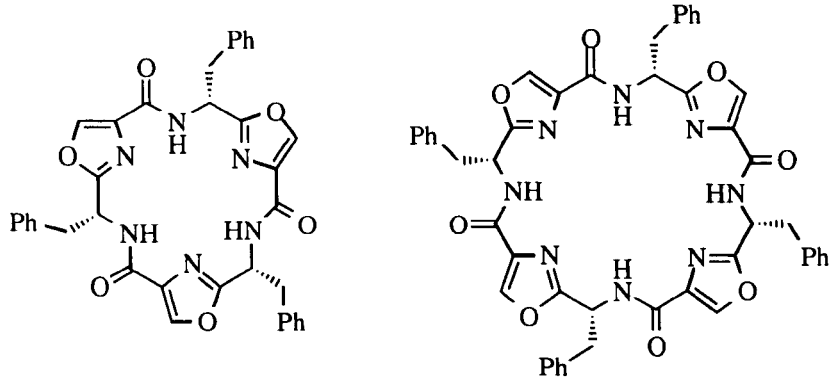
dried ($MgSO_4$) and evaporated *in vacuo* to leave the acid (1.0 g, 5.16 mmol, 96 %) as a yellow foam; $[\alpha]_D^{301} = +12.6$ (c = 0.4, $CHCl_3$); υ_{max} ($CHCl_3$)/cm^{-1} 3696, 3437, 2930, 1713, 1601, 1494, 1455, 1368, 1158, 1109, 984; δ_H (360 MHz, $CDCl_3$) 1.39 (9H, s, Bu^t), 3.24 (2H, d, *J* 6.9 Hz, C*H*$_2$Ph), 5.10-5.29 (1H, m, C*H*CH_2Ph), 5.94 (1H, d, *J* 8.2 Hz, N*H*Boc), 7.08-7.27 (5H, m, Ar*H*), 8.21 (1H, s, C*H*O); δ_C (90.5 MHz, $CDCl_3$) 28.2 (q), 40.3 (t), 50.3 (d), 80.2 (s), 127.1 (d), 128.6 (d), 129.2 (d), 133.0 (s), 135.2 (s), 144.7 (d), 164.1 (s), 182.1 (s).

1'(*R*)-2-(1-Amino-2-phenyl-ethyl)-oxazole-4-carboxylic acid (151)



A 4M solution of hydrochloric acid in dioxane (16 ml) was added to the oxazole **150** (500 mg, 1.5 mmol) at room temperature under an atmosphere of nitrogen. The solution was stirred for 2 h and the dioxane was then removed under reduced pressure using toluene as an azeotrope. The crude amine was triturated with acetonitrile, filtered and dried by suction to give the amine (350 mg, 1.3 mmol, 87 %) as a cream/white solid; mp. 78-90 °C (from methanol); δ_H (360 MHz, CD_3OD) 3.34-3.45 (2H, m, C*H*$_2$Ph), 4.88-4.90 (1H, m, C*H*CH_2Ph), 7.21-7.23 (2H, m, Ar*H*), 7.31-7.38 (3H, m, Ar*H*), 8.58 (1H, s, C*H*O); δ_C (90.5 MHz, CD_3OD) 39.1 (t), 51.1 (d), 128.9 (d), 130.1 (d), 130.4 (d), 135.1 (s), 147.1 (d), 161.5 (s), 163.6 (s).

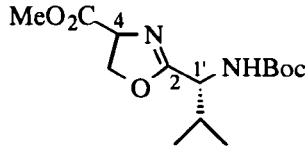
Cyclic-*tris*-(*R*),(*R*),(*R*)-phenylalanine-oxazole (163) and cyclic-*tetra*-(*R*),(*R*),(*R*),(*R*)-phenylalanine-oxazole (164)



Diisopropylethylamine (0.35 ml, 2 mmol) and FDPP (392 mg, 1.02 mmol) were added to a suspension of the oxazole **151** (180 mg, 0.68 mmol) in anhydrous acetonitrile (15 ml) the solution was then stirred at room temperature for 3 days before being evaporated to dryness *in vacuo*. The residue was partitioned between dichloromethane (30 ml) and 2M hydrochloric acid solution (30 ml) the separated organic layer was then washed with 2M hydrochloric acid solution (3 x 30 ml). The combined aqueous solutions were re-extracted with dichloromethane (2 x 30 ml) and the combined organic solutions were washed successively with 2M sodium hydroxide solution (3 x 30 ml), water (3 x 30 ml) and brine (2 x 30 ml). The organic solution was dried ($MgSO_4$) and the solvent removed under reduced pressure to leave the *cyclic peptide* **163** as a mixture with **164** (0.21 mmol, 31 %). The *cyclic peptide* containing residue was separated by chromatography on silica using 70 % ethyl acetate in light petroleum (40-60 °C) as eluant to give, i) the cyclic trimer (6 mg, 0.026 mmol, 15 %) as a white powder; mp. 239-241 °C (from dichloromethane); $[\alpha]_D^{296}$ = -0.80 (c = 0.50, $CHCl_3$); υ_{max} ($CHCl_3$)/cm^{-1} 3697, 3604, 2926, 2855, 1721, 1673, 1600, 1490, 1456, 1295, 1162, 1052, 1010, 966, 903; δ_H (360 MHz, $CDCl_3$) 3.30-3.44 (2H, m, C*H*$_2$Ph), 5.37-5.41 (1H, m, C*H*CH$_2$Ph), 6.95-6.97 (2H, m, Ar*H*), 7.24-7.30 (3H, m, Ar*H*), 8.16-8.18 (1H, m, N*H*CO), 8.16-8.18 (1H, m, C*H*=O); δ_C (90.5 MHz, $CDCl_3$) 28.9 (t), 60.4 (d), 120.1 (d), 125.3 (s), 125.6 (d), 129.8 (s), 129.9 (d), 157.4 (s), 167.1 (s), and ii) the cyclic

tetramer (9 mg, 0.028 mmol, 16 %) as a white powder; mp. 241-244 °C (from dichloromethane); $[\alpha]_D^{301}$ = -21.6 (c = 1.50, $CHCl_3$); λ_{max} (EtOH)/nm 271 (ε/dm^3 mol^{-1}cm^{-1} 64409); υ_{max} ($CHCl_3$)/cm^{-1} 3385, 2958, 2928, 2857, 1719, 1678, 1600, 1494, 1456, 1377, 1297, 1102, 1078, 863; δ_H (360 MHz, $CDCl_3$) 3.30-3.49 (2H, m, C*H*$_2$Ph), 5.37-5.47 (1H, m, C*H*CH$_2$Ph), 6.95-6.97 (2H, m, Ar*H*), 7.22-7.32 (3H, m, Ar*H*), 8.12 (1H, s, C*H*O), 8.17 (1H, d, *J* 6.3 Hz, N*H*CO); δ_C (90.5 MHz, $CDCl_3$) 40.1 (t), 49.7 (d), 127.1 (d), 128.4 (d), 129.3 (d), 135.0 (s), 141.5 (d), 159.2 (s), 162.8 (s).

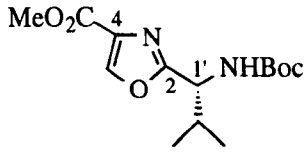
1'(*R*)-2-(1-*tert*-Butoxycarbonylamino-2-methylpropyl)-4,5-dihydro-oxazole-4-carboxylic acid methyl ester (202)[143a]



DAST (0.55 ml, 4.2 mmol) was added dropwise over 2 min to a stirred solution of the dipeptide (1.2 g, 3.7 mmol) in anhydrous dichloromethane (20 ml) at -78 °C under an atmosphere of nitrogen. The reaction was stirred at -78 °C for 1 h, and aqueous ammonia (*ca.* 40 ml) was then added, and the solution allowed to warm to room temperature. The separated aqueous layer was extracted with dichloromethane (4 x 30 ml), and the combined organic extracts were then washed with brine (3 x 20 ml), dried ($MgSO_4$) and evaporated *in vacuo* to leave a yellow oil. Chromatography on silica using 50 % ethyl acetate in light petroleum (40-60 °C) as eluant gave the oxazoline (550 mg, 1.9 mmol, 50 %) as a colourless oil; δ_H (360 MHz, $CDCl_3$) 0.72 (6H, t, *J* 7.5 Hz, (C*H*$_3$)$_2$CH), 1.21 (9H, s, Bu^t), 1.83-1.88 (1H, br. m, C*H*(CH$_3$)$_2$), 3.54 (3H, s, C*H*$_3$O), 4.10 (1H, dd, *J* 5.6, 8.9 Hz, C*H*NH), 4.28 (1H, dd, *J* 8.1 Hz, C*H*$_2$O), 4.34 (1H, dd, *J* 8.1 Hz, C*H*$_2$O), 4.54 (1H, dd, *J* 8.1, 10.4 Hz, C*H*CO$_2$Me), 5.07 (1H, d, *J* 9.2 Hz,

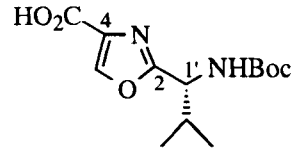
N*H*Boc); δ_C (90.5 MHz, $CDCl_3$) 17.2 (q), 18.2 (q), 27.7 (q), 31.2 (d), 52.0 (d), 53.5 (q), 67.2 (d), 69.3 (t), 79.5 (s), 154.9 (s), 168.9 (s), 170.7 (s).

1'(*R*)-2-(1-*tert*-Butoxycarbonylamino-2-methylpropyl)-oxazole-4-carboxylic acid methyl ester (203)



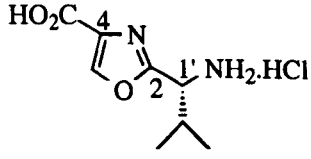
Bromotrichloromethane (0.21 ml, 2.1 mmol) was added dropwise over 5 min to a stirred solution of the oxazoline **202** (550 mg, 1.9 mmol) in anhydrous dichloromethane (10 ml) at 0 °C under an atmosphere of nitrogen. The solution was stirred at 0 °C for 15 min, and then 1,8-diazabicyclo[5.4.0]undec-7-ene (0.31 ml, 2.1 mmol) was added dropwise over 10 min. The resulting solution was stirred at room temperature for a further 2 h and then ammonium chloride solution (*ca* 20 ml) was added. The separated aqueous layer was extracted with dichloromethane (3 x 20 ml) and the combined organic extracts were washed with brine (2 x 20 ml), dried ($MgSO_4$) and evaporated *in vacuo*. The residue was purified by chromatography on silica using 30 % ethyl acetate in light petroleum (40-60 °C) as eluant to give the oxazole **203** (432 mg, 1.5 mmol, 76 %) as a white solid; mp. 125-127 °C (from dichloromethane) (Lit.[146a] mp. 120-123 °C); $[\alpha]_D^{302}$ + 5.6 (c = 1.0, $CHCl_3$); Found : C, 56.1; H, 7.4; N, 9.2 %; calc. for $C_{14}H_{22}O_5N_2$: C, 56.4; H, 7.4; N, 9.4 %; δ_H (360 MHz, $CDCl_3$) 0.70 (6H, t, *J* 6.9 Hz, $CH(CH_3)_2$), 1.19 (9H, s, Bu^t), 1.94-2.03 (1H, br. m, $CH(CH_3)_2$), 3.68 (3H, s, CH_3O), 4.58 (1H, dd, *J* 6.3, 9.2 Hz, CHNHBoc), 5.32 (1H, d, *J* 9.2 Hz, NHBoc), 8.07 (1H, s, CHO); δ_C (90.5 MHz, $CDCl_3$) 17.4 (q), 18.2 (q), 27.7 (q), 32.3 (d), 51.6 (q), 53.8 (d), 79.2 (s), 132.6 (s), 143.6 (d), 154.9 (s), 161.0 (s), 164.6 (s); *m/z* (FAB) Found: 321.3279 (MH^++Na^+, $C_{14}H_{22}O_5N_2Na$ requires 321.3288).

1'(*R*)-2-(1-*tert*-Butoxycarbonylamino-2-methyl-propyl)-oxazole-4-carboxylic acid (204)



Sodium hydroxide (410 mg, 0.22 mmol) was added to a stirred solution of the oxazole (381 mg, 1.28 mmol) in a 5:4 mixture of THF:H_2O (5:4) mixture (7 ml) at room temperature and this mixture was stirred at room temperaure for 14 h. The separated aqueous layer was acidified to pH4 with citric acid and then extracted with ethyl acetate (4 x 20 ml). The combined organic extracts were dried ($MgSO_4$) and evaporated *in vacuo* to leave the acid (349 mg, 1.22 mmol, 96 %) as a white foam which crystallised to give a white powder; mp. 141-142 °C (from dichloromethane) (Lit.[150] mp. 145-147 °C); υ_{max} ($CHCl_3$)/cm^{-1} 3682, 3439, 2974, 2398, 1714, 1601, 1498, 1368, 1326, 1160, 1111, 1001; δ_H (360 MHz, $CDCl_3$) 0.90 (3H, d, *J* 6.7 Hz, $(CH_3)_2CH$), 0.99 (3H, d, *J* 6.7 Hz, Bu^t), 1.44 (9H, s, Bu^t), 2.17-2.25 (1H, m, $CH(CH_3)_3$), 4.65 (1H, d, *J* 6.7 Hz, CHNHBoc), 7.12 (1H, d, *J* 8.8 Hz, NHBoc), 8.49 (1H, c, CHO); δ_C (90.5 MHz, $CDCl_3$) 18.8 (q), 19.3 (q), 28.6 (q), 43.7 (d), 55.9 (d), 80.6 (s), 134.2 (s), 145.7 (d), 163.7 (s), 166.5 (s), 176.6 (s); *m/z* (FAB) Found: 307.1306 (MH^++Na^+, $C_{13}H_{20}O_5N_2Na$ requires 307.1270)

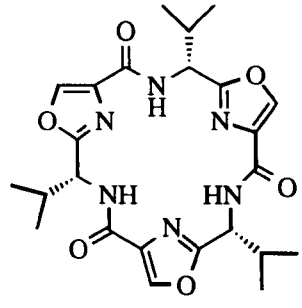
1'(*R*)-2-(1-Amino-2-methylpropyl)-oxazole-4-carboxylic acid (165)



The oxazole **204** (440 mg, 1.6 mmol) was stirred with a 4M solution of hydrochloric acid in dioxane (5 ml) at room temperature for 3 h under an atmosphere of nitrogen. The

dioxane was removed under reduced pressure by azeotroping with toluene to leave the amine (250 mg, 1.04 mmol, 65%) as a sticky pale yellow solid; δ_H (360 MHz, CD_3OD) 1.03 (3H, d, *J* 6.9 Hz, $(CH_3)_2CH$), 1.15 (3H, d, *J* 7.2 Hz, $(CH_3)_2CH$), 2.45-2.53 (1H, br. m, $CH(CH_3)_2$), 4.50 (1H, d, *J* 7.2 Hz, $CHNH_2.HCl$), 8.75 (1H, s, CHO), 8.85-8.96 (1H, br. s, NH); δ_C (90.5 MHz, CD_3OD) 18.0 (q), 18.9 (q), 32.3 (d), 55.2 (d), 134.7 (s), 147.2 (d), 163.6 (s), 173.4 (s); *m/z* (FAB) Found: 185.0930 (MH^+-Cl^-, $C_8H_{13}O_3N_2$ requires 185.0926)

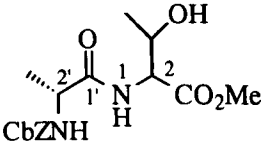
Cyclic-*tris*-(*R*),(*R*),(*R*)-valine-thiazole (166)



Diisopropylethylamine (0.53 ml, 3 mmol) was added to a stirred suspension of the oxazole **165** (220 mg, 1 mmol) in anhydrous acetonitrile (20 ml) at room temperature under an atmosphere of nitrogen. The mixture was stirred for 5 min and FDPP (576 mg, 1.5 mmol) was added in one portion, the resulting solution was then stirred for 3 days at room temperature. The mixture was concentrated *in vacuo* to leave a brown residue which was taken up in dichloromethane (*ca* 30 ml) and washed with 2M hydrochloric acid solution (2 x 20 ml), 2M sodium hydroxide solution (2 x 30 ml), and brine (30 ml). The separated organic layer was dried ($MgSO_4$) and evaporated *in vacuo* to leave a white residue which was purified by chromatography on silica using 70 % ethyl acetate in light petroleum (40-60 °C) as eluant to give the *cyclic peptide* (8 mg, 0.05 mmol, 5 %) as a white powder; mp. 176-179 °C (from dichloromethane); $[\alpha]_D^{301}$ = -57.1 (c = 0.7, $CHCl_3$); λ_{max} (EtOH)/nm 215 (ε/dm^3 mol^{-1}cm^{-1} 152678); υ_{max} ($CHCl_3$)/cm^{-1} 3389,

2968, 2934, 1681, 1600, 1575, 1373, 1304, 1129, 1097, 882; δ_H (360 MHz, $CDCl_3$) 1.05 (3H, d, *J* 6.9 Hz, $(CH_3)_2CH$), 1.09 (3H, d, *J* 6.9 Hz, $(CH_3)_2CH$), 2.34-2.41 (1H, m, $CH(CH_3)_2$), 5.15 (1H, dd, *J* 4.9, 7.9 Hz, CHNH), 8.20 (1H, s, CHO), 8.25 (1H, d, *J* 7.9 Hz, NHCO); δ_C (125 MHz, $CDCl_3$) 18.3 (q), 33.6 (d), 53.3 (d), 135.4 (s), 141.4 (d), 159.6 (s), 163.6 (s); *m/z* (FAB) Found: 521.2189 (MH^++Na^+, $C_{24}H_{30}O_6N_6Na$ requires 521.2125).

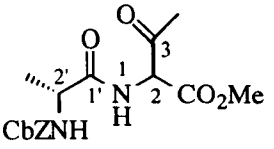
2'(*R*)-2-(2-Benzyloxycarbonylamino-propionylamino)-3-hydroxy-butyric acid methyl ester (157)[149]



N-Methylmorpholine (2.6 ml, 23.7 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (4.5 g, 23.7 mmol) and *N* - hydroxybenzotriazole (3.2 g, 23.7 mmol) were added to a stirred solution of CbZ-protected (*D*)-alanine (4.8 g, 21.5 mmol) at 0 °C under an atmosphere of nitrogen. The resulting mixture was stirred for 20 min and a precooled solution of (*D, L*) - threonine methyl ester hydrochloride (3.6 g, 21.5 mmol) deprotonated with *N*-Methylmorpholine (2.6 ml, 23.7 mmol) in DMF (5 ml) was added dropwise over 5 min. The mixture was stirred at 0 °C for 1 h and at room temperature for 14 h. Water (*ca* 50 ml) was added, the aqueous layer separated and extracted with ethyl acetate (6 x 50 ml). The combined organic extracts were washed with sodium hydrogen carbonate solution (3 x 40 ml), 10 % aqueous citric acid solution (3 x 40 ml), and brine (3 x 40 ml), were dried ($MgSO_4$) and evaporated *in vacuo*. The yellow residue was purified by chromatography on silica using 70 % ethyl acetate in light petroleum (40-60 °C) as eluant to give the dipeptide (5.75 g, 17.0 mmol, 79 %) as a viscous oil; υ_{max} ($CHCl_3$)/cm^{-1} 3429, 2954, 1730, 1682, 1494, 1455, 1321, 1069, 908; δ_H (360 MHz, $CDCl_3$) 1.20 (3H, d, *J* 6.4 Hz, CH_3CHOH), 1.44 (3H, d, *J* 7.1 Hz, CH_3CH), 3.76 (3H, s, CH_3O), 4.34-4.37 (1H, br. m, $CH(OH)CH_3$), 4.34-4.37

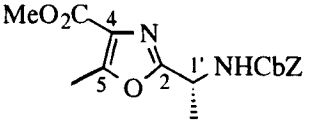
(1H, m, C*H*CH_3), 4.58 (1H, dd, *J* 2.4, 8.9 Hz, C*H*CO_2Me), 5.13 (2H, s, CH_2Ph), 5.40-5.47 (1H, br. s, N*H*CbZ), 6.83 (1H, d, *J* 8.9 Hz, N*H*CO), 7.30-7.39 (5H, m, ArH); δ_C (90.5 MHz, CD_3OD) 18.7 (q), 19.5 (q), 50.5 (d), 52.3 (q), 57.3 (d), 66.6 (t), 67.3 (d), 127.7 (d), 127.9 (d), 128.1 (d), 155.8 (s), 171.3 (s), 173.5 (s); *m/z* (FAB) Found: 361.1377 (M^++Na^+, $C_{16}H_{22}O_6N_2Na$ requires 361.1376);

2'(*R*)-2-(2-Benzyloxycarbonylamino-propionylamino)-3-oxo-butyric acid methyl ester (158)



Dess Martin Periodinane (17.6 g, 41.5 mmol) was added in one portion to a solution of the dipeptide **157** (10.8 g, 31.9 mmol) in anhydrous dichloromethane (100 ml) at room temperature under an atmosphere of nitrogen. The resulting brown solution was stirred for 4 h at room temperature, the volatiles were then removed *in vacuo* leaving a solid yellow residue. The residue was purified by chromatography on silica using 70 % ethyl acetate in light petroleum (40-60 °C) as eluant to give the dipeptide (9.2 g, 27.5 mmol, 86 %) as a white solid; mp. 117-118 °C (from dichloromethane); υ_{max} ($CHCl_3$)/cm^{-1} 3422, 2957, 1757, 1729, 1682, 1492, 1454, 1359, 1069, 973; δ_H (360 MHz, $CDCl_3$) 1.42 (3H, d, *J* 7.1 Hz, CH_3CH), 2.38 (3H, s, CH_3COCH), 3.81 (3H, s, CH_3OCO), 4.36-4.40 (1H, m, C*H*CH_3), 5.13 (2H, s, CH_2Ph), 5.24 (1H, d, *J* 6.3 Hz, C*H*CO_2Me), 5.36 (1H, d, *J* 6.9 Hz, N*H*CbZ), 7.15-7.20 (1H, br. s, N*H*CO), 7.31-7.36 (5H, m, Ar*H*); δ_C (90.5 MHz, CD_3OD) 18.2 (q), 27.5 (q), 50.0 (d), 52.1 (q), 62.6 (d), 66.6 (t), 127.7 (d), 127.8 (d), 128.2 (d), 155.8 (s), 166.3 (s), 172.6 (s), 198.4 (s); *m/z* (FAB) Found: 359.1219 (M^++Na^+, $C_{16}H_{20}O_6N_2Na$ requires 359.1219);

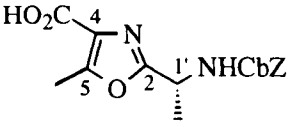
1'(*R*)-2-(1-Benzyloxycarbonylamino-ethyl)-5-methyl-oxazole-4-carboxylic acid methyl ester (159)



A solution of the dipeptide **158** (1.2 g, 3.6 mmol) in anhydrous THF (3.5 ml) at -78 °C under an atmosphere of nitrogen was added dropwise, over 5 min, to a solution of triphenylphosphine (2.1 g, 7.85 mmol), iodine (0.9 g, 7.14 mmol) and triethylamine (1.8 ml, 12.96 mmol) in anhydrous THF (12 ml) at -78 °C. The resulting yellow solution was allowed to stir at -78 °C for 4 h and at room temperature for a further 14 h.

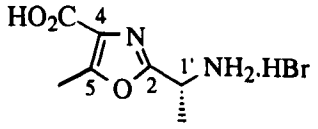
Water (*ca* 50 ml) was added and the separated aqueous layer was extracted with dichloromethane (4 x 40 ml). The combined organic extracts were washed with sodium hydrogen carbonate solution (2 x 40 ml), 2M hydrochloric acid solution (2 x 40 ml) and brine (2 x 40 ml), dried ($MgSO_4$) and evaporated *in vacuo*. The solid yellow residue was purified by chromatography on silica using 70 % ethyl acetate in light petroleum (40-60 °C) as eluant to leave the oxazole (960 mg, 3.02 mmol, 84 %) as a white solid; mp. 126-128 °C (from dichloromethane); $[\alpha]_D^{295}$ = +29.4 (c = 1.0, $CHCl_3$); υ_{max} ($CHCl_3$)/cm^{-1} 3433, 2954, 1722, 1624, 1454, 1353, 1101, 1066, 980; δ_H (360 MHz, $CDCl_3$) 1.57 (3H, d, *J* 6.9 Hz, C*H*$_3$CH), 2.62 (3H, s, C*H*$_3$CO), 3.91 (3H, s, C*H*$_3$O), 5.03-5.09 (1H, m, C*H*CH$_3$), 5.12-5.13 (2H, m, C*H*$_2$Ar), 5.31 (1H, br. s, N*H*CbZ), 7.30-7.35 (5H, m, Ar*H*); δ_C (90.5 MHz, $CDCl_3$); 11.9 (q), 20.0 (q), 45.0 (d), 51.9 (q), 67.0 (t), 128.0 (d), 128.1 (d), 128.4 (d), 136.1 (s), 155.4 (s), 156.6 (s), 162.5 (s), 167.1 (s); *m/z* (FAB) Found: 341.1086 (M^++Na^+, $C_{16}H_{18}O_5N_2Na$ requires 341.1113).

1'(*R*)-2-(1-Benzyloxycarbonylamino-ethyl)-5-methyl-oxazole-4-carboxylic acid (205)



A solution of sodium hydroxide (201 mg, 5 mmol) in water (2 ml) was added to a solution of the oxazole **159** (200 mg, 0.63 mmol) in ethanol (6 ml) at room temperature. The solution was stirred for 3 h, the volatiles were removed *in vacuo* and the resulting oil was flooded with 2M hydrochloric acid solution. The oxazole acid was collected by suction filtration (176 mg, 0.58 mmol, 92 %) as a white solid; mp. 248-250 °C (from diethyl ether); υ_{max} ($CHCl_3$)/cm^{-1} 3437, 2980, 1721, 1632, 1454, 1362, 1120, 1066; δ_H (360 MHz, $CDCl_3$) 1.56 (3H, d, *J* 6.9 Hz, CH_3CH), 2.53 (3H, s, CH_3CO), 5.02-5.12 (1H, m, $CHCH_3$), 5.02-5.12 (2H, m, CH_2Ar), 6.48-6.55 (1H, br. s, NHCbZ) 7.20-7.35 (5H, m, Ar*H*); δ_C (90.5 MHz, $CDCl_3$); 11.7 (q), 20.0 (q), 45.0 (d), 66.9 (t), 128.0 (d), 128.3 (d), 128.6 (d), 136.2 (s), 155.5 (s), 156.0 (s), 163.8 (s), 165.0 (s); *m/z* (FAB) Found: 327.0953 (M^++Na^+, $C_{15}H_{16}O_5N_2Na$ requires 327.0957).

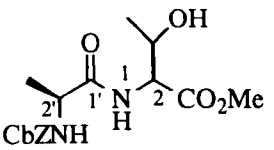
1'(*R*)-2-(1-Amino-ethyl)-5-methyl-oxazole-4-carboxylic acid hydrobromide (160)



A 33 % solution of hydrobromic acid in acetic acid (1.5 ml) and glacial acetic acid (1 ml) were added to the oxazole **205** (250 mg, 0.84 mmol) at room temperature under an atmosphere of nitrogen. The brown solution was stirred for 2 h and the volatiles removed *in vacuo* to leave the amine (193 mg, 0.76 mmol, 91 %) as an orange hygroscopic solid; $[\alpha]_D^{295}$ = +7.4 (c = 1.0, EtOH); δ_H (360 MHz, CD_3OD) 1.73

(3H, d, *J* 7.0 Hz, CH_3CH), 2.69 (3H, s, CH_3CO), 4.68-4.74 (1H, m, C*H*CH$_3$); δ_C (90.5 MHz, CD_3OD); 13.2 (q), 18.5 (q), 46.8 (d), 134.9 (s), 160.1 (s), 160.8 (s), 165.8 (s).

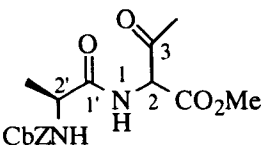
2'(*S*)-2-(2-Benzyloxycarbonylamino-propionylamino)-3-hydroxy-butyric acid methyl ester (206)



N-Methylmorpholine (3.4 ml, 31.2 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (6.0 g, 31.2 mmol) and *N* - hydroxybenzotriazole (4.2 g, 31.2 mmol) were added to a stirred solution of CbZ-protected (*L*)-alanine (6.3 g, 28.4 mmol) at 0 °C under an atmosphere of nitrogen. The resulting mixture was stirred for 20 min at 0 °C and a precooled solution of (*D, L*) - threonine methyl ester hydrochloride (4.8 g, 28.4 mmol) deprotonated with *N*-Methylmorpholine (3.4 ml, 31.2 mmol) in DMF (4 ml) was added dropwise over 5 min. The mixture was stirred at 0 °C for 1 h and at room temperature for 14 h. Water (*ca* 40 ml) was added, the aqueous layer separated and extracted with ethyl acetate (4 x 40 ml). The combined organic extracts were washed with sodium hydrogen carbonate solution (3 x 40 ml), water (3 x 40 ml), and brine (3 x 40 ml), dried ($MgSO_4$) and evaporated *in vacuo*. The yellow residue was purified by chromatography on silica using 70 % ethyl acetate in light petroleum (40-60 °C) as eluant to give the dipeptide (7.88 g, 23.3 mmol, 82 %) as a viscous oil; υ_{max} ($CHCl_3$)/cm^{-1} 3428, 2541, 1715, 1682, 1497, 1455, 1356, 1072; δ_H (360 MHz, $CDCl_3$) 1.16 (3H, d, *J* 6.4 Hz, CH_3CHOH), 1.40 (3H, d, *J* 7.1 Hz, CH_3CH), 3.73 (3H, s, CH_3O), 4.31-4.41 (1H, br. m, C*H*(OH)CH$_3$), 4.31-4.41 (1H, m, C*H*CH$_3$), 4.58-4.61 (1H, m, C*H*CO$_2$Me), 5.03-5.08 (2H, m, CH_2Ph), 5.84 (1H, d, *J* 7.8 Hz, N*H*CbZ), 7.22 (1H, d, *J* 8.9 Hz, N*H*CO), 7.29-7.40 (5H, m, ArH); δ_C (90.5 MHz, CD_3OD) 16.3 (q), 18.2 (q), 49.8 (q), 50.8 (d), 56.9 (d), 65.5 (t), 66.3 (d), 126.7 (d), 126.8 (d), 127.3 (d),

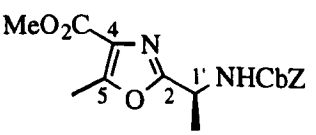
155.8 (s), 171.3 (s), 173.5 (s); *m/z* (FAB) Found: 361.1336 ($M^+ + Na^+$, $C_{16}H_{22}O_6N_2Na$ requires 361.1376);

2'(*S*)-2-(2-Benzyloxycarbonylamino-propionylamino)-3-oxo-butyric acid methyl ester (207)



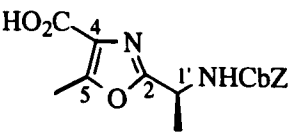
Dess Martin Periodinane (7.8 g, 18.4 mmol) was added in one portion to a solution of the dipeptide **206** (5.0 g, 14.2 mmol) in anhydrous dichloromethane (80 ml) at room temperature under an atmosphere of nitrogen. The resulting yellow solution was stirred for 4 h at room temperature, the volatiles were then removed *in vacuo* leaving a solid orange residue. The residue was purified by chromatography on silica using 50 % ethyl acetate in light petroleum (40-60 °C) as eluant to give the dipeptide (3.1 g, 9.1 mmol, 64 %) as a white solid; mp. 118-119 °C (from dichloromethane) (Lit.[151] mp. 122-123 °C); υ_{max} ($CHCl_3$)/cm^{-1} 3423, 2957, 1757, 1729, 1685, 1493, 1455, 1358, 1070; δ_H (360 MHz, $CDCl_3$) 1.42 (3H, d, *J* 7.1 Hz, C*H*$_3$CH), 2.39 (3H, s, C*H*$_3$COCH), 3.82 (3H, s, C*H*$_3$OCO), 4.35-4.40 (1H, m, C*H*CH$_3$), 5.14 (2H, s, C*H*$_2$Ph), 5.24 (1H, d, *J* 6.5 Hz, C*H*CO$_2$Me), 5.31 (1H, d, *J* 7.6 Hz, N*H*CbZ), 7.15-7.21 (1H, br. s, N*H*CO), 7.32-7.46 (5H, m, Ar*H*); δ_C (90.5 MHz, $CDCl_3$) 18.3 (q), 27.7 (q), 50.1 (d), 53.1 (q), 62.7 (d), 66.8 (t), 127.8 (d), 127.9 (d), 128.3 (d), 155.8 (s), 166.3 (s), 172.6 (s), 198.3 (s); *m/z* (FAB) Found: 359.1219 (M^++Na^+, $C_{16}H_{20}O_6N_2Na$ requires 359.1219);

1'(*S*)-2-(1-Benzyloxycarbonylamino-ethyl)-5-methyl-oxazole-4-carboxylic acid methyl ester (208)



A solution of the dipeptide **207** (3.1 g, 9.3 mmol) in anhydrous THF (9 ml) at -78 °C was added dropwise over 5 min to a solution of triphenylphosphine (5.4 g, 20.5 mmol), iodine (2.4 g, 18.6 mmol) and triethylamine (4.7 ml, 33.5 mmol) in anhydrous THF (32 ml) at -78 °C under an atmosphere of nitrogen. The resulting yellow solution was allowed to stir at -78 °C for 5 h and at room temperature for 15 h. Water (*ca.* 50 ml) was added and the separated aqueous layer was then extracted with dichloromethane (4 x 40 ml). The combined organic extracts were washed with sodium hydrogen carbonate solution (2 x 40 ml), 2M hydrochloric acid solution (2 x 40 ml) and brine (2 x 40 ml), dried ($MgSO_4$) and evaporated *in vacuo*. The solid yellow residue was purified by chromatography on silica using 70 % ethyl acetate in light petroleum (40-60 °C) as eluant to leave the oxazole (2.2 g, 7.0 mmol, 75 %) as a white solid; mp. 126-128 °C (from dichloromethane) (Lit.[151] mp. 125.5-126 °C); $[\alpha]_D^{295}$ = -33.6 (c = 1.0, $CHCl_3$); υ_{max} ($CHCl_3$)/cm^{-1} 3433, 2954, 1722, 1624, 1454, 1353, 1101, 1066, 9803436, 2954, 1722, 1623, 1499, 1454, 1353, 1101, 1066; δ_H (360 MHz, $CDCl_3$) 1.57 (3H, d, *J* 7.0 Hz, C*H*$_3$CH), 2.61 (3H, s, C*H*$_3$CO), 3.91 (3H, s, C*H*$_3$O), 5.02 (1H, dq, *J* 5.5, 7.9 Hz, C*H*CH$_3$), 5.12-5.13 (2H, m, C*H*$_2$Ar), 5.42 (1H, d, *J* 7.9 Hz, N*H*CbZ), 7.29-7.37 (5H, m, Ar*H*); δ_C (90.5 MHz, $CDCl_3$); 11.9 (q), 19.7(q), 44.8 (d), 51.7 (q), 66.7 (t), 127.8 (d), 127.9 (d), 128.2 (d), 135.9 (s), 155.3 (s), 156.3 (s), 162.3 (s), 162.3 (s); *m/z* (FAB) Found: 341.1118 (M^++Na^+, $C_{16}H_{18}O_5N_2Na$ requires 341.1113).

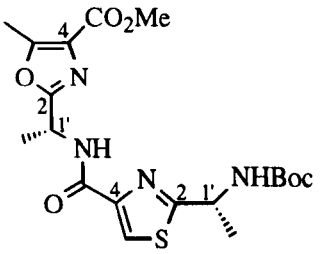
1'(*S*)-2-(1-Benzyloxycarbonylamino-ethyl)-5-methyl-oxazole-4-carboxylic acid (209)



A solution of sodium hydroxide (900 mg, 22.6 mmol) in water (9 ml) was added to a solution of the oxazole **208** (0.9 g, 2.83 mmol) in ethanol (27 ml) at room temperature. The solution was stirred for 3 h, the volatiles were removed *in vacuo* and the resulting

oil was flooded with 2M hydrochloric acid solution. The oxazole acid was collected by suction filtration (792 mg, 2.6 mmol, 92 %) as a white solid; mp. 166-168 °C (from diethyl ether); $[\alpha]_D^{295}$ = -68.7 (c = 1.0, $CHCl_3$); υ_{max} ($CHCl_3$)/cm^{-1} 3441, 2998, 1723, 1454, 1361, 1064; δ_H (360 MHz, CD_3OD) 1.55 (3H, d, *J* 7.1 Hz, C*H*$_3$CH), 2.61 (3H, s, C*H*$_3$CO), 4.91-4.97 (1H, m, C*H*CH$_3$), 5.12 (2H, br. s, C*H*$_2$Ar), 6.48-6.55 (1H, br. s, N*H*CbZ) 7.31-7.39 (5H, m, Ar*H*); δ_C (125 MHz, CD_3OD); 13.0 (q), 20.2 (q), 47.3 (d), 68.7 (t), 129.9 (d), 130.1 (d), 130.2 (d), 139.2 (s), 158.3 (s), 159.1 (s), 165.3 (s), 166.5 (s); *m/z* (FAB) Found: 327.0952 (M^++Na^+, $C_{15}H_{16}O_5N_2Na$ requires 327.0957).

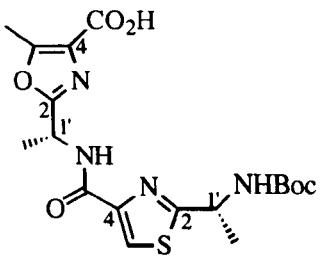
1'(*R*)-(1-{[1'(*R*)-(-*tert*-Butoxycarbonylaminoethyl)-thiazole-4-carbonyl]-amino}-ethyl)-5-methyl-oxazole carboxylic acid methyl ester (179)



N-Methylmorpholine (0.19 ml, 1.76 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (338 mg, 1.76 mmol) and *N*-hydroxybenzotriazole (238 mg, 1.76 mmol) were added to a stirred solution of the alanine thiazole acid **177** (436 mg, 1.6 mmol) in anhydrous dichloromethane (20 ml) at 0 °C under an atmosphere of nitrogen. The resulting solution was stirred at 0 °C for a further 15 min and a precooled solution of the oxazole amine **178** (353 mg, 1.6 mmol) deprotonated with *N*-methylmorpholine (0.19 ml, 1.76 mmol) in DMF (3 ml) was added dropwise over 5 min. The orange/opaque solution was stirred at 0 °C for 1 h and at room temperature for 15 h . 10 % aqueous citric acid solution (30 ml) was added and the separated aqueous layer was extracted with dichloromethane (5 x 30 ml), the combined organic extracts were then washed with sodium hydrogen carbonate solution (3 x 30 ml), water

(3 x 30 ml), and brine (3 x 30 ml), were dried ($MgSO_4$) and evaporated *in vacuo*. The brown residue was purified by chromatography on silica using 60 % ethyl actetate in light petroleum (40-60 °C) as eluant to leave the tetrapeptide (560 mg, 1.28 mmol, 80 %) as a white foam; $[\alpha]_D^{299}$ = +29.6 (c = 1.0, $CHCl_3$); υ_{max} ($CHCl_3$)/cm^{-1} 3688, 3872, 3606, 3441, 3402, 2981, 2955, 2934, 1716, 1672, 1622, 1545, 1490, 1454, 1389, 1369, 1353, 1299, 1153, 1099, 1059, 984, 908; δ_H (360 MHz, $CDCl_3$) 1.46 (9H, s, Bu^t), 1.60 (3H, d, *J* 6.8 Hz, CH_3CH), 1.68 (3H, d, *J* 7.1 Hz, CH_3CH), 2.62 (3H, s, CH_3CCO), 3.90 (3H, s, CH_3CO), 5.06-5.14 (1H, br. m. $CHCH_3$), 5.06-5.14 (1H, br. m. NHBoc), 5.41-5.50 (1H, m, $CHCH_3$), 7.74 (1H, d, *J* 8.5 Hz, NHCO), 8.03 (1H, s, CHS); δ_C (90.5 MHz, $CDCl_3$) 11.3 (q), 18.7 (q), 20.4 (q), 27.7 (d), 30.7 (d), 42.5 (q), 51.1 (q), 79.6 (s), 124.3 (d), 126.7 (s), 148.3 (s), 155.1 (s), 160.1 (s), 161.8 (s), 161.9 (s), 162.2 (s), 162.3 (s).

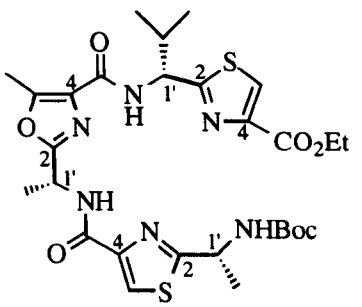
1'(*R*)-2-1'(*R*)-2-(1-{[2-(-*tert*-Butoxycarbonylaminoethyl)-thiazole-4-carbonyl]-amino}-ethyl)-5-methyl-oxazole carboxylic acid (180)



Sodium hydroxide (365 mg, 9.12 mmol) was added to a stirred solution of **179** (500 mg, 1.14 mmol) in a THF:H_2O (5:4) mixture (11 ml) at room temperature and the resulting solution was stirred at room temperature for 14 h. The separated aqueous layer was acidified to pH4 by the addition of citric acid and was extracted with ethyl acetate (5 x 20 ml). The combined organic extracts were then washed with brine (3 x 20 ml), dried ($MgSO_4$) and evaporated *in vacuo* to leave the acid (470 mg, 1.11 mmol, 97%) as a cream foam which crystallised to give a cream powder; mp 76-78 °C (from diethyl

ether); $[\alpha]_D^{298}$ +36.4 (c = 1.0, $CHCl_3$); υ_{max} ($CHCl_3$)/cm^{-1} 3441, 3399, 3206, 2980, 2933, 2629, 1706, 1668, 1622, 1549, 1490, 1454, 1393, 1369, 1323, 1152, 1101, 1058, 984, 897; δ_H (360 MHz, $CDCl_3$) 1.45 (9H, s, Bu^t), 1.57 (3H, d, *J* 6.9 Hz, CH_3CH), 1.70 (3H, d, *J* 6.9 Hz, CH_3CH), 2.63 (3H, s, CH_3CO), 5.06-5.13 (1H, br. s, $CHCH_3$), 5.18-5.33 (1H, br. s, NHBoc), 5.42-5.50 (1H, m, $CHCH_3$), 7.95-7.98 (1H, br. s, NHCO), 8.06 (1H, s, CHS); δ_C (90.5 MHz, $CDCl_3$) 11.6 (q), 18.7 (q), 20.8 (q), 27.9 (q), 42.7 (d), 48.2 (d), 79.7 (s), 124.0 (d), 126.9 (s), 148.1 (s), 154.9 (s), 156.0 (s), 160.6 (s), 162.4 (s), 164.0 (s), 172.7 (s); *m/z* (FAB) Found: 447.1275 (MH^++Na^+, $C_{18}H_{24}O_6N_4SNa$ requires 447.1314).

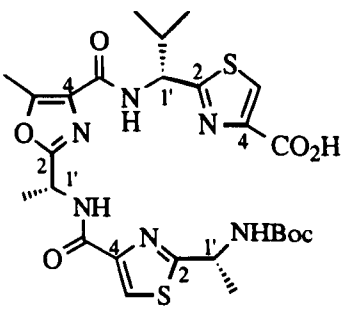
1'(*R*)-2-(1-{[1'(*R*)-2-(1-*tert*-Butoxycarbonylaminoethyl)-thiazole-4-carbonyl]-amino}-ethyl)-5-methyl-oxazole-4-carbonyl]-amino}-1'(*R*)-2-methylpropyl)-thiazole-4-carboxylic acid ethyl ester (182)



N-Methylmorpholine (0.14 ml, 1.26 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (242 mg, 1.26 mmol) and *N*-hydroxybenzotriazole (170 mg, 1.26 mmol) were added to a stirred solution of the dimer **180** in anhydrous dichloromethane (12 ml) at 0 °C under an atmosphere of nitrogen. The cream/opaque solution was stirred at 0 °C for a further 15 min and a precooled solution of the thiazole hydrochloride **181** (304 mg, 1.15 mmol) deprotonated with *N*-methylmorpholine (0.14 ml, 1.26 mmol) in DMF (3 ml) was added dropwise over 5 min. The resulting orange solution was stirred at 0 °C for 1 h and at room temperature for 16 h. 10% aqueous

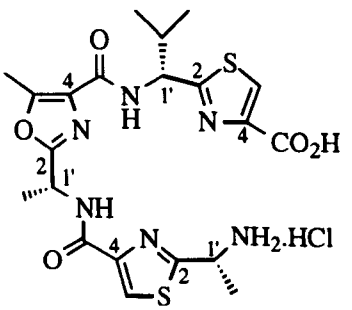
citric acid solution (20 ml) was added, and the separated aqueous layer was extracted with dichloromethane (4 x 30 ml), the combined organic extracts were washed with sodium hydrogen carbonate solution (3 x 20 ml), water (3 x 20 ml), and brine (3 x 20 ml), were dried ($MgSO_4$) and evaporated *in vacuo.* The orange residue was purified by chromatography on silica using 50 % ethyl actetate in light petroleum (40-60 °C) as eluant to leave the hexapeptide (497 mg, 0.8 mmol, 70 %) as a white foam; $[\alpha]_D^{296}$ +46.4 (c = 1.0, $CHCl_3$); υ_{max} ($CHCl_3$)/cm^{-1} 3400, 2979, 1714, 1668, 1635, 1544, 1494, 1369, 1059, 908; δ_H (360 MHz, $CDCl_3$) 0.99 (3H, d, *J* 6.8 Hz, $(CH_3)_2CH$), 1.02 (3H, d, *J* 6.8 Hz, $(CH_3)_2CH$), 1.39 (3H, t, *J* 7.0 Hz, CH_3CH_2), 1.45 (9H, s, Bu^t), 1.63 (3H, d, *J* 6.9 Hz, CH_3CH), 1.68 (3H, d, *J* 7.0 Hz, CH_3CH), 2.55-2.64 (1H, m, $CH(CH_3)_2$), 2.62 (3H, s, CH_3CO), 4.41 (2H, q, *J* 7.0 Hz, CH_2CH_3), 5.01-5.11 (1H, br. s, $CHCH_3$), 5.11-5.26 (1H, br. s, NHBoc), 5.29 (1H, dd, *J* 6.7, 9.2 Hz, $CHCHCH_3$), 5.42 (1H, dt, *J* 7.1, 15.4 Hz, $CHCH_3$), 7.57 (1H, d, *J* 9.2 Hz, NHCO), 7.76 (1H, d, *J* 8.4 Hz, NHCO), 8.06 (1H, s, CHS), 8.06 (1H, s, CHS); δ_C (90.5 MHz, $CDCl_3$) 11.1 (q), 13.8 (q), 17.4 (q), 18.7 (q), 19.1 (q), 20.2 (q), 27.7 (t), 32.4 (d), 42.4 (d), 48.2 (d), 55.4 (d), 60.7 (q), 79.2 (s), 123.4 (d), 126.6 (s), 127.9 (d), 146.7 (s), 148.3 (s), 153.3 (s), 154.2 (s), 159.9 (s), 160.6 (s), 160.8 (s), 160.9 (s), 171.2 (s), 174.5 (s); *m/z* (FAB) Found: 657.2186 (MH^++Na^+, $C_{28}H_{34}O_7N_6S_2Na$ requires 657.2141).

1'(*R*)-2-(1-{[1'(*R*)-2-(1-*tert*-Butoxycarbonylamino-ethyl)-thiazole-4-carbonyl]-amino}-ethyl)-5-methyl-oxazole-4-carbonyl]-amino}-1'(*R*)-2-methyl-propyl)-thiazole-4-carboxylic acid (210)



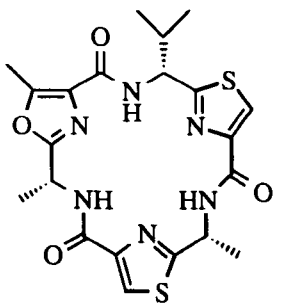
Sodium hydroxide (247 mg, 6.2 mmol) was added to a solution of the trimer **182** (480 mg, 0.77 mmol) in a THF:H_2O (5:4) mixture (8 ml) at room temperature and the reaction was stirred for 15 h. The separated aqueous layer was acidified to pH4 with citric acid and the aqueous layer was extracted with dichloromethane (5 x 20 ml), the combined organic extracts were dried ($MgSO_4$) and evaporated *in vacuo* to leave the acid (446 mg, 0.75 mmol, 97 %) as a white foam which crystallised to give a white powder; mp. 108-110 °C (from dichloromethane); $[\alpha]_D^{296}$ = +42.8 (c = 1.0, $CHCl_3$); υ_{max} ($CHCl_3$)/cm^{-1} 3401, 3246, 2970, 2933, 1714, 1667, 1634, 1546, 1490, 1454, 1394, 1369, 1154, 1059, 952, 908; δ_H (360 MHz, $CDCl_3$) 0.96-1.06 (6H, m, $(CH_3)_2CH$), 1.47 (9H, s, Bu^t), 1.60-1.62 (3H, m, CH_3CH), 1.68 (3H, d, *J* 7.1 Hz, CH_3CH), 2.51-2.55 (1H, m, $CH(CH_3)_2$), 2.62 (3H, s, CH_3CO), 5.02-5.10 (1H, br. m, $CHCH_3$), 5.22-5.28 (1H, br. m, $CHCH(CH_3)_2$), 5.22-5.28 (1H, br. m, NHBoc), 5.44-5.47 (1H, m, $CHCH_3$), 7.70-7.79 (1H, br. s, NHCO), 7.87-7.91 (1H, br. s, NHCO), 8.07 (1H, s, CHS), 8.09 (1H, CHS); δ_C (90.5 MHz, $CDCl_3$) 11.6 (q), 18.1 (q), 19.2 (q), 19.5 (q), 21.1 (q), 28.1 (q), 32 .7 (d), 43.0 (d), 48.7 (d), 55.9 (d), 80.2 (s), 121.1 (s), 124.6 (d), 128.3 (d), 146.7 (s), 148.4 (s), 154.2 (s), 155.2 (s), 160.8 (s), 161.2 (s), 161.6 (s), 163.5 (s), 173.2 (s), 175.9 (s); *m/z* (FAB) Found: 629.1823 (MH^++Na^+, $C_{26}H_{34}O_7N_6S_2Na$ requires 629.1828).

1'(*R*)-2-(1-{[1'(*R*)-2-(1-{[2-(1-Amino-ethyl)-thiazole-4-carbonyl]-amino}-ethyl)-5-methyl-oxazole-4-carbonyl]-amino}-1'(*R*)-2-methylpropyl)-thiazole-4-carboxylic acid hydrochloride (183)



The linear trimer **210** (400 mg, 0.67 mmol) was stirred with a 4M solution of hydrochloric acid in dioxane (3 ml) at room temperature for 2 h under an atmosphere of nitrogen. The reaction was flooded with diethyl ether and the precipitate collected by suction filtration to leave the amine (348 mg, 0.64 mmol, 96 %) as a white solid; mp. 72-74 °C (from dichloromethane); $[\alpha]_D^{299}$ +24.4 (c = 1.0, EtOH); δ_H (360 MHz, CD_3OD) 0.99 (3H, d, *J* 6.7 Hz, $(CH_3)_2CH$), 1.10 (3H, d, *J* 6.7 Hz, $(CH_3)_2CH$), 1.74 (3H, d, *J* 7.0 Hz, CH_3CH), 1.82 (3H, d, *J* 6.9 Hz, CH_3CH), 2.46-2.52 (1H, m, $CH(CH_3)_2$), 2.62 (3H, s, CH_3CO), 4.93-4.98 (1H, m, $CHCH_3$), 5.24 (1H, d, *J* 7.8 Hz, $CHCH(CH_3)_2$), 5.42 (1H, q, *J* 7.0 Hz, $CHCH_3$), 8.36 (1H, s, CHS), 8.42 (1H, s, CHS); δ_C (90.5 MHz, CD_3OD) 18.3 (q), 19.0 (q), 19.9 (q), 20.5 (q), 33.9 (q), 44.4 (d), 49.1 (d), 57.5 (d), 58.1 (d)127.5 (d), 129.3 (s), 129.6 (d), 147.4 (s), 149.6 (s), 153.3 (s), 161.7 (s), 162.6 (s), 163.1 (s), 163.3 (s), 168.6 (s), 173.7 (s); *m/z* (FAB) Found: 507.1451 (MH^+-Cl^-, $C_{21}H_{27}O_5N_6S_2$ requires 507.1484).

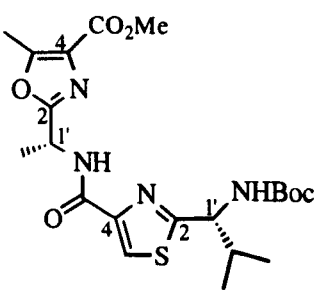
Dendroamide (14)



Diisopropylethylamine (0.15 ml, 0.84 mmol) was added to a stirred suspension of the linear trimer **183** (150 mg, 0.28 mmol) in anhydrous acetonitrile (6 ml) at room temperature under a nitrogen atmosphere. The resulting solution was stirred for 5 min, FDPP (160 mg, 0.42 mmol) was added and the reaction stirred at room temperature for a further 2 h. Water (*ca.* 20 ml) was added and the separated aqueous layer was extracted with dichloromethane (5 x 20 ml). The combined organic extracts were washed with 2M sodium hydroxide solution (3 x 20 ml), 2M hydrochloric acid solution (3 x 20

ml), and brine (2 x 20 ml), were dried ($MgSO_4$) and evaporated *in vacuo* to leave an orange residue which was purified by chromatography on silica using 80 % ethyl acetate in light petroleum (40-60 °C) as eluant to give dendroamide A (125 mg, 0.26 mmol, 91 %) as a white solid; mp. 146-148 °C (from dichloromethane) (Lit.[23] no mp. given); $[\alpha]_D^{296}$ +66.9 (c = 0.7, $CHCl_3$) (Lit.[23] $[\alpha]_D^{296}$ +40.5 (c = 3.5, CH_2Cl_2); λ_{max} (MeOH)/nm 224 (ε/dm^3 mol^{-1}cm^{-1} 279136); υ_{max} ($CHCl_3$)/cm^{-1} 3396, 2963, 2929, 1716, 1666, 1639, 1600, 1546, 1497, 1450, 1363, 1300, 1129, 1065, 976, 895; δ_H (360 MHz, $CDCl_3$) 0.99 (3H, d, *J* 6.8 Hz, $(CH_3)_2CH$), 1.08 (3H, d, *J* 6.8 Hz, $(CH_3)_2CH$), 1.71 (3H, d, *J* 6.8 Hz, CH_3CH), 1.74 (3H, d, *J* 6.8 Hz, CH_3CH), 2.28-2.37 (1H, m, $CH(CH_3)_2$), 2.68 (3H, s, CH_3CO), 5.21 (1H, app. quintet, *J* 6.4 Hz, $CHCH_3$), 5.32 (1H, dd, *J* 4.8, 8.2 Hz, $CHCH(CH_3)_2$), 5.72 (1H, dq, *J* 6.8, 8.2 Hz, $CHCH_3$), 8.14 (1H, s, CHS), 8.15 (1H, s, CHS), 8.49 (1H, d, *J* 8.2 Hz, NHCO), 8.56 (1H, d, *J* 8.2 Hz, NHCO), 8.65 (1H, d, *J* 6.4 Hz, NHCO); δ_C (125 MHz, CD_2Cl_2); 11.7 (q), 18.3 (q), 18.5 (q), 21.1 (q), 25.1 (q), 35.6 (d), 44.6 (d), 47.4 (d), 56.2 (d), 123.8 (d), 124.1 (d), 128.9 (s), 149.2 (s), 149.3 (s), 154.0 (s), 159.7 (s), 160.0 (s), 160.7 (s), 162.2 (s), 168.9 (s), 171.8 (s); *m/z* (FAB) Found: 511.1169 (MH^++Na^+, $C_{21}H_{24}O_4N_6S_2Na$ requires 511.1198).

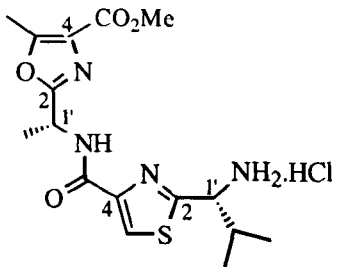
1'(*R*)-2-(1-{[1'(*R*)-2-(1-*tert*-Butoxycarbonylamino-2-methyl-propyl)-thiazole-4-carbonyl]-amino}-ethyl)-5-methyl-oxazole-4-carboxylic acid methyl ester (211)



N-Methylmorpholine (0.1 ml, 0.9 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (173 mg, 0.9 mmol) and *N*-hydroxybenzotriazole (122

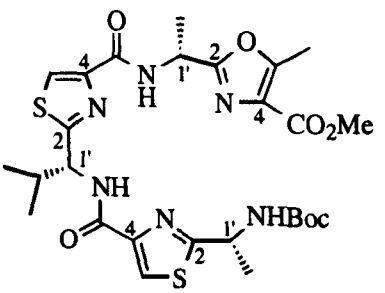
mg, 0.9 mmol) were added to a solution of the valine thiazole acid (235 mg, 0.82 mmol) in anhydrous dichloromethane (10 ml) at 0 °C under an atmosphere of nitrogen. The resulting solution was stirred at 0 °C for 15 min, and a precooled solution of the oxazole amine hydrobromide **178** (218 mg, 0.82 mmol) deprotonated with *N*-methylmorpholine (0.1 ml, 0.9 mmol) in DMF (2 ml) was added dropwise over 5 min. The resulting solution was stirred at 0 °C for 1 h, and at room temperature for 16 h. Water (*ca* 30 ml) was added and the separated aqueous layer was extracted with dichloromethane (4 x 20 ml), the combined organic extracts were washed with 10% aqueous citric acid solution (3 x 20 ml), saturated aqueous sodium bicarbonate solution (3 x 20 ml), were dried ($MgSO_4$) and evaporated *in vacuo*. Purification by chromatography on silica using 30 % ethyl acetate in light petroleum (40-60 °C) as eluant gave the tetrapeptide (244 mg, 0.52 mmol, 64 %) as a white foam; $[\alpha]_D^{295}$ = +19.6 (c = 1.0, $CHCl_3$); υ_{max} ($CHCl_3$)/cm^{-1} 3696, 3605, 3442, 3401, 2967, 2933, 2874, 1715, 1669, 1622, 1544, 1490, 1455, 1392, 1369, 1332, 1158, 1100, 983, 908, 870; δ_H (500 MHz, $CDCl_3$) 0.94 (3H, d, *J* 6.9 Hz, $(CH_3)_2CH$), 1.00 (3H, d, *J* 6.9 Hz, $(CH_3)_2CH$), 1.47 (9H, s, Bu^t), 1.70 (3H, d, *J* 7.1 Hz, CH_3CH), 2.34-2.41 (1H, m, $(CH(CH_3)_2)$), 2.63 (3H, s, CH_3CO), 3.91 (3H, s, CH_3OCO), 4.84-4.88 (1H, m, $CHCH(CH_3)_2$), 5.16 (1H, d, *J* 8.4 Hz, NHBoc), 5.46 (1H, dq, *J* 7.1, 8.6 Hz, $CHCH_3$), 7.69 (1H, d, *J* 8.6 Hz, NHCO), 8.04 (1H, s, CHS); δ_C (125 MHz, $CDCl_3$) 11.8 (q), 17.5 (q), 19.3 (q), 19.4 (q), 28.1 (q), 32.7 (d), 42.8 (q), 51.7 (d), 57.7 (d), 79.9 (s), 123.3 (d), 127.2 (s), 149.1 (s), 155.2 (s), 156.3 (s), 160.3 (s), 162.3 (s), 162.4 (s), 172.4 (s); *m/z* (FAB) Found: 489.1759 ($MH^+ + Na^+$, $C_{21}H_{31}O_6N_4SNa$ requires 489.1784);.

1'(*R*)-2-(1-{[1'(*R*)-2-(1-Amino-2-methyl-propyl)-thiazole-4-carbonyl]-amino)-5-methyl-oxazole-4-carboxylic acid methyl ester hydrochloride (212)



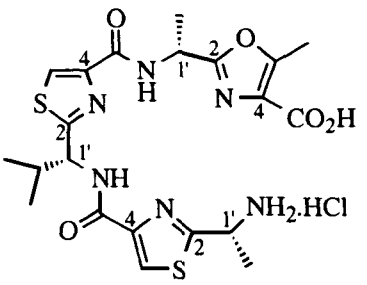
A 4M solution of hydrochloric acid in dioxane (2 ml) was added to the dimer **211** at room temperature under an atmosphere of nitrogen. The orange solution was stirred for 1 h and the dioxane removed *in vacuo* using toluene as an azeotrope to give the amine (225 mg, 0.5 mmol, 98 %) as a pale yellow foam: $[\alpha]_D^{299}$ = +2.4 (c = 0.5, EtOH); δ_H (360 MHz, CD_3OD) 1.07 (3H, d, *J* 6.4 Hz, $(CH_3)_2CH$), 1.18 (3H, d, *J* 6.4 Hz, $(CH_3)_2CH$), 1.73 (3H, d, *J* 6.9 Hz, CH_3CH), 2.45-2.48 (1H, m, $CH(CH_3)_2$), 2.64 (3H, s, CH_3CO), 3.91 (3H, s, CH_3OCO), 4.77-4.79 (1H, m, $CHCH_3$), 5.38-5.40 (1H, m, $CH(CH_3)_2$), 8.41 (1H, s, CHS), 8.75 (1H, br. s, NHCO); δ_C (90.5 MHz, CD_3OD) 10.1 (q), 16.5 (q), 16.7 (q), 16.9 (q), 31.5 (q), 42.6 (d), 50.4 (d), 56.8 (d), 125.3 (d), 126.1 (s), 147.7 (s), 156.0 (s), 160.1 (s), 161.7 (s), 162.0 (s), 164.1 (s); *m/z* (FAB) Found: 367.1459 (MH^++Na^+, $C_{16}H_{23}O_4N_4S$ requires 367.1440).

1'(*R*)-2-(1-{[1'(*R*)-2-(1-{[1'(*R*)-2-(1-*tert*-Butoxycarbonylamino-ethyl)-thiazole-4-carbonyl]-amino}-2-methyl-propyl)-thiazole-4-carbonyl]-amino}-ethyl)-5-methyl-oxazole-4-carboxylic acid methyl ester (213)



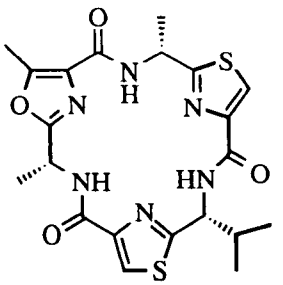
N-Methylmorpholine (0.06 ml, 0.57 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (110 mg, 0.57 mmol) and *N*-hydroxybenzotriazole (77 mg, 0.57 mmol) were added to a stirred solution of the thiazole acid **197** (142 mg, 0.52 mmol) in anhydrous dichloromethane (5 ml) at 0 °C under an atmosphere of nitrogen. The resulting solution was stirred for a further 20 min at 0 °C, and a precooled solution of the dimer **212** (210 mg, 0.52 mmol) deprotonated with *N*-methylmorpholine (0.06 ml, 0.57 mmol) in DMF (2 ml) was added dropwise over 5 min. The resulting solution was stirred for 1 h at 0 °C, and then at room temperature for 16 h. Water (*ca* 20 ml) was added and the separated aqueous layer extracted with dichloromethane (4 x 20 ml). The combined organic extracts were washed with 10% aqueous citric acid solution (3 x 10 ml), saturated aqueous sodium bicarbonate solution (3 x 10 ml), were dried ($MgSO_4$) and evaporated *in vacuo* . Purification by chromatography on silica using 60 % ethyl acetate in light petroleum (40-60 °C) as eluant gave the hexapeptide (207 mg, 0.32 mmol, 62 %) as a white foam which crystallised to give a white powder; mp. 78-84 °C (from dichloromethane); $[\alpha]_D^{297}$ = +6.4 (c = 0.5, $CHCl_3$); υ_{max} ($CHCl_3$)/cm^{-1} 3401, 2970, 1716, 1672, 1622, 1544, 1391, 1369, 1352, 1153, 1099, 1057; δ_H (360 MHz, $CDCl_3$) 1.05 (6H, d, *J* 6.4 Hz, $(CH_3)_2CH$), 1.46 (9H, s, Bu^t), 1.63 (3H, d, *J* 6.5 Hz, CH_3CH), 1.70 (3H, d, *J* 7.1 Hz, CH_3CH), 2.52-2.62 (1H, m, $CH(CH_3)_2$), 2.62 (3H, s, CH_3CO), 3.90 (3H, s, CH_3OCO), 5.11 (1H, br. s, NHBoc), 5.11 (1H, br. s, $CHCH_3$), 5.33 (1H, dd, *J* 6.4, 9.1 Hz, $CHCH(CH_3)_2$), 5.41-5.49 (1H, m, $CHCH_3$), 7.71 (1H, d, *J* 8.3 Hz, NHCO), 7.83 (1H, d, *J* 9.1 Hz, NHCO), 8.04 (1H, s, CHS), 8.06 (1H, s, CHS); δ_C (90.5 MHz, $CDCl_3$) 11.8 (q), 17.7 (q), 19.4 (q), 19.5 (q), 28.1 (d), 32.6 (d), 42.8 (d), 51.7 (q), 56.1 (d), 79.9 (s), 123.5 (d), 123.7 (d), 127.2 (s), 148.8 (s), 149.1 (s), 154.8 (s), 154.9 (s), 156.3 (s), 160.2 (s), 160.6 (s), 162.3 (s), 171.4 (s), 175.0 (s); *m/z* (FAB) Found: 643.1958 (MH^++Na^+, $C_{27}H_{36}O_7N_6S_2Na$ requires 643.1985);

1'(*R*)-2-(1-{[1'(*R*)-2-(1-{[1'(*R*)-(1-Amino-ethyl)-thiazole-4-carbonyl]-amino}-2-methyl-propyl)-thiazole-4-carbonyl]-amino}-ethyl)-5-methyl-oxazole-4-carboxylic acid hydrochloride (214)



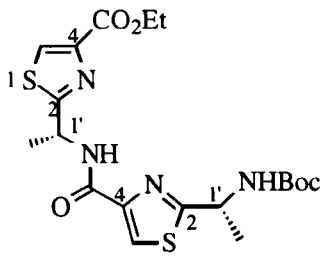
Sodium hydroxide (51 mg, 1.28 mmol) was added to a solution of the trimer **213** (207 mg, 0.32 mmol) in a THF:H_2O (5:4) mixture (4 ml) at room temperature. The solution was stirred for 5 h, the separated aqueous layer was acidified to pH4 by the addition of citric acid and extracted with dichloromethane (4 x 15 ml). The combined organic extracts were washed with water (2 x 10 ml) and brine (2 x 10 ml), were dried ($MgSO_4$) and evaporated *in vacuo* to leave a yellow oil. This yellow oil was subsequently stirred with a 4M solution of hydrochloric acid in dioxane (4 ml) for 2 h at room temperature under an atmosphere of nitrogen. The dioxane was removed *in vacuo* using toluene as an azeotrope to give the hexapeptide (115mg, 0.26 mmol, 82 %) as a hygroscopic cream solid; $[\alpha]_D^{297}$ = -129.1 (c = 0.37, EtOH); δ_H (360 MHz, CD_3OD) 1.05 (3H, d, *J* 6.5 Hz, $(CH_3)_2CH$), 1.14 (3H, d, *J* 6.5 Hz, $(CH_3)_2CH$), 1.72 (3H, d, *J* 6.9 Hz, CH_3CH), 1.84 (3H, d, *J* 6.8 Hz, CH_3CH), 2.46-2.60 (1H, m, $CH(CH_3)_2$), 2.65 (3H, s, CH_3CO), 4.95-5.03 (1H, m, $CHCH_3$), 5.22-5.25 (1H, m, $CHCH(CH_3)_2$), 5.38-5.42 (1H, m, $CHCH_3$), 8.25 (1H, s, CHS), 8.41 (1H, s, CHS); *m/z* (FAB) Found: 507.1455 (M^+-Cl^-, $C_{21}H_{27}O_5N_6S_2$ requires 507.1484).

Structural isomer of dendroamide A (171)



Diisopropylethylamine (0.1 ml, 0.6 mmol) and FDPP (115 mg, 0.3 mmol) were added to a suspension of **214** (100 mg, 0.3 mmol) in anhydrous acetonitrile (3 ml) at room temperature under an atmosphere of nitrogen. The solution was stirred for 14 h at room temperature and water (*ca* 20 ml) was added, the separated aqueous layer was extracted with dichloromethane (5 x 10 ml), the combined organic extracts were then washed with 2M hydrochloric acid solution (3 x 10 ml) and aqueous sodium hydrogen carbonate solution (3 x 10 ml), were dried ($MgSO_4$), and evaporated *in vacuo* to leave a white solid residue. The residue was chromatographed using 70 % ethyl acetate in light petroleum (40-60 °C) as eluant to give the *cyclic peptide* (68 mg, 0.14 mmol, 76 %) as a white powder; mp. 145-147 °C (from dichloromethane); $[\alpha]_D^{295}$ = +61.1 (c = 0.7, $CHCl_3$); υ_{max} ($CHCl_3$)/cm^{-1} 3395, 2969, 1687, 1640, 1546, 1495, 1449, 1360, 1131, 1044, 883; δ_H (360 MHz, $CDCl_3$) 1.02 (3H, d, *J* 6.8 Hz, $(CH_3)_2CH$), 1.09 (3H, d, *J* 6.8 Hz, $(CH_3)_2CH$), 1.69 (3H, d, *J* 6.8 Hz, CH_3CH), 1.73 (3H, d, *J* 6.8 Hz, CH_3CH), 2.29 (1H, dq, *J* 6.8, 13.5 Hz, $CH(CH_3)_2$), 2.69 (3H, s, CH_3CO), 5.24-5.31 (1H, m, $CHCH_3$), 5.39-5.49 (1H, m, $CHCH_3$), 5.39-5.49 (1H, m, $CHCH(CH_3)_2$), 8.11 (1H, s, CHS), 8.15 (1H, s, CHS), 8.44 (1H, d, *J* 9.1 Hz, NHCO), 8.60-8.65 (1H, m, NHCO); δ_C (90.5 MHz, $CDCl_3$) 11.6 (q), 18.5 (q), 18.8 (q), 20.8 (q), 24.9 (q), 35.5 (d), 43.9 (d), 47.5 (d), 55.7 (d), 123.2 (d), 124.2 (d), 131.6 (s), 148.6 (s), 149.2 (s), 153.9 (s), 159.6 (s), 159.8 (s), 160.5 (s), 161.6 (s), 168.3 (s), 171.3 (s); *m/z* (FAB) Found: 511.1231 (M^++Na^+, $C_{21}H_{24}O_4N_6S_2Na$ requires 511.1198);

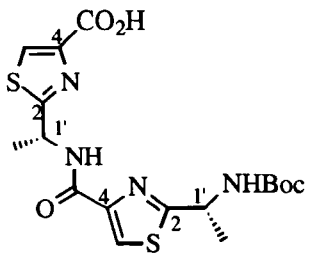
1'(*R*)-2-(1-{[1'(*R*)-2-(1'-*tert*-Butoxycarbonylamino-ethyl)-thiazole-4-carbonyl]-amino}-ethyl)-thiazole-4-carboxylic acid ethyl ester (215)



N-Methylmorpholine (0.07 ml, 0.66 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (127 mg, 0.66 mmol) and *N*-hydroxybenzotriazole (89 mg, 0.66 mmol) were added to a stirred solution of the alanine thiazole acid **197** (163 mg, 0.6 mmol) in anhydrous dichloromethane (7 ml) at 0 °C under an atmosphere of nitrogen. The mixture was stirred at 0 °C for 20 min and a precooled solution of the alanine thiazole amine **89a** (142 mg, 0.6 mmol) deprotonated with *N*-methylmorpholine (0.07 ml, 0.66 mmol) in DMF (1 ml) was added dropwise over 5 min. The solution was stirred at 0 °C for 1 h, and at room temperature for 17 h. 10 % aqueous citric acid solution (20 ml) was added, and the separated aqueous layer was extracted with ethyl acetate (5 x 20 ml). The combined organic extracts were washed with sodium hydrogen carbonate solution (3 x 15 ml), 10 % aqueous citric acid solution (3 x 15 ml), and brine (2 x 15 ml), were dried ($MgSO_4$) and evaporated *in vacuo* to leave a yellow residue. Purification by chromatography on silica using 50 % ethyl acetate in light petroleum (40-60 °C) as eluant gave the tetrapeptide (119 mg, 0.3 mmol, 50 %) as a pale yellow foam; $[\alpha]_D^{296}$ +30.0 (c = 1.0, $CHCl_3$); υ_{max} ($CHCl_3$)/cm^{-1} 3442, 3398, 2981, 2935, 1716, 1668, 1543, 1489, 1453, 1369, 1322, 1154, 1098, 1060, 908, 877; δ_H (360 MHz, $CDCl_3$) 1.41 (3H, t, *J* 7.1 Hz, $CH_3CH_2CO_2$), 1.47 (9H, s, Bu^t), 1.62 (3H, d, *J* 6.6 Hz, CH_3CH), 1.81 (3H, d, *J* 7.0 Hz, CH_3CH), 4.43 (2H, q, *J* 7.1 Hz, $CH_3CH_2CO_2$), 5.61 (1H, br. s, NHBoc), 5.61 (1H, br. s, $CHCH_3$), 5.62 (1H, dq, *J* 7.0, 7.0 Hz, $CHCH_3$), 7.85 (1H, d, *J* 7.9 Hz, NHCO), 8.05 (1H, s, CHS), 8.10 (1H, s, CHS); δ_C (90.5 MHz, $CDCl_3$) 14.2 (q), 20.8 (q), 21.2 (q), 28.2 (q), 47.0 (d), 48.6 (d), 61.3 (t), 80.1 (s), 123.8

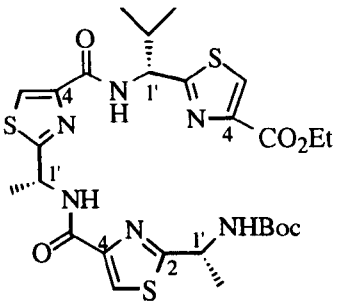
(d), 127.3 (d), 146.9 (s), 148.8 (s), 154.8 (s), 160.4 (s), 161.1 (s), 172.9 (s), 174.6 (s); *m/z* (FAB) Found: 477.1200 (MH^++Na^+, $C_{19}H_{26}O_5N_4S_2Na$ requires 477.1242).

1'(*R*)-2-(1-{[1'(*R*)-2-(1'-*tert*-Butoxycarbonylamino-ethyl)-thiazole-4-carbonyl]-amino}-ethyl)-thiazole-4-carboxylic acid (216)



Sodium hydroxide (96 mg, 2.4 mmol) was added in one portion to a solution of the dimer **215** (119 mg, 0.3 mmol) in a THF:H_2O (6:3) mixture (5 ml) at room temperature. The solution was stirred at room temperature for 6 h, the separated aqueous layer was then acidified to pH4 by the addition of citric acid. The separated aqueous layer was extracted with dichloromethane (5 x 20 ml) and the combined organic extracts were washed with water (3 x 20 ml), and brine (3 x 20 ml), were dried ($MgSO_4$) and evaporated *in vacuo* to give the acid (100 mg, 0.25 mmol, 84 %) as a pale yellow foam; $[\alpha]_D^{294}$ +30.4 (c = 1.0, $CHCl_3$); υ_{max} ($CHCl_3$)/cm^{-1} 3440, 3398, 3125, 2933, 2873, 1758, 1712, 1668, 1544, 1489, 1454, 1369, 1295, 1061, 997, 897; δ_H (360 MHz, $CDCl_3$) 1.46 (9H, s, Bu^t), 1.62 (3H, d, *J* 6.9 Hz, C*H*$_3$CH), 1.81 (3H, d, *J* 6.9 Hz, C*H*$_3$CH), 5.09 (2H, br. d, *J* 6.9, C*H*CH_3 and C*H*CH_3), 7.87 (1H, br. s, N*H*CO), 8.09 (1H, s, C*H*S), 8.10 (1H, s, C*H*S); δ_C (90.5 MHz, $CDCl_3$) 20.7 (q), 21.4 (q), 28.2 (q), 46.9 (d), 47.1 (d), 80.3 (s), 124.2 (d), 128.7 (d), 146.5 (s), 148.6 (s), 160.7 (s), 164.0 (s), 173.1 (s), 174.2 (s),176.4 (s); *m/z* (FAB) Found: 449.0915 (MH^+ + Na^+, $C_{17}H_{22}O_5N_4S_2Na$ requires 449.0929).

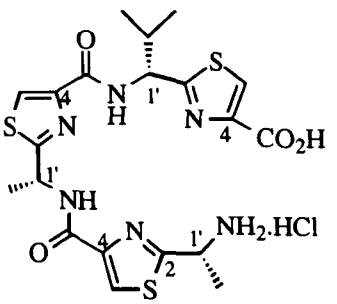
1'(*R*)-2-(1-{[1'(*R*)-2-(1-{[1'-(1'-*tert*-Butoxycarbonylamino-ethyl)-thiazole-4-carbonyl]-amino}-ethyl)-thiazole-4-carbonyl]-amino}-1'(*R*)-2-methylpropyl)-thiazole-4-carboxylic acid ethyl ester (217)



N-Methylmorpholine (0.03 ml, 0.27 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (52 mg, 0.27 mmol) and *N*-hydroxybenzotriazole (37 mg, 0.27 mmol) were added to a solution of the dimer acid **216** (94 mg, 0.24 mmol) in anhydrous dichloromethane (4 ml) at 0 °C under an atmosphere of nitrogen. The mixture was stirred at 0 °C for 20 min and a precooled solution of the valine thiazole amine (64 mg, 0.24 mmol) deprotonated with *N*-methylmorpholine (0.03 ml, 0.27 mmol) in DMF (1 ml) was added dropwise over 5 min. The solution was stirred at 0 °C for 1 h, and then at room temperature for 15 h. 10 % aqueous citric acid solution (20 ml) was added, and the separated aqueous layer was extracted with ethyl acetate (5 x 20 ml), the combined organic extracts were washed with sodium bicarbonate solution (3 x 20 ml), 10 % aqueous citric acid solution (3 x 20 ml), and brine (2 x 20 ml), were dried ($MgSO_4$) and evaporated *in vacuo* to leave a residue. Purification by chromatography on silica using 50 % ethyl acetate in light petroleum (40-60 °C) as eluant gave the hexapeptide (97 mg, 0.15 mmol, 63 %) as a pale yellow foam; $[\alpha]_D^{293}$ = +28.0 (c = 2.0, $CHCl_3$); υ_{max} ($CHCl_3$)/cm^{-1} 3399, 2987, 2939, 2838, 1716, 1668, 1542, 1489, 1370, 1322, 1161, 1098, 1016; δ_H (360 MHz, $CDCl_3$) 1.01 (3H, d, *J* 6.8 Hz, $(CH_3)_2CH$), 1.05 (3H, d, *J* 6.8 Hz, $(CH_3)_2CH$), 1.40 (3H, t, *J* 7.1 Hz, CH_3CH_2), 1.45 (9H, s, Bu^t), 1.62 (3H, d, *J* 6.8 Hz, CH_3CH), 1.80 (3H, d, *J* 6.9 Hz, CH_3CH), 2.65 (1H, app. quintet, *J* 6.8Hz, $CH(CH_3)_2$), 4.42 (2H, q, *J* 7.1 Hz, CH_2CH_3), 5.06-5.10 (1H, m,

C*H*CH_3), 5.10-5.18 (1H, br. s, N*H*Boc), 5.34 (1H, dd, *J* 6.8, 9.2 Hz, C*H*$CH(CH_3)_2$), 5.59 (1H, dq, *J* 6.9, 6.9 Hz, C*H*CH_3), 7.79 (1H, d, *J* 8.4 Hz, N*H*CO), 7.97 (1H, d, *J* 9.3 Hz, N*H*CO), 8.06 (1H, s, C*H*S), 8.07 (1H, s, C*H*S), 8.08 (1H, s, C*H*S); δ_C (90.5 MHz, $CDCl_3$) 14.2 (q), 17.9 (q), 19.6 (q), 21.0 (q), 21.2 (q), 28.2 (q), 32.9 (d), 47.0 (d), 56.4 (d), 61.3 (t), 80.2 (s), 123.9 (d), 124.0 (d), 126.9 (d), 147.3 (s), 148.9 (s), 149.0 (s), 160.4 (s), 160.6 (s), 161.2 (s), 171.6 (s), 172.9 (s), 175.0 (s), 179.8 (s); *m/z* (FAB) Found: 659.1773 ($MH^+ + Na^+$, $C_{27}H_{36}O_6N_6S_3Na$ requires 659.1756).

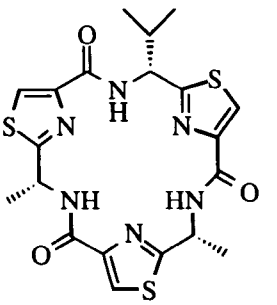
1'(*R*)-2-(1-{[1'(*R*)-2-(1-{[2-(1'-Amino-ethyl)-thiazole-4-carbonyl]-amino}-ethyl)-thiazole-4-carbonyl]-amino}-1'(*R*)-2-methyl-propyl)-thiazole-4-carboxylic acid hydrochloride (218)



Sodium hydroxide (48 mg, 1.2 mmol) was added in one portion to a solution of **217** (97 mg, 0.15 mmol) in a THF:H_2O (5:4) mixture (3 ml) at room temperature. The solution was stirred for 5 h, and the separated aqueous layer was acidified to pH4 by the addition of citric acid, and extracted with ethyl acetate (6 x 15 ml). The combined organic extracts were washed with water (3 x 10 ml) and brine (3 x 10 ml), were dried ($MgSO_4$) and evaporated *in vacuo*. The yellow oil was subsequently stirred with a 4M solution of hydrochloric acid in dioxane (1 ml) for 4 h at room temperature under an atmosphere of nitrogen. The solvent was removed *in vacuo* by azeotroping with toluene to leave the hexapeptide (63 mg, 0.11 mmol, 73 %) as a viscous oil; $[\alpha]_D^{295}$ = +2.0 (c = 0.8, EtOH); δ_H (360 MHz, CD_3OD) 1.01 (3H, d, *J* 7.7 Hz, C*H*$_3$CH), 1.13 (3H, d, *J* 6.7 Hz, C*H*$_3$CH), 1.83 (3H, d, *J* 6.9 Hz, (CH_3)$_2$CH), 1.84 (3H, d, *J* 6.9 Hz,

(CH_3)$_2$CH), 2.50-2.60 (1H, m, C*H*(CH_3)$_2$), 4.99-5.13 (1H, m, C*H*CH_3), 5.28 (1H, d, *J* 8.0 Hz, C*H*CH(CH_3)$_2$), 5.62-5.65 (1H, m, C*H*CH_3), 8.25 (1H, s, C*H*S), 8.37 (1H, s, C*H*S), 8.43 (1H, s, C*H*S); δ_C (90.5 MHz, CD_3OD) 19.1 (q), 20.0 (q), 20.3 (q), 21.1 (q), 34.3 (d), 43.8 (d), 58.3 (d), 74.1 (d), 125.9 (d), 127.4 (d), 129.4 (d), 148.0 (s), 149.8 (s), 149.9 (s), 162.3 (s), 162.9 (s), 163.9 (s), 168.9 (s), 173.4 (s), 176.8 (s); *m/z* (FAB) Found: 509.1102 (MH^+-Cl^-, $C_{20}H_{25}O_4N_6S_3$ requires 509.1099).

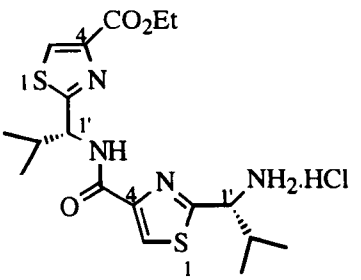
Cyclic-*bis*-(*R*)-Alanine-thiazole-(*R*)-valine-thiazole (172)



Diisopropylethylamine (0.03 ml, 0.17 mmol) was added to a solution of **218** (30 mg, 0.055 mmol) in anhydrous acetonitrile (3 ml) at room temperature under an atmosphere of nitrogen. The solution was stirred for 5 min and FDPP (32 mg, 0.08 mmol) was added in one portion. The resulting mixture was stirred for 3 days at room temperature, water (*ca* 10 ml) was added and the separated aqueous layer extracted with dichloromethane (5 x 15 ml). The combined organic extracts were washed with 2M hydrochloric acid solution (3 x 10 ml), 2M sodium hydroxide solution (3 x 10 ml) and brine (2 x 10 ml), were dried ($MgSO_4$) and evaporated *in vacuo* to leave a yellow residue. The residue was purified by chromatography on silica using 80 % ethyl acetate in light petroleum (40-60 °C) as eluant to give the *cyclic peptide* (22 mg, 0.041 mmol, 82 %) as a white powder; mp. 254-256 °C (from dichloromethane); $[\alpha]_D^{295}$ = +36.0 (c = 0.4, $CHCl_3$); λ_{max} (MeOH)/nm 231 (e/dm^3 mol^{-1}cm^{-1} 19510); υ_{max} ($CHCl_3$)/cm^{-1} 3403, 2930, 1665, 1544, 1496, 1298, 1047, 974; δ_H (360 MHz, $CDCl_3$) 1.04 (3H, d, *J*

6.8 Hz, $(CH_3)_2CH$), 1.09 (3H, d, *J* 6.8 Hz, $(CH_3)_2CH$), 1.75 (3H, d, *J* 6.8 Hz, CH_3CH), 1.75 (3H, d, *J* 6.7 Hz, CH_3CH), 2.26-2.35 (1H, m, $CH(CH_3)_2$), 5.46 (1H, dd, *J* 5.6, 9.1 Hz, $CHCH(CH_3)_2$), 5.58-5.71 (2H, m, $CHCH_3$ and $CHCH_3$), 8.13 (1H, s, CHS), 8.13 (1H, s, CHS), 8.17 (1H, s, CHS), 8.53 (1H, d, *J* 9.1 Hz, NHCO), 8.64 (2H, d, *J* 7.8 Hz, NHCO and NHCO); δ_C (125 MHz, $CDCl_3$); 18.4 (q), 18.8 (q), 24.9 (q), 29.7 (q), 35.5 (d), 47.2 (d), 47.3 (d), 55.8 (d), 123.6 (d), 123.8 (d), 124.1 (d), 148.7 (s), 149.0 (s), 149.1 (s), 159.5 (s), 159.6 (s), 159.7 (s), 168.6 (s), 171.0 (s), 171.4 (s); *m/z* (FAB) Found: 513.0890 ($MH^+ + Na^+$, $C_{20}H_{22}O_3N_6S_3Na$ requires 513.0813).

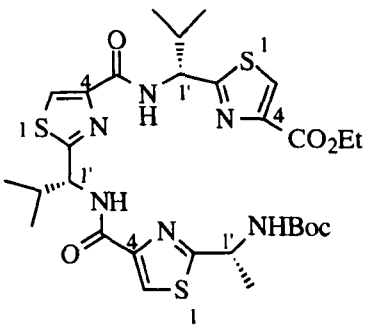
1'(*R*)-2-(1'-{[1'(*R*)-2-(1,2-Dimethylpropyl)-thiazole-4-carbonyl]-amino-}-2-methylpropyl)-thiazole-4-carboxylic acid ethyl ester (219)



A 4M solution of hydrochloric acid in dioxane (1 ml) was added to the dimer (117 mg, 0.26 mmol) at room temperature and the solution was stirred for 8 h under an atmosphere of nitrogen. The solvent was removed *in vacuo* using toluene as an azeotrope to leave the amine (93 mg, 0.24 mmol, 93 %) as a viscous oil; $[\alpha]_D^{296}$ +5.6 (c = 1.5, EtOH); δ_H (360 MHz, CD_3OD) 1.02-1.04 (3H, m, $CH(CH_3)_2$), 1.06-1.10 (3H, m, $CH(CH_3)_2$), 1.12 (3H, d, *J* 6.8 Hz, $CH(CH_3)_2$), 1.16-1.19 (3H, m, $CH(CH_3)_2$), 1.43 (3H, t, *J* 7.1 Hz, $CH_3CH_2CO_2$), 2.41-2.49 (1H, m, $CH(CH_3)_2$), 2.41-2.49 (1H, m, $CH(CH_3)_2$), 4.43 (2H, q, *J* 7.1 Hz, $CO_2CH_2CH_3$), 4.78 (1H, dd, *J* 2.4, 6.2 Hz, $CHCH(CH_3)_2$), 5.33 (1H, dd, *J* 4.7, 7.5 Hz, $CHCH(CH_3)_2$, 8.42 (2H, s, CHS and CHS); δ_C (90.5 MHz, CD_3OD) 14.6 (q), 18.4 (q), 18.6 (q), 19.1 (q), 19.8 (q), 33.6 (d), 34.4 (d), 58.3 (d), 58.6 (d), 62.8 (t), 127.4 (d), 129.8 (d), 146.4 (s), 149.7 (s), 162.0 (s),

162.4 (s), 166.3 (s), 174.4 (s); *m/z* (FAB) Found: 411.155 ($MH^+ + Na^+$, $C_{18}H_{27}ON_4S_2N$ requires 411.1525).

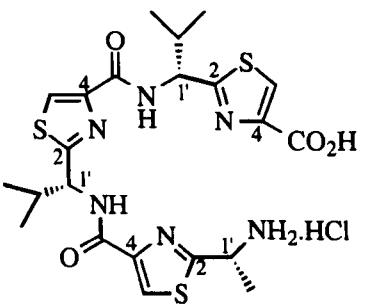
1'(*R*)-2-(1'-{[1'(*R*)-2-(1-{[1'(*R*)-2-(1-*tert*-Butoxycarbonylamino-ethyl)-thiazole-4-carbonyl]-amino}-2-methylpropyl)-thiazole-4-carbonyl]-amino}-2-methylpropyl)-thiazole-4-carboxylic acid ethyl ester (220)



N-Methylmorpholine (0.03 ml, 0.22 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride 942 mg, 0.22 mmol) and *N*-hydroxybenzotriazole (30 mg, 0.22 mmol) were added to a stirred solution of the alanine thiazole acid **197** (55 mg, 0.2 mmol) in anhydrous dichloromethane (4 ml) at 0 °C under an atmosphere of nitrogen. The resulting mixture was stirred at 0 °C for 20 min and a precooled solution of the valine thiazole dimer **219** (77 mg, 0.2 mmol) deprotected with *N*-methylmorpholine (0.024 ml, 0.22 mmol) in DMF (1 ml) was added dropwise over 5 min. The mixture was stirred at 0 °C for 1 h, and at room temperature for 14 h. 10 % aqueous citric acid solution (20 ml) was added, and the separated aqueous layer was extracted with ethyl acetate (5 x 20 ml). The combined organic extracts were washed with sodium bicarbonate solution (3 x 20 ml), 10 % aqueous citric acid solution (3 x 20 ml), and brine (2 x 20 ml), dried ($MgSO_4$) and evaporated *in vacuo* to leave a residue. Purification by chromatography on silica using 50 % ethyl acetate in light petroleum (40-60 °C) as eluant gave the hexapeptide (97 mg, 0.15 mmol, 63 %) as a pale yellow foam; $[\alpha]_D^{294}$ +31.3 (c = 0.6, $CHCl_3$); υ_{max} ($CHCl_3$)/cm^{-1} 3398, 3124, 2967, 2992,

2874, 1721, 1682, 1666, 1545, 1488, 1462, 1392, 1370, 1321, 1153, 1098, 1057, 908, 878; δ_H (360 MHz, $CDCl_3$) 1.02 (3H, d, *J* 6.8 Hz, $(CH_3)_2CH$), 1.05 (3H, d, *J* 6.8 Hz, $(CH_3)_2CH$), 1.06 (3H, d, *J* 6.8 Hz, $(CH_3)_2CH$), 1.08 (3H, d, *J* 6.8 Hz, $(CH_3)_2CH$), 1.40 (3H, t, *J* 7.1 Hz, $CH_3CH_2CO_2$), 1.47 (9H, s, Bu^t), 1.64 (3H, d, *J* 6.7 Hz, CH_3CH), 2.53-2.69 (2H, m, $CH(CH_3)_2$, and $CH(CH_3)_2$) 4.42 (2H, q, *J* 7.1 Hz, CH_2CH_3), 5.02-5.12 (1H, m, $CHCH_3$), 5.13-5.18 (1H, br. s, NHBoc), 5.35 (1H, dd, *J* 6.0, 11.8 Hz, $CHCH(CH_3)_2$), 5.37 (1H, dd, *J* 6.0, 11.8 Hz, $CHCH(CH_3)_2$), 7.89 (1H, d, *J* 8.9 Hz, NHCO), 7.94 (1H, d, *J* 9.2 Hz, NHCO), 8.05 (1H, s, CHS), 8.07 (1H, s, CHS), 8.07 (1H, s, CHS); δ_C (90.5 MHz, $CDCl_3$) 14.3 (q), 17.7 (q), 17.9 (q), 19.4 (q), 19.6 (q), 21.0 (q), 28.2 (q), 29.6 (d), 32.9 (d), 33.1 (d), 56.1 (d), 56.4 (d), 61.3 (t), 80.3 (s), 123.6(d), 123.8 (d), 126.9 (d), 147.4 (s), 149.1 (s), 149.3 (s), 154.9 (s), 160.7 (s), 160.8 (s), 161.2 (s), 171.6 (s), 171.7 (s), 175.1 (s); *m/z* (FAB) Found: 687.2012 (MH^+ + Na^+, $C_{29}H_{40}O_6N_6S_3Na$ requires 687.2069).

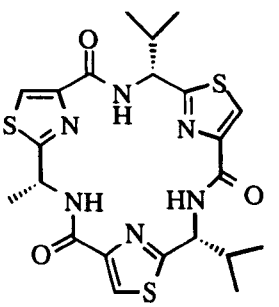
1'(*R*)-2-(1'-{[1'(*R*)-2-(1-{[2-(1-Amino-ethyl)-thiazole-4-carbonyl]-amino}2-methylpropyl)-thiazole-4-carbonyl]-amino}-1'(*R*)-2-methylpropyl)-thiazole-4-carboxylic acid (221)



Sodium hydroxide (51 mg, 1.28 mmol) was added in one portion to a stirred solution of **220** (105 mg, 0.16 mmol) in a THF:H_2O (5:4) mixture (3 ml) at room temperature. The solution was stirred for 9 h, and the separated aqueous layer acidified to pH4 by the addition of citric acid, the acidified aqueous layer was then extracted with ethyl acetate (5 x 20 ml). The combined organic extracts were washed with water (3 x 10 ml) and

brine (3 x 10 ml), were dried ($MgSO_4$) and evaporated *in vacuo*. The yellow oil was subsequently stirred with a 4M solution of hydrochloric acid in dioxane (1 ml) at room temperature under an atmosphere of nitrogen for 3 h. The solvent was removed *in vacuo* by azeotroping with toluene to leave the amine (52 mg, 0.09 mmol, 56 %) as a viscous oil; $[\alpha]_D^{295}$ +8.4 (c = 1.0, EtOH); δ_H (360 MHz, CD_3OD) 1.01 (3H, d, *J* 6.7 Hz, $(CH_3)_2CH$), 1.08 (3H, d, *J* 6.7 Hz, $(CH_3)_2CH$), 1.12-1.15 (6H, m, $(CH_3)_2CH$), 1.82 (3H, d, *J* 6.9 Hz, CH_3CH), 2.50-2.63 (1H, m, $CH(CH_3)_2$), 2.50-2.63 (1H, m, $CH(CH_3)_2$), 5.00-5.06 (1H, m, $CHCH_3$), 5.28-5.39 (1H, m, $CHCH(CH_3)_2$), 5.28-5.39 (1H, m, $CHCH(CH_3)_2$), 8.27 (1H, s, CHS), 8.27 (1H, s, CHS), 8.36 (1H, s, CHS); δ_C (90.5 MHz, CD_3OD) 18.8(q), 19.1 (q), 19.9 (q), 19.9 (q), 20.3 (q), 34.4 (d), 34.5 (d), 34.5 (d), 58.1 (d), 58.3 (d), 125.8 (d), 127.5 (d), 129.5 (d), 147.2 (s), 149.9 (s), 150.0 (s), 162.4 (s), 162.8 (s), 167.2 (s), 169.1 (s), 172.4 (s), 172.6 (s).

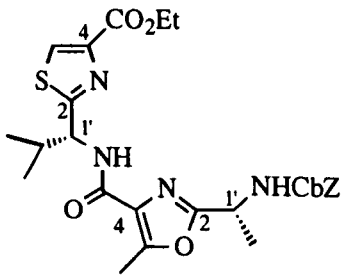
Cyclic-*bis*-(*R*)-Valine-thiazole-(*R*)-alanine-thiazole (173)



Diisopropylethylamine (0.04 ml, 0.26 mmol) and FDPP (50 mg, 0.13 mmol) were added to a suspension of **221** (50 mg, 0.09 mmol) in anhydrous acetonitrile (3 ml) at room temperature under an atmosphere of nitrogen. This solution was stirred at room temperature for 2 days, water (*ca* 10 ml) was added and the separated aqueous layer was then extracted with dichloromethane (4 x 20 ml). The combined organic extracts were washed successively with 2M hydrochloric acid solution (2 x 10 ml), sodium hydrogen carbonate solution (2 x 10 ml) and brine (2 x 10 ml), then dried ($MgSO_4$) and

evaporated *in vacuo* to leave a solid residue. The residue was purified by chromatography using 80 % ethyl acetate in light petroleum (40-60 °C) as eluant to give the *cyclic peptide* (37 mg, 0.07 mmol, 79 %) as a white powder; $[\alpha]_D^{294}$ +34.0 (c = 0.2, $CHCl_3$); υ_{max} ($CHCl_3$)/cm^{-1} 32928, 2926, 1666, 1544, 1045; δ_H (360 MHz, $CDCl_3$) 1.04 (3H, d, *J* 6.8 Hz, $(CH_3)_2CH$), 1.06 (3H, d, *J* 6.8 Hz, $(CH_3)_2CH$), 1.12 (3H, d, *J* 6.8 Hz, $(CH_3)_2CH$), 1.75 (3H, d, *J* 6.8 Hz, (CH_3CH), 2.24-2.37 (1H, m, $CH(CH_3)_2$), 2.24-2.37 (1H, m, $CH(CH_3)_2$), 5.38-5.42 (1H, m, $CH(CH_3)_2$), 5.40 (1H, dd, *J* 6.1, 8.9 Hz, $CHCH(CH_3)_2$), 5.51 (1H, dd, *J* 5.3, 9.4 Hz, $CHCH(CH_3)_2$), 5.61-5.69 (1H, m, $CHCH_3$), 8.10 (1H, s, CHS), 8.13 (1H, s, CHS), 8.13 (1H, s, CHS), 8.47 (1H, d, *J* 8.9 Hz, NHCO), 8.51 (1H, d, *J* 9.4 Hz, NHCO), 8.59 (1H, d, *J* 7.6 Hz, NHCO); δ_C (90.5 MHz, $CDCl_3$) 20.3 (q), 27.3 (q), 28.6 (q), 28.8 (q), 29.7 (q), 30.3 (d), 30.7 (d), 35.2 (d), 35.3 (d), 36.0 (d), 123.4 (d), 128.6 (d), 130.5 (d), 136.3 (s), 144.2 (s), 149.5 (s), 155.7 (s), 159.8 (s), 159.9 (s), 169.1 (s), 177.0 (s), 180.9 (s); *m/z* (FAB) Found: 541.111 ($MH^+ + Na^+$, $C_{22}H_{26}O_3N_6S_3Na$ requires 541.1126).

1'(*R*)-2-(1-{[1'(*R*)-2-(1-Benzyloxycarbonylamino-ethyl)-5-methyl-oxazole-4-carbonyl]-amino}-2-methyl-propyl)-thiazole-4-carboxylic acid ethyl ester (222)

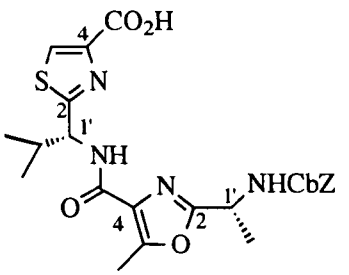


N-Methylmorpholine (0.08 ml, 0.73 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (139 mg, 0.73 mmol) and *N*-hydroxybenzotriazole (99 mg, 0.73 mmol) were added to a solution of the oxazole acid (200 mg, 0.66 mmol) in anhydrous dichloromethane (7 ml) at 0 °C under an atmosphere of nitrogen. This solution was stirred at 0 °C for 20 min and a precooled solution of the valine thiazole

amine (175 mg, 0.66 mmol) deprotonated with *N*-methylmorpholine (0.08 ml, 0.73 mmol) in anhydrous DMF (3 ml) was added dropwise over 5 min. The resulting solution was stirred at 0 °C for 1 h and at room temperature for 15 h.

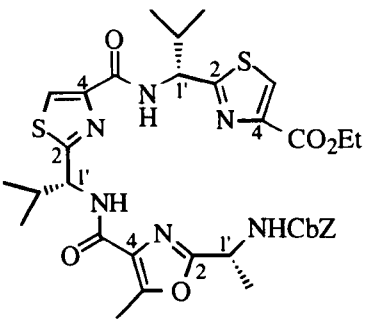
Water (*ca* 30 ml) was added and the separated aqueous layer was extracted with dichloromethane (4 x 25 ml). The combined organic extracts were washed with 10 % aqueous citric acid solution (3 x 20 ml), saturated sodium hydrogen carbonate solution (3 x 20 ml) and brine (2 x 20 ml), were dried ($MgSO_4$) and evaporated *in vacuo.* The yellow residue was purified by chromatography on silica using 60 % ethyl acetate in light petroleum (40-60 °C) as eluant to give the tetrapeptide (260 mg, 0.51 mmol, 77 %) as a cream foam which crystallised to give a white powder; $[\alpha]_D^{294}$ +32.8 (c = 1.0, $CHCl_3$); υ_{max} ($CHCl_3$)/cm^{-1} 3438, 2966, 1723, 1668, 1635, 1498, 1454, 1371, 1341, 1323, 1098, 1066, 908; δ_H (360 MHz, $CDCl_3$) 1.00 (3H, d, *J* 6.8 Hz, $(CH_3)_2CH$), 1.03 (3H, d, *J* 6.8 Hz, $(CH_3)_2CH$), 1.40 (3H, t, *J* 7.1 Hz, CH_3CH_2O), 1.57 (3H, d, *J* 7.0 Hz, CH_3CH), 2.59-2.66 (1H, m, $CH(CH_3)_2$), 2.61 (3H, s, CH_3CO), 4.42 (2H, q, *J* 7.1 Hz, CH_2CH_3), 4.99-5.09 (1H, m, $CHCH_3$), 5.16 (2H, m, CH_2Ph), 5.29 (1H, dd, *J* 6.7, 9.2 Hz, CHNH), 5.34-5.36 (1H, br. m, NHCbZ), 7.35-7.38 (5H, m, ArH), 7.52 (1H, d, *J* 8.9 Hz, NHCO), 8.07 (1H, s, CHS); δ_C (90.5 MHz, $CDCl_3$) 11.4 (q), 14.1 (q), 17.8 (q), 19.4 (q), 19.6 (q), 32.7 (d), 44.9 (d), 55.7 (d), 61.1 (t), 66.8 (t), 126.7 (d), 127.8 (d), 127.9 (d), 128.2 (d), 136.0 (s), 147.2 (s), 153.5 (s), 155.5 (s), 161.0 (s), 161.2 (s), 161.3 (s), 171.7 (s); *m/z* (FAB) Found: 537.1725 ($M^+ + Na^+$, $C_{25}H_{30}O_6N_4SNa$ requires 537.1784).

1'(*R*)-2-(1-{[1'(*R*)-2-(1-Benzyloxycarbonylamino-ethyl)-5-methyl-oxazole-4-carbonyl]-amino}-2-methyl-propyl)-thiazole-4-carboxylic acid ethyl ester (223)



Sodium hydroxide (82 mg, 2.07 mmol) was added to a solution of the dimer **222** (260 mg, 0.51 mmol) in a THF:H_2O (5:3) mixture (5 ml) at room temperature. The solution was stirred for 14 h and the separated aqueous layer acidified to pH 4 with citric acid. The aqueous layer was then extracted with ethyl acetate (5 x 20 ml) and the combined organic extracts washed with brine (2 x 20 ml), were dried ($MgSO_4$) and evaporated *in vacuo* to leave the acid (2.06 mg, 0.42 mmol, 83 %) as a pale yellow foam: mp. 70-73 °C (from dichloromethane); $[\alpha]_D^{294}$ +50.4 (c = 1.0, EtOH); υ_{max} ($CHCl_3$)/cm^{-1} 3437, 1723, 1667, 1452, 1343, 1063; δ_H (360 MHz, CD_3OD) 1.00 (3H, d, *J* 6.7 Hz, (CH_3)$_2$CH), 1.08 (3H, d, *J* 6.7 Hz, (CH_3)$_2$CH), 1.58 (3H, d, *J* 7.2 Hz, CH_3CH), 2.47-2.57 (1H, m, C*H*(CH_3)$_2$), 2.60 (3H, s, CH_3CO), 4.91-4.95 (1H, m, C*H*CH_3), 5.14 (2H, br. s, CH_2Ph), 5.21-5.26 (1H, m, C*H*CH(CH_3)$_2$), 7.28-7.38 (5H, m, Ar*H*), 8.34 (1H, s, C*H*S); δ_C (90.5 MHz, $CDCl_3$) 11.7 (q), 18.8 (q), 19.2 (q), 19.9 (q), 34.1 (d), 43.8 (t), 46.2 (d), 57.8 (d), 128.8 (d), 129.0 (d), 129.1 (d), 129.4 (d), 138.0 (s), 148.3 (s), 155.4 (s), 158.0 (s), 163.6 (s), 163.9 (s), 176.7 (s); *m/z* (FAB) Found: 509.1509 ($M^+ + Na^+$, $C_{23}H_{26}O_6N_4SNa$ requires 509.1471).

1'(*R*)-2-(1-{[1'(*R*)-2-(1-{[1'(*R*)-2-(1-Benzyloxycarbonylamino-ethyl)-5-methyl-oxazole-4-carbonyl]-amino}-2-methyl-propyl)-thiazole-4-carbonyl]-amino}-2-methyl-propyl)-thiazole-4-carboxylic acid ethyl ester (224)

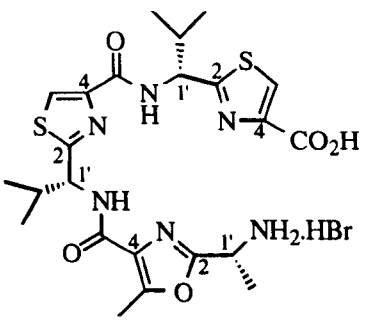


N-Methylmorpholine (0.03 ml, 0.23 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (44 mg, 0.23 mmol) and *N*-hydroxybenzotriazole (31 mg, 0.23 mmol) were added to a solution of the dimer acid (100 mg, 0.21 mmol) in anhydrous dichloromethane (5 ml) at 0 °C under an atmosphere of nitrogen. This solution was stirred for 20 min at 0 °C and a precooled solution of the thiazole amine deprotonated with *N*-methylmorpholine (0.03 ml, 0.23 mmol) in DMF (1 ml) was added dropwise over 5 min. The resulting solution was stirred at 0 °C for 1 h and at room temperature for 17 h.

Water (*ca* 20 ml) was added and the separated aqueous layer was extracted with dichloromethane (4 x 20 ml). The combined organic extracts were washed with 10 % aqueous citric acid solution (3 x 20 ml) and brine (2 x 20 ml), were dried ($MgSO_4$) and evaporated *in vacuo*. The residue was purified by chromatography on silica using 50 % ethyl acetate in light petroleum (40-60 °C) as eluant to leave the hexapeptide (96 mg, 0.4 mmol, 66 %) as a cream foam; $[\alpha]_D^{294}$ +34.6 (c = 0.9, $CHCl_3$); υ_{max} ($CHCl_3$)/cm^{-1} 3399, 2966, 1722, 1668, 1635, 1543, 1489, 1455, 1372, 1322, 1069; δ_H (360 MHz, $CDCl_3$) 1.00-1.06 (6H, m, $(CH_3)_2CH$), 1.00-1.06 (6H, m, $(CH_3)_2CH$), 1.37 (3H, t, *J* 7.1 Hz, CH_3CH_2O), 1.59 (3H, d, *J* 7.0 Hz, CH_3CH), 2.48-2.55 (1H, m, $CH(CH_3)_2$), 2.57-2.66 (1H, m, $CH(CH_3)_2$), 2.62 (3H, s, CH_3CO), 4.39 (2H, q, *J* 7.1 Hz, CH_2CH_3), 5.02-5.09 (1H, m, $CHCH_3$), 5.12-5.14 (2H, m, CH_2Ph), 5.30-5.36 (1H, m,

C*H*CH(CH_3)$_2$), 5.30-5.36 (1H, m, C*H*CH(CH_3)$_2$), 5.48 (1H, d, *J* 8.3 Hz, N*H*CbZ), 7.35-7.39 (5H, m, Ar*H*), 7.48 (1H, d, *J* 9.4 Hz, N*H*CO), 7.98 (1H, d, *J* 9.3 Hz, N*H*CO), 8.03 (1H, s, C*H*S), 8.04 (1H, s, C*H*S); δ_C (90.5 MHz, $CDCl_3$) 11.6 (q), 14.3 (q), 17.8 (q), 17.9 (q), 19.4 (q), 19.6 (q), 19.7 (q), 33.0 (d), 33.1 (d), 45.0 (d), 55.8 (d), 56.4 (d), 61.3 (t), 67.1 (t), 123.5 (d), 126.9 (d), 128.1 (d), 128.2 (d), 128.5 (d), 136.1 (s), 147.4 (s), 149.3 (s), 153.9 (s), 160.7 (s), 161.2 (s), 161.4 (s), 161.5 (s), 171.6 (s), 171.7 (s); *m/z* (FAB) Found: 719.2368 ($M^+ + Na^+$, $C_{33}H_{40}O_7N_6S_2Na$ requires 719.2297).

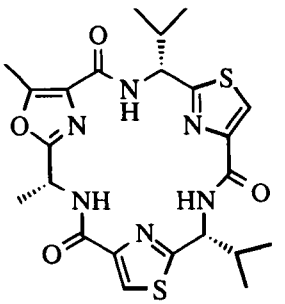
1'(*R*)-2-(1-{[1'(*R*)-2-(1-{[1'(*R*)-2-(1-Amino-ethyl)-5-methyl-oxazole-4-carbonyl]-amino}-2-methyl-propyl)-thiazole-4-carbonyl]-amino}-2-methyl-propyl)-thiazole-4-carboxylic acid (225)



A solution of sodium hydroxide (20 mg, 0.5 mmol) in water (0.5 ml) was added to a solution of the trimer **224** (88 mg, 0.13 mmol) in ethanol (2 ml) and this solution was stirred for 4 h. The ethanol was removed *in vacuo* and the residue acidified with 2M hydrochloric acid solution (30 ml), the amine was then collected by suction filtration as a white solid. This solid was stirred with a 33 % solution of hydrobromic acid in acetic acid (1 ml) and glacial acetic acid (0.6 ml) for 3 h at room temperature under an atmosphere of nitrogen. The volatiles were removed *in vacuo* to leave the amine (65 mg, 0.1 mmol, 77 %) as a brown foam; $[\alpha]_D^{295}$ +4.0 (c = 0.7, EtOH); δ_H (360 MHz, CD_3OD) 1.00-1.04 (3H, m, (C*H*$_3$)$_2$CH), 1.00-1.04 (3H, m, (C*H*$_3$)$_2$CH), 1.09-1.13 (3H, m, (C*H*$_3$)$_2$)CH), 1.09-1.13 (3H, m, (C*H*$_3$)$_2$CH), 1.76 (3H, d, *J* 7.0 Hz, C*H*$_3$CH), 2.51-2.60 (1H, m, C*H*(CH_3)$_2$), 2.51-2.60 (1H, m, C*H*(CH_3)$_2$), 2.67 (3H, s, C*H*$_3$CO), 4.76-

4.80 (1H, m, C*H*CH_3), 5.15-5.28 (1H, m, C*H*$CH(CH_3)_2$), 5.15-5.28 (1H, m, C*H*$CH(CH_3)_2$), 8.26 (1H, s, C*H*S), 8.41 (1H, s, C*H*S); δ_C (90.5 MHz, CD_3OD) 14.9 (q), 15.1 (q), 20.7 (q), 21.5 (q), 21.7 (q), 22.3 (q), 35.6 (q), 48.4 (d), 59.3 (d), 60.0 (d), 60.7 (d), 128.8 (d), 131.5 (d), 133.2 (s), 147.6 (s), 147.8 (s), 158.1 (s), 158.2 (s), 160.4 (s), 164.2 (s), 164.3 (s), 164.4 (s), 176.2 (s); *m/z* (FAB) Found: 552.1777 (M^+ - HBr + H_2O, $C_{23}H_{32}O_6N_6S_2$ requires 552.1825).

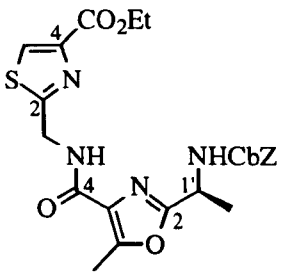
Cyclic-*bis*-(*R*)-valine-thiazole-(*R*)-alanine-oxazole (176)



Diisopropylethylamine (0.4 ml, 0.3 mmol) and FDPP (576 mg, 0.15 mmol) were added to a suspension of **225** (65 mg, 0.1 mmol) in anhydrous acetonitrile at room temperature under an atmosphere of nitrogen. This solution was stirred for 1 day, water (*ca* 10 ml) was added and the separated aqueous layer was extracted with dichloromethane (4 x 15 ml). The combined organic extracts were washed with 2M hydrochloric acid solution (3 x 10 ml), sodium hydrogen carbonate solution (3 x 10 ml), were dried ($MgSO_4$) and evaporated *in vacuo* to leave a solid white residue. This residue was purified by chromatography on silica using 70 % ethyl acetate in light petroleum (40-60 °C) as eluant to give the *cyclic peptide* (35 mg, 0.068 mmol, 68 %) as a white foam; $[\alpha]_D^{297}$ +46.0 (c = 0.3, $CHCl_3$); υ_{max} ($CHCl_3$)/cm^{-1} 3396, 2928, 1668, 1545, 1494, 1372, 1066; δ_H (360 MHz, $CDCl_3$) 1.00 (3H, d, *J* 6.8 Hz, $(CH_3)_2CH$), 1.06 (3H, d, *J* 6.8 Hz, $(CH_3)_2CH$), 1.08 (3H, d, *J* 7.2 Hz, $(CH_3)_2CH$), 1.10 (3H, d, *J* 7.2 Hz, $(CH_3)_2CH$), 1.70 (3H, d, *J* 6.8 Hz, CH_3CH), 2.28-2.38 (1H, m, C*H*$(CH_3)_2$), 2.28-2.38 (1H, m,

C*H*(CH_3)$_2$), 2.67 (3H, s, C*H*$_3$O), 5.21-5.31 (1H, m, C*H*CH_3), 5.21-5.31 (1H, m, C*H*CH(CH_3)$_2$), 5.52 (1H, dd, *J* 5.7, 9.5 Hz, C*H*CH(CH_3)$_2$), 8.11 (1H, s, C*H*S), 8.11 (1H, s, C*H*S), 8.44 (1H, d, *J* 5.7 Hz, N*H*CO), 8.45 (1H, d, *J* 9.0 Hz, N*H*CO), 8.60 (1H, d, *J* 6.6 Hz, N*H*CO); δ_C (90.5 MHz, $CDCl_3$) 11.7 (q), 18.3 (q), 18.6 (q), 19.0 (q), 21.0 (q), 34.9 (q), 35.6 (d), 44.1 (d), 55.4 (d), 55.9 (d), 77.3 (d), 123.4 (d), 123.7 (d), 128.4 (s), 128.5 (s), 148.9 (s), 149.0 (s), 153.8 (s), 159.9 (s), 160.6 (s), 161.7 (s), 168.1 (s), 168.7 (s); *m/z* (FAB) Found: 517.1703 ($M^+ + H^+$, $C_{23}H_{29}O_4N_6S_2$ requires 517.1692).

1'(*R*)-2-({[1'(*R*)-2-(1-Benzyloxycarbonylamino-ethyl)-5-methyl-oxazole-4-carbonyl]-amino}-methyl)-thiazole-4-carboxylic acid ethyl ester (188)

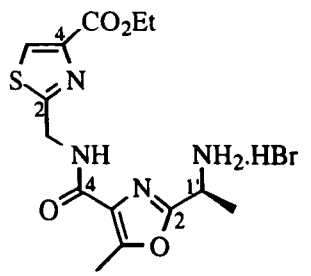


N-Methylmorpholine (0.08 ml, 0.76 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (146 mg, 0.76 mmol) and *N*-hydroxybenzotriazole (103 mg, 0.76 mmol) were added to a solution of the oxazole acid **186** (210 mg, 0.7 mmol) in anhydrous dichloromethane (8 ml) at 0 °C under an atmosphere of nitrogen. The resulting solution was stirred for 20 min and a precooled solution of the glycine thiazole amine hydrochloride **187** (183 mg, 0.82 mmol) deprotonated with *N*-methylmorpholine (0.08 ml, 0.76 mmol) in DMF (1 ml) was added dropwise over 5 min. This solution was stirred at 0 °C for a further 1 h and at room temperature for 18 h.

Water (*ca* 20 ml) was added and the separated aqueous layer was extracted with dichloromethane (3 x 20 ml). The combined organic extracts were washed with 10 % aqueous citric acid solution (3 x 10 ml), sodium hydrogen carbonate solution (3 x 10 ml)

and brine (3 x 10 ml), were dried ($MgSO_4$) and evaporated *in vacuo*. Purification of the residue by chromatography on silica using 40 % ethyl acetate in light petroleum (40-60 °C) yielded the tetrapeptide (208 mg, 0.44 mmol, 60 %) as a cream foam; $[\alpha]_D^{298}$ -231.7 (c = 0.53, CH_3Cl); υ_{max} ($CHCl_3$): 3422, 2983, 2938, 1715, 1668, 1635, 1493, 1454, 1387, 1371, 1343, 1322, 1096, 1065, 972, 908, 878; δ_H (360 MHz, $CHCl_3$) 1.36 (3H, t, *J* 7.1 Hz, C*H*$_3$CH$_2$O), 1.49 (3H, d, *J* 7.0 Hz, C*H*$_3$CH), 2.57 (3H, s, C*H*$_3$CO), 4.38 (2H, q, *J* 7.1 Hz, C*H*$_2$CH$_3$), 4.85 (2H, d, *J* 6.5 Hz, C*H*$_2$NH), 4.86-4.95 (1H, m, C*H*CH$_3$), 5.08 (2H, s, C*H*$_2$Ph), 5.62 (1H, br. s, N*H*CbZ), 7.28-7.31 (5H, m, Ar*H*), 8.09 (1H, s, C*H*S); δ_C (90.5 MHz, $CHCl_3$) 11.1 (q), 13.8 (q), 18.9 (q), 30.8 (d), 39.9 (t), 60.9 (t), 66.3 (d), 127.5 (d), 127.6 (d), 127.6 (d), 127.9 (d), 135.9 (s), 146.3 (s), 153.3 (s), 155.3 (s), 160.7 (s), 161.3 (s), 161.6 (s), 168.7 (s); *m/z* (FAB) Found: 473.1517 ($M^+ + H^+$, $C_{22}H_{25}O_6N_4S$ requires 473.1495).

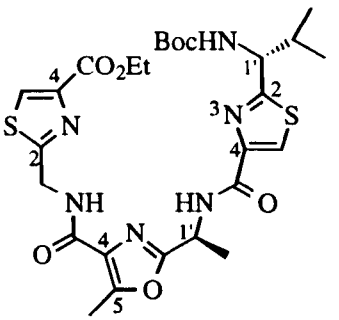
1'(*R*)-2-({[1'(*R*)-2-(1-Amino-ethyl)-5-methyl-oxazole-4-carbonyl]-amino}-methyl)-thiazole-4-carboxylic acid ethyl ester (189)



The tetrapeptide **188** (208 mg, 0.44 mmol) was stirred in a 33 % solution of hydrobromic acid in acetic acid (1 ml) and glacial acetic acid (0.6 ml) for 3 h at room temperature under an atmosphere of nitrogen. The volatiles were removed *in vacuo* to leave the hydrobromide (168 mg, 0.4 mmol, 91 %) as a brown, highly hygroscopic powder; $[\alpha]_D^{302}$ -18.0 (c = 0.4, EtOH); δ_H (360 MHz, CD_3OD) 1.44 (3H, t, *J* 7.1 Hz, C*H*$_3$CH$_2$O), 1.77 (3H, d, *J* 6.9 Hz, C*H*$_3$CH), 2.69 (3H, s, C*H*$_3$CO), 4.45 (2H, q, *J* 7.1 Hz, C*H*$_2$CH$_3$), 4.69-4.87 (1H, m, C*H*CH$_3$), 4.97-5.01 (2H, m, C*H*$_2$NH), 8.51 (1H, s,

C*H*S); δ_C (90.5 MHz, CD_3OD) 12.1 (q), 14.5 (q), 17.8 (q), 40.0 (t), 45.5 (d), 63.9 (t), 129.1 (s), 131.8 (d), 140.0 (s), 156.4 (s), 158.5 (s), 158.6 (s), 163.3 (s), 177.2 (s); *m/z* (FAB) Found: 361.0948 (M^+ - HBr + Na^+, $C_{14}H_{18}O_4N_4SNa$ requires 361.0946).

1'(*R*)-2-({[1'(*R*)-2-(1-{[1'(*R*)-2-(1-*tert*-Butoxycarbonylamino-2-methyl-propyl)-thiazole-4-carbonyl]-amino}-ethyl)-5-methyl-oxazole-4-carbonyl]-amino}-methyl)-thiazole-4-carboxylic acid ethyl ester (191)

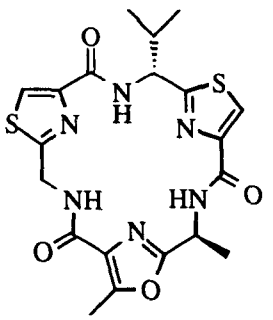


N-Methylmorpholine (0.06 ml, 0.53 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (102 mg, 0.53 mmol) and *N*-hydroxybenzotriazole (72 mg, 0.53 mmol) were added to a solution of the valine thiazole **190** (143 mg, 0.48 mmol) in anhydrous dichloromethane (5 ml) at 0 °C under an atmosphere of nitrogen. This solution was stirred for 20 min at 0 °C and a precooled solution of the dimer **189** (200 mg, 0.48 mmol) deprotonated with *N*-methylmorpholine (0.06 ml, 0.53 mmol) in DMF (1 ml) was added dropwise over 5 min. This solution was stirred at 0 °C for 1 h and at room temperature for 15 h.

Water (*ca* 20 ml) was added and the separated aqueous layer was extracted with dichloromethane (3 x 30 ml). The combined organic extracts were washed with 10 % aqueous citric acid solution (3 x 20 ml), sodium hydrogen carbonate solution (3 x 20 ml) and brine (2 x 10 ml), were dried ($MgSO_4$) and evaporated *in vacuo*. Chromatography on silica using 70 % ethyl acetate in light petroleum (40-60 °C) as eluant gave the

hexapeptide (20 mg, 0.03 mmol, 26 %) as a white foam; $[\alpha]_D^{297}$ +5.0 (c = 0.4, $CHCl_3$); υ_{max} ($CHCl_3$)/cm^{-1} 3408, 2929, 1716, 1669, 1635, 1544, 1490, 1455, 1369, 1096; δ_H (360 MHz, $CDCl_3$) 0.93 (3H, d, *J* 6.8 Hz, $(CH_3)_2CH$), 1.00 (3H, d, *J* 6.8 Hz, $(CH_3)_2CH$), 1.40 (3H, t, *J* 7.1 Hz, CH_3CH_2O), 1.46 (9H, s, Bu^t), 1.66 (3H, s, CH_3CO), 4.43 (2H, q, *J* 7.1 Hz, CH_2CH_3), 4.80-4.87 (1H, m, $CHCH_3$), 4.92 (2H, d, *J* 6.5 Hz, CH_2NH), 5.14 (1H, d, *J* 8.2 Hz, NHBoc), 5.38-5.43 (1H, m, $CHCH(CH_3)_2$), 7.61-7.65 (1H, m, NHCO), 7.61-7.65 (1H, m, NHCO), 8.05 (1H, s, CHS), 8.13 (1H, s, CHS); δ_C (90.5 MHz, $CDCl_3$) 11.7 (q), 14.4 (q), 17.4 (q), 19.4 (q), 28.3 (q), 33.2 (q), 40.4 (d), 42.3 (d), 58.1 (d), 61.5 (d), 123.5 (d), 128.1 (d), 128.4 (s), 134.3 (s), 138.2 (s), 146.9 (s), 154.1 (s), 155.4 (s), 160.3 (s), 161.3 (s), 162.0 (s); *m/z* (FAB) Found: 643.1928 ($M^+ + Na^+$, $C_{27}H_{36}O_7N_6S_2Na$ requires 643.1985).

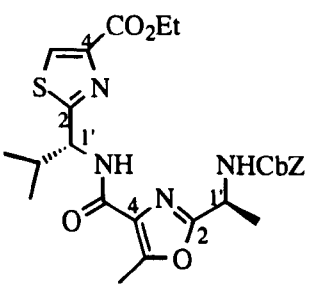
Nostacyclamide (16)



Sodium hydroxide (12 mg, 0.26 mmol) was added to a solution of the dimer **222** (40 mg, 0.06 mmol) in a THF:H_2O (5:3) mixture (2 ml) at room temperature. The solution was stirred for 15 h and the separated aqueous layer acidified to pH 4 with citric acid. The aqueous layer was then extracted with ethyl acetate (5 x 20 ml) and the combined organic extracts washed with brine (2 x 20 ml), were dried ($MgSO_4$) and evaporated *in vacuo* to leave the acid as a pale yellow foam. The acid was then stirred with a 4M solution of hydrochloric acid in dioxane (1 ml) at room temperature for 1 h under an atmosphere of nitrogen. The dioxane was removed *in vacuo* using toluene as an

azeotrope to leave a highly hygroscopic yellow solid which was immediately subjected to the coupling conditions. Diisopropylethylamine (0.02 ml, 0.12 mmol), and FDPP (23 mg, 0.06 mmol) were added to the chloride in anhydrous acetonitrile (1.5 ml) at room temperature under an atmosphere of nitrogen. The solution was stirred for 14 h, water (*ca* 10 ml) was added, the separated aqueous layer was extracted with dichloromethane (4 x 10 ml) and the combined organic extracts were washed with 10 % aqueous citric acid solution (3 x 10 ml), sodium hydrogen carbonate solution (3 x 10 ml) and brine (2 x 10 ml), were dried ($MgSO_4$) and evaporated *in vacuo* to leave a white solid residue which was purified by chromatography on silica using 80 % ethyl acetate in light petroleum (40-60 °C) as eluant to leave nostacyclamide (3 mg, 0.006 mmol, 10 %) as a white solid; mp (255-257 °C) (Lit.[140] mp. 259-260 °C); $[\alpha]_D^{297}$ +28.0 (c = 0.2, $CHCl_3$) (Lit.[139] $[\alpha]_D^{296}$ +51.3 (0.84, $CHCl_3$)); υ_{max} ($CHCl_3$)/cm^{-1} 3396, 3125, 3006, 2966, 2929, 1666, 1642, 1542, 1094; δ_H (360 MHz, $CDCl_3$) 0.95 (3H, d, *J* 7.1 Hz, (CH_3)$_2$CH), 0.97 (3H, d, *J* 7.1 Hz, (CH_3)$_2$CH), 1.70 (3H, d, *J* 6.6 Hz, CH_3CH), 2.31-2.35 (1H, m, C*H*(CH_3)$_2$), 2.71 (3H, s, CH_3O), 4.79 (1H, d, *J* 17.6, CH_2NH), 4.99 (1H, d, *J* 17.6 CH_2NH), 5.10-5.14 (1H, m, C*H*CH_3), 5.65 (1H, d, *J* 3.3 Hz, C*H*CH(CH_3)$_2$), 7.53 (1H, d, *J* 6.1 Hz, N*H*CO), 7.81 (1H, d, *J* 7.1 Hz, N*H*CO), 7.84 (1H, d, *J* 7.1 Hz, N*H*CO), 8.16 (1H, s, C*H*S), 8.19 (1H, s, C*H*S).

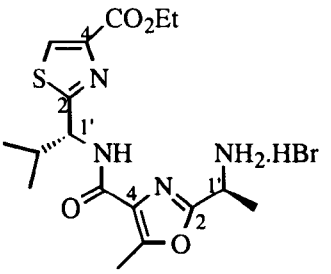
1'(*R*)-2-(1-{[1'(*R*)-2-(1-Benzyloxycarbonylamino-ethyl)-5-methyl-oxazole-4-carbonyl]-amino}-2-methyl-propyl)-thiazole-4-carboxylic acid ethyl ester (226)



N-Methylmorpholine (0.084 ml, 0.76 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (146 mg, 0.76 mmol) and *N*-hydroxybenzotriazole (103 mg, 0.76 mmol) were added to a solution of the oxazole **186** (210 mg, 0.7 mmol) in anhydrous dichloromethane (8 ml) at 0 °C under an atmosphere of nitrogen. This solution was stirred at 0 °C for a further 20 min and a precooled solution of the valine thiazole amine **181** (279 mg, 1.06 mmol) deprotected with *N*-methylmorpholine (0.084 ml, 0.76 mmol) in DMF (1 ml) was added dropwise over 5 min. The resulting solution was stirred at 0 °C for a further 1 h and at room temperature for 16 h.

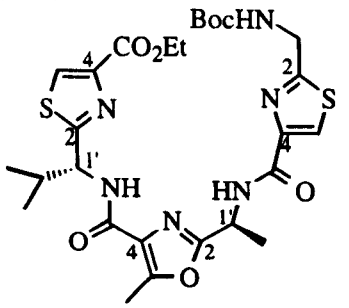
Water (*ca* 20 ml) was added and the separated aqueous layer was extracted with dichloromethane (3 x 20 ml). The combined organic extracts were washed with 10 % aqueous citric acid solution (3 x 10 ml) and sodium hydrogen carbonate solution (3 x 10 ml), were dried ($MgSO_4$) and evaporated *in vacuo* to leave the tetrapeptide (240 mg, 0.54 mmol, 77 %) as a white foam; $[\alpha]_D^{298}$ -101.9 (c = 0.5, $CHCl_3$); υ_{max} ($CHCl_3$)/cm^{-1} 3402, 2965, 1723, 1668, 1634, 1497, 1454, 1371, 1323, 1098, 1066, 908; δ_H (360 MHz, $CDCl_3$) 0.98 (3H, d, *J* 6.8 Hz, $(CH_3)_2CH$), 1.01 (3H, d, *J* 6.8 Hz, $(CH_3)_2CH$), 1.39 (3H, t, *J* 7.1 Hz, CH_3CH_2O), 1.55 (3H, d, *J* 7.0 Hz, CH_3CH), 2.55-2.65 (1H, m, $CH(CH_3)_2$), 2.60 (3H, s, CH_3CO), 4.41 (2H, q, *J* 7.1 Hz, OCH_2CH_3), 4.94-5.01 (1H, m, $CHCH_3$), 5.15 (2H, s, Ar*H*), 5.28 (1H, dd, *J* 6.8, 9.2 Hz, $CHCH(CH_3)_2$), 5.41 (1H, br. s, N*H*CbZ), 7.32-7.37 (5H, m, Ar*H*), 7.51 (1H, d, *J* 9.2 Hz, N*H*CO), 8.07 (1H, s, C*H*S); δ_C (90.5 MHz, $CDCl_3$) 11.4 (q), 14.1 (q), 17.8 (q), 19.4 (q), 32.7 (d), 44.9 (d), 55.7 (d), 61.1 (t), 66.7 (t), 126.7 (d), 127.8 (d), 127.9 (d), 128.2 (d), 136.0 (s), 147.2 (s), 153.5 (s), 155.4 (s), 161.0 (s), 161.2 (s), 161.3 (s), 171.6 (s); *m/z* (FAB) Found: 515.1946 ($M^+ + H^+$, $C_{25}H_{30}O_6N_4S$ requires 515.1964).

1'(*R*)-2-(1-{[1'(*R*)-2-(1-Amino-ethyl)-5-methyl-oxazole-4-carbonyl]-amino}-2-methyl-propyl)-thiazole-4-carboxylic acid ethyl ester hydrobromide (227)



A 33 % solution of hydrobromic acid in acetic acid (1 ml) and glacial acetic acid (0.6 ml) were added to the tetrapeptide **226** at room temperature under an atmosphere of nitrogen. The resulting solution was stirred for 3 h and the volatiles were removed *in vacuo* to leave the bromide (250 mg, 0.54 mmol, 99 %) as a brown foam; $[\alpha]_D^{298}$ +218.4 (c = 0.5, EtOH); δ_H (360 MHz, CD_3OD) 0.99 (3H, d, *J* 6.5 Hz, $(CH_3)_2CH$), 1.09 (3H, d, *J* 6.4 Hz, $(CH_3)_2CH$), 1.20 (3H, t, *J* 7.0 Hz, CH_3CH_2O), 1.77 (3H, d, *J* 6.4 Hz, CH_3CH), 2.50-2.58 (1H, m, $CH(CH_3)_2$), 2.65 (3H, s, CH_3CO), 3.64 (2H, q, *J* 7.0 Hz, CH_2CH_3), 4.79-4.81 (1H, m, $CHCH_3$), 5.19-5.25 (1H, m, $CHCH(CH_3)_2$), 8.45 (1H, s, CHS); δ_C (90.5 MHz, CD_3OD) 12.1 (q), 17.6 (q), 18.0 (q), 18.9 (q), 19.8 (q), 33.6 (q), 45.6 (d), 57.2 (d), 58.0 (t), 129.2 (d), 138.8 (s), 144.5 (s), 156.0 (s), 158.4 (s), 161.2 (s), 162.4 (s); *m/z* (FAB) Found: 381.1597 ($M^+ - Br^-$, $C_{17}H_{25}O_4N_4S$ requires 381.1597).

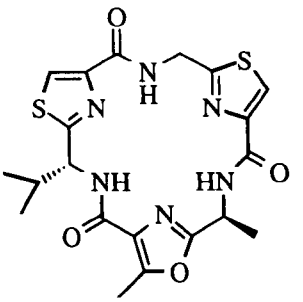
1'(*R*)-2-(1-{[2-(1-{[2-(*tert*-Butoxycarbonylamino-methyl)-thiazole-4-carbonyl]-amino}-ethyl)-5-methyl)-oxazole-4-carbonyl]-amino}-2-methyl-propyl)-thiazole-4-carboxylic acid ethyl ester (228)



N-Methylmorpholine (0.07 ml, 0.59 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (114 mg, 0.59 mmol) and *N*-hydroxybenzotriazole (80 mg, 0.59 mmol) were added to a solution of the valine thiazole acid (140 mg, 0.54 mmol) in anhydrous dichloromethane (6 ml) at 0°C under an atmosphere of nitrogen. This solution was stirred for a further 20 min and a solution of the tetrapeptide **227** (250 mg, 0.54 mmol) deprotected with *N*-methylmorpholine (0.07 ml, 0.59 mmol) in DMF (1 ml) was added dropwise over 5 min. The resulting solution was stirred at 0 °C for 1 h and at room temperature for 15 h.

Water (*ca* 30 ml) was added and the separated aqueous layer was extracted with dichloromethane (3 x 30 ml). The combined organic extracts were washed with a 10 % aqueous citric acid solution (2 x 30 ml) and sodium hydrogen carbonate solution (2 x 30 ml), were dried ($MgSO_4$) and evaporated *in vacuo*. Purification by chromatography on silica using 70 % ethyl acetate in light petroleum (40-60 °C) led to the isolation of the hexapeptide (235 mg, 0.38 mmol, 70 %) as a cream foam; $[\alpha]_D^{295}$ +5.1 (c = 0.7, $CHCl_3$); δ_H (360 MHz, $CHCl_3$) 1.01 (3H, d, *J* 6.7 Hz, $(CH_3)_2CH$), 1.06 (3H, d, *J* 6.7 Hz, $(CH_3)_2CH$), 1.50 (9H, s, Bu^t), 1.70 (3H, d, *J* 7.0 Hz, CH_3CH), 2.65 (3H, s, CH_3CO), 4.65 (2H, d, *J* 5.7 Hz, CH_2NH), 5.30 (1H, dd, *J* 7.0, 9.1 Hz, $CHCH(CH_3)_2$), 5.43-5.50 (1H, m, $CHCH_3$), 7.62 (1H, d, *J* 8.7 Hz, NHCO), 7.77 (1H, d, *J* 8.2 Hz, CHS), 8.13 (1H, s, CHS); δ_C (90.5 MHz, $CHCl_3$) 11.7 (q), 18.0 (q), 19.3 (q), 19.7 (q), 29.3 (q), 29.7 (q), 31.9 (d), 43.0 (d), 56.0 (d), 124.3 (d), 127.2 (q), 128.6 (s), 147.1 (s), 149.1 (s), 154.0 (s), 155.6 (s), 160.2 (s), 161.1 (s), 161.6 (s), 161.8 (s), 169.6 (q), 172.1 (s); *m/z* (FAB) Found: 643.1913 ($M^+ + Na^+$, $C_{27}H_{36}O_7N_6S_2$ requires 643.1985).

Structural isomer of nostacyclamide (194)



Sodium hydroxide (60 mg, 1.42 mmol) was added to a solution of the dimer **222** (215 mg, 0.38 mmol) in a THF:H_2O (5:3) mixture (4 ml) at room temperature. The solution was stirred for 15 h and the separated aqueous layer acidified to pH 4 with citric acid. The aqueous layer was then extracted with ethyl acetate (5 x 20 ml) and the combined organic extracts washed with brine (2 x 20 ml), were dried ($MgSO_4$) and evaporated *in vacuo* to leave the acid as a pale yellow foam. The acid was then stirred with a 4M solution of hydrochloric acid in dioxane (1 ml) at room temperature for 1 h under an atmosphere of nitrogen. The dioxane was removed *in vacuo* using toluene as an azeotrope to leave a highly hygroscopic yellow solid which was immediately subjected to the coupling conditions. Diisopropylethylamine (0.05 ml, 0.28 mmol) and FDPP (54 mg, 0.14 mmol) were added to a suspension of **228** (50 mg, 0.095 mmol) in anhydrous acetonitrile (4 ml) at room temperature under an atmosphere of nitrogen. This solution was stirred for 1 day and water (*ca* 10 ml) was added and the separated aqueous layer was extracted with dichloromethane (5 x 10 ml). The combined organic extracts were washed with 2M hydrochloric acid solution (2 x 10 ml), sodium hydrogen carbonate solution (2 x 10 ml), were dried ($MgSO_4$) and evaporated *in vacuo* to leave a solid white residue. This residue was purified by chromatography on silica using 70 % ethyl acetate in light petroleum (40-60 °C) as eluant to give the *cyclic peptide* (23 mg, 0.049 mmol, 52 %) as a white powder; mp. 263-264 °C (from dichloromethane); $[\alpha]_D^{295}$ +21.1 (c = 1.0, $CHCl_3$); υ_{max} ($CHCl_3$)/cm^{-1} 3394, 2964, 2929, 1667, 1639, 1549, 1488, 1452, 1358, 1303, 1094, 1055, 997; δ_H (360 MHz, $CDCl_3$) 0.90 (3H, d, *J* 6.7 Hz,

(CH_3)$_2$CH), 1.04 (3H, d, *J* 6.7 Hz, (CH_3)$_2$CH), 1.68 (3H, d, *J* 6.7 Hz, CH_3CH), 2.33-2.38 (1H, m, C*H*(CH_3)$_2$), 2.70 (3H, s, CH_3CO), 4.88 (1H, dd, *J* 3.1, 18.2 Hz, CH_2NH), 5.17 (1H, dd, *J* 5.4, 18.2 Hz, CH_2NH), 5.23 (1H, app. quintet, *J* 6.7 Hz, C*H*CH_3), 5.32 (1H, dd, *J* 4.5, 7.7 Hz, C*H*CH(CH_3)$_2$), 8.17 (1H, s, C*H*S), 8.18 (1H, s, C*H*S), 8.43 (1H, br. s, N*H*CH_2), 8.52 (1H, d, *J* 7.7 Hz, N*H*CHCH(CH_3)$_2$), 8.69 (1H, d, *J* 6.7 Hz, N*H*CHCH_3); δ_C (125 MHz, $CDCl_3$) 11.7 (q), 17.8 (q), 18.5 (q), 19.7 (q), 34.7 (d), 41.6 (t), 44.5 (d), 56.2 (d), 123.7 (d), 123.8 (d), 128.5 (s), 148.5 (s), 149.0 (s), 154.2 (s), 159.7 (s), 160.5 (s), 160.6 (s), 161.2 (s), 165.0 (s), 167.9 (s); *m/z* (FAB) Found: 497.1033 ($M^+ + Na^+$, $C_{20}H_{22}O_4N_6S_2$ requires 497.1042).

APPENDIX

Appendix 4.1 *- Reprints of Publications*

SYNLETT

Accounts and
Rapid Communications in
Synthetic Organic Chemistry

REPRINT

With Compliments of the Author

Thieme

The Synthesis of Novel Thiazole Containing Cyclic Peptides *via* Cyclooligomerisation Reactions

Anna Bertram, Jeffery S. Hannam, Katrina A. Jolliffe, Felix González-López de Turiso, Gerald Pattenden*

School of Chemistry, University of Nottingham, Nottingham, UK, NG7 2RD

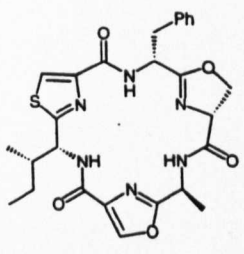
Fax +44 (0)115 951 3564; E-mail: GP@nottingham.ac.uk

Received 23 August 1999

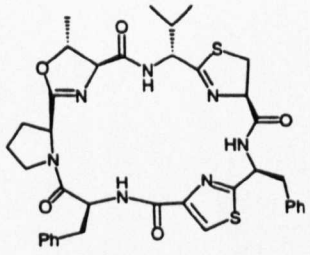
Abstract: Thiazole containing cyclic peptides have been synthesised in high yields by cyclooligomerisation reactions of amino acid substituted thiazole monomers.

Key words: cyclic peptides, macrocycles, cyclizations, heterocycles

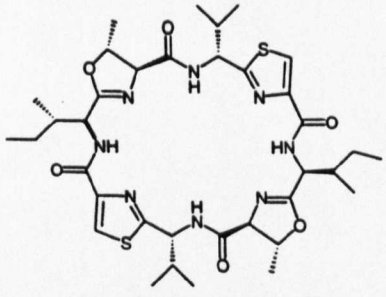
A large number of cyclic peptides containing 'unnatural' D-amino acids, together with modified amino acids in the form of azole heterocycles have been isolated from marine organisms and algae in recent years. The *Lissoclinum* class of cyclic peptides *e.g.* raocyclamide **1**,[1] lissoclinamide 4 **2**,[2] ascidiacyclamide **3**,[3] is one such group, characterised by the presence of oxazoline/oxazole/thiazoline/thiazole heterocycles alternating with amino acid residues.[4] The size and conformations of these macrocycles and the functional groups they possess have suggested that they have potential for metal ion chelation and transport *in vivo*.[5] This, together with the cytotoxic and antineoplastic properties observed for a number of these compounds,[4] has inspired work towards their synthesis, usually *via* a linear approach followed by a macrocyclisation step.[1-4] We now wish to report the preparation of some novel thiazole-based analogues of natural cyclic peptides using a concise high-yielding cyclooligomerisation procedure from appropriate amino acid substituted thiazole precursors.



1



2



3

Thus, the fully protected amino acid thiazoles **4** were first prepared using a modified Hantzsch synthesis.[6,7] Saponification of the thiazole esters, **4** with NaOH next gave the corresponding carboxylic acids **5**, which were then immediately subjected to amine deprotection using a 4 M solution of HCl in dioxane, leading to the amino acids **6** as their hydrochloride salts in essentially quantitative yield.[8]

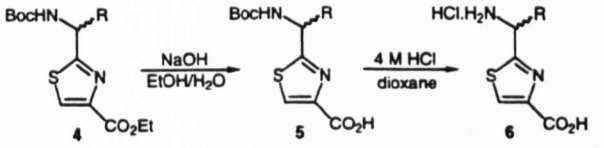
Cyclooligomerisation experiments with **6** were performed initially using the D-valine derived thiazole **6a**. Cyclooligomerisation of **6a** was attempted using a number of peptide coupling reagents (Table 1) and pentafluorophenyl diphenylphosphinate (FDPP) was found to give remarkably consistent high yields (80 - 96 %) of cyclic products.[9] The optimum concentration for this cyclooligomerisation reaction was found to be in the range 10 - 50 mM (Table 1). At lower concentrations (1 mM) the yield was markedly lower (60 %) whilst at higher concentrations (0.5 M) the formation of insoluble polymeric products was found to increase.

Table 1 Cyclooligomerisation reactions of **6a**.

Coupling Reagent	Concentration	Yield of Cyclic Products
DPPA[a]	0.05 M	47 %
DPPCl[b]	0.05 M	55 %
EDC[c]	0.05 M	43 %
FDPP[d]	0.5 M	60 %
FDPP	0.1 M	62 %
FDPP	0.05 M	91 %
FDPP	0.01 M	93 %
FDPP	0.005 M	60 %

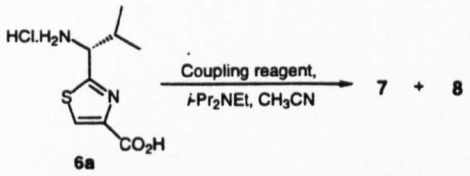
a) diphenyl phosphorazidate; b) diphenylphosphinyl chloride;
c) 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride;
d) pentafluorophenyl diphenylphosphinate.

Under the optimum conditions, *i.e.* yields > 80 %, the major isolated cyclic products resulting from the cyclooligomerisation of **6a** were found to be the cyclic trimer **7** and the cyclic tetramer **8** which were produced in a 5:2 ratio



a, R = D-CH(CH$_3$)$_2$; b, R = D,L-CH(CH$_3$)$_2$; c, R = D-CH$_2$Ph; d, R = D,L-CH$_2$Ph.

(measured by ^{1}H nmr and HPLC).[10] Trace amounts of higher oligomers (pentamer, hexamer, heptamer, octamer and nonamer) were also observed by FAB mass spectrometry, although these compounds were not isolated. The ratio of the trimer **7** and the tetramer **8** did not vary significantly when the reaction was carried out at different concentrations. The ^{1}H nmr spectra of the separated cyclic peptides **7** and **8** in $CDCl_3$ at 22 °C indicated that they were C_3 and C_4 symmetric, respectively. The ^{1}H nmr spectra of **7** and **8** were similar, although the signal attributable to the amide protons in **7** is 0.6 ppm further downfield from that in **8**, suggesting that intramolecular hydrogen bonding may be stronger in **7** than in **8**. The vicinal $^3J_{NHCH}$ values of 9.3 Hz and 9.1 Hz for **7** and **8**, respectively correspond to dihedral angles of $150° < \theta < 180°$ in both the macrocycles.[11]

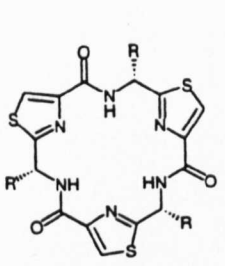


X-ray quality crystals of **7** were obtained by slow diffusion of diethyl ether into a dichloromethane solution of the cyclic peptide. The x-ray structure indicates that **7** is a rigid molecule in which all of the nitrogen atoms point towards the centre of the macrocycle and the valine side chains all lie on the same face of the molecule and adopt axial positions.[12] The NHαCH dihedral angles of the three amide linkages in **7** were found to be between 159 and 167° which is consistent with the values determined by ^{1}H nmr spectroscopy. This correlation suggests that the cyclic tetramer **8** is also a flat molecule in which the thiazole units form the corners of a square with all side chains on the same face of the molecule.[13]

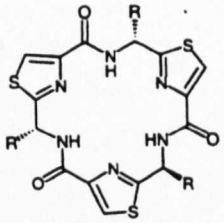
The successful outcome of the cyclooligomerisation of **6a** prompted us to investigate further reactions with other thiazole amino acid based monomers. When the racemic thiazole **6b** was subjected to the same cyclooligomerisation reaction, a statistical mixture of diastereomeric trimers, **7** and **9**, and tetramers, **8**, **10**, **11** and **12** was obtained. This mixture was separated by preparative HPLC and the relative stereochemistry of each of the pure compounds was assigned on the basis of their symmetry as determined by ^{1}H nmr. The overall yield of the cyclic products **7** - **12** was 75 % and the trimer:tetramer ratio was found to be 1:1, which is substantially different to that observed using the homochiral thiazole precursor **6a**. This difference may reflect the relative ease of cyclisation of linear precursors containing a mixture of D- and L-amino acid residues.[14]

The cyclooligomerisations of the phenylalanine derived thiazoles **6c** and **6d** were also studied. Interestingly, when the homochiral precursor **6c** was subjected to the cyclooligomerisation conditions, none of the expected cyclic trimer **13** was produced, although a small amount of its diastereomer **14** (21 %) together with the expected tetramer **15** (10 %) and a diastereomeric tetramer **16** (23 %) were obtained. The formation of both **14** and **16** can be attributed to the presence of the opposite enantiomer (enantiomeric excess as determined by the formation of a Mosher's amide derivative = 60 %)[15] in the starting material. When the racemic phenylalanine derived thiazole **6d** was subjected to the cyclooligomerisation reaction a non-statistical mixture of the trimer **14** and the diastereomeric tetramers **15** - **18** was isolated. The major product isolated was the tetramer **16** where alternating phenylmethyl side chains were positioned on opposite faces of the macrocycle. The overall trimer:tetramer ratio (1:1) observed in this cyclooligomerisation was substantially different to that obtained for the enantiomerically enriched thiazole **6c** (1:3), suggesting that the unsymmetrical trimer **14**, in which one of the side chains lies on the opposite face of the macrocycle is formed more readily than its C_3 symmetrical analogue **13**, where all of the side chains must lie on the same face of the macrocycle. This outcome may be a result of the steric congestion imposed upon **13** by the rigid backbone of the cyclic trimer. We are currently investigating the trimer:tetramer ratio obtained when thiazole monomers bearing different side chains are subjected to the cyclooligomerisation reaction, to determine whether steric congestion is responsible for this difference.

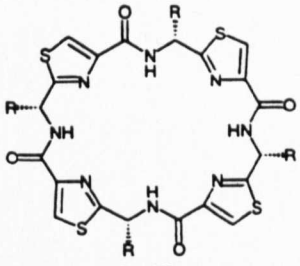
The cyclooligomerisation of an oxazoline based amino acid monomer has previously been employed in the formation of the C_3 symmetric natural product westelliamide (or cyclooxazoline) together with a C_4 symmetric analogue,[13] and in contemporaneous studies the synthesis of a number of thiazole containing symmetrical macrocycles *via* the cyclooligomerisation of a thiazole containing tetrapeptide has been reported.[16] However, in both of these examples the yields of cyclic oligomers obtained were found to be less than 50 %. We have now shown that the cyclooligomerisations of amino acid substituted thiazole monomers can be achieved in high yields (typically >80 %), thereby providing rapid access to large amounts of novel and unusual cyclic peptide analogues. Applications of this reaction to other heterocyclic monomers for the purpose of preparing libraries of novel modified cyclic peptides are now in progress in our laboratories. These macrocycles will then be examined to determine their conformations, biological activity and metal chelating properties together with their potential applications as templates for synthetic receptors or scaffolds for the development of macromolecular devices.



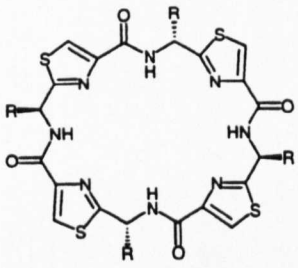
7 R = *i*-Pr
13 R = CH_2Ph



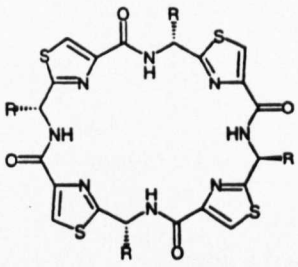
9 R = *i*-Pr
14 R = CH_2Ph



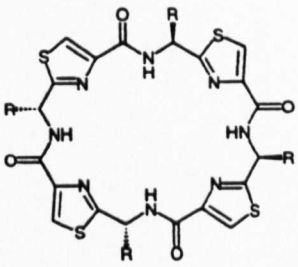
8 R = *i*-Pr
15 R = CH_2Ph



10 R = *i*-Pr
16 R = CH_2Ph



11 R = *i*-Pr
17 R = CH_2Ph



12 R = *i*-Pr
18 R = CH_2Ph

Acknowledgement

We thank the EPSRC for a studentship (to JSH), and Astra Charnwood, SmithKline Beecham (CASE award to JSH) and Pfizer Ltd for their support of this work.

References and Notes

(1) a) V. Admi, U. Afek and S. Carmeli, *J. Nat. Prod.*, 1996, **59**, 396; b) D. J. Freeman and G. Pattenden, *Tetrahedron Lett.*, 1998, **39**, 3251.

(2) a) B. M. Degnan, C. J. Hawkins, M. F. Lavin, E. J. McCaffery, D. L. Parry, A. L. van den Brenk and D. J. Watters, *J. Med. Chem.*, 1989, **32**, 1349; b) F. J. Schmitz, M. B. Ksetbati, J. S. Chang, J. L. Wang, M. B. Hossain, D. van der Helm, M. H. Engel, A. Serban and J. A. Silfer, *J. Org. Chem.*, 1989, **54**, 3463; c) C. D. J. Boden and G. Pattenden, *Tetrahedron Lett.*, 1995, **36**, 6153.

(3) a) Y. Hamamoto, M. Endo, M. Nagawaka, T. Nakanishi and K. Mizukawa, *J. Chem. Soc., Chem. Commun.*, 1983, 323; b) Y. Hamada, S. Kato and T. Shioiri, *Tetrahedron Lett.*, 1985, **26**, 3223.

(4) For reviews on the isolation, structure and synthesis of the *Lissoclinum* cyclic peptides see a) P. Wipf, in *Alkaloids: Chemical and Biological Perspectives*, ed. S. W. Pelletier, Elsevier, Amsterdam, 1998, vol 12., p. 187; b) P. Wipf, *Chem. Rev.*, 1995, **95**, 2115.

(5) J. P. Michael and G. Pattenden, *Angew. Chem., Int. Ed. Engl.*, 1993, **32**, 1.

(6) a) M. W. Bredenkamp, C. W. Holzapfel and W. J. Zyl, *Synth. Commun.*, 1990, **20**, 2235; b) E. Aguilar and A. I. Meyers, *Tetrahedron Lett.*, 1994, **35**, 2473; c) C. D. J. Boden, G. Pattenden and T. Ye, *Synlett*, 1995, 417.

(7) C. J. Moody and M. C. Bagley, *J. Chem. Soc., Perkin Trans. 1*, 1998, 601.

(8) All new compounds gave satisfactory spectroscopic data together with high resolution mass spectrometric and/or microanalytical data.

(9) The following general cyclooligomerisation procedure is described: Diisopropylethylamine (3 eq.) and FDPP (1.5 eq.) were added to a suspension of **6** (2 mmol) in anhydrous acetonitrile (42 mL) and the solution was stirred at ambient temperature for 18 h before evaporating to dryness *in vacuo*. The residue was partitioned between ethyl acetate (50 mL) and aq. HCl (2 M, 50 mL) and the separated organic layer was washed with aq. HCl (2 M, 50 mL). The combined aqueous solutions were back extracted with ethyl acetate (50 mL) and then the organic solutions were combined and washed successively with aq. NaOH (1 M, 2 x 50 mL), H_2O (50 mL) and brine (50 mL). The solution was dried ($MgSO_4$) and the solvent was then removed under reduced pressure to leave a mixture of cyclic peptide products which were separated by column chromotography (silica gel) or by preparative HPLC (Dynamax Silica Gel Cartridge Column, 30 cm x 10 mm internal diameter).

(10) Spectroscopic data for **7**: mp 258 - 260 °C (from Et_2O); $[\alpha]^{29}_D$ +126.8 (c = 0.53, $CHCl_3$); δ_H (360 MHz, $CDCl_3$) 8.45 (3H, d, *J* 9.3, 3 x N*H*), 8.1 (3H, s, 3 x C*H*=C), 5.4 (3H, dd, *J* 9.3 and 5.8, 3 x NHC*H*), 2.3 (3H, m, 3 x C*H*$(CH_3)_2$), 1.1 (9H, d, *J* 6.8, 3 x CH_3), 1.0 (9H, d, *J* 6.8, 3 x CH_3); δ_C (90 MHz, $CDCl_3$) 168.6 (C=O), 159.7 (Cq), 149.1 (Cq), 123.4 (CH), 55.4 (CH), 35.3 (CH), 18.8 (CH_3), 18.3 (CH_3); *m/z* (ES) 569 $(M^+ Na)^+$; Found C, 52.5; H, 5.6; N, 15.0 %. $C_{24}H_{30}N_6O_3S_3$ requires C, 52.7; H, 5.5; N, 15.0 %.
Spectroscopic data for **8**: mp 152 - 154 °C (from Et_2O); $[\alpha]^{29}_D$ +204.6 (c = 0.57, $CHCl_3$); δ_H (360 MHz, $CDCl_3$) 8.0 (4H, s, 4 x C*H*=C), 7.85 (4H, d, *J* 9.1, 4 x N*H*), 5.2 (4H, dd, *J* 9.1 and 8.2, 4 x NHC*H*), 2.6 (4H, m, 4 x C*H*$(CH_3)_2$), 1.2 (12H, d, *J* 6.7, 4 x CH_3), 1.0 (12H, d, *J* 6.6, 4 x CH_3); δ_C (90 MHz, $CDCl_3$) 169.3 (C=O), 160.3 (Cq), 148.9 (Cq), 124.2 (CH), 55.3 (CH), 32.6 (CH), 19.6 (CH_3), 18.9 (CH_3); *m/z* (ES) 751 $(M + Na)^+$, 1479 $(2M + Na)^+$; Found C, 50.9; H, 5.5; N, 14.6 %. $C_{32}H_{40}N_8O_4S_4.0.5\ H_2O$ requires C, 50.9; H, 5.7; N, 14.8 %.

(11) V. F. Bystrov, V. T. Ivanov, S. L. Portnova, T. A. Balshova and Y. A. Ovchinnikov, *Tetrahedron*, 1973, **29**, 873.

(12) We thank Dr A. J. Blake of this Department for this information. Full details will be presented in a forthcoming full paper.
(13) P. Wipf and C. P. Miller, *J. Am. Chem. Soc.*, 1992, **114**, 10975.
(14) K. D. Kopple, *J. Pharm. Sci.*, 1972, **61**, 1345.
(15) J. A. Dale, D. L. Dull and H. S. Mosher, *J. Org. Chem.*, 1969, **34**, 2543.
(16) N. Sokolenko, G. Abbenante, M. J. Scanlon, A. Jones, L. R. Gahan, G. R. Hanson and D. P. Fairlie, *J. Am. Chem. Soc.*, 1999, **121**, 2603.

Article Identifier:
1437-2096,E;1999,0,11,1723,1726,ftx,en;L12799ST.pdf

SYNLETT Accounts and Rapid Communications in Synthetic Organic Chemistry **REPRINT**

With Compliments of the Author

Thieme

Self-Assembly of Amino Acid-Based Thiazoles and Oxazoles. Total Synthesis of Dendroamide A, a Cyclic Hexapeptide from the Cyanobacterium *Stigonema dedroideum*

Anna Bertram, Gerald Pattenden*

School of Chemistry, The University of Nottingham, Nottingham, England NG7 2RD, UK

Fax +44 (0) 115951 3564; E-mail: gp@nottingham.ac.uk

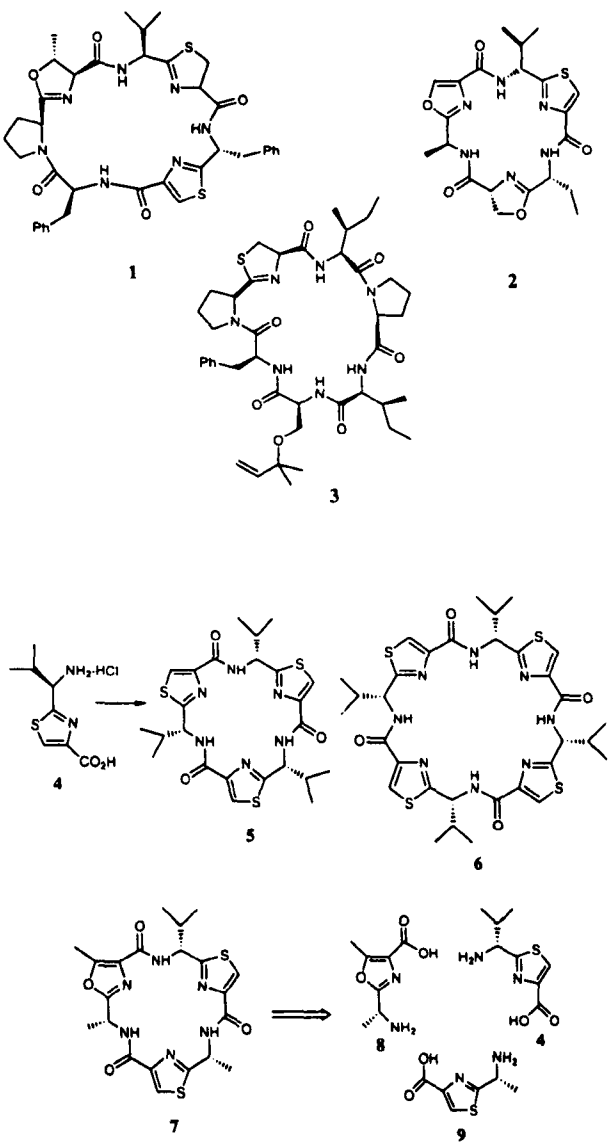
Received 3 August 2000

Abstract: Treatment of a solution containing a 1:1:1 mixture of the chiral thiazole and oxazole-based amino acids **4**, **8** and **9** with pentafluorodiphenylphosphinate in diisopropyethylamine in acetonitrile at room temperature for 2 h resulted in the formation of dendroamide A **7** (23%) and its positional isomer **15** (22%), together with symmetrical thiazole-based macrocyclic trimers in a combined yield of 30%. An independent, linear, synthesis of dendroamide A confirmed the structure and stereochemistry assigned to this natural cyclic hexapeptide.

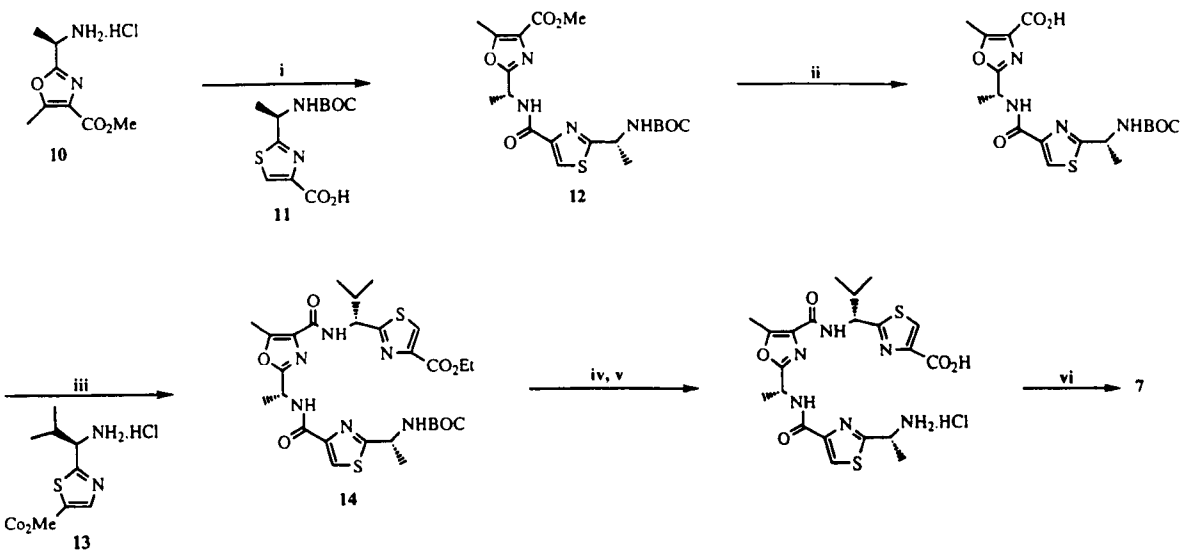
Key words: self-assembly, cyclopeptides, dendroamide A.

The marine environment has delivered an amazing variety of structurally novel and unusual peptidic alkaloids with macrocyclic structures accommodating thiazole and oxazole rings in recent years;[1] representative examples are lissoclinamide 4 (**1**),[2] raocyclamide **2**[3] and mollamide **3**.[4] The characteristic sequence of alternating heterocyclic and amino acid residues present in these compounds is clearly reminiscent of macrocyclic ligands such as azacrown ethers and extended porphyrins, and this has led to speculation that this class of marine metabolite is capable of metal complexation and transport in vivo.[5] It is also possible that metal ions provide a template for the biological assembly of these cyclopeptide metabolites, or even the interesting biological activity observed for several of their number. However, we know very little about the 'order' of the assembly of the amino acid units and the configurational and conformation requirements of acyclic peptide precursors during cyclopeptide biosynthesis. In the case of thiazole and oxazole based marine cyclopeptides we do not even know whether they are produced from preformed oxazole/thiazole based amino acid residues, or whether these heterocyclic rings are elaborated in vivo from substituted serine/cysteine intermediates following macrocyclisation to the cyclopeptide core.

It was against this background that recently we examined the cyclooligomerisations of several valine and phenylalanine-based thiazole amino acids which were found to lead to the formation of cyclic trimers and cyclic tetramers in remarkably high yields e.g. **4** → **5**+**6** (85%).[6] We have now extended these studies and investigated the scope for the 'self-assembly' of thiazole and oxazole based cyclopeptides from more than one heterocycle based amino acid. In this Letter we summarise our first excursions in this area, with a synthesis of dendroamide A (**7**) from the valine and alanine-based thiazoles, **4** and **9** and the alanine-based oxazole **8**.

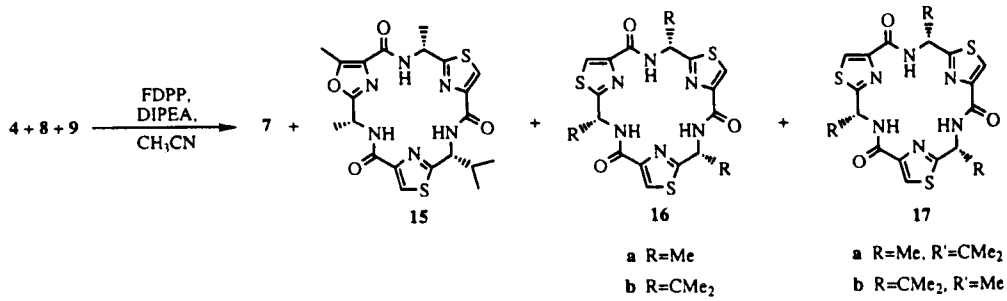


Dendroamide A (**7**) is a bistratamide type cyclic hexapeptide isolated from the cyanobacterium *Stigonema dendroideum* which exhibits multidrug-resistance reversing



Reagents: i, EDC, HOBt, NMM, DCM, 10h, rt, 80%; ii, NaOH, THF, H_2O, 10h, rt, 97%; iii, EDC, HOBt, NMM, DCM, 10h, rt, 70%; iv, NaOH, THF, H_2O, 10h, rt, 98%; v, 4M HCl, dioxane, 2h, rt, 96%; vi, FDPP, DIPEA, CH_3CN, 91%.

Scheme



activity.[7] Its structure is made up of the units **4** (from *D*-valine and cysteine), **9** (from *D*-alanine and cysteine), and **8** (from *D*-alanine and threonine). As a prelude to our self-assembly investigations we first synthesised dendroamide A totally, in a linear fashion, from the methyl ester **10**, the BOC amine **11**, and the methyl ester **13** and proceeding via the oxazole-thiazole **12** and the oxazole *bis*-thiazole **14** as shown in the Scheme. The synthetic material had chiroptical and NMR* spectroscopic data which were found to be identical to those reported for the natural product isolated from *S. dendroideum*.[8]

When a solution containing a 1:1:1 mixture of the heterocyclic amino acids **4**, **8**, and **9** in acetonitrile was treated with pentafluorodiphenylphosphinate (FDPP) and diisopropylethylamine (DIPEA), chromatography led to the isolation of dendroamide A **7** (23%) together with its positional isomer (**15**, 22%) accompanied by the products **16** and **17** resulting from homo-coupling of the thiazole amino acids **4** and **9** in a combined yield of 30%.[9] The structures of these compounds followed from their independent synthesis, using a linear approach similar to that shown in Scheme for dendroamide A, and also from NMR and X-ray cyrstallographic analysis.[10] Investigations are now in progress to optimise and discipline the self-assembly of the amino-acids **4**, **8** and **9** alongside related systems, to produce alternative novel heterocyclic-based cyclic peptides, and to evaluate certain of their number in asymmetric synthesis and in the construction of novel molecular arrays. These results will be published in due course.

Acknowledgement

We thank AstraZeneca and Pfizer Ltd for financial assistance in support of this project, and Dr K A. Joliffe for helpful discussions.

References and Notes

(1) For reviews, see: a) Faulkner, D. J., *J. Nat. Prod.* **2000**, *17*, 1; b) Davidson, B. S., *Chem. Rev.* **1993**, *93*, 1771.

(2) a) Degnan, B. M.; Hawkins, C. J.; Lavin, M. F.; McCaffrey, E. J.; Parry, D. L.; van den Brenk, A. L.; Watters, D. J., *J. Med. Chem.* **1989**, *32*, 1349; b) Chang, J. S.; van der Helm, D.; Hossain, M. B.; Ksebati, M. B.; Schmitz, F. J.; Wang, J. L., *J. Org. Chem.* **1989**, *54*, 3463; c) Boden, C. D. J.; Pattenden, G., *J. Chem. Soc., Perkin Trans. 1* **2000**, 875.

(3) a) Admi, V.; Afek, U.; Carmeli, S., *J. Nat. Prod.* **1996**, *59*, 396; b) Freeman, D. J.; Pattenden, G., *Tetrahedron Lett.* **1998**, *39*, 3251.

(4) a) Bowden, B. F.; Carroll, A. R.; Coll, J. C.; Hockless, D. C. R.; Skelton, B. W.; White, A. H., *Aust. J. Chem.* **1994**, *47*, 61; b) McKeever, B.; Pattenden, G., *Tetrahedron Lett.* **1999**, *40*, 9317.

(5) Michael, J. P.; Pattenden, G., *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 1.

(6) a) Bertram, A.; Hannam, J. S.; Jolliffe, K. A.; González-Lopez de Turisó, F.; Pattenden, G., *Synlett* **1999**, *11*, 1723. b) see also accompanying paper Blake, A. J.; Hannam, J. S.; Jolliffe, K. A.; Pattenden, G., *Synlett* **2000**, 1515.

(7) Moore, R. E.; Ogino, J.; Patterson, G. M.l.; Smith, C. D., *J. Nat. Prod.* **1996**, *59*, 581.

(8) Satisfactory spectroscopic and mass spectrometry data were obtained for all new compounds. Spectroscopic data for synthetic **7**: m.p. 146-148 °C (from CH_2Cl_2); $[\alpha]_D^{296}$+53.9 (c = 0.2, $CHCl_3$); λ_{max}(MeOH)/nm 224 (ε/dm^{3}mol^{-1}cm^{-1} 28,000); IR(cm^{-1}): 3396, 1666, 1546; δ_H (360 MHz, CD_2Cl_2) 8.61 (1H, d, J = 7.4 Hz), 8.52 (1H, d, J = 7.5 Hz), 8.44 (1H, d, J = 7.4 Hz), 8.11 (1H, s), 8.09 (1H, s), 5.68 (1H, dq, J = 8.1 and 6.8 Hz), 5.32 (1H, masked dd), 5.14 (1H, quintet, J = 6.6 Hz), 2.64 (3H, s), 2.27 (1H, m), 1.66 (3H, d, J = 6.7 Hz), 1.66 (3H, d, J = 6.7 Hz), 1.03 (3H, d, J = 6.8 Hz), 0.96 (3H, d, J 6.7 Hz); δ_C (360 MHz, CD_2Cl_2) 11.7 (q), 18.3 (q), 18.5 (q), 21.1 (q), 25.1 (q), 35.6 (d), 44.6 (d), 47.4 (d), 56.2 (d), 123.8 (d), 124.1 (d), 128.9 (s), 149.2 (s), 149.3 (s), 154.0 (s), 159.7 (s), 160.0 (s), 160.7 (s), 162.2 (s), 168.9 (s), 171.8 (s); HRMS (ES) m/z 511.1169; calcd for $C_{21}H_{24}O_4N_6S_2Na$ ([M+Na]$^+$): 511.1198.

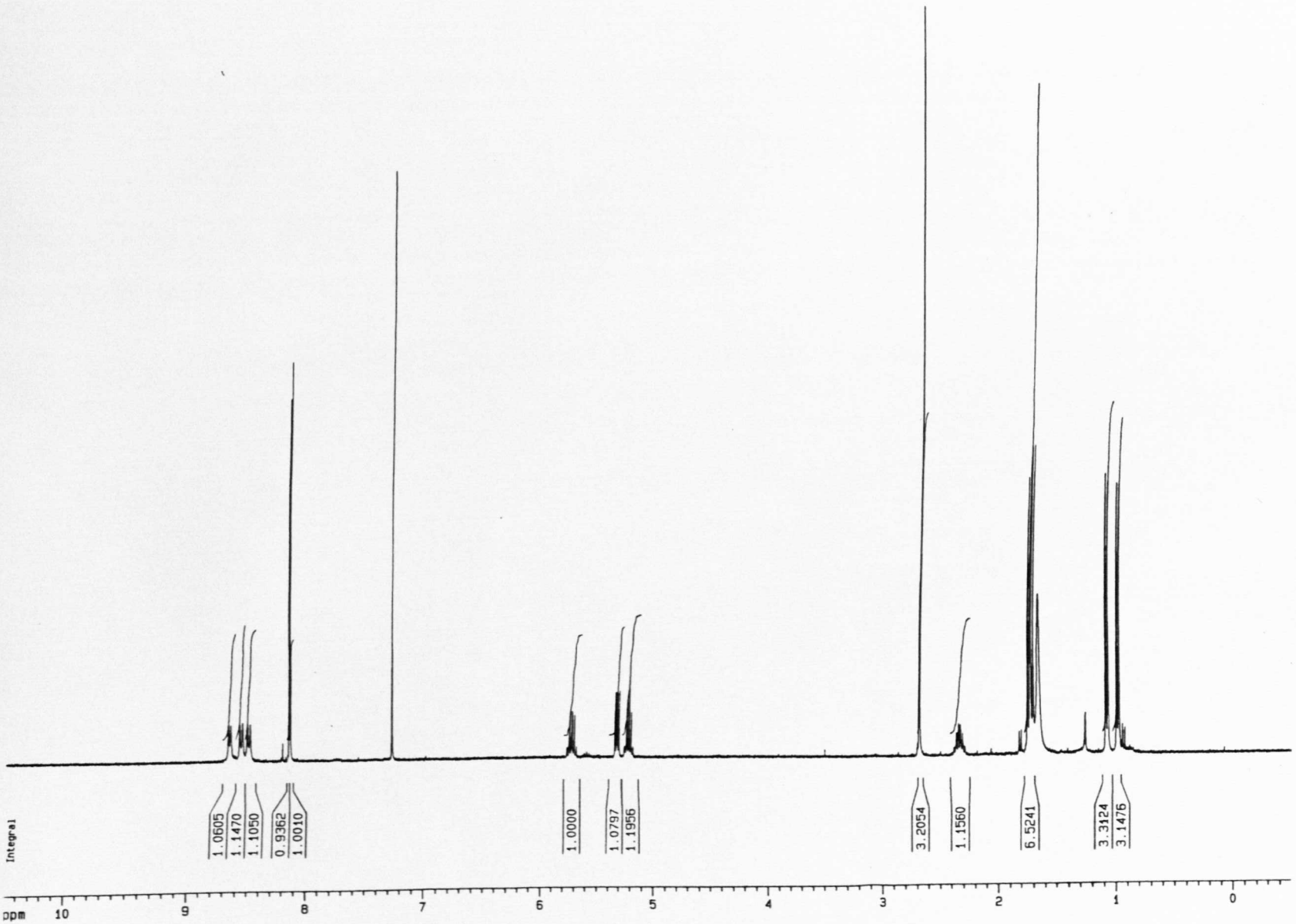
(9) There is a possibility of 11 symmetrical and unsymmetrical thiazole/oxazole cyclopeptides being produced from **4**, **8**, and **9**, i.e. the statistical yield of each cyclopeptide product would be 6.8% based on 75% yield.

(10) We thank Dr A. J. Blake of this School for these data which will be published separately in the full paper.

Article Identifier:
1437-2096,E;2000,0,10,1519,1521,ftx,en;L13800ST.pdf

Appendix 4.2 *- Spectroscopic Data for Dendroamide A*

Dendroamide



Current Data Parameters

NAME	se30ab
EXPNO	5
PROCNO	1

F2 - Acquisition Parameters

Date_	990930
Time	16.54
INSTRUM	spect
PROBHD	5 mm Dual 13
PULPROG	zg30
TD	32768
SOLVENT	CDCl3
NS	16
DS	2
SWH	4310.345 Hz
FIDRES	0.131541 Hz
AQ	3.8011379 sec
RG	912.3
DW	116.000 usec
DE	4.50 usec
TE	300.0 K
D1	1.00000000 sec

========== CHANNEL f1 ==========

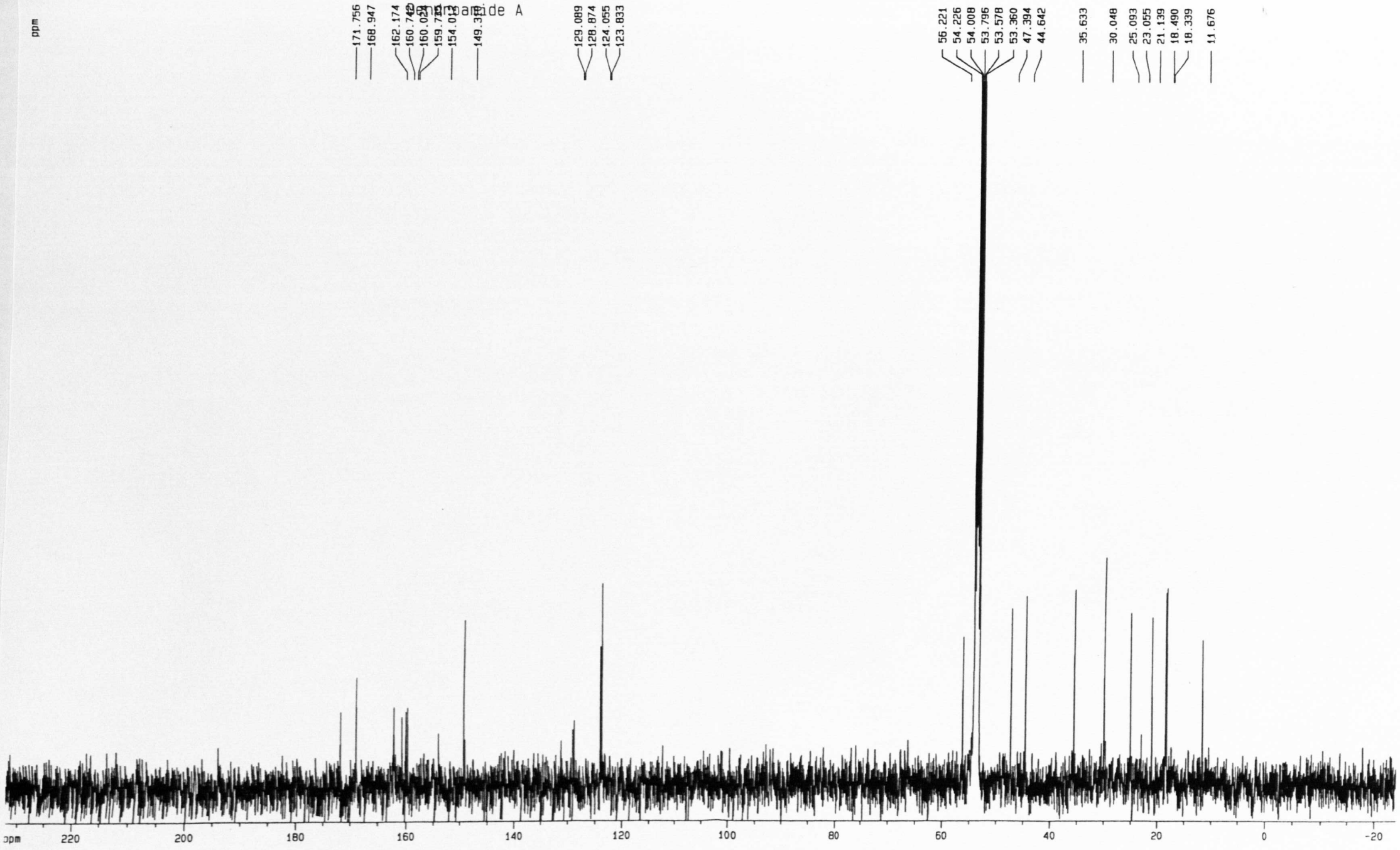
NUC1	1H
P1	9.80 usec
PL1	-6.00 dB
SFO1	360.1319807 MHz

F2 - Processing parameters

SI	16384
SF	360.1300100 MHz
WDW	EM
SSB	0
LB	0.00 Hz
GB	0
PC	1.00

1D NMR plot parameters

CX	30.00 cm
F1P	10.500 ppm
F1	3781.36 Hz
F2P	-0.500 ppm
F2	-180.07 Hz
PPMCM	0.36667 ppm/cm
HZCM	132.04767 Hz/cm


ppm
171.756
168.947
162.174
149.358
129.089
128.874
124.055
123.833
56.221
54.226
54.008
53.796
53.578
53.360
47.394
44.642
35.633
30.048
25.093
23.055
21.139
18.490
18.339
11.676
220
200
180
160
140
120
100
80
60
40
20
0
-20

REFERENCES

References

1. Fleming, A, *Brit. J. Exp. Path.*, 1929, **10**, 226.
2. Crowfoot, D.; Bunn, C. W.; Rogers-Low, B. W.; Turner-Jones, A., in *The Chem. of Penicillin*, Princeton University Press, Princeton, NJ., 1949, 310.
3. a) Sheehan, J. C.; Henery-Logan, K. R., *J. Am. Chem. Soc.*, 1957, **79**, 1262, b) Sheehan, J. C.; Henery-Logan, K. R., *ibid.*, 1959, **81**, 3089.
4. Wani, M. C.; Taylor, H. L.; Wall, M. E.; Coggon, P.; McPhail, A. T., *J. Am. Chem. Soc.*, 1971, **93**, 2325.
5. Nicolaou, K. C.; Dai, W. -M.; Guy, R. K., *Angew. Chem., Int. Ed. Engl.*, 1994, **33**, 15.
6. a) Nicolaou, K. C.; Yang, Z.; Liu, J. -J.; Ueno, H.; Nantarmet, P. G.; Guy, R. K.; Claiborne, C. F.; Renaud, J.; Couladouros, E. A.; Paulvannan, K.; Sorensen, E. J., *Nature*, 1994, **367**, 630; b) Nicolaou, K. C.; Nantermet, P. G.; Ueno, H.; Guy, R. K.; Couladouros, E. A.; Sorensen, E. J., *J. Am. Chem. Soc.*, 1995, **117**, 624; c) Nicolaou, K. C.; Liu, J. -J.; Yang, Z.; Ueno, H.; Sorensen, E. J.; Claiborne, C. F.; Guy, R. K.; Hwang, C. -K.; Nakada, M.; Nantermet, P. G., *J. Am. Chem. Soc.*, 1995, **117**, 634; d) Nicolaou, K. C.; Yang, Z.; Liu, J. -J.; Nantermet, P. G.; Clairborne, C. F.; Renaud, J.; Guy, R. K.; Shibayama, K., *J. Am. Chem. Soc.*, 1995, **117**, 645; e) Nicolaou, K. C.; Ueno, H.; Liu, J. -J.; Nantermet, P. G.; Yang, Z.; Renaud, J.; Paulvannan, K.; Chadha, R.; f) Nicolaou, K. C.; Guy, R. K., *Angew. Chem.*, 1995, **107**, 2047. *Angew. Chem. Int. Ed. Engl.*, 1995, **34**, 2079.
7. Holton, R. A.; Somoza, C.; Kim, K. B.; Liang, F.; Biediger, R. J.; Boatman, P. D.; Shindo, M.; Smith, C. C.; Kim, S.; Nadizadeh, H.; Suzuki, Y.; Tao, C.; Vu, P.; Tang, S.; Zhang, P.; Murthi, K. K.; Gentile, L. N.; Liu, J. H., *J. Am. Chem. Soc.*, 1994, **116**, 1597; b) Holton, R. A.; Kim, K. B.; Somoza, C.; Liang, F.; Biediger, R. J.; Boatman, P. D.; Shindo, M.; Smith, C. C.; Kim, S.; Nadizadeh,

H.; Suzuki, Y.; Tao, C.; Vu, P.; Tang, S.; Zhang, P.; Murthi, K. K.; Gentile, L. N.; Liu, J. H., *J. Am. Chem. Soc.*, 1994, **116**, 1599.

8. Rinehart, K. L.; Gloer, J. B.; Cook, J. C.; Mizsak, S. A.; Scahill, T. A., *J. Am. Chem Soc.*, 1981, **103**, 1857.

9. Gloer, J. B; Bozich, F. A; Kishore, V.; Lake, R. J.; Maleczka, R. E.; Munro, M. H. G.; Nagarajan, S.; Rinehart, K. L.; Sakai, R.; Sullins, D. W.; Todsen, W. L.; Li, K. -M., *J. Am. Chem. Soc.*, 1987, **109**, 6846.

10. Carroll, A. R.; Bowden, B. F.; Coll, J. C.; Hockless, D. C. R.; Skelton, B. W.; White, A. H., *Aust. J. Chem.*, 1994, **47**, 61.

11. Bourne, D. J.; Bowden, B. F.; Carroll, A. R.; Coll, J. C.; Ireland, C. M.; MacLeod, J. K.; Zabriskie, T. M., *Aust. J. Chem.*, 1996, **49**, 659.

12. McKeever, B.; Pattenden, G., *Tetrahedron Lett.*, 1999, **40**, 9317.

13. Uto, Y.; Wipf, P., *J. Org. Chem.*, 2000, **65**, 1037.

14. Ireland, C. M.; Durso, A. R.; Newman, R. A.; Hacker, M. P., *J. Org. Chem.*, 1982, **47**, 1807.

15. Ireland, C.; Scheuer, P. J., *J. Am. Chem. Soc.*, 1980, **102**, 5688.

16. Hamamoto, Y.; Endo, M.; Nakagawa, M.; Nakanishi, T.; Mizukawa, K, *Chem. Commun.*, 1983, 323.

17. Davidson, B. S., *Chem. Rev.*, 1993, **93**, 1771.

18. a) Wasylyk, J. M.; Biskupiak, J. E.; Costello, C. E.; Ireland, C. M., *J. Org. Chem.*, 1983, **48**, 4445.

b) Degnan, B. M.; Hawkins, C. J.; Lavin, M. F.; McCaffrey, E. J.; Parry, D. L.; van den Brenk, A. L.; Watters, D. J., *J. Med. Chem.*, 1989, **32**, 1349.

c) Schmitz, F. J.; Ksebati, M. B.; Chang, J. S.; Wang, J. L.; Hossain, M. B.; van der Helm, D.; Engel, M. H.; Serban, A.; Silfer, J. A., *J. Org. Chem.*, 1989, **54**, 3463

d) Hawkins, C. J.; Lavin, M. F.; Marshall, K. A.; Van den Brenk, A. L.; Watters, D. J., *J. Med. Chem.*, 1990, **33**, 1634.

19. Degnan, B. M.; Hawkins, C. J.; Lavin, M. F.; McCaffrey, E. J.; Parry, D. L.; Watters, D. J., *J. Med. Chem.*, 1989, **32**, 1354.

20. Foster, M. P.; Concepcion, G. P.; Caraan, G. B.; Ireland, C. M., *J. Org. Chem.*, 1992, **57**, 6671.

21. Hambley, T. W.; Hawkins, C. J.; Lavin, M. F.; van den Brenk, A.; Watters, D. J., *Tetrahedron*, 1992, **48**, 341.

22. Prinsep, M. P.; Moore, R. E.; Levine, I. A.; Patterson, G. M. L., *J. Nat. Prod.*, 1992, **55**, 140.

23. Ogino, J.; Moore, R. E.; Patterson, G. M. L.; Smith, C. D., *J. Nat. Prod.*, 1996, **59**, 581.

24. Todorova, A. K.; Jüttner, F.; Linden, A.; Plüss, T.; von Philipsborn, W., *J. Org. Chem.*, 1995, **60**, 7891.

25. Admi, V.; Afek, U.; Carmeli, S., *J. Nat. Prod.*, 1996, **59**, 396.

26. Fusetani, N.; Hashimoto, K.; Kato, Y.; Matsunaga, S., *J. Am. Chem. Soc.*, 1986, **108**, 2780.

27. Evans, D. A.; Gage, J. R.; Leighton, J. L.; *J. Am. Chem. Soc.*, 1992, **114**, 9434.

28. Filla, S. A.; Masamune, S., *Angew. Chem., Int. Ed. Engl.*, 1994, **33**, 673.

29. Smith, A. B., III; Friestad, G. K.; Duan, J. J.-W.; Barbosa, J.; Hull, K. G.; Iwashima, M.; Qiu, Y.; Spoors, P. G.; Bertounesque, E.; Salvatore, B. A., *J. Org. Chem.*, 1998, **63**, 7596.

30. Jansen, R.; Kunze, B.; Wray, V.; Reichenbach, H.; Juriewic, Z. E.; Hunsmann, G.; Holfe, G., *Liebigs Ann. Chem.*, 1991, 707.

31. a) Ishibashi, Y.; Ohba, S.; Nishiyama, S.; Yamamura, S., *Tetrahedron Lett.*, 1996, **37**, 2997.; b) Garey, D.; Ramirez, M.; Gonzales, S.; Wertsching, A.; Tith, S.; Keefe, K.; Pena, M. R., *J. Org. Chem.*, 1996, **61**, 4853.

32. Searle, P. A.; Molinski, T. F., *J. Am. Chem. Soc.*, 1995, **117**, 8126.

33. Forsyth, C. J.; Ahmed, F.; Cink, R. D.; Lee, C. S., *J. Am. Chem. Soc.*, 1998, **120**, 5597.

34. Evans, D. A.; Cee, V. J.; Smith, T. E.; Fitch, D. M.; Cho, P. S., *Angew. Chem. Int. Ed.*, 2000, **39**, 2533; b) Evans, D. A.; Fitch, D. M., *Angew. Chem. Int. Ed.*, 2000, **39**, 2536.

35. a) Cocito, C., *Microbiol. Rev.*, 1979, **43**, 145; b) Paris, J. M.; Barriére, J. C.; Smith, C.; Bost, P. E., In *Recent Progress in the Chemical Synthesis of Antibiotics*, Lucas, G.; Ohno, M., Eds, Springer Verlag, Berlin 1990, 183.

36. Lam, Y. K.; Bogen, D.; Chang, R. S.; Faust, K. A.; Hensens, O. D.; Zink, D. L.; Schwarz, C. D.; Zitano, L.; Garrity, G. M.; Gagliardi, M. M.; Currie, S. A.; Woodruff, H. B., *J. Antibiot.*, 1991, **44**, 613.

37. Schlessinger, R. H.; Li, Y-J., *J. Am. Chem. Soc.*, 1996, **118**, 3301.

38. Tavares, F.; Lawson, J. P.; Meyers, A. I., *J. Am. Chem. Soc.*, 1996, **118**, 3303.

39. Entwistle, D. A.; Jordon, S. I.; Montgomery, J.; Pattenden, G., *Synthesis*, 1998, 603.

40. Ichiba, T.; Yoshida, W. Y.; Scheuer, P. J., *J. Am. Chem. Soc.*, 1991, **113**, 3173.

41. Wipf, P.; Lim, S., *J. Am. Chem. Soc.*, 1995, **117**, 558.

42. Williams, D. R.; Brooks, D. A.; Berliner, M. A., *J. Am. Chem. Soc.*, 1999, **121**, 4924.

43. Nagatsu, A.; Kajitami, H.; Sakakibara, J., *Tetrahedron Lett.*, 1995, **36**, 4097.

44. Wipf, P.; Venkatraman, S., *J. Org. Chem.*, 1996, **61**, 6517.

45. Muir, J. C.; Pattenden, G.; Thomas, R. M., *Synthesis*, 1998, 613.

46. a) Kernan, M. R.; Molinski, T. F.; Faulkner, D. J., *J. Org. Chem.*, 1988, **53**, 5014; b) Matsunaga, S.; Fusetani, N.; Hashimoto, K.; Koseki, K.; Noma, M.; Noguchi, H.; Sankawa, U., *ibid.*, 1989, **54**, 1360.

47. Matsunaga, S.; Fusetani, N.; Hashimoto, K.; Koseki, K.; Noma, M., *J. Am. Chem. Soc.*, 1986, **108**, 847.

48. Fusetani, N.; Yasumuro, K.; Matsunaga, S.; Hashimoto, K., *Tetrahedron Lett.*, 1989, **30**, 2809.

49. Roesener, J. A.; Scheuer, P. J., *J. Am. Chem. Soc.*, 1986, **108**, 846.

50. Chattopadhyay, S. K.; Pattenden, G., *Tetrahedron Lett.*, 1998, **39**, 6095.

51. Kempson, J.; Pattenden, G., *Synlett*, 1999, 533.

52. Carmeli, S.; Moore, R. E.; Patterson, G. M. L.; Corbetti, T. H.; Valeriote, F. A., *J. Am. Chem. Soc.*, 1990, **112**, 8195.

53. Jansen, R.; Kunze, B.; Reichenback, H.; Jurkiewicz, E.; Hunsmann, G.; Höfle, G., *Liebigs. Ann. Chem.*, 1992, 357.

54. Pattenden, G.; Boyce, R. S., *Synlett*, 1994, 587.

55. Boyce, R. J.; Mulqueen, G. C.; Pattenden, G., *Tetrahedron*, 1995, **51**, 7321.

56. Ehrler, J.; Farooq, S., *Synlett*, 1994, **9**, 702.

57. Heathcock, C. H.; Walker, M. A., *J. Org. Chem.*, 1992, **57**, 5566.

58. Parsons, R. L.; Heathcock, C. H., *J.Org. Chem.*, 1994, **59**, 4733.

59. a) Wipf, P.; Venkatraman, S., *J. Org. Chem.*, 1995, **60**, 7224; b) Wipf, P.; Venkatraman, S., *Synlett*, 1997, 1.

60. Abbenante, G.; Fairlie, D. P.; Gahan, L. R.; Hanson, G. R.; Pierens, G. K.; van den Brenk, A. L., *J. Am. Chem. Soc.*, 1996, **118**, 10384.

61. Chang, J. S; Hossain, M. B.; Schmitz, F. J.; van der Helm, D.; Ksebati, M. B.; Wang, J. L., *J. Org. Chem.*, 1989, **54**, 3463.

62. Mocek, U.; Zeg, Z.; O'Hagan, D.; Zhou, P.; L-D, G, Fan.; Beale, J. M.; Floss, H. G., *J. Am. Chem. Soc.*, 1993, **115**, 7992.

63. Astanina, A. N.; Ismailova, M. A.; Offengenden, E. Y.; Rudenko, A. P.; Yakubov, H. M., *Inorg. Chim. Acta.*, 1983, **79**, 284.

64. Garson, M. J., *Nat. Prod.*, 1989, **6**, 143.

65. Okami, Y., *Pure Appl. Chem.*, 1982, **54**, 1951.

66. Crews, P.; Inman, W.; McDowell, R., *J. Org. Chem.*, 1989, **54**, 2523.

67. Fu, X.; Kelly-Borges, M.; Schmitz, F. J.; Taner, R.S., *J. Nat. Prod.*, 1998, **61**, 384.

68. van den Brenk, A.L.; Fairlie, D. P.; Gahan, L. R.; Hanson, G. R.; Hawkins, C.J.; Jones, A., *Inorg. Chem.*, 1994, **33**, 2280.

69. van Den Brenk, A. L.; Byriel, K. A.; Fairlie, D. P.; Gahan, L. R.; Hanson, G. R.; Hawkins, C. J.; Jones, A.; Kennard, C. H. L.; Moubaraki, B.; Murray, K. S., *Inorg. Chem.*, 1994, **33**, 3549.

70. Abbenante, G.; Fairlie, D. P.; Gahan, L. R.; Grøndahl, L.; Hanson, G. R.; Sololenko, N., *Dalton Trans*, 1999, 1227.

71. van den Brenk, A. L.; Fairlie, D. P.; Gahan, L. R.; Hanson, G. R.; Hambley, T. W., *Inorg. Chem.*, 1996, **35**, 1095.

72. Drake, A. F.; Freeman, D. J.; Pattenden, G.; Siligardi, G., *J. Chem. Soc. Perkin Trans.*, 1998, 129.

73. Geib, S. J.; Miller, C. P.; Venkatraman, S.; Wipf, P., *Angew. Chem. Int. Ed. Engl.*, 1994, **33**, 1516.

74. Blake, A. J.; Hannam, J. S.; Jolliffe, K. A.; Pattenden, G., *Synlett*, **2000**, In Press

75. Holzapfel, C. W.; Pettit, G. R., *J. Org. Chem.*, 1985, **50**, 2323.

76. Houssain, R.; Lohez, M.; Bernier, J.; Hemichart, J., *J. Org. Chem.*, 1985, **50**, 2787.

77. Scheibye, S.; Peterson, B. S.; Lawesson, S. O., *Bull. Soc. Chim. Belg.*, 1978, **87**, 229.

78. Schmidt, U.; Gleich, P.; Gleisser, H.; Utz, R., *Synthesis*, 1986, 992.

79. Kelly, R. C.; Gebhard, I.; Wicnienski, N., *J. Org. Chem.*, 1986, **51**, 4590.

80. Bredankemp, M. W.; Holzapfel, C. W.; Vanzyl, W. J., *Syn. Comm*, 1990, **20**, 2235.

81. Aguilar, E.; Meyers, A. I., *Tetrahedron Lett.*, 1994, **35**, 2473.

82. Aguilar, E.; Meyers, A. I., *Tetrahedron Lett.*, 1994, **35**, 2477.

83. Houck, D. R.; Chen, L. C.; Keller, P. J.; Beale, J. M.; Floss, H. G., *J. Am. Chem. Soc.*, 1988, **110**, 5800.

84. Mocek, U.; Zeng, Z.; O'Hagan, D.; Zhou, P.; Fan, L. D. G.; Beale, J. M.; Floss, H. G., *J. Am. Chem. Soc.*, 1993, **115**, 7992.

85. Hirotsu, Y.; Shiba, T.; Kaneko, T., *Bull. Chem. Soc. Japan*, 1967, **40**, 2945.

86. North, M.; Pattenden, G., *Tetrahedron*, 1990, **46**, 8267.

87. Boyce, R. S.; Pattenden, G., *Synlett*, 1994, 587.

88. Boyce, R. S.; Mulqueen, G. C.; Pattenden, G., *Tetrahedron*, 1995, **51**, 7321.

89. a) Hamada, Y.; Kohia, K.; Shioiri, T., *Tetrahedron Lett.*, 1984, **25**, 5303.; b) Hamada, Y.; Shibata, M.; Shioiri, T., *Tetrahedron Lett.*, 1985, **26**, 5159.; c) Hamada, Y.; Shibata, M.; Sugiura, T.; Kata, S.; Shioiri, T., *J. Org. Chem.*, 1987, **52**, 1252.

90. Attenburrow, J.; Elliott, D. F.; Penny, G. F., *J.Chem. Soc.*, 1948, 310.

91. Elliott, D. F., *J. Chem. Soc.*, 1949, 589, *ibid.* 1950, 62.

92. Vorbrüggen, H.; Krolikiewicz, K., *Tetrahedron*, 1993, **49**, 9353.

93. Wipf, P.; Miller, C. P., *Tetrahedron Lett.*, 1992, **33**, 6267.

94. a) Mitsunobu, O., *Synthesis*, 1981, 1.; b) Hughes, D. L., *Org. React.*, 1992, **42**, 335.

95. Atkins, G. M.; Burgess, E. M., *J. Am. Chem. Soc.*, 1968, **90**, 4744.

96. Boden, C. D. J.; Pattenden, G., *Tetrahedron Lett.*, 1995, **36**, 6153.

97. Guenot, P.; Larfargue, P.; Lellouche, J. P., *Synlett*, 1995, 171.

98. Rutt, J. E., *PhD Thesis, University of Nottingham*, 1998.

99. Evans, D. L.; Minster, D. K.; Jordis, U.; Hecht, S. M.; Mazzu, A. L.; Meyers, A. I., *J. Org. Chem.*, 1979, **44**, 497.

100. Fritch, P. C.; Miller, C. P.; Venkatraman, S.; Wipf, P., *Tetrahedron Lett.*, 1995, **36**, 6395.

101. Rawlinson, D. J.; Sosnovsky, G., *Synthesis*, 1972, 1.; *ibid.*, 1973, 567.

102. Meyers, A. I.; Tavares, F. X., *J. Org. Chem.*, 1996, **61**, 8207.

103. Williams, D. R.; Lowder, P. D.; Gu, Y. G.; Brooks, D. A., *Tetrahedron Lett.*, 1997, **38**, 331.

104. Videnov, G.; Kaiser, D.; Kempter, C.; Jung, G., *Angew. Chem. Int. Ed. Engl.*, 1996, **35**, 1503.

105. Minster, D. K.; Jordis, U.; Evans, D. L.; Hecht, S. M., *J. Org. Chem.*, 1978, **48**, 1624.

106. Mosher, H. S.; Dale, J. A., *J. Am. Chem. Soc.*, 1973, **95**, 512.

107. Bertram, A.; Hannam, J. S.; Jolliffe, K. A.; González-López de Turiso, F.; Pattenden, G., *Synlett*, 1999, 1723.

108. Solenko, N.; Abbenante, G.; Scanlon, M. J.; Jones, A.; Gahan, L. R.; Hanson, G. R.; Fairlie, D. P., *J. Am. Chem. Soc.*, 1999, **121**, 2603.

109. Ercolani, G.; Mandolini, L.; Masci, B., *J. Am. Chem. Soc.*, 1981, **103**, 2780.

110. Brady, P. A.; Sanders, J. K. M., *J. Chem. Soc., Perkin Trans. 1*, 1997, 3237.

111. a) Robinson, R., *J. Chem. Soc.*, 1909, **95**, 2167; b) Gabriel, S., *Ber.*, 1910, **43**, 1283.

112. Cornforth, J. W.; Cornforth, R. H., *J. Chem. Soc.*, 1947, 96.

113. a) Doyle, M. P.; Buhro, W. E.; Davidson, J. G.; Elliot, R. C.; Hoekstra, J. W.; Oppenhuizen, M., *J. Org. Chem.*, 1980, **45**, 3657; b) Bagley, M. C.; Buck, R. T.; Hind, S. L.; Moody, C. J., *J. Chem. Soc., Perkin Trans 1*, 1998, 591.

114. Konopelski, J. P.; Hottenroth, J. M.; Oltra, H. M.; Véliz, E. A.; Yang, Z-C., *Synlett*, 1996, 609.

115. a) Lakhan, R.; Ternai, B., *Heterocycl. Chem.*, 1974, **17**, 99; b) Vaccaro, H.; Levy, D. E.; Sawade, A.; Jaetsch, T.; Masamune, S., *Tetrahedron Lett.*, 1992, **33**, 1937.

116. Panek, J. S.; Beresis, R. T., *J. Org. Chem.*, 1996, **61**, 6496.

117. Hamada, Y.; Shibata, M.; Shioira, T., *Tetrahedron Lett.*, 1985, **26**, 6501.

118. Misra, R. N.; Brown, B. R.; Sher, P. M.; Patel, M. M.; Hall, S. F.; Han, W-C.; Barrish, J. C.; Kocy, O.; Harris, D. N.; Goldenberg, H. J.; Michael, I. M.; Schumacher, W. A.; Webbs, M. L.; Monshizadegan, H.; Ogletree, M. L., *J. Med. Chem.*, 1993, **36**, 1401.

119. Galeotti, N.; Montagne, C.; Poncet, J.; Jouin, P., *Tetrahedron Lett.*, 1996, **33**, 2807.

120. Dahuron, N.; Langois, N.; Chiaroni, A.; Riche, C., *Heterocycles*, 1996, **142**, 635.

121. a) Burrell, G.; Evans, S. M.; Jones, G. E.; Stemp, G., *Tetrahedron Lett.*, 1990, **31**, 3649; b) Wipf, P.; Miller, C. P., *Tetrahedron Lett.*, 1992, **33**, 907.

122. Nakajima, K.; Kawai, H.; Takai, M.; Okawa, K., *Bull. Chem. Soc. Jpn.*, 1977, **50**, 917.

123. Hamada, Y.; Kato, S.; Shioiri, T., *Tetrahedron Lett.*, 1985, **26**, 3223.

124. Hamada, Y.; Shibata, M.; Shioiri, T., *Tetrahedron Lett.*, 1985, **26**, 5155.

125. Freeman, D. J.; Pattenden, G., *Tetrahedron Lett.*, 1998, **39**, 3251.

126. Wipf, P.; Miller, C. P., *J. Org. Chem.*, 1993, **58**, 3604.

127. a) Dess, D. B.; Martin, J. C., *J. Am. Chem. Soc.*, 1991, **113**, 7277.; For an improved procedure for the preperation of this reagent see: b) Ireland, R. E.; Liu, L, *J. Org. Chem.*, 1993, **58**, 2899.

128. Wipf, P.; Lim, S., *J. Am. Chem. Soc.*, 1995, **117**, 558.

129. Wipf, P.; Venkatraman, S., *J. Org. Chem.*, 1996, **61**, 6517.

130. Williams, D. R.; Brooks, D. A.; Berliner, M. A., *J. Am. Chem. Soc.*, 1999, **121**, 4924.

131. Muir, J. C.; Pattenden, G.; Thomas, R. M., *Synthesis*, 1998, 613.

132. Barrish, J. C.; Singh, J.; Spergel, S. H.; Han, W. C.; Kissick, T. P.; Kronenthal, D. R.; Muetter, R. H., *J. Org. Chem.*, 1993, **58**, 4494.

133. Evans, D. A.; Guge, J. R.; Leighton, J. L.; Kim, A. S., *J. Org. Chem.*, 1992, **57**, 1961.

134. Wipf, P.; Miller, C. P., *J. Am. Chem. Soc.*, 1992, **114**, 10975.

135. Guenot, P.; Lafargue, P.; Lellouche, J. -P., *Heterocycles*, 1995, **41**, 947.

136. Miller, C. P.; Wipf, P., *Tetrahedron Lett.*, 1992, **33**, 907.

137. Phillips, A. J.; Uto, Y.; Wipf, P.; Reno, M. J.; Williams, D. R., *Organic Lett.*, 2000, **2**, 1165.

138. Bertram, A.; Pattenden, G.; Synlett, *In Press*

139. Moody, C. J.; Bagley, M. C., *J. Chem. Soc., Perkin, Trans. 1*, 1998, 601.

140. Kigoshi, H.; Yamada, S., *Tetrahedron*, 1999, 12301.

141. Kohrt, A.; Hartke, K., J. Med. Chem., 1989, **32**, 1886.

142. a) Aguilar, E.; Meyers, A. I., *Tetrahedron Lett.*, 1994, **35**, 2473; b) Schmidt, U.; Griesser, H.; Gleich, P.; Utz, R., *Synthesis*, 1986, 992; c) Kigoshi, H.; Yamada, S., *Tetrahedron*, 1999, **42**, 1230.

143. Okamura, K.; Ito, A.; Saito, H.; Nakamura, Y.; Shin, C-G., *Bull. Chem. Soc. Jpn.*, 1996, **69**, 2309.

144. Cross, D. F. W., *J. Chem. Soc.*, 1963, 2143.

145. a) Bredenkamp, M. W.; Holzapfel, C. W.; Snyman, R. M.; van Zyl, W. J., *Synth. Commun.*, 1992, **22**, 3029; b) Brain, C. T.; Hallett, A.; Ko, S. Y., *J. Org. Chem.*, 1997, **62**, 3808.

146. a) Meyers, A. I.; Tavares, F. X., , *J. Org. Chem.*, 1996, **61**, 8207; b) Pozdnev, V. F., *Tetrahedron Lett.*, 1995, **36**, 7115.

147. Houssain, R.; Lohez, M.; Bernier, J. -L.; Hénichart, J. -P., *J. Org. Chem.*, 1985, **50**, 2787.

148. Cross, D. F.W.; Kenner, G. W.; Sheppard, R. C.; Stehr, C. E., *J. Chem. Soc.*, 1963, 2143.

149. Downing, S. V.; Aguilar, E.; Meyers, A. I., *J. Org. Chem.*, 1999, **64**, 826.

150. Williams, D. R.; Lowder, P. D.; Gu, Y. G.; Brooks, D. A., *Liebigs Ann. Chem.*, 1968, 201.

151. Bagley, M. C.; Buck, R. T.; Hind, S. L.; Moody, C. J., *J. Chem. Soc. Perkin Trans. 1.*, 1998, 591.