Applications of the Bohlmann-Rahtz Reaction in the Synthesis of Thiopeptide Antibiotics

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This thesis is dedicated to Dr. M. A. Ledlie.

ABSTRACT

Chemical degradation studies on micrococcin P1 have established that the stereochemistry of the valine residue in the natural product is *S* and not *R* as previously assigned in the literature. This confirms Ciufolini's hypothesis that the Bycroft-Gowland structure of MP1 differs from the natural product on stereochemical grounds.

A range of conditions for the synthesis of amino acid-derived thiazoles have been investigated and previously reported conditions found to be unreliable. Evidence has been provided to suggest that racemisation in the Hantzsch thiazole synthesis occurs in the elimination step of the reaction. The peptide backbone of micrococcin P1 has been prepared in 10 steps and 44% overall yield, incorporating *S*-valine based on the results of our degradation studies.

A microwave-assisted method for the conversion of sterically hindered β-ketoesters to enamines has been devised, which is suitable for single- and multimode instruments, and for scale-up to produce multigram quantities of material. The central heterocyclic domain of the micrococcin thiopeptides has been prepared in 15 steps and 9% overall yield *via* a highly convergent strategy incorporating the Bohlmann-Rahtz pyridine synthesis and featuring an efficient two-directional thiazole elaboration.

The micrococcin thiopeptide macrocycle has been synthesised in 20 steps and 4% overall yield. Three micrococcin P1 side chain dipeptides have been prepared, each incorporating different protecting groups, enabling a range of different approaches to the natural product to be investigated.

HPLC analysis of degradation products from newly isolated cyclothiazomycin thiopeptides and comparison with synthetic material has provided evidence that the alanine-derived residue in the cyclothiazomycin core domain is of *S* stereochemistry, and not *R* as previously assigned in the literature.

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ABBREVIATIONS

aa	Amino acid
Ac	Acetyl
Alloc	Allyloxycarbonyl
AOP	(7-azabenzotriazol-1-yl)oxytris-(dimethylamino)phosphonium hexafluorophosphate
APCI	Atmospheric Pressure Chemical Ionization
aq	Aqueous
Ar	Unspecified aryl substituent
Arg	Arginine
Asn	Asparagine
Boc	*tert*–Butoxycarbonyl
Boc_2O	Di-*tert*-butyldicarboxylate
Bn	Benzyl
BOI	2-(Benzotriazol-1-yl)oxy-1,3-dimethylimidazolinium hexafluorophosphate
BOP	Benzotriazol-1-yl-dimethylaminophosphonium hexafluorophosphate
bp	Boiling point
Bpoc	2-(4-Biphenylyl)isopropoxycarbonyl
BroP	Bromotris(dimethylamino)phosphonium hexafluorophosphate
Bu	Butyl
BuLi	Butyl lithium
c	Concentration
cat.	Catalytic/catalyst
CBMIT	1,1'-Carbonylbis(3-methylimidazolium) triflate
Cbz	Benzyloxycarbonyl
CDI	1,1'-Carbonyldiimidazole
CI	Chemical Ionisation
CIP	2-Chloro-1,3-dimethylimidazolinium hexafluorophosphate
CloP	Chlorotris(dimethylamino)phosphonium hexafluorophosphate
COSY	Correlation Spectroscopy
DAA	2,4-Dinitrophenyl-(*S*)-alaninamide
DAST	Diethylaminosulphur-trifluoride
Dba	Dibenzylidineacetone
DBU	1,8–Diaza[5.4.0]undec–7–ene
DCC	Dicyclohexylcarbodiimide
DCE	1,2-Dichloroethane

DDQ	Dichlorodicyanobenzoquinone
DEPC	Diethylcyanophosphonate
DEPT	Distorntionless enhancement by polarisation transfer
DHAA	Dehydrohomoalanine
DIPEA	Diisopropylethylamine
DMAP	*N,N*–Dimethylaminopyridine
DME	1,2-Dimethoxyethane
DMF	*N,N*–Dimethylformamide
DMP	Dimethoxypropane
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DPPA	Diphenylphosphoryl azide
EDCI	1-[3-(Dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride
ee	Enantiomeric excess
EI	Electron Impact
Enz	Enzyme
eq.	Equivalent
equiv.	Equivalent
ES	Electrospray
Et	Ethyl
exch.	Exchange
FAB	Fast Atom Bombardment
FDAA	1-Fluoro-2,4-dinitrophenyl-5-(*S*)-alanine amide
FDPP	Pentafluorophenyl diphenylphosphinate
Fmoc	9-Fluorenylmethoxycarbonyl
FTIR	Fourier Transform Infra Red
g	Grams
GC-MS	Gas xhromatography mass spectrometry
GDP	Guanosine-5'-diphosphate
Gln	Glutamine
Glu	Glutamic acid
GTP	Guanosine-5'-triphosphate
h	Hour(s)
HATU	*O*-(7-Azabenzotriazol-1-yl)-*N*, *N*, *N'*, *N'*-tetramethyluronium hexafluorophosphate
HBTU	*O*-(Benzotriazol-1-yl)-*N*, *N*, *N'*, *N'*-tetramethyluronium hexafluorophosphate

HOAt	1-Hydroxy-7-azabenzotriazole
HOBt	1-Hydroxybenzotriazole
HOCt	Ethyl 1-hydroxy-1,2,3-triazole-4-carboxylate
HPLC	High pressure liquid chromatography
HMBC	Heteronuclear multiple bond correlation
HMPA	Hexamethylphosphoramide
HRMS	High resolution mass spectrometry
HSQC	Heteronuclear single quantum coherence
Hz	Hertz
IBX	*o*-Iodoxybenzoic acid
IC_{50}	Concentration of an inhibitor required for 50% inhibition of an enzyme *in vitro*
IR	Infra Red
J	Coupling constant (in Hz)
kg	Kilograms
L	Laevorotatory
LDA	Lithium diisopropylamine
Leu	Leucine
lit.	Literature
LRMS	Low Resolution Mass Spectrometry
Lu	Lutidine
M	Molar
MALDI	Matrix-Assisted Laser Desorption
MDa	Megadaltons
Me	Methyl
mg	Milligrams
µg	Micrograms
MHz	Megahertz
min	Minutes
MinC	Minimum induction Concentration
mL	Millilitres
µL	Microlitres
mM	Millimolar
µM	Micromolar
Mol	Mole
mp	Melting point
mRNA	Messenger ribonucleic acid

MRSA	Methicillin resistant *Staphylococcus aureus*
MS	Mass Spectrometry
Ms	Mesyl (methanesulfonyl)
MW	Microwave
NAD	Nicotinamide adenine dinucleotide
NIS	*N*–Iodosuccinimide
nM	Nanomolar
nm	Nanometre
NMR	Nuclear Magnetic Resonance
nOe	Nuclear Overhauser effect
NOSEY	Nuclear Overhauser and Exchange Spectroscopy
p	*Para*
PG	Protecting group
Ph	Phenyl
PhMe	Toluene
PP	Pyrophosphate
ppm	Parts per million
PPTS	Pyridinium *p*–toluenesulphonate
Pr	Propyl
Pro	Proline
p-Tol	*p*-Tolyl
PTSA	*p*–Toluenesulphonic acid
py	Pyridine
PyAOP	[(7-Azabenzotriazol-1-yl)oyx]tris-(pyrrolidino)phosphonium hexafluorophosphate
PyBOP®	Benzotriazol-1-yl-oxytris-pyrrolidinophosphonium hexafluorophosphate
PyBroP	Bromotri(pyrrolidino)phosphonium hexafluorophosphate
PyCloP	Chlorotri(pyrrolidino)phosphonium hexafluorophosphate
quant.	Quantitative
R	Specified substituent
rac	Racemic
R_f	Retention factor
RNA	Ribonucleic acid
RNAP	Ribonucleic acid polymerase
rRNA	Ribosomal ribonucleic acid
ROESY	Rotating-frame Overhauser Enhancement Spectroscopy

r.t.	Room temperature
Ser	Serine
Silica/SiO_2	Merck Kieselgel 60 H silica or Matrex silica 60
TATU	*O*-Benzotriazol-1-yl-1,1,3,3-tetramethyluronium tetrafluoroborate
TBAF	Tetra-*n*-butylammonium fluoride
TBDMS	*tert*–Butyldimethylsilyl
TBS	*tert*–Butyldimethylsilyl
TBTU	*O*-(7-Azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate
TEAP	Triethylammonium phosphate
Teoc	(Trimethylsilyl)ethoxycarbonyl
Tert	Tertiary
TES	Triethylsilyl
Tf	Triflate (trifluoromethanesulfonate)
TFA	Trifluoroacetic acid
TFAA	Trifluoroacetic anhydride
TFFH	Tetramethylfluoroformamidinium hexafluorophosphate
TFP	Tri(2-furyl)phosphane
THF	Tetrahydrofuran
Thr	Threonine
Tip	Thiostrepton-induced protein
TLC	Thin Layer Chromatography
TMS	Trimethylsilyl
TMSE	2-(Trimethylsilyl)ethyl
TOF	Time of Flight
tRNA	Transfer ribonucleic acid
Ts	Tosyl (*para*-toluenesulfonyl)
Tyr	Tyrosine
UV	Ultraviolet
Val	Valine
vs.	Versus
W	Watts
2D	2-Dimensional

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Chapter One – Introduction

The Thiopeptide Antibiotics

1 – The Thiopeptide Antibiotics

1.1 – Introduction

The thiopeptide antibiotics are naturally occurring, sulfur containing, highly modified, macrocyclic peptides, nearly all of which inhibit protein synthesis in bacteria.[1] These complex natural products, grouped as thiazolyl peptides for Bérdy's classification of antibiotics according to chemical structure,[2] share a number of common structural features: a tri- or tetrasubstituted nitrogen heterocycle clustered in a central polyazole domain that is part of a macrocyclic framework consisting of modified heterocyclic residues, including thiazoles, oxazoles and indoles, and dehydroamino acids. These biologically active substances are secondary metabolites produced by actinomycetes, Gram-positive mycelial sporulating bacteria, largely of the genus *Streptomyces* that can be subdivided into 29 different antibiotic families containing well over 76 structurally distinct entities. In spite of their chemical and taxonomical diversity, many of them broadly seem to share a similar biological profile, displaying almost no activity against Gram-negative bacteria, whereas against Gram-positive bacteria they are highly active inhibitors of protein synthesis and are, in many cases, effective against methicillin resistant *Staphylococcus aureus* (MRSA), a bacterial strain that is resistant to most conventional treatments. This biological property, as well as an increased understanding of their mode of action and the failure of traditional therapies to counter the emergence of bacterial resistance, has led to renewed interest in this antibiotic class.

1.2 – Classification by Central Heterocyclic Domain

The individual chemical identity of structurally distinct compounds isolated from natural sources and classified as thiopeptide or thiazolylpeptide antibiotics is relatively diverse. In spite of many similarities in biosynthetic origin, these secondary metabolites have been isolated from a number of different strains of actinomycetes, predominantly soil bacteria but also from marine sources, and elicit a wide range of different biological responses. The complexity of the many mechanisms employed by pathogens to avoid intoxication, and used by these metabolites in order to inhibit bacterial protein synthesis, are only now coming to light, facilitated by advances in crystallography, NMR spectroscopy and our understanding of *Streptomyces* transcriptional mechanisms and ribosomal function. Although further research may reveal that many of these processes are interrelated, at present most can be attributed to specific regions or motifs in metabolite structure particular to certain families or groups of families related by functional group commonality. For this reason, classification of thiopeptide antibiotics by structure, in particular in the nature of the central heterocyclic domain, also categorises their biological properties and is useful for highlighting structural relationships between the 29 different antibiotic families identified

to date. Examining the structure of individual thiopeptides reveals that there are five distinct classes of these natural products assigned according to the oxidation state of the central heterocyclic domain. Each class can be further subdivided into families that group cyclic peptides with a high degree of structural homology or in some cases that were isolated from the same antibiotic producing organism (Table 1).

Table 1 Thiopeptide antibiotic families classified according to their central heterocyclic domain

Series *a* and *b*	Series *c*	Series *d*	Series *e*
Bryamycin (A-8506)[a]	Sch 40832	A10255	Glycothiohexide α
Sch 18640 (68-1147)		Amythiamicin	MJ347-81F4
Siomycin		Berninamycin	Multiomycin[b]
Thiactin[a]		Cyclothiazomycin	Nocathiacin
Thiopeptin		GE2270	Nosiheptide
Thiostrepton		GE37468	S-54832
		Geninthiocin	
		Methylsulfomycin	
		Micrococcin	
		Promoinducin	
		Promothiocin	
		QN3323	
		Radamycin	
		Sulfomycin	
		Thioactin	
		Thiocillin	
		Thiotipin	
		Thioxamycin	
		YM-226 183-4	

[a] Shown to be identical to thiostrepton; [b] Shown to be identical to nosiheptide

Hensens first suggested grouping thiopeptide antibiotics according to the structure and oxidation state of the central heterocyclic domain, in order to distinguish between different constituents of the thiopeptins (**2**).[3] This classification system has since been extended to describe the five distinct heterocyclic domains, representative examples of which are illustrated in Figure 1. The parent of the thiopeptide antibiotics, thiostrepton (**1**), is classified as a '*b*' series thiopeptide, whereas some of the thiopeptin factors (**2**) possess the fully reduced '*a*' series central domain, tetrasubstituted dehydropiperidine or saturated piperidine heterocycles, respectively. The related '*c*' series of thiopeptides to date only consists of a single antibiotic, Sch 40832 (**3**), and has an unusual imidazopiperidine core. With increasing unsaturation, in line with Hensens' classification, by far the most prolific thiopeptide class is the 'series *d*' antibiotics, possessing a 2,3,6-trisubstituted pyridine domain. This domain is shared by 19 different families including the first thiopeptide to be isolated, micrococcin P1 (**4**). Finally 'series *e*' thiopeptides such as nosiheptide (**5**), exhibit a structurally related central motif, oxidised in comparison with their series *d* counterparts, containing a tetrasubstituted hydroxypyridine.

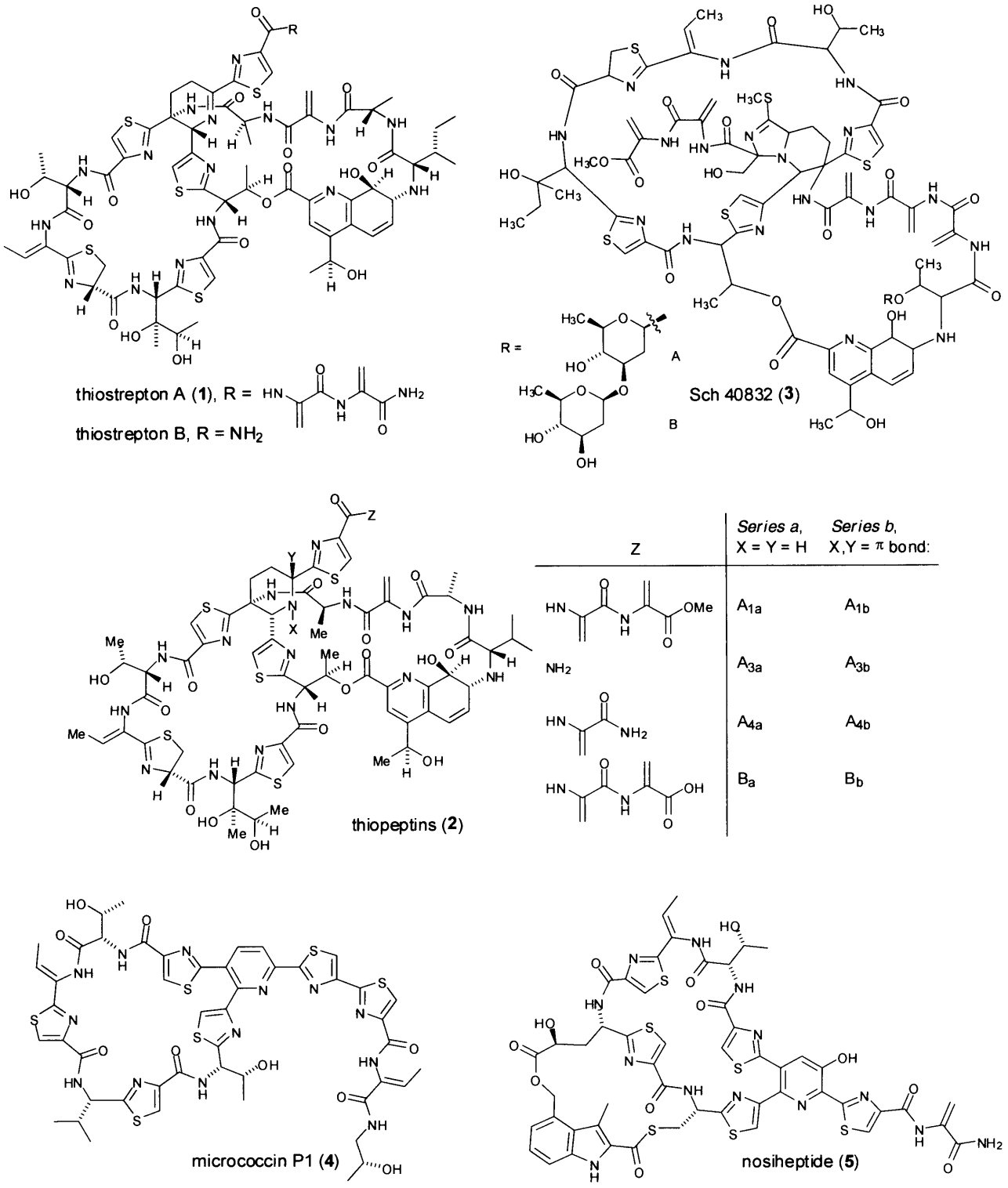


Figure 1 Representative examples of the five thiopeptide families

1.3 – Biological Properties

1.3.1 – Biological Activity

The thiopeptide antibiotics largely inhibit the growth of Gram-positive bacteria, although the activity of some of these metabolites as antifungal or anticancer agents, against Gram-negative

bacteria, as renin inhibitors or against *Plasmodium falciparum*, the malaria parasite, has also been reported. In spite of considerable structural homology the site and mode of action for these antibiotics actually varies in different thiopeptide families and can be categorised, broadly, into two classes: those that bind to a region of the 23S ribosomal RNA (rRNA) known as the L11 binding domain (L11BD) and those that bind to a protein (Ef-Tu) complex involved in the elongation cycle.

The antibacterial activity of the thiopeptide antibiotics *in vitro* is comparable to that of the penicillins, with little or no adverse toxicological effects in mammalian cells, disrupting protein synthesis in the bacterial cell's protein factory, the ribosome. Prokaryotic and eukaryotic ribosomes interpret the information in messenger RNA (mRNA) and use it to assemble the corresponding sequence of amino acids in a protein.[4] Although bacterial and mammalian ribosomes do exhibit many structural similarities, they differ considerably in size, the latter being approximately 30% larger and containing so-called expansion sequences in the rRNA as well as a number of additional ribosomal proteins. The job of the ribosome is translation, that is to read each codon of the mRNA in turn and match it with the anticodon of the corresponding transfer RNA (tRNA) bound amino acid, assembled by the respective synthetase, and thus build up the protein, residue by residue, that it encodes. All ribosomes are composed of two subunits of unequal size; the bacterial ribosome, with a relative sedimentary rate of 70S, consisting of a large 50S and a small 30S subunit. These two subunits are associated through non-covalent interactions and organise to give a ribonucleoprotein particle 2.6-2.8 MDa in size, with a diameter of 200-250 Å, that functions as a platform for bacterial protein synthesis. In *Escherichia coli* each subunit consists of proteins and rRNA fragments, the small 30S subunit containing 21 proteins (S1-S21) and a 16S rRNA strand whereas the 50S subunit comprises 34 proteins (L1-L34) and two strands of rRNA, the 23S and 5S (Figure 2).

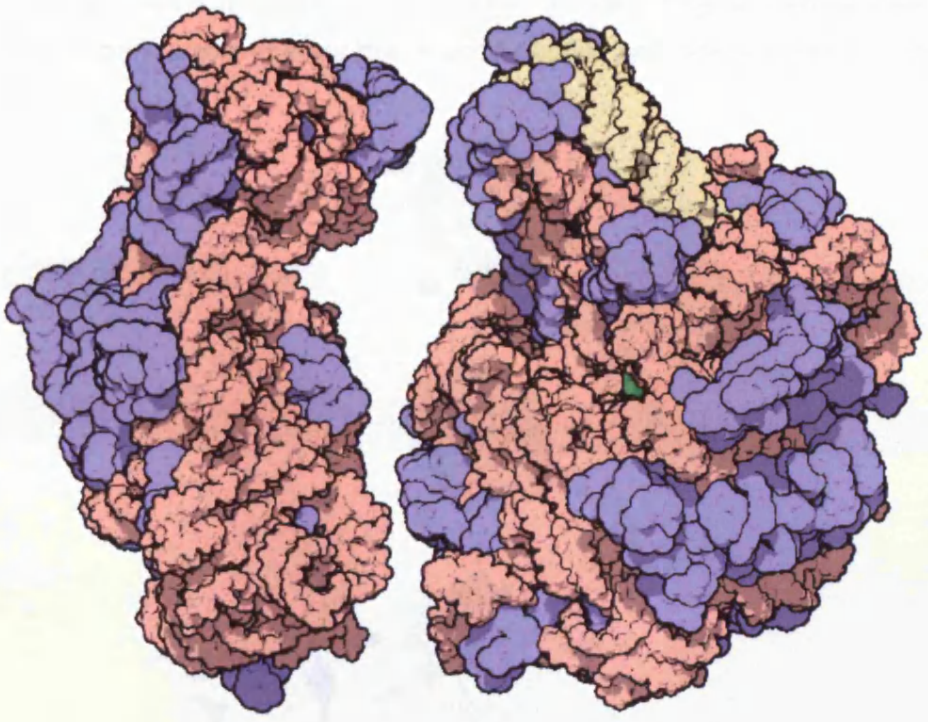
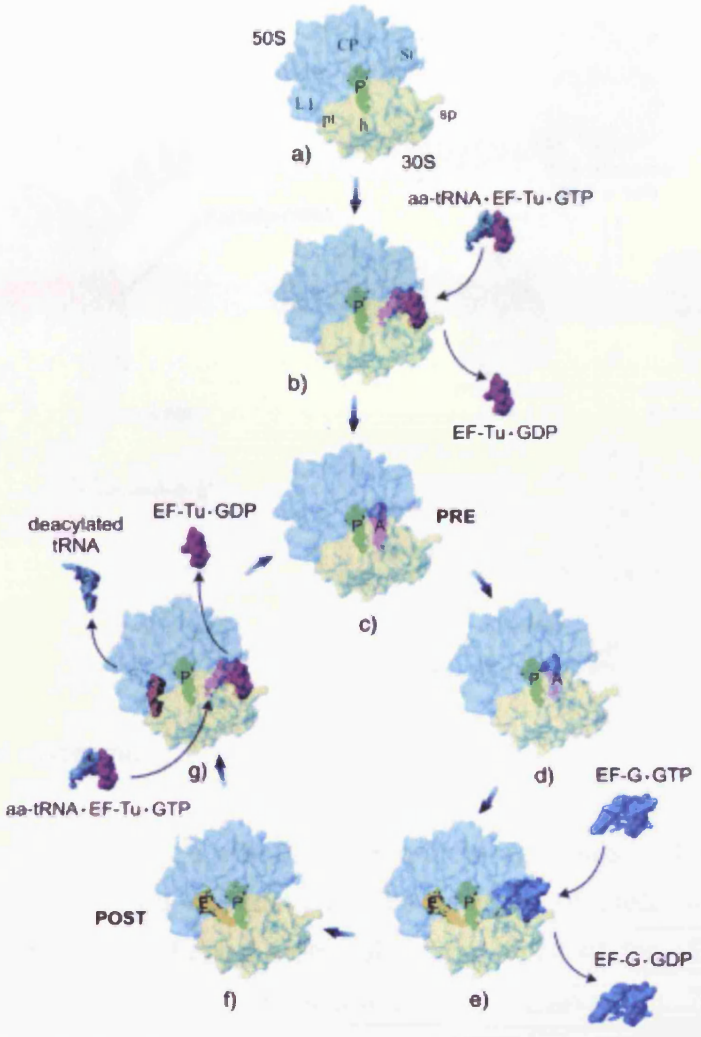


Figure 2 Composition of the 70S bacterial ribosome[5,6]

Colour key. Left: small 30S subunit; proteins S1-S21 (blue) and 16S rRNA strand (pink). Right: large 50S subunit; proteins L1-L34 (blue), 23S rRNA (pink) and 5S rRNA (yellow). Credit to David S. Goodsell of The Scripps Research Institute.

The sites on the ribosome involved in the sequential construction of the nascent protein from the individual amino acid components are denoted as the A site, where aminoacyl-tRNA (aa-tRNA) containing the next amino acid residue docks on instruction from the codon of its corresponding mRNA, the P site, where the growing peptide chain waits in readiness to form the next peptide bond, and the E site, that receives the tRNA for its exit at the end of the sequence (Scheme 1). Once bacterial protein synthesis has been initiated by interaction of the 3' end of the 16S rRNA in the 30S subunit with a complementary sequence on mRNA,[7] the initiator tRNA binds directly to the P site (Scheme 1a) and each aa-tRNA in accordance with its corresponding codon is delivered to the A site as a ternary complex (Scheme 1b) formed by combination with the elongation factor Tu (Ef-Tu) and GTP. GTP hydrolysis causes a conformational change in the ternary complex that releases Ef-Tu·GDP from the ribosome to be recycled back to Ef-Tu·GTP, leaving the aa-tRNA bound in the A site (Scheme 1c). The next peptide bond is then formed on the large ribosomal subunit by the transfer of the peptide to the A site, generating a peptidyl-tRNA whilst leaving behind its own tRNA in the P site (Scheme 1d). Translocation of the peptidyl-tRNA from the A site back to the P site is then mediated by a different elongation factor, Ef-G (Scheme 1e), which vacates the A site and moves the deacylated tRNA to the E site ready for exit (scheme 1f). When the next aa-tRNA-containing ternary complex binds (Scheme 1g), the

tRNA docked in the E site is released and the protein elongation cycle repeats itself (Scheme 1c) until protein termination factors liberate the finished peptide and dissociates the ribosome.



Scheme 1 Overview of protein translation

The small 30S subunit is depicted in yellow, the 50S subunit is depicted in blue. A, P and E denotes sites on the ribosome that can be occupied by tRNA. The A site is where aa-tRNA binds, the P site is where peptidyl-tRNA binds before peptide bond formation and the E site is the exit site for deacylated tRNA. Translation cycle consists of: (a) initiator tRNA binds in the P site; (b) aa-tRNA·Ef-Tu·GTP is delivered to the A site; (c) aa-tRNA is bound in the A site; (d) peptide is transferred to the A site with formation of next peptide bond; (e) translocation of peptidyl-tRNA from the A site back to the P site is mediated by Ef-G·GTP; (f) deacylated tRNA waits in the E-site; (g) aa-tRNA·Ef-Tu·GTP is delivered to the A site releasing the deacylated tRNA from the E site. Reprinted from ref 4. Copyright 2003 Wiley-VCH Verlag GmbH & Co. KgaA, Weinheim.

1.3.2 – Ribosomal Inhibitors

Seven different classes of antibiotics in clinical practice target the bacterial ribosome, including the aminoglycosides, tetracyclines, macrolides and streptogramins. Ribosomal inhibitors that

interact with rRNA may exhibit a favourable resistance profile in the clinic as most pathogens have multiple copies of the *rrn* operons that encode rRNA thus resistance-inducing mutations are rarely dominant.[8]

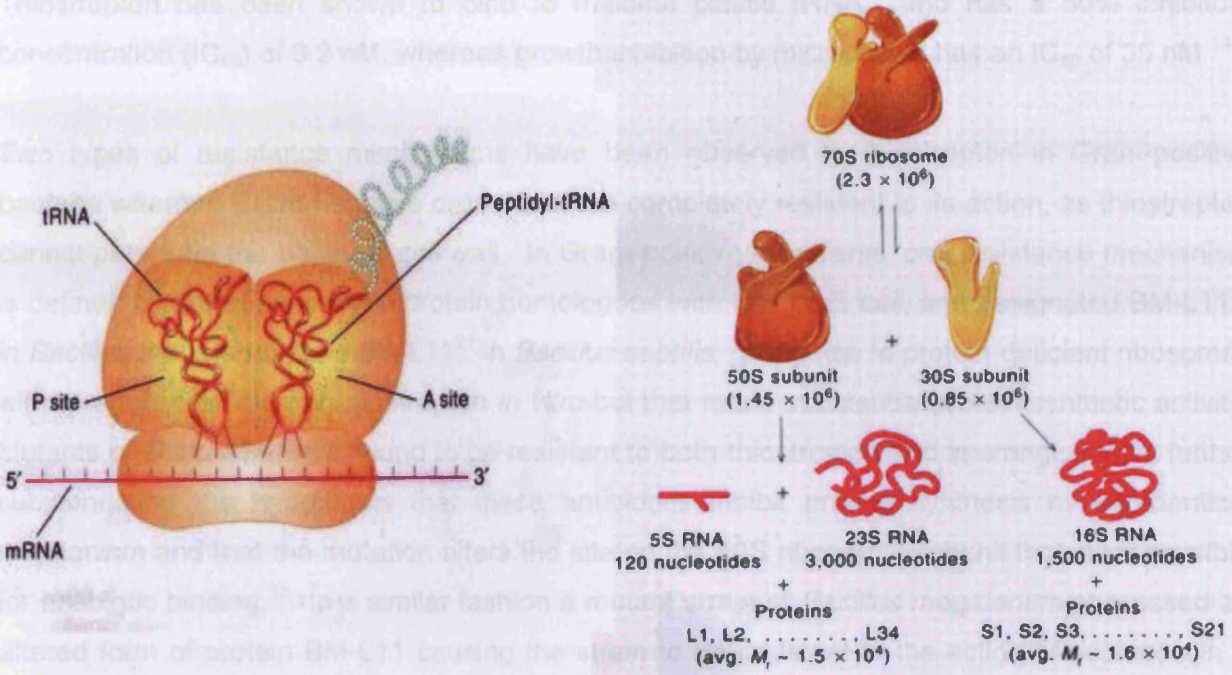


Figure 3 The bacterial ribosome

Many thiopeptide antibiotics interfere with bacterial protein synthesis on the ribosome, although the precise inhibitory mechanism operating for the majority of these agents has not been established. The most closely studied mode of action of all of the thiopeptides is that of thiostrepton (**1**), which has been applied in rational structure-based drug design in an attempt to address problems with this antibiotic's low solubility and poor bioavailability.[9] *In vivo* thiostrepton inhibits the binding of the aminoacyl-tRNA-containing ternary complex to the ribosomal A site (Figure 3).[10] The energy for protein translation is provided by the action of the elongation factors Ef-Tu and Ef-G, these hydrolysis reactions taking place on the large ribosomal subunit at a GTPase centre located on a double hairpin structure within domain II of 23S rRNA,[11] where ribosomal protein L11 and the pentameric complex L10.$(L12)_4$ assemble cooperatively, and on a ribotoxin hairpin loop within domain VI of 23S rRNA.[12] The action of thiostrepton inhibits peptide elongation probably by impeding a conformational change within protein L11, when bound in a region of the 23S rRNA known as the L11 binding domain (L11BD).[13] The RNA-binding domain of protein L11 recognises an rRNA tertiary structure that is stabilised by thiostrepton,[14] the antibiotic preventing one or more conformational transitions critical for stimulating the GTPase action of the elongation factors,[15] necessary to drive the directional movement of transfer and messenger RNA on the ribosome.[16]

Both thiostrepton (**1**) and micrococcin (**4**) inhibit the growth of the malaria parasite *Plasmodium falciparum*, inhibiting organellar protein synthesis by targeting the large subunit encoded by a 35-kb organelle, which is one of two extrachromosomal DNAs possessed by the parasite.[17] Thiostrepton has been shown to bind to malarial plastid rRNA[18] and has a 50% inhibitory concentration (IC_{50}) of 3.2 nM, whereas growth inhibition by micrococcin has an IC_{50} of 35 nM.[19]

Two types of resistance mechanisms have been observed to thiostrepton in Gram-positive bacteria whereas Gram-negative organisms are completely resistant to its action, as thiostrepton cannot penetrate the bacterial cell wall. In Gram-positive organisms, one resistance mechanism is defined by the absence of a protein homologous with L11 in *E. coli*, and designated BM-L11[20] in *Bacillus megaterium* and BS-L11[21] in *Bacillus subtilis*, giving rise to protein-deficient ribosomes with a reduced affinity for thiostrepton *in vitro* but that retain substantial protein synthetic activity. Mutants of *B. subtilis* were found to be resistant to both thiostrepton and sporangiomycin, further substantiating the hypothesis that these antibiotics inhibit protein synthesis by an identical mechanism and that the mutation alters the site on the 50S ribosomal subunit that is responsible for antibiotic binding.[22] In a similar fashion a mutant strain of *Bacillus megaterium* possessed an altered form of protein BM-L11 causing the strain to be resistant to the action of micrococcin.[23] However, the tRNA uncoupled hydrolysis reaction catalysed jointly by the ribosome and the protein factor Ef-G, which is inhibited by thiostrepton, is markedly stimulated by micrococcin upon organisms sensitive to this drug,[24] a disparity worthy of note in considering the seemingly common mode of action of thiopeptide ribosomal inhibitors. Both thiostrepton and micrococcin bind to the GTPase region in domain II of 23S rRNA and in so doing they alter the accessibility of adenosine (A)-1067 and A1095 in the 23S rRNA towards chemical reagents, indicating that thiopeptide ribosomal inhibitors interact directly with these nucleotides.[25] These two drugs had different effects on the chemical reactivity of A1067 in a terminal loop of *E. coli* ribosomes *in vitro*, micrococcin enhanced its reactivity whereas thiostrepton protected the N-1 position reducing reactivity, a difference that correlates with the opposite effects of the two antibiotics on GTPase activity.[26] This can be rationalised in a model for the tRNA uncoupled system where the dissociation of Ef-G·GDP from the ribosome is the rate-limiting step of Ef-G-dependent GTP hydrolysis.[27] Thiostrepton and micrococcin act by increasing the dissociation rates of both Ef-G·GTP and Ef-G·GDP, although micrococcin with lesser potency has a weaker dissociating effect on Ef-G·GTP than thiostrepton. In this model, the action of micrococcin would increase the turnover of Ef-G, increasing the rate of GTP hydrolysis, whereas Ef-G-GTP dissociation induced by thiostrepton would be too rapid to allow for GTP hydrolysis, reducing the rate, thus explaining the apparently different inhibitory effects of the two drugs. The structural basis for the contrasting activities of ribosome binding thiazole antibiotics has been studied using NMR and a thiostrepton-resistance methyltransferase assay, which rationalised the different binding profiles of thiostrepton, nosiheptide (**5**), siomycin (**68**, section 1.4.5) and micrococcin based upon the

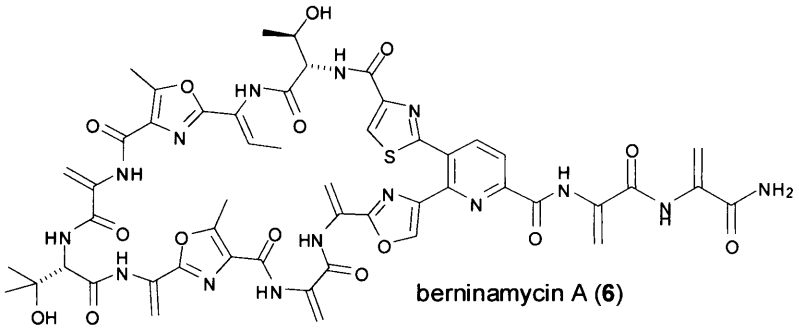
interaction of the quinaldic acid residue in thiostrepton, or an equivalent aromatic group, with the A1067 residue located in the L11BD.[28] However, it may well be the case that the key step in protein synthesis inhibition differs from organism to organism and depends upon cellular growth conditions, although clearly the conformational constraint of protein L11 perturbs the function of the ribosomal factor-guanosine nucleotide complexes and thus inhibits cell growth.[27]

Actinomycetes antibiotic producers have to adopt a resistance mechanism to defend themselves against their own self-intoxication.[29] In general *Streptomyces* are quite sensitive to thiostrepton and yet the producing organism, *S. azureus*, is totally unaffected by the drug.[30] An RNA-pentose methylase enzyme,[31] produced constitutively from its own promoter, is responsible for the autoimmunity in thiostrepton producers[32] and this has been demonstrated *in vitro* upon ribosomal RNA from other bacteria. The methylase enzyme, thiostrepton resistance methylase, introduces a single methyl group into the A1067 residue of the 23S rRNA in *Escherichia coli* to give a modified 2'-*O*-methyladenosine-containing ribosome that is completely resistant to the antibiotic,[33] a phenomenon that has been observed in 23S rRNA mutants of *Halobacterium halobium*.[34] Base changes at position 1159 in halobacteria, which corresponds to A1067 of the *E. coli* rRNA, from A to U or G, as well as base methylation causes high-level resistance to thiostrepton.[35] The thiostrepton-resistance (*tsr*) gene hybridises with a sulfomycin resistance determinant from *S. viridochromogenes* ATCC 29776,[36] encodes the 23S rRNA A1067 methyltransferase in *S. laurentii* and is located within a cluster of ribosomal protein operons, not clustered with genes that encode the biosynthetic enzymes.[37] The *tsr* gene product has been overexpressed from *S. azureus* in *E. coli* to characterise the enzymic reaction and establish that recognition is dependent upon the secondary structure of the ribosomal hairpin loop that contains nucleotide 1067.[38] The study of thiostrepton-resistant mutants is thus not only of relevance to account for the origin and determinants of antibiotic resistance, but also as a valuable tool in establishing the complex function and specific dynamic processes in operation during bacterial protein synthesis to provide new leads and focus for structure-based drug-design.

RNA-pentose methylation confers resistance in other actinomycetes, including *S. laurentii*, another thiostrepton producer, *P. parontospora*, from which sporangiomycin was isolated, and *S. sioyaensis*, the producer of the siomycins.[39] Radiographical studies on the inhibition of bacterial protein synthesis by siomycin supports many of the mechanistic findings for thiostrepton and lends credence to a common, or very closely related, mode of action that operates for all thiopeptide ribosomal inhibitors. The incorporation of radioactive amino acids and base pairs, into the nascent peptide or mRNA, respectively, was studied in the presence of siomycin.[40] Although the action of the antibiotic prevented the incorporation of radioactive amino acids into *Bacillus subtilis* cells, ^{14}C base pairs were incorporated into the mRNA, indicating that the agent acted as a ribosomal rather than transcription inhibitor, that was selective for Gram-positive and

mycobacteria. Antibiotic binding at the ribosomal G site could cause a distortion of the A site, which would explain why siomycin inhibits translocation of the peptidyl-tRNA from the A to the P site, and prevents the binding of both Ef-G·GTP and the ternary complex aa-tRNA·Ef-Tu·GTP to the ribosome at the G and A sites, respectively.[41]

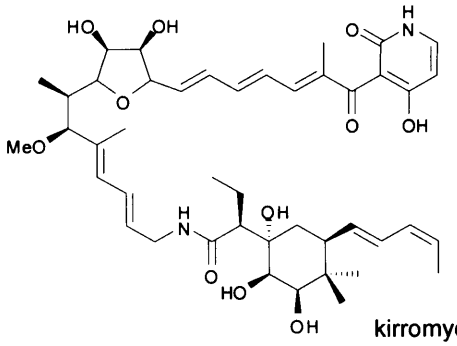
Actinomycetes that produce thiopeptide metabolites with very different chemical structures have been shown to adopt the same resistance mechanism and to give rise to antibiotics that function by a common mode of action. The growth of *Streptomyces bernensis*, the producer of the berninamycins (**6**), is totally resistant to the action of thiostrepton, even though structurally these two antibiotics are grouped in a different thiopeptide series.[42] Similarly, nosiheptide has been shown to partially inhibit both the enzymatic binding of aminoacyl-tRNA to the ribosome and the simultaneous hydrolysis of GTP, but only when present at quite a high molar excess over the ribosomes.[43] In spite of differences in antibiotic structure, all of these compounds bind to the complex of 23S RNA and protein L11 and affect various functions of the ribosomal A site, although thiostrepton is considerably more potent in its action than nosiheptide.[44] The specific pentose-methylation of the 23S rRNA renders the ribosomes of *S. bernensis* and *S. actuosus* totally refractory to berninamycin and nosiheptide, respectively, and prevents the binding of thiostrepton,[45] indicating both a common mode of action of these drugs and a common mechanism of self-defence employed by the respective producing organisms.



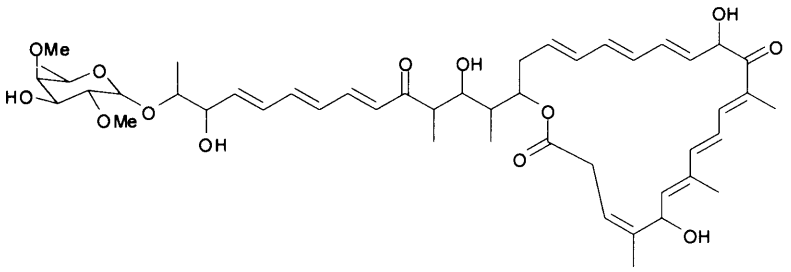
berninamycin A (**6**)

1.3.3 – Inhibition of Elongation Factor Tu

Elongation factor Tu (Ef-Tu) is the most abundant protein of the bacterial cell and participates in peptide elongation by mediating the recognition and delivery of noninitiator aminoacyl-tRNA, as a ternary complex with GTP, to the mRNA codon in the acceptor site of the bacterial ribosome. When its GTPase action is triggered, Ef-Tu·GDP, which does not bind aa-tRNA, dissociates from the ribosomal complex, leaving behind the aa-tRNA in the ribosomal A site in readiness for peptide translocation. Many types of antibiotics act by binding to Ef-Tu, which either prevents the dissociation of Ef-Tu·GDP from the ribosomal complex, as is the case for the polyketide kirromycin (**7**), or inhibits the binding of aa-tRNA to Ef-Tu·GTP, exemplified by pulvomycin (**8**).



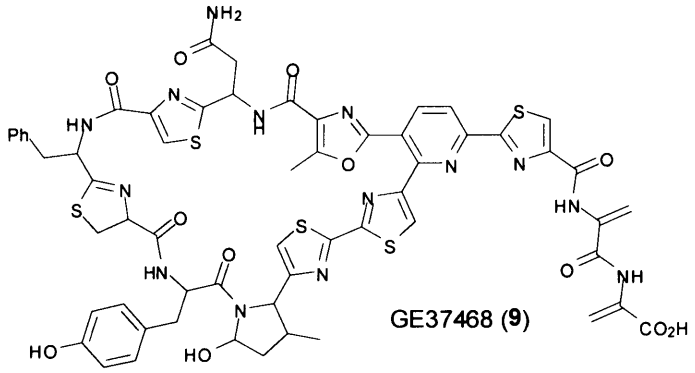
kirromycin (**7**)



pulvomycin (**8**)

The thiopeptide GE2270A (MDL 62,879) (**42**, section 1.4.4) inhibits bacterial protein synthesis at an IC_{50} of 0.4 μM by binding to Ef-Tu at a distinct site from kirromycin and so differs in its mode of action from thiopeptide ribosomal inhibitors.[46] GE2270A interacts directly with the GTP-bound form of Ef-Tu and this inhibits the formation of a stable ternary complex by preventing the binding of aa-tRNA to Ef-Tu·GTP.[47] Although it does not change the GTPase activity of Ef-Tu, antibiotic binding does prevent the ribosomal catalysis of this process and slows down the dissociation of the Ef-Tu·GTP complex to even a greater degree than aa-tRNA. GE2270A forms a strongly bound 1:1 molar complex with Ef-Tu, which can be observed by a mobility shift in an electric field, only reversed by protein denaturation. The strong affinity between *Escherichia coli* Ef-Tu·GDP and GE2270A has been explained by X-ray diffraction studies of the complex at a resolution of 2.35 Å, which showed, in addition to 18 protein-antibiotic Van der Waals interactions (<3.5 Å), a highly unusual salt bridge between Arg 223 and Glu 259 that folds over the GE2270A side chain when bound in a pocket formed by three segments of amino acids, Glu 215-Arg 230, Thr 256-Leu 264 and Asn 273-Leu 277, located in the second domain of Ef-Tu·GTP.[188] The exchange of guanine nucleotides induces conformational changes in Ef-Tu complexes, as confirmed by proteolytic cleavage experiments. The superposition of the Ef-Tu·GDP·GE2270A and Ef-Tu·GTP structures suggests that antibiotic binding causes steric constraints that prevent complete formation of the GTP conformation. Thus, it seems likely that on binding to domain II, GE2270A impedes the GDP to GTP conformational change and prevents the formation of an Ef-Tu·GTP·aa-tRNA ternary complex by blocking the aa-tRNA binding site in the antibiotic-bound complex.[48]

This mode of action has been shown to operate for the GE2270 thiopeptides, GE37468 (**9**) and the amythiamicins (**23**, section 1.4.2) and has been used to develop a screening programme for thiopeptide antibiotics with similar binding properties, by selecting activities antagonised by exogenous Ef-Tu.[49,50] Inhibitors of this GTP-dependent translation factor have been shown to possess antimalarial activity in blood cultures of *Plasmodium falciparum*, the most active being amythiamicin A with an IC_{50} of 0.01 μM, and to bind to recombinant *P. falciparum* plastid Ef-Tu, indicating that endogenous plastid protein synthesis is a potential target for thiopeptides that inhibit prokaryotic protein synthesis by this mechanism.[51]



GE37468 (**9**)

Little is known about how the producers of Ef-Tu binding thiopeptides avoid self-intoxication, although resistance mechanisms are complicated by the fact that some actinomycetes possess more than one *tuf* gene to encode Ef-Tu translation factors. In *Planobispora rosea*, the GE2270A producer, both the *tuf1* and *tuf3* genes encode Ef-Tu-like proteins, the former encoding the regular elongation factor EF-Tu1.[52] This bacterial protein is totally resistant to GE2270A, ten times more resistant to kirromycin than Ef-Tu1 of *Streptomyces coelicolor* and not at all resistant to pulvomycin, an antibiotic that also inhibits the formation of the aa-tRNA·EF-Tu·GTP complex. Kirromycin resistance is accounted for by the replacement of Tyr 160 with a Gln at the kirromycin binding site, the interface of domains 1 and 3 of Ef-Tu in its GTP-bound conformation. The mutations that confer GE2270A resistance would appear to map to a number of amino acids in domain 2, located at the GE2270A binding pocket, in close proximity to residue 226 at the domain 1-2 interface, which is part of the binding site of the 3'-end of aa-tRNA. Ef-Tu mutants of *Bacillus subtilis* that were resistant to Ef-Tu-binding thiopeptide antibiotics also showed significant changes of conserved amino acids, which may account for their similar resistance behaviour.[49,53]

1.3.4 – *TipA* Promotion

Actinomycetes multidrug resistance mechanisms often rely upon transport proteins or modifying enzymes and transcriptional regulatory proteins to recognise multiple drugs and respond to stress-response signals. *Streptomyces lividans* 1326, a strain that is not known as a thiazolylpeptide producer, reacts to thiostrepton by inducing resistance to a number of structurally heterogeneous antibiotics with the accumulation of thiostrepton-induced proteins (Tip), two of which, TipAL (253 amino acids) and TipAS (144 amino acids), the latter corresponding to the *C*-terminal region of TipAL, are in-frame translation products of the same gene, *tipA*.[54] TipAS, the independently translated thiopeptide binding domain present in vast molar excess (>20:1) over TipAL, renders the organism resistant by sequestering the drug in the cytosol and by modulating a drug-dependent positive feedback loop that controls its own expression.[55] Their transcription is induced by a number of thiopeptide antibiotics, including

thiostrepton, promothiocin (**15**, section 1.4.1) and nosiheptide, by complex formation with TipAL in the absence of added co-factors, that activates transcription of a monocistronic mRNA from the tipA promoter (*ptipA*) and so elicits autogenous expression of its own promoter. The TipAL protein is a dimer in solution with an *N*-terminal domain (residues 1-109), containing both an helix-turn-helix that binds DNA (residues 5-25) and a long coiled-coil dimerisation region (residues 74-109), and a *C*-terminal domain (residues 110-253), represented by TipAS, for thiopeptide recognition (Figure 4A). The formation of a covalent bond between cysteine 214 (not cysteine 207)[56] and a dehydroalanine residue in the thiopeptide irreversibly generates a ligand·TipAL complex (Figure 4B) and enhances the affinity of the bound protein for its operator site, inducing the recruitment of RNA polymerase (RNAP) to the promoter *ptipA*[57] and activating its transcription at least 200-fold. On binding thiostrepton, conformational changes within TipAL enhance its association with *ptipA* and lower the rate of dissociation from the binding site, increasing the affinity of RNAP for *ptipA* in an alternative mechanism of transcriptional activation.[58] By analogy with the mercury resistance regulator (MerR) protein, the ligand-bound TipAL dimer activates transcription and increases the affinity of the ligand-bound TipAL to the *tipA* promoter, by inducing folding of the unstructured *N*-terminal part of apo TipAS, a globin-like α-helical fold with a deep surface cleft and an unfolded *N*-terminal region that is the linker to the DNA-binding domain of TipAL.[59] TipAL and other MerR regulators bind as dimers to an inverted repeat sequence located within the spacer region of their promoters. In contrast to the MerR regulatory proteins, TipAL can bind to its target site and activate transcription in the absence of the ligand, elevated external osmolarity causing an increase in intracellular negative DNA supercoiling that enhances *ptipA* expression.[60] On binding thiopeptide antibiotics, the *N*-terminal coiled-coil linker parts of the TipAL complex become rigid. This conformational change clamps the DNA-binding domains of the dimer and twists the DNA helix of the promoter, bringing the two consensus recognition sequences into alignment to allow RNAP to initiate transcription (Scheme 2).

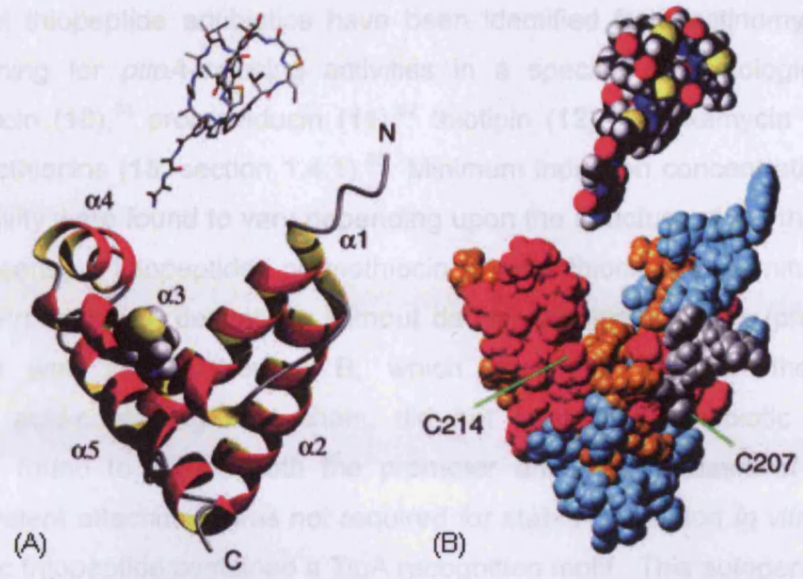
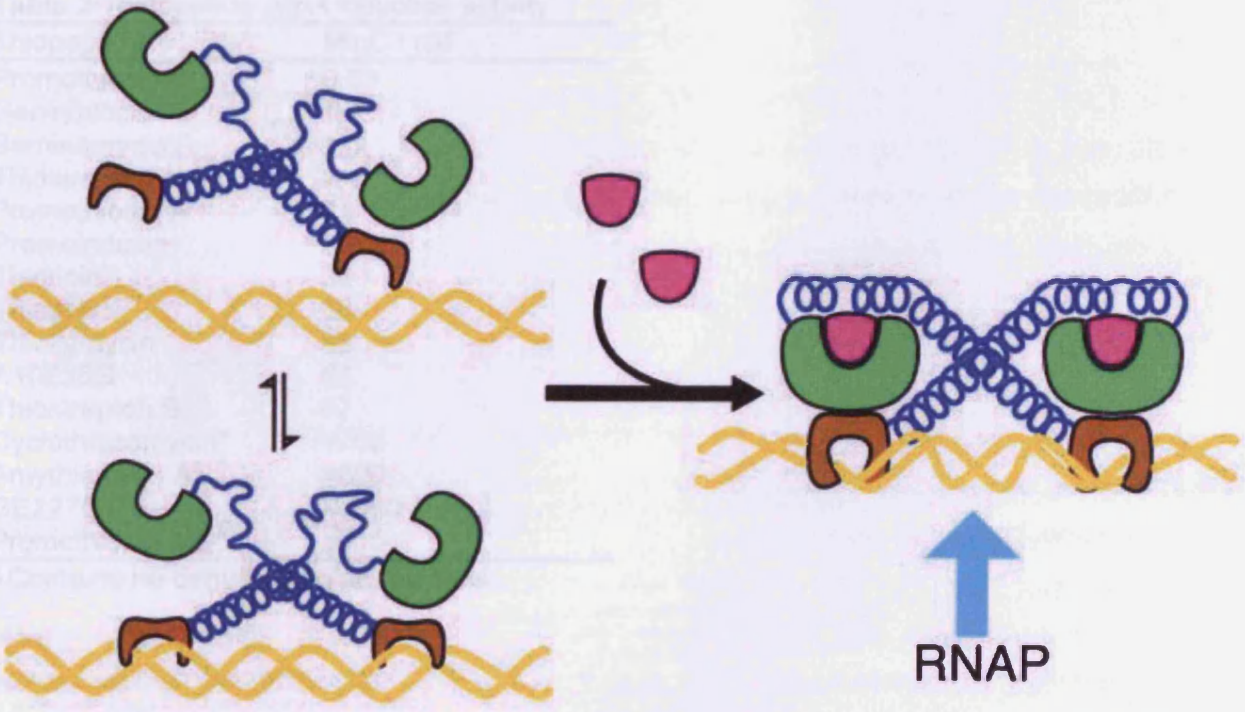


Figure 4 Structure of the folded part of apo TipAS

Left: ribbon representation showing α-helices, *N*- and *C*-termini and ligand binding residue C214 as a space-filling model. Right: colour map of ligand-induced chemical shift changes on TipAS-thiostrepton complex formation. Red indicates strongly affected residues, orange indicates moderately affected residues and blue indicates weakly affected residues. The thiopeptide antibiotic shown alongside the TipAS-ligand binding site is thiostrepton. Reprinted from ref. 59. Copyright 2003 European Molecular Biology Organization.



Scheme 2 Suggested mechanism of *TipA*-induction by TipAL·thiopeptide complex formation

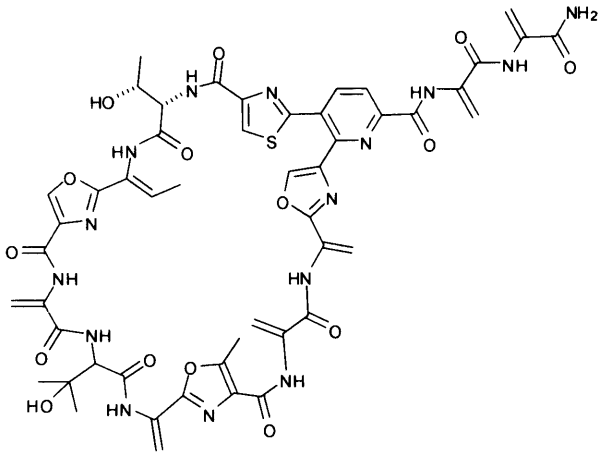
Cartoon colour coding: thiopeptide antibiotic, red; DNA, yellow; TipAL, green, blue and brown. Reprinted from ref. 59. Copyright 2003 European Molecular Biology Organization.

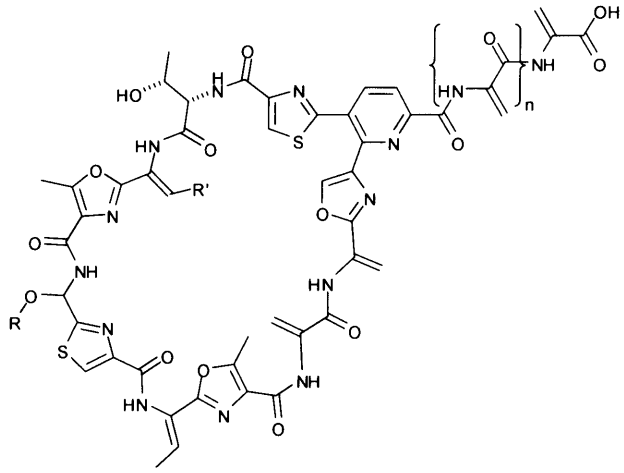
A number of novel thiopeptide antibiotics have been identified from actinomycete metabolite libraries by screening for *ptipA*-inducing activities in a specific microbiological disc assay, including geninthiocin (**10**),[61] promoinducin (**11**),[62] thiotipin (**12**),[63] thioxamycin (**13**),[64] thioactin (**14**) and the promothiocins (**15**, section 1.4.1).[65] Minimum induction concentrations (MinCs) for *ptipA* induction activity were found to vary depending upon the structure of the thiopeptide ligand (Table 2) with the series *d* thiopeptides promothiocin B, geninthiocin and berninamycin A being the most active.. Promothiocin derivatives without dehydroalanine residues (promothiocin MO: methyl ester), as well as thiostrepton B, which does not possess the characteristic polydehydroamino acid-containing side chain, did not form TipAS·antibiotic complexes but nevertheless were found to induce both the promoter and the synthesis of TipAS protein, confirming that covalent attachment was not required for stable interaction *in vitro* or *in vivo* and that the macrocyclic thiopeptide contained a TipA recognition motif. This autogenously controlled antibiotic resistance system responds to these antibiotics by synthesising a single mRNA that includes TipAS, to sequester the antibiotic and thus limit activation of the TipAL-dependent promoter. It may well be the case that these proteins, in addition to providing a low-level antibiotic resistance system, regulate resistance to other stress-response signals, such as those induced by heavy metals or changes in redox potentials, indicating a biological relevance far beyond growth inhibition in competing organisms.

Table 2 Thiopeptide *ptipA* induction activity

Thiopeptide	MinC / nM
Promothiocin B	0.63
Geninthiocin	1.0
Berninamycin A	1.0
Thiostrepton A	1.4
Promothiocin A	24
Promoinducin	30
Thiotipin	32
Thioactin	38
Thioxamycin	63
A10255G	66
Thiostrepton B	67
Cyclothiazomycin[a]	>700
Amythiamicin A[a]	>800
GE2270A[a]	>1000
Promothiocin MO[a]	3300

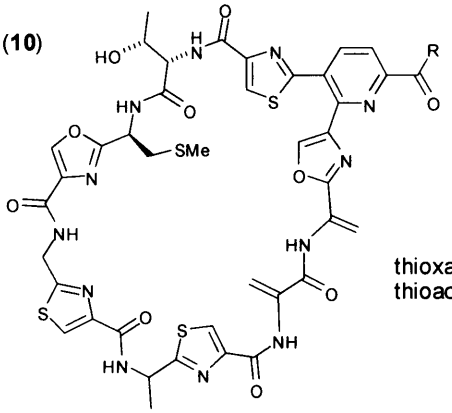
[a] Contains no dehydroalanine residues

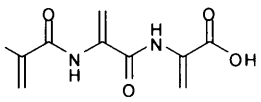




promoinducin (**11**) R = Me, R' = CH(OH)Me, n = 3;
thiotipin (**12**) R = H, R' = Me, n = 2

geninthiocin (**10**)



thioxamycin (**13**) R = 
thioactin (**14**) R = NH_2

1.4 – Total Syntheses of Thiopeptide Antibiotics

In recent years, considerable advances have been made towards the synthesis of the thiopeptide antibiotics. The complex molecular architecture of this family of antibiotics has attracted significant interest from research groups worldwide, and culminated in the successful total syntheses of promothiocin A, amythiamicin D, thiostrepton, GE2270A and T and siomycin A.

1.4.1 – Promothiocin A

The promothiocin thiopeptides were isolated by Seto *et al.* from *Streptomyces* sp. SF2741.. These compounds were identified as promothiocin A (**15a**) ($C_{36}H_{37}N_{11}O_8S_2$) and promothiocin B (**15b**) ($C_{42}H_{43}N_{13}O_{10}S_2$) following a combination of IR spectroscopy, high-resolution FAB mass spectrometry, 2D NMR experiments and amino acid analyses, which provided one mole each of alanine, glycine and valine and led to the elucidation of the constitution, but not the stereochemistry of both of the promothiocin factors. The total synthesis of promothiocin A by Moody and Bagley[66,67] established unequivocally the (*S*)-configuration of the three stereogenic centers in the natural product and this was subsequently supported by degradation studies and molecular modelling (Figure 5).[68] Chiral-HPLC analysis of the acid hydrolysates confirmed the L-configuration of both alanine and valine, whereas the (*S*)-2-(1-amino-1-ethyl)thiazole-4-carboxylate residue provided the best calculated fit in a DADAS90 conformational study.

Minimum *tipA* promoter induction concentrations of promothiocins A and B were reported as 0.2 and 0.1 μg/mL, respectively.

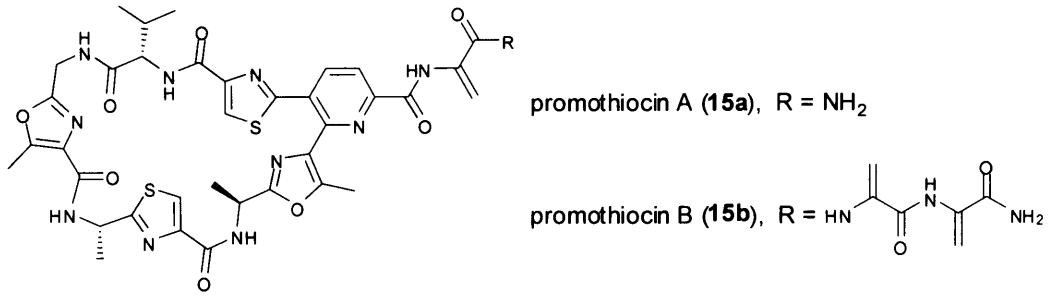
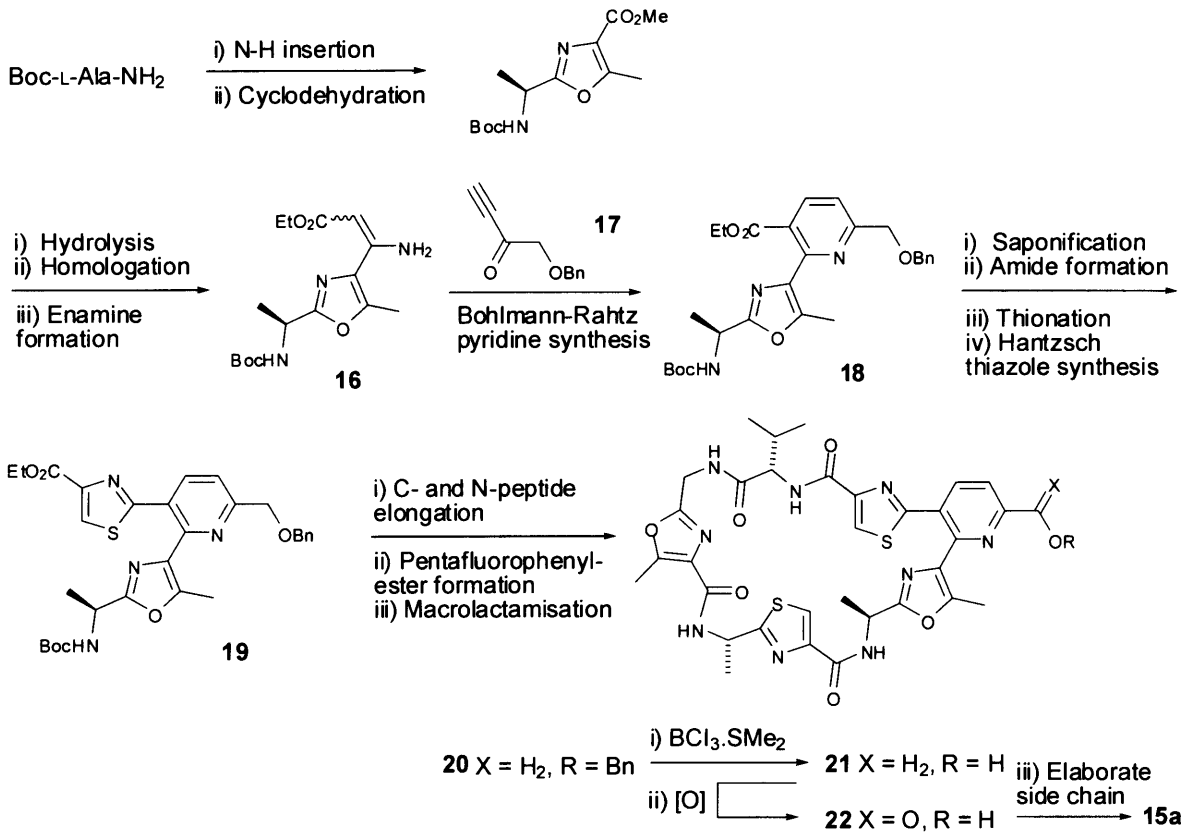


Figure 5 Structure of the promothiocins

The first chemical synthesis of a thiopeptide natural product was the preparation of promothiocin A by Moody and Bagley.[66,67] This landmark synthesis featured the then little-used Bohlmann-Rahtz heteroannulation reaction to establish the central oxazole–thiazole–pyridine domain **19**,[69] by saponification, amide formation, thionation and Hantzsch thiazole synthesis. The Bohlmann-Rahtz synthesis of pyridine **18** from ethyl 3-amino-3-(4-oxazolyl)propenoate **16**, prepared from (*S*)-*N*-tert-butoxycarbonylalaninamide by dirhodium(II)-catalysed carbenoid insertion into the amide N–H followed by cyclodehydration with triphenylphosphine-iodine under basic conditions, saponification, homologation using the magnesium enolate of ethyl potassium malonate and enamine formation, and 1-benzyloxybut-3-yn-2-one (**17**), obtained by Grignard addition and propargylic oxidation, proceeds in two steps by initial Michael addition at 50 °C and subsequent double bond isomerisation-cyclisation at 140 °C in the absence of solvent (Scheme 3). The elongation of the linear peptide by *N*- and *C*-terminus functionalisation, followed by macrolactamisation under basic conditions *via* the pentafluorophenylester gave macrocycle **20**. Benzyl ether cleavage with boron trichloride dimethyl sulfide complex gave alcohol **21**, which was oxidised to carboxylic acid **22** using *o*-iodoxybenzoic acid (IBX) in DMSO followed by treatment with sodium chlorite, and then coupled with an *O*-protected serinamide derivative using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI). Protodesilylation and dehydration by mesylate formation followed by treatment with triethylamine, installed the dehydroalanine side chain, completing the first total synthesis of one of the thiopeptide antibiotics and verifying the (*S*)-stereochemistry of all three stereogenic centres in the metabolite isolated from *Streptomyces* sp. SF2741.



Scheme 3 Total synthesis of promothiocin A

1.4.2 – Amythiamicin D

The amythiamicins (**23**) were obtained from the fermentation broth of *Amycolatopsis* sp. MI481-42F4, isolated from soil samples collected in Tokyo, Japan and detected by a paper disc diffusion screen based upon their *in vitro* antibacterial activity against *Staphylococcus aureus*.[70] These antibiotics, although inactive against most Gram-negative bacteria and fungi, inhibit the growth of Gram-positive bacteria, including multi-drug resistant strains such as *Staphylococci aureus* MS9610 and methicillin-resistant *S. aureus* (MRSA),[71] and show no signs of toxicity when administered intraperitoneally to mice at a dose of 100 mg/kg.. The structure of the different components was elucidated by a combination of NMR spectroscopic techniques, chemical degradation and FAB mass spectrometry.[72,73] Amino acid analyses of amythiamicin A ($C_{50}H_{51}N_{15}O_8S_6$), B ($C_{50}H_{53}N_{15}O_9S_6$) and C ($C_{50}H_{50}N_{14}O_9S_6$) found them to contain one mole of glycine and one extra mole of both L-proline and L-serine, determined by chiral HPLC, with respect to amythiamicin D ($C_{43}H_{42}N_{12}O_7S_6$) (Figure 6).

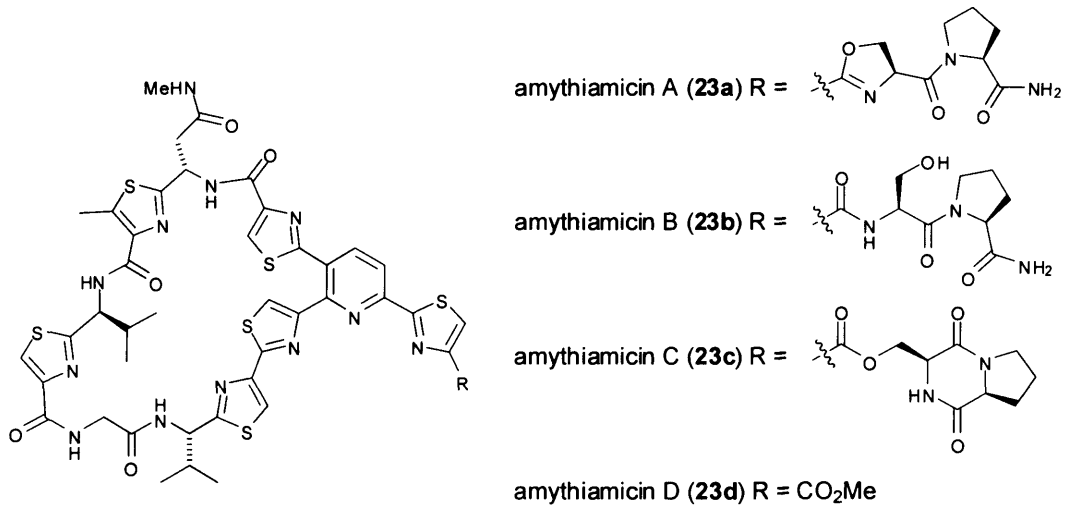
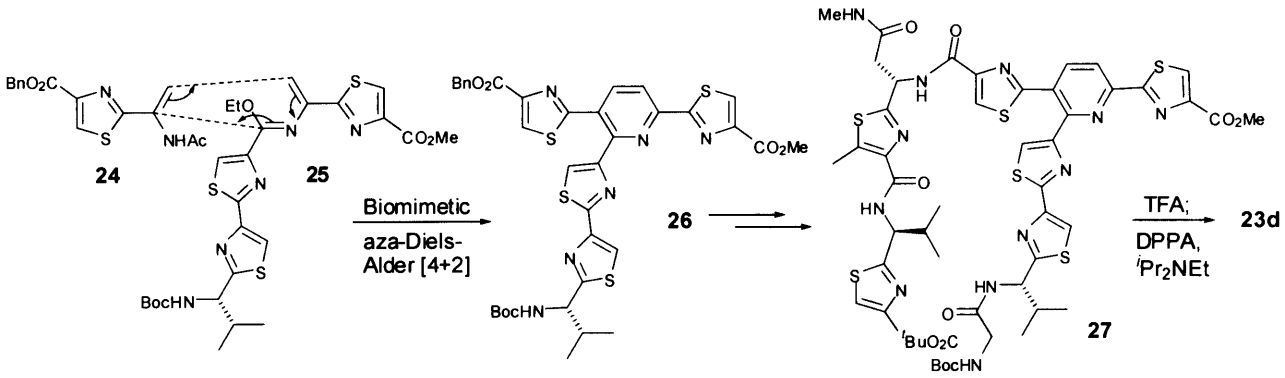


Figure 6 Structure of the amythiamicins

As the composition of the macrocyclic loop was found to be the same for all four thiopeptides, differences in their amino acid analyses were attributed to variations in the peptide side chain. Although the absolute configuration of the two valine-derived and one aspartate-derived thiazole residues in the amythiamicin macrocycle could not be determined from the isolated natural product, due to racemisation under acid-catalysed hydrolysis conditions, the total synthesis of amythiamicin D by Moody *et al.* has since verified the structure and absolute stereochemistry of this thiopeptide family, substantiated by X-ray crystallographic data, confirming the L-stereochemistry of all constituent amino acids.[74,75] Moody *et al.* employed a biomimetic strategy for the synthesis of the 2,3,6-tris(thiazolyl)pyridine domain **26** of the amythiamicins which was further elaborated to amythiamicin D, establishing the (*S*)-stereochemistry of the three stereogenic centres of the natural product (Scheme 4). The formal aza-Diels-Alder cycloaddition of dehydroalanine dienophile **24** and 2-azadiene **25** proceeded in modest yield by microwave irradiation[76] in toluene at 120 °C for 12 hours. Elongation of the central pyridine domain **26** gave linear peptide **27**, which was cyclised by deprotection of both the *C*- and *N*-termini using trifluoroacetic acid (TFA) followed by treatment with diphenylphosphoryl azide (DPPA) and Hünig's base in DMF to give the macrocyclic natural product amythiamicin D.



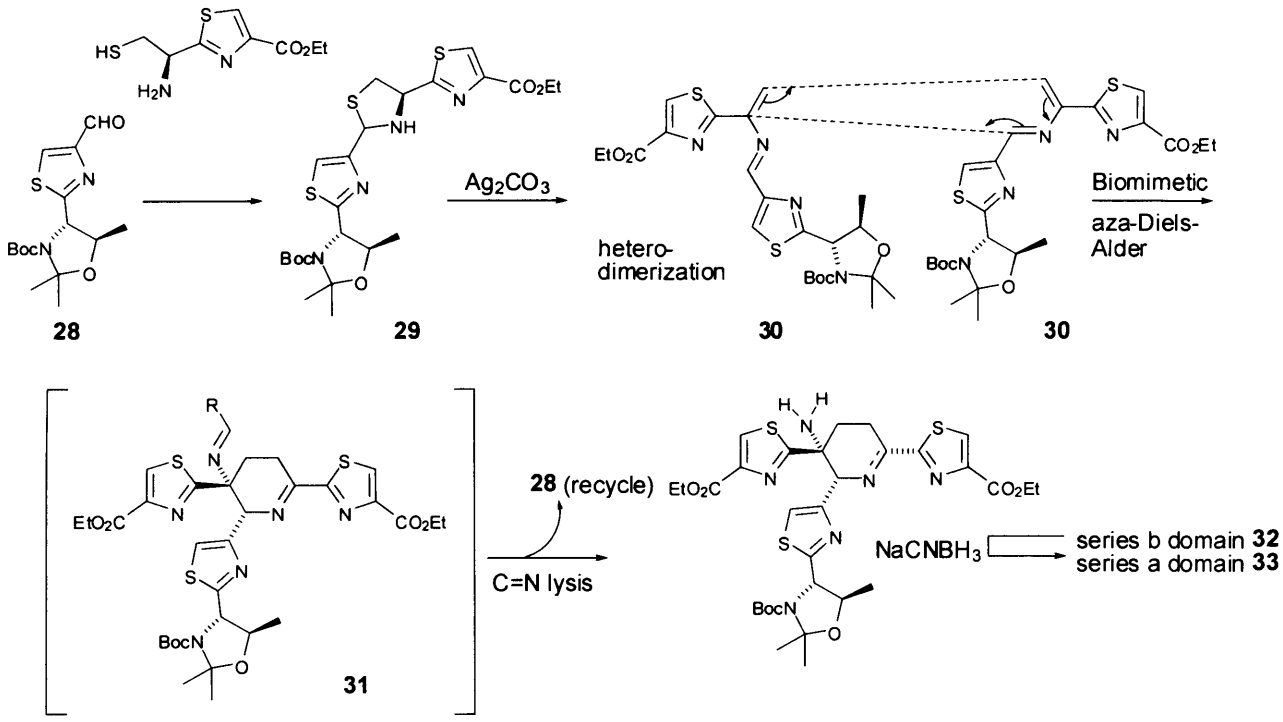
Scheme 4 Total synthesis of amythiamicin D

1.4.3 – Thiostrepton

Thiostrepton (**1**), the parent compound of the thiopeptide antibiotics, was first isolated in 1954 from *Streptomyces azureus*[77,78,79] and *S. hawaiiensis*,[80] and later from *S. laurentii* (1977).[81] The substance isolated from *S. hawaiiensis* was originally named bryamycin but was later shown to be identical to thiostrepton.[82]

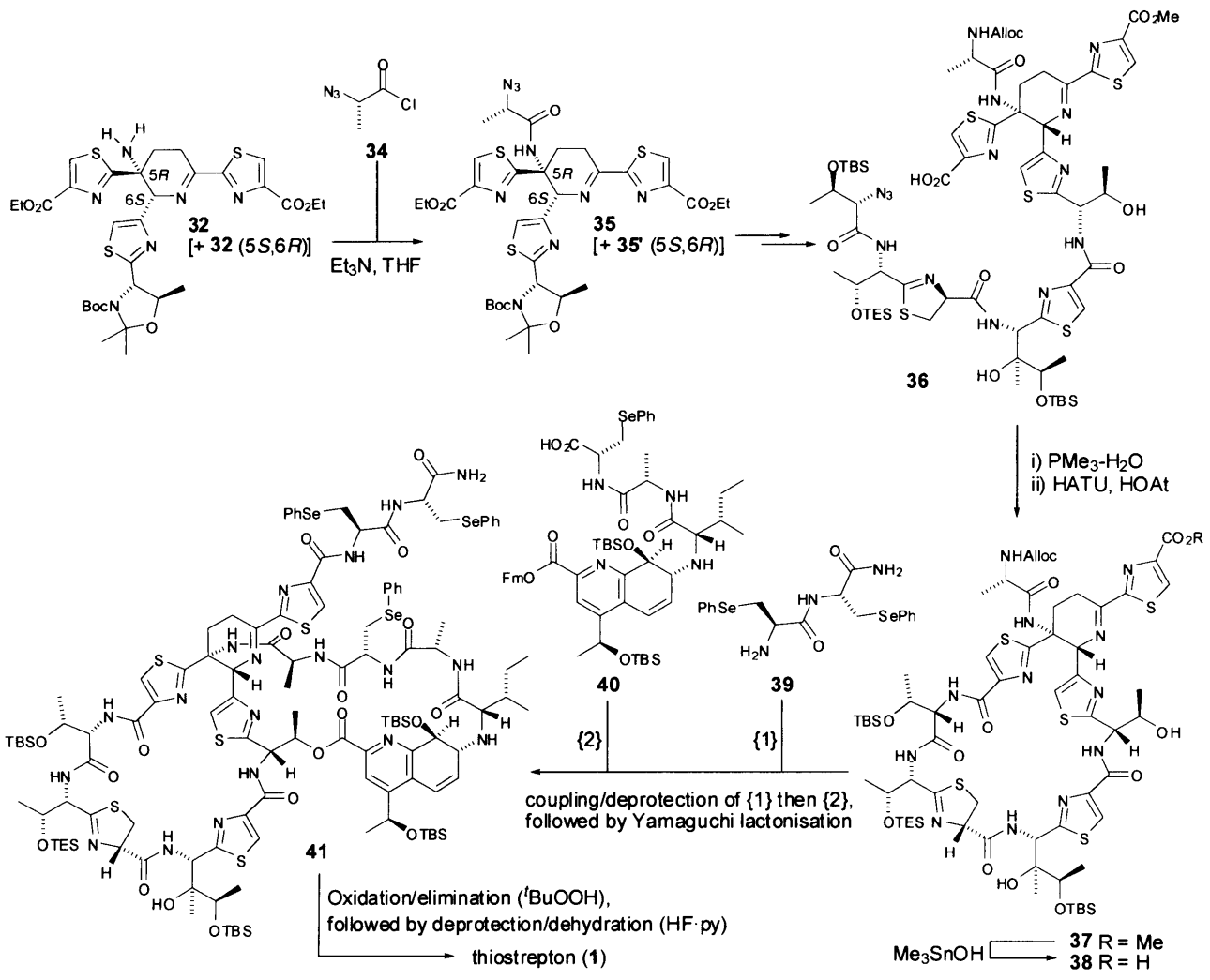
The majority of the structure of thiostrepton was determined by Dorothy Crowfoot Hodgkin through X-ray crystallography in 1970,[83] although several degradation studies were conducted in the years between the isolation of the natural product and Hodgkin's study in an attempt to elucidate the structure. Since that date, further X-ray crystallographic studies on tetragonal crystals using the anomalous dispersive signal from sulphur collected at the Cu Kα wavelength enabled Hunter and co-workers to solve the structure of the macrocycle of thiostrepton[84] and the landmark total synthesis of **1** by Nicolaou *et al.* has verified its structural identity.[85]

Nicolaou and co-workers utilised an elegant biomimetic strategy to establish the dehydropiperidine domain of thiostrepton. With the stereoselective synthesis of the quinaldic acid-containing macrocycle[86] and construction of all requisite components,[87,88] a highly convergent total synthesis of this complex antibiotic was realised.[89] The regioselective and *endo*-selective hetero-Diels-Alder dimerisation of 2-azadiene **30**, obtained from thiazolidine **29** by treatment with silver carbonate, proceeded without facial selectivity to give dehydropiperidine **32** as a 1:1 mixture of diastereomers in a cascade sequence with *in situ* lysis of imine intermediate **31** and release of aldehyde **28** to be recycled to dimerisation precursor **29** (Scheme 5).[90] Stereospecific reduction of cycloadduct **32** using sodium cyanoborohydride generated piperidine **33** and demonstrated that a biomimetic heterodimerisation approach can provide expedient access to the central domain of either series *a* (**33**) or *b* (**32**) thiopeptide antibiotics.



Scheme 5 Biomimetic synthesis of series *a* or *b* piperidine domain

The application of this work in the total synthesis of thiostrepton was realised by capturing the free amino group of the dehydropiperidine intermediate with the acyl chloride of an azide derivative of alanine (**34**), to produce imine **35** exclusively, as a diastereomeric mixture (Scheme 6).. After peptide elaboration, closure of the thiazoline-containing macrocycle was successful for only one (**36**) of two monoacids, formed by the action of trimethyltin hydroxide in 1,2-dichloroethane, to give the desired macrocycle **37** following reduction with PMe_3–H_2O and treatment with HATU–HOAt–iPr$_2$NEt. The construction of the two macrocyclic domains was effected by attachment of a phenylseleno-disubstituted peptide **39**, as a precursor for the dehydroalanine subunits in the side chain, to acid **38**, followed by *N*-terminal deprotection, coupling with quinaldic acid linear peptide **40** and macrolactonisation under Yamaguchi conditions with 2,4,6-trichlorobenzoyl chloride.. The synthesis was completed by *t*BuOOH-mediated oxidation of all three phenylseleno groups in bis-macrocycle **41**, which brought about spontaneous selenoxide *syn* elimination, followed by silyl ether deprotection with hydrogen fluoride-pyridine, with concomitant elimination to form the thiazoline-conjugated *Z* double bond. This landmark route gave synthetic thiostrepton with identical physical properties to an authentic sample, constituting a highly convergent and stereoselective synthesis of a complex thiopeptide antibiotic that may pave the way for related synthetic studies in the future.

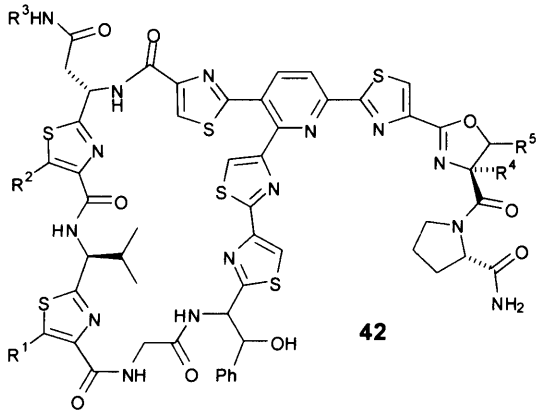


Scheme 6 Total synthesis of thiostrepton

1.4.4 – GE2270A and GE2270T

The GE2270 (MDL 62,879) class of series *d* thiopeptides is the largest family of these natural products with over 10 structurally-related components (Figure 7).[91] The main factor of this complex, GE2270A ($C_{56}H_{55}N_{15}O_{10}S_6$), isolated in a screening programme designed to detect inhibitors of protein synthesis from the fermentation broth of *Planobispora rosea* ATCC 53773, was extracted from the mycelium and purified by column chromatography on silica gel.[92] The original structure was proposed following chemical degradation, UV, IR and NMR spectroscopic studies[93] and mass spectrometric techniques,[94] but was subsequently revised on further investigation, correcting the sequence of thiazole amino acids in the cyclic peptide through analysis of the hydrolysates[95] with some assignment of absolute stereochemistry.[96] The isolation and characterisation of 9 of the minor components, separated from the GE2270 antibiotic complex by HPLC, was reported in 1995 and facilitated by altering the fermentation conditions, a study that showed that *P. rosea* modifies the GE2270 backbone by introducing a variable number of methylene units.[97] The structures of the individual factors, E, D1, D2, C1, C2a and

C2b (not to be confused with subscripts *a* and *b*, used to denote differences in the central domain in the thiopeptin factors), B1, B2 and T were determined by 2D NMR spectroscopy and differ from GE2270A in the nature of the 5-substituent on two thiazole residues in the peptide backbone, the asparagine *N'*-substituent and the oxidation state of the azole in the 6-pyridine side chain. All of the GE2270 factors were found to inhibit protein synthesis in Gram-positive microorganisms and anaerobes, with particular activity noted against *Propionibacterium acnes* (MIC for GE2270A against L1014 ATCC 6919 <0.004 μg/ml) and *Mycobacterium tuberculosis* (MIC for GE2270A of 1 μg/ml), as well as exhibiting some activity against Gram-negative bacteria. These antibiotics inhibit bacterial protein synthesis by acting specifically on the GTP-bound form of Ef-Tu, the elongation factor required for the binding of aminoacyl-tRNA to the ribosomal A site and so function in a similar fashion to kirromycin-like antibiotics and pulvomycin (section 1.3).

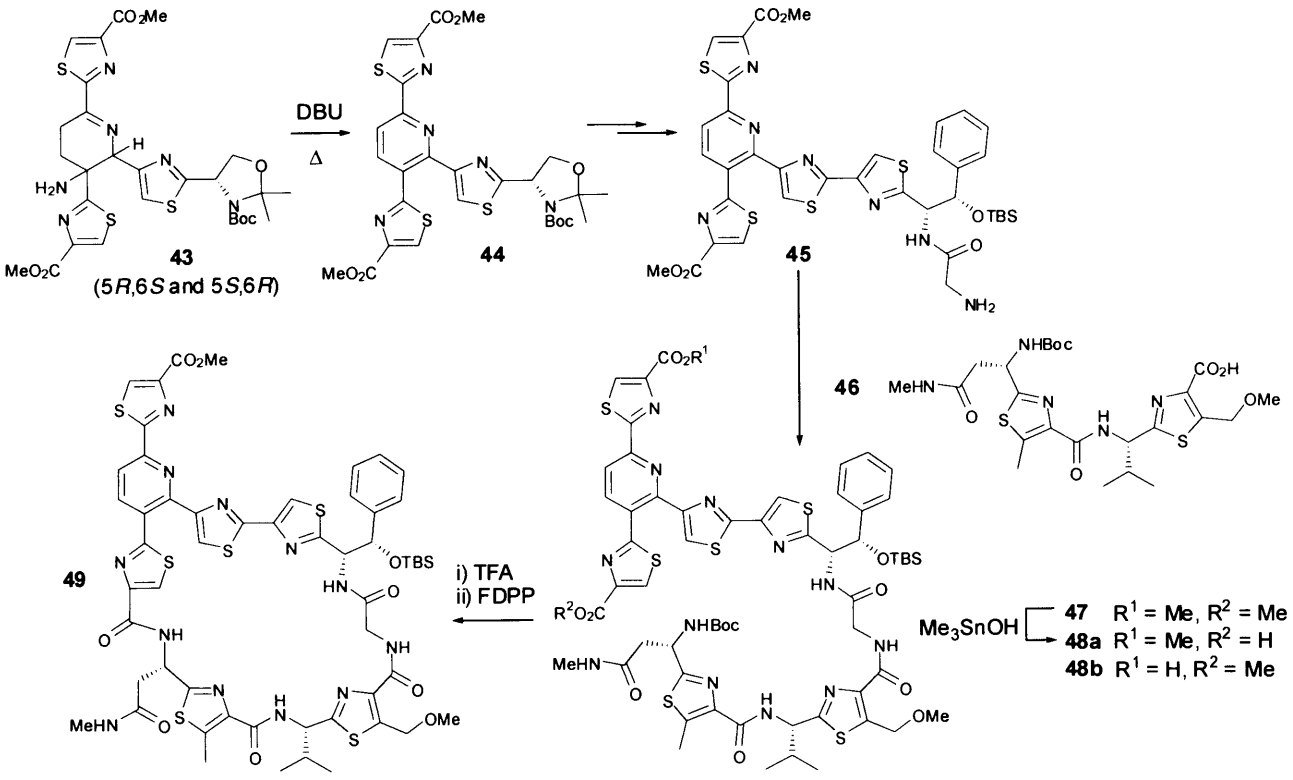


42

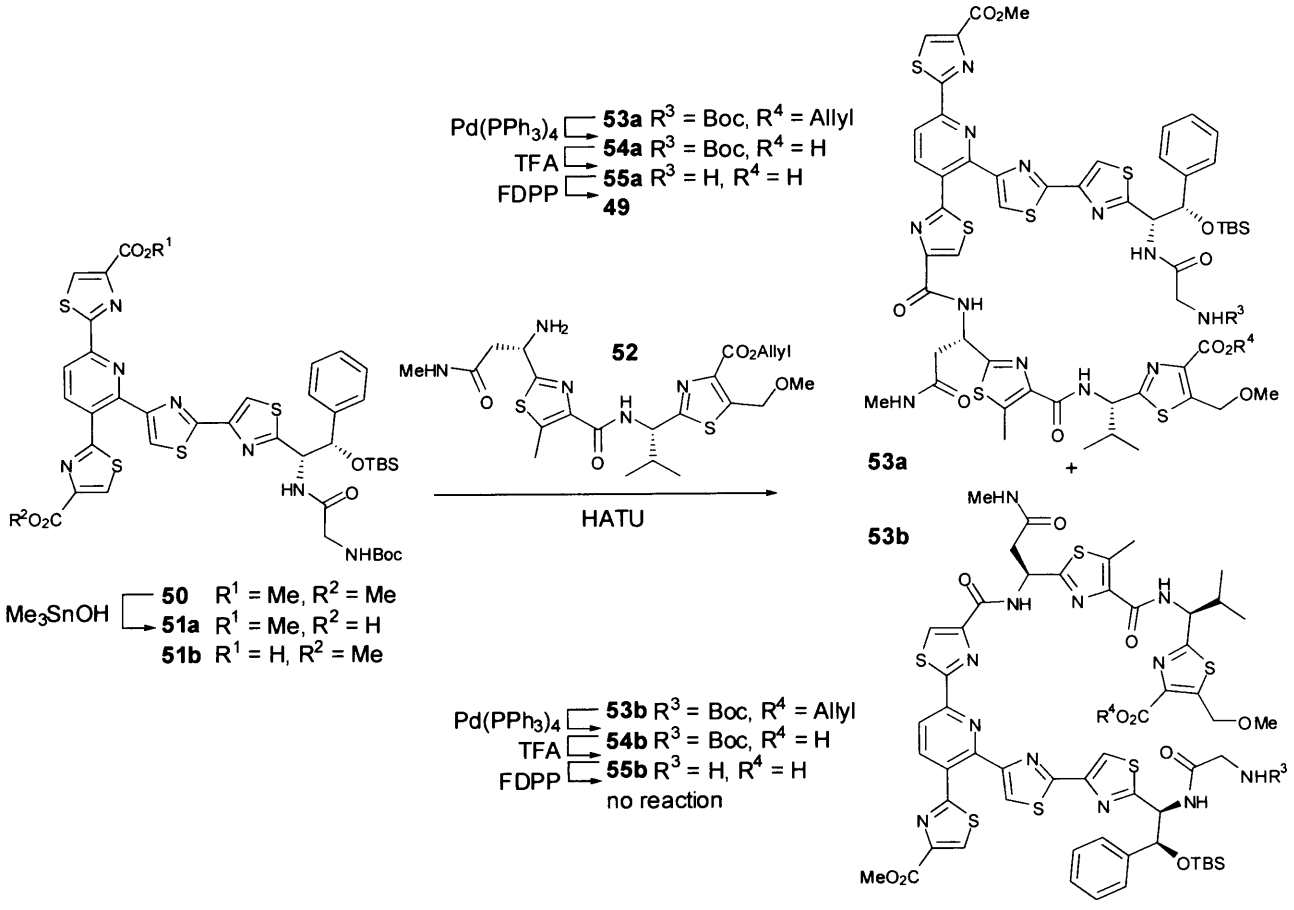
Factor	R^1	R^2	R^3	R^4, R^5
A	CH_2OMe	CH_3	CH_3	H
B1	CH_2OMe	CH_3	H	H
B2	CH_3	CH_3	CH_3	H
C1	H	CH_3	CH_3	H
C2a	CH_2OMe	CH_2OH	CH_3	H
C2b	CH_2OMe	H	CH_3	H
D1	H	CH_3	H	H
D2	CH_2OH	CH_3	CH_3	H
E	CH_2OH	CH_3	H	H
T	CH_2OMe	CH_3	CH_3	π-bond

Figure 7 Structure of the GE2270 factors

The first chemical syntheses of thiopeptides GE2270A and GE2270T were realised by Nicolaou *et al.* in 2006.[98] The route to both total syntheses proceeded *via* dehydropiperidine **43**, prepared by the biomimetic hetero-Diels-Alder dimerisation already successfully utilised in the synthesis thiostrepton. Elimination of ammonia and dehydrogenation of **43** furnished the pyridine core domain **44**, which was subsequently elaborated to **45**; HATU-mediated peptide coupling with **46** followed by ester hydrolysis with Me_3SnOH afforded esters **48a** and **48b**, only one of which underwent macrolactamisation on *N*-deprotection and treatment with FDPP to furnish macrocycle **49** (Scheme 7). An alternative approach to compound **49** was also investigated wherein pyridine domain **50** was hydrolysed to give a mixture of esters **51a** and **51b**. Coupling with peptide **52** and deprotection furnished **55a** and **55b**, and as previously observed, only the desired macrocycle **49** was formed, **55b** failing to cyclise (Scheme 8).

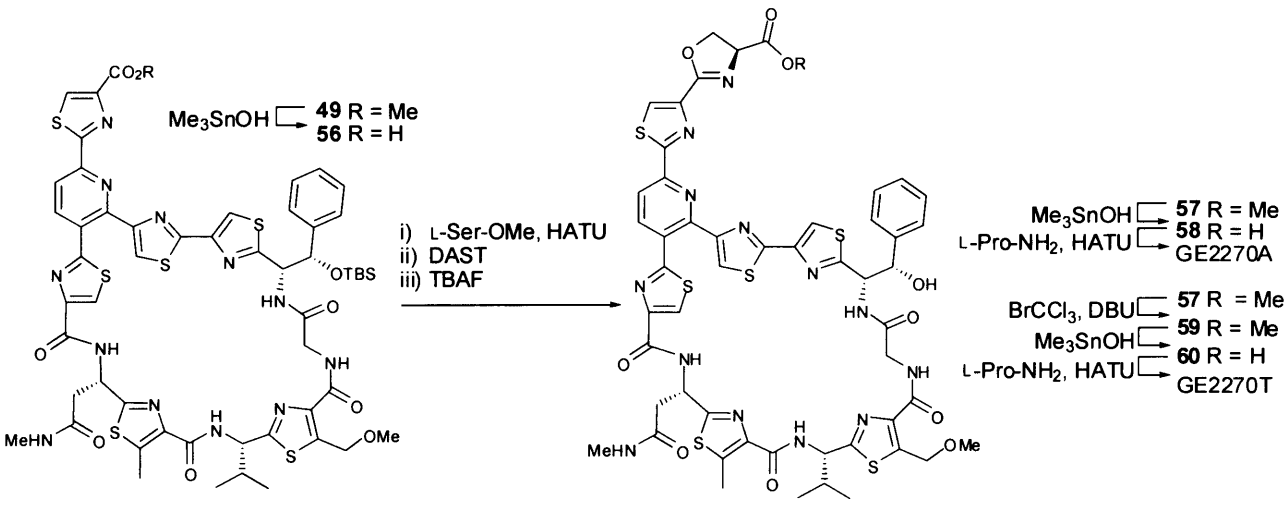


Scheme 7 Synthesis of GE2270 macrocycle **49**



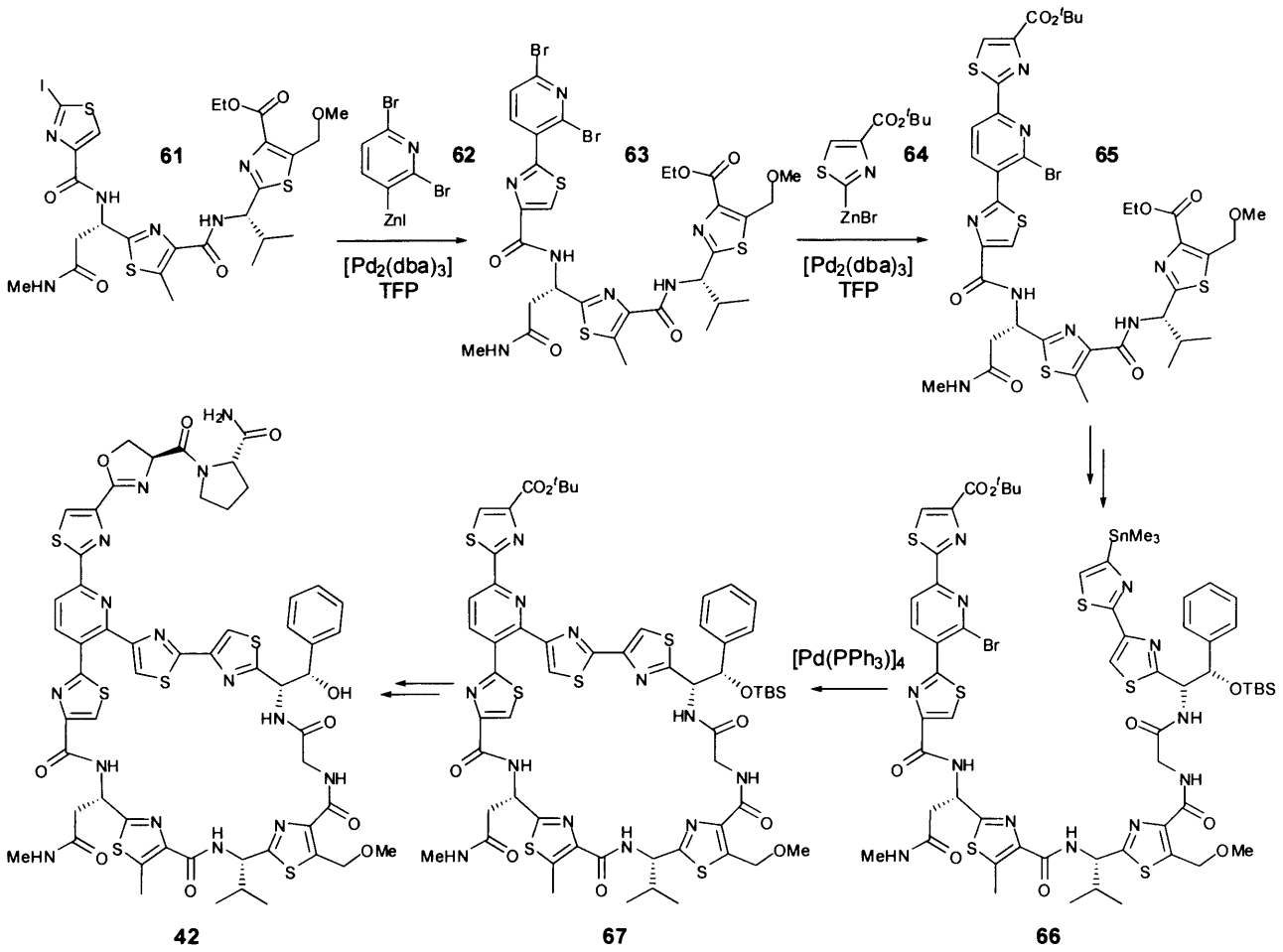
Scheme 8 Alternative approach to macrocycle **49**

Macrocycle **49** was then elaborated to oxazoline **57** by ester hydrolysis, coupling with serine methyl ester, treatment with DAST and deprotection. Advanced intermediate **57** was subsequently converted to both GE2270A and GE2270T (Scheme 9).



Scheme 9 Elaboration of **49** to GE2270A and GE2270T

GE2270A was also synthesised by Bach and co-workers in 2007 using an elegant strategy which differed markedly from those used in previous thiopeptide syntheses, in that the thiazole moieties were appended to the pyridine core by way of metal-catalysed cross-coupling reactions (Scheme 10).[99] Peptide building block **61** was coupled to organozinc reagent **62** in a Negishi cross coupling. The resulting fragment was submitted to a second Negishi coupling with thiazole **64** to afford **65**. Formation of organotin reagent **66** and intramolecular Stille coupling furnished macrocycle **67** from which the natural product was prepared in four further steps.

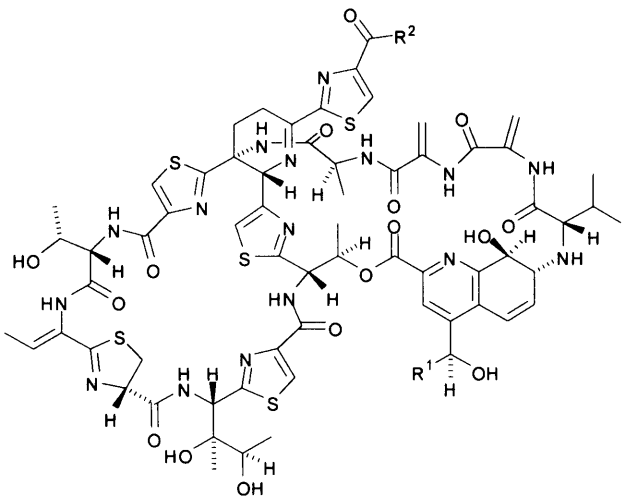


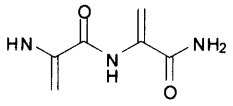
Scheme 10 Metal-catalysed cross coupling approach to GE2270A

1.4.5 – Siomycin A

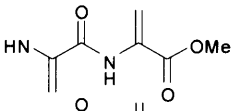
The siomycins (**68**) are a family of four thiopeptides with structures closely related to that of thiostrepton (**1**) (Figure 8). The siomycin antibiotic complex was first isolated from cultures of *Streptomyces sioyaensis* in 1959 and was found to be active against Gram-positive bacteria and mycobacteria.[100] Further purification of the crude preparation ten years later[101] found that this sulfur-containing peptide antibiotic actually consisted of one major component, siomycin A (**68a**), and three minor components, siomycin B, C and D_1, the latter of which was discovered 21 years after isolation of the original antibiotic complex.[102] Siomycin B (**68b**) (SIM-B, $C_{65}H_{75}N_{17}O_{16}S_5$) is derived from siomycin A (SIM-A, $C_{71}H_{81}N_{19}O_{18}S_5$) during storage whereas siomycin C (**68c**) (SIM-C, $C_{72}H_{82}N_{18}O_{19}S_5$) and D_1 (**68d**) (SIM-D_1, $C_{70}H_{79}N_{19}O_{18}S_5$) are true natural products of *Streptomyces sioyaensis*. Chemical degradation studies[103] in conjunction with a range of spectroscopic experiments, in particular ^{13}C and ^{1}H NMR studies,[104,105,106] were carried out in order to determine the relationship between the siomycin structure and that of thiostrepton, which had recently been characterised by X-ray crystallography, and also to explain the similar physicochemical and biological properties of these antibiotics. The isolation and identification of

the degradation products formed by reduction, oxidation, acidic hydrolysis and ammonolysis of the natural product was supported by extensive ^{1}H, ^{13}C and nOe NMR spectroscopic data, as well as ^{15}N NMR spectroscopy, and eventually confirmed that the siomycins differ from thiostrepton only in a dehydroalanine-valine unit attached to the quinaldic acid in place of an alanine-isoleucine residue.



Siomycin A (**68a**), R^1 = Me, R^2 = 

Siomycin B (**68b**), R^1 = Me, R^2 = NH_2

Siomycin C (**68c**), R^1 = Me, R^2 = 

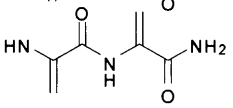
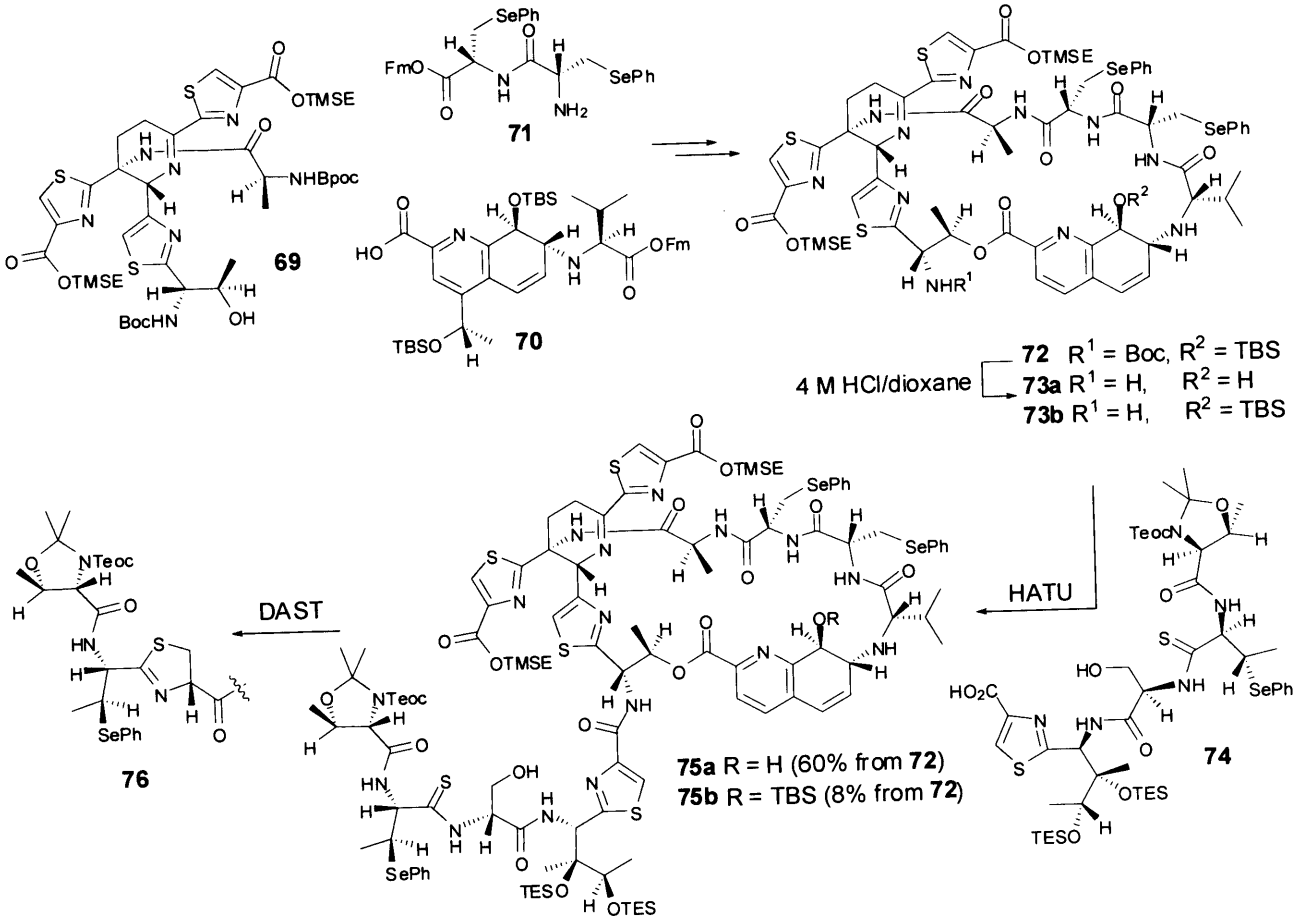
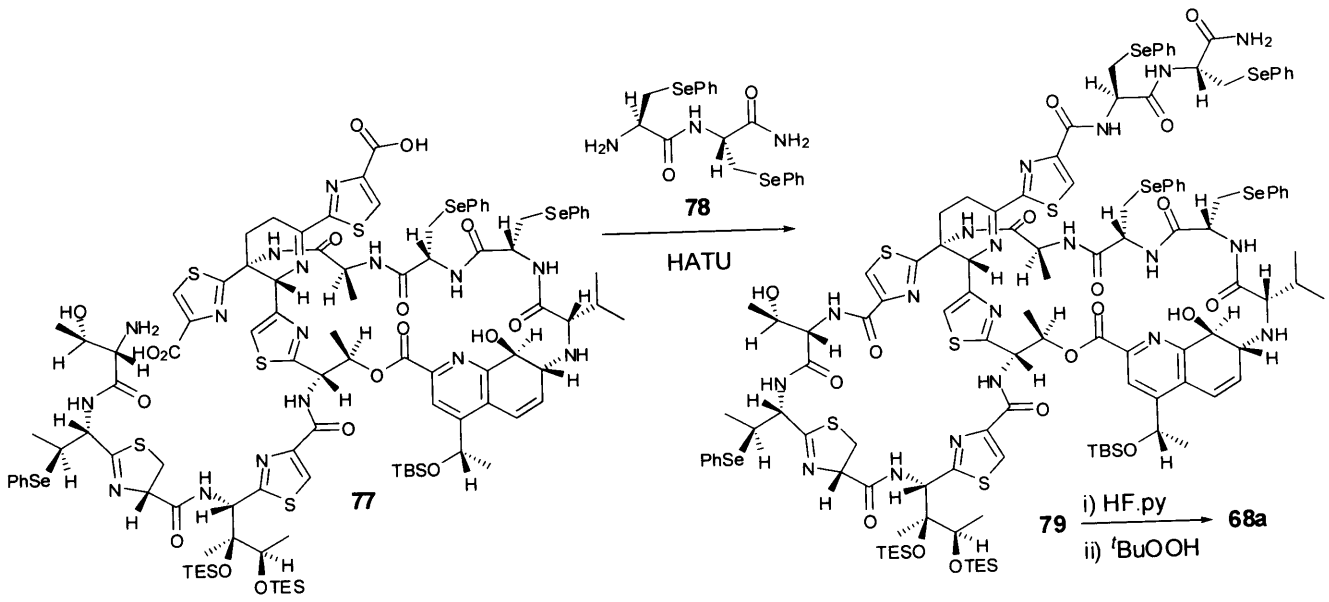
Siomycin D_1 (**68d**), R^1 = H, R^2 = 

Figure 8 Structure of the siomycins

Siomycin A (**68a**) was successfully synthesised by Hashimoto *et al.* in 2006.[107] Macrocyclic precursor **72**[108] was prepared by the coupling of dehydropiperidine **69**,[109] dihydroquinoline **70**[110] and peptide **71**.. Compound **72** was then deprotected and submitted to HATU-mediated peptide coupling with pentapeptide **74** (Scheme 11).[111] Treatment of the mixture of **75a** and **75b** with DAST effected cyclisation of the serine residue and adjacent thioamide to give the desired thiazoline **76**. Subsequent cleavage of the Teoc, acetonide and TMSE protecting groups with zinc(II) chloride furnished **77** in readiness for the second macrolactamisation and attachment of the side chain (Scheme 12). Cyclisation of **77** and coupling with peptide **78** was achieved in a one pot procedure by stirring **77** with HATU and DIPEA, followed by addition of **78**. The structural identity of reaction **79** could not be verified at this stage, but was confirmed following global deprotection with HF.pyridine and oxidative elimination with *tert*-butylhydroperoxide, as the natural product was isolated in 7% yield from **76**.



Scheme 11 Synthesis of siomycin A precursor **76**



Scheme 12 Completion of the total synthesis of siomycin A

1.5 – The Micrococcin Thiopeptides

Micrococcin was the first thiopeptide antibiotic to be isolated, and is one of the least structurally complex members of this class of compounds. Despite their relatively low molecular weight and lack of the intricate molecular architecture possessed by thiostrepton, the micrococcin thiopeptides have posed a challenge to synthetic chemists for more than half a century. What follows is an account of their isolation and the many attempts made to assign the correct structure by chemical degradation, spectroscopy and even the total synthesis of two epimeric micrococcin analogues.

1.5.1 – Isolation

The micrococcin antibiotic complex was first isolated in 1948 by Su from a newly identified strain of *Micrococcus* (later named *Micrococcus* NCTC 7128), found in a sample of sewage from the city of Oxford.[112] Micrococcin was reported to be 'an odourless, tasteless, amorphous, cream-coloured powder' and no studies were undertaken to determine the structural identity of the compound. Indeed, only a melting point and UV spectrum were published, and the optical rotation reported to be 'between +50° and +75°'. Following the first isolation of this antibiotic, methods for purification[113] and large scale production were investigated, the most successful of which utilised a Marmite™-containing culture medium.[114,115] An antibiotic isolated from a spore-bearing bacillus of the *B. pumilis* group found in soil collected from East Africa[116] was found to exhibit similar biological and physical properties to Su's micrococcin, and was named micrococcin P.[117] More recently, micrococcin P1 has been isolated from *Staphylococcus equorum* WS 2733, a strain of bacteria present on the surface of French Raclette cheese.[118,119]

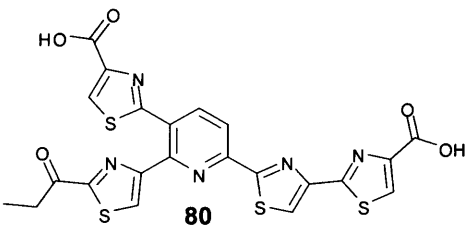
1.5.2 – Early Biological Studies

Initial studies on the biological activity of Su's micrococcin were hampered by the extremely low solubility in water and high molecular weight of the antibiotic.[120] Su had reported that micrococcin exhibited antibacterial activity against Gram-positive microorganisms *in vitro* but the aforementioned lack of solubility and consequent poor bioavailability *in vivo* immediately limited the usefulness of micrococcin clinically. Nevertheless, a small number of *in vivo* studies were conducted, the first of which entailed administration of a suspension of the antibiotic by injection into mice.[121] It was found that although micrococcin was removed from the bloodstream by phagocytic cells, it remained in the body and was not excreted. Whilst inside the organism, micrococcin retained antibiotic activity and exhibited no toxic effects. The effect of injection of a suspension of micrococcin on rabbits infected with tuberculosis was also studied.[122] The rabbits that were treated with micrococcin suspensions died earlier than the control animals, but were found to have fewer lesions in their lungs, liver and kidneys. It was also noted that despite

promising *in vitro* activity against several strains of tuberculosis bacteria, resistance to micrococcin developed rapidly and before an effective concentration could be reached. In the light of such discouraging results, micrococcin has found no clinical applications to date.

1.5.3 – Degradation Studies

Prior to the advent of NMR spectroscopy as a technique for routine use, chemical degradation studies were frequently employed as a means of structure determination for natural products. Micrococcin was the subject of a number of such investigations conducted by Walker *et al.* in the 1950's and 1960's. Acidic hydrolysis of Fuller's micrococcin P and analysis of the resulting fragments led to the proposal of a molecular weight in the region of 2200.[123] Further studies on the acid-soluble degradation products of micrococcin P led to the identification of aminoacetone and D-2-aminopropan-1-ol (D-alaninol),[124] although this was subsequently found to be D-(*R*)-isoalaninol in NMR studies (section 1.5.4). After investigation of the acid-insoluble fraction of micrococcin degradation products, it was concluded that the natural product possesses an extended chromophore containing several thiazoles.[125] The acid-insoluble component was named micrococcinic acid (**80**), which could be converted into dimethyl micrococcinate (**81**) on treatment with methanolic hydrogen chloride.[126] The structures of **80** and **81** were confirmed following ^{1}H-NMR spectroscopic[127] and X-ray crystallographic studies.[128]



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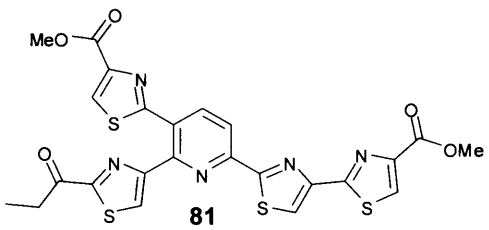

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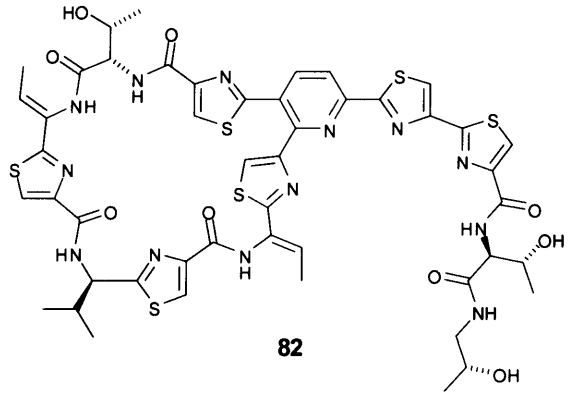
1.5.4 – The Walker-Lukacs Structure

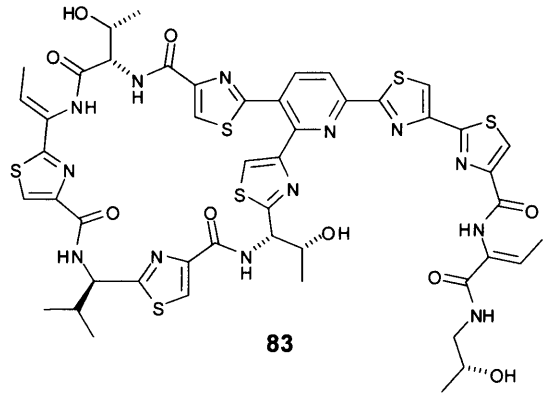
Walker's numerous degradation studies on micrococcin P were followed by a ^{13}C-NMR investigation, culminating in the 'Walker-Lukacs' structure of micrococcin P (**82**).[129] The assignment of the absolute stereochemistry of the amino acids was made based on amino acids isolated on acidic hydrolysis of the natural product; the connectivity of the residues was assigned based on assumed structural homology with thiostrepton (**1**) and nosiheptide (**5**).



82

1.5.5 – The Bycroft-Gowland Structure

Prompted by the publication of the Walker-Lukacs structure of micrococcin P, Bycroft and Gowland proposed an alternative structure following their own investigation of micrococcin P using ^{1}H- and ^{13}C-NMR spectroscopy in conjunction with degradation studies.[130] It transpired that micrococcin P was in fact a mixture of two components present in a ratio of approximately 7:1. The major component was named micrococcin P_1 (**83**; also referred to as micrococcin P1 or MP1) and the minor component micrococcin P_2 (**84**; micrococcin P2, MP2), and it was found that reduction of MP2 with sodium borohydride furnished MP1. The identification of the two components of the micrococcin antibiotic complex is in agreement with Walker's earlier degradation studies, accounting for the isolation of both D-(*R*)-isoalaninol and aminoacetone on acidic hydrolysis of the natural product. As with the Walker-Lukacs structure, the assignment of absolute stereochemistry was based on Walker's original degradation studies.



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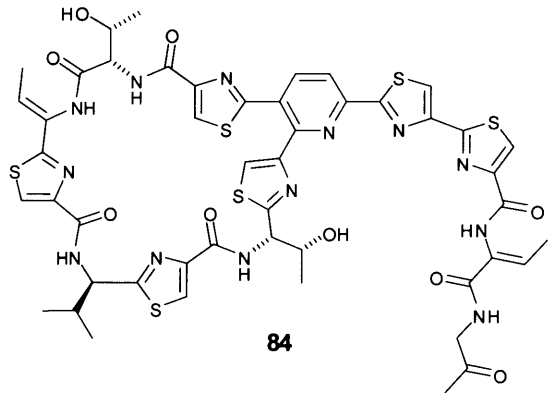
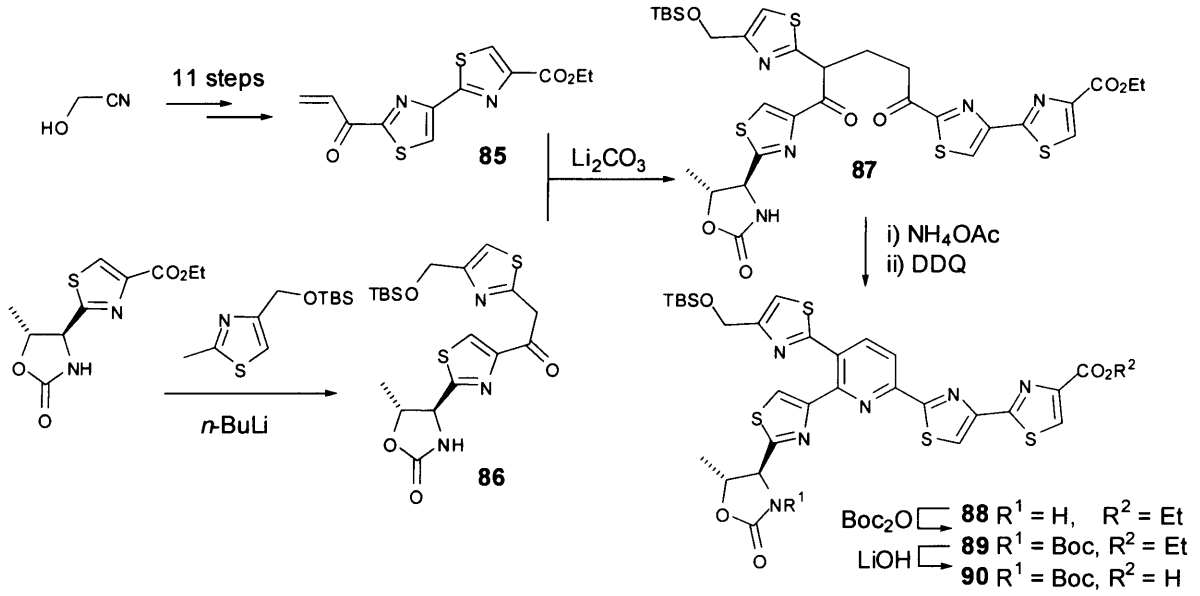


84

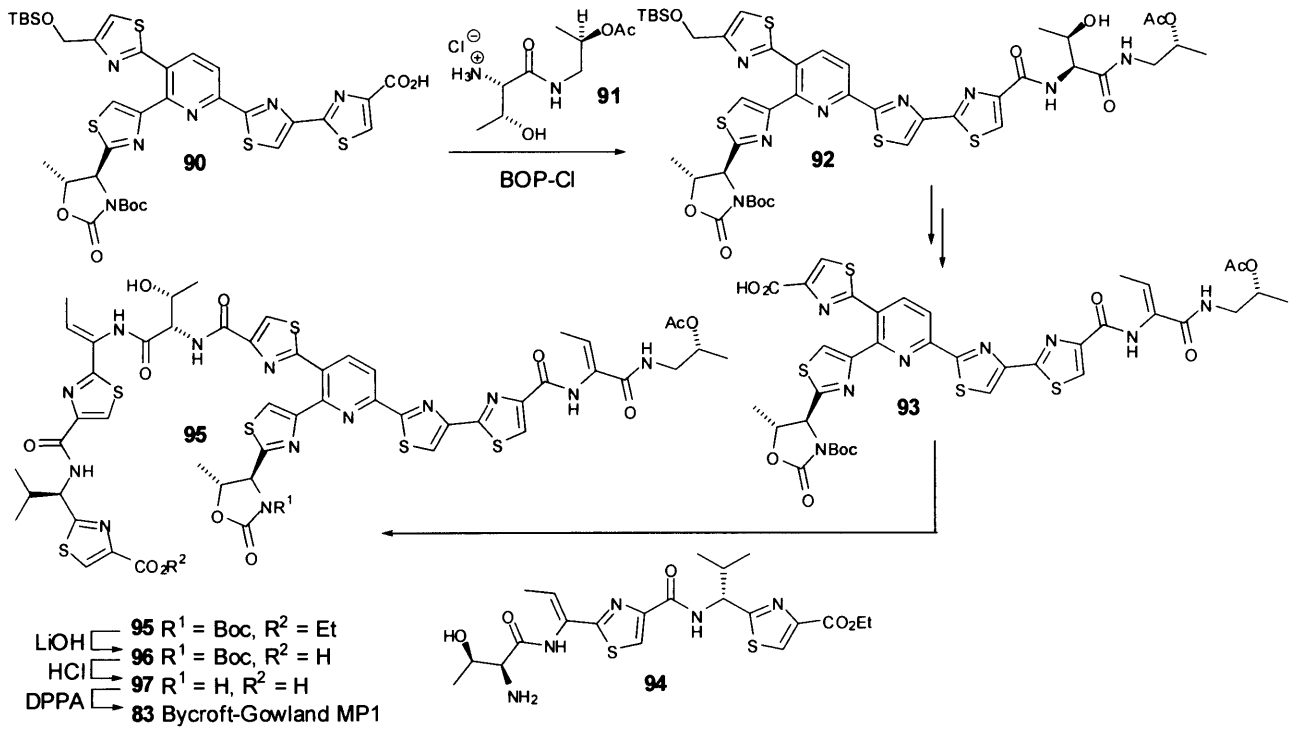
1.5.6 – Ciufolini's Synthesis of Bycroft-Gowland MP1

The Bycroft-Gowland structure of micrococcin P1 was accepted for more than 20 years, until Ciufolini's total synthesis of **83** demonstrated that Bycroft-Gowland MP1 is not identical to the natural product. Ciufolini's landmark total synthesis of **83** began with the preparation of pyridine core **90** (Scheme 13).



Scheme 13 Synthesis of pyridine core domain **89**

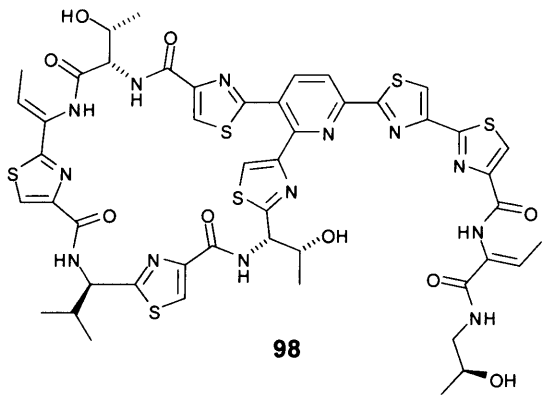
Bis-thiazole enone **85** and ketone **86** were combined to form 1,5-diketone **87** *via* Michael addition. Treatment with ammonium acetate followed by DDQ oxidation furnished the central pyridine moiety; *N*-Boc protection and hydrolysis of the ethyl ester afforded core domain **90** in readiness for elaboration to the natural product.[131] BOP-mediated coupling with side chain unit **91** gave fragment **92** which was further elaborated to yield **93**, with the dehydrodemethylvaline unit present in the side chain and free carboxylic acid on the 3-thiazole in preparation for coupling with tripeptide sector **94** (Scheme 14). Coupling afforded advanced intermediate **95**, which on deprotection and macrolactamisation gave Bycroft-Gowland micrococcin P1 (**83**) in 23 steps and 5.7% yield (longest linear route).[132] Careful examination of the spectroscopic data of **83** and comparison with that of the natural material (**4**) showed that the proposed structure, accepted for more than two decades was incorrect and set in motion many studies to rectify this problem.



Scheme 14 Completion of the synthesis of Bycroft-Gowland MP1

1.5.7 – Shin's Synthetic Epimers of MP1

The confusion surrounding the structure of the micrococcin thiopeptides was heightened with the publication of two total syntheses by Shin *et al.* of 'micrococcin P_1',[133] in fact an epimer of Bycroft-Gowland MP1 containing L-(*S*)-isoalaninol in place of the correct D-(*R*)-isoalaninol residue (**98**), and 'micrococcin P' (**99**),[134] which is an epimer of Walker-Lukacs micrococcin, again possessing L-(*S*)-isoalaninol in the side chain. Despite the claim that these compounds exhibited spectroscopic and physical properties identical to the natural material, neither **98** nor **99** represent the correct structure of natural MP1, the (*R*)-stereochemistry of the isoalaninol residue having been conclusively established in degradation studies by Walker and Bycroft.[130]



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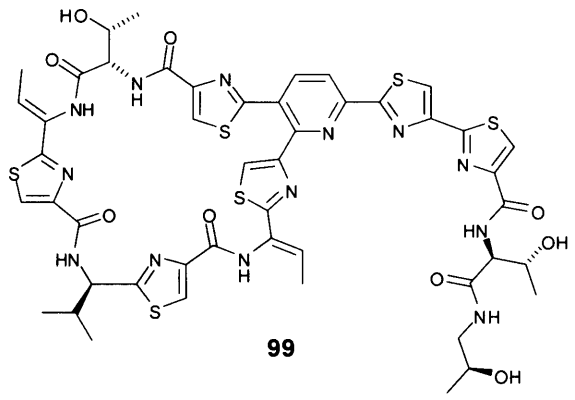
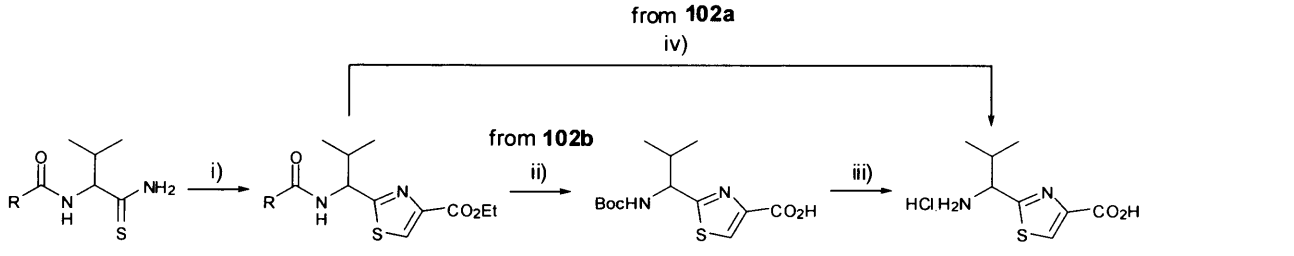


99

1.5.8 – The Stereochemistry of MP1 Reinvestigated

Following Ciufolini's discovery that Bycroft-Gowland micrococcin P1 was not spectroscopically identical to the natural product, it was necessary to ascertain where the structural differences between the two compounds lay. Extensive NMR studies using HSQC and HMBC techniques were used to validate the Bycroft-Golwland hypothesis with regard to the order of the amino acid residues, and it was concluded that the difference between natural MP1 and Bycroft-Gowland MP1 was purely stereochemical.[135] A re-examination of the literature with respect to the assignment of the stereochemistry of the amino acids reveals that the valine residue was initially assigned as being 'probably of the D-configuration' on the basis of comparison of degradation product **100** with synthetic material prepared from L-valine.[136] Walker *et al.* were able to reproducibly isolate **100** from the acidic hydrolysis of micrococcin with an optical rotation of -6.2 (*c* 0.5, H_2O). Walker then prepared **100** from L-valine and recorded values for optical rotation varying between 0 and -20.8, leading the conclusion that the natural product contains D-valine. Walker's synthesis of **100** employed the Hantzsch thiazole synthesis, a reaction which is known to racemise α-aminothioamides (**101**),[137,138] and also harsh acidic conditions to deprotect thiazole **102a**. In view of these ambiguous results, a reinvestigation of Walker's study was undertaken[139] whereby both enantiomers of **100** were prepared *via* a known alternative route,[140] and the enantiomeric purity of key intermediates (*S*)- and (*R*)-**102b** confirmed by HPLC (Scheme 15).

The close agreement in the optical rotations recorded for (*S*)-**100** isolated from micrococcin P1 and (*S*)-**100** prepared from enantiomerically pure (*S*)-**102b**, and the equal but opposite optical rotation of (*R*)-**100** from (*R*)-**102b** led to the proposal that the valine residue in micrococcin P1 should be reassigned as *S*. Figure 9 provides a summary of the various structures of the micrococcin thiopeptides proposed over the past fifty years, a long-standing mystery which, due to the scarcity of the natural product, awaits chemical synthesis to provide a solution.



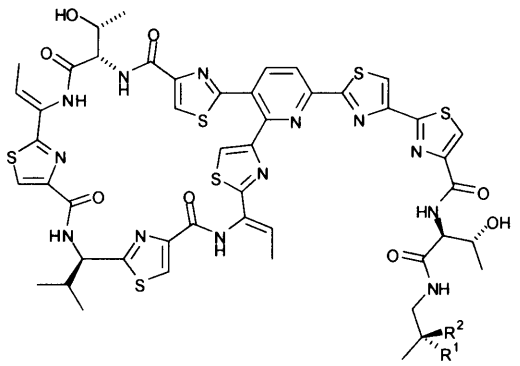
101a R = Ph
101b R = O^{t}Bu

(*S*)-**102a** R = Ph
(*S*)-**102b** R = O^{t}Bu, 97% *ee*
(*R*)-**102b** R = O^{t}Bu, 97% *ee*

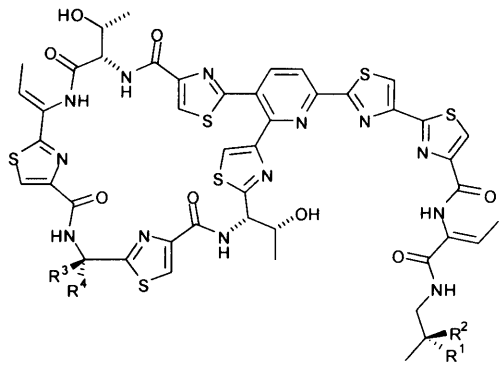
(*S*)-**100** $[\alpha]_D^{23}$ -7.2 (*c* 0.5, H_2O)
(*R*)-**100** $[\alpha]_D^{23}$ +7.2 (*c* 0.5, H_2O)
100 from MP1 $[\alpha]_D$ -6.2 (*c* 0.5, H_2O)
(*S*)-**100** from **102a** $[\alpha]_D^{25}$ +5.2 (*c* 0.5, H_2O)

Reagents and conditions: i) From **101a**, ethyl bromopyruvate, EtOH, reflux; From **101b**, $KHCO_3$, ethyl bromopyruvate, DME, then TFAA, 2,6-lutidine; ii) From **102b**, LiOH, H_2O, MeOH, r.t.; iii) HCl in dioxane, r.t.; iv) From **102a**, 20% HCl (aq), reflux.

Scheme 15 Reinvestigation of the stereochemistry of the valine-derived thiazole



Walker-Lukacs micrococcin (**82**) R^1 = H, R^2 = OH
Shin's micrococcin P (**99**) R^1 = OH, R^2 = H



Bycroft-Gowland MP1 (**83**) R^1 = OH, R^2 = H, R^3 = $CHMe_2$, R^4 = H
Bycroft-Gowland MP2 (**84**) R^1, R^2 = O (π-bond), R^3 = $CHMe_2$, R^4 = H
Shin's micrococcin P1 (**98**) R^1 = H, R^2 = OH, R^3 = $CHMe_2$, R^4 = H
Proposed structure MP1 (**4**) R^1 = OH, R^2 = H, R^3 = H, R^4 = $CHMe_2$
Proposed structure MP2 (**103**) R^1, R^2 = O (π-bond), R^3 = $CHMe_2$, R^4 = H

Figure 9 Structures of the micrococcin thiopeptides

Chapter Two – Results and Discussion

Reinvestigation of MP1 Stereochemistry by Chemical Degradation Studies

2.1 – Introduction

2.2 – Walker's Degradation Studies

2.3 – Determination of Stereochemistry in Cyclic Peptides

2.4 – Synthesis and Degradation of Model Peptide **115**

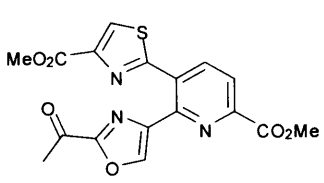
2.5 – Degradation of Natural MP1

2.6 – Conclusion

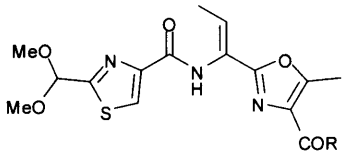
2 – Reinvestigation of MP1 Stereochemistry by Chemical Degradation Studies

2.1 – Introduction

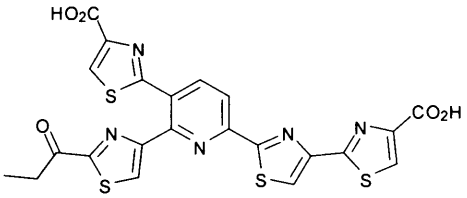
Degradation studies were widely used as a means of structure determination for the thiopeptide antibiotics prior to the advent of sophisticated spectroscopic techniques such as COSY, NOESY, HSQC and HMBC-NMR spectroscopy and high resolution FAB-MS which can now be used routinely. As well as yielding an array of smaller compounds of synthetic interest to chemists (Figure 10),[141,142,143,144,145,146] degradation studies can provide valuable information unavailable by conventional spectroscopic techniques, in particular the absolute stereochemistry of stereogeneic centres within a natural product.



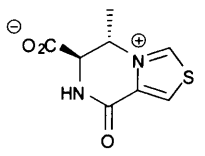
dimethyl sulfomycinamate



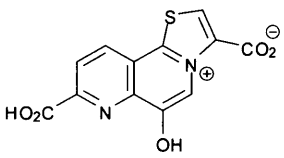
methyl sulfomycinate, R = OMe
sulfomycinic amide, R = NH_2



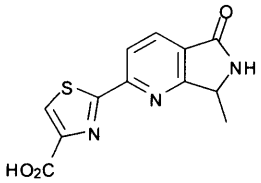
micrococcinic acid



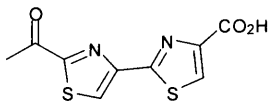
sulfomycinine



berninamycinic acid



cyclothiazomycin lactam



saramycetic acid

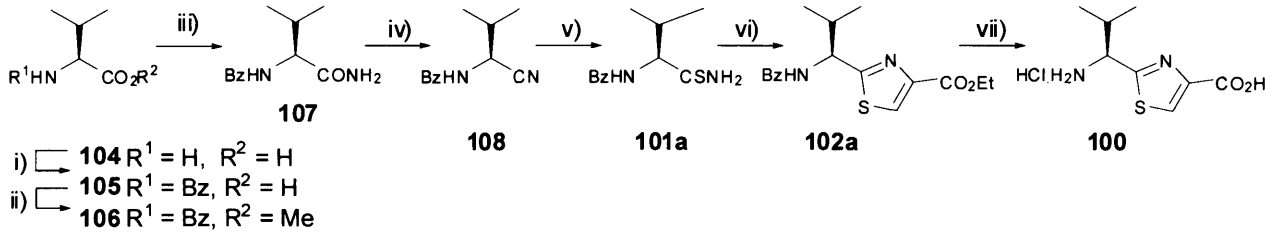
Figure 10 Thiopeptide degradation products

2.2 – Walker's degradation studies

As discussed previously (sections 1.5.3 and 1.5.8), the chemical degradation of micrococcin P1 was studied extensively by Walker *et al.* between 1957 and 1967. Walker and co-workers reported the reproducible isolation of laevorotatory 2-(1-amino-2-methylpropyl)thiazole-4-carboxylic acid (**100**) from the acidic hydrolysis of the natural product.[136] Subsequent synthesis of **100** from L-valine and comparison of the optical rotation of the synthetic and isolated material led to the assignment of the valine-derived stereogenic centre in MP1 as '*probably of the D-configuration*'.

2.2.1 – Walker's synthesis of thiazole 100

Walker's synthesis of thiazole **100** (Scheme 16) began with L-valine (**104**), which was benzoylated using Schotten-Baumann conditions, esterified with diazomethane and treated with methanolic ammonia to afford amide **107**. Dehydration to nitrile **108** followed by thionation with liquid hydrogen sulphide furnished thiazole precursor **101a**. As discussed previously (section 1.5.8) the Hantzsch thiazole synthesis was employed in the preparation of **102a**. Walker reports an optical rotation of -3.7 (*c* 2.5, ethanol) for thiazole (*S*)-**102a**, significantly lower than that reported by Holzapfel [-42.0 (*c* 2.6, chloroform)][137] and Meyers [-37.5 (*c* 2.6, chloroform)].[138]

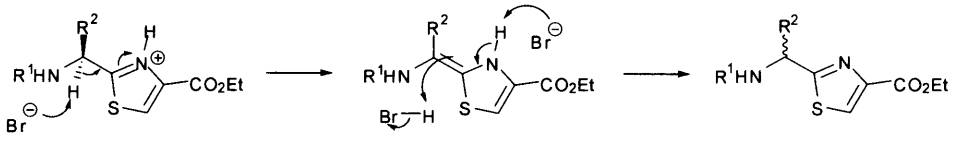


Reagents and Conditions i) Schotten-Baumann conditions; ii) Diazomethane; iii) NH_3 in MeOH, 110 °C, 8 h, 58%; iv) $POCl_3$, pyridine, 0 °C, 30 mins, 72%; v) liquid H_2S, 55 °C, 8 h, 89%; vi) ethyl bromopyruvate, ethanol, reflux, 96%; vii) 20% w/v HCl, reflux, 4 h, 75%.

Scheme 16 Walker's synthesis of thiazole **100**

2.2.2 – Racemisation of amino acid-derived thiazoles

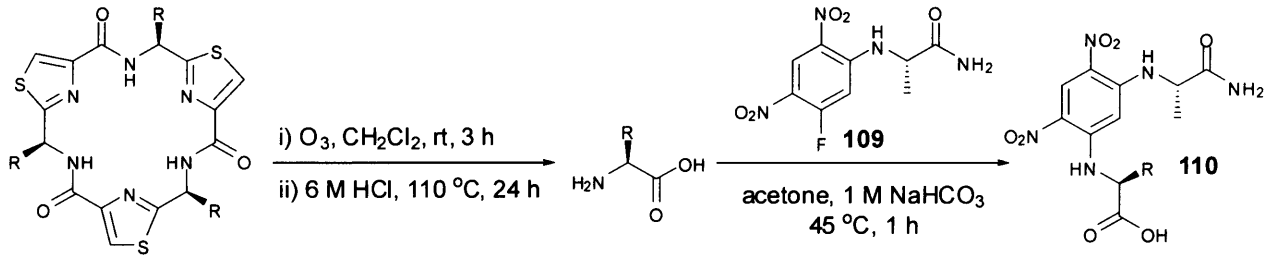
The Hantzsch synthesis of thiazoles by reaction of an amino acid-derived thioamide and α-bromocarbonyl compound at reflux in ethanol[147,148] is known to result in epimerisation at the α-stereogenic centre as a result of the concomitant formation of one equivalent of hydrogen bromide (Scheme 17).[137,138] Hence, Walker's use of these conditions in the preparation of thiazole **102a** is the likely cause of the erroneous assignment of the stereochemistry of the valine residue in micrococcin P1. Modifications of the original Hantzsch synthesis to circumvent the problem of racemisation will be discussed in detail in the next chapter. Furthermore, deprotection of thiazole **102a** using refluxing hydrochloric acid would exacerbate the problem, as thiazole hydrochloride salt **100** is known to racemise completely in aqueous solution at room temperature in 3 hours.[139]



Scheme 17 Racemisation of amino acid-derived thiazoles in the Hantzsch thiazole synthesis

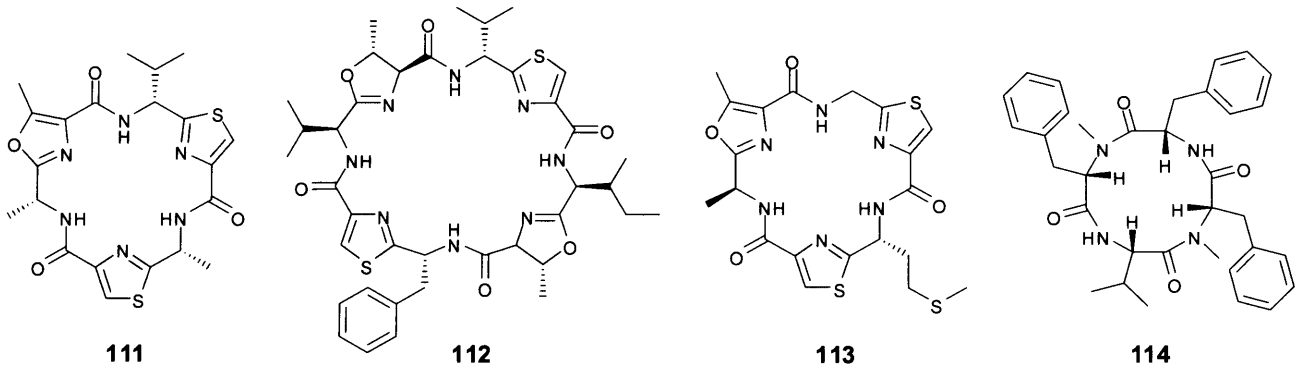
2.3 – Determination of stereochemistry in cyclic peptides

In view of the problematic racemisation encountered when treating α-amino acid-derived thiazoles with acids, a method for the degradation and analysis of macrocyclic heterocyclic peptides needed to be established which does not result in racemisation of stereogenic centres adjacent to thiazoles, thus enabling the assignment of absolute stereochemistry of the amino acid residues present in the natural product. The general method is outlined in Scheme 18 using a model cyclic peptide.



Scheme 18 General method for the racemisation-free degradation of cyclic peptides

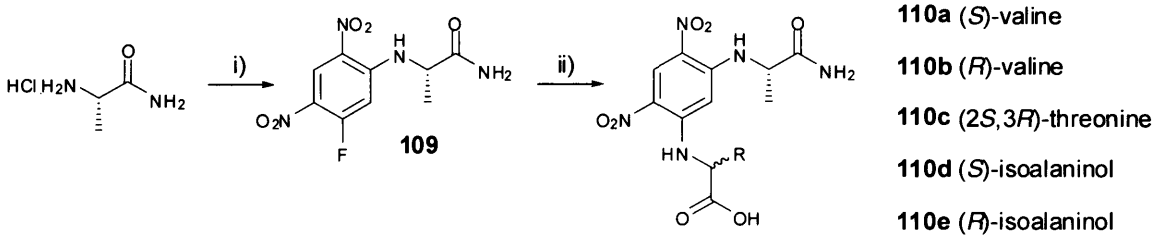
The first step in the sequence is treatment of the natural product with ozone, in order to derivatise the thiazoles,[149] destroying their aromaticity and preventing racemisation.[150] The residue from the ozonolysis step is then subjected to acidic hydrolysis with 6 M hydrochloric acid to afford the constituent amino acids. Treatment of the hydrolysis products with Marfey's reagent (1-fluoro-2,4-dinitrophenyl-5-L-alanine amide, FDAA, **109**)[151] affords compounds with the general structure **110**, containing a chromophore which makes the derivatives readily detectable by UV spectroscopy and ideal for analysis by HPLC. This method has been used to determine the absolute stereochemistry of amino acid residues in numerous cyclic peptides including dendroamide A (**111**), patellamide E (**112**), nostocyclamide M (**113**),[152] and hirsutide (**114**).[153]



111 **112** **113** **114**

2.3.1 – Synthesis and use of Marfey's Reagent

Marfey's reagent was prepared according to literature procedures by the reaction of L-alaninamide with 1,5-difluoro-2,4-dinitrobenzene (Scheme 19). The product was then reacted with a range of amino acids to form diastereomeric derivatives **110a-e**.



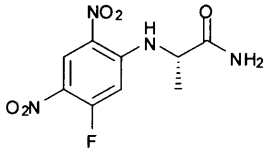
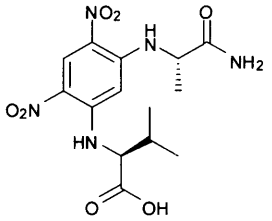
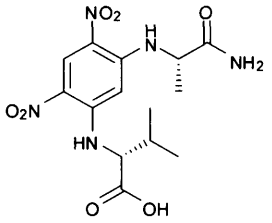
Reagents and Conditions i) 1 M NaOH, acetone, $MgSO_4$, r.t., 3 h, then 1,5-difluoro-2,4-dinitrobenzene, acetone, r.t., 30 min, 67%; ii) amino acid, water-acetone, 1 M $NaHCO_3$, 35 °C, 1 h then 2 M HCl.

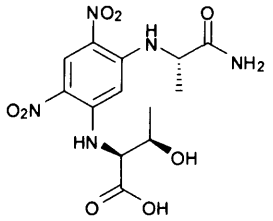
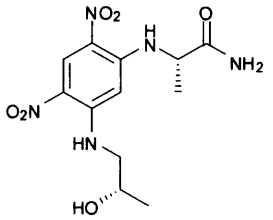
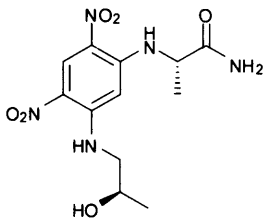
Scheme 19 Synthesis and use of Marfey's reagent

2.3.2 – HPLC analysis of derivatised amino acids

Amino acid derivatives **110a-e** were analysed by reverse-phase HPLC using a chiral Hypersil 5μ reverse phase column, gradient eluting with 10% - 50% acetonitrile in 50 mM TEAP buffer (pH 3) at a flow rate of 0.25 ml min^{-1} and detected at 340 nm. The retention times are given in Table 3.

Table 3 Retention times of FDAA-derivatised amino acids

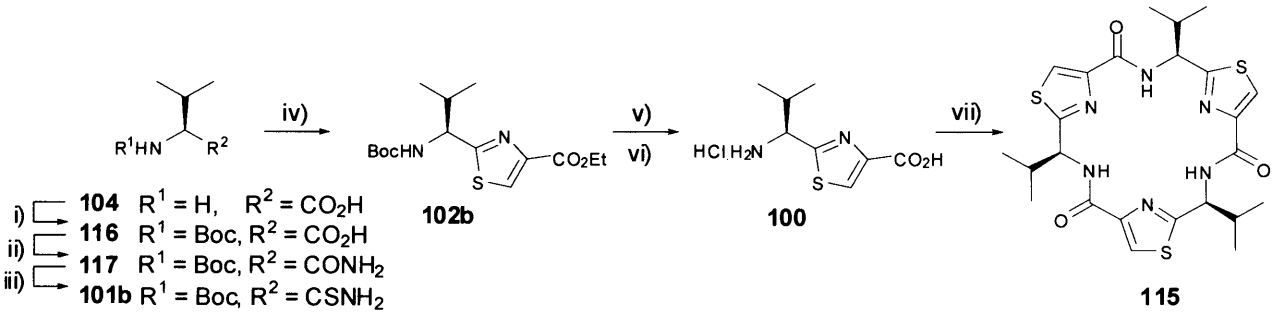
Compound	Structure	Retention time /mins
109		35
110a		40
110b		46

Compound	Structure	Retention time /mins
110c		28
110d		32
110e		33

2.4 – Synthesis and degradation of model peptide 115

2.4.1 – Synthesis of cyclic peptide 115

In order to verify that the degradation method outlined in section 2.3 was suitable for the analysis of the valine residue in micrococcin P1, cyclic hexapeptide **115** was prepared in 7 steps from (*S*)-valine according to Pattenden's method (Scheme 20).[140] Valine was Boc-protected and converted to amide **117**. Thionation of the amide with Lawesson's reagent afforded thioamide **101b** which was elaborated to thiazole **102b** using Meyers' modification of the Hantzsch thiazole synthesis.[138] *N*- and *C*-terminus deprotection furnished (*S*)-**100** and subsequent treatment with pentafluorophenyl diphenylphosphinate (FDPP) gave the desired cyclic peptide in 30% yield.

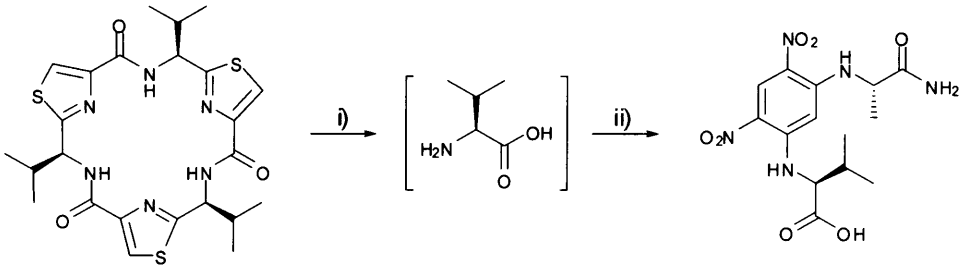


Reagents and Conditions i) Boc_2O, *t*-BuOH-H_2O, r.t., 16 h, 98%; ii) EtOCOCl, Et_3N, THF, 0 °C, 1 h, then 35% aq NH_3, 0 °C, 1 h, 95%; iii) Lawesson's Reagent, CH_2Cl_2, reflux, 24 h, 66%; iv) Ethyl bromopyruvate, $KHCO_3$, DME, -20 °C, 16 h, then TFAA, 2,6-lutidine, -20 °C, 30 mins, 59%; v) LiOH, MeOH-H_2O, r.t., 16 h, 90%; vi) 4 M HCl in dioxane, r.t., 2 h, 96%; vii) FDPP, DIPEA, MeCN, r.t., 18 h, 30%.

Scheme 20 Synthesis of cyclic hexapeptide **115**

2.4.2 – Degradation and analysis of hexapeptide 115

Cyclic hexapeptide **115** was subjected to the degradation conditions previously applied to a number of cyclic peptide natural products (section 2.3). It was hoped that the resulting HPLC trace would show only the presence of (*S*)-valine thus indicating that the degradation proceeded without racemisation and that the same conditions could be applied to micrococcin P1. In order to accurately replicate conditions which would be used for the degradation of the natural product and verify the sensitivity of the method, one milligram of hexapeptide **115** was analysed as illustrated in Scheme 21.



115 **110a**

Reagents and Conditions: i) Ozone, CH_2Cl_2, r.t., 3 h, then 6 M HCl, 110 °C, 24 h; ii) Marfey's reagent, water-acetone, 1 M $NaHCO_3$, 45 °C, 1 h, then 2 M HCl.

Scheme 21 Degradation and analysis of model peptide **115**

Analysis of the resulting solution by reverse-phase HPLC[i] showed the presence of (*S*)-valine-DAA (**110a**, retention time 39.5 minutes) and unreacted Marfey's reagent (**109**, retention time 37 minutes) (Figure 11). Spiking the sample with a solution of racemic valine-DAA (**110a** and **110b**) resulted in an increase in intensity of the peak at 39.5 minutes and the appearance of a new peak at 46 minutes, indicating the absence of (*R*)-valine in the original sample and thus confirming that the degradation of **115** proceeded without racemisation of the valine residues.

[i] Chiral Hypersil 5μ reverse phase column, gradient eluting with 10% - 50% acetonitrile in 50 mM TEAP buffer (pH 3), 0.25 ml min^{-1}, detected at 340 nm.

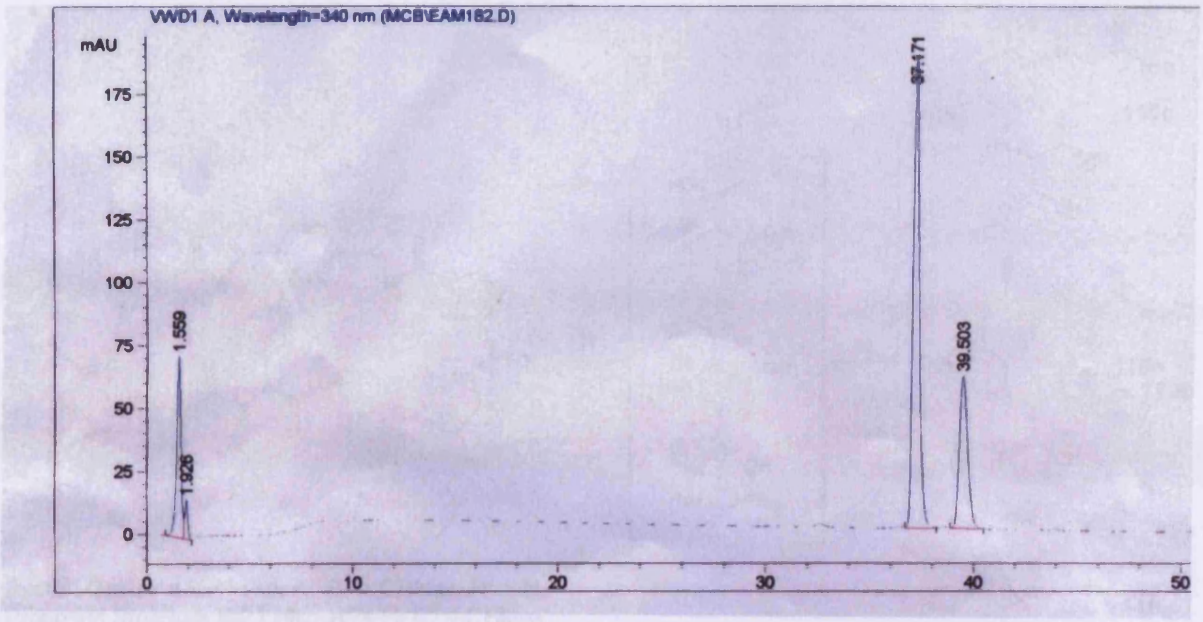
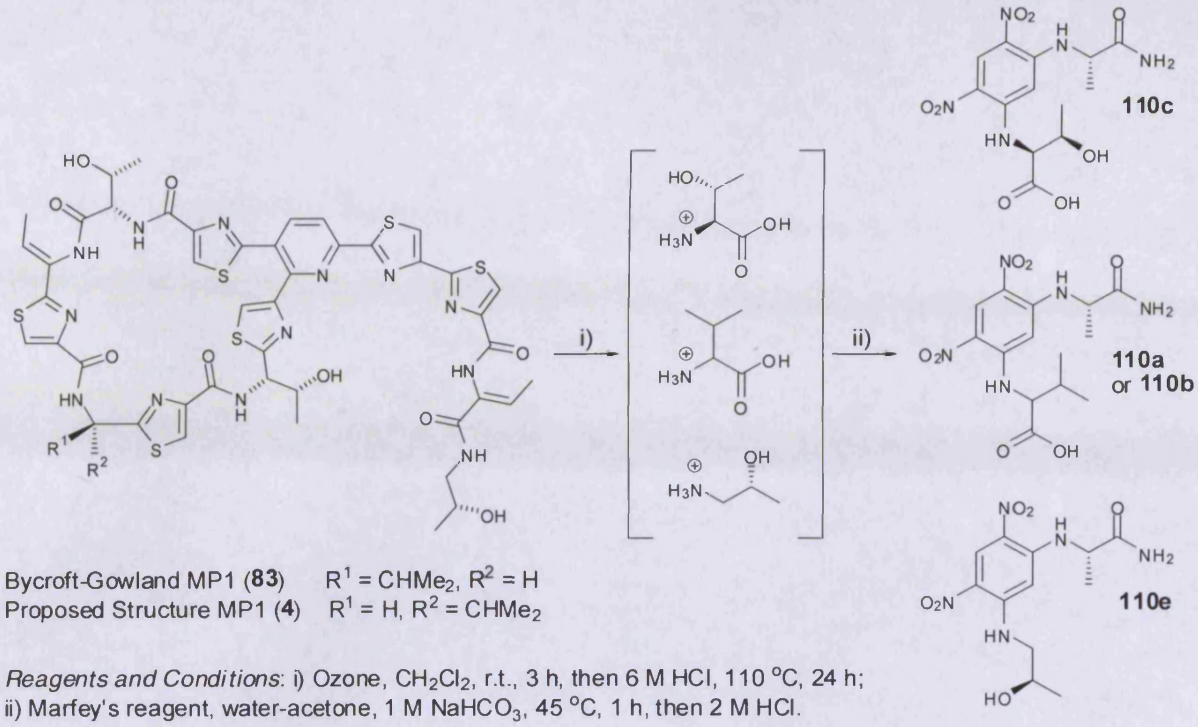


Figure 11 HPLC analysis of FDAA-derivatised **115** degradation products

2.5 – Degradation of natural MP1

2.5.1 – Chemical degradation of MP1

Encouraged by the successful degradation of model cyclic peptide **115**, the same method was applied to micrococcin P1 in order to confirm our hypothesis that the Bycroft-Gowland structure of MP1 (**83**) differs from the natural product in the stereochemical configuration of the valine residue.[139] To this end, one milligram of micrococcin P1 was subjected to ozonolysis, acidic hydrolysis and finally FDAA derivatisation (Scheme 22).



Bycroft-Gowland MP1 (**83**) $R^1 = CHMe_2$, $R^2 = H$
Proposed Structure MP1 (**4**) $R^1 = H$, $R^2 = CHMe_2$

Reagents and Conditions: i) Ozone, CH_2Cl_2, r.t., 3 h, then 6 M HCl, 110 °C, 24 h;
ii) Marfey's reagent, water-acetone, 1 M $NaHCO_3$, 45 °C, 1 h, then 2 M HCl.

Scheme 22 Degradation of natural MP1

2.5.2 – HPLC analysis of MP1 degradation products

The following figures show the HPLC traces obtained after the degradation of MP1 and the subsequent spiking experiment performed on the natural product samples in order to confirm the identities of the peaks. Figure 12a shows the derivatised degradation products of MP1, with peaks at 26 minutes, 32 minutes, 33 minutes, 35 minutes, 37 minutes and 40 minutes.

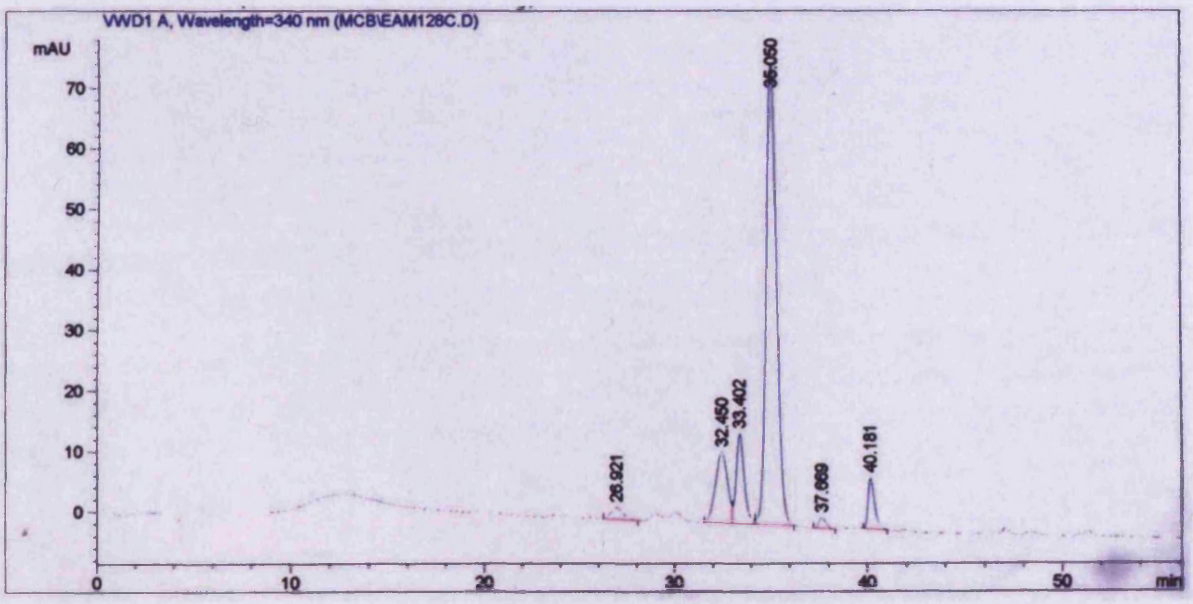


Figure 12a HPLC analysis of FDAA-derivatised MP1 degradation products

On spiking with (*S*)-valine-DAA (**110a**) (Figure 12b), the peak at 40 minutes shows a marked increase in intensity, confirming the presence of (*S*)-valine in natural micrococcin P1. Spiking with *rac*-valine-DAA (Figure 12c) results in the same increase in intensity of the peak at 40 minutes (**110a**) and also gives rise to a new peak at 46 minutes (**110b**; (*R*)-val-DAA) which is absent in the natural product. This confirms the presence of (*S*)-valine and also the absence of (*R*)-valine in the natural product, and is in keeping with earlier findings regarding the stereochemistry of the valine residue.[139]

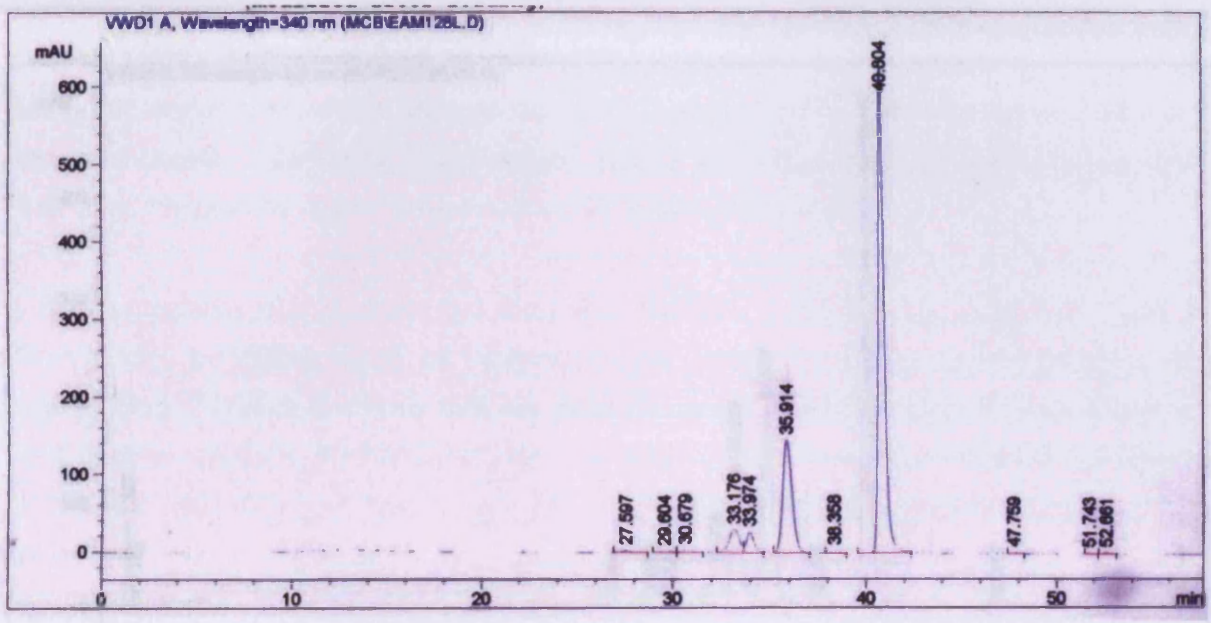


Figure 12b HPLC of MP1 degradation products spiked with **110a**

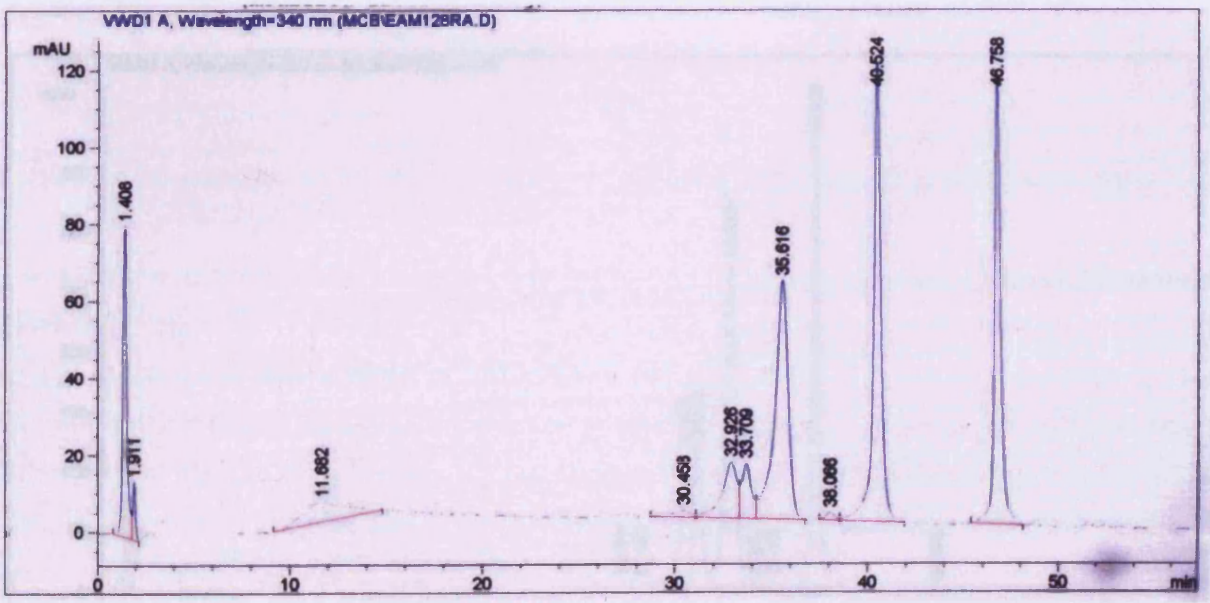


Figure 12c HPLC of MP1 degradation products spiked with **110a** and **110b**

The stereochemistry of the isoalaninol residue had been confirmed as *R* by Walker *et al.* during the course of their degradation studies on micrococcin and is not in doubt.[124,130] Spiking with **110d** resulted in an increase in intensity of the peak at 33 minutes, confirming, as expected, the presence of (*R*)-isoalaninol in micrococcin P1 (Figure 12d). Surprisingly, (*S*)-isoalaninol was also identified in the mixture of degradation products (Figure 12e), demonstrating that although this method of cyclic peptide degradation prevents racemisation at stereogenic centres adjacent to thiazoles, it is not always reliable for the determination of stereochemistry of all the stereogenic centres present in a natural product. A possible mechanism for the racemisation of the isoalaninol side chain is illustrated in Scheme 23.

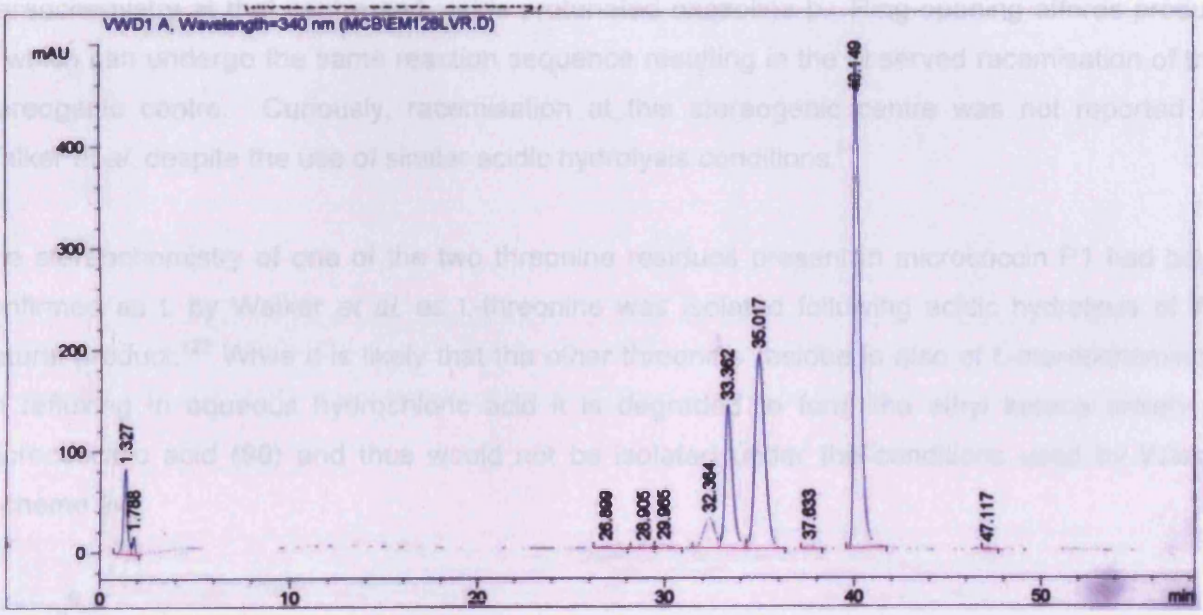


Figure 12d HPLC of MP1 degradation products spiked with **110a**, **110b** and **110d**

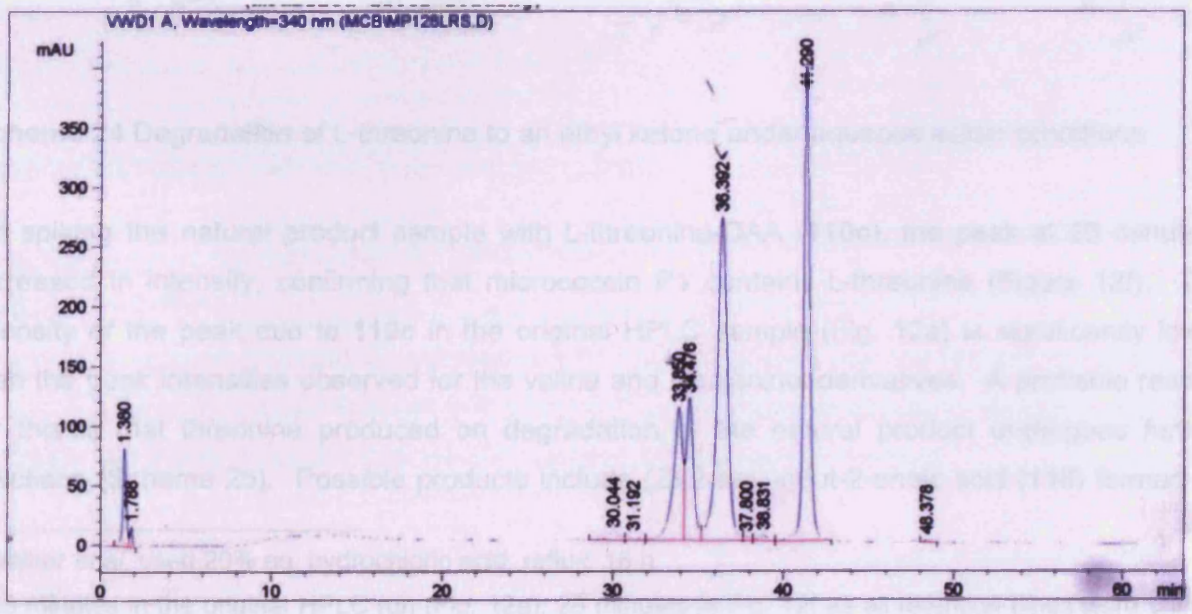
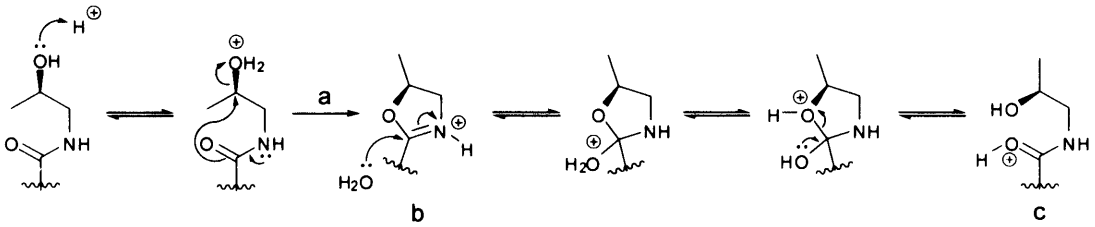


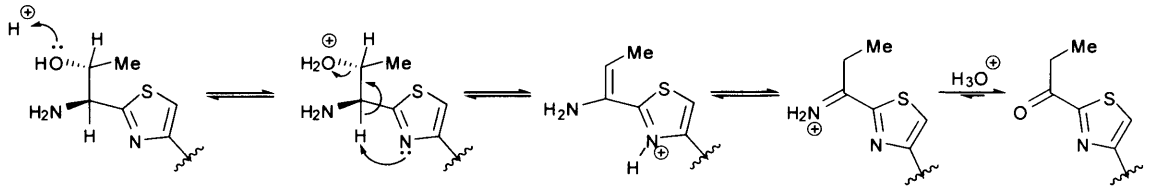
Figure 12e HPLC of MP1 degradation products spiked with **110a**, **110b**, **110d** and **110e**



Scheme 23 Acid-catalysed racemisation of the (*R*)-isoalaninol side chain of MP1

The key step in the above mechanism is the S_N2 attack of the carbonyl oxygen atom on the protonated alcohol of the isoalaninol residue (step a). This results in inversion of the stereochemistry at that centre and yields protonated oxazoline b. Ring-opening affords product c, which can undergo the same reaction sequence resulting in the observed racemisation of this stereogenic centre. Curiously, racemisation at this stereogenic centre was not reported by Walker *et al.* despite the use of similar acidic hydrolysis conditions.[ii]

The stereochemistry of one of the two threonine residues present in micrococcin P1 had been confirmed as L by Walker *et al.* as L-threonine was isolated following acidic hydrolysis of the natural product.[123] While it is likely that the other threonine residue is also of L-stereochemistry, on refluxing in aqueous hydrochloric acid it is degraded to form the ethyl ketone moiety of micrococcinic acid (**80**) and thus would not be isolated under the conditions used by Walker (Scheme 24).



Scheme 24 Degradation of L-threonine to an ethyl ketone under aqueous acidic conditions

On spiking the natural product sample with L-threonine-DAA (**110c**), the peak at 26 minutes[iii] increased in intensity, confirming that micrococcin P1 contains L-threonine (Figure 12f). The intensity of the peak due to **110c** in the original HPLC sample (Fig. 12a) is significantly lower than the peak intensities observed for the valine and isoalaninol derivatives. A probable reason for this is that threonine produced on degradation of the natural product undergoes further reactions (Scheme 25). Possible products include (*Z*)-2-aminobut-2-enoic acid (**118**) formed by

[ii] Walker *et al.* used 20% aq. hydrochloric acid, reflux, 16 h.

[iii] 26 minutes in the original HPLC run (Fig. 12a); 28 minutes in Fig. 12f as all retention times were shifted by + 2 minutes in this run

elimination of water, and 2-oxobutanoic acid (**119**) formed by the same mechanism as the ethyl ketone moiety of micrococcinic acid (Scheme 24). 2-Oxobutanoic acid cannot undergo the S_NAr reaction with Marfey's reagent, and would therefore remain non-derivatised and appear absent by HPLC analysis at the detection wavelength used.

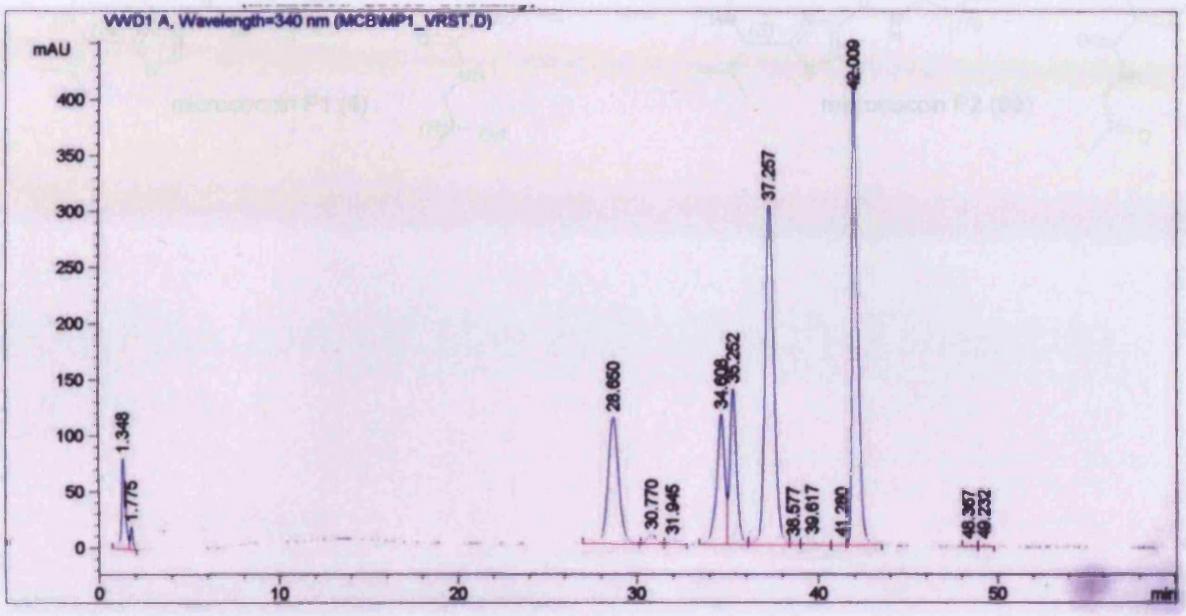
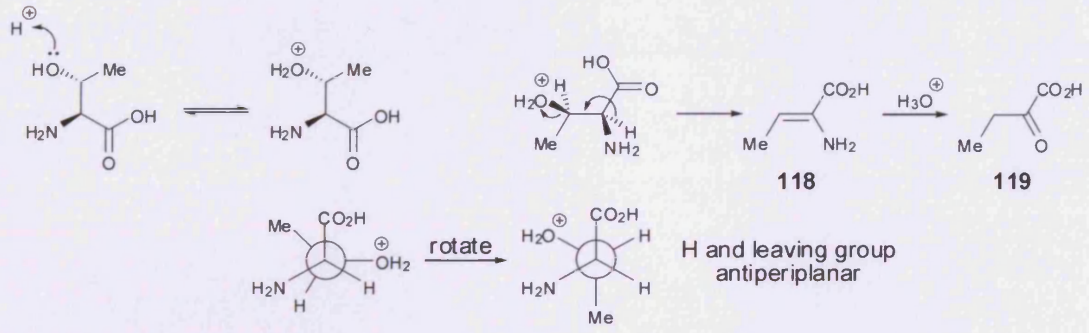


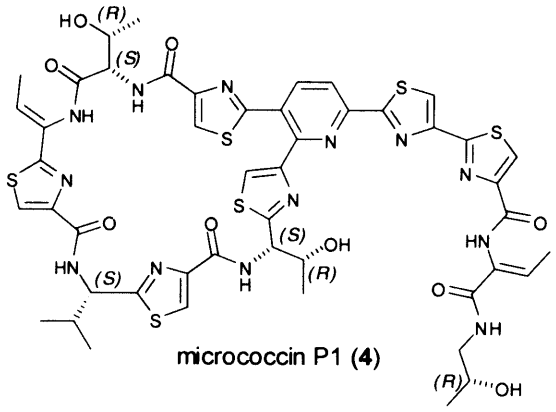
Figure 12f HPLC of MP1 degradation products spiked with **110a**, **110b**, **110d, 110e** and **110c**



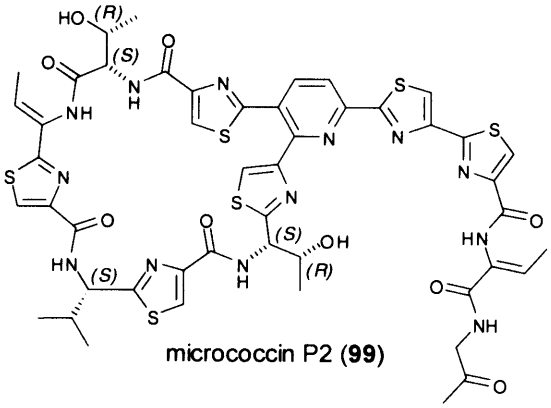
Scheme 25 Further reaction of threonine under aqueous acidic conditions

2.6 – Conclusion

Chemical degradation studies on micrococcin P1 have confirmed that the stereochemistry of the valine residue in the natural product is *S* and not *R* as previously assigned in the literature. Thus the likely structures of the micrococcin thiopeptides are as illustrated below.



micrococcin P1 (**4**)



micrococcin P2 (**99**)

Chapter Three – Results and Discussion

Modification of the Hantzsch Thiazole Synthesis

3.1 – Introduction

3.2 – The Hantzsch Thiazole Synthesis

3.3 – Reinvestigation of the Hantzsch Thiazole Synthesis

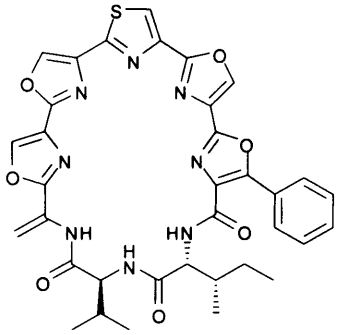
3.4 – Synthesis of the Peptide Backbone of MP1

3.5 – Conclusion

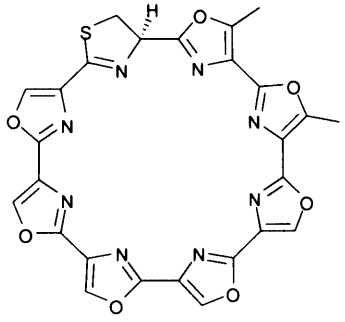
3 – Modification of the Hantzsch Thiazole Synthesis

3.1 – Introduction

Thiazoles occur widely in nature, and many thiazole-containing natural products possess important biological properties. In addition to the thiazolyl peptide antibiotics already discussed (section 1.3), compounds such as mechercharmycin A[154] (**120**) and thiazoline-containing telomestatin[155] (**121**) exhibit potent cytotoxicity.

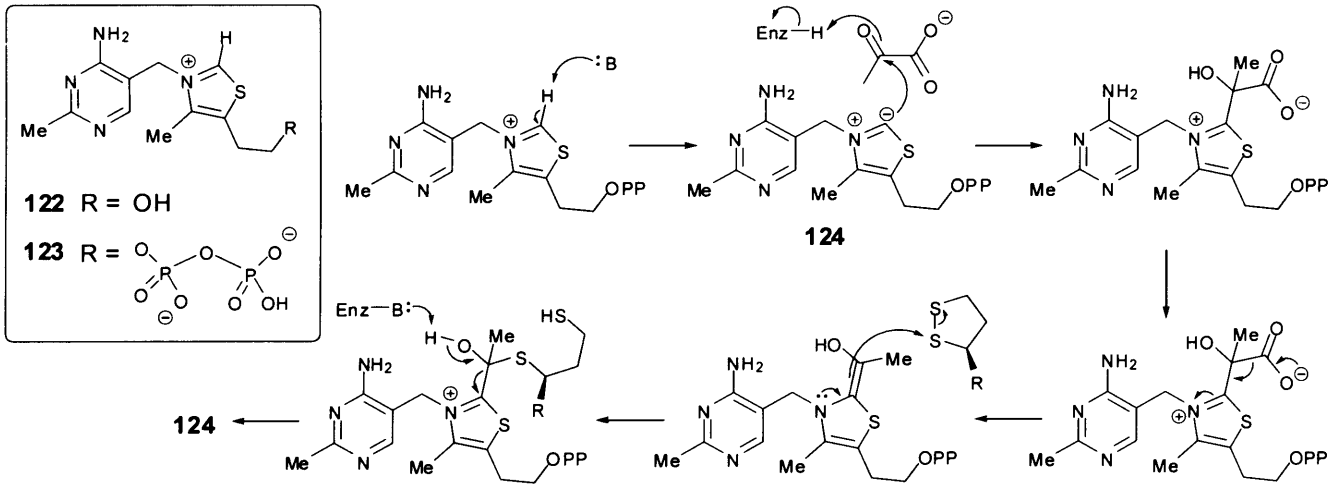


120



121

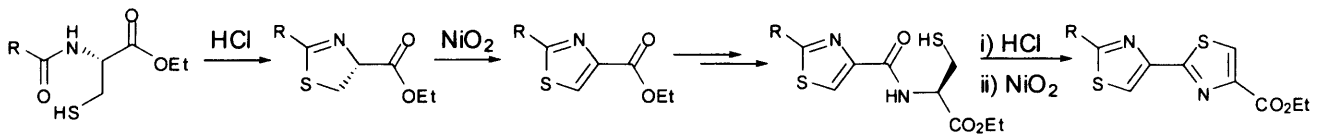
Thiamine (vitamin B_1, **122**) occurs naturally in yeast, rice husks and cereals, and a lack of thiamine in the diet results in beriberi and damage to the nervous system (polyneuritis). Thiamine pyrophosphate (**123**) is a coenzyme involved in the decarboxylation of pyruvate; the quaternised thiazole ring is essential to its biological activity (Scheme 26).



Scheme 26 Thiamine pyrophosphate-catalysed decarboxylation of pyruvate

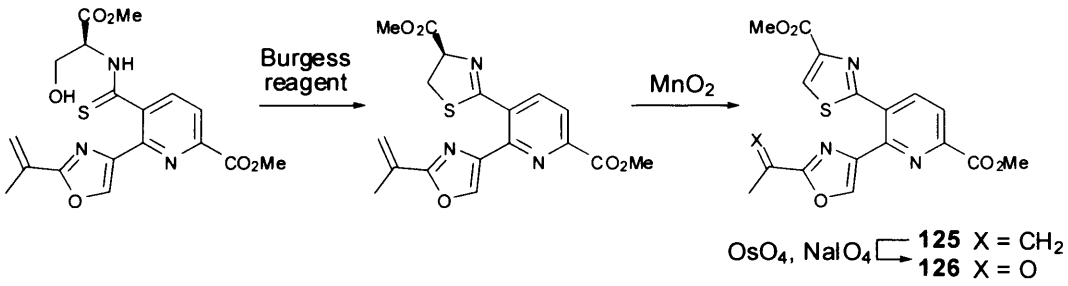
3.1.1 – Biomimetic synthesis of thiazoles

In nature, thiazoles present in heterocyclic peptides are formed by the cyclisation of a cysteine residue either during the growth of the peptide chain, or by subsequent modification of the completed linear peptide.[156] A biomimetic strategy can be employed in the synthesis of heterocyclic peptides whereby a cysteine residue is cyclised and the resulting thiazoline oxidised to afford the desired thiazole (Scheme 27).[157,158]



Scheme 27 Biomimetic thiazole synthesis

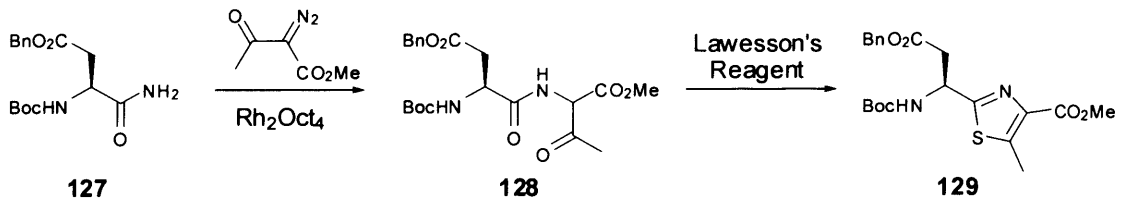
A similar approach was utilised by Bagley *et al.* in their synthesis of dimethyl sulfomycinamate (**126**) in which a serine residue was cyclised onto a thioamide (Scheme 28).[141]



Scheme 28 Cyclisation of serine onto a thioamide

3.1.2 – Rhodium carbenoid insertion

In the course of their total synthesis of amythiamycin D,[74,75] Moody and co-workers employed rhodium carbene N-H insertion methodology to furnish aspartate-derived thiazole **129** (Scheme 29) [159]. Amide **127** underwent chemoselective N-H insertion with the rhodium carbenoid formed by the reaction of dirhodium tetraoctanoate and 2-diazo-3-oxobutanoate. The resulting ketoamide **128** was converted to the desired thiazole on heating with Lawesson's reagent.



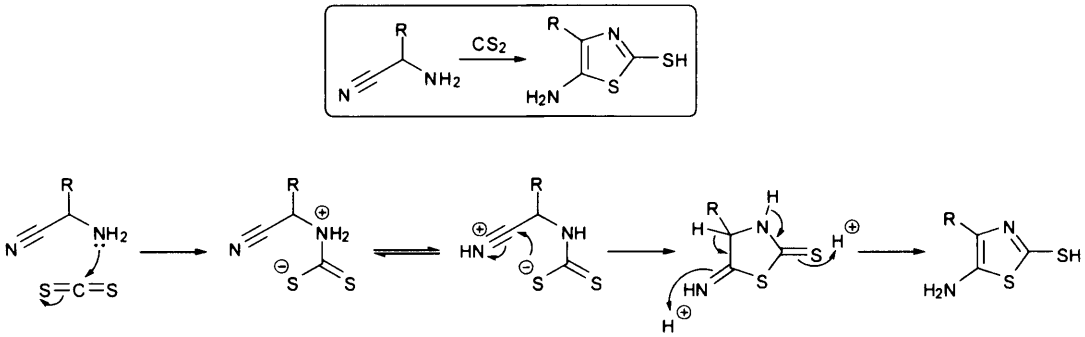
127 **128** **129**

Scheme 29 Synthesis of thiazoles using rhodium carbene methodology

3.1.3 – Alternative synthetic routes to thiazoles

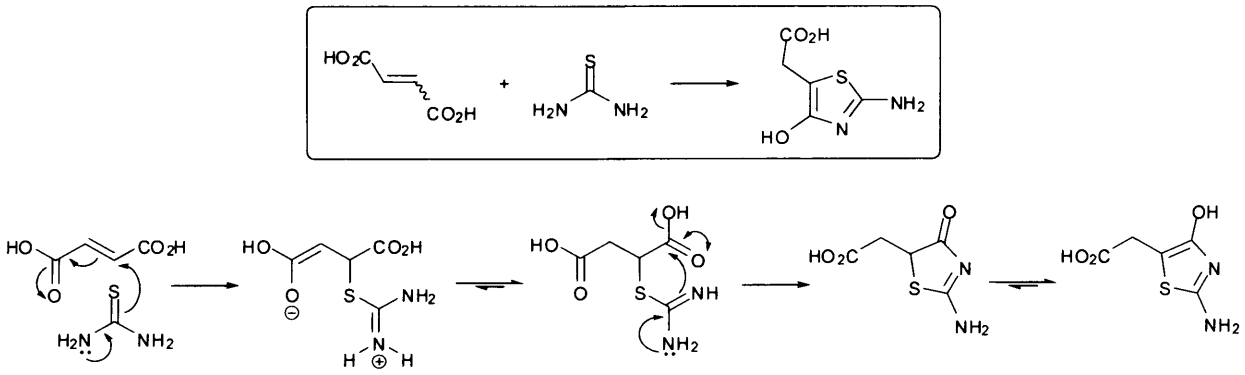
In addition to the reactions discussed in the preceding sections, there are alternative literature procedures for the preparation of thiazoles which are of limited use in the synthesis of thiopeptide antibiotics due to the substitution pattern of the thiazoles they produce, but are outlined below for completeness.

The Cook-Heilbron thiazole synthesis (Scheme 30) is the reaction of an α-aminonitrile with CS_2, COS or an isothiocyanate to give a 2,4-disubstituted 5-aminothiazole.[160]



Scheme 30 The Cook-Heilbron thiazole synthesis

The Andreasch thiazole synthesis is the reaction between maleic or fumaric acid and thiourea (Scheme 31).[161]

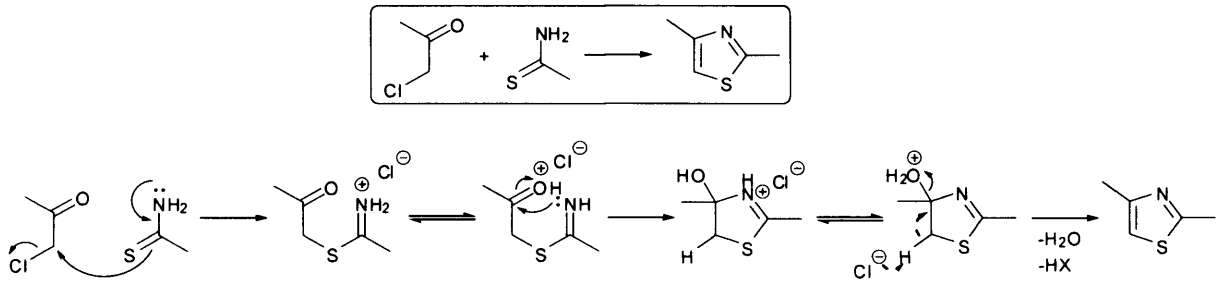


Scheme 31 The Andreasch thiazole synthesis

3.2 – The Hantzsch Thiazole Synthesis

3.2.1 – The Hantzsch thiazole synthesis

Discovered in 1889 by Arthur Hantzsch, the original reaction entails heating a thioamide in the presence of an α-halocarbonyl compound in benzene (Scheme 32).[147]

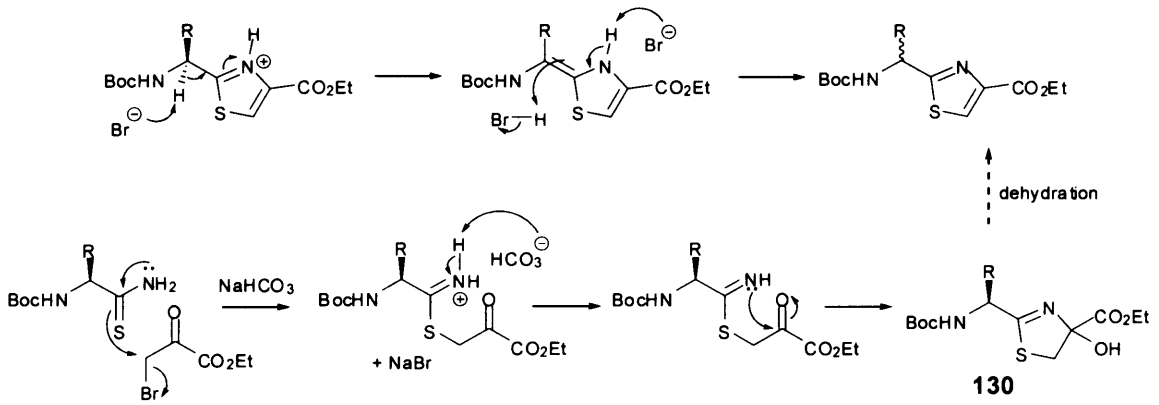


Scheme 32 The original Hantzsch thiazole synthesis

Modifications of the original Hantzsch protocol have been numerous, including formation of the thioamide *in situ*.[148] Many of the recent adaptations of this reaction have been focussed towards enabling the use of racemisation-prone amino acid derived substrates. These modifications have led to the widespread use of the Hantzsch thiazole synthesis in thiopeptide synthesis and will be discussed in detail in this chapter.

3.2.2 – Racemisation of amino acid-derived substrates

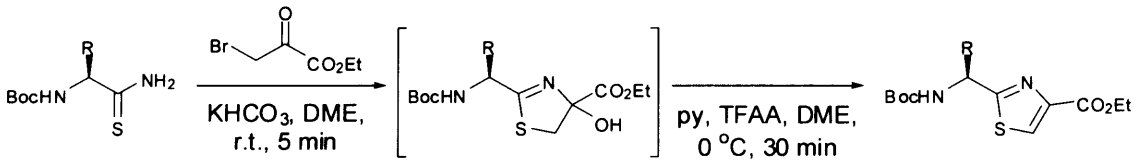
The successful total synthesis of natural products such as micrococcin P1 (**4**) which contain amino acid-derived thiazoles is reliant upon the ability to prepare these structural motifs in high yield and enantiomeric purity. The Hantzsch thiazole synthesis is a useful method for the synthesis of such thiazoles as the necessary thioamide precursors are readily prepared from commercially available, enantiopure starting materials. However, a means of circumventing the problematic racemisation caused by the concomitant liberation of an equivalent of hydrogen bromide is necessary (Scheme 33). Conducting the reaction in the presence of a base neutralises the acid liberated, but results in the formation of hydroxythiazoline intermediate **130** which needs to be activated in order to undergo elimination to the thiazole product (Scheme 33).



Scheme 33 The effect of base on the Hantzsch thiazole synthesis

3.2.3 – Modifications made by Holzapfel

Holzapfel *et al.* reported the first modified version of the Hantzsch thiazole synthesis in 1990 as a result of their need for optically pure glutamine-derived thiazoles.[137] The Holzapfel modification of the Hantzsch thiazole synthesis is outlined in Scheme 34.



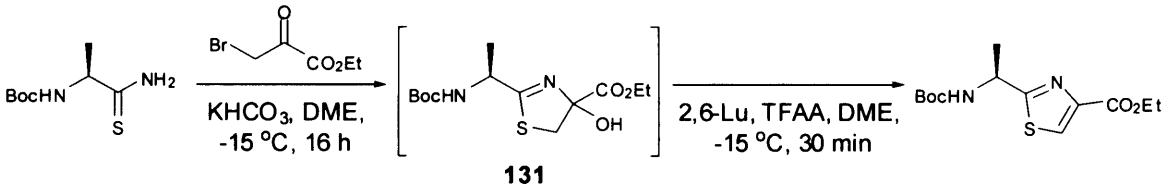
Scheme 34 The Holzapfel-Hantzsch thiazole synthesis

The thioamide is stirred with solid potassium hydrogen carbonate in DME and ethyl bromopyruvate added in order to form the intermediate hydroxythiazoline. The mixture is then cooled to 0 °C and a solution of pyridine and trifluoroacetic anhydride in DME added to effect elimination to the thiazole product. The use of a low reaction temperature and basic conditions prevent racemisation. Holzapfel *et al.* successfully used this method to prepare enantiomerically pure thiazoles derived from (*R*)-glutamine, (*S*)-glutamine, (*S*)-alanine, (*S*)-valine, (*S*)-leucine, (*S*)-isoleucine and (*S*)-phenylalanine.

3.2.4 – Modifications made by Meyers

The next modification of the Hantzsch thiazole synthesis was made by Aguilar and Meyers in 1994.[138] They reported that in their hands, the reaction conditions developed by Holzapfel *et al.* afforded either partially or completely racemised thiazoles. In the light of these unexpected findings, the reaction was reinvestigated. The initial hydroxythiazoline formation was conducted at -15 °C in DME with solid potassium hydrogen carbonate, and a wide range of bases and

temperatures screened for the subsequent dehydration step. The optimum conditions for the dehydration of alanine-derived hydroxythiazoline **131** were found to be treatment with a solution of 2,6-lutidine and trifluoroacetic anhydride at -15 °C (Scheme 35).

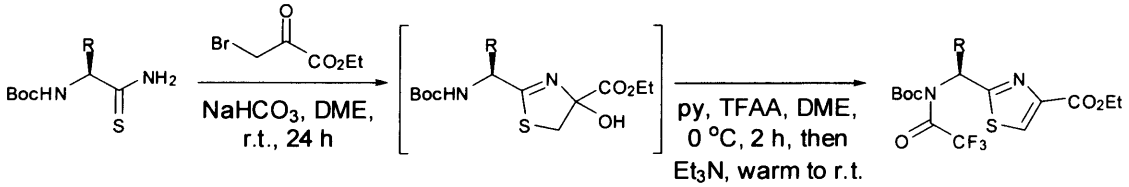


Scheme 35 The Meyers-Hantzsch thiazole synthesis

The Meyers modification of the Hantzsch thiazole synthesis has been utilised successfully in the synthesis of many natural products including (+)-nostocyclamide[162] and promothiocin A (**15a**).[66,67] The Meyers-Hantzsch reaction conditions employed in the syntheses of both (+)-nostocyclamide and promothiocin A differ slightly from those published by Meyers in that the addition of ethyl bromopyruvate in the first step, and the addition of the trifluoroacetic anhydride - 2,6-lutidine solution in the second step were conducted at -40 °C, and the reaction mixture allowed to warm to -20 °C, then stirred for the times indicated in Scheme 35.

3.2.5 – Modifications made by Nicolaou

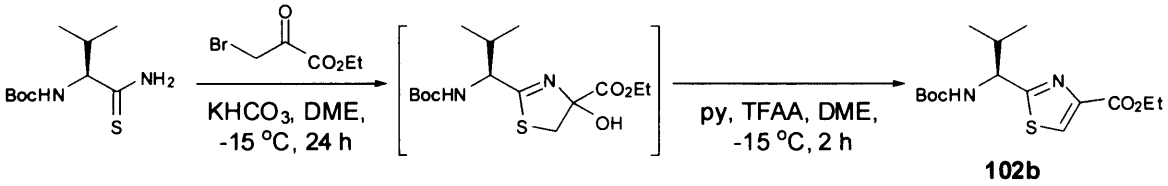
In the course of his landmark total synthesis of thiostrepton (**1**), Nicolaou *et al.* revisited the procedures developed by Holzapfel and Meyers.[85] The Nicolaou-Hantzsch thiazole synthesis is conducted at a higher temperature than the Meyers-Hantzsch reaction and employs both pyridine and triethylamine in the elimination step of the reaction (Scheme 36). Also, the pyridine and trifluoroacetic anhydride are added separately and sequentially to the reaction mixture, not as a pre-mixed solution as used by Holzapfel and Meyers. The Nicolaou-Hantzsch thiazole synthesis leads to the trifluoroacetylation of any free NH groups in the target molecule.



Scheme 36 The Nicolaou-Hantzsch thiazole synthesis

3.2.6 – Modifications made by Bach

Bach and co-workers employed a further variation of the Hantzsch thiazole synthesis to prepare valine-derived thiazole **102b** en route to the total synthesis of GE2270A (**42**).[99] The Bach-Hantzsch thiazole synthesis (Scheme 37) utilises the longer reaction times of the Nicolaou modification, the low reaction temperature as used by Meyers, and pyridine as the base for elimination, as used by Holzapfel.



Scheme 37 The Bach-Hantzsch thiazole synthesis

3.3 – Reinvestigation of the Hantzsch Thiazole Synthesis

While the Hantzsch thiazole synthesis is a valuable addition to the heterocyclic chemist's toolkit, it is clear that this reaction is somewhat capricious when applied to amino acid-derived substrates. Indeed three of the successful thiopeptide total syntheses (promothiocin A, thiostrepton and GE2270A) use a different modification of the Hantzsch thiazole synthesis. Following the discovery that the stereochemistry of the valine residue in micrococcin P1 had been misassigned in the literature (Chapter 2), it was decided that results from the chemical degradation studies should be verified by total synthesis of the natural product. The total synthesis of micrococcin P1 requires the synthesis of enantiomerically pure thiazole building blocks **102b** and **132**.

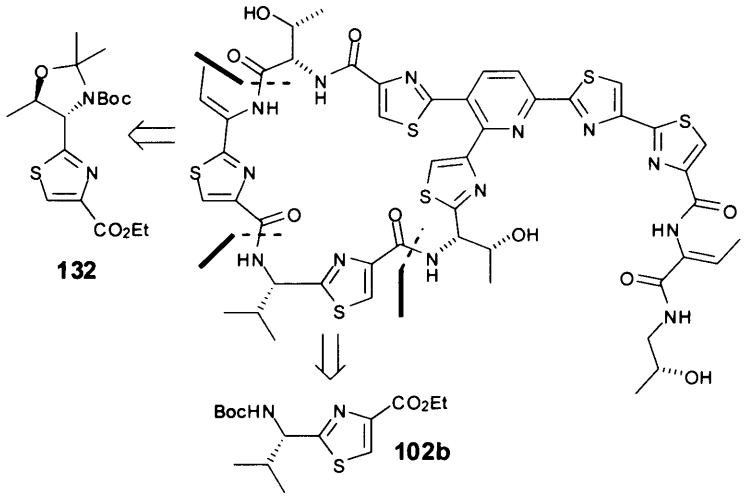
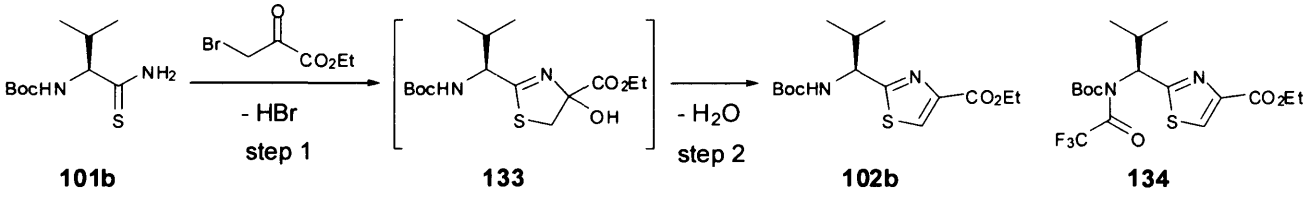


Figure 13 Disconnection of MP1 illustrating amino acid-derived thiazole building blocks

3.3.1 – Comparison of the literature methods for the synthesis of 102b

(*S*)-*N*-*tert*-(Butoxycarbonyl)thiovalinamide (**101b**) was prepared in 3 steps from (*S*)-valine (Scheme 20) and subjected, it turn, to the Hantzsch, Holzapfel, Meyers and Nicolaou methods for thiazole synthesis, and the yield and optical purity of the product **102b** recorded for each set of conditions (Table 4). The acidic reaction conditions of the traditional Hantzsch thiazole synthesis (entry 1) were not suitable for an acid-sensitive substrate and failed to yield the product. The Holzapfel and Meyers modifications afforded the desired thiazole albeit in low yield on a small scale (entries 2 and 3), or compromised enantiomeric purity on a larger scale (entry 4). The failure of the Meyers-Hantzsch conditions to provide optically pure product in good yield on a large scale (>1 g) has been previously reported in a study by Pattenden *et al.*, the problems being attributed to the decomposition of ethyl bromopyruvate under the reaction conditions.[163] Nicolaou's conditions were also investigated and afforded *N*-trifluoroacetamide **134** as the sole reaction product, which on treatment with sodium ethoxide gave thiazole **102b** in 88% ee.



101b **133** **102b** **134**

Table 4 Application of literature methodology to the synthesis of thiazole **102b**

			Step 1		Step 2			
	Method	Quantity of **101b** /g	Solvent	Temp. /°C	Temp. /°C	Reagents	Yield (%)	ee[a] (%)
1	Hantzsch		EtOH	reflux	reflux	-	0[b]	-
2	Holzapfel	0.05	DME	0	0	TFAA, 2,6-Lu	33	>98
3	Meyers	0.22	DME	-40 → -20	-40 → -20	TFAA, 2,6-Lu	9	>98
4	Meyers	1.00	DME	-40 → -20	-40 → -20	TFAA, 2,6-Lu	59[c]	88
5	Nicolaou	0.30	DME	r.t.	0	TFAA, py, Et_3N	85[d]	88[d]

[a] The ee values were determined by HPLC on a chiral stationary phase, using a ChiralPac AD column; flow rate 1 mL min^{-1}; *i*-PrOH:hexane, 5:95; detected at 269 nm.

[b] No product was isolated, probably as a result of HBr-catalysed Boc deprotection and subsequent loss of product during work-up and purification.

[c] Recrystallisation furnished optically pure **102b** (ee >98%)

[d] Only **134** was isolated; ee value was determined following subsequent solvolysis to **102b**.

3.3.2 – Modification of literature procedures

As the established literature procedures failed to provide a satisfactory and reproducible route to thiazole **102b** in terms of both yield and optical purity, further modifications to the reaction conditions were investigated (Table 5).

Table 5 Modified procedures for the synthesis of **102b**

	Step 1			Step 2			
	Solvent	Temp. /°C	Base	Temp. /°C	Reagents	Yield (%)	ee[a] (%)
1	DME	r.t.	$NaHCO_3$	0	TFAA, 2,6-Lu, Et_3N	88[b]	91[b]
2	DME	-18	$KHCO_3$	-18	TFAA, 2,6-Lu	97[c]	>98

[a] The ee values were determined by HPLC on a chiral stationary phase, using a ChiralPac AD column; flow rate 1 mL min^{-1}; *i*-PrOH:hexane, 5:95; detected at 269 nm.

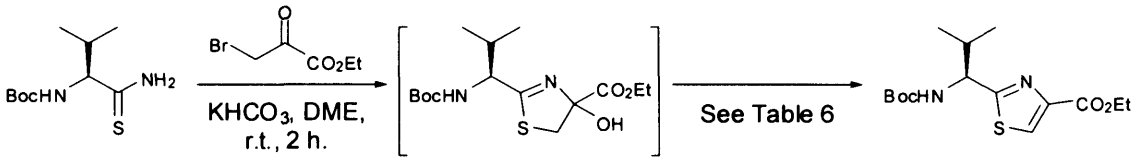
[b] Isolated **102b** (18% yield, 91% ee) and **134** (70% yield, 91% ee); the ee value for **134** was determined following subsequent solvolysis to **102b**.

[c] Only **134** was isolated; ee value was determined following subsequent solvolysis to **102b**.

Modification of Nicolaou's conditions by changing the base used for the elimination of **133** from pyridine to 2,6-lutidine led to the isolation of a 1:4 mixture of the desired product **102b** and the *N*-trifluoroacetyl derivative **134** (entry 1), both in 91% ee, demonstrating that no racemisation occurred during the removal of the trifluoroacetyl group. The Meyers modification of the Hantzsch thiazole synthesis was then performed at -18 °C rather than -40 → -20 °C and resulted in a dramatic increase in the yield from 59% to 97% but curiously delivered only the trifluoroacetamide **134** (entry 2). Treatment of **134** with sodium ethoxide in ethanol according to Nicolaou's procedure led to the isolation of **102b** as a single enantiomer.[85] Despite the additional solvolysis step, this method has proved a high yielding and reliable route to optically pure valine-derived thiazole **102b**.

3.3.3 – Activation using methanesulfonyl chloride

With a method in hand for the synthesis of optically pure thiazoles, the reagents used to effect the dehydration of hydroxythiazoline **133** were varied in order to circumvent the formation of *N*-trifluoroacetyl thiazole **134** and thus remove the need for a subsequent solvolysis step. The use of methanesulfonyl chloride and triethylamine in the dehydration of alcohols is a well documented and reliable procedure, so these reagents were applied to the dehydration of hydroxythiazoline **133** (Table 6).



101b **133** **102b**

Table 6 Reaction conditions for the methanesulfonyl chloride-triethylamine mediated elimination of hydroxythiazoline **133**

	MsCl (equiv.)	Temp. /°C	Time /h	Aq. work-up	Yield (%)	ee[a] (%)
1	1.1	r.t.	1	No	70	38
2	1.1	0 → r.t.	2	No	33	>98
3	1.1	0 → r.t.	2	Yes	15	>98
4	1.1	0 → r.t.	48	No	21	>98
5	1.1	0	3.5	No	35	92
6	2	0 → r.t.	60	No	28	>98
7	5	r.t.	2+1	No	36	38
8	10	r.t.	2+1	No	68	36
9[b]	10	-1	0.5	No	68	12
10[c]	10	-1	0.5	No	26	>98

[a] The ee values were determined by HPLC on a chiral stationary phase, using a ChiralPac AD column; flow rate 1 mL min^{-1}; *i*-PrOH:hexane, 5:95; detected at 269 nm.

[b] Reaction conducted using a CEM Discover® CoolMate apparatus, with concurrent heating and cooling.

[c] Reaction conducted under conductive heating conditions.

Reaction of thiovalinamide **101b** with ethyl bromopyruvate at room temperature in the presence of solid potassium hydrogen carbonate afforded hydroxythiazoline **133**, which was treated *in situ* with methanesulfonyl chloride and triethylamine as described in Table 6. These findings demonstrate that it is possible to prepare thiazole **102b** as a single enantiomer following dehydration, even when **133** is prepared at room temperature (entries 2-4 and 6). This would suggest that under basic reaction conditions, racemisation in the Hantzsch thiazole synthesis occurs in the elimination step of the procedure. This finding contradicts earlier results obtained by Meyers *et al.* which imply that racemisation occurs in the hydroxythiazoline formation step, further demonstrating the capricious nature of this reaction.[138] Addition of methanesulfonyl chloride and triethylamine to **133** at room temperature and stirring for one hour furnished **102b** in 70% yield and 38% ee (entry 1). The probable cause of the observed racemisation was the exothermic reaction which occurred on addition of methanesulfonyl chloride and triethylamine

and caused the reaction temperature to reach up to 90 °C. Repetition of the reaction with addition of the reagents at 0 °C prevented the exotherm and the reaction temperature increased by only 7 °C. The product was found to be optically pure by HPLC but the yield was drastically reduced (entry 2). Purification of the reaction mixture by aqueous work-up further reduced the yield (entry 3), and increasing the reaction time to 48 h also failed to improve the yield (entry 4).

Based on these findings it was concluded that the exotherm responsible for the racemisation was necessary to improve the yield of the reaction. Addition of two equivalents of methanesulfonyl chloride and prolonged reaction times (60 hours, entry 6) afforded a slight improvement in yield over entry 4 (28% vs. 21%). Addition of five equivalents of methanesulfonyl chloride, stirring for two hours, followed by addition of triethylamine resulted in a poor yield and low optical purity (entry 7); increasing to 10 equivalents of methanesulfonyl chloride gave a moderate yield of **102b** (68%) albeit with low optical purity (36%, entry 8).

Following the limited success afforded by increasing the reaction time, the effect of low temperature microwave irradiation with concurrent heating and cooling was investigated, as this technique had been successfully applied to pyrazole synthesis within our laboratory.[164] Hydroxythiazoline **133** was prepared by irradiation of a mixture of thioamide **101b**, ethyl bromopyruvate and solid potassium hydrogen carbonate in DME for 20 minutes at -20 °C with an initial power of 300 W, using a CEM Discover® CoolMate Sub-Ambient microwave synthesiser (Figure 14). The mixture was then treated as shown in Table 6. Irradiation at -1 °C for 30 minutes furnished the desired thiazole **102b** in moderate yield, however the enantiomeric excess was only 12% (entry 9). Finally, the CoolMate elimination step was repeated under thermal conditions (entry 10) and afforded **102b** as a single enantiomer albeit in poor yield (26%). This finding also supports the previous results in suggesting that racemisation occurs in the elimination step of the Hantzsch thiazole synthesis, rather than the hydroxythiazoline formation step.

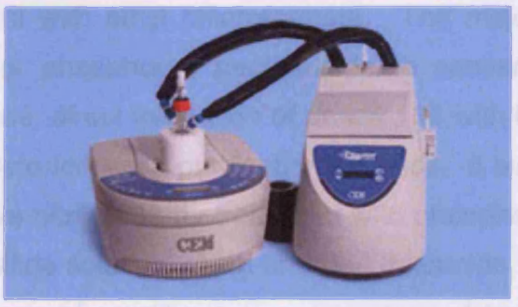


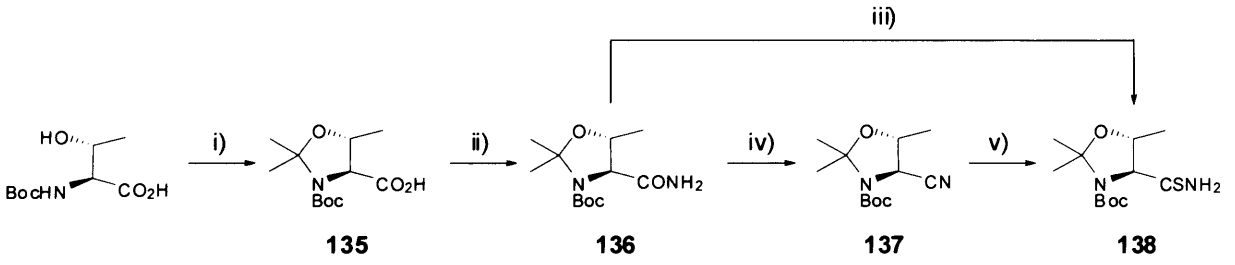
Figure 14 The CEM Discover® CoolMate Sub-Ambient microwave synthesiser

3.3.4 – Activation using other reagents

As no suitable method for the synthesis of optically pure thiazoles in high yield had been discovered using methanesulfonyl chloride, other activating agents were investigated. No thiazole was formed on treatment of hydroxythiazoline **133** with either one or five equivalents of toluenesulfonyl chloride and triethylamine, even after prolonged reaction times (96 hours) at room temperature. Reactions using trifluoromethanesulfonic anhydride failed due to the polymerisation of the solvent. On the basis of these findings it was concluded that the most efficient route to enantiopure valine-derived thiazole **120b** was *via* the trifluoroacetamide derivative **134** using the conditions shown in Table 5, entry 2.

3.3.5 – Synthesis of threonine-derived thiazole 132

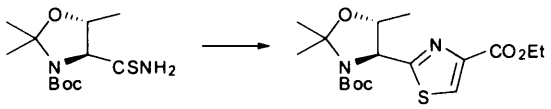
With a route to valine-derived thiazole building block **102b** now in hand, the synthesis of threonine-derived thiazole **132** was investigated. Threonine-derived thioamide **138** was prepared as illustrated in Scheme 38.



Reagents and Conditions i) 2,2-DMP, PPTS, THF, reflux, 6 h, 100%; ii) EtOCOCl, Et_3N, THF, 0 °C, 1 h, then 35% aq. NH_3, r.t., 24 h, 78%; iii) Lawesson's Reagent, PhMe, 80 °C, 1.5 h, 24%; iv) $POCl_3$, pyridine, 0 °C, 2 h, 86%; v) $(NH_4)_2S$, MeOH, r.t., 24 h, 100%.

Scheme 38 Synthesis of threonine-derived thioamide **138**

N-Boc-(2*S*,3*R*)-threonine was protected as oxazolidine **135** then converted to amide **136** *via* the mixed anhydride by treatment with ethyl chloroformate. The majority of literature procedures utilise Lawesson's reagent or phosphorus pentasulfide to convert an amide to a thioamide directly.[85] However, in this case, direct thionation of amide **136** with Lawesson's reagent afforded thioamide **138** in only 24% yield following purification on silica. It was found to be more efficient to dehydrate the amide to give nitrile **137** by treatment with phosphorus oxychloride, followed by thionation with ammonium sulfide solution which afforded thioamide **138** in excellent yield with no need for purification.[165] A range of conditions were investigated for the conversion of thioamide **138** into thiazole **132** (Table 7).



138 **132**

Table 7 Synthesis of threonine-derived thiazole **132** from thioamide **138**

	Method	Yield (%)
1	Meyers (Table 4, entries 2 and 3)	0
2	MsCl-Et_3N[a]	21
3	Nicolaou (Table 4, entry 5)	87

[a] Reagents and conditions: Ethyl bromopyruvate, $NaHCO_3$, DME, r.t., 24 h; then MsCl (10 equiv., r.t., 16 h), Et_3N (r.t., 4 h).

Meyers' original conditions failed to give the desired product (entry 1); the methanesulfonyl chloride-triethylamine conditions afforded thiazole **132** in poor yield (entry 2). Nicolaou's original conditions furnished **132** as a single diastereoisomer in very good yield (entry 3). The lack of a free NH in thiazole **132** eliminates the possibility of formation of an *N*-trifluoroacetyl derivative, as observed in the valine example (Table 4, entry 5), thus making the Nicolaou modification of the Hantzsch thiazole synthesis particularly useful for such substrates.

3.4 – Synthesis of the peptide backbone of MP1

Having successfully prepared thiazole building blocks **102b** and **132**, the synthesis of the backbone peptide fragment of micrococcin P1 (**139**) was undertaken. The disconnection of the natural product and of the peptide backbone is illustrated in Figure 15.

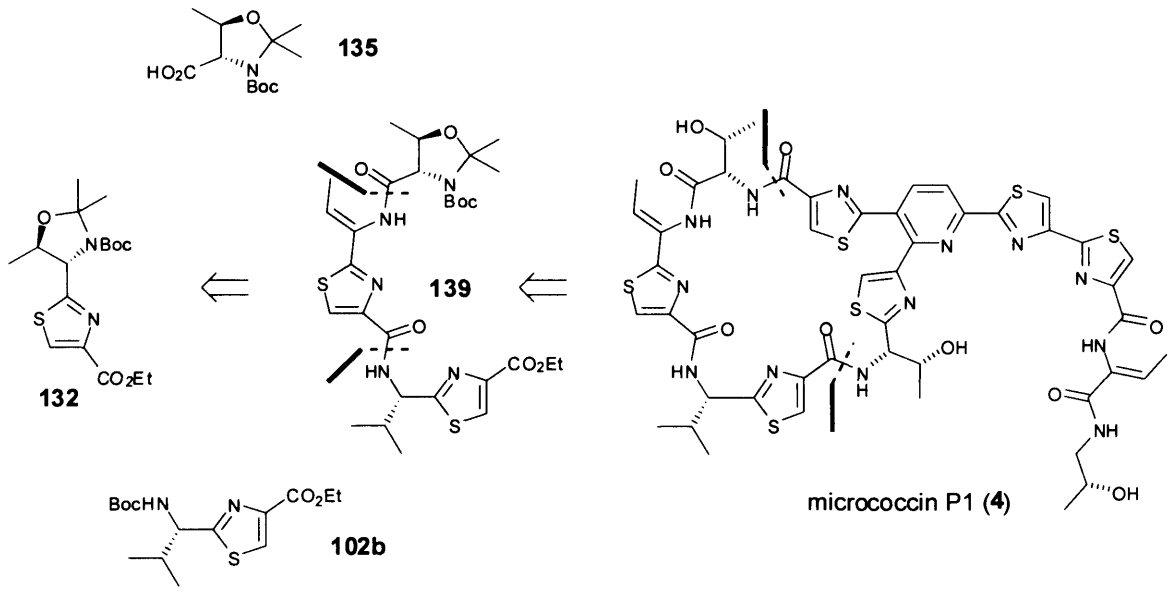
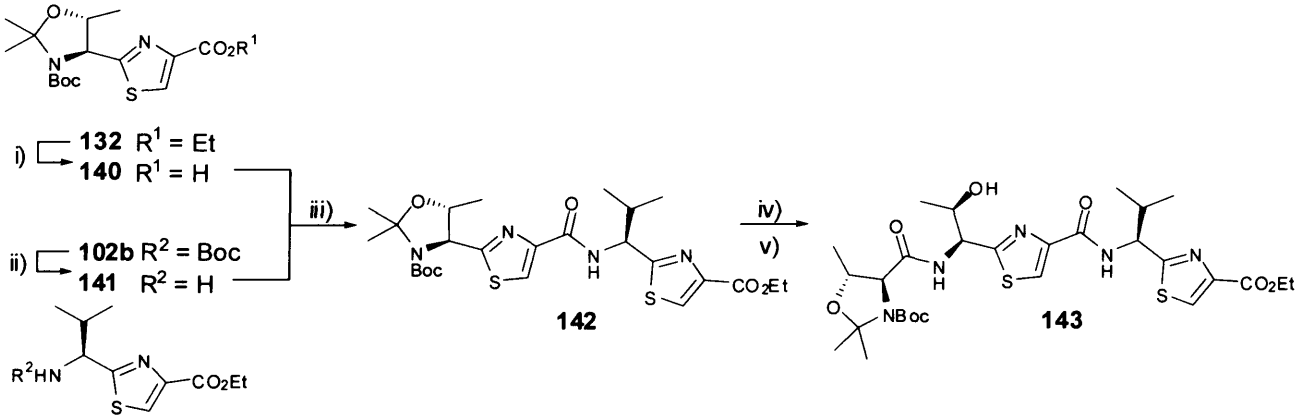


Figure 15 Disconnection of the peptide backbone fragment of MP1

3.4.1 – Synthesis of backbone pentapeptide 143

The synthesis of backbone pentapeptide **143** is illustrated in Scheme 39. Deprotection of thiazole **132** was effected in quantitative yield by stirring with five equivalents of lithium hydroxide for three hours. The resulting carboxylic acid **140** was converted to the ethyl chloroformate mixed anhydride and coupled with *N*-deprotected valine-derived thiazole **141** to furnish tetrapeptide **142** in good yield. *N*-Deprotection of peptide **142** was achieved using trifluoroacetic acid in dichloromethane, and the residue immediately neutralised and subjected to PyBOP®-mediated peptide coupling with protected threonine **135** to afford the peptide backbone **143** in 93% yield. Use of HATU for this peptide coupling furnished pentapeptide **143** in 84% yield.

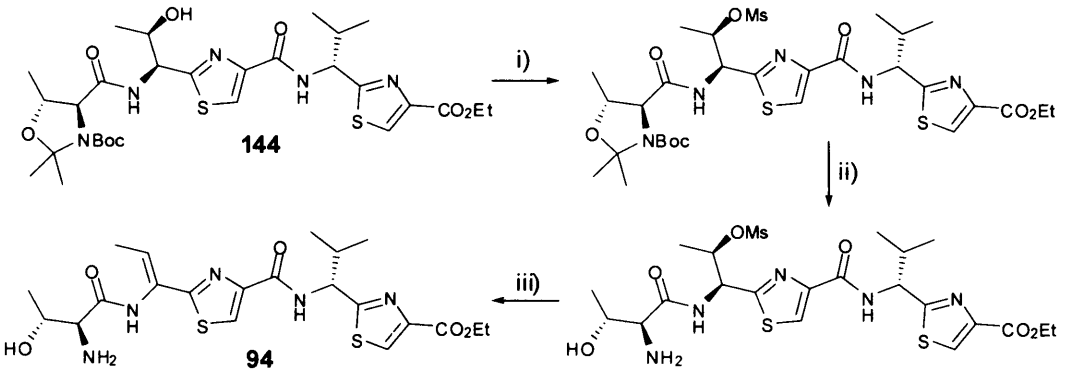


Reagents and Conditions i) LiOH, MeOH-H_2O, r.t., 3 h, 100%; ii) 4 M HCl in dioxone, r.t., 2 h, 98%; iii) **140**, EtOCOCl, Et_3N, THF, 0 °C, 1 h; then **141**, Et_3N, THF, r.t., 2 h, 82%; iv) TFA, CH_2Cl_2, r.t., 1 h; v) **135**, PyBOP®, Et_3N, CH_2Cl_2, r.t., 4 h, 93%.

Scheme 39 Synthesis of backbone pentapeptide **143**

3.4.2 – Elimination to form protected backbone fragment 139

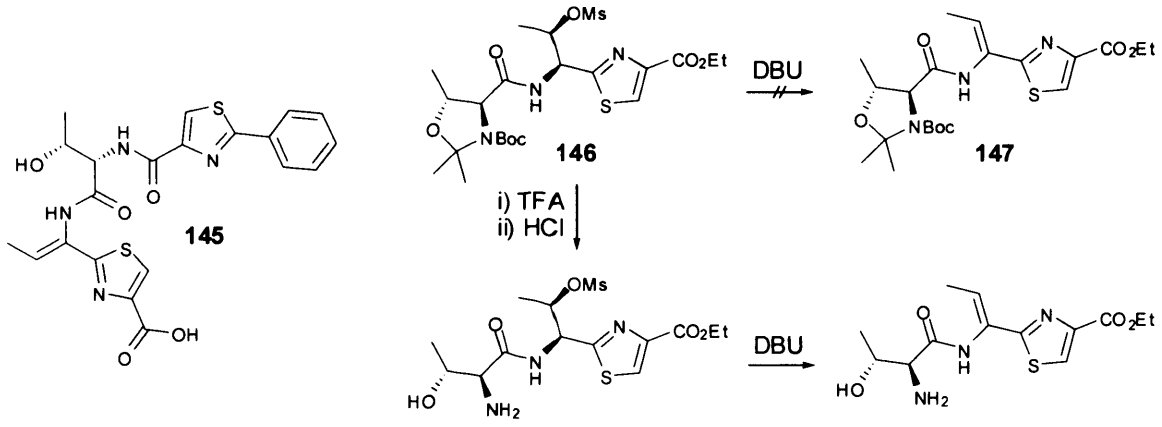
During the course of their total synthesis of the Bycroft-Gowland structure of micrococcin P1, Ciufolini *et al.* prepared backbone peptide **144**, which differs from pentapeptide **143** only in the stereochemical configuration of the valine residue.[132] Ciufolini's peptide **144** was treated with methanesulfonyl chloride, then Boc-deprotected with trifluoroacetic acid. The remaining acetonide protecting group was cleaved using aqueous hydrochloric acid, prior to addition of DBU to effect elimination of the mesylate, furnishing peptide **94** in readiness for coupling to core domain **93** (Scheme 40, Scheme 14).



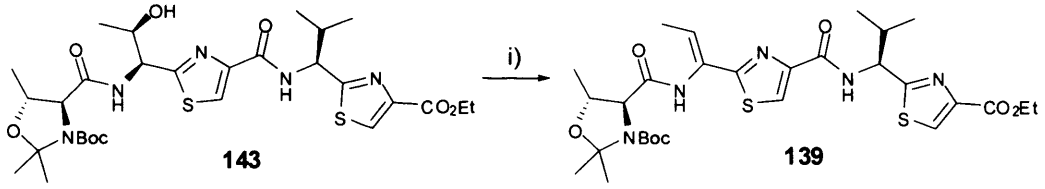
Reagents and Conditions i) MsCl, Et_3N, CH_2Cl_2, 0 °C, 10 mins, 91%; ii) TFA, CH_2Cl_2, r.t., 10 mins; then 0.2 M HCl, THF, r.t., 30 mins; iii) DBU, $CHCl_3$, r.t., 30 mins, 88%

Scheme 40 Ciufolini's modification of peptide backbone **144**

In our hands, all attempts at reproducing these transformations failed, producing an intractable mixture of products. In the light of these problems it was decided to attempt the elimination of the central threonine residue whilst leaving all of the protecting groups intact. Previous reports by Bower and co-workers suggested that elimination cannot occur on such a substrate as the cyclic acetal protecting group forms a rigid scaffold, restricting the conformation and preventing E2 elimination.[166] For the synthesis of thiostrepton binding fragment **145**, Bower *et al.* employed Ciufolini's mesylation-deprotection-elimination procedure after finding that *O*-mesylated peptide **146** failed to undergo elimination to give **147** on treatment with DBU (Scheme 41). Gratifyingly, this was not the case for peptide **143** and the micrococcin peptide backbone **139** was prepared in 98% yield (Scheme 42).



Scheme 41 Bower's attempted synthesis of dehydrodemethylvaline-containing peptide **147**



Reagents and Conditions i) MsCl, Et_3N, CH_2Cl_2, 0 °C, 16 h; then DBU $CHCl_3$, r.t., 2 h, 98%.

Scheme 42 Mesylation and elimination of peptide **143** to form MP1 backbone peptide **139**

3.5 – Conclusion

A range of conditions have been investigated for the conversion of amino acid-derived thioamides into thiazoles without racemisation and previously reported conditions for this transformation have been found to be unreliable. Modifications to the reaction temperature and reagents used have provided evidence which suggests that racemisation in the Hantzsch thiazole synthesis occurs in the elimination step of the reaction. Valine-derived thiazole **102b** has been prepared in excellent yield and with complete stereocontrol. The synthesis of threonine-derived thiazole **132** was also investigated and it was found that the Nicolaou-Hantzsch reaction conditions provided the optimum route to this thiazole building block. The pentapeptide backbone of the micrococcin thiopeptides (**139**) has been prepared from threonine in 10 steps and 44% overall yield.

Chapter Four – Results and Discussion

Synthesis of the Central Heterocyclic Domain of MP1

4.1 – Introduction

4.2 – Synthetic Approaches to Thiopeptide Core Domains

4.3 – Synthesis of β-Ketoester **172**

4.4 – Synthesis of Enamine **169**

4.5 – Alternative Routes to Enamine **169**

4.6 – Synthesis of Thiazole Alkynone **170**

4.7 – Synthesis of Protected Core Domain **168**

4.8 – Synthetic Approaches to Micrococcinic Acid

4.9 – Conclusion

4 – Synthesis of the Central Heterocyclic Domain of MP1

4.1 – Introduction

Many biologically important molecules contain pyridine rings, and range from simple structures such as the B-vitamins depicted in Figure 16 to the complex molecular architecture of the series *d* and *e* thiopeptides (section 1.2, Table 1, Figure 1).

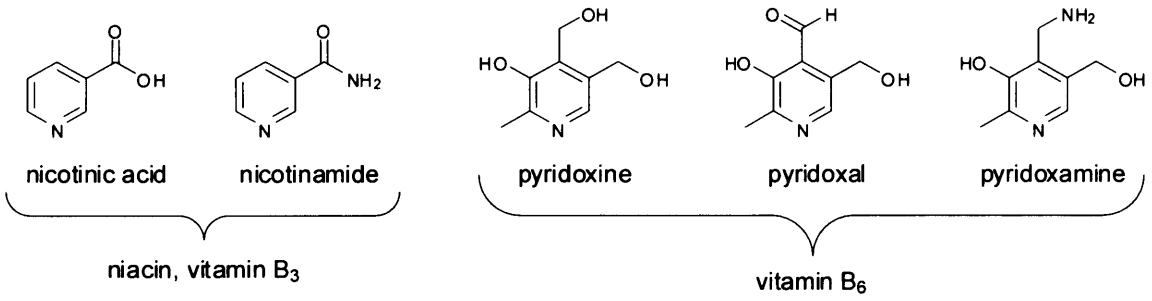


Figure 16 Pyridine-containing vitamins B_3 and B_6

The coenzyme nicotinamide adenine dinucleotide (NAD^+, **148**) and its reduced form NADH (**149**) are components of oxidoreductase enzymes, enabling biological redox reactions to proceed with reversible transfer of hydrogen *via* the 4-positions of **148** and **149** (Figure 17).

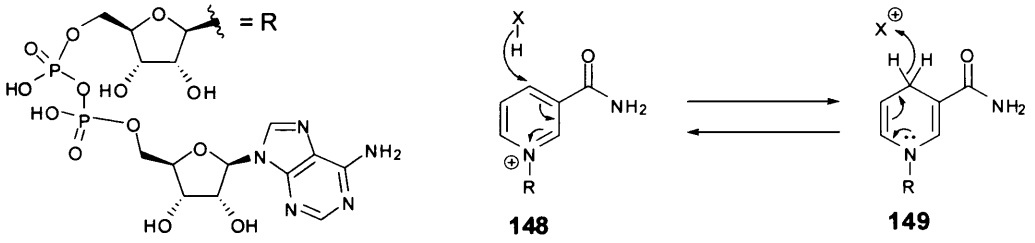


Figure 17 Nicotinamide adenine dinucleotide

Synthetic routes to pyridine-containing target molecules vary widely, as the core pyridine can be disconnected in several different ways (Figure 18). The most commonly used approaches are *via* cyclocondensation reactions (e.g. the Hantzsch pyridine synthesis, section 4.1.1 and the Bohlmann-Rahtz reaction, section 4.2.6) or cycloaddition reactions (e.g. the Wakatsuki-Yamazaki-Bönnemann reaction, section 4.1.2 and the Boger-Panek reaction, section 4.1.3). Specific routes towards thiopeptide pyridine domains will be discussed in detail in section 4.2.

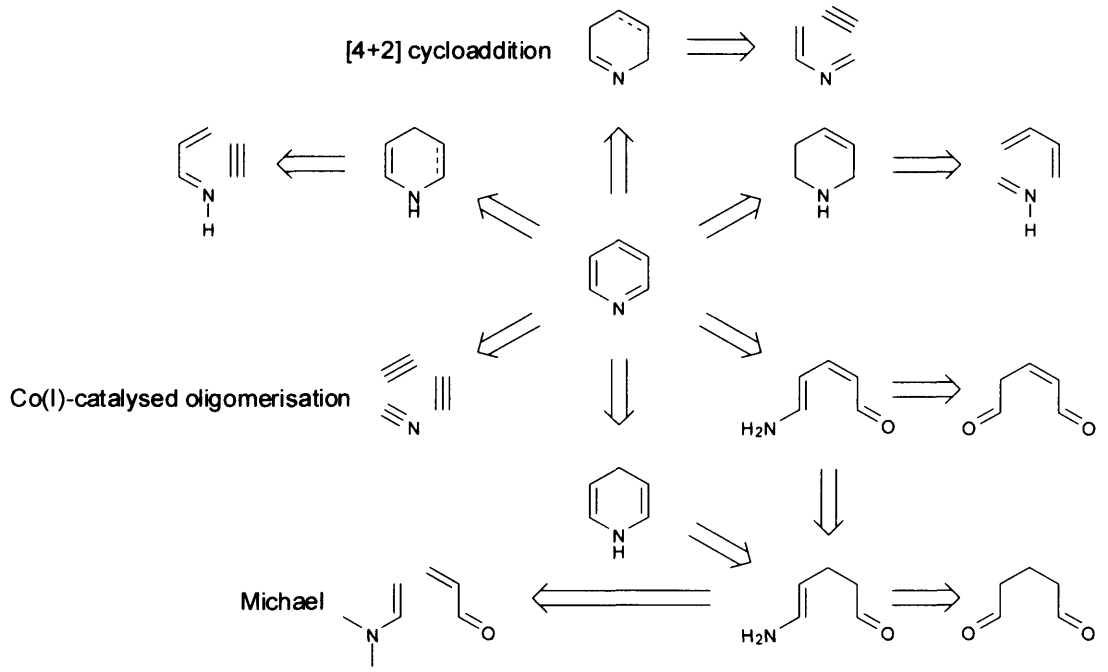
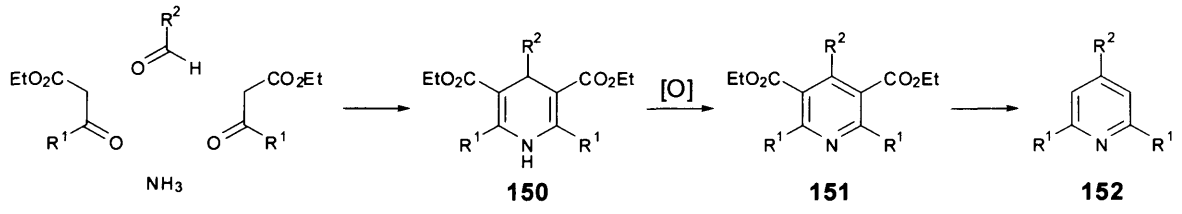


Figure 18 Retrosynthetic analysis of pyridine

4.1.1 – The Hantzsch pyridine synthesis

First reported in 1882 by Arthur Hantzsch, this pyridine synthesis involves the condensation of four components, two equivalents of a β-dicarbonyl compound, an aldehyde and ammonia (Scheme 43).[167] The initial product of the Hantzsch reaction is a 1,4-dihydropyridine (**150**). This dihydropyridine can be readily oxidised to pentasubstituted pyridine **151**. Hydrolysis and decarboxylation of the ester functionalities in the 3- and 5- positions affords 2,4,6-trisubstituted pyridines **152**. The Hantzsch thiazole synthesis is a versatile and flexible route to pyridines and 1,4-dihydropyridines, given the array of commercially available aldehyde starting materials, and the ease of synthesis of β-ketoesters with a range of substituents in the R^1 position.

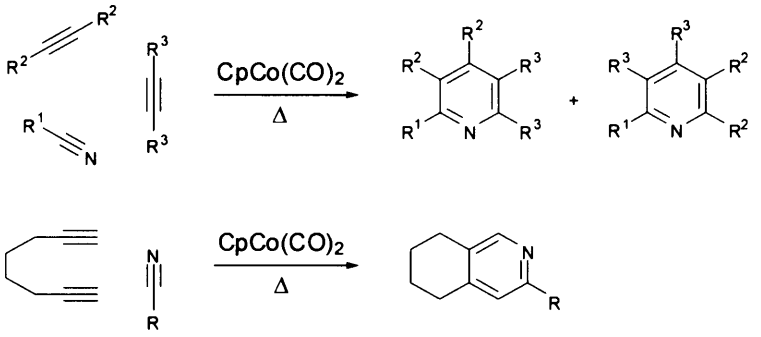


Scheme 43 The Hantzsch pyridine synthesis

4.1.2 – The Wakatsuki-Yamazaki-Bönnemann reaction

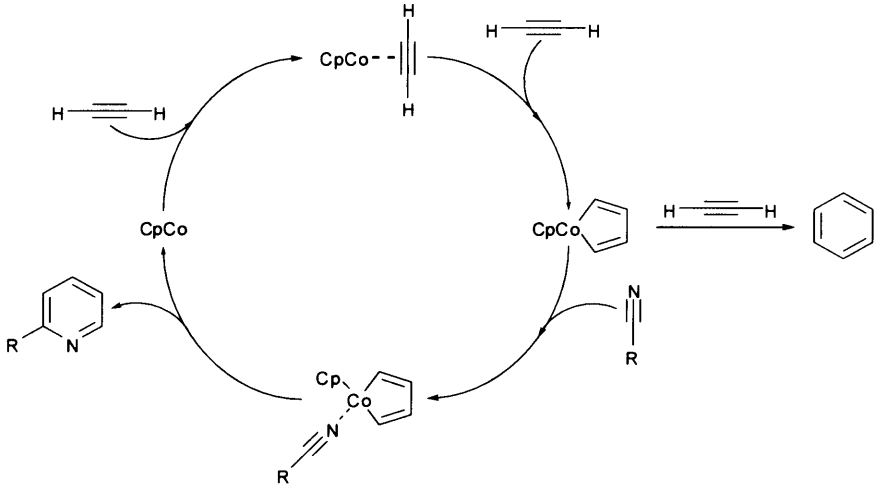
The Wakatsuki-Yamazaki-Bönnemann reaction is a cobalt(I)-catalysed co-oligomerisation of a nitrile with alkynes (Scheme 44).[168,169] If two different symmetrically substituted alkynes are used,

a mixture of products is obtained. 1,7-Diynes can be cyclised with nitriles to afford 5,6,7,8-tetrahydroisoquinolines using this methodology.



Scheme 44 The Wakatsuki-Yamazaki-Bönnemann reaction

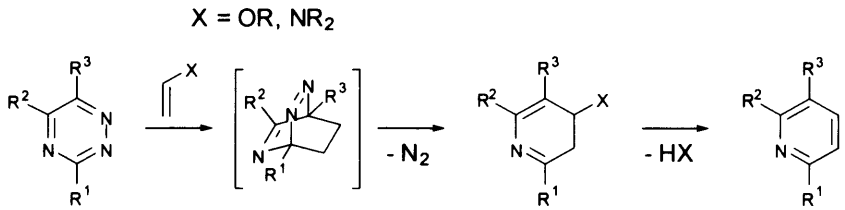
The proposed catalytic cycle for this reaction is illustrated in Scheme 45. The competing side reaction (co-trimerisation of the acetylene) can be suppressed by the use of an excess of the nitrile component.



Scheme 45 Proposed catalytic cycle for the Wakatsuki-Yamazaki-Bönnemann reaction

4.1.3 – The Boger-Panek pyridine synthesis

The Boger-Panek pyridine synthesis is a [4+2] cycloaddition involving a 1,2,4-triazine and an enamine or enol ether (Scheme 46).[170,171] The initial steps is an inverse electron demand Diels-Alder reaction between the triazine diene and the electron-rich dienophile. The initial product **153** loses nitrogen in a retro-Diels-Alder reaction to form 3,4-dihydropyridine **154** which subsequently eliminates amine or alcohol to form the pyridine product **155**.



153 **154** **155**

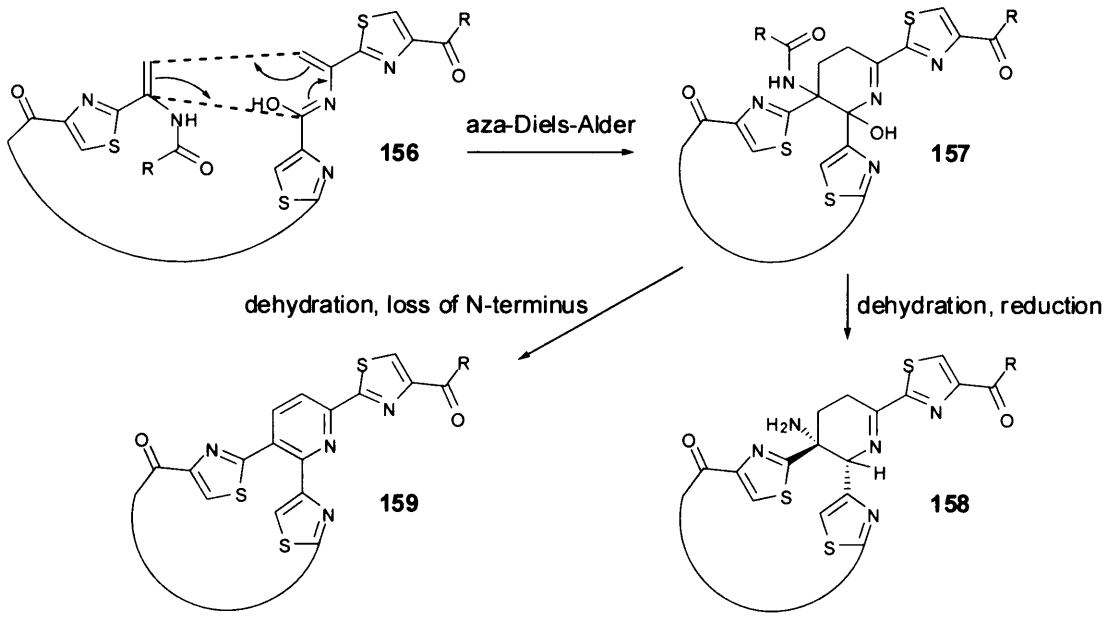
Scheme 46 The Boger-Panek pyridine synthesis

4.2 – Synthetic approaches to thiopeptide core domains

Each of the thiopeptide total syntheses successfully completed to date has utilised a different method for the synthesis of the central heterocyclic domain. These routes are outlined in the following section, together with the proposed biosynthetic pathway to the trisubstituted pyridine domain of micrococcin P1.

4.2.1 – Proposed biosynthetic pathway to the pyridine and tetrahydropyridine cores of the thiopeptide antibiotics

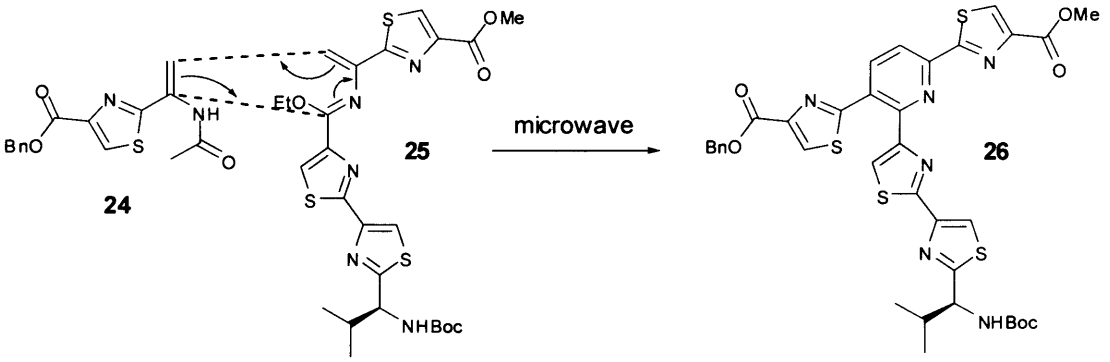
In their 1978 paper reporting the structures of micrococcins P1 and P2, Bycroft and Gowland also proposed that the pyridine ring could be biosynthesised from a pair of dehydroalanine units, formed by the dehydration of two serine residues in the peptide precursor.[130] This proposal was confirmed by Floss *et al.* fifteen years later following isotopic labelling studies.[172,173] Floss and co-workers proposed a cycloaddition mechanism as shown in Scheme 47. Intramolecular aza-Diels-Alder reaction of 2-azadiene **156** affords hydroxytetrahydropyridine **157** which can undergo dehydration and 1,4-reduction to form the tetrahydropyridine core of thiostrepton (**158**). Alternatively, intermediate **157** can undergo aromatisation and loss of the –NHCOR unit to form the trisubstituted pyridine core of the micrococcin thiopeptides (**159**).



Scheme 47 The Bycroft-Gowland-Floss proposal for the biosynthesis of thiopeptide core domains

4.2.2 – Aza-Diels-Alder approach

Moody *et al.* sought to directly mimic the Bycroft-Gowland-Floss mechanistic proposal in their total synthesis of amythiamicin D.[74,75] 1-Ethoxyazadiene **25** was reacted with *N*-acetyldehydroalanine **24** under microwave irradiation to yield the desired trisubstituted pyridine domain **26** in 33% yield (Scheme 48).

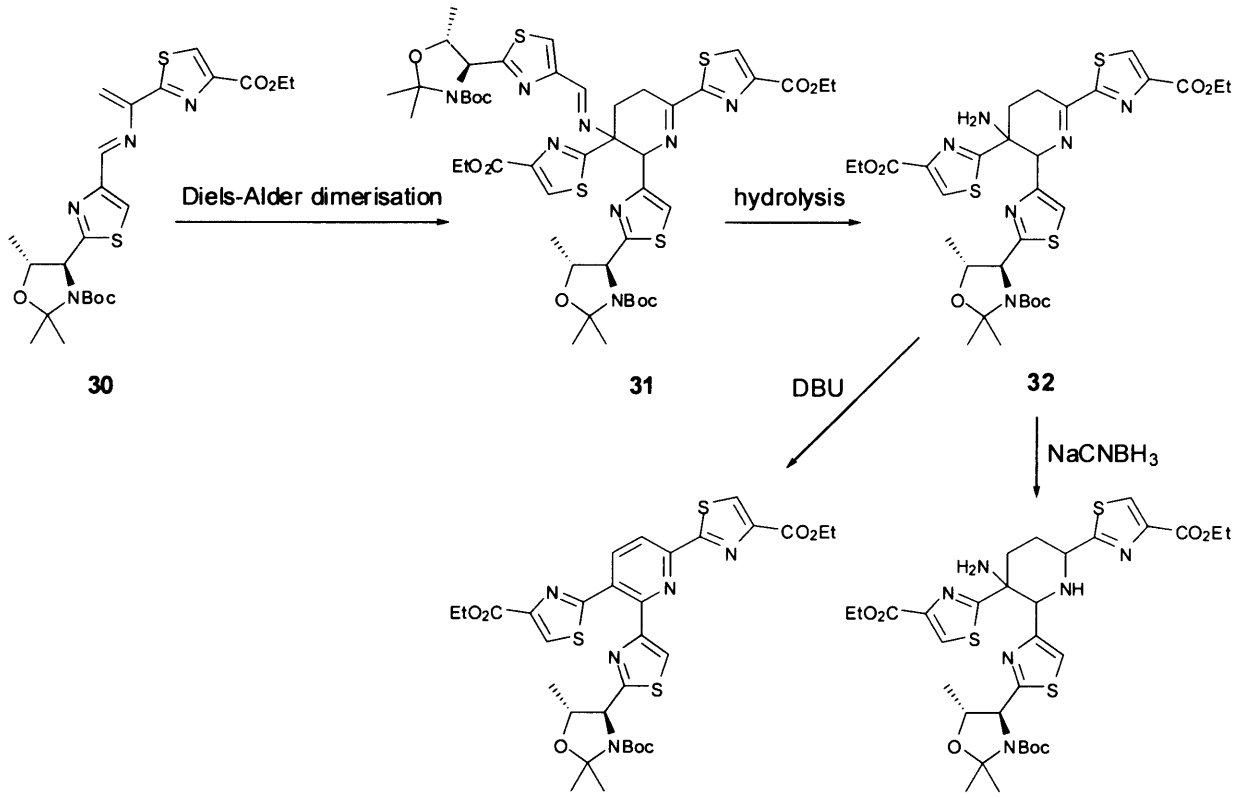


Scheme 48 Biomimetic aza-Diels-Alder approach to the core domain of amythiamicin D

4.2.3 – Diels-Alder dimerisation

Nicolaou and co-workers also employed the aza-Diels-Alder reaction in the total syntheses of thiostrepton, GE2270A and GE2270T. For all three thiopeptide antibiotics, the core domain was derived from building block **30**, which underwent a Diels-Alder dimerisation (section 1.4.3,

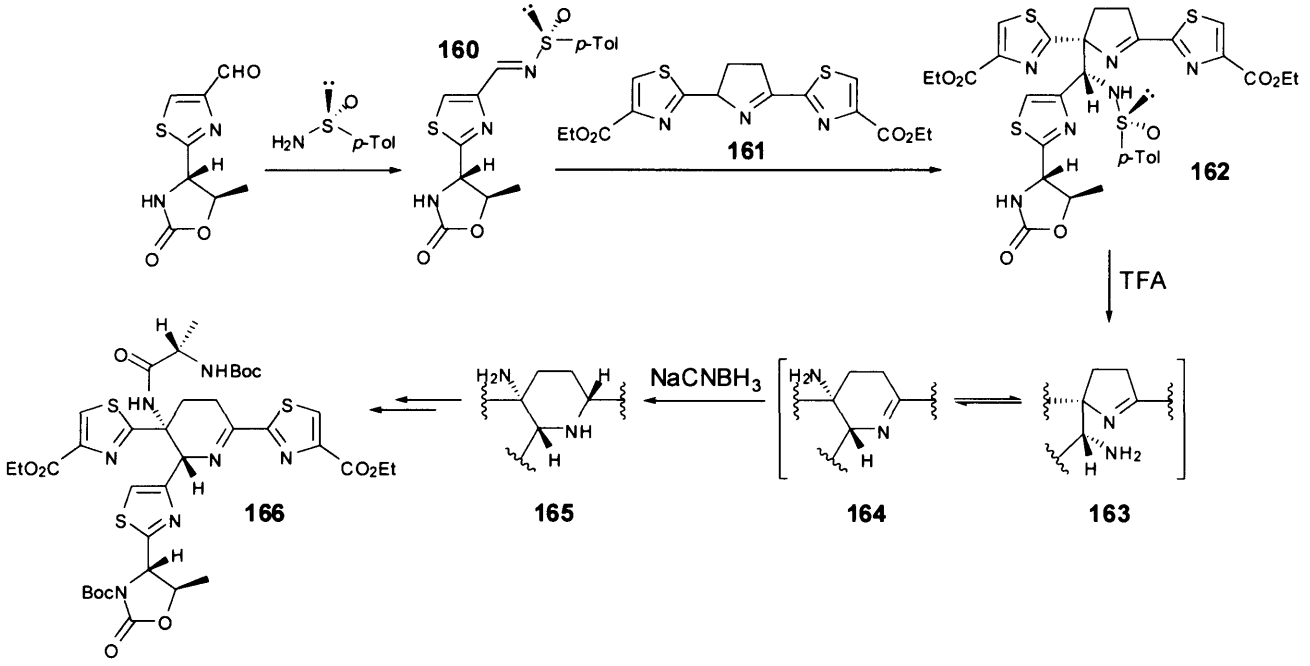
Scheme 5) to form intermediate **31**. Subsequent hydrolysis of the imine functionality afforded the thiostrepton tetrahydropyridine domain **32** which could be converted to the corresponding pyridine by heating in the presence of DBU, or to a piperidine by treatment with sodium cyanoborohydride (Scheme 49).



Scheme 49 Diels-Alder dimerisation approach to the core domains of thiostrepton and GE2270A

4.2.4 – Coupling of an azomethine ylide and a chiral sulfinimine

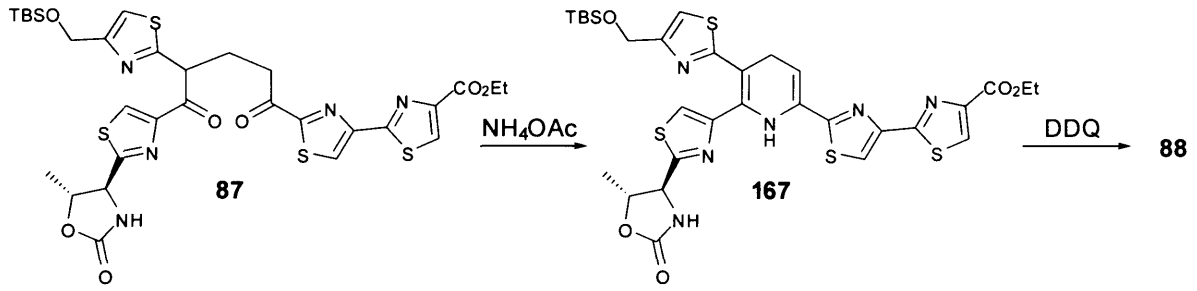
Hashimoto *et al.* utilised a different and to date unique approach in the field of thiopeptide synthesis to prepare the dehydropiperidine domain of siomycin A (Scheme 50).[107,109] The azomethine ylide derived from **161** was coupled with chiral sulfinimine **160** to form imine **162**. Removal of the sulfinyl group furnished an equilibrium mixture of five-membered imine **163** and six-membered imine **164**, the six-membered imine being stereoselectively reduced with sodium cyanoborohydride to piperidine **165**. The tetrasubstituted dehydropiperidine domain of siomycin A (**166**) was prepared in three further steps.



Scheme 50 Preparation of the core domain of siomycin A

4.2.5 – Synthesis *via* a 1,5-diketone

Ciufolini and Shen employed a cyclocondensation method for the synthesis of the central heterocyclic domain of the micrococcin thiopeptides.[131] In this Hantzsch-like procedure, 1,5-diketone **87** was prepared (section 1.5.6, Scheme 13) and treated with ammonium acetate to form 1,4-dihydropyridine **167**. Oxidation with DDQ afforded the trisubstituted pyridine core domain **88** (Scheme 51).

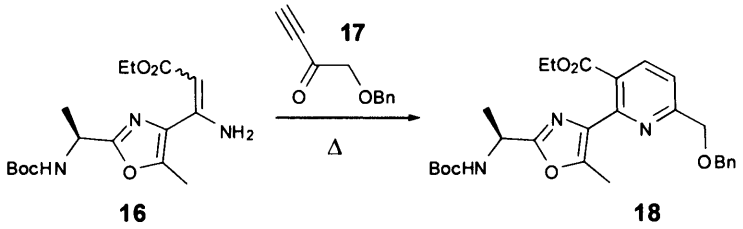


Scheme 51 Ciufolini's synthesis of the pyridine domain of the micrococcin thiopeptides

4.2.6 – The Bohlmann-Rahtz pyridine synthesis

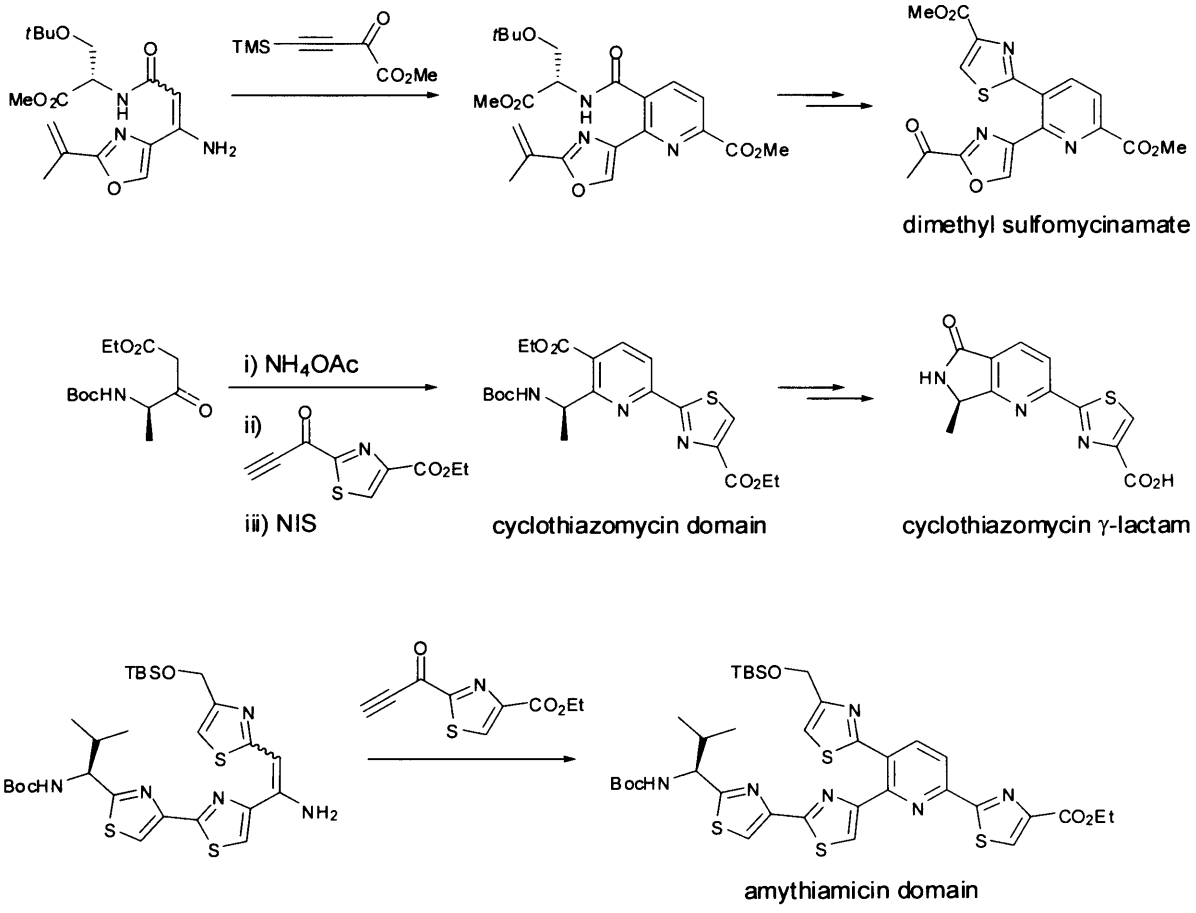
The total synthesis of promothiocin A by Moody and Bagley utilised the then little known Bohlmann-Rahtz pyridine synthesis to prepare the trisubstituted pyridine domain of the natural product (Scheme 52).[66,67] While related to the Hantzsch pyridine synthesis, the Bohlmann-Rahtz

reaction has the advantage that the pyridine is formed directly, without the need for an additional oxidation step as a result of employing an ynone rather than an enone to react with the enamine.[69]



Scheme 52 The Bohlmann-Rahtz pyridine synthesis of the core domain of promothiocin A

The Bohlmann-Rahtz reaction has been studied extensively within our laboratory[174] and has been successfully applied in the synthesis of several thiopeptide fragments and degradation products (Scheme 53).[141,143,175]



Scheme 53 Thiopeptide fragments synthesised using the Bohlmann-Rahtz reaction

4.2.7 – Retrosynthetic analysis of the central heterocyclic domain of MP1

With the backbone peptide sector of the micrococcin thiopeptides now in hand, a route to the central heterocyclic domain (**168**) was devised (Figure 19). Enamine **169** would be prepared from threonine-derived thiazole **132** and reacted with propargylic ketone **170** to furnish trisubstituted pyridine **171**. Subsequent simultaneous elaboration of both ester moieties to the corresponding thiazole ethyl esters would yield the desired heterocyclic motif, completing the synthesis of **168**.

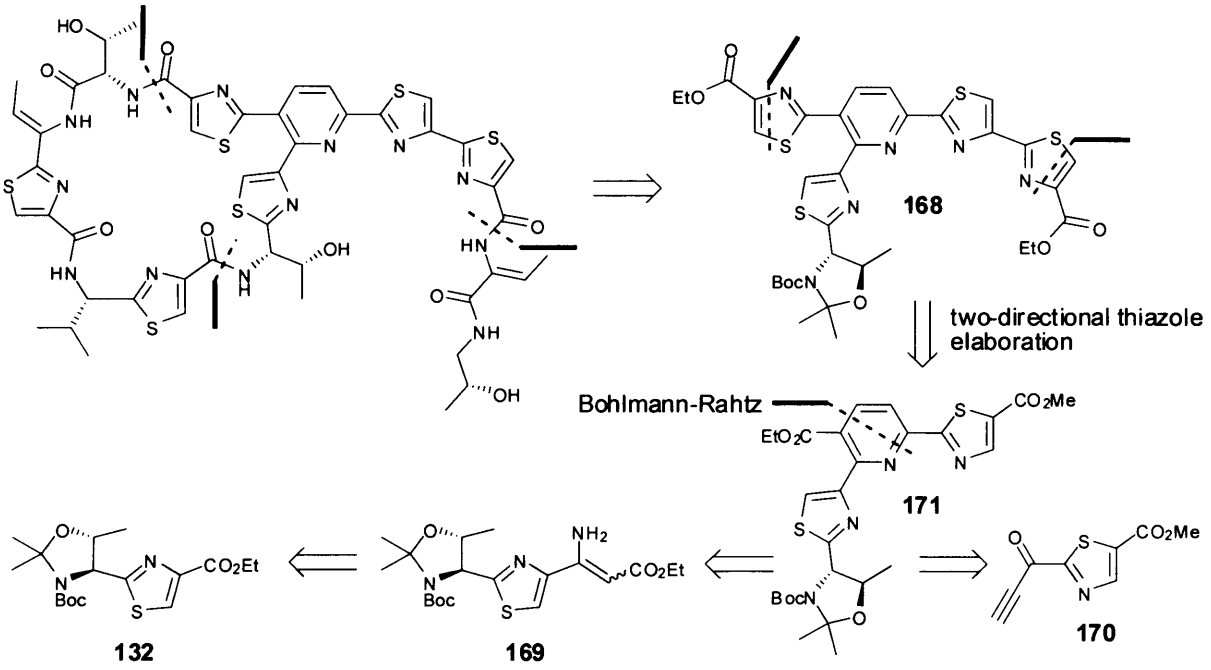
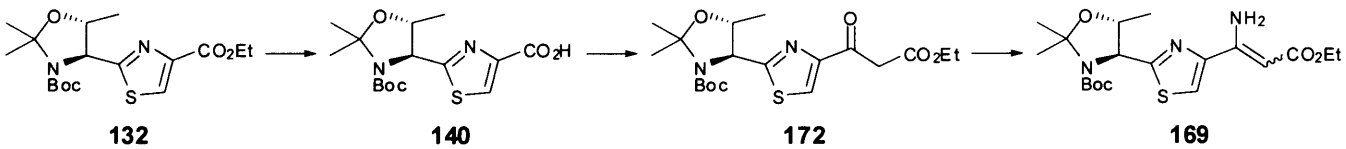


Figure 19 Disconnection of the central heterocyclic domain of micrococcin P1

4.3 – Synthesis of β-ketoester 172

4.3.1 – Conversion of carboxylic acid 140 to β-ketoester 172

It was proposed that enamine building block **169** would be prepared from threonine-derived thiazole **132** as illustrated in Scheme 54. Central to this approach is the ability to prepare β-ketoester **172** in high yield. A range of different procedures were employed in order to effect the transformation of **132** or **140** into **172** (Table 8).



Scheme 54 Proposed synthesis of enamine **169**

Table 8 Synthesis of β-ketoester **172**

	Substrate	Activating reagents	Enolate source	Time /h	Yield (%)
1	**132**	-	MeOAc / NaOMe	2	0[a]
2	**140**	EtOCOCl, Et_3N	EtOAc / LDA	0.5	0[b]
3	**140**	DCC, DMAP	Meldrum's acid, EtOH	4.5	0[b]
4	**140**	EtOCOCl, Et_3N	Potassium ethyl malonate / MeMgBr	16	33
5	**140**	EtOCOCl, Et_3N	Potassium ethyl malonate / MeMgBr	2	72

[a] Only starting material **132** recovered.

[b] Only starting material **140** recovered.

Direct formation of β-ketoester **172** from threonine-derived thiazole ester **132** was attempted by the addition of sodium methoxide to a solution of **132** in methyl acetate (entry 1). Monitoring of the reaction by thin layer chromatography suggested the consumption of all of the starting material and formation of a single product, but on work up and NMR, only the starting ester was present, recovered in quantitative yield. Treatment of carboxylic acid **140** (activated as a mixed anhydride) with the lithium enolate of ethyl acetate failed to yield the desire product, again returning the starting material in quantitative yield (entry 2). Activation of **140** with dicyclohexylcarbodiimide and DMAP followed by reaction with Meldrum's acid for 1.5 hours at room temperature then 3 hours at 65 °C again returned only starting material (entry 3). It was found that reaction of the mixed anhydride of **140** with a pre-mixed solution of potassium ethyl malonate and methyl magnesium bromide furnished the desired product in 33% yield if allowed to stir overnight at room temperature (entry 4). Encouraged by this result, the reaction was repeated, but stirred at room temperature for two hours (entry 5). This shortening of the reaction time led to the isolation of β-ketoester **172** in 72% yield after work up and purification by chromatography. It was possible to scale this reaction up with no decrease in yield, allowing access to multi-gram quantities of **172** in readiness for conversion to enamine **169**.

4.4 – Synthesis of enamine 169

The conversion of β-ketoester **172** into enamine **169** proved to be extremely challenging, failing under all but the harshest reaction conditions. The unexpected inertness of **172** to reaction with sources of ammonia is attributed in part to the steric bulk and conformational constraints imposed by the protecting groups used, and also to the stability of the enol tautomer of **172**,

which is clearly visible in the proton NMR spectrum, accounting for 22% of the material present by ^{1}H-NMR integration in $CDCl_3$.

4.4.1 – Ammonia-saturated solvents

The reaction of **172** with ammonia-saturated solvents at room temperature or under microwave irradiation conditions (Table 9, entries 1-3) failed to yield enamine **169**. Addition of a Lewis acid catalyst also failed to furnish the desired product (entry 4), unreacted starting material being returned in quantitative yield in all cases.

Table 9 Reaction of β-ketoester **172** with ammonia-saturated solvents

	Solvent	Temperature /°C	Catalyst	Time	Yield (%)
1	NH_3 in EtOH	r.t.	-	24 h	0
2	NH_3 in $CHCl_3$	r.t.	-	96 h	0
3	NH_3 in EtOH	100[a]	-	30 min	0
4	NH_3 in CH_2Cl_2	100[b]	$Sc(OTf)_3$	10 min	0

[a] CEM Discover® single-mode microwave synthesiser, initial power 300 W.

[b] CEM Discover® single-mode microwave synthesiser, initial power 100 W.

4.4.2 – Ammonium acetate

Following the negative results obtained by treating **172** with ammonia-saturated solvents (Table 9), a new ammonia source was employed. Enamine formation by the reaction of a β-ketoester with ammonium acetate in a mixture of benzene and acetic acid (5:1) is a well established procedure which has been utilised successfully in our laboratory in previous studies.[176] Unfortunately, β-ketoester **172** failed to react with ammonium acetate in toluene-acetic acid either at room temperature or at reflux (Table 10).

Table 10 Reaction of β-ketoester **172** with ammonium acetate under thermal conditions

	Solvent	NH_4OAc (equiv)	Temperature /°C	Time	Yield (%)
1	PhMe-AcOH (5:1)	10	r.t.	6 days	0[a]
2	PhMe-AcOH (5:1)	10	reflux	24 h	0[a]

[a] Only starting material **172** recovered.

4.4.3 – Conductive heating vs. microwave conditions

The failure of the reaction of **172** with ammonium acetate in toluene-acetic acid when heated at reflux was partially attributed to the sublimation of ammonium acetate which occurs at 115 °C. This led to the build up of large quantities of solid ammonium acetate in the condenser, thus it was unable to act as an ammonia source in the reaction mixture. In order to circumvent this problem, the reaction was conducted in a sealed tube with microwave irradiation. It was hoped that the more efficient heating, coupled with the increased temperature and pressure would force **172** to undergo the desired reaction (Table 11).

Table 11 Reaction of β-ketoester **172** with ammonium acetate under microwave irradiation

	Solvent	NH_4OAc (equiv)	Temperature /°C	Time	Heat source	Yield (%)
1	PhMe-AcOH (5:1)	50	150	30 min	MW, 300 W[b]	0[a]
2	PhMe-AcOH (5:1)	120	150	30 min	MW, 300 W[b]	38
3	Mesitylene	120	150	2 h	thermal	28
4	PhMe-AcOH (5:1)	120	150	15 min	MW, 300 W[b]	49
5	PhMe-AcOH (5:1)	120	150	10 min	MW, 300 W[b]	69
6	PhMe-AcOH (5:1)	120	150	10 min	MW, 300 W[c]	68

[a] Only starting material **172** recovered.

[b] CEM Discover® single-mode synthesiser. Power is moderated throughout the course of the experiment to maintain a constant reaction temperature. 100 mg of **172** used, 3 mL solvent.

[c] Milestone BatchSYNTH® multimode synthesiser. Power is moderated throughout the course of the experiment to maintain a constant reaction temperature. 1 g of **172** used, 30 mL solvent.

Microwave irradiation at 150 °C in the presence of 50 equivalents of ammonium acetate led to the recovery of the starting material (entry 1) but on increasing to 120 equivalents of ammonium acetate, the elusive enamine was finally obtained, albeit in 38% yield (entry 2). It was concluded that the high reaction temperature was necessary in order to provide a sufficiently high pressure of ammonia in the sealed microwave tube to force the reaction to occur. Evidence for this is provided by the observed pressure jump within the microwave vessel between 145 °C and 150 °C (Figure 20). The reaction was then attempted under thermal conditions using mesitylene as the solvent in the hope of providing a method whereby the reaction could be scaled up (entry 3). Thermal reaction at 150 °C did furnish the desired product but the yield was poor, most likely as a result of the sublimation of large quantities of ammonium acetate out of the reaction mixture, or the evaporation of much of the ammonia generated. Faced with the poor yield of the thermal reaction (entry 3), the microwave methodology was optimised further, and it was found that

reducing the reaction time increased the yield of **169** (entries 4 and 5). The optimum reaction time was found to be ten minutes, as shorter times led to the isolation of unreacted starting material which proved difficult to separate from the product.

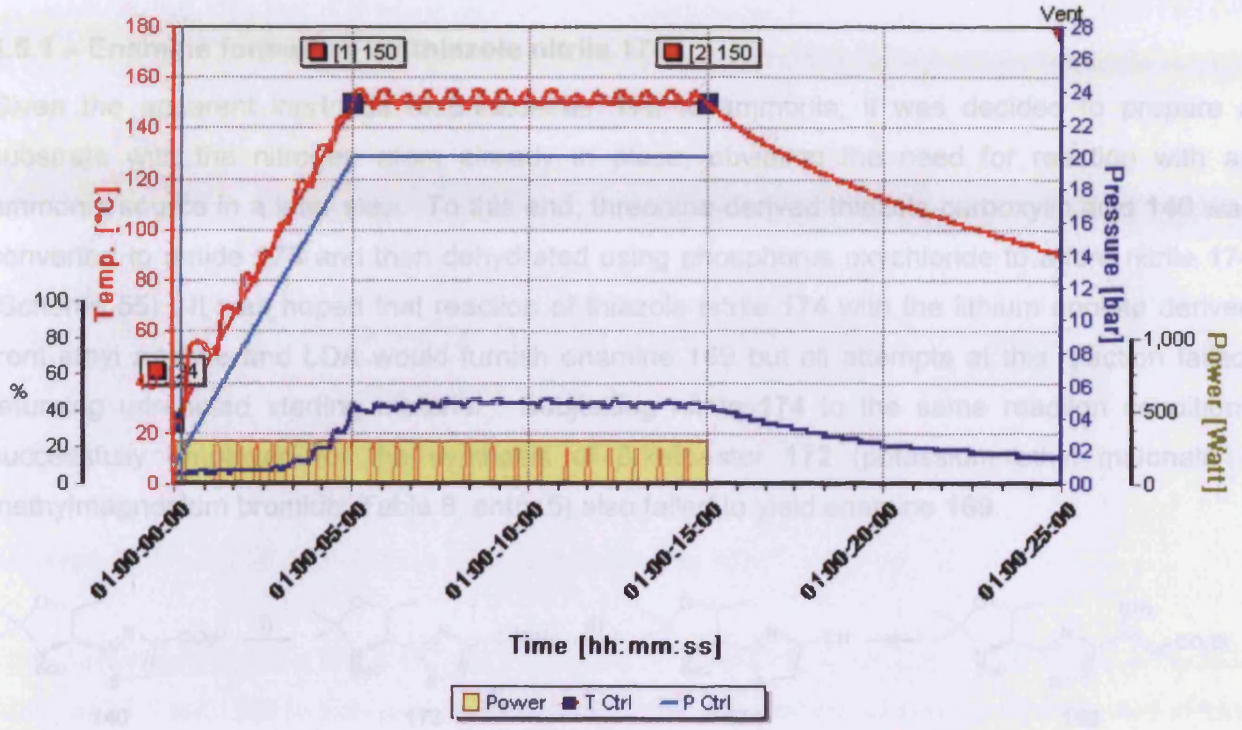


Figure 20 Reaction temperature, pressure and power profile under microwave irradiation conditions for the conversion of **172** to **169** using the Milestone BatchSYNTH® multimode synthesiser (Table 11, entry 6). Colour coding: Red = reaction temperature, yellow = power, light blue = pressure safety limit, dark blue = pressure inside reaction vessel.

4.4.4 – Scale-up using a multimodal microwave

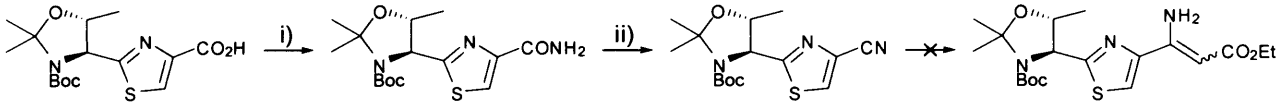
With a method for the formation of **169** now in hand, it was necessary to scale up the reaction in order to continue the synthesis of target domain **168**. However, the need for such a vast excess of ammonium acetate meant that only 100 mg of **172** could be reacted at a time in the standard 10 mL microwave vessels. Continuous flow systems could not be employed for the scale-up of this reaction due to the use of solid ammonium acetate. Fortunately, the use of a large capacity multimodal microwave synthesiser with a 60 mL reaction vessel provided the opportunity to scale up the reaction, using 1 gram of β-ketoester **172** and 22 grams of ammonium acetate (Table 11, entry 6). This enabled the preparation of enamine **169** in 680 mg batches with a reproducible and almost identical yield (68%) to the small scale monomodal reaction (Table 11, entry 5, 69%).

4.5 – Alternative routes to enamine 169

In parallel with the development of the microwave-assisted route to enamine **169** discussed in the preceding section, alternative routes to **169** and related compounds were investigated due to the initial difficulties in obtaining the desired enamine, and in scaling up of the reaction.

4.5.1 – Enamine formation *via* thiazole nitrile 174

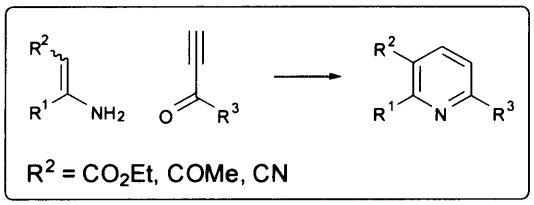
Given the apparent inertness of β-ketoester **172** to ammonia, it was decided to prepare a substrate with the nitrogen atom already in place, obviating the need for reaction with an ammonia source in a later step. To this end, threonine-derived thiazole carboxylic acid **140** was converted to amide **173** and then dehydrated using phosphorus oxychloride to afford nitrile **174** (Scheme 55). It was hoped that reaction of thiazole nitrile **174** with the lithium enolate derived from ethyl acetate and LDA would furnish enamine **169** but all attempts at this reaction failed, returning unreacted starting material. Subjecting nitrile **174** to the same reaction conditions successfully employed for the synthesis of β-ketoester **172** (potassium ethyl malonate – methylmagnesium bromide; Table 8, entry 5) also failed to yield enamine **169**.

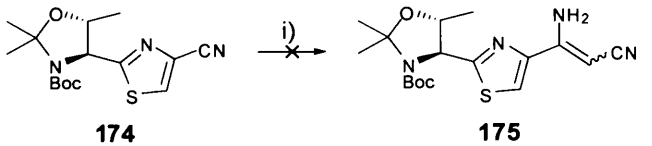


Reagents and Conditions: i) EtOCOCl, Et_3N, THF, 0 °C, 1 h, then 35% aq. NH_3, r.t., 3 h, 87%; ii) $POCl_3$, pyridine, 0 °C, 2 h, 48%.

Scheme 55 Proposed synthesis of enamine **169** *via* nitrile **174**

The Bohlmann-Rahtz pyridine synthesis can be performed using an enamine with a nitrile group in place of the more common ester substituent (Scheme 56).[69] It was hoped that nitrile **174** would react with the anion of acetonitrile to form **175** which could be used in the Bohlmann-Rahtz pyridine synthesis. Addition of sodium hydride to a solution of **174** in acetonitrile failed to yield the desired product, the starting material persisting even after 96 hours.



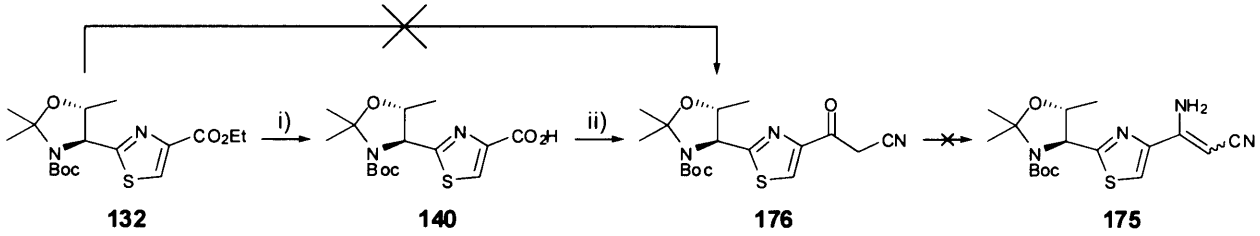


Reagents and Conditions: i) NaH, MeCN, reflux, 96 h.

Scheme 56 Attempted synthesis of enamine nitrile **175**

4.5.2 – Enamine formation *via* β-ketonitrile 176

Although the conversion of nitrile **174** into **175** failed, a different synthetic approach was investigated, this time accessing **175** *via* β-ketonitrile **176** (Scheme 57). Initially, the synthesis of **176** directly from thiazole ester **132** was investigated, based on good literature precedent.[177] Addition of ester **132** to a mixture of sodium hydride and acetonitrile in THF failed to give the product, the starting material being recovered in quantitative yield, even after 5 days with heating at reflux. Addition of sodium hydride to a solution of ester **132** in acetonitrile also failed to yield the product instead producing a mixture of products.



Reagents and Conditions: i) LiOH, MeOH-H_2O, r.t., 3 h, 100%; ii) EtOCOCl, Et_3N, MeCN, r.t., 2 h; then NaH, r.t., 2 h, 78%.

Scheme 57 Synthetic approach to **175** *via* β-ketonitrile **176**

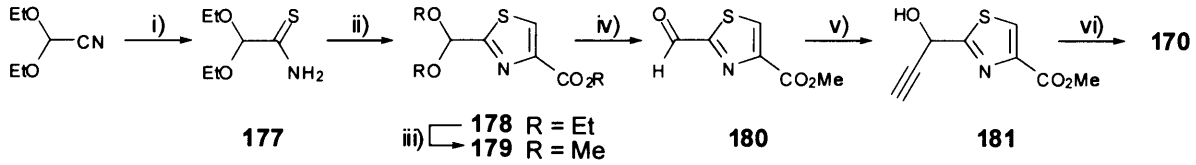
The elusive β-ketonitrile **176** was finally obtained by preparing a solution of the mixed anhydride of carboxylic acid **140** in acetonitrile, then adding five equivalents of sodium hydride and stirring for a further two hours. Quenching of the reaction mixture and purification by chromatography led to the isolation of **176** in 78% yield. It was apparent from the proton NMR and infrared spectra of compound **176** that the β-ketonitrile existed entirely as the enol tautomer, and the lack of reactivity towards any sources of ammonia was attributed to this. Literature procedures for the conversion of β-ketonitriles to enamines by refluxing with ammonium acetate,[178] or heating in ethanolic ammonia in a sealed vessel at 130 °C,[179] failed to afford the product, returning only starting material. Even the harsh conditions devised for the conversion of β-ketoester **172** to an enamine failed to force **176** to react, so the synthesis of nitrile **175** was not pursued further.

4.6 – Synthesis of thiazole alkynone 170

With a reliable and reproducible route to enamine building block **169** now established, our attention was turned to the preparation of propargylic ketone **170**. The ethyl ester homologue of **170** had previously been synthesised within our laboratory (Scheme 53)[143,175] and could have also been employed in this synthesis, but the methyl ester was required for the synthesis of another target (Chapter 6) so the same alkynone was used in the synthesis of **168**.

4.6.1 – Synthesis of thiazole alkynone 170

Propargylic ketone **170** was prepared in six steps and 51% overall yield by the route shown in Scheme 58. Ammonium sulfide thionation of diethoxyacetonitrile afforded 2,2-diethoxythioacetamide **177**, which underwent the Hantzsch thiazole synthesis in the presence of 4 Å molecular sieves in order to prevent concomitant acetal deprotection by trapping the equivalent of hydrogen bromide liberated in the course of the reaction. Thiazole acetal **178** was then trans-esterified to form **179** and acetal-deprotected using aqueous acid to liberate the aldehyde moiety. Treatment of aldehyde **180** with ethynylmagnesium bromide in tetrahydrofuran furnished propargylic alcohol **181**, which was oxidised to the desired propargylic ketone **170** using manganese dioxide.



Reagents and Conditions: i) $(NH_4)_2S$, MeOH, r.t., 24 h, 100%; ii) Ethyl bromopyruvate, 4 Å molecular sieves, EtOH, reflux, 1 h, 100%; iii) PTSA, MeOH, reflux, 48 h, 96%; iv) 2 M aq HCl, acetone, reflux, 2 h, 86%; v) Ethynylmagnesium bromide, THF, 0 °C, 1 h, 81%; vi) Activated MnO_2, CH_2Cl_2, r.t., 2 h, 77%.

Scheme 58 Synthesis of propargylic ketone **170**

Alkynone **170** was found to be extremely unstable, decomposing to a black tar-like substance overnight, even when stored at -18 °C under an inert atmosphere. For this reason, the propargylic alcohol precursor **181** was prepared in large quantities and only oxidised immediately prior to use in the subsequent Bohlmann-Rahtz reaction.

4.6.2 – Scale-up limitations with the oxidation of propargylic alcohol 181

While the route depicted in Scheme 58 proved successful in furnishing multi-gram quantities of propargylic alcohol **181**, subsequent oxidation to thiazole alkynone **170** consistently failed when undertaken using more than 800 mg of **181**. The starting material was all consumed during the oxidation, and in most cases a single product spot was observed by thin layer chromatography. However, on filtration through Celite® the characteristic orange band of product could be seen to turn black, and TLC and ^{1}H-NMR spectroscopic analysis of the filtrate revealed the absence of the previously observed alkynone (Figure 21).

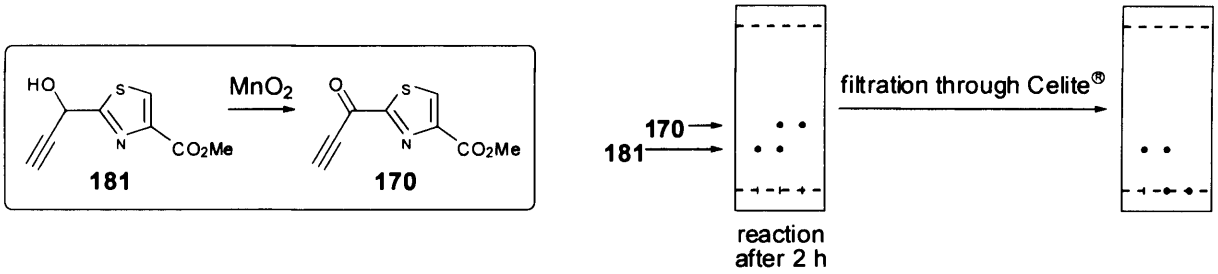


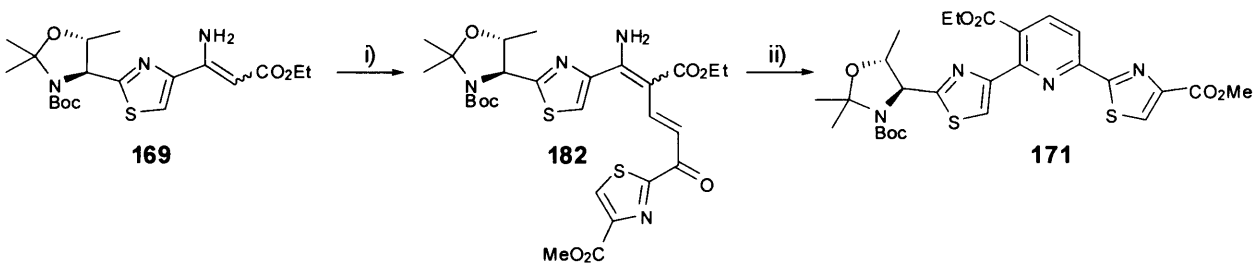
Figure 21 Observed decomposition of **170** when prepared on a large scale

Given the difficulties in storing alkynone **170**, conducting the reaction in a number of small batches and combining the product was not a viable alternative to a single large scale reaction. Instead, a different oxidising agent was sought in order to circumvent the apparently problematic filtration step. A test reaction on 100 mg of **181** using IBX as the oxidising agent was found to be successful, furnishing the product in moderate yield (56%). On scale-up to 3.50 grams of **181**, the yield fell to 34%, the probable cause being difficulty in removal of the product from 350 mL of DMSO during the work-up of the reaction. Despite the poor yield, IBX oxidation was the only method by which gram quantities of alkynone **170** could be reproducibly obtained.

4.7 – Synthesis of protected core domain 168

4.7.1 – The Bohlmann-Rahtz pyridine synthesis

With building blocks **169** and **170** now in hand, the assembly of pyridine core **171** was now attempted *via* the proposed Bohlmann-Rahtz methodology (Scheme 59). The success of the initial Michael addition of enamine **169** to propargylic ketone **170** was hampered by the instability of **170**, the reaction yielding aminodienone **182** in 58% yield with 40% recovery of unreacted enamine **169** when 1.5 equivalents of alkynone **170** were used. Increasing to 2 equivalents of alkynone substantially improved the efficiency of the reaction, furnishing aminodienone **182** in 81% yield with 12% recovery of enamine **169**.



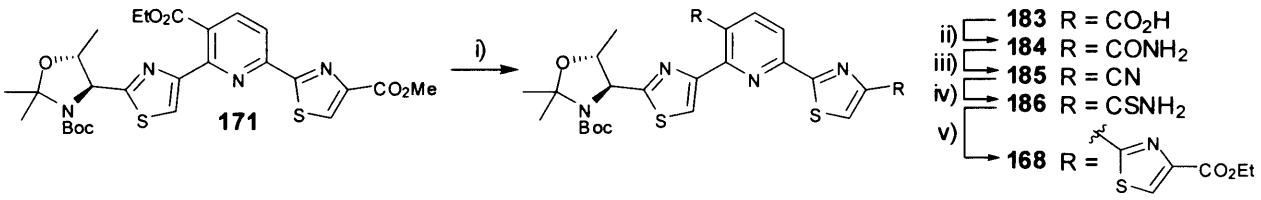
Reagents and Conditions: i) **170**, EtOH, r.t., 18 h, 58-81%; ii) I_2, r.t., 2 h, 100%.

Scheme 59 Bohlmann-Rahtz synthesis of pyridine **171**

Numerous methods for the cyclodehydration of aminodienones to pyridines have been developed within our laboratory, including the use of Brønsted acids,[180] Lewis acids,[181] *N*-halosuccinimides,[182] and iodine.[183] For the synthesis of pyridine **171**, iodine was the cyclodehydration reagent of choice due to the extremely mild reaction conditions and high yield and purity of the product obtained, column chromatography being unnecessary following aqueous work up with sodium thiosulfate.

4.7.2 – Two-directional thiazole elaboration

The final stage in our synthesis of the central heterocyclic domain of micrococcin P1 was the two-directional elaboration of pyridine **171**, installing both of the remaining thiazoles simultaneously (Scheme 60).



Reagents and Conditions: i) LiOH, MeOH-H_2O, reflux, 72 h, 100%; ii) EtOCOCl, Et_3N, THF, 0 °C, 18 h, then 35% aq. NH_3, r.t., 24 h, 62%; iii) $POCl_3$, pyridine, 0 °C, 2 h, 87%; iv) $(NH_4)_2S$, MeOH, r.t., 18 h, 34-70%; v) Ethyl bromopyruvate, $KHCO_3$, DME, r.t., 24 h, then pyridine, TFAA, 0 °C → r.t., 3 h, then Et_3N, r.t., 3 h, 96%.

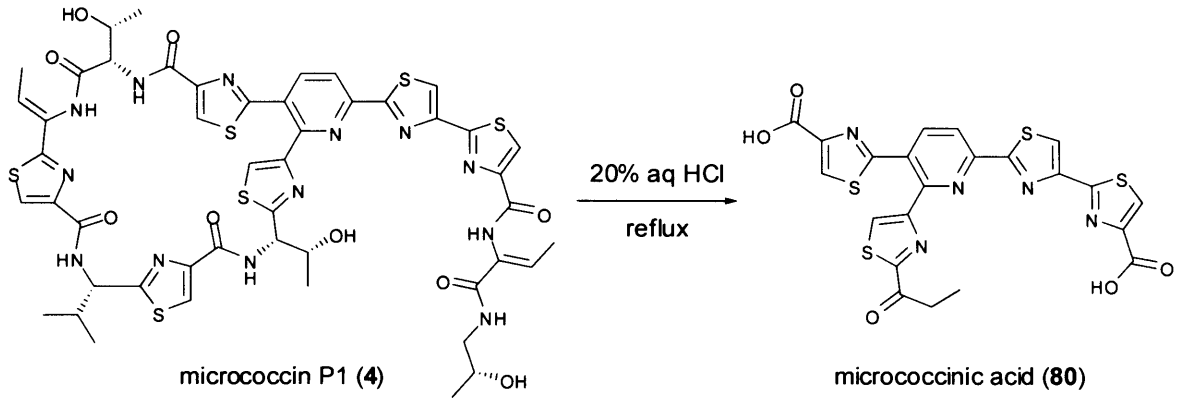
Scheme 60 Simultaneous elaboration of the 3- and 6-position carboxylate groups of **171**

Initial attempts to hydrolyse both esters using lithium hydroxide at room temperature failed, the ethyl ester remaining intact under all but the harshest hydrolysis conditions. The desired bis-acid **183** was only formed on prolonged heating (72 hours or more) in the presence of 20 equivalents of lithium hydroxide. Bis-amide **184** was prepared in modest yield by the reaction of bis-acid **183** with ethyl chloroformate followed by addition of 35% aqueous ammonia. As with the synthesis of threonine-derived thiazole **132** (section 3.3.5), direct thionation of the amide with Lawesson's reagent was not used, as **184** failed to react with Lawesson's reagent and recovery of the unreacted starting material proved difficult. Instead, bis-nitrile **185** was prepared and thionated using ammonium sulfide. This procedure proved to be uncharacteristically capricious when applied to nitrile **185**, affording thioamide **186** in yields ranging from 34% to 70% after column chromatography. The final thiazole formation was successfully achieved using the Nicolaou modification of the Hantzsch thiazole synthesis (section 3.2.5), furnishing target molecule **168** in excellent yield.

4.8 – Synthetic approaches to micrococcinic acid

4.8.1 – Degradation of micrococcin P1 to micrococcinic acid

Acidic hydrolysis of micrococcin by Walker *et al.*[123] led to the isolation of an acid-insoluble compound which contained many thiazoles,[125] and was later named micrococcinic acid (**80**).[126] Walker's degradation of 'micrococcin P' was performed by dissolving 5.4 grams of the natural product in 150 mL of 20% hydrochloric acid and heating the solution at reflux for 16 hours. Addition of the solution to water precipitated the 'acid-insoluble fraction' which was subsequently identified as micrococcinic acid (Scheme 61).

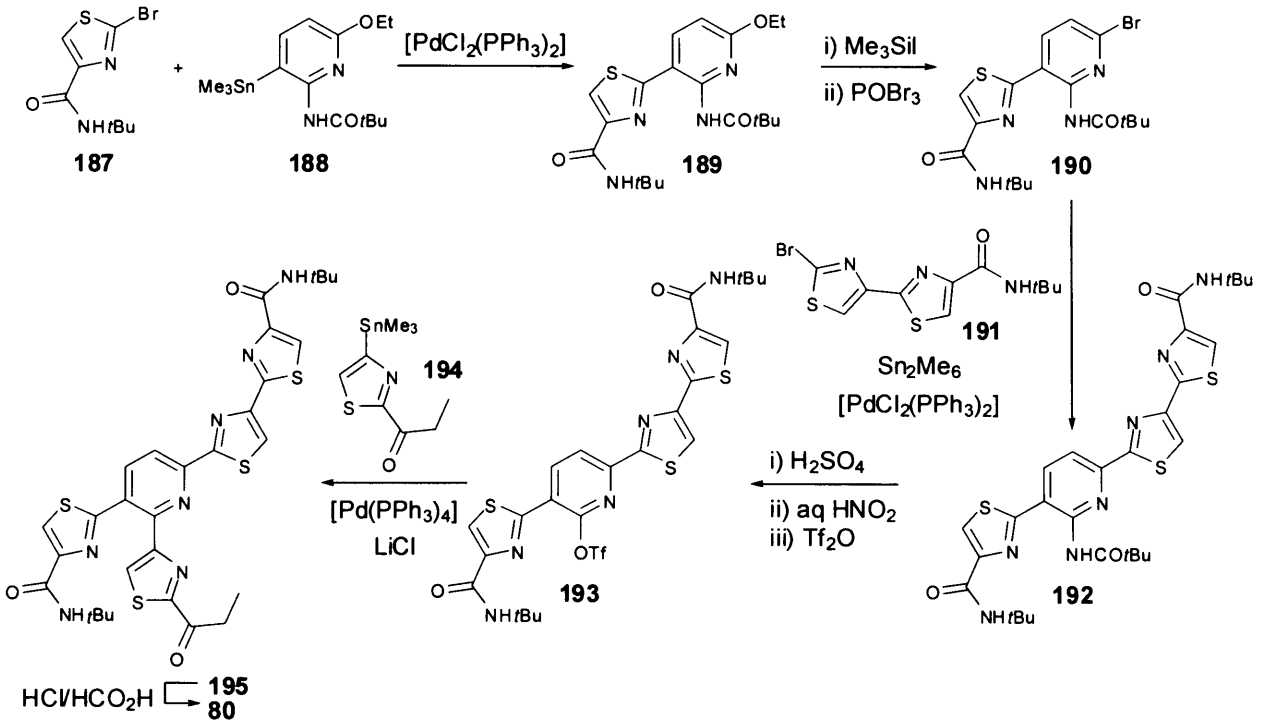


micrococcin P1 (**4**) micrococcinic acid (**80**)

Scheme 61 Acidic hydrolysis of micrococcin P1 to micrococcinic acid

4.8.2 – Kelly's synthesis of micrococcinic acid

The first and indeed only synthesis of micrococcinic acid was reported by Kelly and co-workers in 1991.[184] Using a strategy similar to that recently employed by Bach *et al.* in their elegant total synthesis of GE2270A (section 1.4.4),[99] individually synthesised thiazole building blocks were appended to a pyridine core by means of palladium-catalysed coupling reactions (Scheme 62).

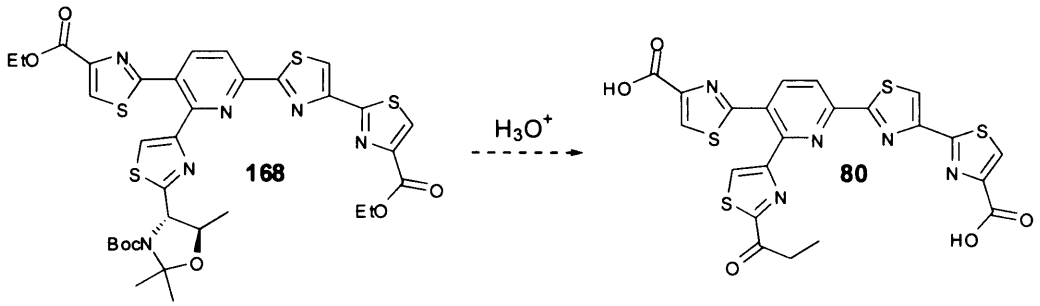


Scheme 62 Kelly's synthesis of micrococcinic acid

Bromothiazole **187** and 3-trimethylstannylpyridine **188** were coupled to form intermediate pyridine-thiazole **189**. Subsequent conversion to **190** and coupling with bromo-bis-thiazole **191** in the presence of Sn_2Me_6 and $PdCl_2(PPh_3)_2$ afforded compound **192** which was converted to triflate **193** in readiness for the final biaryl coupling. The final coupling between pyridine triflate **193** and 4-trimethylstannylthiazole **194** yielded micrococcinic acid precursor **195**, which was successfully hydrolysed to furnish the target molecule with identical physical and spectroscopic properties to that isolated from the natural product.

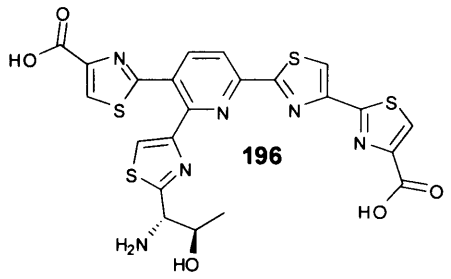
4.8.3 – Our synthetic approach to micrococcinic acid

Given the structural similarities between our protected micrococcin core domain **168** and micrococcinic acid **80**, it was hoped that acidic hydrolysis of **168** using Walker's original conditions would furnish the thiopeptide degradation product (Scheme 63). It was postulated that the harsh aqueous acidic conditions would cleave both *N*-terminus protecting groups, hydrolyse the ethyl esters, and convert the remaining threonine residue into the ethyl ketone, as observed in the degradation of the natural product (section 2.5.2, Scheme 24).



Scheme 63 Attempted conversion of **168** into micrococcinic acid

Micrococcin core domain **168** was dissolved in 6 M hydrochloric acid and heated at reflux for 2 hours. On addition of the reaction mixture to water, a yellow acid-insoluble precipitate was observed, as described in the literature, but this was found to be deprotected core domain **196** and not micrococcinic acid as hoped.



In order to effect the conversion of the still intact threonine residue into the ethyl ketone moiety of micrococcinic acid, the precipitate was heated in 6 M hydrochloric acid for a further 16 hours. This also failed to yield micrococcinic acid, instead returning a mixture of decomposition products unidentifiable by ^{1}H-NMR spectroscopy due to the small scale of the reaction.

Undeterred, an alternative degradation method was employed, also used successfully by Walker *et al.* to hydrolyse micrococcin to micrococcinic acid.[126] Accordingly, **168** was heated at reflux for 6 hours in a 1:1 mixture of concentrated hydrochloric acid and 98% formic acid. Addition of the reaction mixture to water induced precipitation of a yellow solid, which was again unidentifiable by NMR spectroscopy, although it appeared to contain a monosubstituted pyridine. Following the lack of success with both sets of hydrolysis conditions, the synthesis of micrococcinic acid from **168** was not pursued further, as the central heterocyclic domain was required for the proposed total synthesis of micrococcin P1.

4.9 – Conclusion

A range of conditions for the conversion of sterically hindered β-ketoesters to enamines have been investigated, and a reproducible microwave-assisted method devised, which is suitable for both single- and multimode instruments, and for scale-up to produce multi-gram quantities of material. The central heterocyclic domain (**168**) of the micrococcin thiopeptides has been prepared from *N*-Boc-(2*S*,3*R*)-threonine in 15 steps and 9% overall yield *via* a highly convergent strategy featuring an efficient two-directional thiazole elaboration.

Chapter Five – Results and Discussion

Assembly of the MP1 Macrocycle

5.1 – Introduction

5.2 – Deprotection of Core Domain **168**

5.3 – Deprotection of Backbone Peptide **139**

5.4 – Construction of Macrocycle **251**

5.5 – Synthesis of the MP1 Side Chain

5.6 – Elaboration of macrocycle **251** to MP1

5.7 – Conclusion

5 – Assembly of the MP1 Macrocycle

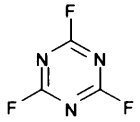
5.1 – Introduction

The amide bond is ubiquitous in nature, proteins and peptides being present and abundant within every living organism on Earth. Indeed, the importance of proteins is recognised in their name, derived from the Greek πρωτεοσ, meaning ranked first. In the synthesis of any peptide-based target, be it an enzyme containing perhaps hundreds of amino acid residues or a modified heterocyclic peptide such as those under investigation in this thesis, the ability to synthesise amide bonds in high yield and without racemisation/epimerisation is of paramount importance. What follows is a brief overview of peptide coupling reagents, and a discussion of the use of such reagents in the macrocyclisation steps of thiopeptide antibiotic syntheses.

5.1.1 – Acid halogenating reagents

The activation of an amino acid by conversion to the corresponding acid chloride was introduced by Emil Fischer in 1903.[185] Traditionally, acid chlorides are prepared using thionyl chloride, a reagent which is too vigorous for use with complex or sensitive substrates. While useful for the coupling of achiral or extremely hindered amino acids, activation as the acid chloride is prone to side reactions and racemisation, and thus unlikely to be the method of choice in the synthesis of a complex target molecule.

Acid fluorides, which are more tolerant of moisture and of acid-labile functionalities than their chloride counterparts, can be prepared using reagents such as cyanuric fluoride (**197**)[186] and tetramethylfluoroformamidinium hexafluorophosphate (TFFH, **198**).[187]



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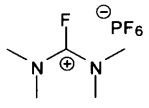
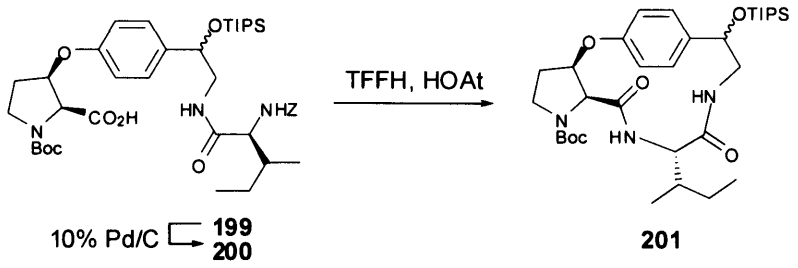


198

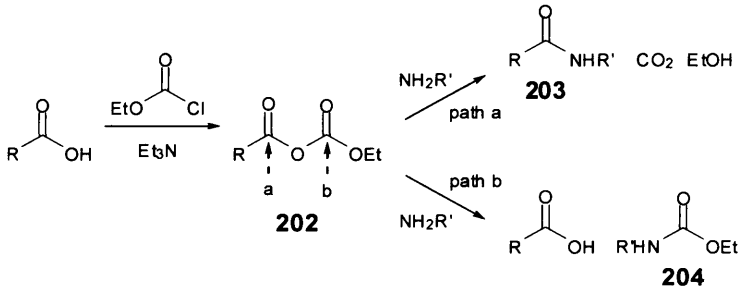
Acid fluoride methodology has been successfully employed in macrolactamisation reactions (Scheme 64). In their synthetic approach to C3-epimauritine D, Han *et al.* generated the acid fluoride of **200** *in situ* using TFFH in the presence of HOAt, and formed the desired macrocyclic product **201** in 75% yield over two steps.[188]



Scheme 64 TFFH-mediated macrolactamisation

5.1.2 – Chloroformates

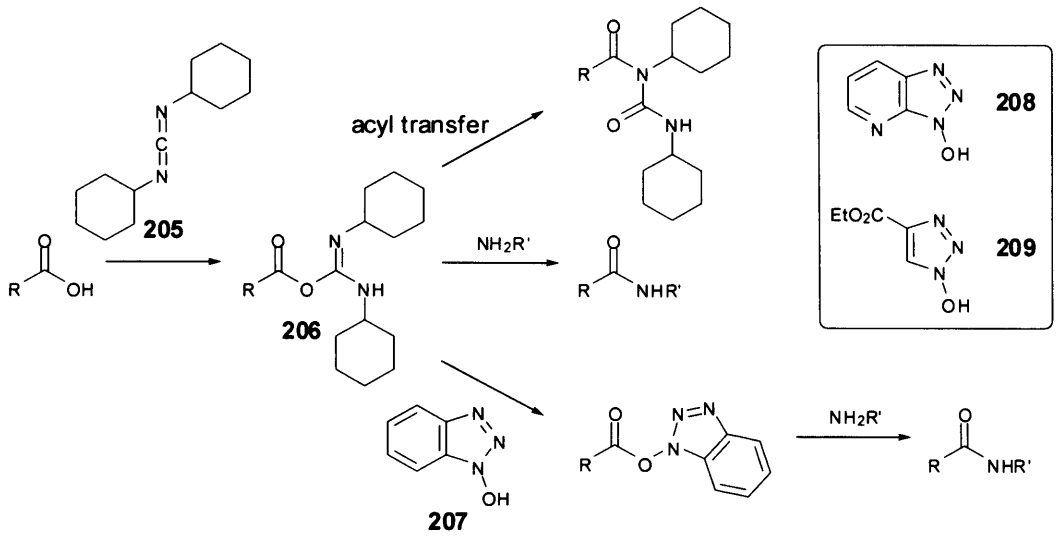
Chloroformates are widely used in peptide coupling reactions due to their low cost and the ease of purification of the product. For example, the by-products of an ethyl chlorofomate-mediated peptide coupling are ethanol, carbon dioxide and the hydrochloride salt of the tertiary amine base used (Scheme 65, path a). Disadvantages of chloroformate reactions include side reactions due to a lack of regioselectivity in the nucleophilic attack of the amine on the mixed anhydride intermediate **202**, leading to acetylation of the amine (path b, **204**).[189] Also, it is necessary to protect any free alcohols present in the starting material, a step which may be omitted with the use of other peptide coupling reagents.



Scheme 65 Possible side reactions in chloroformate-mediated peptide coupling reactions

5.1.3 – Carbodiimide reagents

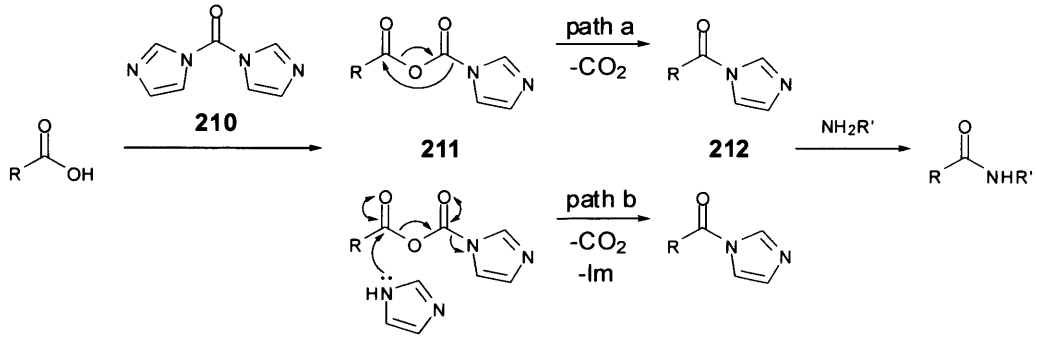
Carbodiimide reagents such as dicyclohexylcarbodiimide (DCC, **205**) were first reported by Sheehan and Hess in 1955 and have been widely used in peptide synthesis ever since (Scheme 66).[190] The by-product of DCC coupling reactions is dicyclohexylurea, which is readily separated from the reaction product due to its poor solubility in most organic solvents. The intermediate *O*-acylisourea **206** is prone to side reactions (intramolecular acyl transfer) and racemisation, so an additional nucleophile is often added to react with **206** before any such reactions can occur. Common additives include 1-hydroxybenzotriazole (HOBt, **207**), 1-hydroxy-7-azabenzotriazole (HOAt, **208**) and ethyl-1-hydroxy-1*H*-1,2,3-triazole-4-carboxylate (HOCt, **209**).



Scheme 66 DCC-mediated peptide coupling

5.1.4 – Imidazolium reagents

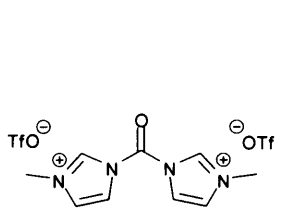
Imidazolium reagents were first developed in the 1950s in the search for improved coupling reagents based on DCC (**205**).[191] The parent reagent of this class of compounds is 1,1'-carbonyldiimidazole (CDI, **210**). There are two possible routes to the active imidazolide species (**212**) in a CDI-mediated peptide coupling, namely rearrangement of the anhydride intermediate **211** (Scheme 67, path a), or nucleophilic attack of the previously displaced imidazole (path b).



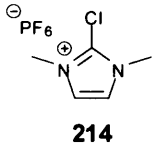
Scheme 67 Formation of the active intermediate in CDI-mediated peptide coupling

More recent additions to the imidazolium family of reagents include 1,1'-carbonylbis(3-methylimidazolium) triflate (CBMIT, **213**),[192] which has proved useful in coupling sterically hindered amino acids, but suffers from considerable drawbacks regarding air and moisture sensitivity, and restricted choice of solvents as a result of its high polarity.[193] Other imidazolium reagents such as 2-chloro-1,3-dimethylimidazolinium hexafluorophosphate (CIP, **214**) and 2-

(benzotriazol-1-yl)oxy-1,3-dimethylimidazolidinium tetrafluoroborate (BOI, **215**) have found application as both peptide coupling reagents and esterification reagents.[194,189]



213



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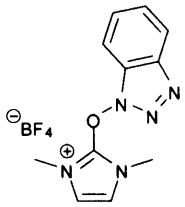

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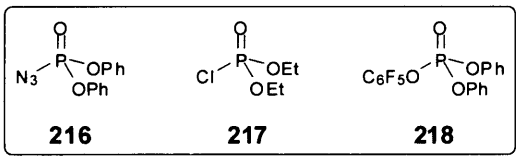
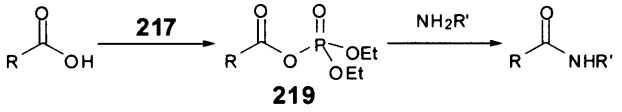
5.1.5 – Organophosphorus reagents

The first reported use of an organophosphorus reagent as a peptide coupling reagent was by Yamada *et al.* in 1972.[195] Yamada and co-workers prepared diphenylphosphoryl azide (DPPA, **216**) by treatment of diphenylphosphorochloridate with sodium azide. Reaction of a carboxylic acid with an organophosphorus reagent (e.g. DEPC, **217**) generates a mixed carboxylic and phosphoric anhydride intermediate (**219**), which exhibits superior regioselectivity towards nucleophilic attack than the corresponding mixed anhydride **202** (Scheme 68).[196] An alternative mechanism is that the displaced leaving group (azide, chloride or pentafluorophenoxide for reagents **216**, **217** and **218** respectively) could attack intermediate **219**, forming another activated species which is in turn attacked by the amine nucleophile to afford the product (as in Scheme 67).



216 **217** **218**

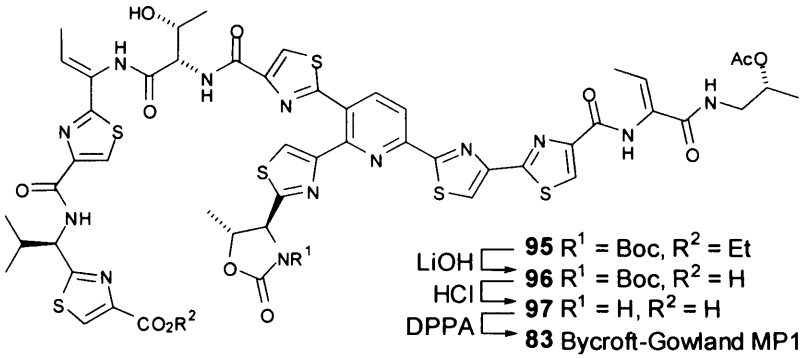


219

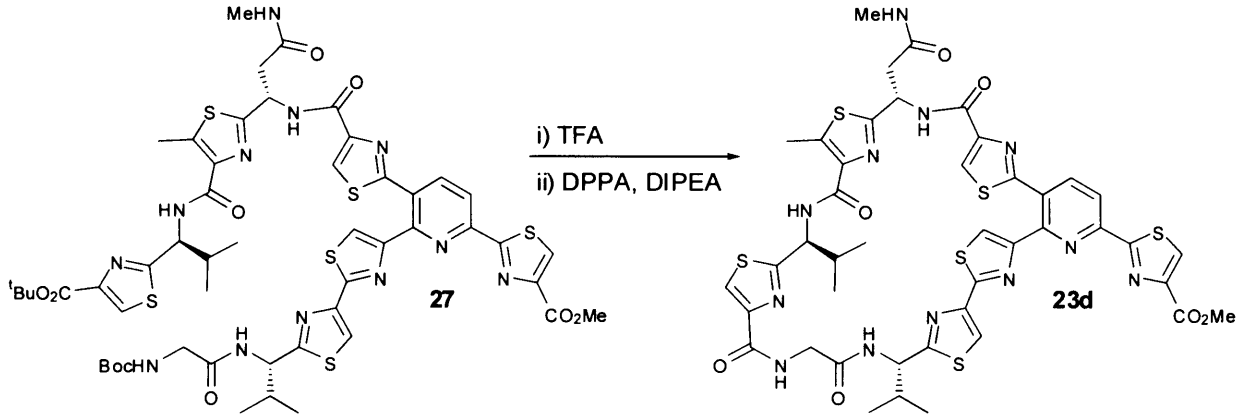
Scheme 68 Organophosphorus peptide coupling reagents

Both Ciufolini's synthesis of Bycroft-Gowland micrococcin P1 and Moody's synthesis of amythiamicin D utilised DPPA in their macrocyclisation steps. In Ciufolini's synthesis of BG-MP1 (**83**),[132] DPPA-mediated macrolactamisation was the final step in the total synthesis, proceeding in 65% yield over 3 steps from precursor **95** (Scheme 69).

Macrolactamisation was also the final step in the synthesis of amythiamicin D (**23d**).[75] *N*- and C-terminus deprotection of precursor **27** followed by treatment with DPPA and diisopropylethylamine furnished the natural product in excellent yield (73%) over two steps (Scheme 70).

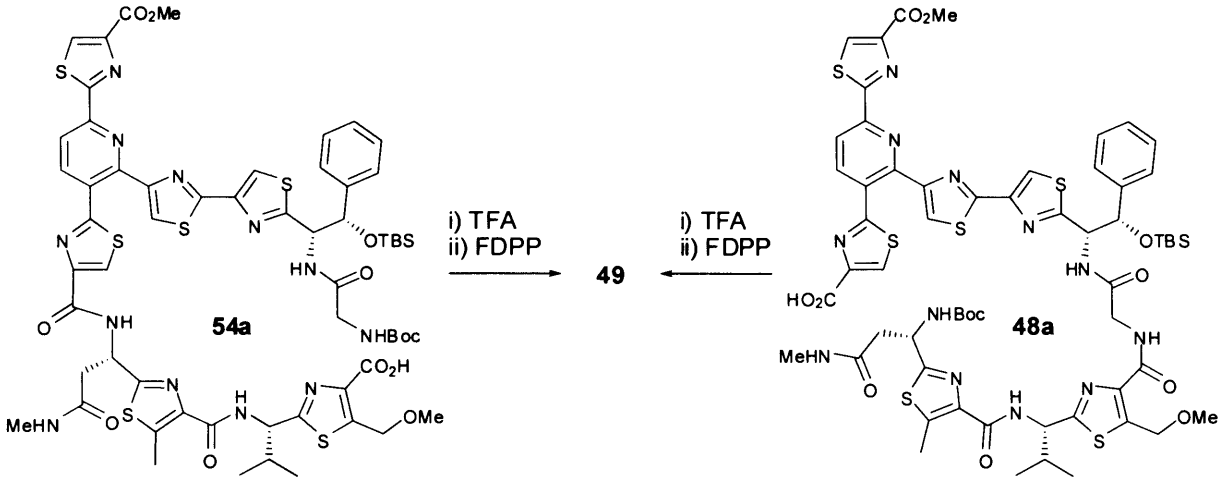


Scheme 69 Synthesis of the Bycroft-Gowland MP1 macrocycle



Scheme 70 Synthesis of the amythiamicin D macrocycle

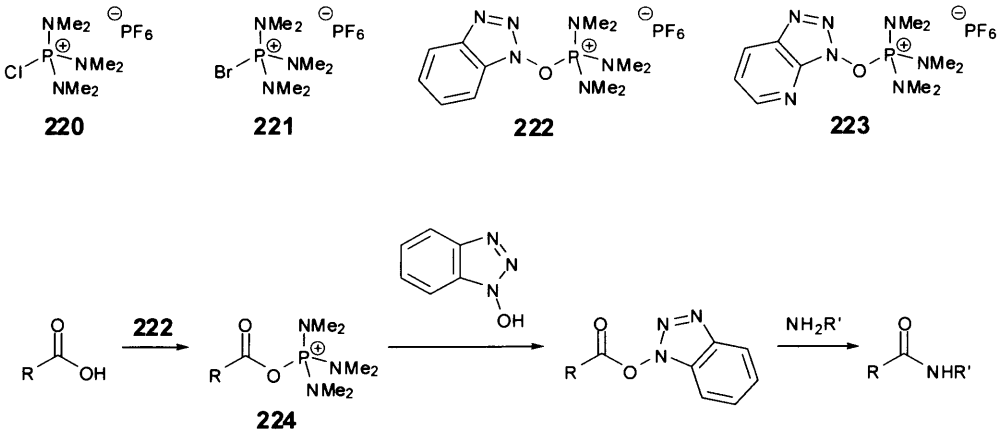
Pentafluorophenyl diphenylphosphinate (FDPP, **218**) was developed in 1991 by Chen and Xu[197] and has found many applications to date, most notably in macrolactamisation reactions which are notoriously difficult to perform in high yield. Indeed, FDPP was used as the macrolactamisation reagent in the synthesis of the macrocyclic precursor (**49**) to GE2270A and GE2270T.[98] Nicolaou *et al.* prepared macrocycle **49** from both **48a** and **54a** following Boc-deprotection and subsequent treatment of the linear peptides with FDPP at high dilution in DMF (Scheme 71).



Scheme 71 Macrolactamisation approaches to GE2270A and GE2270T

5.1.6 – Phosphonium reagents

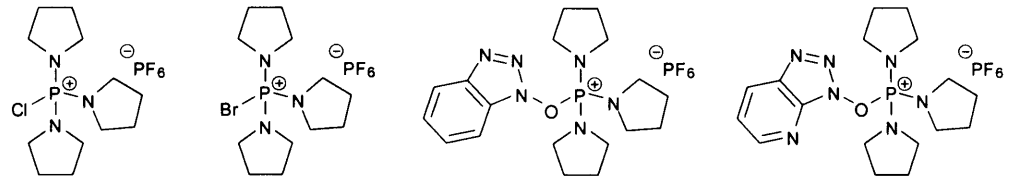
Castro introduced the first phosphonium reagents (ClоP, **220** and BroP, **221**) in the early 1970s.[198,199] Amino acids coupled using ClоP and BroP tended to exhibit significant degrees of racemisation and these reagents were soon superseded by the combined ClоP-HOBt and ClоP-HOAt reagents, BOP (**222**) and AOP (**223**). These reagents contain an inbuilt HOBt or HOAt nucleophile which reacts with intermediate **224** before racemisation can occur (Scheme 72; see also section 5.1.3).[200]



Scheme 72 Early phosphonium reagents

All of the phosphonium reagents **220-223** possess one major drawback, namely the generation of extremely toxic and carcinogenic HMPA as a side product. For this reason, pyrrolidine analogues PyClоP (**225**), PyBroP (**226**), PyBOP® (**227**) and PyAOP (**228**) were introduced.[201] PyBOP® in particular is widely used in peptide synthesis due to ease of handling, few side

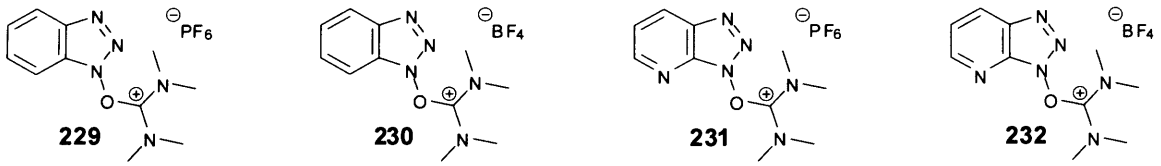
reactions, the racemisation-suppressing capabilities of the HOBt nucleophile and formation of non-toxic by-products.



225 **226** **227** **228**

5.1.7 – Uronium reagents

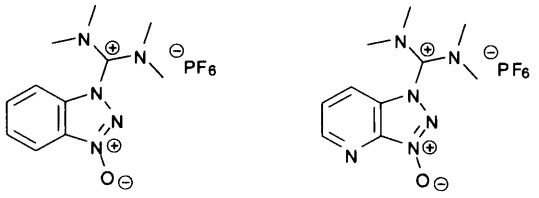
The so-called uronium salts are amongst the most popular peptide coupling reagents in use today, functioning well in a wide range of applications including coupling of sterically hindered residues, macrolactamisation and even solid phase peptide synthesis.[189,202] The first uronium reagent was *O*-(benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU, **229**), introduced by Gross *et al.* in 1978.[203] Since then, a range of analogues have been prepared, including the tetrafluoroborate anion analogue (TBTU, **230**) and the HOAt analogues HATU (**231**) and TATU (**232**). It has since been demonstrated that the non-nucleophilic counterion has no effect on either reaction rate or degree of racemisation.[204]



229 O-HBTU **230** O-TBTU **231** O-HATU **232** O-TATU

Figure 22 Initially proposed uronium structures for HBTU and its analogues

The structure of HBTU was initially assigned as the uronium salt **229** by analogy with the phosphonium salts such as BOP (**222**). HATU was synthesised in 1993 and assigned the analogous structure (**231**).[205] However, further studies indicated that the true structures in both the crystalline state[206] and in solution were in fact guanidinium salts (N-HBTU, **233** and N-HATU, **234**, Figure 23).



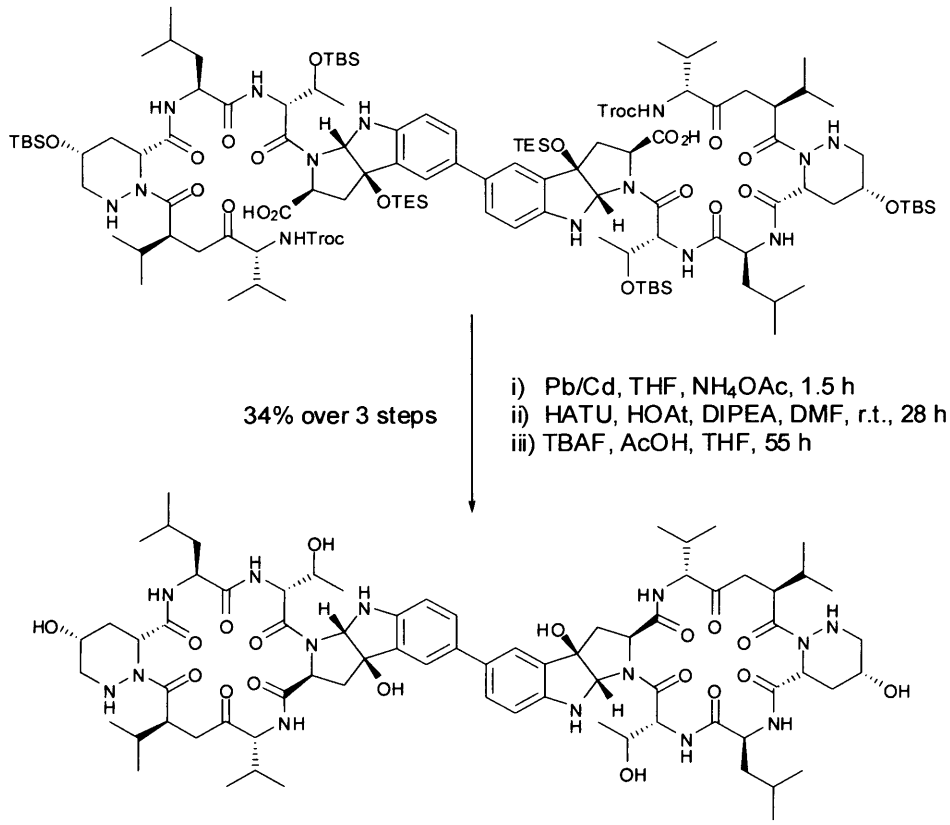
233 N-HBTU

234 N-HATU

Figure 23 Guanidinium structures of HBTU and HATU

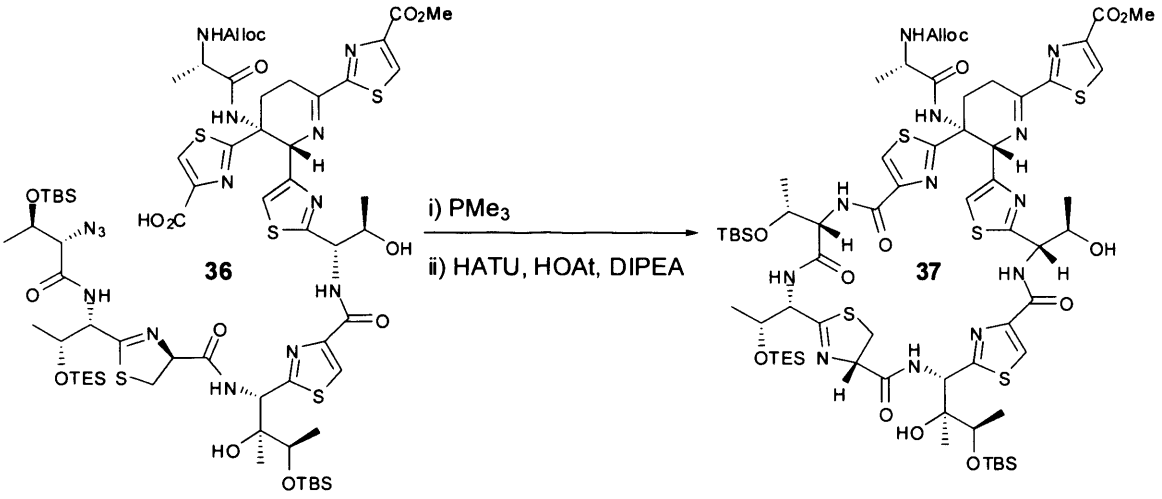
Preparation of the originally postulated uronium salts of HBTU and HATU (O-HBTU and O-HATU, Figure 22) revealed spectroscopic discrepancies with commercially available N-HBTU and N-HATU, as well as reactivity differences, confirming the true structures as being the those of the guanidinium species (Figure 23).[207] Despite this revelation, the acronyms HBTU and HATU remain in use and these reagents are still frequently referred to as uronium salts.

HATU is widely regarded as the best peptide coupling reagent prepared to date, performing exceptionally well in macrolactamisation reactions.[202,208] This superiority is illustrated by the use of HATU to simultaneously form two peptide macrocycles in Danishefsky's synthesis of hemastatin (Scheme 73).[209]



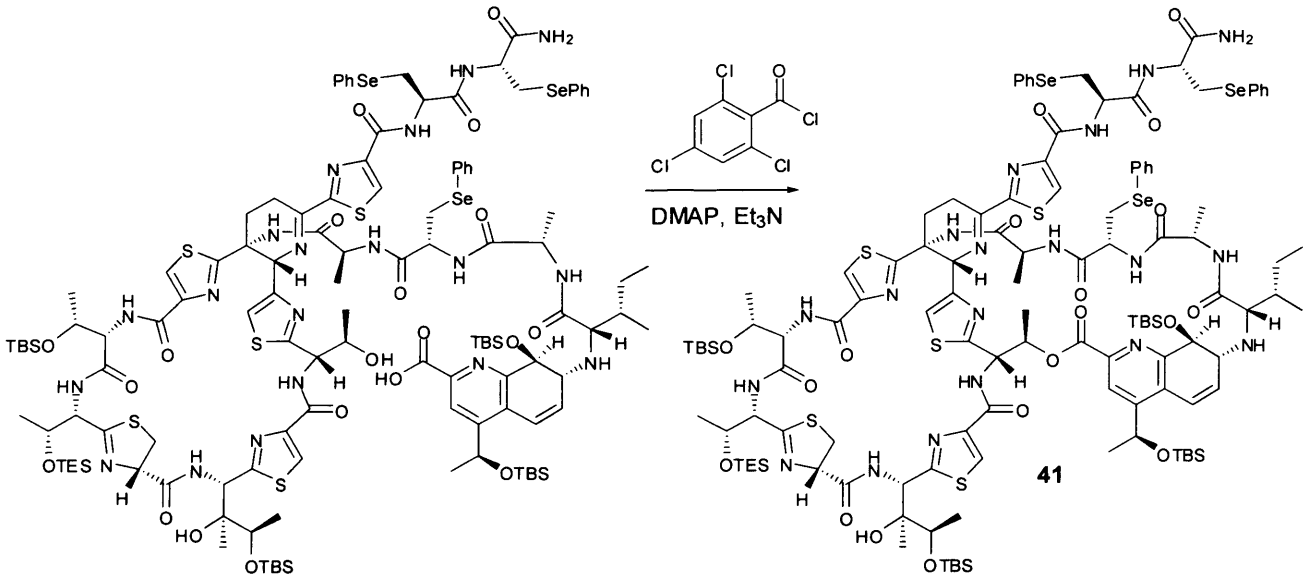
Scheme 73 Use of HATU in the bis-macrocyclisation step of Danishefsky's hemastatin synthesis

Thiostrepton (**1**) possesses two macrocycles, the first of which was cyclised using HATU in Nicolaou's total synthesis of **1**.[89] Staudinger reaction of **36** followed by treatment of the product with HATU, HOAt and diisopropylethylamine, furnished macrocycle **37** in 32% yield from **36** (Scheme 74).



Scheme 74 Formation of the first thiostrepton macrocycle

The second thiostrepton macrocycle was closed using the Yamaguchi macrolactonisation protocol, affording advanced intermediate **41** (Scheme 75).[210]



Scheme 75 Formation of the second thiostrepton macrocycle

Both of the macrocycles of siomycin A (**68a**) were closed using HATU-mediated peptide couplings (Figure 24). The closure of the second macrocycle was conducted in the presence of dipeptide fragment **77** and an excess of HATU, enabling macrolactamisation and attachment of the side chain to occur in one pot (section 1.4.5, Scheme 12).[107]

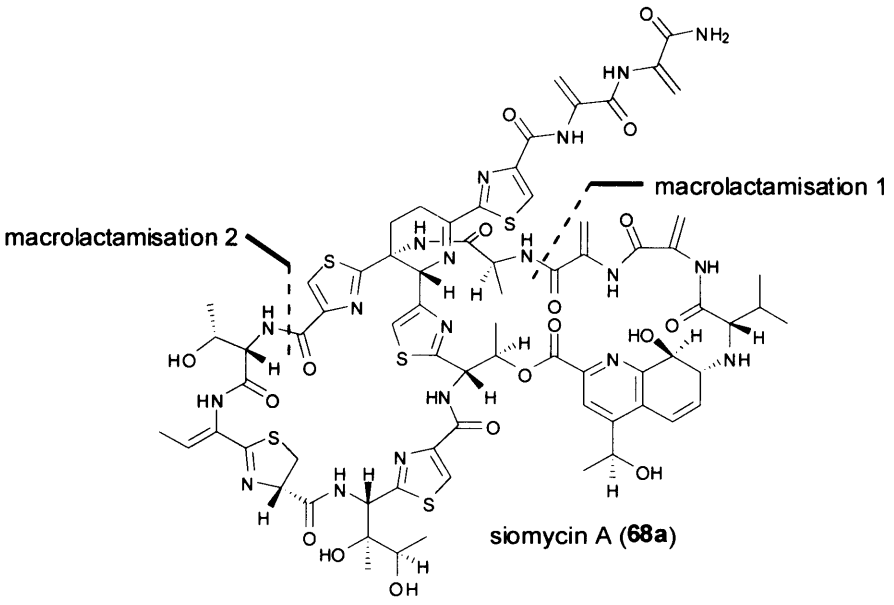


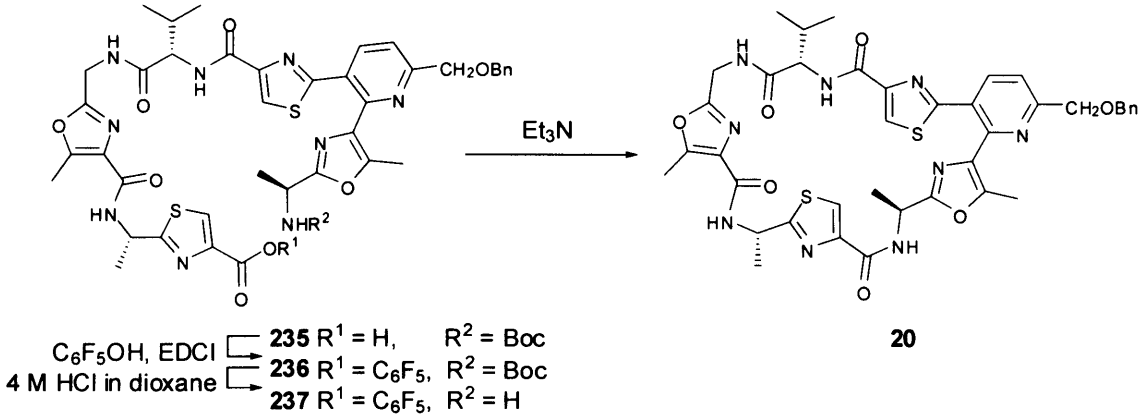
Figure 24 Positions of HATU-mediated macrolactamisations in the synthesis of siomycin A

5.1.8 – Alternative macrocyclisation strategies used in thiopeptide synthesis

While the majority of the thiopeptide total syntheses completed to date make use of peptide coupling reagents discussed in the preceding sections to form the macrocycle(s), there are exceptions to this. The promothiocin A macrocycle was prepared using the Schmidt protocol, and Bach's synthesis of GE2270A features an intramolecular Stille reaction.

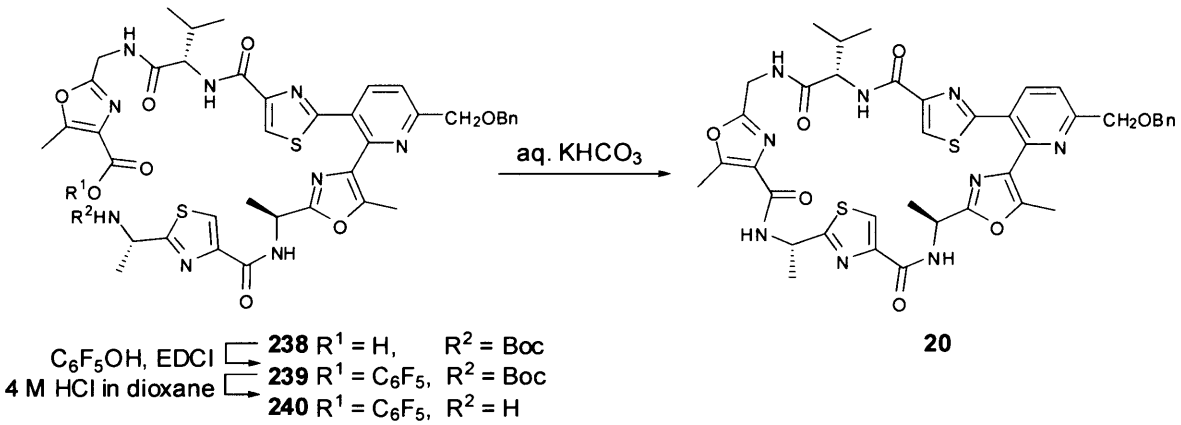
5.1.8.1 – The Schmidt protocol

Moody and Bagley's total synthesis of promothiocin A (**15a**) employed the three-step Schmidt macrolactamisation protocol[211] which had previously been used in their successful total synthesis of (+)-nostocyclamide.[162] The first step in this method is conversion of the linear peptide precursor **235** into the activated pentafluorophenyl ester **236** by coupling with pentafluorophenol in the presence of EDCI (Scheme 76). Boc-deprotection of the resulting peptide **236** followed by neutralisation with potassium hydrogen carbonate and subsequent basification with triethylamine furnished macrocycle **20** in good yield (55%) from **236**.[66]



Scheme 76 Formation of the promothiocin A macrocycle

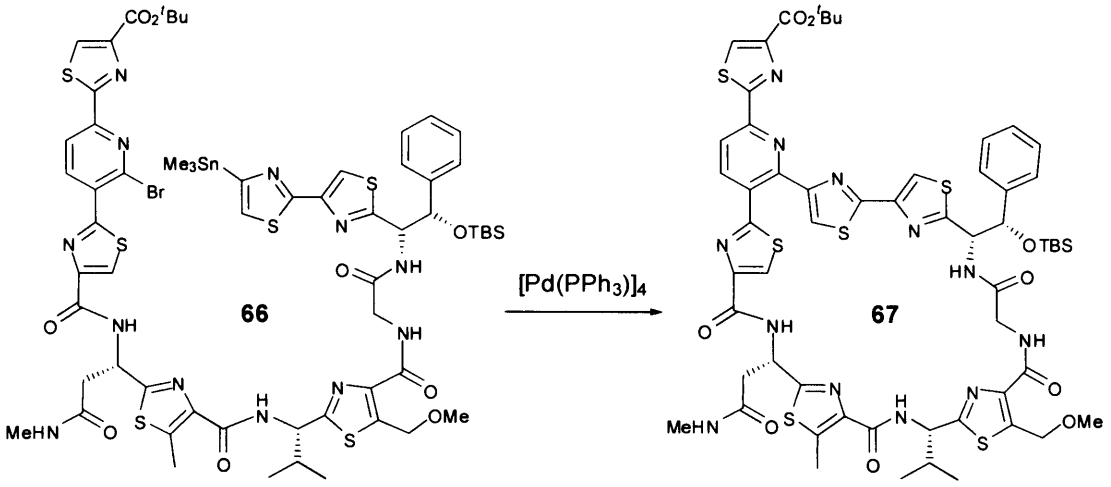
An alternative route to the promothiocin A macrocycle **20** was also successfully realised by Moody *et al.* utilising the same Schmidt protocol (Scheme 77).[67] Cyclisation in this position proved more efficient, proceeding in excellent yield (71% overall from **238**).



Scheme 77 Alternative promothiocin A macrolactamisation strategy

5.1.8.2 – Intramolecular Stille coupling

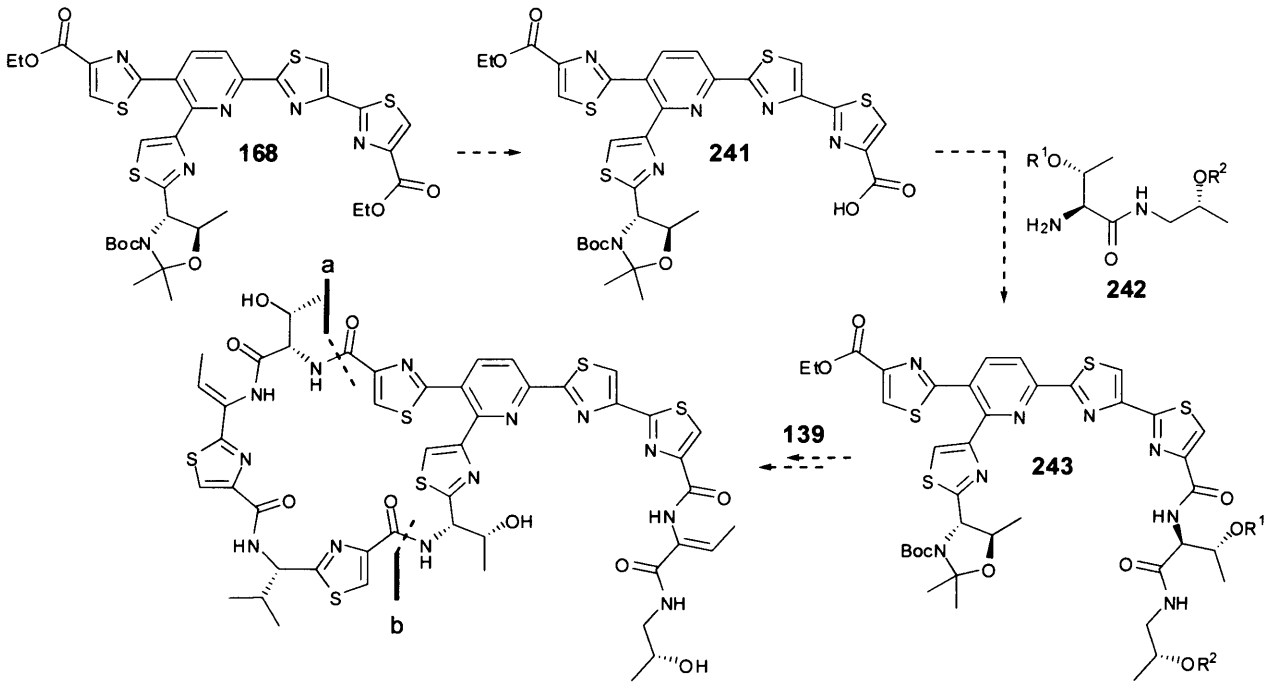
In keeping with their unique approach to GE2270A *via* metal-catalysed coupling of thiazole moieties to a central pyridine core,[99] Bach *et al.* made use of an elegant intramolecular Stille coupling reaction[212] to form macrocycle **67** from linear precursor **66** in excellent yield (75%).



Scheme 78 Intramolecular Stille macrocyclisation in Bach's synthesis of GE2270A

5.2 – Deprotection of core domain 168

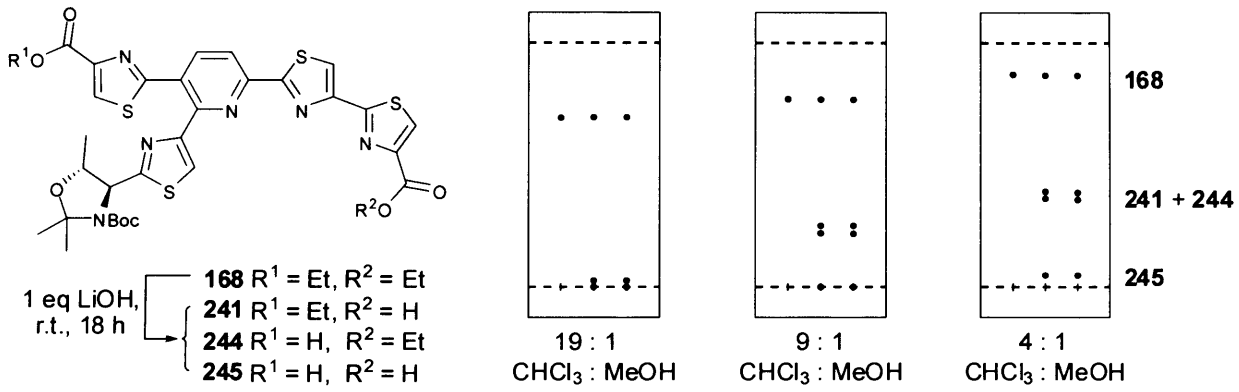
Our initial synthetic route to micrococcin P1 is outlined in Scheme 79. Based on the discovery that the ester in the 6-position of pyridine **171** was readily hydrolysed, whereas the hydrolysis of the ester in the 3-position required harsh reaction conditions (section 4.7.2) it was hoped that despite the additional thiazoles in the core domain, selective ester hydrolysis would still be possible. This would permit coupling of the resulting core domain monoacid (**241**) with a side chain fragment (**242**), forming advanced intermediate **243**, and permitting two possible macrolactamisation positions (a and b), depending on whether the backbone segment **139** was attached to the *N*- or C- terminus of **243**.



Scheme 79 Proposed route to MP1 *via* core-side chain coupling

5.2.1 – Ester hydrolysis with lithium hydroxide

Our initial attempt at the selective hydrolysis of the 6-position ester of **168** used one equivalent of lithium hydroxide in methanol-water. It was hoped that the use of a single equivalent of base would minimise any hydrolysis of the more sterically hindered 3-ester. Monitoring of this reaction by TLC was straightforward due to the bright blue fluorescence exhibited by the core domain chromophore under ultraviolet irradiation at both 254 nm and 360 nm. The reaction appeared sluggish, starting material remaining after 18 hours. However, on changing to a more polar TLC solvent system, it became apparent that the remaining starting material was due to partial hydrolysis of both the 3- and 6-esters, consuming all of the base added (Scheme 80). The two isomeric mono-acids formed (**241** and **244**) proved to be inseparable by chromatography, prohibiting the isolation of the desired product.



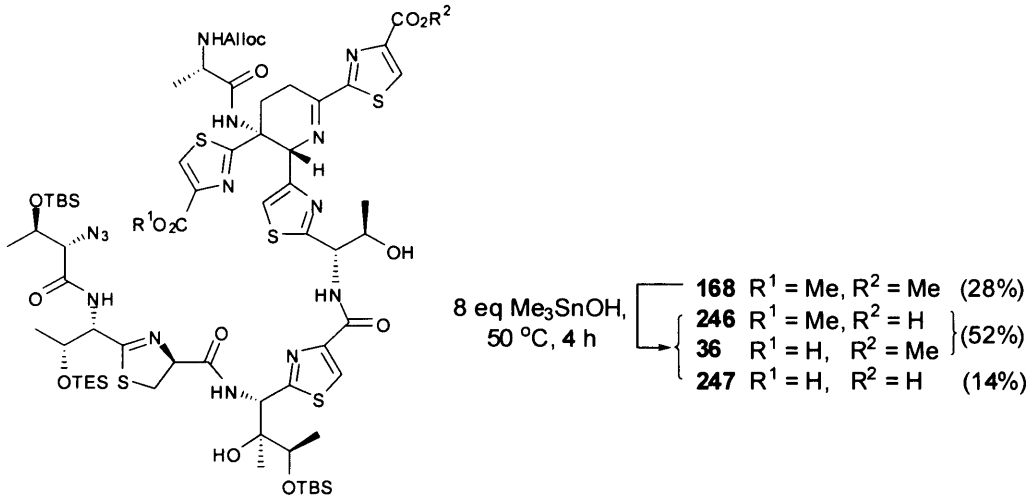
Scheme 80 TLC analysis of the reaction between **168** and one equivalent of lithium hydroxide

The same result was also observed on conducting the reaction in THF-water, **168**, **241**, **244** and **245** all present in the reaction mixture even after two weeks. Treatment of **168** with two or more equivalents of lithium hydroxide led to the formation of bis-acid **245** as the sole reaction product.

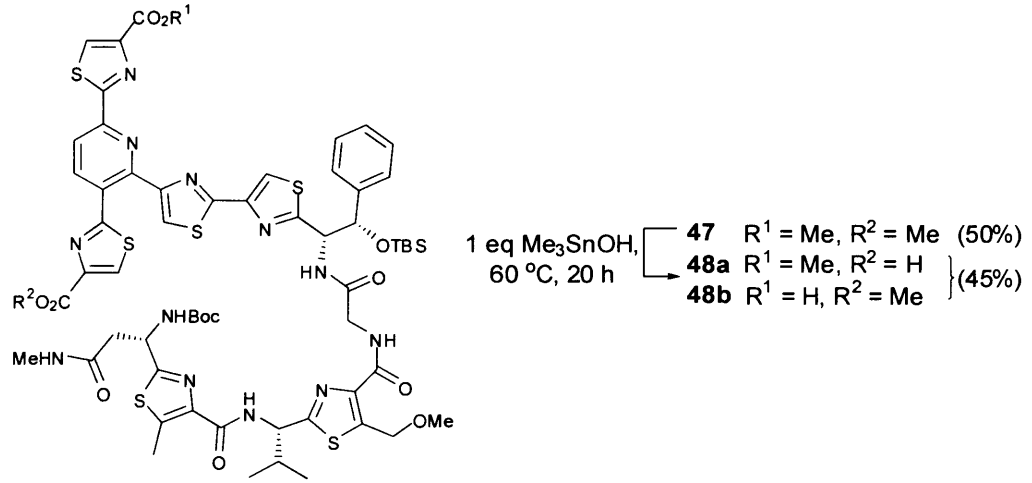
5.2.2 – Ester hydrolysis with trimethyltin hydroxide

Following the lack of selectivity between the 3- and 6-esters when lithium hydroxide was used to perform the hydrolysis, an alternative reagent was sought. In their syntheses of thiostrepton, GE2270A and GE2270E, Nicolaou *et al.* employed trimethyltin hydroxide to hydrolyse esters, finding it to be less harsh than the more traditional lithium hydroxide.[213] In both the thiostrepton synthesis (Scheme 81), and the GE2270 syntheses (Scheme 82), an intermediate bis-ester was formed which necessitated selective ester hydrolysis prior to macrocyclisation. In neither case was selective hydrolysis achieved, a mixture of mono-acids, starting material and bis-acid resulting in all cases. Ultimately, this lack of selectivity did not hamper the success of the

syntheses, as only the desired macrocycles were formed, the unwanted mono-acids (**246** and **48b**) failing to cyclise.

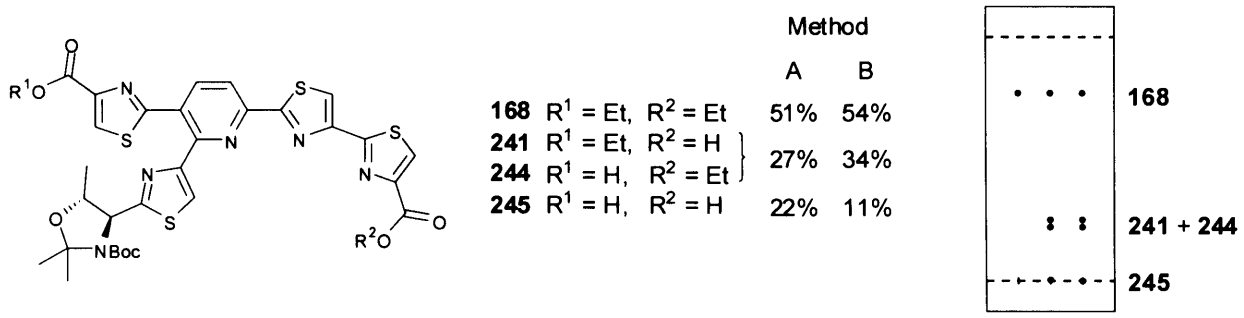


Scheme 81 Attempted selective ester hydrolysis during Nicolaou's thiostrepton synthesis



Scheme 82 Attempted selective ester hydrolysis during Nicolaou's GE2270A and E syntheses

Trimethyltin hydroxide also failed to discriminate between the 3- and 6-esters in **168**, returning a mixture of compounds similar to that observed by Nicolaou and co-workers (Scheme 83). Addition of two equivalents of trimethyltin hydroxide to a solution of **168** at 50 °C at hourly intervals (method A) resulted in 51% recovery of **168**, a 27% yield of the mixed mono-acids **241** and **244**, and 22% conversion to bis-acid **245**. Prolonged reaction with 2 equivalents of trimethyltin hydroxide at 50 °C (method B) afforded a mixture of **241** and **244** in 34% yield with 11% bis-acid **245**, the remainder of the material isolated being starting bis-ester **168**. Repetition of method B using 1.2 equivalents of trimethyltin hydroxide afforded a similar mixture of components.



Method A: Me_3SnOH (2 eq), 1,2-DCE, 50 °C; then Me_3SnOH (2 eq) added after 1 h, 2 h, 3 h, stirred for 1 h more.
Method B: Me_3SnOH (2 eq), 1,2-DCE, 50 °C, 4 days

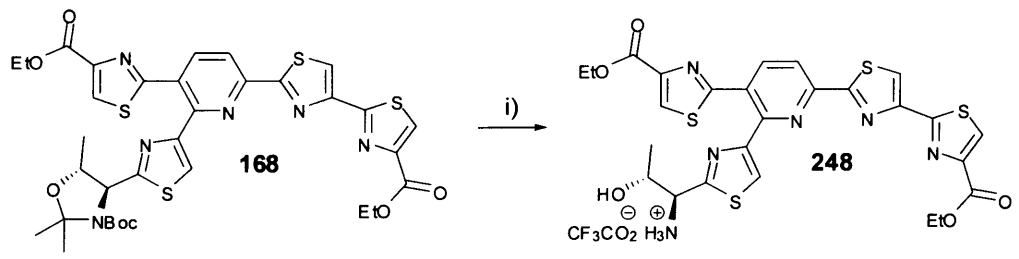
Scheme 83 Ester hydrolysis using trimethyltin hydroxide

All attempts at coupling either the mixture of esters (**241** and **244**) or bis-acid **245** with various side chain fragments (section 5.5) failed to yield the desired product (**243**). Separation of the numerous products by chromatography proved impossible, and analysis of the crude reaction mixtures by mass spectrometry confirmed that no coupling had occurred. Faced with a lack of selectivity between the esters and our inability to couple even the bis-acid with side chain fragments (**242**), the proposed synthetic route to micrococcin P1 had to be revised.

5.2.3 – *N*-terminus deprotection of 168

Our alternative approach to MP1 is illustrated in Figure 25. This route begins with coupling of *N*-deprotected central heterocyclic domain **248** and *C*-deprotected backbone pentapeptide **249** to furnish intermediate peptide **250**. Due to the aforementioned issues with selective ester hydrolysis, both the 3-and 6-esters would then be cleaved, followed by *N*-terminus deprotection. Based on the literature precedent established by Nicolaou's syntheses of thiostrepton and the GE2270 thiopeptides, it was postulated that the linear peptide would cyclise to form only the desired macrocycle **251** which could then be elaborated to the natural product.

N-deprotection of **168** proved to be unusually facile, furnishing **248** in good yield (Scheme 84).



Reagents and Condtions: i) TFA, CH_2Cl_2, r.t., 1 h, 75%

Scheme 84 *N*-deprotection of **168**

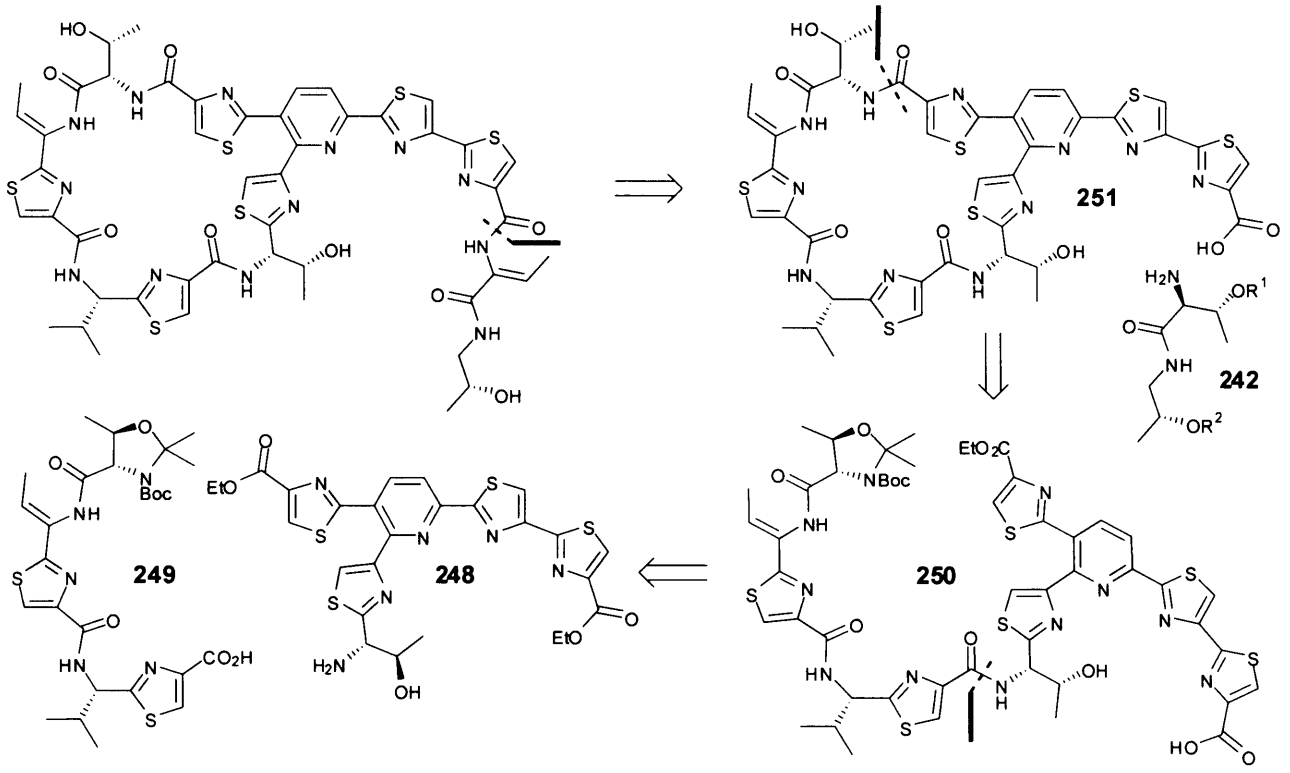


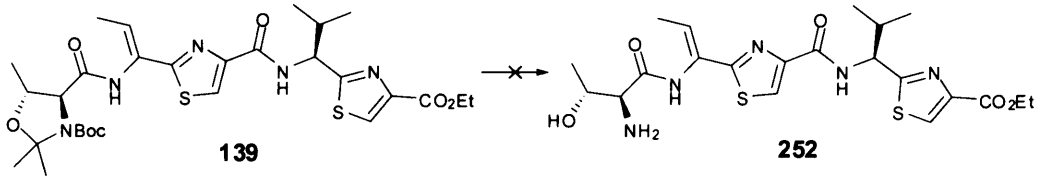
Figure 25 Revised disconnection of micrococcin P1

5.3 – Deprotection of backbone peptide 139

5.3.1 – Literature methods for *N*-deprotection

Had the initial synthetic approach to the core-side chain fragment of micrococcin (**243**, Scheme 79) proved successful, then coupling with backbone pentapeptide **139** could have taken place at two possible sites (Scheme 79), requiring either or *C*- or *N*-deprotection of **139**.

The difficulties in *N*-deprotection of backbone precursor **143** using Ciufolini's method[132] have already been discussed (section 3.4.2). It was hoped that eliminated peptide **139** would undergo deprotection more readily (Scheme 85). Use of aqueous hydrochloric acid failed to effect deprotection, as did treatment with aqueous hydrochloric acid followed by TFA, both methods resulting in the disappearance of most of the material, likely due to the formation of water-soluble fragments which were lost during aqueous work up. Following the facile *N*-deprotection of core domain **168** using a 1:1 solution of TFA and dichloromethane, it was hoped that these conditions would be applicable to substrate **139**, but as with all other attempts, an intractable mixture of products was formed.

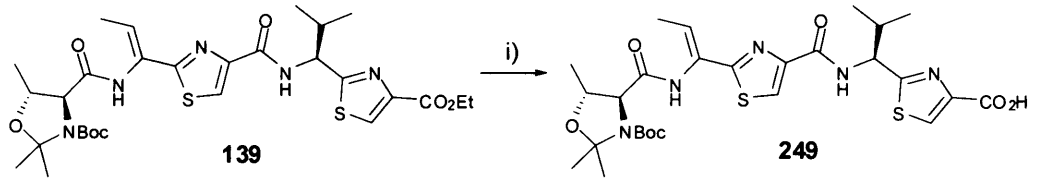


Scheme 85 Failed *N*-deprotection of pentapeptide **139**

Fortunately, due to the failure of the selective ester hydrolysis of core domain **168**, *N*-deprotection of **139** was no longer necessary, and C-deprotection was investigated instead.

5.3.2 – *C*-terminus deprotection

Gratifyingly, *C*-terminus deprotection of pentapeptide **139** proved to be both facile and reliable, with carboxylic acid **249** formed in excellent yield (Scheme 86) in readiness for coupling with core domain **248**.



Reagents and Conditions i) LiOH, MeOH-H_2O, r.t., 16 h, 89%.

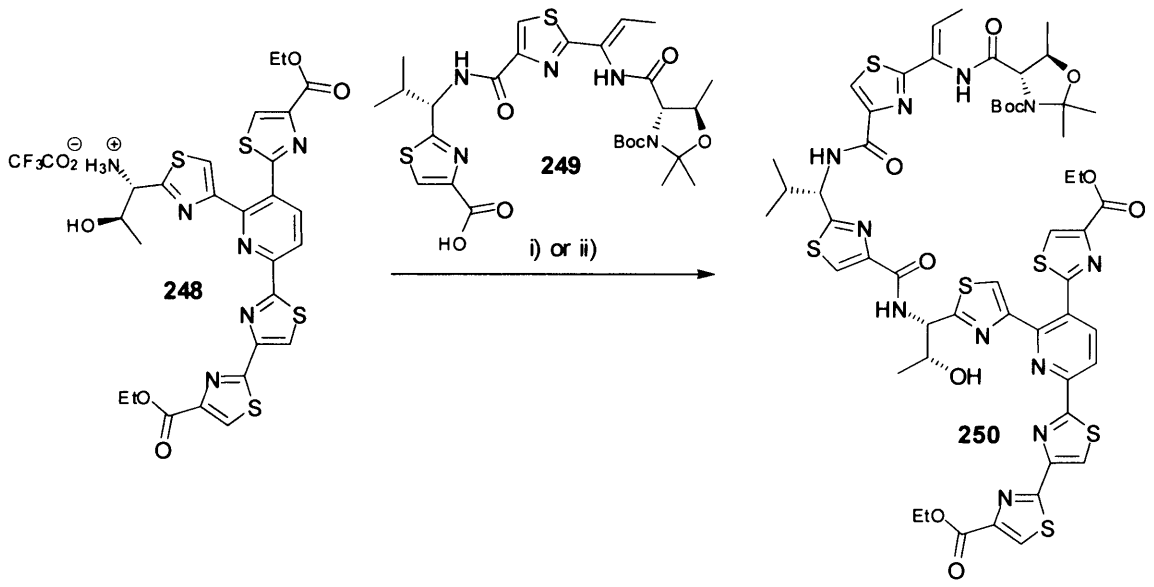
Scheme 86 *C*-terminus deprotection of backbone peptide **139**

5.4 – Construction of macrocycle 251

With both the central heterocyclic domain and the peptide backbone segment successfully deprotected, the stage was set for the assembly of the micrococcin thiopeptide macrocycle (**251**).

5.4.1 – Coupling of core domain 248 and backbone peptide 249

The preliminary attempt at coupling fragments **248** and **249** was made using PyBOP® (**227**) as this reagent had already been used successfully in the synthesis of peptide **143** (section 3.4.1, Scheme 39). Addition of a solution of **248** and triethylamine in dichloromethane to a solution of **249** and PyBOP® in dichloromethane, followed by stirring overnight at room temperature afforded the desired product **250** in 33% yield (Scheme 87).



Reagents and Conditions i) **249**, PyBOP®, CH_2Cl_2, 0 °C, 5 min; then **248**, Et_3N, CH_2Cl_2, r.t., 16 h, 33%; ii) **249**, DIPEA, HATU, CH_2Cl_2, r.t., 1 h; then **248**, DIPEA, CH_2Cl_2, r.t., 3.5 h, 77%

Scheme 87 Synthesis of intermediate **250**

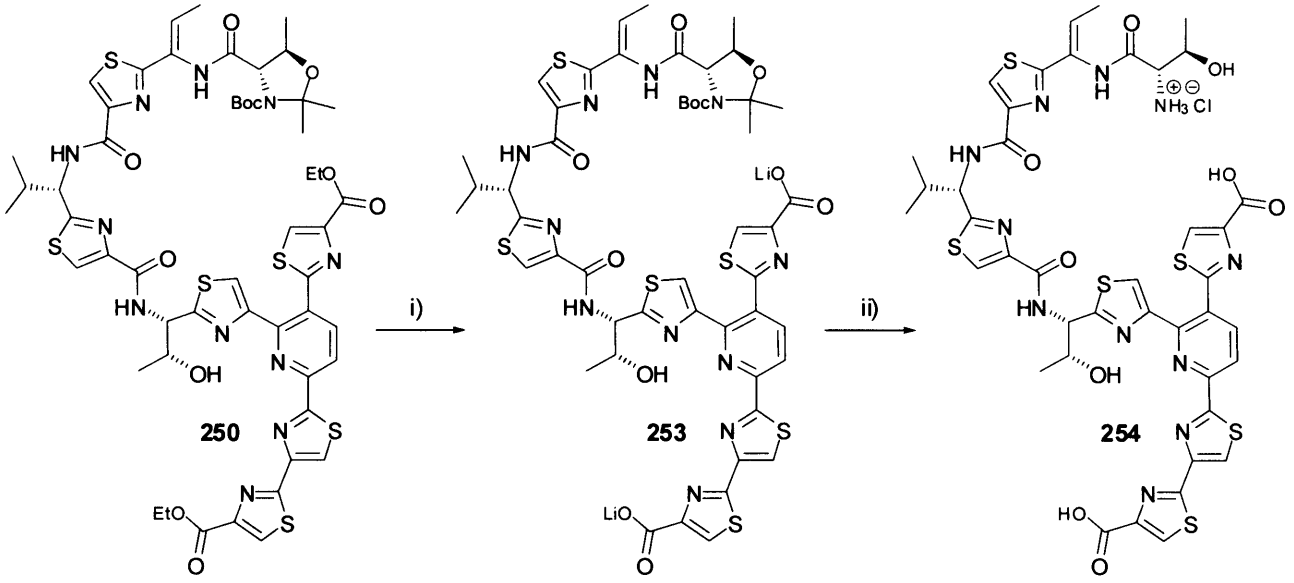
Repetition of this reaction on the same scale (~40 mg of **249**) using HATU as the coupling reagent increased the yield dramatically to 77%. Although consistently more efficient than PyBOP®, the yield of the HATU-mediated coupling ranged between 50-77% when scaled up to between 40 mg and 130 mg of **249**. However, on two occasions, coupling failed completely, returning an inseparable mixture of compounds, probably containing both starting materials, HATU, the tetramethylguanidinium derivative of **248**, HOAt, and numerous other species, none of which were the reaction product.

5.4.2 – Deprotection of fragment 250

Despite the occasional failure of the coupling of **248** and **249**, sufficient quantities of advanced intermediate **250** were obtained to continue with the synthesis of macrocycle **251** (Scheme 88). It was known from the earlier investigation into ester hydrolysis (sections 5.2.1 and 5.2.2) that both the 3- and 6-positions could be readily deprotected, and lithium hydroxide was the reagent of choice owing to the high toxicity of trimethyltin hydroxide. Accordingly, **250** was stirred at room temperature with 10 equivalents of lithium hydroxide for 14 hours. Isolation of the product proved difficult, and it was decided that on completion of the hydrolysis, the reaction mixture would be evaporated and not purified prior to *N*-deprotection in the next step, the lithium salt being protonated to form the acid under the *N*-deprotection conditions.

Following the difficulties in *N*-deprotection of peptides **143** and **139**, it was with some trepidation that the next step was approached. Fortunately, deprotection was effected using HCl-saturated

ethyl acetate (see section 5.5.4), although the success of the reaction was difficult to verify. Bis-acid **253** was insoluble in ethyl acetate, but on sonication formed a fine, pale yellow precipitate. Addition of HCl-ethyl acetate resulted in a colour change to give a bright yellow solid, which was insoluble in all solvents with the exception of DMF and DMSO. This prevented analysis of compound **254** by mass spectrometry, and due to the scarcity of material, NMR analysis in d_6-DMSO was not undertaken.



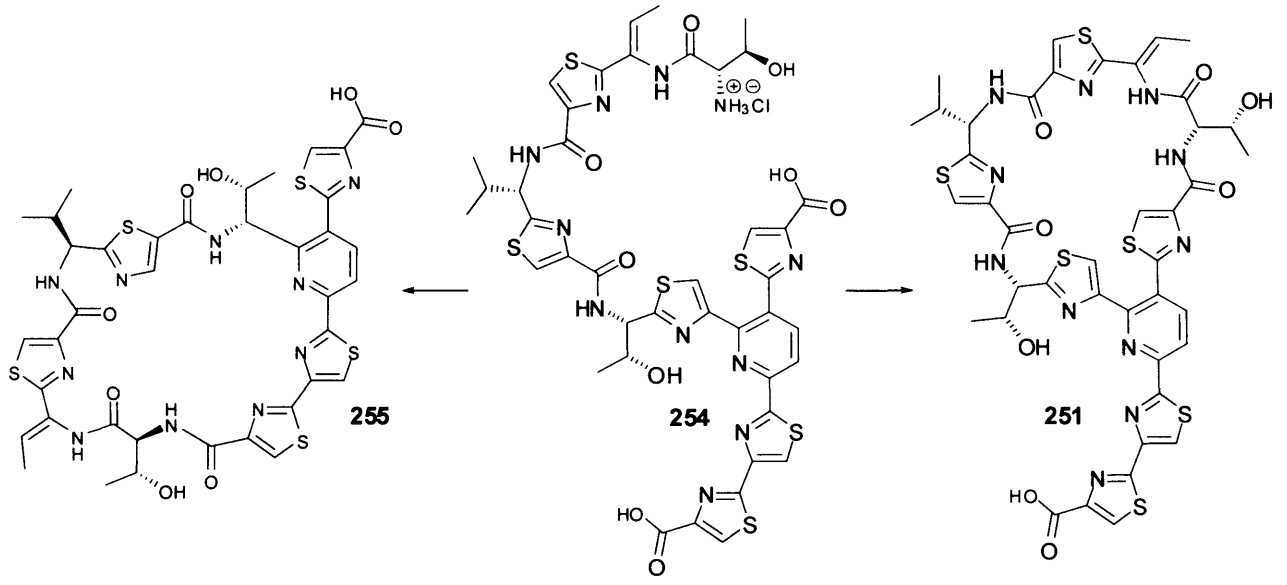
Reagents and Conditions i) LiOH, MeOH-H_2O, r.t., 14 h; ii) HCl-EtOAc, EtOAc, r.t., 1 h.

Scheme 88 Deprotection of fragment **250**

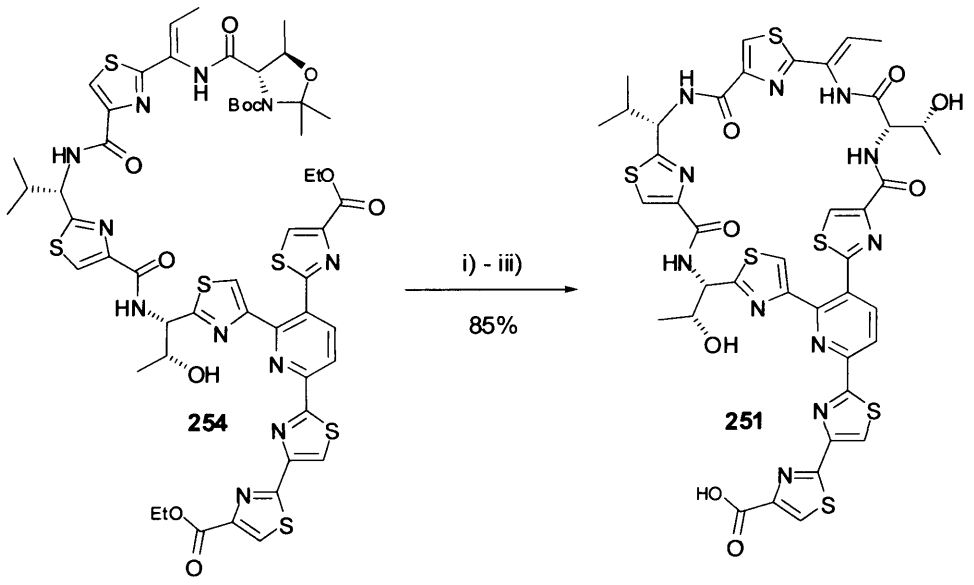
5.4.3 – Macrolactamisation

Based on literature precedent, it was hoped that precursor **254** would cyclise to form macrocycle **251**, and not **255** (Scheme 89). In their synthesis of the GE2270 macrocycle, Nicolaou *et al.* used FDPP to effect cyclisation, and obtained only the correct macrocycle. For this reason, cyclisation of **254** with FDPP was investigated. Monitoring of the reaction proved difficult due to the extremely polar nature of the starting material. Proton NMR analysis of the crude mixture following evaporation of the solvent revealed only FDPP. As no product was observed, it was decided that HATU would be used as the macrolactamisation reagent, based on its successful use in the syntheses of thiostrepton and siomycin A, and also in the coupling of fragments **248** and **249**. A solution of **254**, diisopropylethylamine and HATU in DMF at high dilution (1 mM) was stirred at room temperature for 45 hours, then evaporated *in vacuo*. Analysis of the reaction mixture by mass spectrometry showed a peak at m/z 1004 (Appendix 1), corresponding to the $[M+H]^+$ of macrocycle **251**. The residue was triturated with ethyl acetate, leaving an off-white solid. In order to remove the HATU and base, the solid was partitioned between chloroform and 5% acetic acid solution. A pale brown solid remained between the organic and aqueous layers,

which was subsequently found to be the macrocycle, formed in excellent yield (85% over three steps, Scheme 90). Unfortunately, the macrocycle was insoluble in all solvents except DMSO, DMF and pyridine, making characterisation and verification of its identity as either **251** or **255** impossible. It was hoped, as found in the syntheses of thiostrepton and siomycin A, that the identity of the macrocycle would be confirmed following elaboration to the natural product and spectroscopic comparison with natural material.



Scheme 89 Possible macrocycles formed from linear precursor **254**

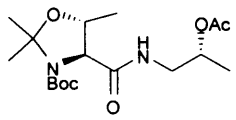


Reagents and Conditions i) LiOH, MeOH-H_2O, r.t., 14 h; ii) HCl-EtOAc, EtOAc, r.t., 1 h; iii) HATU, DIPEA, DMF, r.t., 24 h, 85% (3 steps)

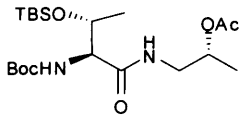
Scheme 90 Synthesis of macrocycle **251**

5.5 – Synthesis of the MP1 side chain

The dipeptide side chain of micrococcin P1 was synthesised adorned with different combinations of protecting groups in order to allow greater flexibility once attached to macrocycle **251** for elaboration to the natural product.



256



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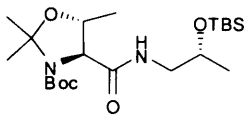
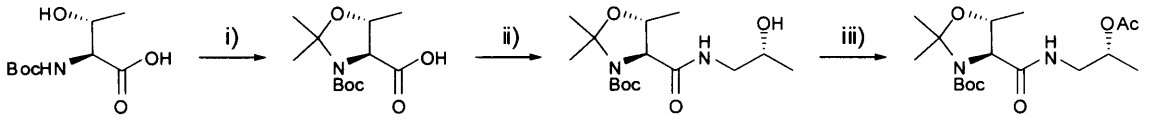


258

5.5.1 – Synthesis of side chain fragment 256

Protected dipeptide **256** was synthesised from *N*-Boc-(2*S*,3*R*)-threonine in three steps and 98% overall yield (Scheme 91).



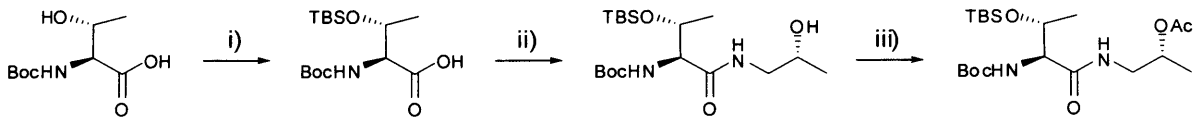
135 **259** **256**

Reagents and Conditions: i) 2,2-DMP, PPTS, THF, reflux, 6 h, 100%; ii) (*R*)-1-amino-2-propanol, PyBOP®, Et_3N, CH_2Cl_2, r.t., 18 h, 100%; iii) Ac_2O, pyridine, r.t., 48 h, 98%.

Scheme 91 Synthesis of side chain fragment **256**

5.5.2 – Synthesis of side chain fragment 257

Protected dipeptide **257** was synthesised from *N*-Boc-(2*S*,3*R*)-threonine in three steps and 55% overall yield (Scheme 92). TBDMS-protection of threonine was achieved using TBDMS-triflate in dichloromethane, followed by treatment with aqueous base to hydrolyse the intermediate silyl ester to the desired carboxylic acid **260**.



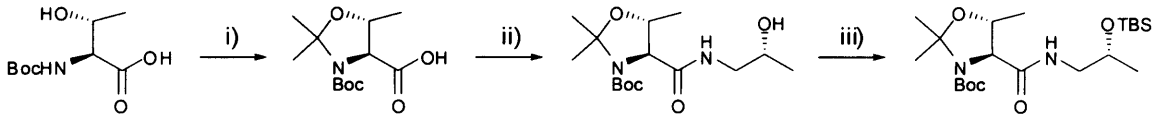
260 **261** **257**

Reagents and Conditions: i) TBDMSOTf, Et_3N, CH_2Cl_2, r.t., 2 h; then K_2CO_3, MeOH-H_2O, r.t., 2 h, 100%; ii) (*R*)-1-amino-2-propanol, PyBOP®, Et_3N, CH_2Cl_2, r.t., 18 h, 65%; iii) Ac_2O, pyridine, r.t., 48 h, 84%.

Scheme 92 Synthesis of side chain fragment **257**

5.5.3 – Synthesis of side chain fragment 258

Side chain peptide **258** was synthesised in three steps and 98% overall yield from *N*-Boc-(2*S*,3*R*)-threonine (Scheme 93), *via* intermediate **259** which had previously been used to prepare fragment **256**.



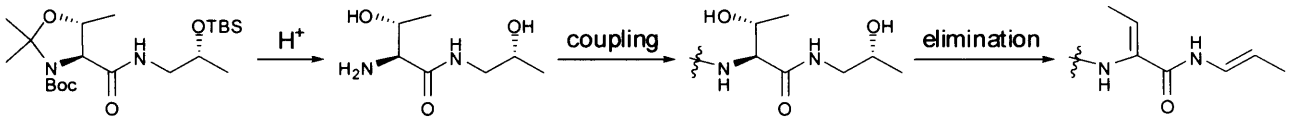
135 **259** **258**

Reagents and Conditions: i) 2,2-DMP, PPTS, THF, reflux, 6 h, 100%; ii) (*R*)-1-amino-2-propanol, PyBOP®, Et_3N, CH_2Cl_2, r.t., 18 h, 100%; iii) Imidazole, DMAP, TBDMSCl, DMF, 36 h, 98%.

Scheme 93 Synthesis of side chain fragment **258**

5.5.4 – Selective removal of Boc in the presence of TBDMS

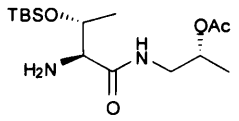
Side chain fragments **257** and **258** contain *tert*-butoxycarbonyl and *tert*-butyldimethylsilyl protecting groups, both of which are acid-labile. Prior to coupling with the micrococcin macrocycle, Boc-deprotection is necessary, but this must be conducted in such a way that the TBDMS group remains intact. Failure to do so would result in a fragment containing two unprotected secondary alcohols (**262**), thereby preventing selective elimination of the threonine residue to the dehydrodemethylvaline unit present in the natural product.



258 **262**

Scheme 94 Problems caused by accidental TBDMS deprotection.

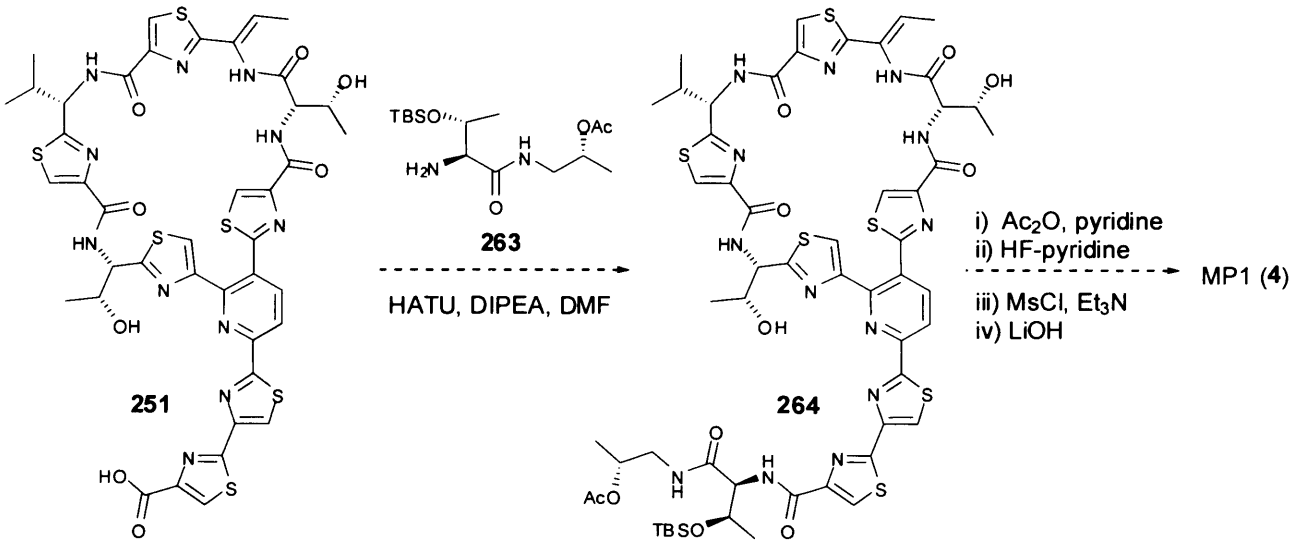
Fortunately, a detailed investigation into the selective *N*-Boc deprotection in peptides containing TBDMS-protected alcohols had been undertaken by Cavelier and Enjalbal, in which a wide range of reagents and conditions were screened.[214] The most effective reagent was found to be a saturated solution of HCl in ethyl acetate. This reagent was prepared and used in the Boc-deprotection of side chain fragment **257**; analysis of the reaction mixture by mass spectrometry indicated that after 30 minutes at room temperature, the major component of the reaction mixture was the desired product **263** (Appendix Two). This reagent was also successful in *N*-terminus deprotection of macrocycle precursor **253**.



263

5.6 – Elaboration of macrocycle 251 to MP1

With both the micrococcin P1 side chain and the macrocycle in hand, the final approach towards the natural product was undertaken. It was hoped that coupling of macrocycle **251** and fragment **263** (derived from dipeptide **257**) would afford the carbon framework of the natural product (**264**) and that this intermediate could be readily elaborated to micrococcin P1 in four further steps (Scheme 95).



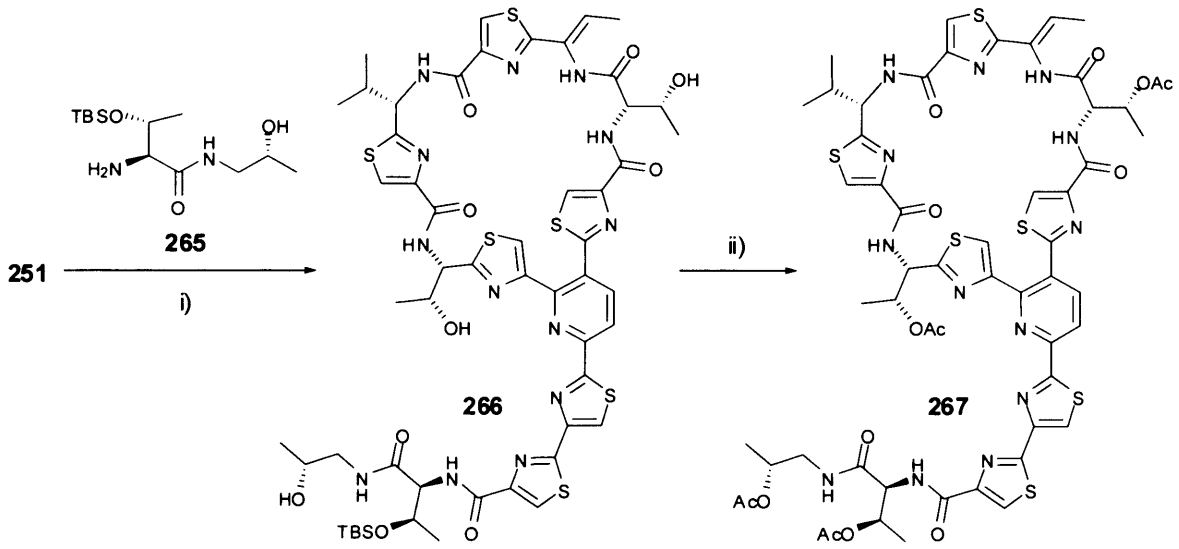
Scheme 95 Initial proposal for the elaboration of **251** to micrococcin P1

Following coupling of the side chain dipeptide, acetate protection of the two free alcohols and removal of the TBDMS group using a fluoride source would afford the substrate for mesylation and elimination. This would install the final dehydrodemethylvaline residue, and subsequent global deprotection of the three remaining acetate groups would furnish the natural product.

5.6.1 – One-pot macrolactamisation and side chain coupling

The first attempt at preparation of intermediate **264** was *via* a one-pot cyclisation-side chain elongation strategy as used by Hashimoto and co-workers in the final stages of their total synthesis of siomycin A.[107] To this end, a solution of macrocycle precursor **254**, side chain fragment **263**, HATU and DIPEA in DMF was stirred at room temperature and the reaction monitored by TLC. The only identifiable product from this reaction was macrocycle **251**, isolated

in 35% yield. Treatment of **251** with HATU, DIPEA and a solution of side chain fragment **265** afforded a mixture of unidentifiable products, which appeared to possess a TBDMS protecting group. It was therefore decided that the products should be subjected to acetate protection using acetic anhydride in pyridine (Scheme 96), in an attempt to improve the solubility and thus aid purification and identification. By ^{1}H-NMR it was determined that these reaction conditions had cleaved the TBDMS protecting group, which would leave all four secondary alcohols protected identically (**267**), preventing successful completion of the total synthesis (Appendix Three).



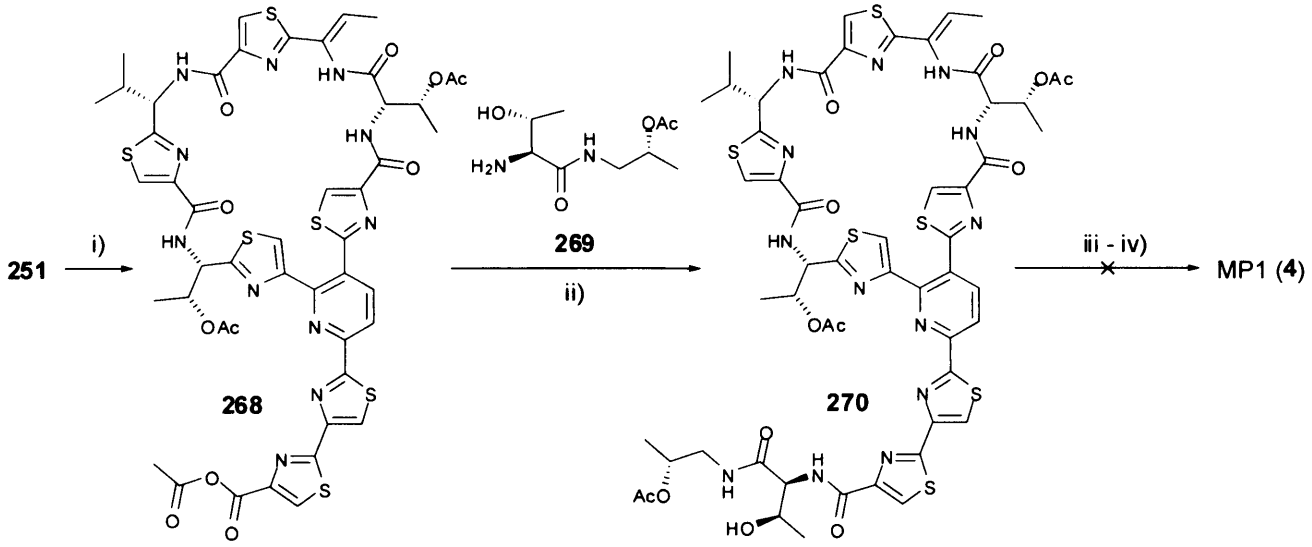
Reagents and Conditions: i) **251**, HATU, DIPEA, DMF, r.t., 1 h; then **265**, DIPEA, DMF, r.t., 72 h; ii) Ac_2O, pyridine, r.t., 62 h.

Scheme 96 Attempted acetate protection of intermediate **266**

5.6.2 – Acetate protection of macrocycle 251

Armed with the knowledge that the conditions used for acetate protection could cleave a TBDMS protecting group, it was decided that macrocycle **251** would be protected prior to coupling with side chain peptide **269** (derived from **256**), the resulting product **270** then requiring only mesylation-elimination and acetate removal to furnish the natural product (Scheme 97). Due to the poor solubility and scarcity of material, verification of the success of acetate protection of **251** by ^{1}H-NMR was not possible. It was hoped that submitting the material to the remaining steps in the synthesis would afford the natural product, thus confirming the identities of all intermediate species. Analysis of the reaction mixture by thin layer chromatography prior to the final deprotection step indicated that there was a single compound present, which exhibited characteristic blue fluorescence under UV irradiation (Figure 26). Encouragingly, this compound had a higher R_f than micrococcin P1, as predicted due to the three hydroxyl groups being protected in proposed structure **271**. Unfortunately, the natural product remained elusive and

was not identified among the mixture of compounds produced on treatment with lithium hydroxide.



Reagents and Conditions: i) **251**, Ac_2O, pyridine, r.t., 24 h; ii) **268**, HATU, DIPEA, DMF, r.t., 1 h; then **269**, DIPEA, DMF, r.t., 24 h; iii) MsCl, Et_3N, CH_2Cl_2, r.t., 1 h; then DBU, $CHCl_3$, r.t., 2 h; iv) $LiOH.H_2O$, $MeOH\text{-}H_2O$, r.t., 12 h.

Scheme 97 Acetate protection of **251** and attempted elaboration to the natural product

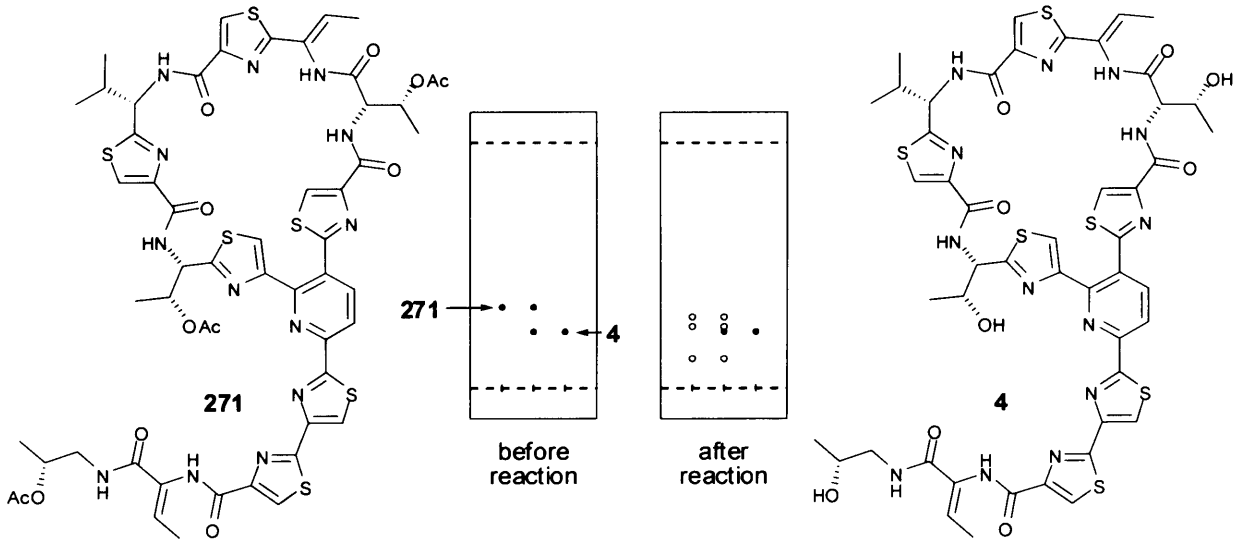
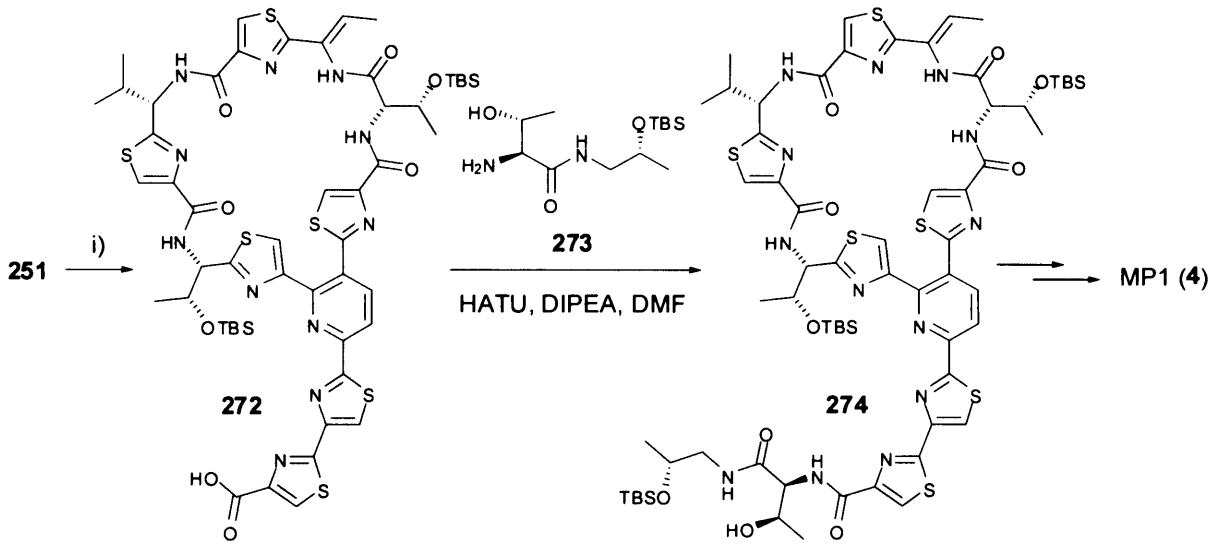


Figure 26 Attempted deprotection of **271** to form micrococcin P1

5.6.3 – TBDMS-protection of macrocycle 251

Following the failure of the acetate-protected macrocycle route (Scheme 97), TBDMS-protection of the alcohols in **251** was investigated. The proposed synthetic route is outlined in Scheme 98.



Reagents and Conditions: i) TBDMSOTf, Et_3N, DMF, r.t., 18 h; or TBDMSOTf, imidazole, DMF, 50 °C, 24 h.

Scheme 98 Proposed synthesis of MP1 *via* TBDMS-protected macrocycle **272**

Treatment of macrocycle **251** with *tert*-butyldimethylsilyl triflate and triethylamine in DMF at room temperature failed to yield **272**, the starting material being recovered in 67% yield. Repetition of the reaction using imidazole in place of triethylamine, and heating the mixture at 50 °C resulted in the apparent decomposition of the macrocycle.

5.7 - Conclusion

The micrococcin thiopeptide macrocycle has been synthesised in 20 steps and 4% overall yield from *N*-Boc-(2*S*,3*R*)-threonine. Three micrococcin P1 side chain dipeptides have been prepared, each bearing different protecting groups, allowing for a range of synthetic strategies in the final stages of the total synthesis.

Chapter Six – Results and Discussion

Reinvestigation of the Stereochemistry of Cyclothiazomycin

6.1 – Introduction

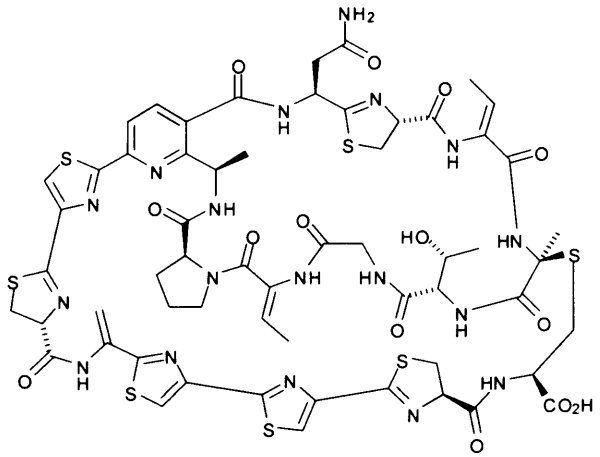
6.2 – Reinvestigation of the Stereochemistry of Cyclothiazomycin

6.3 – Conclusion

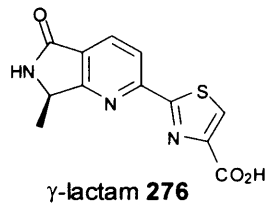
6 – Reinvestigation of the Stereochemistry of Cyclothiazomycin

6.1 – Introduction

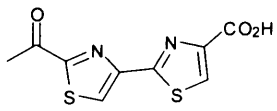
Cyclothiazomycin (**275**) is an unusual series *d* thiopeptide that possesses a number of unique structural features. First isolated from the fermentation broth of *Streptomyces* sp. NR0516 from a soil sample collected at Kanagawa, Japan,[215] initial work towards structure determination used high-resolution FAB mass spectrometry, elemental analysis and ^{13}C and ^{1}H NMR spectroscopy. The spectroscopic data was supported by chemical degradation studies, acidic hydrolysis generating an unusual pyridine-containing γ-amino acid as lactam **276** and saramycetic acid (**277**).[216] NOESY experimental data was used in the elucidation of both the structure and stereochemistry of cyclothiazomycin, the latter supported by amino acid analyses, and showed this unique series *d* thiazolyl peptide lacked the characteristic 2- and 3-azole substituents on the central domain. Cyclothiazomycin was found to possess an alanine-derived heterocyclic residue, reportedly of (*R*)-configuration, quaternary sulfide and two macrocyclic peptide loops (Figure 27).[217]



cyclothiazomycin (**275**)



γ-lactam **276**



saramycetic acid (**277**)

Figure 27 Proposed structure of cyclothiazomycin and its degradation products

Although no antibacterial data has been associated with cyclothiazomycin, which also lacks the characteristic polydehydroalanine side chain implicated in *tipA* promoter activity, this thiopeptide is biologically active, functioning as an inhibitor of human plasma renin, with an IC_{50} of 1.7 μM.

6.1.1 – Chemical degradation of cyclothiazomycin

Acidic hydrolysis of cyclothiazomycin was conducted by heating **275** in 6 M hydrochloric acid at 110 °C for 18 hours. In addition to isolating **276** and **277**, one equivalent each of glycine, L-

threonine, L-proline and L-aspartic acid were isolated, along with four equivalents of L-cysteine, determined by HPLC.[216,217] The optical rotation of γ-lactam **276** was not reported, and the configuration of its alanine-derived stereogenic centre assigned purely on the basis of NOE data.

6.1.2 – Isolation of new cyclothiazomycins

In 2006, Hashimoto and co-workers reported the isolation of two new cyclothiazomycin thiopeptides, named cyclothiazomycin B1 (**278**) and cyclothiazomycin B2 (**279**), from *Streptomyces* sp. A307.[218] Both of these thiopeptides were reported to exhibit antifungal activity, with cyclothiazomycin B1 also acting as a bacterial RNA polymerase inhibitor.

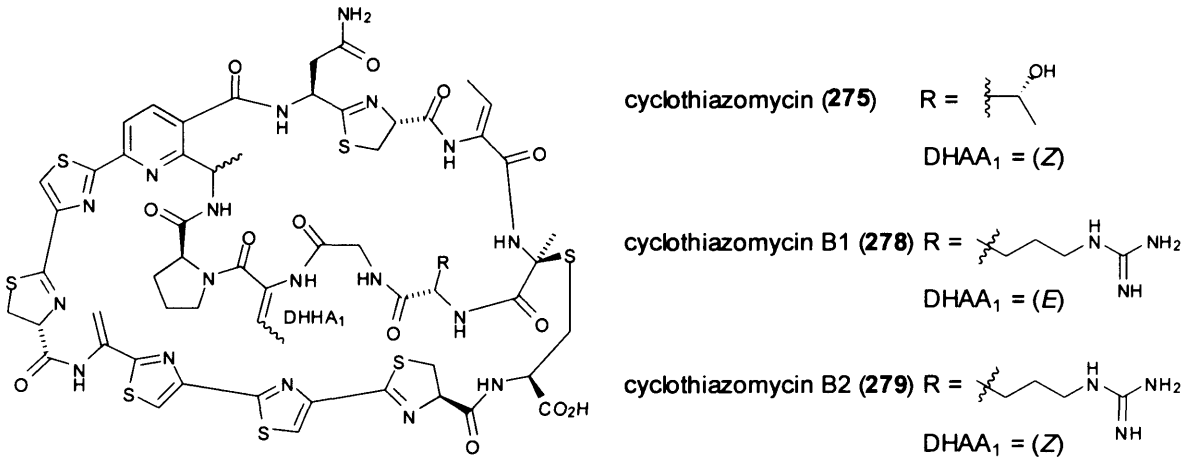


Figure 28 Cyclothiazomycins B1 and B2

Hashimoto *et al.* determined the structures of **278** and **279** using ^{1}H- and ^{13}C-NMR, COSY, NOESY, ROESY and HMBC data in conjunction with tandem MS-MS (MALDI-TOF/TOF) studies. The absolute stereochemical configurations of the amino acids were determined by acidic hydrolysis of the natural products followed by treatment with Marfey's reagent (**109**, section 2.3) and HPLC analysis. Acidic hydrolysis of **278** and **279** under the same conditions employed by Aoki *et al.* in their degradation of cyclothiazomycin **275** afforded saramycetic acid (**277**) and pyridine domain **280** which surprisingly failed to cyclise to lactam **276**. In order to facilitate study by NMR spectroscopy, pyridine bis-acid **280** was esterified to give the dimethyl derivative **281**.

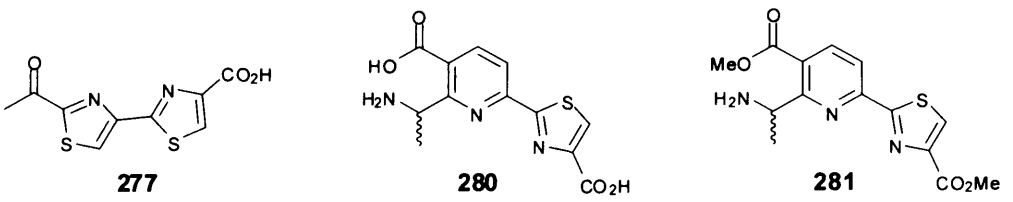
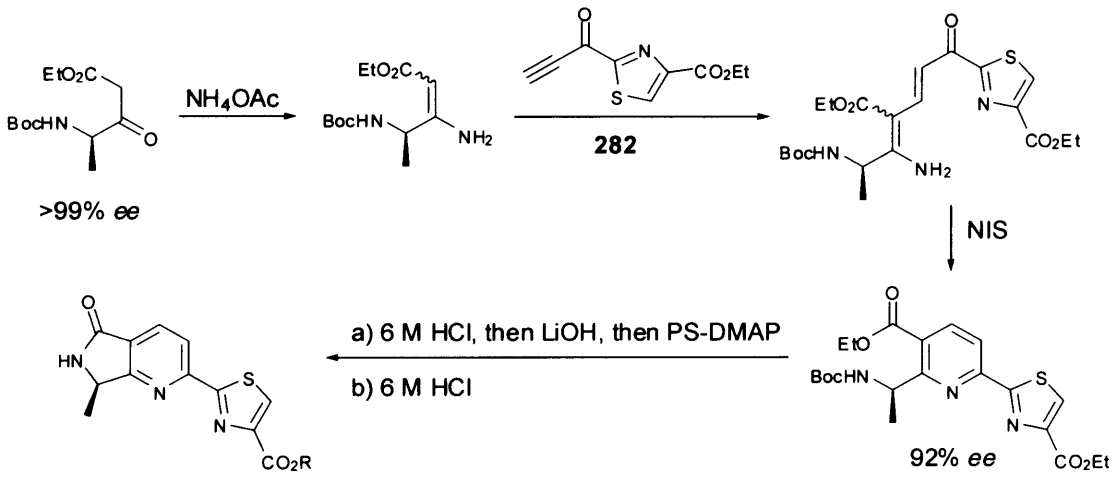


Figure 29 Degradation products from the acidic hydrolysis of cyclothiazomycins B1 and B2

The stereochemistry of the alanine-derived stereogenic centre was not assigned, although it was proposed that the centre should be of (*S*)-configuration, i.e. derived from natural L-alanine. This is at variance with the presently accepted structure of cyclothiazomycin (**275**), and based on our previous findings relating to the valine stereochemistry in the micrococcin thiopeptides, it was deemed worthy of further investigation.

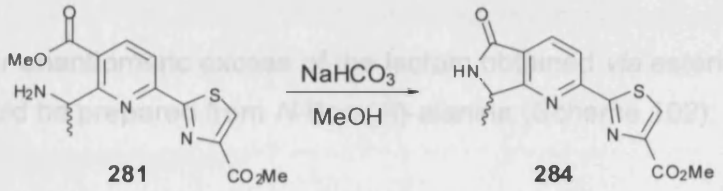
6.2 – Reinvestigation of the stereochemistry of cyclothiazomycin

The synthesis of cyclothiazomycin lactam **276** had previously been completed within our laboratory using the Bohlmann-Rahtz pyridine synthesis (Scheme 99).[143]



Scheme 99 Synthesis of cyclothiazomycin γ-lactam **276**

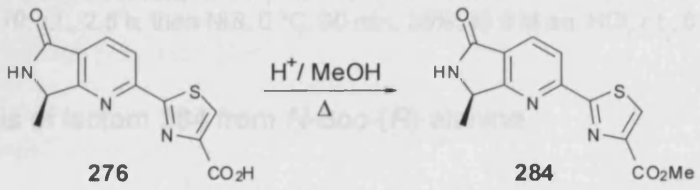
Discussions with Prof. Hashimoto revealed that treatment of pyridine methyl ester **281**, derived from material isolated from the degradation of cyclothiazomycin B1 with sodium hydrogen carbonate in methanol resulted in the formation of lactam **284** (Scheme 100). It was therefore decided that lactam methyl ester **284** would be synthesised *via* the same synthetic route as lactam **276**, using thiazole alkynone **170** (section 4.6.1) in place of **282** (Scheme 99). Deprotection route b) would be employed in order to cleave the *N*-Boc group and form the lactam, leaving the methyl ester intact. Comparison of the material isolated from the natural product with that prepared *via* the route outlined above would permit the determination of the absolute configuration of the alanine residue in the central domain of the cyclothiazomycin thiopeptides.



Scheme 100 Conversion of pyridine **281** into lactam **284**

6.2.1 – Synthesis of lactam 284

Our first synthesis of lactam **284** was achieved by the esterification of a 4 milligram sample of lactam **276** previously prepared in our laboratory (Scheme 101).[iv] Lactam **276** was heated at reflux in methanol with a trace of 6 M hydrochloric acid for three hours. The product was identical to that obtained by Hashimoto *et al.* by ^{1}H-NMR (Appendix Four), but the enantiomeric excess found to be only 6% by HPLC on a chiral stationary phase (Figure 30).[v]



Scheme 101 Preparation of **284** by esterification of **276**

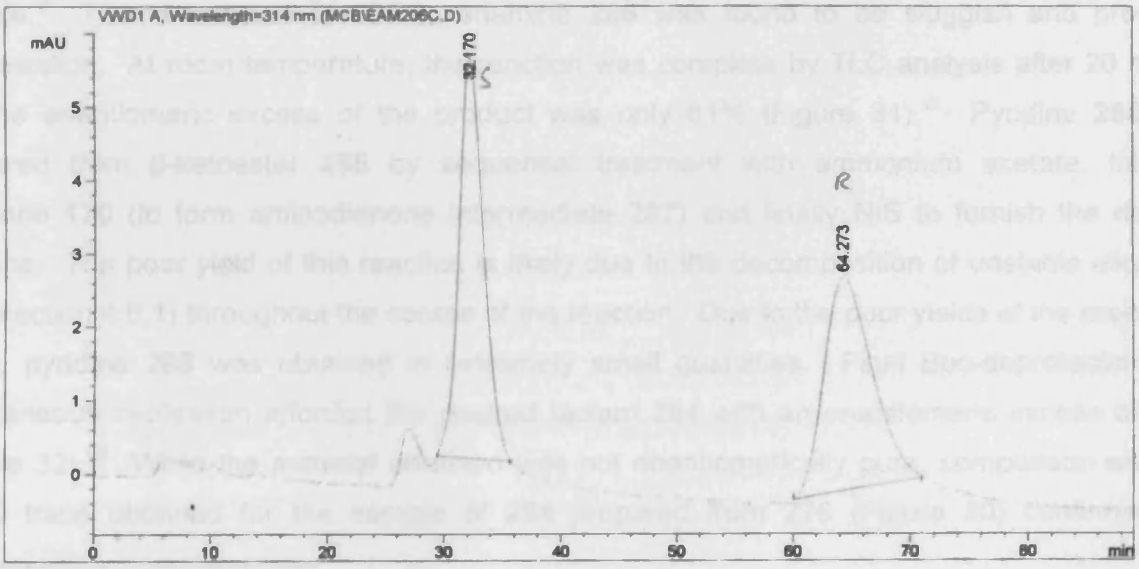
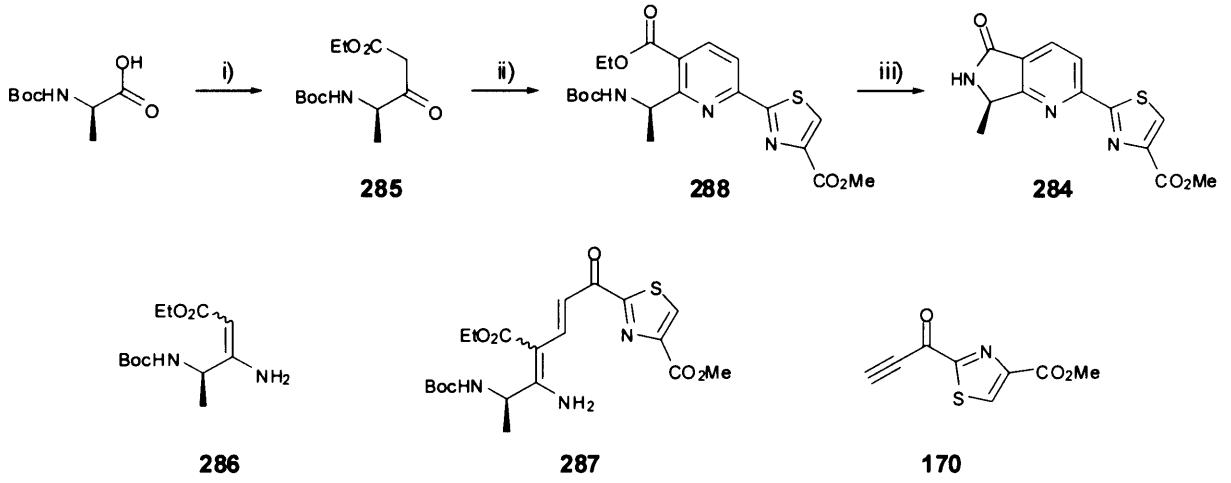


Figure 30 HPLC analysis of lactam **284** prepared by esterification of **276**

[iv] Lactam **276** prepared by Dr. Xin Xiong; for experimental details, see ref. 143

[v] Chiralcel OD column, eluting with *i*-PrOH:hexane (10:90), 1.00 ml min^{-1}, detected at 314 nm, retention times: (*S*)-enantiomer = 32 min, (*R*)-enantiomer = 64 min.

In the light of the poor enantiomeric excess of the lactam obtained *via* esterification of **276**, it was decided that **284** would be prepared from *N*-Boc-(*R*)-alanine (Scheme 102).



Reagents and Conditions: i) EtOCOCl, Et_3N, THF, -10 °C, 30 mins; then LDA, EtOAc, -78 °C, 1 h, 30%; ii) NH_4OAc, EtOH, 50 °C, 20 h; then **170**, r.t., 2.5 h; then NIS, 0 °C, 30 min, 36%; iii) 6 M aq. HCl, r.t., 6 h.

Scheme 102 Synthesis of lactam **284** from *N*-Boc-(*R*)-alanine

Despite its similarity to the route used in the successful synthesis of **276**, the preparation of **284** proved extremely challenging. β-Ketoester **285** was prepared as a single enantiomer from *N*-Boc-(*R*)-alanine in 30% yield by treatment of the mixed anhydride with the lithium enolate of ethyl acetate.[vi] The conversion of **285** to enamine **286** was found to be sluggish and prone to racemisation. At room temperature, the reaction was complete by TLC analysis after 20 hours, but the enantiomeric excess of the product was only 61% (Figure 31).[vii] Pyridine **288** was prepared from β-ketoester **285** by sequential treatment with ammonium acetate, thiazole alkynone **170** (to form aminodienone intermediate **287**) and finally NIS to furnish the desired pyridine. The poor yield of this reaction is likely due to the decomposition of unstable alkynone **170** (section 4.6.1) throughout the course of the reaction. Due to the poor yields of the preceding steps, pyridine **288** was obtained in extremely small quantities. Final Boc-deprotection and spontaneous cyclisation afforded the desired lactam **284** with an enantiomeric excess of 47% (Figure 32).[viii] While the material obtained was not enantiomerically pure, comparison with the HPLC trace obtained for the sample of **284** prepared from **276** (Figure 30) confirmed the

[vi] ChiralPak AD column, eluting with *i*-PrOH:hexane (1:99), 1.00 ml min^{-1}, detected at 244 nm, retention times: (*R*)-enantiomer = 39 min.

[vii] ChiralPak AD column, eluting with *i*-PrOH:hexane (5:95), 1.00 ml min^{-1}, detected at 273 nm, retention times: (*R*)-enantiomer = 16 min, (*S*)-enantiomer = 23 min.

[viii] Chiralcel OD column, eluting with *i*-PrOH:hexane (10:90), 1.00 ml min^{-1}, detected at 314 nm.

retention times of (*S*)- and (*R*)-**284** as 32 and 64 minutes respectively, paving the way for HPLC analysis of the lactam isolated from the natural product.

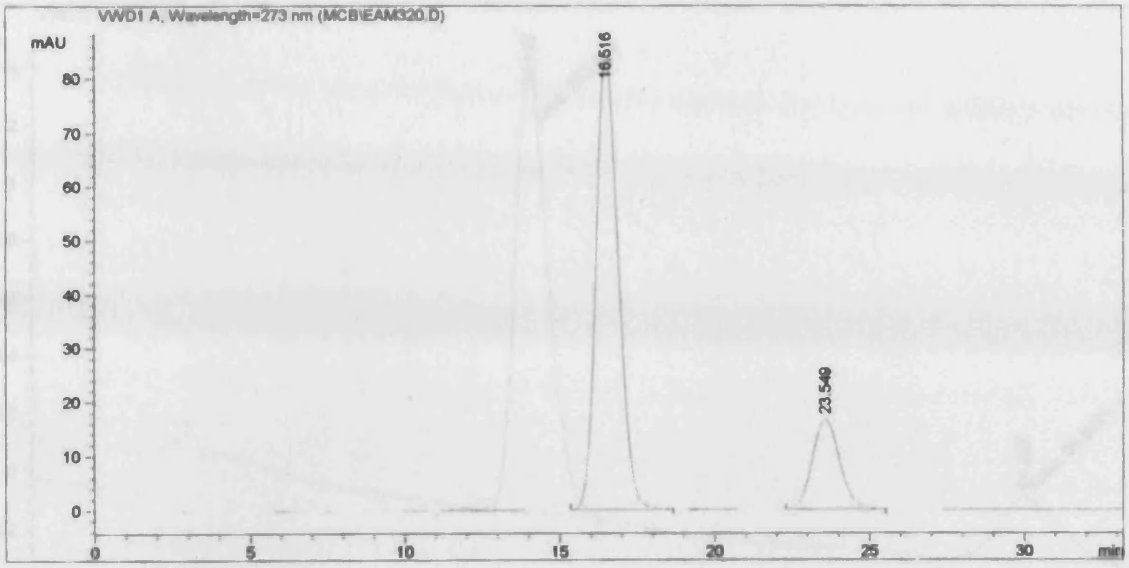


Figure 31 HPLC analysis of enamine **286**

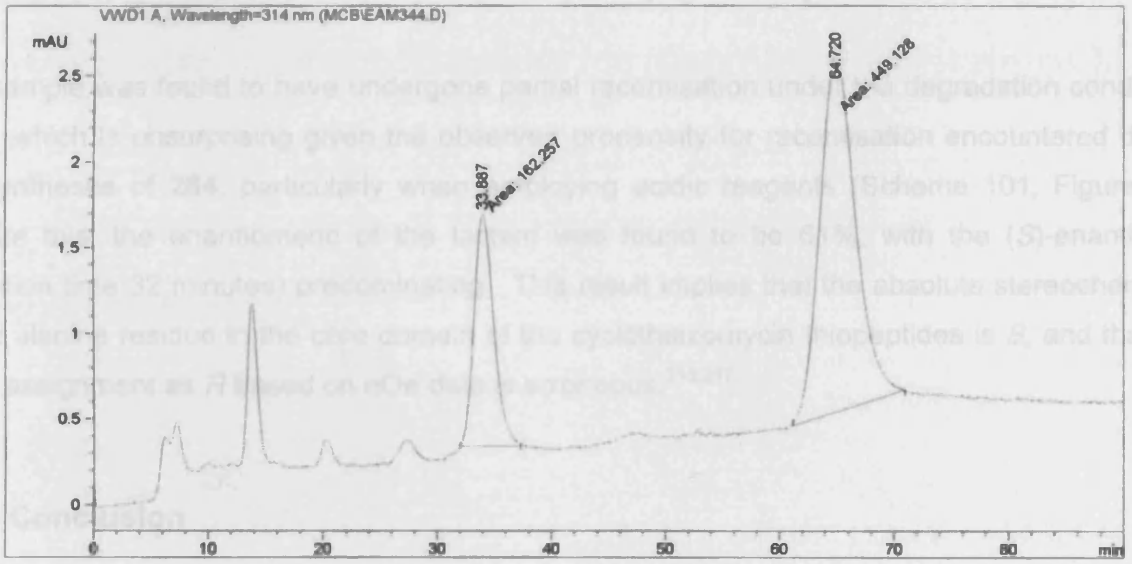


Figure 32 HPLC analysis of lactam **284** prepared from *N*-Boc-(*R*)-alanine

6.2.2 – HPLC analysis of lactam 284

A sample of lactam **284** was provided by Prof. Hashimoto for HPLC analysis and comparison with our synthetic material, in order to verify the stereochemistry of the alanine residue in the core domain of the cyclothiazomycin thiopeptides. HPLC analysis of two synthetic samples of **284** derived from (*R*)-alanine confirmed the retention times of (*S*)- and (*R*)-**284** as 32 and 64

minutes (Figures 30 and 32). The sample derived from the natural product was then analysed under the same conditions (Figure 33).[ix]

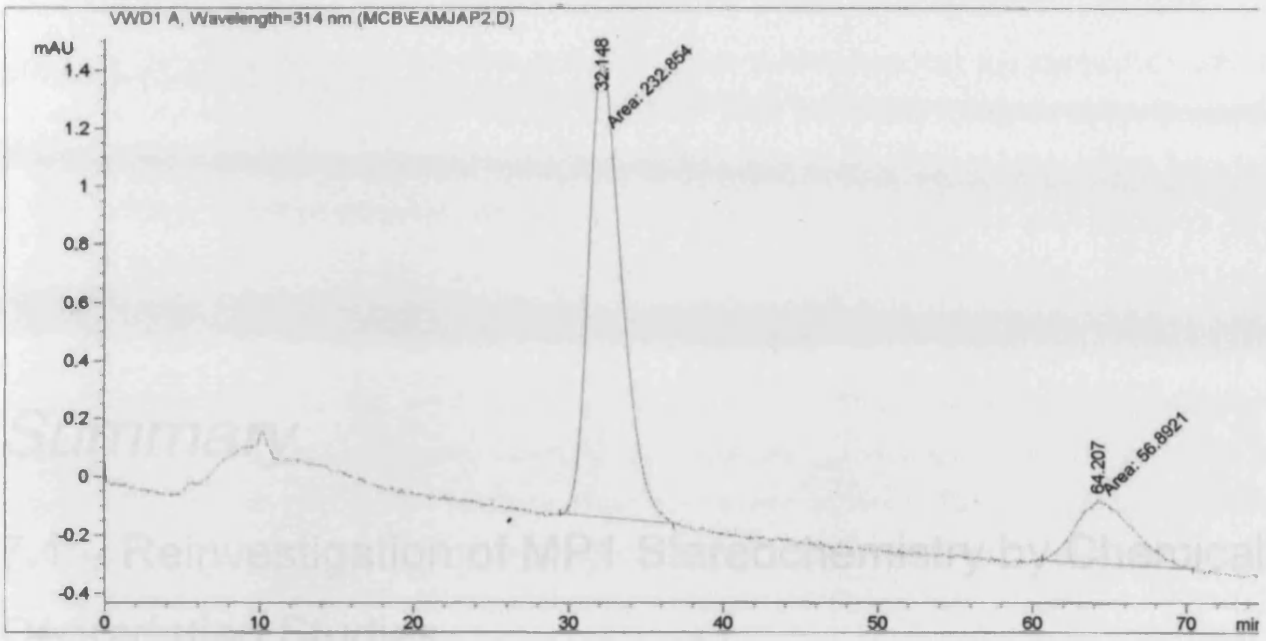


Figure 33 HPLC analysis of lactam **284** derived from cyclothiazomycin B1

The sample was found to have undergone partial racemisation under the degradation conditions used, which is unsurprising given the observed propensity for racemisation encountered during the syntheses of **284**, particularly when employing acidic reagents (Scheme 101, Figure 30). Despite this, the enantiomeric of the lactam was found to be 61%, with the (*S*)-enantiomer (retention time 32 minutes) predominating. This result implies that the absolute stereochemistry of the alanine residue in the core domain of the cyclothiazomycin thiopeptides is *S*, and that the initial assignment as *R* based on nOe data is erroneous.[216,217]

6.3 – Conclusion

HPLC analysis of degradation products from newly isolated cyclothiazomycin thiopeptides and comparison with synthetic material has provided evidence that the stereochemistry of the alanine-derived heterocyclic residue in the cyclothiazomycin core domain has been incorrectly assigned as *R*. On the basis of these findings, it is proposed that the absolute stereochemistry should be revised to *S*.

[ix] Chiralcel OD column, eluting with *i*-PrOH:hexane (10:90), 1.00 ml min^{-1}, detected at 314 nm.

Chapter Seven – Summary

Summary

7.1 – Reinvestigation of MP1 Stereochemistry by Chemical Degradation Studies

7.2 – Modification of the Hantzsch Thiazole Synthesis

7.3 – Synthesis of the Central Heterocyclic Domain of MP1

7.4 – Assembly of the MP1 Macrocycle

7.5 – Reinvestigation of the Stereochemistry of Cyclothiazomycin

7 – Summary

7.1 – Reinvestigation of MP1 stereochemistry by chemical degradation studies

Chemical degradation studies on micrococcin P1 have established that the stereochemistry of the valine residue in the natural product is *S* and not *R* as previously assigned in the literature. This confirms Ciufolini's hypothesis that the Bycroft-Gowland structure of MP1 differs from the natural product on stereochemical grounds.

7.2 – Modification of the Hantzsch thiazole synthesis

A range of conditions for the synthesis of amino acid-derived thiazoles have been investigated and previously reported conditions found to be unreliable. Evidence has been provided to suggest that racemisation in the Hantzsch thiazole synthesis occurs in the elimination step of the reaction. The peptide backbone of micrococcin P1 has been prepared in 10 steps and 44% overall yield, incorporating *S*-valine based on the results of our degradation studies.

7.3 – Synthesis of the central heterocyclic domain of MP1

A microwave-assisted method for the conversion of sterically hindered β-ketoesters to enamines has been devised, which is suitable for single- and multimode instruments, and for scale-up to produce multigram quantities of material. The central heterocyclic domain of the micrococcin thiopeptides has been prepared in 15 steps and 9% overall yield *via* a highly convergent strategy incorporating the Bohlmann-Rahtz pyridine synthesis and featuring an efficient two-directional thiazole elaboration.

7.4 – Assembly of the MP1 macrocycle

The micrococcin thiopeptide macrocycle has been synthesised in 20 steps and 4% overall yield. Three micrococcin P1 side chain dipeptides have been prepared, each incorporating different protecting groups, enabling a range of different approaches to the natural product to be investigated.

7.5 – Reinvestigation of the stereochemistry of cyclothiazomycin

HPLC analysis of degradation products from newly isolated cyclothiazomycin thiopeptides and comparison with synthetic material has provided evidence that the alanine-derived residue in the cyclothiazomycin core domain is of *S* stereochemistry, and not *R* as previously assigned in the literature.

Chapter Eight – Experimental

Experimental

8.1 – Experimental Techniques

8.2 – General Experimental Methods

8.3 – Experimental

8 – Experimental

8.1 – Experimental techniques

Commercially available reagents were used without further purification; solvents were dried by standard procedures. Petroleum ether refers to the fraction with bp 40–60 °C, ether (Et_2O) refers to diethyl ether and EtOAc refers to ethyl acetate. Column chromatography was carried out using Merck Kieselgel 60 H silica or Matrex silica 60. Analytical thin layer chromatography was carried out using aluminium–backed plates coated with Merck Kieselgel 60 GF_{254} that were visualised under UV light (at 254 and/or 360 nm). Melting points (mp) were determined on a Kofler hot stage apparatus and are uncorrected. Infra–red (IR) spectra were recorded in the range 4000–650 cm^{-1} on a Perkin–Elmer 1600 series FTIR spectrometer using KBr disks, as a nujol mull between NaCl plates, or in CH_2Cl_2 for solid samples, or as a thin film between NaCl plates for liquid samples and are reported in cm^{-1}. Nuclear magnetic resonance (NMR) spectra were recorded in $CDCl_3$ at 25 °C unless stated otherwise using a Bruker DPX 400, Bruker Avance 250 or Bruker DRX 500 instrument operating at the frequency stated and are reported in ppm; *J* values are reported in Hz and multiplicities are expressed by the usual conventions (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, app = apparent, m = multiplet). Low–resolution mass spectra (LRMS) were recorded using a Fisons VG Platform II Quadrupole instrument using the ionisation method stated. APCI refers to atmospheric pressure chemical ionisation, ES refers to electrospray ionisation, CI refers to chemical ionisation (ammonia) and EI refers to electron impact ionisation. High-resolution mass spectra were obtained courtesy of the EPSRC Mass Spectrometry Service at Swansea, UK using the ionisation methods specified. Microanalyses were recorded courtesy of Warwick Analytical Services Ltd. at University of Warwick Science Park, UK using an Exeter 440 Elemental Analyser. Specific rotations were measured at the indicated temperature using an AA–1000 (Optical Activity Ltd) polarimeter at the sodium D line and are given in deg $cm^3\ g^{-1}\ dm^{-1}$ with concentration *c* in 10^{-2} g cm^{-3}. HPLC was carried out using a HP-1100 HPLC machine equipped with a variable wavelength UV detector and variable flow pump. *In vacuo* refers to evaporation at reduced pressure using a rotary evaporator and diaphragm pump, followed by the removal of trace volatiles using a vacuum (oil) pump.

8.2 – General experimental methods

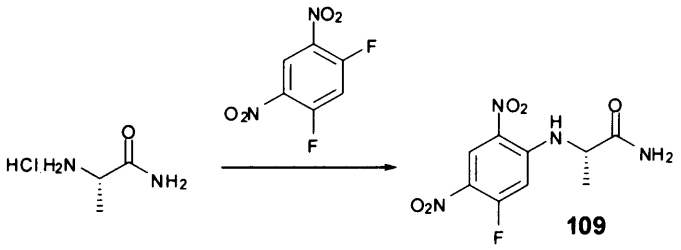
8.2.1 – General method for the derivatisation of amino acids with Marfey's reagent

Amino acid (125 μmol) was dissolved in water (2.5 mL) and a solution of Marfey's reagent (1% in acetone, 47.6 mg, 175 μmol, 1.4 equiv, in 4.76 mL acetone) added. Aqueous sodium hydrogen carbonate (1 M, 1 mL, 1 mmol) was added and the solution stirred at 35 °C for 1 h. The reaction

mixture was allowed to cool to room temperature, aqueous hydrochloric acid (2 M, 0.5 mL, 1 mmol) was added and the solution extracted with ethyl acetate (3 x 2 mL). The combined organic extracts were dried (Na_2SO_4) and the solvent removed *in vacuo* to afford the product.

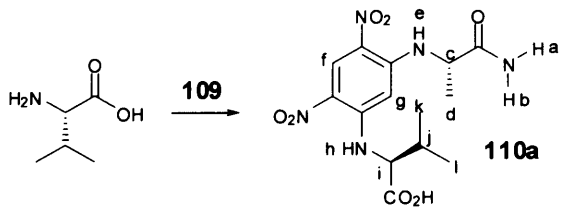
8.3 - Experimental

(*S*)-2-(5-Fluoro-2,4-dinitrophenylamino)propanamide (Marfey's reagent, 109)



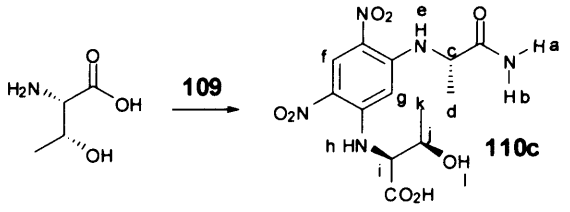
(*S*)-Alaninamide hydrochloride (472 mg, 3.79 mmol) was dissolved in aqueous sodium hydroxide solution (1 M, 3.90 mL, 3.90 mmol) and acetone (60 mL) added. Anhydrous magnesium sulphate (10 g) was added and the solution stirred at room temperature for 3 hours. The solution was filtered and added dropwise to a stirred solution of 1,5-difluoro-2,4-dinitrobenzene (668 mg, 3.27 mmol) in acetone (15 mL). The reaction mixture was stirred at room temperature for 30 minutes, water (75 mL) added and the acetone removed *in vacuo*. Filtration of the resulting aqueous solution afforded the title compound (596 mg, 67%) as a bright yellow solid, mp 235-237 °C (acetone) (Found: $[M+H]^+$, 273.0627. Calc. for $C_9H_{10}N_4O_5F$: 273.0630); $[\alpha]_D^{25}$ +60.4 (*c* 0.50, acetone); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3440, 3339, 3167, 2925, 1687, 1668, 1643, 1632, 1582, 1500, 1423, 1365, 1333, 1287, 1172, 1117, 1057, 1037, 925, 837, 739; δ_H(400 MHz; d_6-DMSO) 9.05 (1 H, d, *J* 6.4, N*H*CHCH$_3$), 8.84 (1 H, d, *J* 8.2, 6-H), 7.68 (1 H, s, NH_2), 7.46 (1 H, s, NH_2), 6.90 (1 H, d, *J* 14.4, 3-H), 4.33 (1 H, app. quin, *J* 6.8, NHC*H*CH$_3$), 1.38 (3 H, d, *J* 6.8, CHC*H*$_3$); δ_C(67 MHz; DMSO) 172.3 (C), 147.7 (C), 147.5 (C), 127.4 (CH), 102.1 (CH, d, *J* 29), 51.6 (CH), 18.3 (CH_3), doublets for *C*F and FC*C*NO$_2$ not observed; *m/z* (ES+) 311 ($[M+K]^+$, 100%), 295 ($[M+Na]^+$, 50%), 228 (19), 140 (12).

(*S*)-2-(5-((*S*)-1-Amino-1-oxopropan-2-ylamino)-2,4-dinitrophenylamino)-3-methylbutanoic acid (110a)



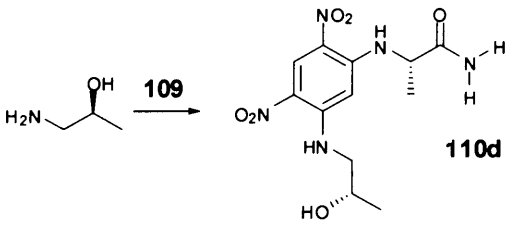
The title compound was prepared according to general method 8.2.1 using (*S*)-valine. Isolated as a bright yellow solid, mp 165-168 °C (acetone) (Found: $[M+Na]^+$, 392.1174. Calc. for $C_{14}H_{19}N_5O_7Na$: 392.1177); $[\alpha]_D^{25}$ -76.0 (*c* 0.25, acetone); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3100-2000 br, 2915, 1674, 1659, 1614, 1559, 1524, 1469, 1398, 1383, 1343, 1248, 1192, 1127, 1087, 1032, 926; UV (MeCN-TEAP [25 mM, pH 3], 1:1)/nm λ_{max} 338 (ε 25902.5 mol^{-1} dm^3 cm^{-1}); δ_H(250 MHz; d_6-DMSO) 9.00 (1 H, s, **f**), 8.92 (1 H, br d, *J* 7.0, **h**), 8.63 (1 H, br d, *J* 6.5, **e**), 7.81 (1 H, s, **a**), 7.41 (1 H, s, **b**), 5.73 (1 H, s, **g**), 4.28 (1 H, br app. quin, *J* 6.5, **c**), 4.07 (1 H, br m, **i**), 2.22 (1 H, br m, **j**), 1.44 (3 H, d, *J* 6.7, **d**), 1.06 (3 H, d, *J* 6.7, **k**), 0.93 (3 H, d, *J* 6.7, **l**); *m/z* (ES+) 408 ($[M+K]^+$, 25%), 392 ($[M+Na]^+$, 100%), 324 (50), 280 (7).

(2*S*,3*R*)-2-(5-((*S*)-1-Amino-1-oxopropan-2-ylamino)-2,4-dinitrophenylamino)-3-hydroxybutanoic acid (110c)



The title compound was prepared according to general method 8.2.1 using (2*S*,3*R*)-threonine. Isolated as a bright yellow solid, mp 141-144 °C (acetone) (Found: $[M+H]^+$, 372.1165. Calc. for $C_{13}H_{18}N_5O_8$: 372.1155); $[\alpha]_D^{25}$ -25.6 (*c* 0.25, acetone); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3100-2000 br, 2915, 2845, 1609, 1564, 1529, 1479, 1408, 1383, 1343, 1248, 1192, 1032, 911, 796; UV (MeCN-TEAP [25 mM, pH 3], 1:1)/nm λ_{max} 342 (ε 27579.5 mol^{-1} dm^3 cm^{-1}); δ_H(250 MHz; d_6-DMSO) 9.02 (1 H, s, **f**), 8.86 (1 H, br d, *J* 6.6, **h**), 8.83 (1 H, br d, *J* 6.6, **e**), 7.77 (1 H, s, **a**), 7.47 (1 H, s, **b**), 5.75 (1 H, s, **g**), 4.40-4.18 (3 H, m, **c**, **i**, **j**), 1.40 (3 H, d, *J* 6.7, **d**), 1.16 (3 H, d, *J* 6.1, **k**); *m/z* (ES+) 394 ($[M+Na]^+$, 100%), 305 (37), 217 (18), 140 (11).

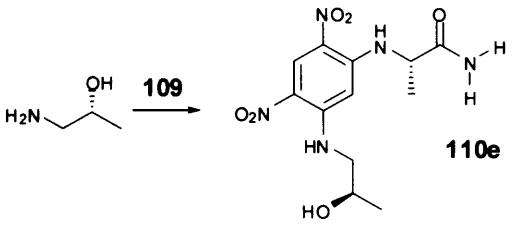
(*S*)-2-(5-((*S*)-2-Hydroxypropylamino)-2,4-dinitrophenylamino)propanamide (110d)



(*S*)-(+)-1-Amino-2-propanol (9.4 mg, 125 μmol) was dissolved in water (2.5 mL) and a solution of Marfey's reagent (1% in acetone, 47.6 mg, 175 μmol, 1.4 equiv, in 4.76 mL acetone) added.

Aqueous sodium hydrogen carbonate (1 M, 1 mL, 1 mmol) was added and the solution stirred at 35 °C for 1 h. The reaction mixture was allowed to cool to room temperature, aqueous hydrochloric acid (2 M, 0.5 mL, 1 mmol) added and the resulting solution analysed by reverse-phase HPLC.

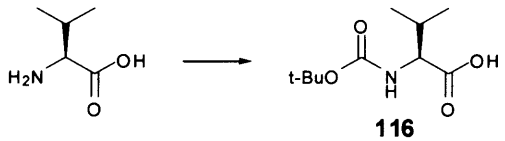
(*S*)-2-(5-((*R*)-2-Hydroxypropylamino)-2,4-dinitrophenylamino)propanamide (110e)



110e

(*R*)-(-)-1-Amino-2-propanol (9.4 mg, 125 µmol) was dissolved in water (2.5 mL) and a solution of Marfey's reagent (1% in acetone, 47.6 mg, 175 µmol, 1.4 equiv, in 4.76 mL acetone) added. Aqueous sodium hydrogen carbonate (1 M, 1 mL, 1 mmol) was added and the solution stirred at 35 °C for 1 h. The reaction mixture was allowed to cool to room temperature, aqueous hydrochloric acid (2 M, 0.5 mL, 1 mmol) added and the resulting solution analysed by reverse-phase HPLC.

(*S*)-*N*-*tert*-(Butoxycarbonyl)valine (116)

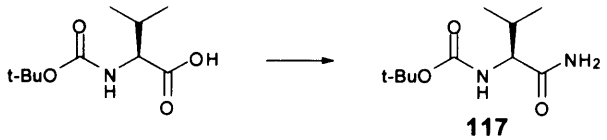


116

Sodium hydroxide (0.75 g, 18.78 mmol, 1.1 equiv) and di-*tert*-butyldicarbonate (4.10 g, 18.78 mmol, 1.1 equiv) were added to an ice-cold solution of (*S*)-valine (2.00 g, 17.07 mmol, 1.0 equiv) in water-*tert*-butanol (1:1, 40 mL), and the mixture stirred at 0 °C for 1 hour, warmed to room temperature and stirred for a further 14 hours. The mixture was concentrated to half-volume and extracted with light petroleum (20 mL) and the aqueous layer was acidified to pH 1-2 with ice-cold 1 M H_2SO_4. The resulting solution was extracted with ethyl acetate (3 x 15 mL) and the combined organic extracts were dried (Na_2SO_4), filtered and evaporated *in vacuo* to give a colourless viscous oil (4.05 g). After drying *in vacuo* for 48 h, the title compound (3.96 g, 98%) was obtained as a colourless solid, mp 78-80 °C (EtOAc) (Found: $[M+H]^+$ 218.1388. Calc. for $C_{10}H_{20}NO_4$: 218.1387); $[\alpha]_D^{17}$ -6.4 (*c* 1.0, AcOH); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3309, 2924, 2854, 1706, 1648, 1458, 1370, 1276, 1161, 1096, 1012, 982, 858, 782; δ_H(400 MHz; $CDCl_3$) 10.46 (1 H, br s, NH), 6.00 (0.37 H, br s, NH), 4.95 (0.63 H, d, *J* 8.9, NH), 4.20 (0.63 H, dd, *J* 8.9, 4.5, α-CH), 3.98

(0.37 H, br s, α-CH), 2.15 (1 H, m, Me_2CH), 1.38 (9 H, s, CMe_3), 0.94 (3 H, d, *J* 6.8, MeCH*Me*), 0.87 (3 H, d, *J* 6.8, *Me*CHMe); δ_C(100 MHz; $CDCl_3$) 177.4 (C), 177.0 (C), 157.1 (C), 155.9 (C), 81.6 (C), 80.1 (C), 60.0 (CH), 58.4 (CH), 31.1 (CH), 30.9 (CH) 28.3 (CH_3), 28.2 (CH_3), 19.1 (CH_3), 19.0 (CH_3) 17.5 (CH_3), 17.4 (CH_3); *m/z* (APCI) 218 ([M+H]$^+$, 80%), 162 (100), 160 (15), 144 (5).

(*S*)-*N*-*tert*-(Butoxycarbonyl)valinamide



117

To a stirred solution of *N*-Boc-(*S*)-valine (3.00 g, 13.81 mmol) and triethylamine (1.94 mL, 1.41 g, 13.94 mmol, 1.01 equiv) in anhydrous tetrahydrofuran (120 mL) at 0 °C was added ethyl chloroformate (1.33 mL, 1.51 g, 13.94 mmol, 1.01 equiv) and the mixture stirred for 1 h. Aqueous ammonia (35%, 10 mL) was added and the solution was allowed to warm to room temperature and stirred for 1 hour. The organic solvent was removed *in vacuo* and the resulting aqueous solution diluted with water (10 mL) and extracted with ethyl acetate (3 x 50 mL). The combined organic extracts were washed sequentially with saturated aqueous sodium hydrogen carbonate solution (50 mL), citric acid (1 M, 50 mL), brine (50 mL), dried (Na_2SO_4) and evaporated *in vacuo* to yield the title compound (2.83 g, 95%) as a colourless solid, mp 159–161 °C (EtOAc) (lit.,[219] 160–161 °C) (Found: [M+H]$^+$ 217.1546. Calc. for $C_{10}H_{21}N_2O_3$: 217.1547); $[\alpha]_D^{20}$ 17.0 (*c* 1.00, MeOH) {lit.,[220] 17.7 (*c* 1.33, DMF)}; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3385, 3342, 3191, 2921, 1681, 1657, 1519, 1461, 1377, 1245, 1170, 1044, 1019, 899, 878, 775, 722; δ_H(250 MHz; $CDCl_3$) 6.05 (1 H, br s, exch. D_2O, NH), 5.72 (1 H, br s, exch. D_2O, NH), 5.05 (1 H, br d, exch. D_2O, NH), 3.90 (1 H, dd, *J* 7.9, 7.9, α-CH), 2.08 (1 H, m, Me_2CH), 1.38 (9 H, s, CMe_3), 0.92 (3H, d, *J* 6.8, MeCH*Me*), 0.88 (3 H, d, *J* 6.8, *Me*CHMe); δ_C(100 MHz; $CDCl_3$) 175.0 (C), 156.1 (C), 80.1 (C), 59.4 (CH), 30.8 (CH), 28.3 (CH_3), 19.3 (CH_3), 17.8 (CH_3); *m/z* (APCI) 217 ([M+H]$^+$, 90%), 161 (100), 151 (15), 149 (57), 139 (10), 129 (22).

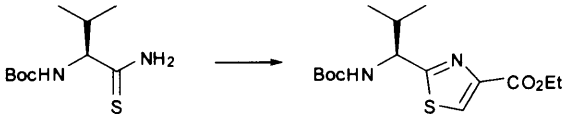
(*S*)-*N*-*tert*-(Butoxycarbonyl)thiovalinamide (101b)

101b

(*S*)-*N*-*tert*-(Butoxycarbonyl)valinamide (2.00 g, 9.25 mmol, 1 equiv) was dissolved in dry dichloromethane (120 mL). Lawesson's reagent (1.87 g, 4.62 mmol, 0.5 equiv) was added and

the resulting solution heated at reflux for 16 h. The solvent was evaporated *in vacuo* and the residue dissolved in ethyl acetate (100 mL) and washed with saturated aqueous sodium hydrogen carbonate solution (100 mL). The organic layer was dried (Na_2SO_4) and evaporated *in vacuo*. Purification on silica eluting with petroleum ether-diethyl ether (3:7), gave the title compound (1.33 g, 62%) as a yellow foam, mp 84–85 °C (light petroleum–diethyl ether) (lit.,[96] 112–113 °C) (Found: $[M+H]^+$ 233.1319. Calc. for $C_{10}H_{21}N_2O_2S$: 233.1318); $[\alpha]_D^{26}$ –46.7 (*c* 0.3, chloroform) {lit.,[96] –43.5 (c 0.7, chloroform)}; R_f 0.30 (petroleum ether-diethyl ether 3:7); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3317, 3146, 2923, 2853, 1684, 1509, 1456, 1366, 1298, 1237, 1164, 1045, 1011, 870, 763, 718; δ_H(400 MHz; $CDCl_3$) 8.20 (1 H, br s, exch. D_2O, NH), 7.70 (1 H, br s, exch. D_2O, NH), 5.24 (1 H, d, *J* 8.9, exch. D_2O, NH), 4.13 (1 H, m, α-CH), 2.10 (1 H, m, Me_2CH), 1.37 (9 H, s, CMe_3), 0.92 (6 H, app. d, *J* 5.9, $CHMe_2$); δ_C(100 MHz; $CDCl_3$) 209.5 (C), 156.0 (C), 80.3 (C), 65.2 (CH), 33.3 (CH), 28.4 (CH_3), 19.6 (CH_3), 18.3 (CH_3); *m/z* (APCI) 233 ($[M+H]^+$, 18%), 177 (100), 133 (75).

(*S*)-Ethyl 2-(1-(*tert*-butoxycarbonylamino)-2-methylpropyl)thiazole-4-carboxylate (102b) [Meyers, Table 4, entry 4]

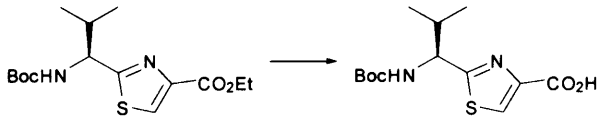


102b

Potassium hydrogen carbonate (1.72 g, 17.2 mmol, 4 equiv) was added to a solution of (*S*)-*N*-*tert*-(butoxycarbonyl)thiovalinamide (1.00 g, 4.3 mmol) in anhydrous 1,2-dimethoxyethane (10 mL) at -40 °C. Ethyl bromopyruvate (2.32 mL, 3.61 g, 18.5 mmol, 3 equiv) was added and the solution was stirred for 16 h at –20 °C. The mixture was allowed to warm to room temperature, filtered through a pad of Celite® and the pad washed with diethyl ether (10 mL). The filtrate was evaporated *in vacuo* and the residue dissolved in anhydrous DME (10 mL). The solution was cooled to –40 °C and a solution of trifluoroacetic anhydride (2.01 mL, 2.98 g, 14.2 mmol, 3.3 equiv) and 2,6-lutidine (3.46 mL, 3.18 g, 29.7 mmol, 6.9 equiv) in anhydrous DME (5 mL) was added. The reaction mixture was stirred at –20 °C for 30 minutes then evaporated *in vacuo*. The residue was dissolved in chloroform (100 mL) and washed with water (100 mL). The aqueous layer was further extracted with chloroform (50 mL) and the combined organic extracts washed with water (100 mL) and brine (100 mL), dried (Na_2SO_4) and evaporated *in vacuo*. Purification of the residue by column chromatography on silica eluting with petroleum ether-ethyl acetate (3:1) afforded the title compound (0.83 g, 59%) as a colourless solid, mp 114–116 °C (ethyl acetate–petroleum ether) (lit.,[162] 114–115 °C) (Found: $[M+H]^+$ 329.1532. Calc. for $C_{15}H_{25}N_2O_4S$: 329.1530); $[\alpha]_D^{23}$ –36.2 (*c* 1.00, chloroform) {lit.,[162] (*R*)-enantiomer; +41.6 (c 1.06, chloroform)}; *ee* 88%; R_f 0.28 (petroleum ether-ethyl acetate 3:1); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3300, 3094, 2922, 2853,

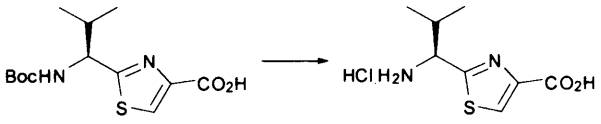
1713, 1699, 1456, 1379, 1339, 1305, 1232, 1174, 1104, 1084, 1031, 999, 878, 777, 718; δ_H(400 MHz; $CDCl_3$) 8.01 (1 H, s, 5-H), 5.25 (1 H, d, *J* 7.7, exch. D_2O, NH), 4.85 (1 H, dd, *J* 7.7, 7.7, α-CH), 4.35 (2 H, q, *J* 7.1, OCH_2CH_3), 2.41 (1 H, m, Me_2CH), 1.38 (9 H, s, CMe_3), 1.34 (3 H, t, *J* 7.1, OCH_2CH_3), 0.92 (3 H, d, *J* 6.7, MeCHMe), 0.83 (3 H, d, *J* 6.7, MeCHMe); δ_C(100 MHz; $CDCl_3$) 173.3 (C), 161.4 (C), 155.5 (C), 147.4 (C), 126.8 (CH), 80.1 (C), 61.4 (CH_2), 58.0 (CH), 33.3 (CH), 28.3 (CH_3), 19.5 (CH_3), 17.3 (CH_3), 14.4 (CH_3); *m/z* (APCI) 329 ($[M+H]^+$, 100%).

(*S*)-2-[1-(*N*-*tert*-Butoxycarbonylamino)-2-methylpropyl]thiazole-4-carboxylic acid



To a stirred solution of (*S*)-ethyl-2-[1-(*N*-*tert*-butoxycarbonylamino)-2-methylpropyl]thiazole-4-carboxylate (328 mg, 1.00 mmol) in methanol-water (5:1, 20 mL) was added lithium hydroxide monohydrate (210 mg, 5.00 mmol, 5 equiv) and the mixture stirred at room temperature for 16 h. The methanol was evaporated *in vacuo* and the solution partitioned between water (20 mL) and diethyl ether (20 mL). The organic layer was further extracted with water (20 mL) and the combined aqueous extracts acidified to pH 3 using citric acid (10% w/v). The acidified aqueous solution was extracted with ethyl acetate (3 x 20 mL) and the combined organic extracts were washed with water (20 mL) and brine (20 mL), dried (Na_2SO_4) and evaporated *in vacuo* to afford the title compound (270 mg, 90%) as a colourless solid, mp 300 °C (lit.,[162] 300-302 °C) (Found: $[M+H]^+$ 301.1219. Calc. for $C_{13}H_{21}N_2O_4S$: 301.1217); $[\alpha]_D^{21}$ –41.0 (*c* 1.00, chloroform) {lit.,[162] (*R*)-enantiomer; +42.0 (c 2.60, chloroform)}; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3400-3000 br, 2922, 2853, 1712, 1461, 1377, 1340, 1229, 1167, 1105, 1001, 722; δ_H(400 MHz; $CDCl_3$) 8.12 (1 H, s, 5-H), 6.20 (0.25 H, br s, exch. D_2O, NH), 5.31 (0.75 H, br s, NH), 4.83 (0.75 H, app br m, α-CH), 4.69 (0.25 H, app br s, α-CH), 2.34 (1 H, m, Me_2CH), 1.38 (9 H, s, CMe_3), 0.90 (3 H, d, *J* 6.6, MeCHMe), 0.86 (3 H, d, *J* 6.6, MeCHMe); δ_C(100 MHz; $CDCl_3$) 173.8 (C), 164.4 (C), 155.5 (C), 146.7 (C), 128.3 (CH), 80.3 (C), 58.0 (CH), 33.2 (CH), 28.3 (CH_3), 19.4 (CH_3), 17.4 (CH_3); *m/z* (APCI) 301 ($[M+H]^+$, 100%), 175 (5), 153 (10), 149 (22), 139 (24), 130 (7).

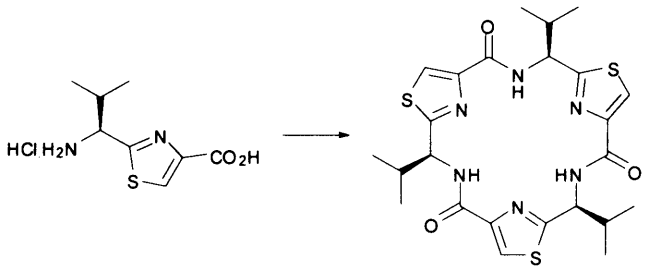
(S)-2-(1-Amino-2-methylpropyl)thiazole-4-carboxylic acid hydrochloride (100)



100

A solution of hydrogen chloride in dioxane (4 M, 2 mL) was added to (*S*)-2-[1-(N-*tert*-butoxycarbonylamino)-2-methylpropyl]thiazole-4-carboxylic acid (200 mg, 0.67 mmol) the mixture stirred at room temperature under an atmosphere of nitrogen for 2 hours. The solvent was removed i*n vacuo* and toluene (2 mL) added. The solvent was removed *in vacuo* and the residue purified by trituration with diethyl ether (2 x 5 mL) to furnish the title compound (152 mg, 96%) as a colourless solid, mp 258-259 °C dec. (lit.,[136] 259-261 °C dec.) (Found: $[M+H]^+$ 201.0692. Calc. for $C_8H_{13}N_2O_2S$: 201.0692); $[\alpha]_D^{23}$ –7.2 (c 0.5, water); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3408, 3316, 2922, 1702, 1589, 1509, 1465, 1377, 1244; δ_H(400 MHz; D_2O) 8.39 (1 H, s, 5-H), 4.55 (1 H, d, *J* 7.5 α-CH), 2.31 (1 H, app qd, *J* 6.7, 7.5, Me_2CH), 1.00 (3 H, d, *J* 6.7, *Me*CHMe), 0.85 (3 H, d, *J* 6.7, MeCH*Me*); δ_C(100 MHz; D_2O) 166.1 (C), 164.2 (C), 146.4 (C), 131.1 (CH), 57.8 (CH), 32.6 (CH), 17.9 (CH_3), 17.8 (CH_3); *m/z* (ES+) 201 ($[M+H]^+$, 15%), 184 (85), 166 (100).

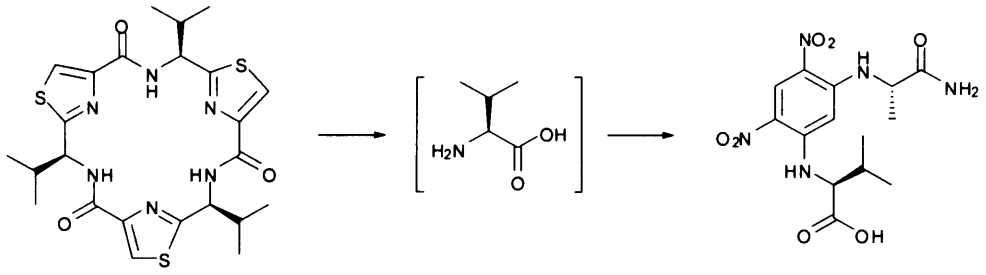
Cyclic hexapeptide 115



115

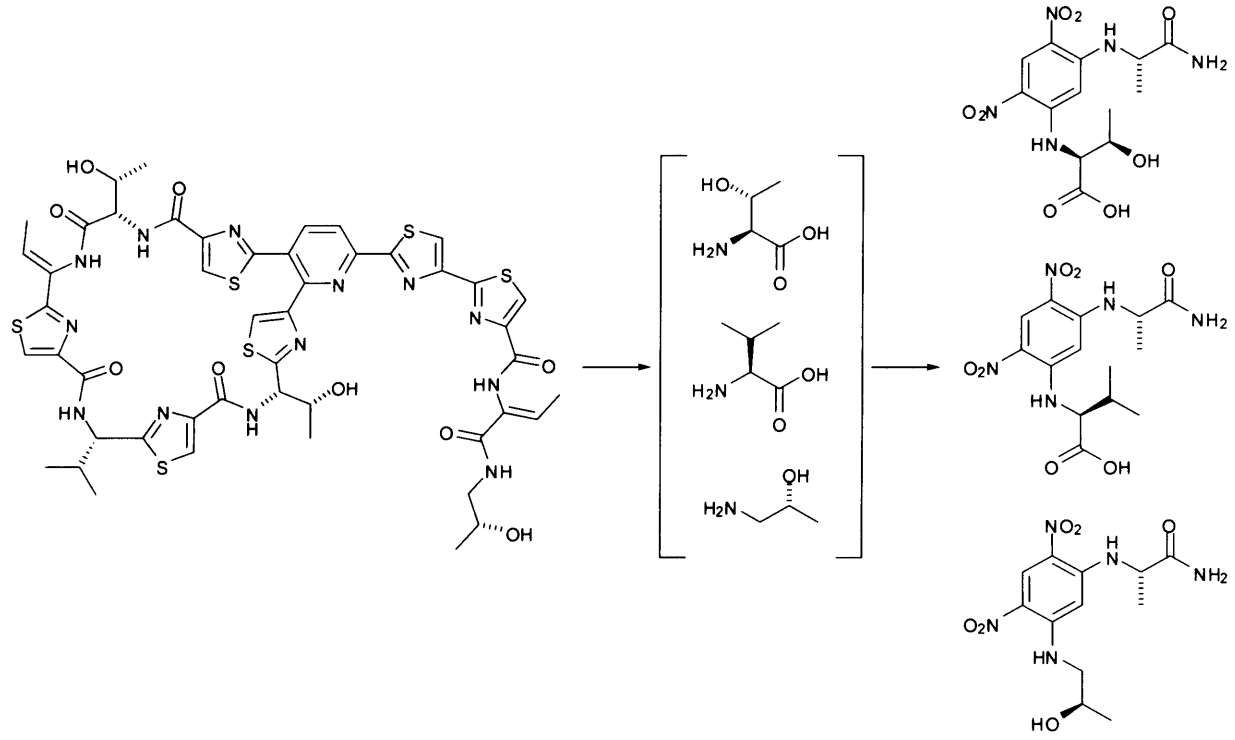
To a suspension of (S)-2-(1-amino-2-methylpropyl)thiazole-4-carboxylic acid hydrochloride (100 mg, 0.42 mmol) in acetonitrile (8.8 mL) under an inert atmosphere was added diisopropylethylamine (0.219 mL, 163 mg, 1.26 mmol, 3 equiv), followed by FDPP (242 mg, 0.63 mmol, 1.5 equiv) and the mixture stirred at room temperature for 18 h. The solvent was removed *in vacuo* and the resiude dissolved in ethyl acetate (25 mL) and washed with hydrochloric acid (2 M, 2 x 25 mL). The combined aqueous layers were extracted with ethyl acetate (25 mL) and the combined organic extracts washed sequentially with aqueous sodium hydroxide (1 M, 2 x 25 mL), water (25 mL) and brine (25 mL), dried (Na_2SO_4) and evaporated *in vacuo*. Purification of the residue by chromatography on silica eluting with petroleum ether-ethyl acetate (3:7) furnished the title compound as a colourless solid, mp 256-259 °C (Et_2O) (lit.,[140] 258-260 °C) (Found: $[M+H]^+$ 547.1618. Calc. for $C_{24}H_{31}N_6O_3S_3$: 547.1614); $[\alpha]_D^{25}$ –110.4 (c 0.25, chloroform) {lit.,[140] (*R*)-enantiomer; +126.8 (c 0.53, chloroform)}; R_f 0.25 (petroleum ether-ethyl acetate 3:7); δ_H(400 MHz; $CDCl_3$) 8.39 (3 H, d, *J* 9.3 NH), 8.02 (3 H, s, 5-H), 5.37 (3 H, dd, *J* 9.3, 6.7 α-CH), 2.21 (3 H, app qd, *J* 6.7, 6.7, Me_2CH), 1.03 (9 H, d, *J* 6.7, *Me*CHMe), 0.98 (9 H, d, *J* 6.7, MeCH*Me*); δ_C(100 MHz; $CDCl_3$) 168.6 (C), 159.8 (C), 149.2 (C), 123.5 (CH), 55.5 (CH), 35.4 (CH), 18.9 (CH_3), 18.4 (CH_3); *m/z* (APCI) 547 ($[M+H]^+$, 65%), 385 (100), 279 (26), 247 (40), 233 (15), 221 (23), 219 (8), 130 (10).

Degradation of hexapeptide 115



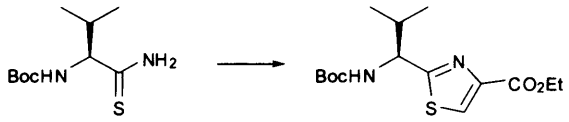
Cyclic hexapeptide **115** (1 mg, 1.8 μmol) was dissolved in dichloromethane (10 mL) and a stream of ozone bubbled through the resulting solution at room temperature for 3 h. The solvent was evaporated under a stream of nitrogen and the residue dissolved in hydrochloric acid (6 M, 0.3 mL) and heated at 110 °C for 24 h. The solvent was removed *in vacuo* and water (0.1 mL) added. A solution of Marfey's reagent (1 mg, 3.7 μmol) in acetone (0.2 mL) was added, followed by sodium hydrogen carbonate solution (1 M, 40 μL, 40 μmol), and the mixture heated at 45 °C for 1 hour. The solution was allowed to cool to room temperature and hydrochloric acid (2 M, 20 μL, 40 μmol) added. The products were identified by reverse-phase HPLC using a chiral Hypersil 5μ reverse phase column, gradient eluting with 10% - 50% acetonitrile in 50 mM TEAP buffer (pH 3), flow rate 0.25 ml min^{-1}, detected at 340 nm.

Degradation of MP1



Micrococcin P1 (1 mg, 0.87 μmol) was dissolved in dichloromethane (5 mL) and a stream of ozone bubbled through the resulting solution at room temperature for 3 h. The solvent was evaporated under a stream of nitrogen and the residue dissolved in hydrochloric acid (6 M, 0.5 mL) and heated at 110 °C for 24 h. The solvent was removed *in vacuo* and water (0.1 mL) added. A solution of Marfey's reagent (2 mg, 7.4 μmol) in acetone (0.2 mL) was added, followed by sodium hydrogen carbonate solution (1 M, 50 μL, 50 μmol), and the mixture heated at 45 °C for 1 hour. The solution was allowed to cool to room temperature and hydrochloric acid (2 M, 25 μL, 50 μmol) added. The products were identified by reverse-phase HPLC using a chiral Hypersil 5μ reverse phase column, gradient eluting with 10% - 50% acetonitrile in 50 mM TEAP buffer (pH 3), flow rate 0.25 ml min^{-1}, detected at 340 nm.

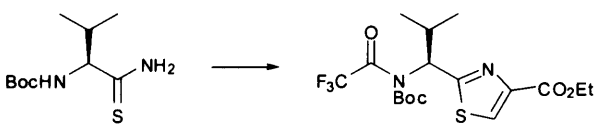
(*S*)-Ethyl 2-(1-(*tert*-butoxycarbonylamino)-2-methylpropyl)thiazole-4-carboxylate (102b) [Holzapfel, Table 4, entry 2]



102b

Potassium hydrogen carbonate (0.176 g, 1.76 mmol, 8 equiv) was added to a solution of (*S*)-*N*-*tert*-(butoxycarbonyl)thiovalinamide (50 mg, 0.22 mmol) in DME (2 mL) and the mixture stirred vigorously for 5 minutes. Ethyl bromopyruvate (83 μL 0.66 mmol, 3 equiv) was added and the solution was stirred for 1 minute then cooled to 0 °C. A solution of trifluoroacetic anhydride (125 μL, 0.89 mmol, 4 equiv) and 2,6-lutidine (0.22 mL, 1.87 mmol, 8.5 equiv) in DME (1 mL) was added and the reaction mixture was allowed to reach ambient temperature before evaporation *in vacuo*. The residue was partitioned between chloroform (10 mL) and water (10 mL), and the organic layer dried (Na_2SO_4) and evaporated *in vacuo*. Purification on silica eluting with light petroleum-diethyl ether (2:1) afforded the title compound (24 mg, 33%) as a pale yellow solid, *ee* >98%.

(*S*)-Ethyl 2-(1-(*N*-(*tert*-butoxycarbonyl)-2,2,2-trifluoroacetamido)-2-methylpropyl)thiazole-4-carboxylate (134) [Table 5, entry 2]

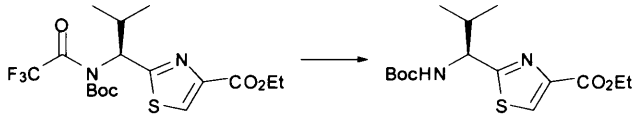


134

Potassium hydrogen carbonate (2.36 g, 23.6 mmol, 8 equiv) was added to a solution of (*S*)-*N*-*tert*-(butoxycarbonyl)thiovalinamide (684 mg, 2.94 mmol) in anhydrous 1,2-dimethoxyethane (7

mL) and the solution cooled to –18 °C. Ethyl bromopyruvate (1.11 mL, 1.72 g, 8.83 mmol, 3 equiv) was filtered through a pad of Celite®, the pad washed with diethyl ether (7 mL) and the combined filtrates evaporated *in vacuo*. The residue was dissolved in anhydrous DME (7 mL) and the solution cooled to –18 °C. A solution of trifluoroacetic anhydride (1.67 mL, 2.48 g, 11.78 mmol, 4 equiv) and 2,6-lutidine (3.09 mL, 2.84 g, 26.50 mmol, 9 equiv) in anhydrous DME (4 mL) was added. The reaction mixture was stirred at –18 °C for 30 minutes then evaporated *in vacuo*. The residue was dissolved in chloroform (20 mL) and washed with water (20 mL). The aqueous layer was further extracted with chloroform (20 mL) and the combined organic extracts washed with water (20 mL) and brine (20 mL), dried (Na_2SO_4) and evaporated *in vacuo*. Purification of the residue by column chromatography on silica eluting with petroleum ether–ethyl acetate (3:1) afforded the title compound (1.21 g, 97%) as a yellow oil; (Found: $[M+H]^+$ 425.1361. Calc. for $C_{17}H_{24}N_2O_5F_3S$: 425.1358); R_f 0.30 (petroleum ether-ethyl acetate 3:1); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 2970, 2925, 1713, 1649, 1508, 1369, 1237, 1202, 1168, 1097, 1017; δ_H(500 MHz; $CDCl_3$) 8.12 (1 H, s, 5-H), 5.39 (1 H, d, *J* 10.9, α-CH), 4.34 (2 H, q, *J* 7.1, OCH_2CH_3), 2.78 (1 H, m, Me_2CH), 1.43 (9 H, s, CMe_3), 1.31 (3 H, t, *J* 7.1, OCH_2CH_3), 0.95 (3 H, d, *J* 6.6, *Me*CHMe), 0.92 (3 H, d, *J* 6.6, MeCH*Me*); δ_C(125 MHz; $CDCl_3$) 173.2 (C), 161.4 (C), 155.2 (C), 147.4 (C), 126.8 (CH), 86.9 (C), 61.4 (CH2), 58.0 (CH), 33.3 (CH), 28.3 (CH3), 27.4 (CH3), 19.4 (CH3), 14.4 (CH3) {signals for F_3CCO and F_3CCO not observed}; *m/z* (ES+) 425 ($[M+H]^+$, 3%), 325 (58), 273 (100), 196 (30), 180 (8).

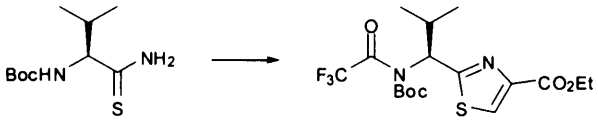
(*S*)-Ethyl 2-(1-(*tert*-butoxycarbonylamino)-2-methylpropyl)thiazole-4-carboxylate (102b) [from Table 5, entry 2]



102b

(*S*)-Ethyl 2-(1-(*N*-(*tert*-butoxycarbonyl)-2,2,2-trifluoroacetamido)-2-methylpropyl)thiazole-4-carboxylate (700 mg, 1.65 mmol) was dissolved in ethanol (16.5 mL) and sodium ethoxide (0.336 mg, 4.95 mmol, 3 equiv) added. The solution was stirred at room temperature for 4 hours then evaporated *in vacuo*. The residue was partitioned between ethyl acetate (20 mL) and water (20 mL). The aqueous layer ws further extracted with ethyl acetate (2 x 10 mL) and the combined organic extracts dried (Na_2SO_4) and evaporated *in vacuo*. Trituration of the residue with petroleum ether afforded the product (440 mg, 81%) as a colourless solid, *ee* >98%.

(*S*)-Ethyl 2-(1-(*N*-(*tert*-butoxycarbonyl)-2,2,2-trifluoroacetamido)-2-methylpropyl)thiazole-4-carboxylate (134) [Nicolaou, Table 4, entry 5]



134

Sodium hydrogen carbonate (868 mg, 10.33 mmol, 8 equiv) was added to a solution of (*S*)-*N*-*tert*-(butoxycarbonyl)thiovalinamide (300 mg, 1.29 mmol) in anhydrous 1,2-dimethoxyethane (15 mL). Ethyl bromopyruvate (0.49 mL, 755 mg, 3.87 mmol, 3 equiv) was added and the solution stirred for 16 h at room temperature. The mixture was evaporated *in vacuo* and the residue partitioned between water (30 mL) and diethyl ether (15 mL). The aqueous layer was further extracted with diethyl ether (15 mL) and the combined organic extracts washed with brine (15 mL), dried (Na_2SO_4) and evaporated *in vacuo*. The residue was dissolved in anhydrous DME (15 mL) and the solution cooled to 0 °C. Pyridine (0.94 mL, 919 mg, 11.61 mmol, 9 equiv) was added slowly followed by dropwise addition of trifluoroacetic anyhdride (0.73 mL, 1.09 g, 5.16 mmol, 4 equiv) and the solution was stirred at 0 °C for 2 h. Triethylamine (0.36 mL, 261 mg, 2.58 mmol, 2 equiv) was added and the reaction mixture was warmed to room temperature and evaporated *in vacuo*. The residue was dissolved in chloroform (20 mL), washed with water (20 mL) and brine (10 mL), dried (Na_2SO_4) and evaporated *in vacuo*. Purification of the residue by column chromatography on silica eluting with petroleum ether-ethyl acetate (3:1) afforded the title compound (467 mg, 85%) as a yellow oil. [*ee* 88% after conversion to (*S*)-ethyl 2-(1-(*tert*-butoxycarbonylamino)-2-methylpropyl)thiazole-4-carboxylate (**102b**) by the method outlined above.]

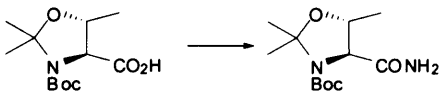
(4*S*,5*R*)-3-(*tert*-Butoxycarbonyl)-2,2,5-trimethyloxazolidine-4-carboxylic acid (135)[85]

135

N-Boc-L–threonine (7.50 g, 34.2 mmol) was dissolved in tetrahydrofuran (200 mL) and 2,2-dimethoxypropane (42.07 mL, 35.64 g, 342.2 mmol, 10 equiv) and PPTS (2.58 g, 10.3 mmol, 0.3 equiv) added. The solution was heated at reflux for 16 hours, allowed to cool and evaporated *in vacuo*. The residue was partitioned between ethyl acetate (200 mL) and water (200 mL) and the aqueous layer further extracted with EtOAc (2 x 100 mL). The combined organic extracts were washed with brine (100 mL), dried (Na_2SO_4) and evaporated *in vacuo* to furnish the title compound (8.81 g, 99%) as an off-white solid, mp 91–92 °C (chloroform) (Found: $[M+H]^+$

260.1491. Calc. for $C_{12}H_{22}NO_5$: 260.1492); $[\alpha]_D^{22}$ –60.0 (*c* 1.00, chloroform); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3468, 3067, 2923, 1754, 1666, 1459, 1367, 1133, 988, 949, 856, 783, 722; δ_H(400 MHz; $CDCl_3$) 10.47 (1 H, br s, exch. D_2O, O*H*), 4.16 (1 H, dq, *J* 8.0, 6.0, 5–H), 3.93 (0.38 H, d, *J* 8.0, 4–H), 3.86 (0.62 H, d, *J* 8.0, 4–H), 1.59 (1.86 H, s, 2–Me), 1.53 (1.14 H, s, 2–Me), 1.51 (1.86 H, s, 2–Me), 1.48 (1.14 H, s, 2–Me), 1.42 (3.43 H, s, CMe_3), 1.37 (3 H, d, *J* 6.0, 5–Me), 1.34 (5.57 H, s, CMe_3); δ_C(100 MHz; $CDCl_3$) 177.1 (C), 175.9 (C), 152.2 (C), 150.9 (C), 95.3 (C), 94.7 (C), 81.3 (C), 80.9 (C), 73.9 (CH), 73.5 (CH), 66.0 (CH), 65.9 (CH), 28.3 (CH_3), 28.2 (CH_3), 27.7 (CH_3), 26.5 (CH_3), 24.8 (CH_3), 23.9 (CH_3), 18.9 (CH_3); *m/z* (APCI) 260 ($[M+H]^+$, 5%), 204 (42), 160 (100), 146 (32).

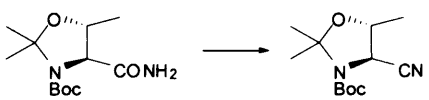
(4*S*,5*R*)-*tert*-Butyl 4-carbamoyl-2,2,5-trimethyloxazolidine-3-carboxylate (136)[85]



136

To a stirred solution of (4*S*,5*R*)-3-(*tert*-butoxycarbonyl)-2,2,5-trimethyloxazolidine-4-carboxylic acid (8.80 g, 33.9 mmol) and triethylamine (4.97 mL, 3.61 g, 35.63 mmol, 1.05 equiv) in anhydrous tetrahydrofuran (200 mL) at 0 °C was added ethyl chloroformate (3.41 mL, 3.87 g, 35.63 mmol, 1.05 equiv.) and the mixture stirred for 1 h. Aqueous ammonia (35%, 100 mL) was added and the solution was allowed to warm to room temperature and stirred for 24 hours. The organic solvent was removed *in vacuo* and the resulting aqueous solution extracted with ethyl acetate (3 x 100 mL). The combined organic extracts were washed sequentially with water (100 mL) and brine (100 mL), dried (Na_2SO_4) and evaporated *in vacuo* to yield the title compound (6.77 g, 77%) as a colourless solid, mp 148–150 °C (chloroform) (Found: $[M+H]^+$ 259.1654. Calc. for $C_{12}H_{23}N_2O_4$: 259.1652); $[\alpha]_D^{22}$ –45.4 (*c* 1.00, chloroform); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3412, 3217, 2922, 1694, 1634, 1456, 1377, 1268, 1221, 1173, 1135, 1089, 987, 942, 861, 784; δ_H(400 MHz; $CDCl_3$) 6.60-5.80 (2 H, br s, exch. D_2O, 2 NH), 4.16 (1 H, app. br s, 5–H), 3.70 (1 H, app. br s, 4–H), 1.55 (3 H, s, 2–Me), 1.52 (3 H, s, 2–Me), 1.39 (9 H, s, CMe_3), 1.33 (3 H, d, *J* 6.1, 5–Me); δ_C(100 MHz; $CDCl_3$) 172.7 (C), 94.8 (C), 81.1 (C), 74.2 (CH), 67.4 (CH), 28.3 (CH_3), 25.3 (CH_3), 18.9 (CH_3); *m/z* (APCI) 259 ($[M+H]^+$, 25%), 221 (17), 204 (22), 203 (70), 159 (100), 145 (5).

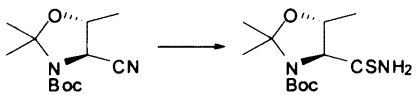
(4*R*,5*R*)-*tert*-Butyl 4-cyano-2,2,5-trimethyloxazolidine-3-carboxylate (137)



137

(4*S*,5*R*)-*tert*-Butyl 4-carbamoyl-2,2,5-trimethyloxazolidine-3-carboxylate (6.70 g, 25.9 mmol) was dissolved in pyridine (150 mL) and the solution cooled to 0 °C. Phosphorus oxychloride (6.04 mL, 9.94 g, 64.9 mmol, 2.5 equiv) was added and the solution stirred at 0 °C for 3 hours. The mixture was poured over ice then diluted with water (150 mL) and extracted with diethyl ether (3 x 100 mL). The combined organic extracts were washed sequentially with hydrochloric acid (2M, 150 mL), water (3 x 150 mL), brine (100 mL), dried (Na_2SO_4) and evaporated *in vacuo* to afford the title compound (4.88 g, 78%) as a colourless solid, mp 44–47 °C (Et_2O) (Found: $[M+H]^+$ 241.1547. Calc. for $C_{12}H_{21}N_2O_3$: 241.1547); $[\alpha]_D^{22}$ –69.4 (*c* 1.00, chloroform); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3058, 2982, 2936, 2245, 1713, 1477, 1458, 1367, 1318, 1266, 1216, 1167, 1132, 1096, 993, 961, 936, 857, 776; δ_H(400 MHz; $CDCl_3$) 4.37 (1 H, m, 5–H), 4.04 (0.3 H, br d, *J* 6.1, 4–H), 3.96 (0.7 H, d, *J* 7.3, 4-H), 1.61 (3 H, s, 2–Me), 1.56 (3 H, s, 2–Me), 1.45 (9 H, s, CMe_3), 1.38 (3 H, d, *J* 6.1, 5–Me); δ_C(100 MHz; $CDCl_3$) 150.5 (C), 117.2 (C), 95.8 (C), 95.2 (C), 82.2 (C), 74.1 (CH), 73.9 (CH), 53.0 (CH), 30.3 (CH_3), 29.7 (CH_3), 28.3 (CH_3), 27.8 (CH_3), 26.5 (CH_3), 25.4 (CH_3), 24.5 (CH_3), 18.3 (CH_3); *m/z* (CI+) 259 ($[M+NH_4]^+$, 100%), 241 ($[M+H]^+$, 40%), 202 (12), 125 (10), 114 (50), 58 (20).

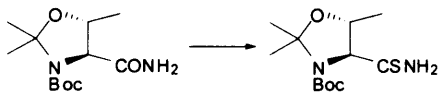
(4*S*,5*R*)-*tert*-Butyl 4-carbamothioyl-2,2,5-trimethyloxazolidine-3-carboxylate (138)[85]



138

(4*R*,5*R*)-*tert*-Butyl 4-cyano-2,2,5-trimethyloxazolidine-3-carboxylate (4.80 g, 20.0 mmol) was dissolved in methanol (100 mL) and ammonium sulfide (50 wt% in H_2O, 5.44 mL, 40.0 mmol, 2 equiv) added. The solution was stirred at room temperature for 24 hours then evaporated *in vacuo*. The residue was partitioned between ethyl acetate (100 mL) and water (100 mL) and the aqueous layer further extracted with ethyl acetate (2 x 50 mL). The combined organic extracts were dried (Na_2SO_4) and evaporated *in vacuo* to yield the title compound (5.40 g, 99%) as a pale yellow solid, mp 106–108 °C (Et_2O) (Found: $[M+H]^+$ 275.1424. Calc. for $C_{12}H_{23}N_2O_3S$: 275.1424); $[\alpha]_D^{22}$ –8.2 (*c* 1.00, chloroform) {lit.,[85] –9.8 (*c* 0.19, chloroform)}; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3307, 3196, 2925, 1672, 1619, 1443, 1368, 1265, 1139, 1090, 978, 940, 855, 736; δ_H(400 MHz; $CDCl_3$) 7.86 (2 H, br s, exch. D_2O, 2 NH), 4.21 (1H, d, *J* 7.7, 5–H), 4.06 (1 H, br s, 4–H), 1.57 (6 H, s, both 2–Me), 1.36 (12 H, app. s, CMe_3 and 5-Me); δ_C(100 MHz; $CDCl_3$) 205.6 (C), 152.2 (C), 125.5 (CH), 94.8 (C), 81.6 (C), 73.9 (CH), 30.3 (CH_3), 28.3 (CH_3), 26.2 (CH_3), 19.2 (CH_3); *m/z* (ES+) 297 ($[M+Na]^+$, 7%), 219 (12), 161 (100), 133 (27), 116 (40).

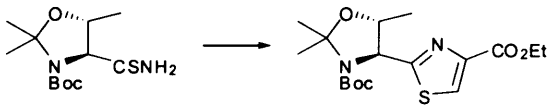
(4*S*,5*R*)-*tert*-Butyl 4-carbamothioyl-2,2,5-trimethyloxazolidine-3-carboxylate (138)



138

To a stirred solution of (4*S*,5*R*)-*tert*-butyl 4-carbamoyl-2,2,5-trimethyloxazolidine-3-carboxylate (12.90 g, 49.94 mmol) in toluene (500 mL) at 80 °C, Lawesson's reagent (10.50 g, 25.97 mmol, 0.52 eq) was added and the mixture stirred at 80 °C for 1.5 hours. The solvent was removed *in vacuo* and the residue purified by chromatography on silica, furnishing the title compound (3.26 g, 24% as a pale yellow solid (R_f 0.28, petroleum ether-diethyl ether 3:7).

(4*S*,5*R*)-*tert*-Butyl 4-(4-(ethoxycarbonyl)thiazol-2-yl)-2,2,5-trimethyloxazolidine-3-carboxylate (132) [Nicolaou, Table 7, entry 3]

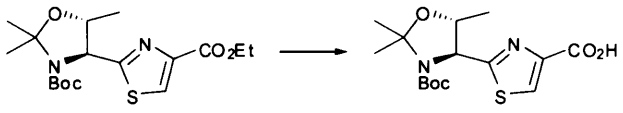


132

Sodium hydrogen carbonate (20.82 g, 247.81 mmol, 8 equiv) was added to a solution of (4*S*,5*R*)-*tert*-butyl 4-carbamothioyl-2,2,5-trimethyloxazolidine-3-carboxylate (8.50 g, 30.98 mmol) in anhydrous 1,2-dimethoxyethane (150 mL). Ethyl bromopyruvate (11.66 mL, 18.12 g, 92.93 mmol, 3 equiv) was added and the solution was stirred for 16 h at room temperature. The mixture was evaporated *in vacuo* and the residue partitioned between water (150 mL) and diethyl ether (150 mL). The aqueous layer was further extracted with diethyl ether (75 mL) and the combined organic extracts washed with brine (150 mL), dried (Na_2SO_4) and evaporated *in vacuo*. The residue was dissolved in anhydrous DME (150 mL) and the solution cooled to 0 °C. Pyridine (22.55 mL, 22.05 g, 278.79 mmol, 9 equiv) was added slowly followed by dropwise addition of trifluoroacetic anyhdride (17.53 mL, 26.06 g, 123.91 mmol, 4 equiv) and the solution was stirred at 0 °C for 2 h. Triethylamine (8.64 mL, 6.27 g, 61.95 mmol, 2 equiv) was added and the reaction mixture warmed to room temperature and evaporated *in vacuo*. The residue was dissolved in chloroform (200 mL), washed with water (200 mL) and brine (100 mL), dried (Na_2SO_4) and evaporated *in vacuo*. Purification of the residue by column chromatography on silica eluting with petroleum ether-ethyl acetate (3:1) afforded the title compound (9.97 g, 87%) as an off-white solid, mp 97–100 °C (petroleum ether–ethyl acetate) (Found: $[M+H]^+$ 371.1633. Calc. for $C_{17}H_{27}N_2O_5S$: 371.1635); $[\alpha]_D^{22}$ –59.6 (*c* 1.00, chloroform) {lit.,[85] –51.0 (*c* 0.33, chloroform)}; R_f 0.25 (petroleum ether-ethyl acetate 3:1); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3400, 2982, 1720, 1699, 1476, 1369, 1302, 1261, 1240, 1214, 1136, 1096, 1020, 942, 784; δ_H(400 MHz; $CDCl_3$)

8.09 (1 H, s, 5'–H), 4.73 (1 H, br d, *J* 6.0, 4–H), 4.36 (2 H, q, *J* 6.7, OCH_2CH_3), 4.09 (1 H, br s, 5–H), 1.63 (6 H, s, both 2–Me), 1.36 (3 H, d, *J* 6.0, 4–Me), 1.33 (3 H, t, *J* 6.7, OCH_2CH_3), 1.12 (9 H, br s, CMe_3); δ_C(100 MHz; $CDCl_3$) 173.4 (C), 161.2 (C), 151.3 (C), 146.7 (C), 127.3 (CH), 95.3 (C), 80.7 (C), 77.9 (CH), 65.9 (CH), 61.5 (CH_2), 28.1 (CH_3), 26.5 (CH_3), 25.9 (CH_3), 17.9 (CH_3), 14.4 (CH_3); *m/z* (ES+) 393 ($[M+Na]^+$, 45%), 371 ($[M+H]^+$, 20%), 271 (100), 257 (75), 213 (52), 167 (30).

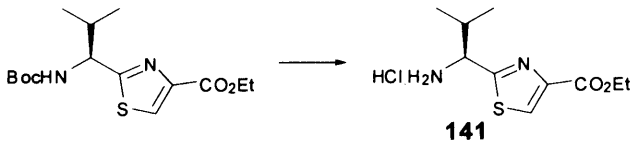
2-((4*S*,5*R*)-3-(*tert*-Butoxycarbonyl)-2,2,5-trimethyloxazolidin-4-yl)thiazole-4-carboxylic acid (140)



140

To a stirred solution of (4*S*,5*R*)-*tert*-butyl 4-(4-(ethoxycarbonyl)thiazol-2-yl)-2,2,5-trimethyloxazolidine-3-carboxylate (3.00 g, 8.10 mmol) in methanol-water (5:1, 150 mL) was added lithium hydroxide monohydrate (1.70 g, 40.50 mmol, 5 equiv) and the mixture stirred at room temperature for 3 h. The methanol was evaporated *in vacuo* and the resulting aqueous solution extracted with diethyl ether (2 x 50 mL). The aqueous layer was acidified to pH 3 using citric acid (10% w/v) and extracted with ethyl acetate (3 x 100 mL). The combined organic extracts were washed with water (100 mL) and brine (100 mL), dried (Na_2SO_4) and evaporated *in vacuo* to afford the title compound (2.77 g, 100%) as a colourless solid, mp 159–160 °C (chloroform) (Found: $[M+H]^+$ 343.1324. Calc. for $C_{15}H_{23}N_2O_5S$: 343.1322); $[\alpha]_D^{22}$ –63.00 (*c* 1.00, chloroform); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3400-2500 br, 3116, 3054, 2984, 1704, 1368, 1281, 1136, 1091, 984, 934, 895, 856; δ_H(400 MHz; $CDCl_3$) 10.48 (1 H, br s, OH), 8.23 (1H, s, 5–H), 4.73 (1 H, d, *J* 7.0, 4'–H), 4.12 (1 H, m, 5'–H), 1.63 (6 H, s, both 2'-Me), 1.37 (3 H, d, *J* 6.0, 5'–Me), 1.13 (9 H, s, CMe_3); δ_C(100 MHz; $CDCl_3$) 173.9 (C), 164.7 (C), 151.3 (C), 145.7 (C), 128.9 (CH), 95.4 (C), 80.9 (C), 77.8 (CH), 65.9 (CH), 28.1 (CH_3), 26.5 (CH_3), 25.9 (CH_3), 17.8 (CH_3); *m/z* (ES+) 365 ($[M+Na]^+$, 32%), 343 ($[M+H]^+$, 7%), 309 (25), 265 (19), 243 (96), 229 (100), 211 (42), 185 (88), 167 (89).

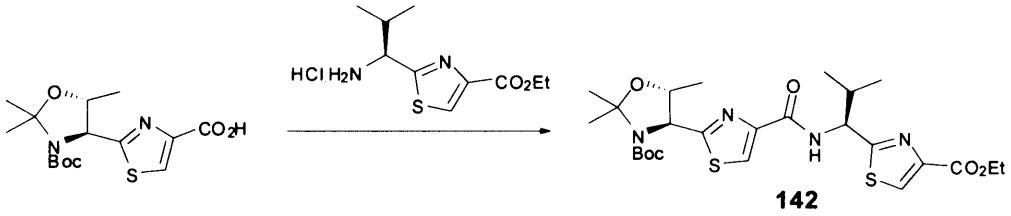
(*S*)-Ethyl 2-(1-amino-2-methylpropyl)thiazole-4-carboxylate hydrochloride (141)



141

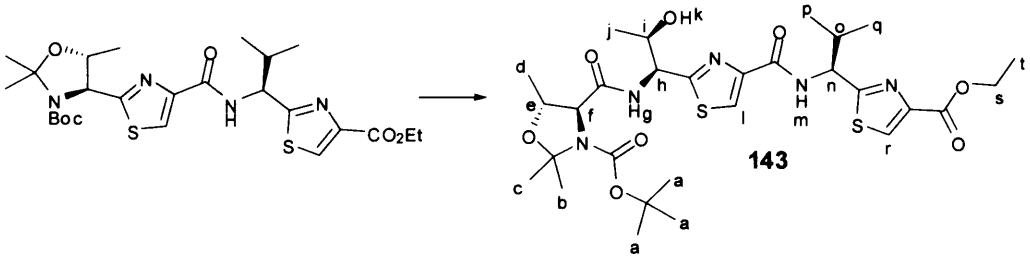
A solution of hydrogen chloride in dioxane (4 M, 1 mL) was added to (*S*)-ethyl-2-[1-(*N*-*tert*-butoxycarbonylamino)-2-methylpropyl]thiazole-4-carboxylate (70 mg, 0.21 mmol) and the solution stirred at room temperature for 2 hours. Toluene (1 mL) was added and the solvent removed *in vacuo*. Purification of the residue by trituration with diethyl ether (2 x 1 mL) afforded the title compound as an off-white solid, mp 110 °C (H_2O evolution), 159-162 ° (ether); δ_H(400 MHz; CD_3OD) 8.41 (1 H, s, 5-H), 4.56 (1 H, d, *J* 6.6, α-H), 4.30 (2 H, q, *J* 7.1, OCH_2CH_3), 2.28 (1 H, m, Me_2CH), 1.29 (3 H, t, *J* 7.1, OCH_2CH_3), 1.02 (3 H, d, *J* 6.8, *Me*CHMe), 0.90 (3 H, d, *J* 6.8); δ_C(100 MHz; CD_3OD) 163.7 (C), 159.5 (C), 145.1 (C), 127.7 (CH), 59.7 (CH_2), 55.6 (CH), 30.9 (CH), 15.6 (CH_3), 11.6 (CH_3); *m/z* (APCI) 229 ($[M+H]^+$, 100%), 212 (78), 158 (22), 72 (26).

(4*S*,5*R*)-*tert*-Butyl 4-(4-((*S*)-1-(4-(ethoxycarbonyl)thiazol-2-yl)-2-methylpropylcarbamoyl)thiazol-2-yl)-2,2,5-trimethyloxazolidine-3-carboxylate (142)



To a stirred solution of carboxylic acid **140** (71 mg, 208 μmol) and triethylamine (29 μL, 208 μmol, 1 equiv) in dry THF (2 mL) at 0 °C, ethyl chloroformate (20 μL, 208 μmol) was added and the mixture stirred for 1 hour. A solution of amine **141** (55 mg, 208 μmol) and triethylamine (29 μL) in dry THF (I mL) was added to the reaction mixture and the resulting solution stirred at room temperature for 2 hours then evaporated *in vacuo*. Purification of the residue on silica eluting with petroleum ether-ethyl acetate (1:1) furnished the title compound (94 mg, 82%) as a pale yellow solid, mp 106-108 °C (chloroform) (Found: $[M+NH_4]^+$, 570.2414. Calc. for $C_{25}H_{40}N_5O_6S_2$: 570.2415); $[\alpha]_D^{25}$ -46.4 (*c* 0.25, acetone); R_f 0.42 (petroleum ether-ethyl acetate 1:1); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3399, 3121, 2977, 2935, 2874, 1711, 1538, 1480, 1367, 1320, 1238, 1211, 1172, 1135, 1091, 1022, 921, 856; δ_H(400 MHz; $CDCl_3$) 8.05 (1 H, br s, Thr-thz H), 8.01 (1 H, s, Val-thz H) 7.89 (1 H, br d, exch. D_2O, *J* 9.3, NH), 5.27 (1 H, dd, *J* 9.3, 6.7, Val α-H), 4.70 (0.33 H, br s, Thr 4-H), 4.60 (0.67 H, br d, *J* 7.5, Thr 4-H), 4.35 (2 H, q, *J* 7.1, OCH_2CH_3), 4.12 (1 H, dq, *J* 7.5, 6.3, Thr 5-H), 2.58 (1 H, m, Me_2CH), 1.65 (6 H, br s, both Thr 2-Me), 1.38 (3 H, d, *J* 6.3, Thr 5-Me), 1.33 (3 H, t, *J* 7.1, OCH_2CH_3), 1.11 (9 H, s, CMe_3), 0.92 (6 H, d, *J* 6.8, *Me*CH*Me*); δ_C(125 MHz; $CDCl_3$) 161.3 (C), 147.6 (C), 126.9 (CH), 123,8 (CH), 77.8 (CH), 65.9 (CH), 61.4 (CH_2), 56.5 (CH), 33.0 (CH), 28.1 (CH_3), 25.5 (CH_3), 19.7 (CH_3), 17.1 (CH_3), 14.4 (CH_3); *m/z* (ES+) 575 ($[M+Na]^+$, 18%), 553 ($[M+H]^+$, 18%), 453 (100), 395 (5), 212 (5).

(4*S*,5*R*)-*tert*-Butyl 4-((1S,2*R*)-1-(4-((*S*)-1-(4-(ethoxycarbonyl)thiazol-2-yl)-2-methylpropylcarbamoyl)thiazol-2-yl)-2-hydroxypropylcarbamoyl)-2,2,5-trimethyloxazolidine-3-carboxylate (143)



PyBOP®-mediated coupling

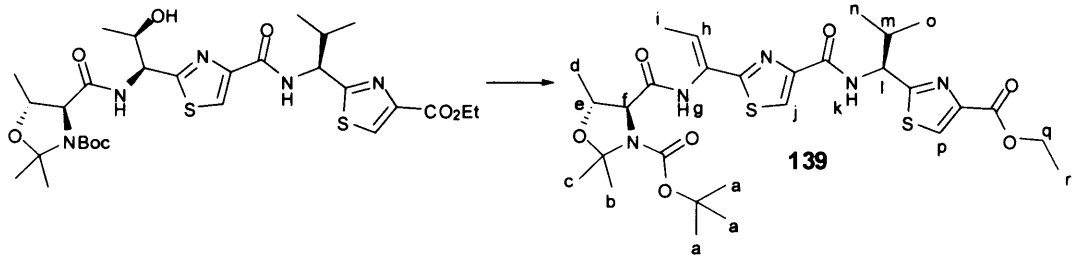
Peptide **142** (399 mg, 0.72 mmol) was dissolved in dichloromethane (2.5 mL) and trifluoroacetic acid (2.5 mL) added. The solution was stirred at room temperature for 1 hour then evaporated *in vacuo*. The residue was dissolved in dichloromethane (5 mL) and triethylamine (1.50 mL, 1.09 g, 10.76 mmol, 14.9 equiv) added. The resulting solution was added dropwise to a stirred solution of (4*S*,5*R*)-3-(*tert*-butoxycarbonyl)-2,2,5-trimethyloxazolidine-4-carboxylic acid (187 mg, 0.72 mmol, 1 equiv) and PyBOP® (413 mg, 0.79 mmol, 1.1 equiv) in dichoromethane (10 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred for 4 hours. Evaporation *in vacuo* and purification by chromatography on silica, eluting with ethyl acetate furnished the title compound (440 mg, 93%) as a yellow solid, mp 123-124 °C (chloroform) (Found: $[M+NH_4]^+$, 671.2892. Calc. for $C_{29}H_{47}N_6O_8S_2$: 671.2891); $[\alpha]_D^{25}$ -64.0 (*c* 0.20, acetone); R_f 0.40 (EtOAc); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3376, 3082, 2978, 2935, 1722, 1682, 1538, 1484, 1392, 1368, 1216, 1175, 1142, 1094, 1020, 917, 874; δ_H(400 MHz; d_6-acetone) 8.23 (1 H, s, **l**), 8.12 (1 H, br s, exch. D_2O, **g**) 8.05 (1 H, s, **r**), 7.76 (1 H, d, *J* 8.0, exch. D_2O, **m**), 5.16 (1 H, dd, *J* 9.0, 7.4, **h**), 5.12 (1 H, dd, *J* 8.0, 6.7, **n**), 4.34 (1 H, app. br m, **f**), 4.22 (2 H, q, *J* 7.1, **s**), 4.04 (2 H, app. br m, **e**, **i**), 2.41 (1 H, m, **o**), 1.45 (6 H, s, **b**, **c**), 1.42 (9 H, s, **a**), 1.29 (3 H, br d, *J* 5.3, **d**), 1.24 (3 H, t, *J* 7.1, **t**), 1.14 (3 H, br d, *J* 4.6, **j**), 0.92 (3 H, d, *J* 6.7, **p**), 0.86 (3 H, d, *J* 6.7, **q**); δ_C(125 MHz; $CDCl_3$) 161.3 (C), 147.3 (C), 127.0 (CH), 125.3 (CH), 77.8 (CH), 67.5 (CH), 61.5 (CH), 56.6 (CH), 33.8 (CH), 28.3 (CH_3), 25.6 (CH_3), 19.7 (CH_3), 18.2 (CH_3), 14.3 (CH_3); *m/z* (ES-) 652 ($[M-H]^+$, 25%), 608 (58), 534 (18), 476 (12), 312 (10), 183 (11), 145 (100), 77 (12).

HATU-mediated coupling

(4*S*,5*R*)-3-(*tert*-Butoxycarbonyl)-2,2,5-trimethyloxazolidine-4-carboxylic acid (199 mg, 0.77 mmol, 1.25 equiv) was dissolved in dichloromethane (10 mL) and diisopropylethylamine (0.54 mL, 398 mg, 3.08 mmol, 5 equiv) added. HATU (292 mg, 0.77 mmol, 1.25 equiv) was added and the solution stirred at room temperature for 1 hour before the dropwise addition of a solution of *N*-deprotected **142** (324 mg, 0.62 mmol, 1 equiv) and diisopropylethylamine (0.54 mL, 398 mg, 3.08 mmol, 5 equiv) in dichloromethane (5 mL). The reaction mixture was stirred at room

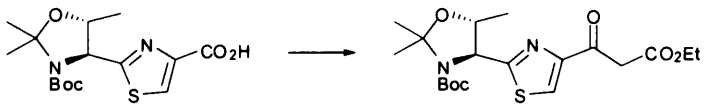
temperature for 4 hours, evaporated *in vacuo* and the residue purified by chromatography on silica, eluting with ethyl acetate to give the title compound (338 mg, 84%) as a yellow solid.

(4*S*,5*R*)-*tert*-Butyl 4-((*Z*)-1-(4-((*S*)-1-(4-(ethoxycarbonyl)thiazol-2-yl)-2-methylpropylcarbamoyl)thiazol-2-yl)prop-1-enylcarbamoyl)-2,2,5-trimethyloxazolidine-3-carboxylate (139)



Peptide **143** (323 mg, 0.49 mmol) was dissolved in dichloromethane (15 mL) and the solution cooled to 0 °C. Triethylamine (1.03 mL, 750 mg, 7.41 mmol, 15 equiv) was added, followed by dropwise addition of methanesulfonyl chloride (0.38 mL, 566 mg, 4.94 mmol, 10 equiv). The solution was stirred at room temperature for 16 hours, evaporated *in vacuo* and partitioned between chloroform (20 mL) and water (10 mL). The organic layer was washed with saturated aqueous sodium hydrogen carbonate (10 mL), brine (10 mL) and dried (Na_2SO_4). DBU (0.22 mL, 226 mg, 1.48 mmol, 3 equiv) was added and the solution stirred at room temperature for 2 hours. Evaporation *in vacuo* and purification on silica eluting with ethyl acetate furnished the title compound (307 mg, 98%) as a pale yellow solid, mp 79-80 °C (chloroform) (Found: $[M+Na]^+$, 658.2334. Calc. for $C_{29}H_{41}N_5O_7S_2Na$: 658.2340); $[\alpha]_D^{25}$ +71.2 (*c* 0.25, acetone); R_f 0.60 (EtOAc); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3450, 3294, 2978, 2935, 1720, 1692, 1537, 1481, 1392, 1368, 1321, 1214, 1173, 1133, 1095, 1021, 918, 857; δ_H(400 MHz; $CDCl_3$) 8.02 (1 H, s, **j**), 7.95 (1 H, s, **p**) 7.84 (1 H, d, *J* 9.1, exch. D_2O, **k**), 7.66 (1 H, s, exch. D_2O, **g**), 6.60 (1 H, app br m, **h**), 5.23 (1 H, dd, *J* 9.1, 7.2, **l**), 4.35 (1 H, app. br m, **e**), 4.35 (2 H, q, *J* 7.1, **q**), 3.99 (1 H, d, *J* 7.2, **f**), 2.58 (1 H, m, **m**), 1.53 (6 H, s, **b**, **c**), 1.41 (3 H, app br m, **i**), 1.40 (9 H, s, **a**), 1.38 (3 H, t, *J* 7.1, **r**), 1.32 (3 H, d, *J* 6.9, **d**), 0.97 (3 H, d, *J* 6.7, **n**), 0.93 (3 H, d, *J* 6.7, **o**); δ_C(125 MHz; $CDCl_3$) 168.4 (C), 161.3 (C), 128.0 (CH), 127.0 (CH), 81.4 (C), 77.3 (CH), 76.6 (CH), 61.4 (CH), 56.7 (CH), 33.0 (CH), 31.5 (CH_3), 30.9 (CH_3), 28.4 (CH_3), 28.3 (CH_3), 26.2 (CH_3), 26.0 (CH_3), 19.7 (CH_3), 19.5 (CH_3), 18.4 (CH_3), 14.4 (CH_3); *m/z* (ES+) 658 ($[M+Na]^+$, 28%), 636 ($[M+H]^+$, 10), 580 (11), 536 (100), 261 (9).

(4''*S*,5''*R*)-*tert*-Butyl 4-(4-(3-ethoxy-3-oxopropanoyl)thiazol-2-yl)-2,2,5-trimethyloxazolidine-3-carboxylate (172)



172

i) Methyl magnesium bromide in diethyl ether (3 M, 6.02 mL, 18.05 mmol, 2.06 equiv) was added to a stirred suspension of potassium ethyl malonate (3.07 g, 18.05 mmol, 2.06 equiv), in dry THF (50 mL) at 0 °C. The mixture was stirred at 0 °C for 30 minutes then warmed to room temperature and stirred for a further 1.5 hours.

ii) Ethyl chloroformate (0.89 mL, 1.01 g, 9.29 mmol, 1.06 equiv) was added dropwise to a stirred solution of carboxylic acid **140** (3.00 g, 8.76 mmol) and triethylamine (1.29 mL, 0.94 g, 9.29 mmol, 1.06 equiv) in dry THF (100 mL) at 0 °C and stirred for 1 hour. The solution prepared in part i) was added and the resulting mixture stirred at room temperature for 2 h. The reaction was quenched with saturated aqueous NH_4Cl (100 mL) and the organic solvent evaporated *in vacuo*. The resulting solution was diluted with water (50 mL) and extracted with chloroform (3 x 75 mL) and the combined organic extracts washed sequentially with acetic acid (5% w/v; 100 mL), water (100 mL), saturated aqueous $NaHCO_3$ (100 mL) and brine (100 mL), dried (Na_2SO_4) and evaporated *in vacuo*. Purification by column chromatography on silica, eluting with light petroleum–EtOAc (1:1) gave the title compound as a pale yellow oil (2.61 g, 72%) (Found: $[M+H]^+$, 413.1738. Calc. for $C_{19}H_{29}N_2O_6S$: 413.1741); $[\alpha]_D^{22}$ –42.6 (*c* 1.00, chloroform); R_f 0.70 (EtOAc); ν_{max}(film)/cm^{-1} 3438, 2986, 2925, 1735, 1694, 1639, 1479, 1366, 1258, 1217, 1167, 1137, 1092, 1027; δ_H(500 MHz; $CDCl_3$) 12.01 (0.22 H, s, exch. D_2O, OH), 8.12 (0.78 H, s, 5'–H), 7.70 (0.22 H, s, 5'–H), 5.99 (0.22 H, s, 2–H), 4.69 (0.22 H, br s, 4''–H), 4.59 (1 H, m, 5''–H), 4.18 (0.4 H, q, *J* 7.1, OCH_2Me), 4.11 (1.6 H, q, *J* 7.1, OCH_2Me), 4.10 (0.78 H, br s, 4''–H), 4.05 (0.78 H, d, *J* 18.1, 2–*H*H), 3.98 (0.78 H, d, *J* 18.1, 2–H*H*), 1.7–1.0 (18H, 2'',2'',5''–Me and CMe_3), 1.28 (0.7 H, t, *J* 7.1, CH_2Me), 1.20 (2.3 H, t, *J* 7.1, CH_2Me); δ_C(125 MHz; $CDCl_3$) 187.5 (C), 173.4 (C), 172.4 (C), 171.2 (C), 167.5 (C), 166.6 (C), 164.8 (C), 153.4 (C), 152.3 (C), 151.3 (C), 149.6 (C), 125.9 (CH), 120.1 (CH), 95.4 (C), 95.2 (C), 94.8 (C), 89.6 (CH), 81.2 (C), 80.7 (C), 80.5 (C), 65.9 (CH), 65.7 (CH), 61.5 (CH_2), 61.2 (CH_2), 60.4 (CH_2), 49.0 (C), 46.9 (CH_2), 41.7 (CH_2), 29.1 (C), 28.1 (C), 26.5 (CH_3), 26.2 (CH_3), 26.1 (CH_3), 25.5 (CH_3), 17.7 (CH_3), 14.3 (CH_3), 14.1 (CH_3); *m/z* (CI+) 413 ($[M+H]^+$, 100%), 357 (12), 297 (10), 158 (8), 58 (18).

(4*S*,5*R*)-*tert*-Butyl 4-(4-(1-amino-3-ethoxy-3-oxoprop-1-enyl)thiazol-2-yl)-2,2,5-trimethyloxazolidine-3-carboxylate (169)

169

CEM Discover® (Table 11, entry 5)

β-Ketoester **172** (100 mg, 0.24 mmol) was dissolved in toluene-acetic acid (5:1, 3 mL) and ammonium acetate (2.24 g, 29.09 mmol, 120 equiv) added. The mixture was irradiated in a sealed tube at 150 °C with continuous cooling for 10 minutes using a CEM Discover® single mode microwave synthesiser (initial power 300 W). The mixture was cooled to room temperature, evaporated *in vacuo* and partitioned between water (20 mL) and ethyl acetate (10 mL). The aqueous layer was further extracted with ethyl acetate (2 x 10 mL) and the combined organic extracts washed sequentially with saturated aqueous sodium hydrogen carbonate (15 mL) and brine (15 mL), dried (Na_2SO_4) and evaporated *in vacuo*. Purification by column chromatography on silica, eluting with petroleum ether-ethyl acetate (3:1) gave the title compound (69 mg, 69%) as a viscous yellow oil (Found: $[M+H]^+$, 412.1899. Calc. for $C_{19}H_{29}N_3O_5S$: 412.1901); $[\alpha]_D^{25}$ -52.5 (*c* 1.50, acetone); R_f 0.34 (petroleum ether-ethyl acetate 3:1); ν_{max}(film)/cm^{-1} 3488, 3407, 3146, 2975, 1694, 1478, 1368, 1258, 1217, 1167, 1132, 1087; δ_H(500 MHz; $CDCl_3$) 7.54 (1 H, br s, thz), 7.10-6.60 (2 H, br s, exch. D_2O, NH) 5.12 (1 H, br s, C=C-*H*), 4.68 (0.4 H, br s, Thr 4-H), 4.57 (0.6 H, br d, *J* 4.2, Thr 4-H), 4.14 (1 H, m, Thr 5-H), 4.12 (2 H, q, *J* 7.0, OCH_2CH_3), 1.64 (7 H, br s), 1.40 (3 H, br s), 1.34 (3 H, br d, *J* 6.2, Thr 5-Me), 1.24 (3 H, t, *J* 7.0, OCH_2CH_3), 1.10 (6 H, br s); δ_C(125 MHz; $CDCl_3$) 171.9 (C), 170.4 (C), 151.8 (C), 151.2 (C), 150.6 (C), 124.9 (CH), 116.9 (CH), 95.2 (C), 81.4 (CH), 80.6 (C), 65.9 (CH), 58.9 (CH_2), 28.1 (CH_3), 26.5 (CH_3), 25.5 (CH_3), 17.7 (CH_3), 14.6 (CH_3); *m/z* (APCI) 412 ($[M+H]^+$, 7%), 356 (17), 312 (30), 285 (10), 241 (30), 227 (25), 183 (14), 134 (100).

Milestone BatchSYNTH® (Table 11, entry 6)

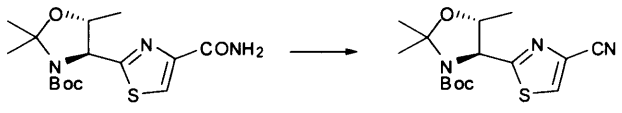
β-Ketoester **172** (1.00 g, 2.43 mmol) was dissolved in toluene-acetic acid (5:1, 30 mL) and ammonium acetate (22.44 g, 291.12 mmol, 120 equiv) added. The mixture was irradiated in a sealed 60 mL Teflon reaction vessel at 150 °C for 10 minutes using a Milestone BatchSYNTH® Dave multimodal microwave synthesiser (initial power 300 W). The mixture was cooled to room temperature, evaporated *in vacuo* and partitioned between water (200 mL) and ethyl acetate (50 mL). The aqueous layer was further extracted with ethyl acetate (2 x 25 mL) and the combined organic extracts washed sequentially with saturated aqueous sodium hydrogen carbonate (150 mL) and brine (100 mL), dried (Na_2SO_4) and evaporated *in vacuo*. Purification by column chromatography on silica, eluting with petroleum ether-ethyl acetate (3:1) gave the title compound (680 mg, 68%) as a viscous yellow oil.

(4*S*,5*R*)-*tert*-Butyl 4-(4-carbamoylthiazol-2-yl)-2,2,5-trimethyloxazolidine-3-carboxylate (173)

173

Carboxylic acid **140** (753 mg, 2.20 mmol) was dissolved in THF (50 mL) and triethylamine (0.32 mL, 234 mg, 2.31 mmol, 1.05 equiv) added. Ethyl chloroformate (0.221 mL, 251 mg, 2.31 mmol, 1.05 equiv) was added and the solution stirred at room temperature for 1 hour. Aqueous ammonia (35%, 50 mL) was added and the solution stirred vigorously at room temperature for 3 hours. The organic solvent was removed *in vacuo* and the resulting aqueous solution diluted with water (20 mL) and extracted with ethyl acetate (3 x 25 mL). The combined organic extracts were washed with brine (25 mL), dried (Na_2SO_4) and evaporated *in vacuo* to furnish the title compound (652 mg, 87%) as a colourless solid, mp 65 °C (EtOAc) (Found: $[M+H]^+$, 342.1485. Calc. for $C_{15}H_{24}N_3O_4S$: 342.1482); $[\alpha]_D^{24}$ –49.0 (*c* 1.02, chloroform); ν_{max}(KBr)/cm^{-1} 3465, 2979, 2925, 1701, 1591, 1458, 1367, 1308, 1261, 1173, 1135, 1089, 984, 946, 857, 757; δ_H(500 MHz; $CDCl_3$) 8.06 (1 H, s, 5–H), 7.08 (1 H, br s, exch D_2O, N*H*H) 6.39 (0.6 H, br s, exch D_2O, NH*H*), 6.26 (0.4 H, br s, exch D_2O, NH*H*), 4.65 (0.4 H, br s, 4'–H), 4.57 (0.6 H, d, *J* 7.3, 4'–H), 4.13 (1 H, m, 5'–H), 1.62 (6 H, s, both 2'-Me), 1.36 (3 H, d, *J* 5.0, 5'–Me), 1.12 (9 H, s, CMe_3); δ_C(125 MHz; $CDCl_3$) 172.1 (C), 171.2 (C), 163.1 (C), 149.1 (C), 124.0 (CH), 95.3 (C), 81.3 (C), 77.6 (CH), 65.8 (CH), 28.3 (CH_3), 26.5 (CH_3), 25.5 (CH_3), 21.1 (CH_3) ; *m/z* (ES+) 364 ($[M+Na]^+$, 36%), 308 (30), 286 (28), 242 (100), 228 (52), 184 (23), 166 (10).

(4*S*,5*R*)-*tert*-Butyl 4-(4-cyanothiazol-2-yl)-2,2,5-trimethyloxazolidine-3-carboxylate (174)

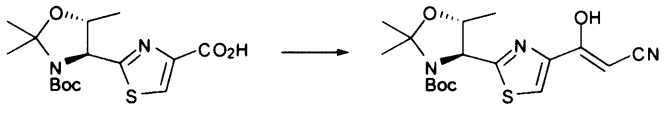


174

Amide **173** (600 mg, 1.76 mmol) was dissolved in pyridine (25 mL) and phosphorus oxychloride (0.41 mL, 674 mg, 4.39 mmol, 2.5 equiv) added. The solution was stirred at 0 °C for 2 hours then poured over ice. Water (50 mL) was added and the aqueous solution extracted with diethyl ether (3 x 20 mL). The combined organic extracts were washed with hydrochloric acid (2 M, 100 mL), water (100 mL) and brine (50 mL), dried (Na_2SO_4) and evaporated *in vacuo* to afford the title compound (271 mg, 48%) as a yellow oil (Found: $[M+H]^+$, 324.1376. Calc. for $C_{15}H_{22}N_3O_3S$: 342.1376); $[\alpha]_D^{23}$ –66.38 (*c* 0.93, chloroform); ν_{max}(film)/cm^{-1} 3020, 2250, 1703, 1369, 1215, 1135,

932, 760, 668; δ_H(400 MHz; $CDCl_3$) 7.95 (1 H, br s, 5–H), 4.72 (0.5 H, m, 4'–H), 4.65 (0.5 H, m, 4'–H), 4.13 (0.5 H, m, 5'–H), 4.10 (0.5 H, m, 5'–H), 1.65 (6 H, s, both 2'-Me), 1.40 (3 H, d, *J* 6.0, 5'–Me), 1.36 (9 H, s, CMe_3); δ_C(100 MHz; $CDCl_3$) 174.5 (C),173.1 (C), 152.3 (C), 151.1 (C), 130.3 (CH), 126.3 (C), 113.8 (C), 95.5 (C), 95.0 (C), 81.5 (C), 81.0 (C), 77.4 (CH), 76.7 (CH), 65.8 (CH), 65.4 CH), 28.1 (CH_3), 26.5 (CH_3), 26.2 (CH_3), 25.8 (CH_3), 17.9 (CH_3), 17.7 (CH_3); m/z (CI+) 324 ([M+H^+], 70%), 285 (35), 268 (30), 114 (15), 58 (100).

(4*S*,5*R*)-*tert*-Butyl 4-(4-((*Z*)-2-cyano-1-hydroxyvinyl)thiazol-2-yl)-2,2,5-trimethyloxazolidine-3-carboxylate (176)



176

Carboxylic acid **140** (175 mg, 0.51 mmol) was dissolved in dry acetonitrile (20 mL) and triethylamine (75 µL, 54 mg, 0.54 mmol, 1.05 equiv) added. Ethyl chloroformate (51 µL, 58 mg, 0.54 mmol, 1.05 equiv) was added and the solution stirred at room temperature for 2 hours. Sodium hydride (60% in mineral oil, 102 mg, 61 mg NaH, 2.56 mmol, 5 equiv) was added under an umbrella of nitrogen and the solution stirred at room temperature for a further 2 h then quenched with saturated aqueous ammonium chloride solution (10 mL) and evaporated *in vacuo*. The resulting aqueous solution was diluted with water (10 mL) and extracted with ethyl acetate (3 x 15 mL). The combined organic extracts were washed with brine (15 mL), dried (Na_2SO_4) and evaporated *in vacuo*. Purification of the residue on silica eluting with diethyl ether furnished the title compound (145 mg, 78%) as a beige solid, mp 63-65 °C (ether); $[\alpha]_D^{25}$ -48.3 (*c* 1.20, acetone); ν_{max}(KBr)/cm^{-1} 3518, 3468, 3397, 2976, 2252, 1683, 1574, 1368, 1258, 1132; δ_H(400 MHz; $CDCl_3$) 8.03 (1 H, s, 5–H), 7.08 (1 H, br s, exch D_2O, OH) 6.00 (1 H, br s, C*H*CN), 4.65 (0.3 H, br s, 4'–H), 4.56 (0.7 H, br d, *J* 6.3, 4'–H), 4.13 (1 H, br m, 5'–H), 1.62 (6 H, s, both 2'-Me), 1.36 (3 H, d, *J* 5.0, 5'–Me), 1.12 (9 H, s, CMe_3); δ_C(125 MHz; $CDCl_3$) 162.8 (C), 149.1 (C), 124.0 (CH), 95.3 (C), 77.6 (CH), 65.8 (CH), 28.1 (CH_3), 26.5 (CH_3), 25.5 (CH_3), 17.7 (CH_3).

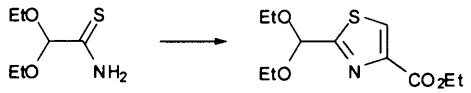
2,2-Diethoxythioacetamide (177)

177

2,2–Diethoxyacetonitrile (5.00 mL, 36.0 mmol) was dissolved in methanol (200 mL) and ammonium sulfide (50 wt. % in H_2O; 9.80 mL, 71.9 mmol) added. The solution was stirred at

room temperature for 18 h then evaporated *in vacuo* to give the title compound (5.87 g, 100%) as a pale yellow solid, mp 94–95 °C (Et_2O) {(lit.[143] mp 93–94 °C)} (Found: $[M+Na]^+$, 186.0560. Calc. for $C_6H_{13}NO_2SNa$: 186.0559); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3371, 3250, 2971, 2884, 1603, 1423, 1245, 1121, 1057, 1011, 960, 912, 823, 730; δ_H(500 MHz; $CDCl_3$) 7.81 (1 H, br s, exch D_2O, NH), 7.56 (1 H, br s, exch D_2O, NH), 4.98 (1 H, s, CH), 3.67 (2 H, dq, *J* 9.5, 7.0, 2 OC*H*HMe), 3.58 (2 H, dq, J 9.5, 7, 2 OCH*H*Me), 1.19 (6 H, t, *J* 7.0, 2 OCH_2*Me*); δ_C(125 MHz; $CDCl_3$) 202.4 (C), 103.2 (CH), 63.0 (CH_2), 15.1 (CH_3); m/z (CI+) 181 ($[M+NH_4]^+$, 20%), 164 ($[M+H]^+$, 65%), 132 (10), 103 (100), 88 (18), 74 (12).

Ethyl 2-(diethoxymethyl)thiazole-4-carboxylate (178)



178

Ethyl bromopyruvate (5.44 ml, 8.45 g, 43.3 mmol, 1.2 equiv) was added to a stirred solution of 2,2–diethoxythioacetamide (5.89 g, 36.1 mmol) in ethanol (100 ml) over 4Å molecular sieves (25 g) and the mixture heated at reflux for 1 h. The solution was allowed to cool, filtered through Celite® and evaporated *in vacuo*. The residue was dissolved in dichloromethane (200 mL) and the solution washed with saturated aqueous sodium hydrogen carbonate (200 mL), brine (200 mL), dried (Na_2SO_4) and evaporated *in vacuo* to furnish the title compound (9.36 g, 100%) as a brown oil, (Found: $[M+Na]^+$, 282.0769. Calc. for $C_{11}H_{17}NO_4SNa$: 282.0770); ν_{max}(film)/cm^{-1} 3113, 2979, 1735, 1484, 1369, 1312, 1241, 1205, 1061, 948, 905, 759; δ_H(400 MHz; $CDCl_3$) 8.14 (1 H, s, 5–H), 5.64 (1 H, s, CH), 4.36 (2 H, q, *J* 7.2, OCH_2Me), 3.69 (2 H, dq, *J* 9.6, 7.2, 2 OC*H*H), 3.59 (2 H, dq, *J* 9.6, 7.2, 2 OCH*H*), 1.34 (3 H, t, *J* 7.2, OCH_2*Me*), 1.19 (6 H, app t, *J* 7.2, 2 OCH_2*Me*); δ_C(100 MHz; $CDCl_3$) 170.2 (C), 161.4 (C), 147.1 (C), 128.5 (CH), 98.8 (CH), 62.8 (CH_2), 61.5 (CH_2), 15.1 (CH_3), 14.4 (CH_3); m/z (ES+) 282 ($[M+Na]^+$, 32%), 214 (72), 186 (93), 157 (41), 139 (100).

Ethyl 2-(dimethoxymethyl)thiazole-4-carboxylate (179)

179

Ethyl 2-(diethoxymethyl)thiazole-4-carboxylate (9.36 g, 36.1 mmol) was dissolved in methanol (250 mL) and *p*-toluenesulfonic acid (1.37 g, 7.22 mmol, 20 mol%). The solution was heated at reflux for 48 h, cooled to room temperature and evaporated *in vacuo*. The residue was dissolved

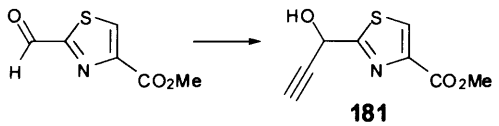
in ethyl acetate (100 mL) and the solution washed with saturated aqueous sodium hydrogen carbonate solution. The aqueous layer was extracted with ethyl acetate (2 x 100 mL) and the combined organic extracts washed with water (100 mL) and brine (100 mL), dried (Na_2SO_4) and evaporated *in vacuo* to give the title compound (7.51 g, 96%) as a red-brown oil (Found: $[M+H]^+$, 218.0480. Calc. for $C_8H_{12}NO_4S$: 218.0482); ν_{max}(film)/cm^{-1} 3118, 2954, 2835, 1729, 1488, 1435, 1327, 1247, 1216, 1068, 974, 919, 864, 754; δ_H(250 MHz; $CDCl_3$) 8.18 (1 H, s, 5–H), 5.54 (1 H, s, CH), 3.89 (3 H, s, OMe), 3.39 (6 H, s, 2 OMe); δ_C(125 MHz; $CDCl_3$) 169.0 (C), 161.8 (C), 147.0 (C), 128.7 (CH), 100.4 (CH), 54.0 (CH_3), 52.5 (CH_3); m/z (ES+) 218 ($[M+H]^+$, 58%), 186 (100).

Methyl 2-formylthiazole-4-carboxylate (180)

180

Methyl 2-(dimethoxymethyl)thiazole-4-carboxylate (7.50 g, 34.53 mmol) was dissolved in acetone (150 mL) and aqueous hydrochloric acid (2 M, 150 mL) added. The solution was heated at reflux for 1 hour, allowed to cool to room temperature and the acetone removed *in vacuo*. The remaining aqueous solution was extracted with chloroform (3 x 100 mL) and the combined organic extracts washed with brine (100 mL), dried (Na_2SO_4) and evaporated *in vacuo* to yield the title compound (5.19 g, 88%) as a yellow-brown solid, mp 111-114 °C (CH_2Cl_2) (Found: $[M+H]^+$, 172.0066. Calc. for $C_6H_6NO_3S$: 172.0063); ν_{max}(CH_2Cl_2)/cm^{-1} 3120, 2956, 2865, 1735, 1699, 1492, 1463, 1429, 1336, 1256, 1217, 1103, 989, 865, 790; δ_H (400 MHz; $CDCl_3$) 10.00 (1 H, s, CHO), 8.72 (1 H, s, 5-H), 3.96 (3 H, s, CO_2Me); δ_C(100 MHz; $CDCl_3$) 183.6 (CH), 166.2 (C), 161.0 (C), 149.1 (C), 133.3 (CH), 53.0 (CH_3); *m/z* (ES+) 172 ($[M+H^+]$, 13%), 158 (40), 140 (100).

Methyl 2-[1-(1-hydroxyprop-2-ynyl)]thiazole-4-carboxylate (181)

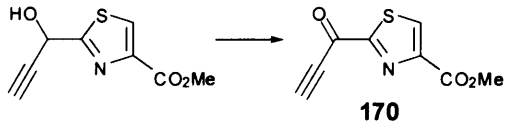


181

To a stirred solution of methyl 2-formylthiazole-4-carboxylate (4.50 g, 26.3 mmol) in anhydrous THF (125 mL) at 0 °C, ethynylmagnesium bromide (0.5 M in THF, 55.2 mL, 27.6 mmol, 1.05 equiv) was added and the mixture stirred at 0 °C for 1 hour. Saturated aqueous ammonium chloride (125 mL) was added and the THF removed *in vacuo*. The resulting aqueous solution was partitioned between chloroform (150 mL) and water (50 mL) and the aqueous layer was

further extracted with chloroform (2 x 75 mL). The combined organic extracts were washed with brine (100 mL), dried (Na_2SO_4) and evaporated *in vacuo*. The residue was purified by recrystallisation (CH_2Cl_2-Et_2O) to afford the title compound (4.19 g, 81%) as a pale brown solid, mp 109-110 °C ($CHCl_3$) (Found: $[M+H]^+$, 198.0217. Calc. for $C_8H_8NO_3S$: 198.0219); R_f 0.30 (petroleum ether-ethyl acetate 1:1); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3428, 3307, 3016, 2393, 2112, 1725, 1503, 1479, 1428, 1328, 1248, 1212, 1092, 1032, 992, 926; δ_H (400 MHz; $CDCl_3$) 8.13 (1 H, s, 5-H), 5.82 (1 H, s, 1'-H), 4.14 (1 H, s, OH), 3.88 (3 H, s, CO_2Me), 2.65 (1 H, d, *J* 2.4, 3'-H); δ_C(125 MHz; $CDCl_3$) 171.2 (C), 161.6 (C), 146.7 (C), 128.7 (CH), 80.9 (C), 75.8 (CH), 61.6 (CH), 52.6 (CH_3); *m/z* (APCI) 220 ([M+Na$^+$], 50%), 198 ([M+H$^+$], 23), 180 (95), 166 (100), 122 (8).

Methyl 2-(propynoyl)thiazole-4-carboxylate (170)



170

MnO_2 oxidation

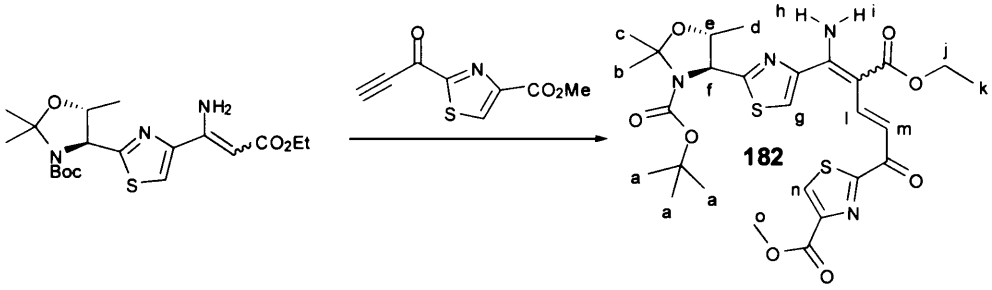
To a stirred solution of methyl 2-[1-(1-hydroxyprop-2-ynyl)]thiazole-4-carboxylate (882 mg, 4.47 mmol) in dichloromethane (50 mL), activated manganese(IV) oxide (1.94 g, 22.36 mmol, 5 equiv) was added and the mixture stirred at room temperature for 30 minutes. A further portion of activated manganese(IV) oxide (1.94 g, 22.36 mmol, 5 equiv) was added and the solution stirred at room temperature for 2 hours then filtered through a pad of Celite® and the pad washed with dichloromethane (25 mL). The solvent was evaporated under a stream of nitrogen and the residue (672 mg, 77%) used immediately without further purification. Recrystallisation afforded the *title compound* as a yellow solid, mp 152 °C (dec.) ($CHCl_3$) (Found: $[M+H]^+$, 196.0068. Calc. for $C_8H_6NO_3S$: 196.0059); R_f 0.40 (petroleum ether-ethyl acetate 1:1); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3209, 3128, 2360, 1742, 1641, 1480, 1461, 1427, 1341, 1265, 1240, 1109, 993, 960, 911, 827; δ_H (250 MHz; $CDCl_3$) 8.46 (1 H, s, 5-H), 3.93 (3 H, s, CO_2Me), 3.66 (1 H, s, 3'-H); δ_C(125 MHz; $CDCl_3$) 169.0 (C), 166.2 (C), 161.1 (C), 149.2 (C), 133.8 (CH), 84.9 (CH), 78.9 (C), 52.8 (CH_3); *m/z* (ES+) 196 ([M+H$^+$], 100%), 156 (15).

IBX oxidation

Methyl 2-[1-(1-hydroxyprop-2-ynyl)]thiazole-4-carboxylate (3.50 g, 17.75 mmol) was dissolved in DMSO (35 mL) and the solution added to a homogenous solution of IBX (12.43 g, 44.37 mmol, 2.5 equiv) in DMSO (315 mL) and the mixture stirred at room temperature for 16 hours. Water (350 mL) was added and the mixture stirred at 0 °C for 10 minutes then partitioned between water (200 mL) and ether (200 mL). The aqueous layer was extracted with ether (4 x 200 mL) and the combined organic extracts washed with water (3 x 200 mL), saturated aqueous sodium hydrogen carbonate (200 mL) and brine (200 mL), dried (Na_2SO_4) and evaporated *in vacuo* to

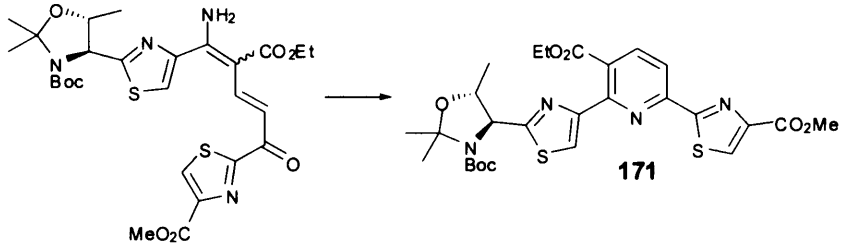
furnish the title compound (1.19 g, 34%) as a yellow-orange solid which was used immediately without further purification.

(4*S*,5*R*)-*tert*-Butyl 4-(4-((3*E*)-1-amino-2-(ethoxycarbonyl)-5-(4-(methoxycarbonyl)thiazol-2-yl)-5-oxopenta-1,3-dienyl)thiazol-2-yl)-2,2,5-trimethyloxazolidine-3-carboxylate (182)



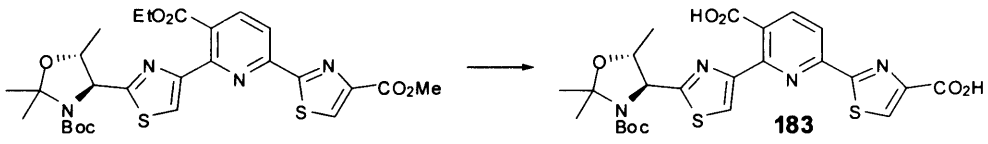
To a stirred suspension of methyl 2-(propynoyl)thiazole-4-carboxylate (670 mg, 3.43 mmol, 1.5 equiv) in ethanol (75 mL) was added a solution of enamine **169** (920 mg, 2.24 mmol, 1 equiv) in ethanol (25 mL) and the mixture stirred at room temperature for 24 h. The solvent was evaporated *in vacuo*. Purification by column chromatography on silica, eluting with petroleum ether–ethyl acetate (1:1) afforded the title compound (800 mg, 58%) as a bright yellow-orange foam, mp 70-72 °C (EtOAc-petrol); (Found: $[M+H]^+$, 607.1898. Calc. for $C_{27}H_{35}N_4O_8S_2$: 607.1891); $[\alpha]_D^{25}$ –45.2 (*c* 1.00, $CHCl_3$); R_f 0.45 (petroleum ether-ethyl acetate 1:1); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3488, 3406, 3128, 2976, 1695, 1641, 1478, 1461, 1368, 1265, 1218, 1167, 1132, 1087; δ_H (400 MHz; $CDCl_3$) 9.71 (1 H, br s, exch D_2O, **i**), 8.31 (1 H, s, **n**), 8.22 (1 H, d, *J* 15.3, **l**), 7.82 (1 H, d, *J* 15.3, **m**), 7.72 (0.5 H, br s, **g**), 7.64 (0.5 H, br s, **g**), 6.66 (1 H, br s, exch D_2O, **h**), 4.70 (0.4 H, br s, **f**), 4.60 (0.6 H, br d, *J* 7.5, **f**), 4.34 (2 H, q, *J* 7.1, **j**), 4.16 (1 H, m, **e**), 3.91 (3 H, s, **o**), 1.64 (6 H, app br s, **b**, **c**), 1.48 (3 H, t, *J* 7.1, **k**), 1.41 (3 H, br s), 1.36 (4 H, br s) 1.12 (5 H, br s); δ_C(100 MHz; $CDCl_3$) 181.8 (C), 170.7 (C), 169.7 (C), 161.7 (C), 151.1 (C), 148.0 (CH), 147.8 (CH), 144.6 (C), 144.2 (C), 132.5 (CH), 125.6 (CH), 115.3 (CH), 114.6 (CH), 97.0 (C), 95.4 (C), 94.9 (C), 81.4 (C), 80.8 (C), 76.8 (CH), 65.9 (CH), 60.8 (CH_2), 52.7 (CH_3), 52.5 (CH_3), 28.1 (CH_3), 26.5 (CH_3), 26.2 (CH_3), 25.6 (CH_3), 18.0 (CH_3), 17.7 (CH_3), 14.2 (CH_3); *m/z* (ES+) 629 ([M+Na^+], 70%), 607 ([M+H^+], 100%), 407 (8), 385 (20), 363 (40), 341 (50), 285 (30), 263 (13), 241 (45).

(4*S*,5*R*)-*tert*-Butyl 4-(4-(3-(ethoxycarbonyl)-6-(4-(methoxycarbonyl)thiazol-2-yl)pyridin-2-yl)thiazol-2-yl)-2,2,5-trimethyloxazolidine-3-carboxylate (171)



Aminodienone **182** (650 mg, 1.07 mmol) was dissolved in ethanol (100 mL) and iodine (136 mg, 0.54 mmol, 0.5 equiv) added. The solution was stirred at room temperature for 2 hours, evaporated *in vacuo* and partitioned between ethyl acetate (50 mL) and aqueous sodium thiosulfate (1 M, 50 mL). The aqueous layer was further extracted with ethyl acetate (2 x 25 mL) and the combined organic extracts washed with water (50 mL), brine (50 mL), dried ($MgSO_4$) and evaporated *in vacuo* to furnish the title compound (630 mg, 100%) as a viscous oil (Found: $[M+H]^+$, 589.1788. Calc. for $C_{27}H_{33}N_4O_7S_2$: 589.1785); $[\alpha]_D^{25}$ -73.6 (*c* 0.50, acetone); R_f 0.30 (petroleum ether-ethyl acetate 1:1); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3117, 2980, 1728, 1583, 1564, 1478, 1454, 1435, 1367, 1249, 1218, 1172, 1134, 1094, 1021, 991, 913, 856, 763, 733; δ_H(400 MHz; $CDCl_3$) 8.26 (1 H, s, 6-thz), 8.21 (1 H, d, *J* 8.0, pyr-4), 8.05 (1 H, br s, 2-thz), 7.91 (1 H, d, *J* 8.0, pyr-5), 4.69 (0.4 H, br s, Thr 4-H), 4.61 (0.6 H, br s, Thr 4-H), 4.31 (2 H, br q, *J* 6.9, OCH_2CH_3), 4.15 (1 H, m, Thr 5-H), 3.93 (3 H, s, OCH_3), 1.65 (6 H, br s, both Thr 2-Me), 1.50-1.15 (15 H, many br s, Thr 5-Me, CMe_3, OCH_2CH_3); δ_C(100 MHz; $CDCl_3$) 161.8 (C), 152.9 (C), 150.5 (C), 148.2 (C), 137.9 (CH), 130.3 (CH), 129.1 (CH), 117.9 (CH), 77.3 (CH), 65.9 (CH), 61.8 (CH_2), 52.6 (CH_3), 28.2 (CH_3), 14.6 (CH_3); *m/z* (ES+) 611 ($[M+Na]^+$, 100%), 589 ($[M+H]^+$, 72%), 533 (10), 489 (73).

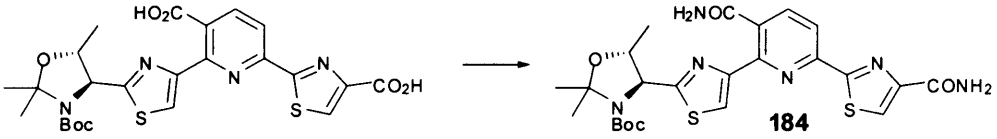
2-(6-(2-((4*S*,5*R*)-3-(*tert*-Butoxycarbonyl)-2,2,5-trimethyloxazolidin-4-yl)thiazol-4-yl)-5-carboxypyridin-2-yl)thiazole-4-carboxylic acid (183)



Pyridine bis-ester **171** (1.30 g, 2.21 mmol) was dissolved in methanol-water (5:1, 120 mL) and lithium hydroxide monohydrate (1.85 g, 44.17 mmol, 20 equiv) added. The solution was heated at reflux for 60 hours, allowed to cool to room temperature and the methanol removed *in vacuo*. The resulting aqueous solution was diluted with water (30 mL) and extracted with ethyl acetate (50 mL). The aqueous layer was acidified to pH 3 (10% w/w aq $KHSO_4$) and extracted with ethyl

acetate (4 x 50 mL). The combined organic extracts were dried ($MgSO_4$) and evaporated *in vacuo* to yield the title compound (1.21 g, 100%) as an orange viscous oil (Found: $[M+H]^+$, 547.1326. Calc. for $C_{24}H_{27}N_4O_7S_2$: 547.1321); $[\alpha]_D^{25}$ -54.8 (*c* 0.50, acetone); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3500-2500 br, 3121, 2980, 2934, 2625, 1713, 1584, 1566, 1504, 1369, 1258, 1170, 1138, 1095, 1023, 984, 912, 856, 772, 732; δ_H(400 MHz; $CDCl_3$) 10.0-9.2 (2 H, br s, 2 CO_2H), 8.73 (1 H, br s, 6-thz), 8.48 (1 H, app br s, pyr-4), 8.40 (1 H, s, 2-thz), 8.35 (1 H, app br s, pyr-5), 4.86 (0.5 H, br s, Thr 4-H), 4.79 (0.5 H, br s, Thr 4-H), 4.28 (1 H, app br s, Thr 5-H), 1.75 (6 H, br s, both Thr 2-Me), 1.45 (9 H, br s, CMe_3), 1.25 (3 H, app br s, Thr 5-Me); δ_C(125 MHz; $CDCl_3$) 168.4 (C), 151.9 (C), 148.0 (C), 147.9 (C), 144.1 (CH), 131.5 (CH), 119.0 (CH), 77.3 (CH), 65.2 (CH), 30.9 (CH_3), 28.3 (CH_3), 20.7 (CH_3), 17.7 (CH_3); *m/z* (ES+) 564 ($[M+NH_4]^+$, 100%), 547 ($[M+H]^+$, 98%), 524 (18), 507 (62).

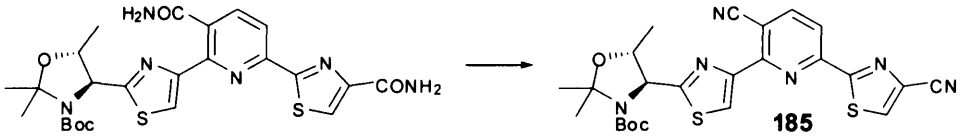
(4*S*,5*R*)-*tert*-Butyl 4-(4-(3-carbamoyl-6-(4-carbamoylthiazol-2-yl)pyridin-2-yl)thiazol-2-yl)-2,2,5-trimethyloxazolidine-3-carboxylate (184)



184

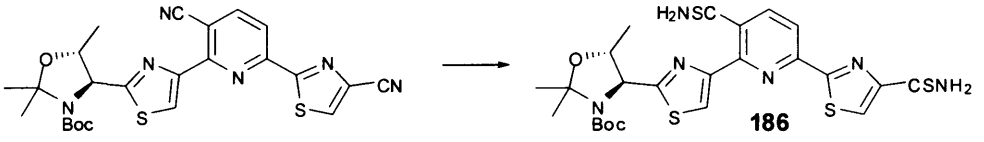
Pyridine bis-acid **183** (1.15 g, 2.10 mmol) was dissolved in THF (100 mL) and triethylamine (0.616 mL, 447 mg, 4.42 mmol, 2.10 equiv) added. Ethyl chloroformate (0.422 mL, 479 mg, 4.42 mmol, 2.10 equiv) was added and the solution stirred at room temperature for 18 hours. Aqueous ammonia (35%, 100 mL) was added and the solution stirred vigorously at room temperature for 24 hours. The organic solvent was removed *in vacuo* and the resulting aqueous solution filtered and the solid obtained dried in a desiccator under reduced pressure to furnish the title compound (710 mg, 62%) as a salmon-coloured solid, mp 139-141 °C (acetone) (Found: $[M+H]^+$, 545.1634. Calc. for $C_{24}H_{29}N_6O_5S_2$: 545.1635); $[\alpha]_D^{22}$ -20.4 (*c* 0.5, methanol); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3367, 3167, 2925, 2845, 1694, 1659, 1584, 1458, 1373, 1303, 1253, 1127, 856; δ_H(400 MHz; d_6-acetone) 8.21 (1 H, s, 6-thz), 8.12 (1 H, d, *J* 7.9, pyr-4), 8.10 (1 H, br s, exch D_2O, NH), 7.92 (1 H, d, *J* 7.9 pyr-5), 7.64 (1 H, br s, exch D_2O, NH), 7.21 (1 H, br s, exch D_2O, NH), 6.82 (1 H, br s, 2-thz), 6.68 (1 H, br s, exch D_2O, NH), 4.56 (1 H, d, *J* 7.6, Thr 4-H), 4.16 (1 H, app br s, Thr 5-H), 1.54 (3 H, s, Thr 2-Me), 1.52 (3 H, s, Thr 2-Me), 1.28 (9 H, s, CMe_3), 1.04 (3 H, app br s, Thr 5-Me); δ_C(125 MHz; DMSO) 167.7 (C), 138.5 (CH), 127.3 (CH), 118.6 (CH), 81.3 (C), 80.2 (C), 73.3 (CH), 65.5 (CH), 28.4 (CH_3), 25.6 (CH_3), 17.9 (CH_3) {signals absent even at high concentrations and with increased numbers of scans}; *m/z* (ES+) 545 ($[M+H]^+$, 100%), 141 (16).

(4*S*,5*R*)-*tert*-Butyl 4-(4-(3-cyano-6-(4-cyanothiazol-2-yl)pyridin-2-yl)thiazol-2-yl)-2,2,5-trimethyloxazolidine-3-carboxylate (185)



Pyridine bis-amide **184** (265 mg, 0.49 mmol) was dissolved in pyridine (20 mL) and phosphorus oxychloride (0.23 mL, 373 mg, 2.43 mmol, 5 equiv) added. The solution was stirred at 0 °C for 2 hours then poured over ice. Water (50 mL) was added and the aqueous solution extracted with diethyl ether (3 x 50 mL). The combined organic extracts were washed with aqueous potassium hydrogen sulfate (10% w/v, 100 mL) and water (100 mL), dried ($MgSO_4$) and evaporated *in vacuo* to afford the title compound (214 mg, 87%) as an off-white solid, mp 53-55 °C ($CHCl_3$) (Found: $[M+Na]^+$, 531.1251. Calc. for $C_{24}H_{24}N_6O_3S_2Na$: 531.1244); $[\alpha]_D^{22}$ -45.6 (*c* 0.5, methanol); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3156, 2986, 2895, 2243, 1815, 1789, 1639, 1559, 1469, 1378, 1292, 1212, 1167, 1092, 926, 901; δ_H(500 MHz; $CDCl_3$) 8.19 (1 H, s, 6-thz), 8.16 (2 H, app br s, pyr-4 and pyr-5), 8.12 (1 H, s, 2-thz), 4.76 (1 H, br s, Thr 4-H), 4.40 (0.4 H, br s, Thr 5-H), 4,23 (0.6 H, br s, Thr 5-H), 1.66 (6 H, br s, both Thr 2-Me), 1.49-1.35 (9 H, br s, CMe_3), 1.18 (3 H, app br s, Thr 5-Me); δ_C(125 MHz; $CDCl_3$) 151.2 (C), 144.5 (CH), 133.5 (CH), 128.6 (C), 117.9 (CH), 113.6 (C), 108.1 (C), 77.3 (CH), 30.3 (CH_3), 28.3 (CH_3), 17.9 (CH_3) {signals absent even at high concentrations and with increased numbers of scans}; *m/z* (ES+) 531 ($[M+Na]^+$, 12%), 138 (32), 82 (19), 63 (38), 60 (100).

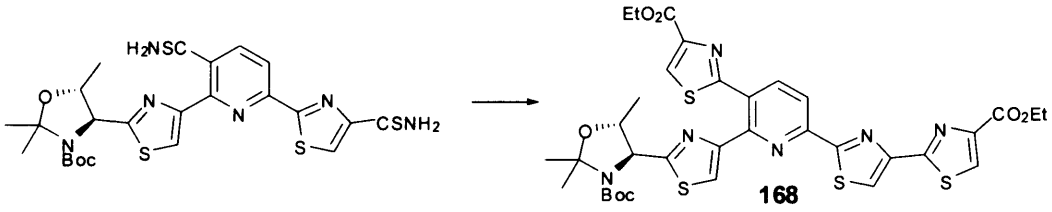
(4*S*,5*R*)-*tert*-Butyl 4-(4-(3-carbamothioyl-6-(4-carbamothioylthiazol-2-yl)pyridin-2-yl)thiazol-2-yl)-2,2,5-trimethyloxazolidine-3-carboxylate (186)



Pyridine bis-nitrile (200 mg, 0.393 mmol) was dissolved in methanol (20 mL) and ammonium sulfide (50 wt. % in H_2O; 0.56 mL, 3.932 mmol, 10 equis) added. The solution was stirred at room temperature for 18 h then evaporated *in vacuo*. Purification of the residue by column chromatography on silica, eluting with chloroform-methanol (19:1) furnished the title compound as a bright yellow solid (160 mg, 70%), mp 114-118 °C (chloroform) (Found: $[M+H]^+$, 577.1183. Calc. for $C_{24}H_{29}N_6O_3S_4$: 577.1178); $[\alpha]_D^{25}$ -32.0 (*c* 0.25, acetone); R_f 0.20 (chloroform-methanol 19:1); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3285, 3168, 2978, 2932, 1691, 1609, 1579, 1493, 1441, 1391, 1368,

1309, 1260, 1215, 1170, 1141, 1089, 1022, 980, 909, 852, 733; δ_H(500 MHz; $CDCl_3$) 8.61 (1 H, s, 6-thz), 8.45 (1 H, br s, exch D_2O, NH), 8.42 (1 H, d, *J* 7.9, pyr-4), 8.33 (1 H, br s, exch D_2O, NH), 8.03 (1 H, br s, exch D_2O, NH), 7.92 (1 H, d, *J* 7.9 pyr-5), 7.85 (1 H, br s, exch D_2O, NH), 7.81 (1 H, br s, 2-thz), 4.68 (0.4 H, br s, Thr 4-H), 4.52 (0.6 H, br s, Thr 4-H), 4.23 (1 H, br s, Thr 5-H), 1.62 (6 H, br s, both Thr 2-Me), 1.38 (9 H, s, CMe_3), 1.18 (3 H, app br s, Thr 5-Me); δ_C(125 MHz; $CDCl_3$) 144.0 (CH), 130.0 (CH), 126.2 (CH), 118.3 (CH), 77.3 (CH), 65.7 (CH), 30.3 (CH_3), 28.4 (CH_3), 17.9 (CH_3); *m/z* (ES+) 577 ($[M+H]^+$, 100%), 143 (17), 141 (47), 130 (28).

(4*S*,5*R*)-*tert*-Butyl 4-(4-(6-(4-(ethoxycarbonyl)-2,4'-bithiazol-2'-yl)-3-(4-(ethoxycarbonyl)thiazol-2-yl)pyridin-2-yl)thiazol-2-yl)-2,2,5-trimethyloxazolidine-3-carboxylate (168)



168

Pyridine bis-thioamide **186** (140 mg, 0.24 mmol) was dissolved in anhydrous DME (20 mL) and dry potassium hydrogen carbonate (389 mg, 3.88 mmol, 16 equiv) added. Ethyl bromopyruvate (0.183 mL, 284 mg, 1.46 mmoL, 6 equiv) was added and the mixture stirred at room temperature for 24 hours. The solvent was removed *in vacuo* and the residue partitioned between water (50 mL) and diethyl ether (50 mL). The organic extract was dried ($MgSO_4$) and evaporated *in vacuo*, and the residue dissolved in anhydrous DME (20 mL). The solution was cooled to 0 °C and pyridine (0.353 mL, 346 mg, 4.37 mmol, 18 equiv) and TFAA (0.275 mL, 408 mg, 1.94 mmol, 8 equiv) added slowly and sequentially. The solution was stirred at 0 °C for 3 hours, allowed to warm to room temperature and triethylamine (0.135 mL, 98 mg, 0.97 mmol, 4 equiv) added. The solution was stirred at room temperature for 3 hours then evaporated *in vacuo*. Purification of the residue by column chromatography on silica eluting with chloroform-methanol (5:1) afforded the title compound (180 mg, 96%) as a yellow solid, mp 49-50 °C (chloroform) (Found: $[M+H]^+$, 769.1604. Calc. for $C_{34}H_{37}N_6O_7S_4$: 769.1601); $[\alpha]_D^{25}$ -46.0 (*c* 0.10, acetone); R_f 0.69 (chloroform-methanol, 19:1); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3438, 2966, 2915, 1734, 1700, 1579, 1464, 1438, 1363, 1298, 1258, 1091, 1022; δ_H(400 MHz; $CDCl_3$) 8.25 (1 H, s, thz), 8.24 (1 H, app br s, pyr-4), 8.20 (1 H, s, thz) 8.17 (1 H, s, thz), 7.95 (1 H, app br s, pyr-5), 6.91 (1 H, s, thz), 4.95 (0.6 H, s, Thr 4-H), 4.83 (0.4 H, d, *J* 11.0, Thr 4-H), 4.39 (2 H, q, *J* 7.0, OCH_2CH_3), 4.36 (2 H, q, *J* 7.0, OCH_2CH_3), 4.35 (1 H, br s, Thr 5-H), 1.61 (3 H, br s), 1.51 (6 H, br s), 1.45 (3 H, br s), 1.38 (3 H, t, *J* 7.0, OCH_2CH_3), 1.31 (3 H, t, *J* 7.0, OCH_2CH_3), 1.18 (6 H, br s); δ_C(125 MHz; $CDCl_3$) 140.8 (CH), 128.0 (CH), 120.5 (CH), 61.8 (CH_2), 28.3 (CH_3), 14.4 (CH_3); *m/z* (ES+) 786 $(M+Na]^+$, 45%), 769 ($[M+H]^+$, 100%), 656 (8), 639 (8), 224 (8), 141 (8).

Synthetic approaches to micrococcinin acid (80) [section 4.8.3]

Method a)

MP1 core domain **168** (15 mg, 21 μmol) was dissolved in hydrochloric acid (6 M, 3 mL) and heated at 110 °C for 2 h. The solution was allowed to cool to room temperature, diluted with water (3 mL) and centrifuged at 9000 rpm for 5 minutes, furnishing a yellow solid (3 mg).

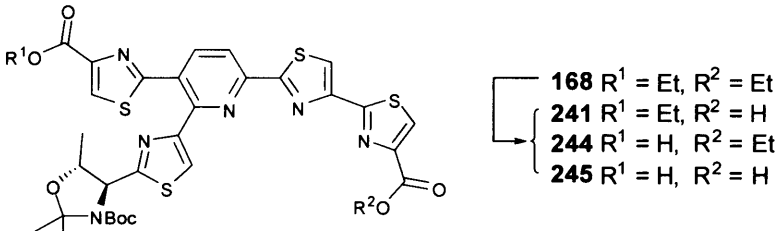
Method b)

The yellow solid from the above reaction was dissolved in hydrochloric acid (6 M, 3 mL) and the solution heated at reflux for 16 hours, cooled to room temperature and diluted with water (3 mL). The resulting yellow precipitate was collected by centrifugation (9000 rpm, 5 mins).

Method c)

MP1 core domain **168** (5 mg, 7 μL) was dissolved a mixture of hydrochloric acid (10.8 M, 1 mL) and formic acid (98%, 1 mL) and the solution heated at 110 °C for 6 hours, allowed to cool to room temperature and diluted with water (3 mL). The resulting yellow precipitate was washed with diethyl ether (2 mL), leaving an off-white solid solid (2 mg).

Attempted selective ester hydrolysis [sections 5.2.1 and 5.2.2]



Lithium hydroxide (1 equiv)

MP1 core domain **168** (30 mg, 39 μmol) was dissolved in methanol-water (5:1, 12 mL) and lithium hydroxide monohydrate (1.7 mg, 41 μmol, 1.05 equiv) added. The solution was stirred at room temperature for 18 h then evaporated *in vacuo*. The residue was partitioned between ethyl acetate (5 mL) and water (5 mL) and the aqueous layer acidified to pH 3 with 10% w/v potassium hydrogen sulphate solution. The acidified aqueous layer was extracted with ethyl acetate (3 x 5 mL) and the combined organic extracts dried ($MgSO_4$) and evaporated *in vacuo* to afford a mixture of compounds **168**, **241**, **244** and **245** (21 mg).

Lithium hydroxide (5 equiv)

MP1 core domain **168** (100 mg, 0.13 mmol) was dissolved in methanol-water (5:1, 10 mL) and lithium hydroxide monohydrate (27 mg, 0.65 mmol, 5 equiv) added. The solution was stirred at

room temperature for 18 h then evaporated *in vacuo*. The residue was partitioned between ethyl acetate (10 mL) and water (10 mL) and the aqueous layer acidified to pH 3 with 10% w/v potassium hydrogen sulphate solution. The acidified aqueous layer was extracted with ethyl acetate (3 x 10 mL) and the combined organic extracts dried ($MgSO_4$) and evaporated *in vacuo* to afford compound **245** (60 mg, 93%) as a pale yellow solid, R_f 0.05 (chloroform-methanol, 4:1); *m/z* (ES+) 713 ($[M+H]^+$, 11%), 117 (26), 100 (100).

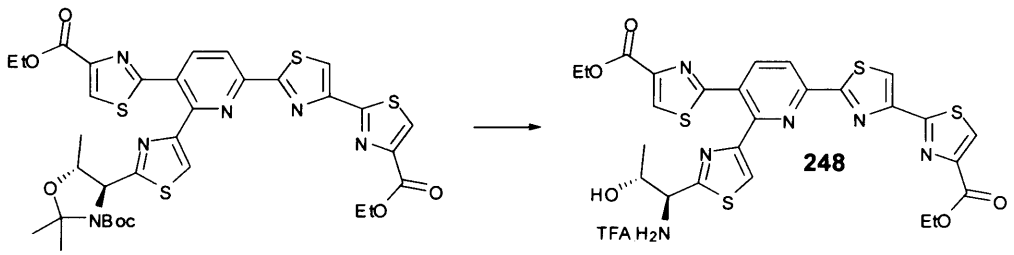
Trimethyltin hydroxide (4 x 2 equiv)

To a stirred solution of MP1 core domain **168** (20 mg, 26 µmol) in 1,2-DCE (2 mL) was added trimethyltin hydroxide (9.4 mg, 52 µmol) and the solution heated at 50 °C for 1 hour. Portions of trimethyltin hydroxide (9.4 mg, 52 µmol) were added after 1 h, 2 h and 3 h, and the solution stirred for one further hour. The solvent was removed *in vacuo* and the residue purified by column chromatography on silica, eluting with chloroform-methanol (9:1) to furnish **168** (10 mg, 50%), **241** and **244** (5 mg, 27%) and **245** (4 mg, 22%).

Trimethyltin hydroxide (2 equiv)

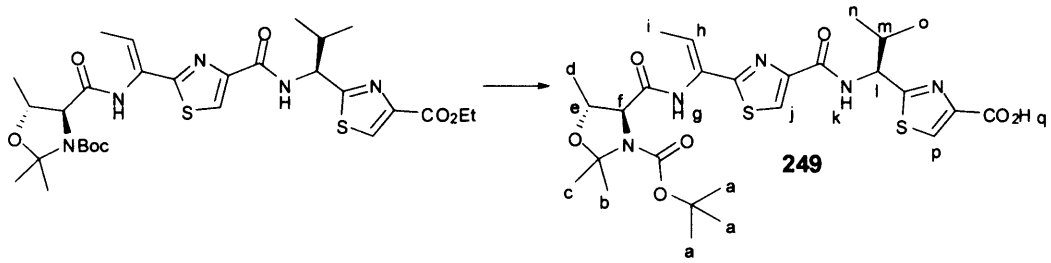
MP1 core domain **168** (50 mg, 65 µmol) was dissolved in 1,2-DCE (5 mL) and trimethyltin hydroxide (23 mg, 130 µmol, 2 equiv) added. The solution was stirred at 50 °C for 96 h then evaporated *in vacuo*. Purification of the residue by column chromatography on silica, eluting with chloroform-methanol (4:1) afforded **168** (27 mg, 54%), **241** and **244** (16 mg, 34%) and **245** (5 mg, 11%).

Ethyl 2'-(6-(2-((1*S*,2*R*)-1-amino-2-hydroxypropyl)thiazol-4-yl)-5-(4-(ethoxycarbonyl)thiazol-2-yl)pyridin-2-yl)-2,4'-bithiazole-4-carboxylate trifluoroacetate (248)



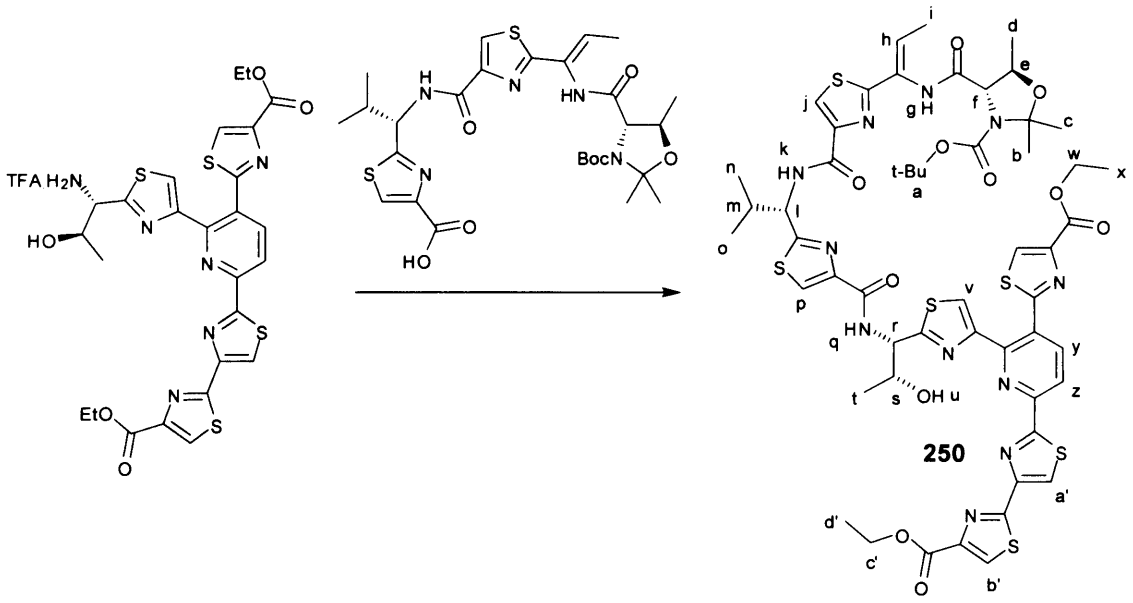
MP1 core domain (90 mg, 117 µmol) was dissolved in dichloromethane (1 mL) and trifluoroacetic acid (1 mL) added. The solution was stirred at room temperature for 1 hour then evaporated under a stream of nitrogen. Trituration of the residue with diethyl ether (2 x 2 mL) furnished the title compound (65 mg, 75%) as a beige solid which was used immediately without further purification.

2-((*S*)-1-(2-((*Z*)-1-((4*S*,5*R*)-3-(*tert*-Butoxycarbonyl)-2,2,5-trimethyloxazolidine-4-carboxamido)prop-1-enyl)thiazole-4-carboxamido)-2-methylpropyl)thiazole-4-carboxylic acid (249)



MP1 backbone peptide **139** (174 mg, 0.27 mmol) was dissolved in methanol-water (5:1, 15 mL) and lithium hydroxide monohydrate (57 mg, 1.37 mmol, 5 equiv) added. The solution was stirred at room temperature for 16 h then evaporated *in vacuo*. The residue was partitioned between ethyl acetate (10 mL) and water (10 mL) and the aqueous layer acidified to pH 3 with 10% w/v potassium hydrogen sulfate solution. The acidified aqueous layer was extracted with ethyl acetate (3 x 10 mL) and the combined organic extracts dried (Na_2SO_4) and evaporated *in vacuo* to afford the title compound (148 mg, 89%) as a colourless foam, mp 111-114 °C (EtOAc) (Found: $[M+H]^+$, 608.2189. Calc. for $C_{27}H_{38}N_5O_7S_2$: 608.2213); $[\alpha]_D^{25}$ +54.4 (*c* 0.25, acetone); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3500-2700 br, 3282, 3122, 2976, 2933, 1690, 1534, 1482, 1391, 1368, 1325, 1215, 1172, 1083, 913; δ_H(500 MHz; $CDCl_3$) 8.12 (1 H, s, **j**), 7.98 (1 H, s, **p**) 7.91 (2 H, app br s, exch. D_2O, **k**, **g**), 6.53 (1 H, app br m, **h**), 5.27 (1 H, d, *J* 7.2, **l**), 4.39 (1 H, app. br m, **e**), 4.03 (1 H, d, *J* 7.2, **f**), 2.48 (1 H, m, **m**), 1.80 (3 H, d, *J* 7.1, **i**), 1.55 (6 H, s, **b**, **c**), 1.38 (9 H, s, **a**), 1.35 (3 H, d, *J* 7.1, **d**), 0.97 (3 H, d, *J* 6.7, **n**), 0.93 (3 H, d, *J* 6.7, **o**); δ_C(125 MHz; $CDCl_3$) 128.0 (CH), 127.0 (CH), 81.7 (C), 77.3 (CH), 56.7 (CH), 28.3 (CH_3), 26.1 (CH_3), 19.6 (CH_3); *m/z* (ES+) 625 ($[M+NH_4]^+$, 100%), 608 ($[M+H]^+$, 30%), 552 (4), 391 (33), 335 (8).

(4*S*,5*R*)-*tert*-Butyl 4-((*Z*)-1-(4-((*S*)-1-(4-((1*S*,2*R*)-1-(4-(6-(4-(ethoxycarbonyl)-2,4'-bithiazol-2'-yl)-3-(4-(ethoxycarbonyl)thiazol-2-yl)pyridin-2-yl)thiazol-2-yl)-2-hydroxypropylcarbamoyl)thiazol-2-yl)-2-methylpropylcarbamoyl)thiazol-2-yl)prop-1-enylcarbamoyl)-2,2,5-trimethyloxazolidine-3-carboxylate (250)



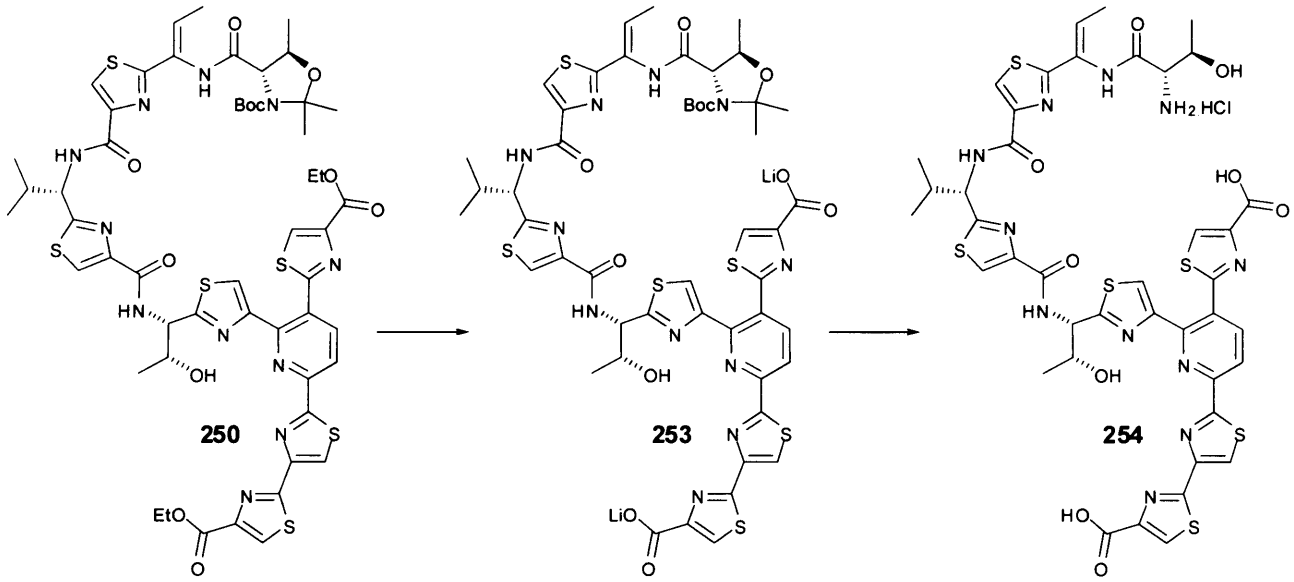
PyBOP®-mediated coupling

MP1 backbone carboxylic acid **249** (40 mg, 66 μmol) was dissolved in dichloromethane (5 mL) and the solution cooled to 0 °C. PyBOP® (41 mg, 79 μmol, 1.2 equiv) was added followed by dropwise addition of a solution of amine **248** (49 mg, 79 μmol, 1.2 equiv) and triethylamine (1 mL) in dichloromethane (2 mL). The solution was stirred at room temperature for 16 h then evaporated *in vacuo*. Purification of the residue by column chromatography on silica, eluting with ethyl acetate furnished the title compound (26 mg, 33%) as a pale yellow solid, mp 141-142 °C (EtOAc) (Found: $[M+H]^+$, 1218.2793. Calc. for $C_{53}H_{60}N_{11}O_{11}S_6$: 1218.2798); $[\alpha]_D^{25}$ +30.0 (*c* 0.10, acetone); R_f 0.38 (ethyl acetate); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3386, 3124, 2979, 1709, 1676, 1582, 1535, 1481, 1422, 1368, 1213, 1096, 1020, 914, 856, 731; δ_H(500 MHz; $CDCl_3$) 8.26 (1 H, d, *J* 8.0, **y**), 8.25 (1 H, s, thz), 8.16 (1 H, d, *J* 8.0, **z**), 8.15 (1 H, s, thz), 8.07 (1 H, s, thz), 8.02 (1 H, s, thz), 7.98 (1 H, s, thz), 7.97 (1 H, br d, *J* 8.9, **q**), 7.76 (1 H, s, thz), 7.80 (1 H, br d, *J* 8.7, **k**), 7.72 (1 H, br s, **g**), 6.58 (1 H, app br s, **h**), 5.27 (1 H, dd, *J* 8.7, 7.0, **l**), 5.17 (1 H, dd, *J* 8.9, 7,2, **r**), 4.40 (2 H, q, *J* 7.1, **w** or **c'**), 4.38 (1 H, d, *J* 7.2, **f**), 4.21 (1 H, dd, *J* 7.2, 6.4, **s**), 4.05 (2 H, q, *J* 7.1, **w** or **c'**), 3.95 (1 H, m, **e**), 2.51 (1 H, m, **m**), 2.50 (1 H, br s, **u**), 1.82 (3 H, d, *J* 7.1, **i**), 1.59 (3 H, s, **b** or **c**), 1.55 (3 H, s, **b** or **c**), 1.35 (15 H, m, **a**, **x**, **d'**), 1.19 (3 H, d, *J* 7.2, **d**), 1.16 (3 H, d, *J* 6.4, **t**), 0.96 (3 H, d, *J* 6.8, **n** or **o**), 0.94 (3 H, d, *J* 6.8, **n** or **o**); *m/z* (ES+) 1240 ($[M+Na]^+$, 29%), 1218 ($[M+H]^+$, 100%), 684 (8), 636 (15), 559 (23), 536 (12), 305 (11), 224 (11), 179 (56).

HATU-mediated coupling

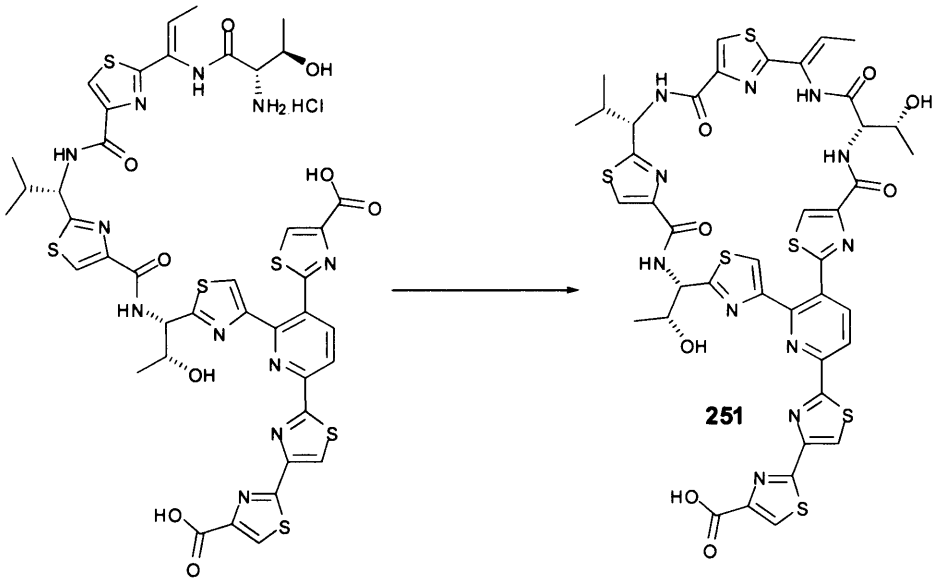
MP1 backbone carboxylic acid **249** (36 mg, 59 μmol, 1.25 equiv) was dissolved in dichloromethane (3 mL) and diisopropylethylamine (41 μL, 30 mg, 236 μmol, 5 equiv) added. HATU (23 mg, 60 μmol, 1.25 equiv) was added and the solution stirred at room temperature for 1 hour before the dropwise addition of a solution of **248** (35 mg, 47 μmol, 1 equiv) and diisopropylethylamine (41 μL, 30 mg, 236 μmol, 5 equiv) in dichloromethane (2 mL). The reaction mixture was stirred at room temperature for 4 hours, evaporated *in vacuo* and the residue purified by chromatography on silica, eluting with ethyl acetate to give the title compound (44 mg, 77%) as a pale yellow solid.

2'-(6-(2-((1*S*,2*R*)-1-(2-((*S*)-1-(2-((*Z*)-1-((2*S*,3*R*)-2-amino-3-hydroxybutanamido)prop-1-enyl)thiazole-4-carboxamido)-2-methylpropyl)thiazole-4-carboxamido)-2-hydroxypropyl)thiazol-4-yl)-5-(4-carboxythiazol-2-yl)pyridin-2-yl)-2,4'-bithiazole-4-carboxylic acid hydrochloride (254)



To a stirred solution of MP1 core-backbone fragment **250** (15 mg, 12 μmol) in methanol-water (5:1, 3 mL) was added lithium hydroxide monohydrate (5 mg, 123 μmol, 10 equiv) and the solution stirred at room temperature for 14 h. The solvent was evaporated *in vacuo* and ethyl acetate (2 mL) added. The mixture was sonicated to produce a fine suspension, to which was added hydrogen chloride in ethyl acetate (2 mL). The resulting mixture was stirred at room temperature for 1 hour then evaporated under a stream of nitrogen to afford the title compound as a bright yellow solid, used immediately without further purification.

Macrocycle 251



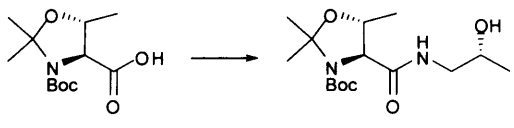
FDPP-mediated macrocyclisation

Deprotected core-backbone fragment **254** (10 mg, 9.8 μmol) was dissolved in DMF (33 mL) and diisopropylethylamine (1 mL) added. FDPP (19 mg, 48.8 μmol, 5 equiv) was added and the solution stirred at room temperature for 48 h. The solvent was removed *in vacuo* and the residue triturated with ethyl acetate (2 mL) to afford an off-white solid which was determined to be FDPP.

HATU-mediated macrocyclisation

Deprotected core-backbone fragment **254** (≤12.8 mg, 12 μmol) was dissolved in DMF (12 mL) and diisopropylethylamine (21 μL, 16 mg, 120 μmol, 10 equiv) added. HATU (19 mg, 60 μmol, 5 equiv) was added and the solution stirred at room temperature for 45 h. The solvent was removed *in vacuo* and the residue triturated with ethyl acetate (2 mL) to afford an off-white solid which was partitioned between chloroform (2 mL) and aqueous acetic acid (5% w/v; 2 mL). The solution was filtered to furnish macrocycle **251** (10.4 mg, 85%) as a pale brown solid, mp 200-204 °C ($CHCl_3$-AcOH-H_2O); *m/z* (ES+) 1021 ($[M+NH_4]^+$, 55%), 1004 ($[M+H]^+$, 87%), 995 (23), 980 (21), 433 (32), 377 (38), 338 (30), 333 (13), 263 (48), 219 (100), 143 (11), 130 (28).

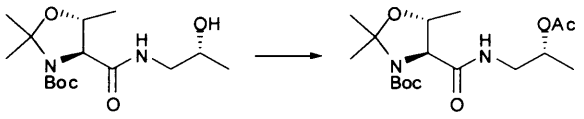
(4*S*,5*R*,2'*R*)–4–[(2–Hydroxypropyl)aminocarbonyl]–2,2,5–trimethyl–3–(*tert*–butoxcarbonyl)oxazolidine (259)



259

Triethylamine (2.78 mL, 2.01 g, 19.9 mmol, 2.07 equiv) was added dropwise over 30 minutes to a stirred solution of (4*S*,5*R*)-3-(*tert*-butoxycarbonyl)-2,2,5-trimethyloxazolidine-4-carboxylic acid (2.49 g, 9.60 mmol, 1.07 equiv), (*R*)–1–amino–2–propanol (675 mg, 9.00 mmol, 1 equiv) and PyBOP® (5.20 g, 10.00 mmol, 1.11 equiv) in dry dichloromethane (19 mL) at 0 °C. The solution was allowed to warm to room temperature and stirred for a further 18 h. After concentrating *in vacuo*, purification by column chromatography on silica, eluting with ethyl acetate gave the title compound (2.84 g, 100%) as a colourless solid, mp 118–119 °C (petroleum ether–Et_2O) (Found: $[M+H]^+$, 317.2072. Calc. for $C_{15}H_{29}N_2O_5$: 317.2071); $[\alpha]_D^{31}$ –62.0 (*c* 1.50, chloroform); R_f 0.29 (EtOAc); ν_{max}(KBr)/cm^{-1} 3488, 3286, 3116, 2978, 2925, 2865, 1672, 1570, 1366, 1259, 1135, 989, 967, 945, 909, 855, 781, 765, 690; δ_H(400 MHz; $CDCl_3$) 6.44 (1 H, br s, exch D_2O, NH), 4.22 (1 H, m, 5–H), 3.91 (1 H, app br s, 2'–H), 3.76 (1 H, d, *J* 7.5, 4–H), 3.40 (1 H, br s, 1'–C*H*H), 3.14 (1 H, m, 1'–CH*H*), 2.97 (1 H, br s, exch D_2O, OH), 1.60 (3 H, s, 2–Me), 1.58 (3 H, s, 2–Me), 1.44 (9 H, s, CMe_3), 1.37 (3 H, d, *J* 6.0, 5–Me), 1.17 (3 H, d, *J* 6.5, 2'–Me); δ_C(100 MHz; $CDCl_3$) 170.2 (C), 152.8 (C), 94.7 (C), 81.5 (C), 73.9, (CH), 67.9 (CH), 66.8 (CH), 47.1 (CH_2), 28.3 (CH_3), 27.6 (CH_3), 25.2 (CH_3), 20.2 (CH_3), 18.6 CH_3); m/z (APCI) 317 ($[M+H]^+$, 100%), 261 (28), 217 (21), 173 (25), 155 (12).

(4*S*,5*R*,2'*R*)–4–[(2–Acetoxypropyl)aminocarbonyl]–2,2,5–trimethyl–3–(*tert*–butoxycarbonyl)oxazolidine (256)

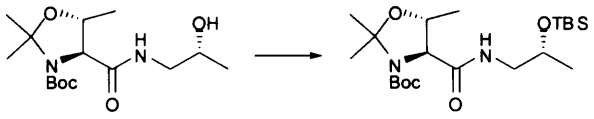


256

Acetic anhydride (0.27 mL, 0.29 g, 2.85 mmol, 3 equiv) was added to a solution of alcohol **259** (300 mg, 0.95 mmol) in dry pyridine (10 mL) and stirred at room temperature for 48 h. The solution was evaporated *in vacuo* and the residue purified by column chromatography on silica, eluting with light petroleum–ethyl acetate (1:1) to furnish the title compound (333 mg, 98%) as a colourless solid, mp 124–125 °C (petroleum ether–Et_2O) (Found: $[M+H]^+$, 359.2179. Calc. for $C_{17}H_{31}N_2O_6$: 359.2177); $[\alpha]_D^{26}$ –37.6 (*c* 1.00, chloroform); R_f 0.24 (petroleum ether-ethyl acetate, 1:1); ν_{max}(KBr)/cm^{-1} 3382, 2985, 2945, 1735, 1711, 1663, 1532, 1478, 1459, 1376, 1241, 1221, 1175, 1126, 1096, 1076, 1057, 1026, 986, 972, 954, 894, 857, 783; δ_H(400 MHz; $CDCl_3$) 6.36 (1H, br s, exch D_2O, NH), 4.99 (1 H, ddq, *J* 6.0, 4.0, 6.0, 2'–H), 4.22 (1 H, br s, 5–H), 3.70 (1 H, d, *J* 6.5, 4–H), 3.55 (1 H, ddd, *J* 14.2, 6.0, 4.0, 1'–C*H*H), 3.37 (1 H, ddd, *J* 14.2, 6.0, 7.0, 1'–CH*H*), 2.05 (3 H, s, Me), 1.60 (3 H, s, 2–Me) 1.55 (3 H, s, 2–Me), 1.49 (9 H, s, CMe_3), 1.42 (3 H, d, *J* 6.0, 2'–Me), 1.30 (3 H, d, *J* 6.5, 5–Me); δ_C(100 MHz; $CDCl_3$) 170.6 (C), 170.0 (C), 166.4 (C), 94.6 (C), 80.7 (C), 73.9 (CH), 69.6 (CH), 67.4 (CH), 43.8 (CH_2), 28.2 (CH_3), 27.7 (CH_3), 25.0

(CH_3), 21.1 (CH_3), 18.8 (CH_3), 17.5 (CH_3); m/z (APCI) 359 ($[M+H]^+$, 100%), 303 (25), 259 (85), 213 (20).

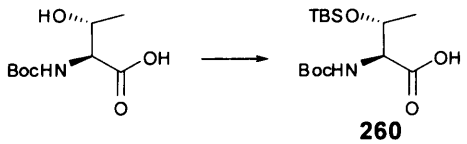
(4*S*,5*R*,2'*R*)–4–[(2–*tert*–Butyldimethylsilyloxy)aminocarbonyl]–2,2,5–trimethyl–3–(*tert*–butoxycarbonyl)oxazolidine (258)



258

Imidazole (400 mg, 5.90 mmol, 1.8 equiv), DMAP (122 mg, 1.00 mmol, 0.3 equiv) and TBDMS-chloride (690 mg, 4.60 mmol, 1.4 equiv) were added successively to a solution of alcohol **259** (1.04 g, 3.27 mmol) in DMF (25 mL). The mixture was stirred at room temperature for 36 h, acidified with hydrochloric acid (1 M, 80 mL) and extracted with diethyl ether (3 x 90 mL). The combined organic extracts were washed with water (3 x 300 mL), dried (Na_2SO_4) and evaporated *in vacuo* to furnish the title compound (1.39 g, 98%) as a pale yellow-green oil (Found: $[M+H]^+$, 431.2934. Calc. for $C_{21}H_{43}N_2O_5Si$: 431.2936); $[\alpha]_D^{23}$ –26.4 (*c* 0.75, chloroform); ν_{max}(film)/cm^{-1} 3329, 2976, 2931, 2858, 2359, 1710, 1669, 1530, 1462, 1367, 1258, 1175, 1133, 1006, 940, 860, 837, 777; δ_H(400 MHz; $CDCl_3$) 6.10 (1 H, br s, exch D_2O, NH), 4.11 (1 H, br s, 5–H), 3.89 (1 H, m, 2'–H), 3.69 (1 H, br s, 4–H), 3.23 (2 H, 1'–CH_2), 1.55 (3 H, s, 2–Me), 1.52 (3 H, s, 2–Me), 1.36 (9 H, s, $OCMe_3$), 1.32 (3 H, d, *J* 6.0, 5–Me), 1.07 (3 H, d, *J* 6.0, 2'–Me), 0.82 (9 H, s, $SiCMe_3$), 0.01 (3 H, s, *Me*SiMe), 0.00 (3 H, s, MeSi*Me*); δ_C(100 MHz; $CDCl_3$) 169.9 (C), 151.5 (C), 94.9 (C), 80.7 (C), 77.3 (C), 74.7 (CH), 68.1 (CH), 67.2 (CH), 46.3 (CH_2), 28.3 (CH_3), 25.8 (CH_3), 21.1 (CH_3), 19.2 (CH_3), 18.0 (CH_3), –3.6 (CH_3), –4.4 (CH_3); m/z (APCI) 431 ($[M+H]^+$, 80%), 375 (100), 331 (75), 285 (45).

***N*-*tert*-(Butoxycarbonyl)-*O*-*tert*-(butyldimethylsilyl)threonine (260)**

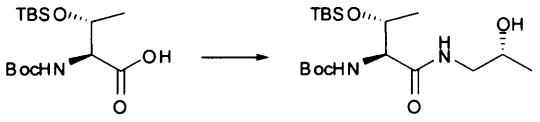


260

N-Boc-L–threonine (5.00 g, 22.81 mmol) was dissolved in dry dichloromethane (75 mL) and triethylamine (10.7 mL, 7.77g, 76.77 mmol, 3.37 equiv) added. The solution was cooled to 0 °C and TBDMS-triflate (15.72 mL, 18.09 g, 68.43 mmol, 3.00 equiv) added slowly. The mixture was stirred at room temperature for 2 hours, quenched with water (75 mL) and the aqueous layer extracted with dichloromethane (2 x 25 mL). The combined organic layers were filtered,

evaporated *in vacuo* and the residue dissolved in methanol (75 mL). Water (32 mL) and potassium carbonate (7.88 g, 57.02 mmol, 2.50 equiv) were added and the solution stirred at room temperature for 2 hours, extracted with hexane (100 mL) and acidified to pH 3 with 10% w/v aqueous potassium hydrogen sulfate . The acidified water-methanol solution was extracted with ethyl acetate (4 x 100 mL) and the combined organic extracts dried (Na_2SO_4) and evaporated *in vacuo* to yield the title compound (7.61 g, 100%) as a viscous, colourless oil (Found: $[M+H]^+$, 334.2049. Calc. for $C_{15}H_{32}NO_5Si$: 334.2050); $[\alpha]_D^{25}$ +11.0 (*c* 4.00, chloroform) {lit.,[221] +10.7 (*c* 3.90, chloroform)}; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3600-2400 br, 3456, 3166, 2955, 2858, 1722, 1694, 1624, 1504, 1472, 1392, 1367, 1254, 1169, 1101, 1072, 1031, 939, 837, 777; δ_H(400 MHz; $CDCl_3$) 5.16 (1 H, d, *J* 8.4, exch D_2O, NH), 4.34 (0.8 H, br m, β-H), 4.30 (0.2 H, br s, β-H), 4.15 (1 H, dd, *J* 8.4, 6.7, α-H), 1.36 (9 H, s, $OCMe_3$), 1.10 (3 H, d, *J* 6.2, HC*Me*), 0.77 (9 H, s, $SiCMe_3$), 0.00 (3 H, s, *Me*SiMe), -0.06 (3 H, s, MeSi*Me*); δ_C(125 MHz; d_6-DMSO) 172.6 (C), 156.0 (C), 78.8 (C), 68.9 (CH), 59.6 (CH), 28.5 (CH_3), 26.1 (CH_3), 20.8 (CH_3), 18.1 (CH_3), -4.6 (CH_3), -5.3 (CH_3); *m/z* (APCI) 334 ($[M+H]^+$, 47%), 278 (100), 275 (17), 234 (35).

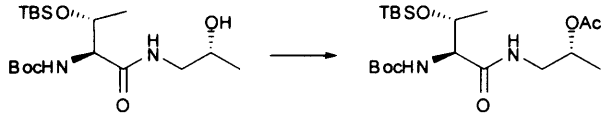
tert-Butyl (2*S*,3*R*)-3-(_tert_-butyldimethylsilyloxy)-1-((*R*)-2-hydroxypropylamino)-1-oxobutan-2-ylcarbamate (261)



261

Triethylamine (0.96 mL, 698 mg, 6.90 mmol, 2.3 equiv) was added dropwise to a stirred solution of *N*-*tert*-(butoxycarbonyl)-*O*-*tert*-(butyldimethylsilyl)threonine (1.00 g, 3.00 mmol, 1 equiv), (*R*)–1–amino–2–propanol (225 mg, 3.00 mmol, 1 equiv) and PyBOP® (1.71 g, 3.30 mmol, 1.1 equiv) in dry dichloromethane (35 mL) and the solution stirred at room temperature for 18 h. After concentrating *in vacuo*, purification by column chromatography on silica, eluting with ethyl acetate gave the title compound (759 mg, 65%) as a colourless oil, $[\alpha]_D^{25}$ -8.8 (*c* 0.50, acetone); R_f 0.50 (EtOAc); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3422, 2955, 2931, 2858, 1702, 1664, 1534, 1500, 1392, 1367, 1255, 1168, 1131, 1100, 1074, 1021, 959, 839, 779; δ_H(400 MHz; d_6-acetone) 7.22 (1 H, app br s, exch D_2O, NH), 5.67 (1 H, br d, *J* 7.2, exch D_2O, NH), 4.31 (1 H, m, Thr β-H), 3.94 (1 H, app d, *J* 7.2, Thr α-H), 3.75 (1 H, m, CH_2CH(OH)Me), 3.26 (1 H, ddd, *J* 12.3, 7.0, 6.0, C*H*H), 2.95 (1 H, ddd, *J* 12.3, 7.0, 5.3, CH*H*), 2.77 (1 H, s, exch D_2O, OH), 1.32 (9 H, s, $OCMe_3$), 1.05 (3 H, d, *J* 6.3, Thr β-Me), 1.02 (3 H, d, *J* 6.2, CH(OH)*Me*), 0.79 (9 H, s, $SiCMe_3$), 0.00 (3 H, s, *Me*SiMe), 0.02 (3 H, s, MeSi*Me*); δ_C(100 MHz; $CDCl_3$) 170.2 (C), 156.0 (C), 79.8 (C), 68.2 (CH), 67.0 (CH), 66.1 (CH), 47.1 (CH_2), 30.8 (CH_3), 28.3 (CH_3), 25.7 (CH_3), 20.7 (CH_3), 17.6 (C), -4.7 (CH_3), -5.6 (CH_3); *m/z* (APCI) 391 ($[M+H]^+$, 31%), 335 (69), 291 (75), 219 (9), 117 (100).

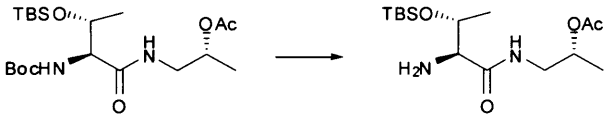
(*R*)-1-((*2S,3R*)-2-(*tert*-Butoxycarbonylamino)-3-(*tert*-butyldimethylsilyloxy)butanamido)propan-2-yl acetate (257)



257

Acetic anhydride (0.18 mL, 196 mg, 2.85 mmol, 3 equiv) was added to a solution of alcohol **261** (250 mg, 0.64 mmol) in dry pyridine (10 mL) and stirred at room temperature for 48 h. The solution was evaporated *in vacuo* and the residue purified by column chromatography on silica, eluting with ethyl acetate to furnish the title compound (234 mg, 84%) as a colourless oil, R_f 0.72 (EtOAc); δ_H(400 MHz; d_6-acetone) 7.26 (1 H, app br s, exch D_2O, NH), 5.63 (1 H, br d, *J* 7.5, exch D_2O, NH), 4.83 (1 H, ddq, *J* 6.3, 4.5, 6.5, CH_2CH(OAc)Me), 4.27 (1 H, dq, *J* 6.2, 2.1, Thr β-H), 3.92 (1 H, dd, *J* 8.0, 2.1 Thr α-H), 3.46 (1 H, ddd, *J* 12.1, 7.2, 4.5, CH*H*), 3.07 (1 H, ddd, *J* 12.1, 6.5, 5.6, CH*H*), 1.87 (3 H, s, CH_3), 1.33 (9 H, s, $OCMe_3$), 1.07 (3 H, d, *J* 6.3, CH(OAc)*Me*), 1.04 (3 H, d, *J* 6.2, Thr β-Me), 0.79 (9 H, s, $SiCMe_3$), 0.00 (3 H, s, *Me*SiMe), 0.02 (3 H, s, MeSi*Me*); δ_C(125 MHz; d_6-acetone) 170.2 (C), 169.7 (C), 155.6 (C), 78.7 (C), 78.3 (C), 69.2 (CH), 68.7 (CH), 59.8 (CH), 43.2 (CH_2), 27.6 (CH_3), 25.3 (CH_3), 23.8 (CH_3), 20.3 (CH_3), 19.3 (CH_3), 17.6 (CH_3), 16.9 (CH_3); *m/z* (APCI) 433 ($[M+H]^+$, 52%), 377 (63), 333 (30), 215 (20), 141 (100), 126 (52), 118 (28).

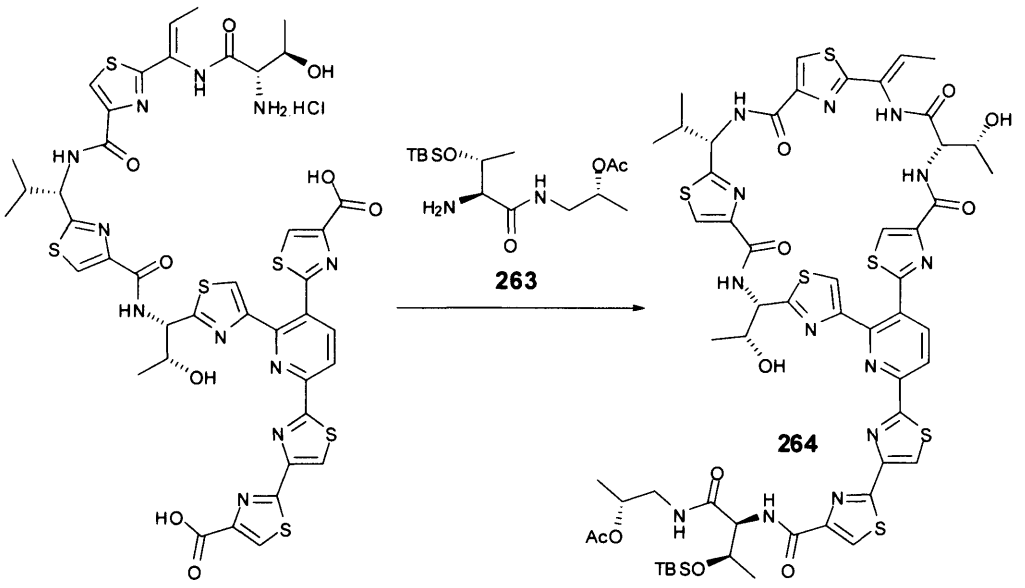
(*R*)-1-((2*S*,3*R*)-2-amino-3-(*tert*-butyldimethylsilyloxy)butanamido)propan-2-yl acetate (263)



263

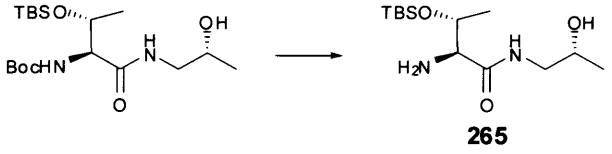
A saturated solution of hydrogen chloride in ethyl acetate (2 mL) was added to side chain dipeptide **257** (20 mg, 46 μmol) and the resulting solution stirred at room temperature for 30 minutes. The solvent was removed *in vacuo* and the residue used immediately without further purification; *m/z* (ES+) 665 ($[2M+H]^+$, 7%), 333 ($[M+H]^+$, 100%), 287 (30), 259 (12), 219 (7), 201 (7), 159 (5).

One-pot macrolactamisation and side chain coupling (264)



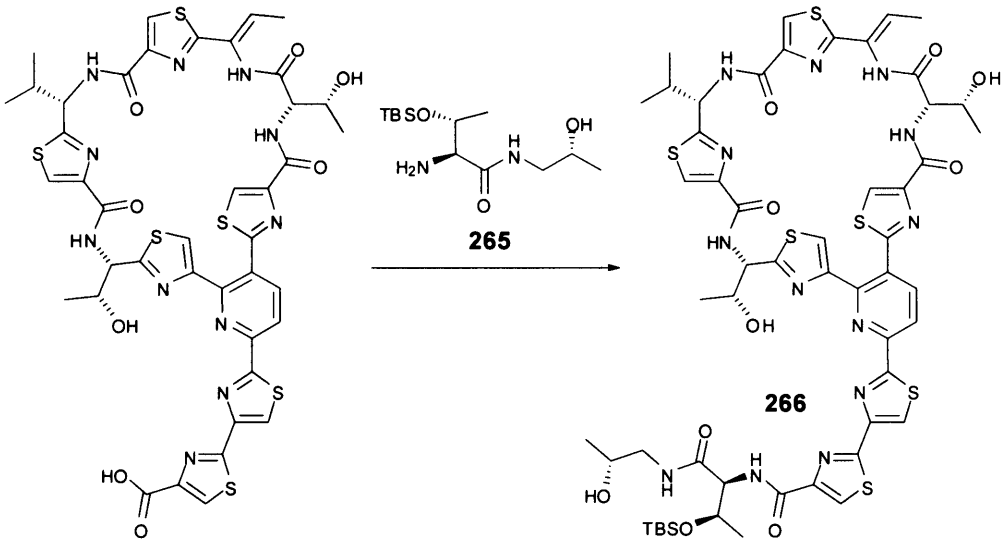
Deprotected core-backbone fragment **254** (60 mg, 57 μmol) was dissolved in DMF (57 mL) and diisopropylethylamine (49 μL, 37 mg, 283 μmol, 5 equiv) added. HATU (108 mg, 283 μmol, 5 equiv) was added and the solution stirred at room temperature for 3 h. A solution of amine **263** (~63 mg, 170 μmol, 3 equiv) and diisopropylethylamine (49 μL, 37 mg, 283 μmol, 5 equiv) in DMF (3 mL) was added and the mixture stirred at room temperature for 36 h. The solvent was removed *in vacuo* and the residue triturated with ethyl acetate (2 mL) to afford an off-white solid which was partitioned between chloroform (2 mL) and aqueous acetic acid (5% w/v; 2 mL). The solution was filtered to furnish macrocycle **251** (20 mg, 35%) as a pale brown solid.

(2*S*,3*R*)-2-Amino-3-(*tert*-butyldimethylsilyloxy)-N-((*R*)-2-hydroxypropyl)butanamide (265)



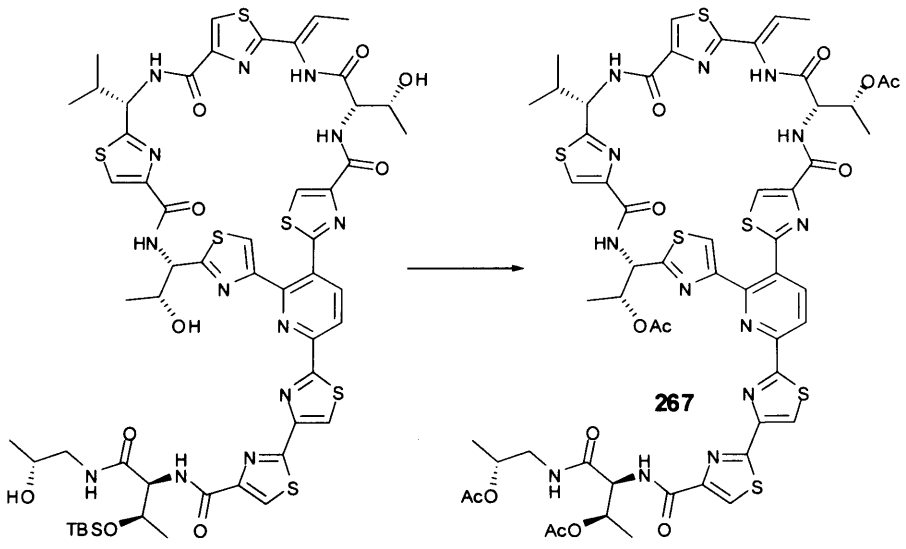
A saturated solution of hydrogen chloride in ethyl acetate (2 mL) was added to side chain dipeptide **261** (24 mg, 61 μmol) and the resulting solution stirred at room temperature for 1 hour. The solvent was removed *in vacuo* and the residue used immediately without further purification.

Macrocycle-side chain coupling (266)



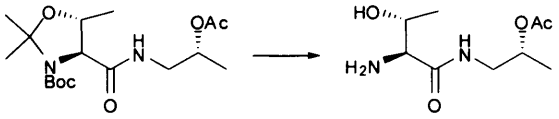
Macrocycle **251** (~20 mg, 20 μmol) was dissolved in DMF (2 mL) and diisopropylethylamine (17 μL, 13 mg, 100 μmol, 5 equiv) added. HATU (15 mg, 40 μmol, 2 equiv) was added and the solution stirred at room temperature for 1 h. A solution of amine **265** (~20 mg, 61 μmol, 3 equiv) and diisopropylethylamine (0.5 mL) in DMF (1 mL) was added and the mixture stirred at room temperature for 72 h. The solvent was removed *in vacuo* and the residue triturated with ethyl acetate (2 mL) to afford an off-white solid which was partitioned between chloroform (2 mL) and aqueous acetic acid (5% w/v; 2 mL). The solution was filtered to furnish an intractable mixture of insoluble products (15 mg) as an off-white solid.

Acetate protection of macrocycle 266



Acetic anhydride (50 µL, 54 mg, 53 µmol, 45 equiv) was added to a solution of **266** (15 mg, 12 µmol) in dry pyridine (1 mL) and stirred at room temperature for 62 h. The solution was evaporated *in vacuo* and the residue purified by column chromatography on silica, eluting with chloroform-methanol (19:1) to furnish **267** (5 mg, 33%) as an off-white solid, R_f 0.10 (chloroform-methanol, 19:1); *m/z* (ES+) 1330 ($[M+H]^+$, 5%), 817 (5), 709 (5), 627 (5), 487 (7), 452 (10), 430 (10), 325 (100), 279 (43), 243 (32), 197 (12), 83 (9).

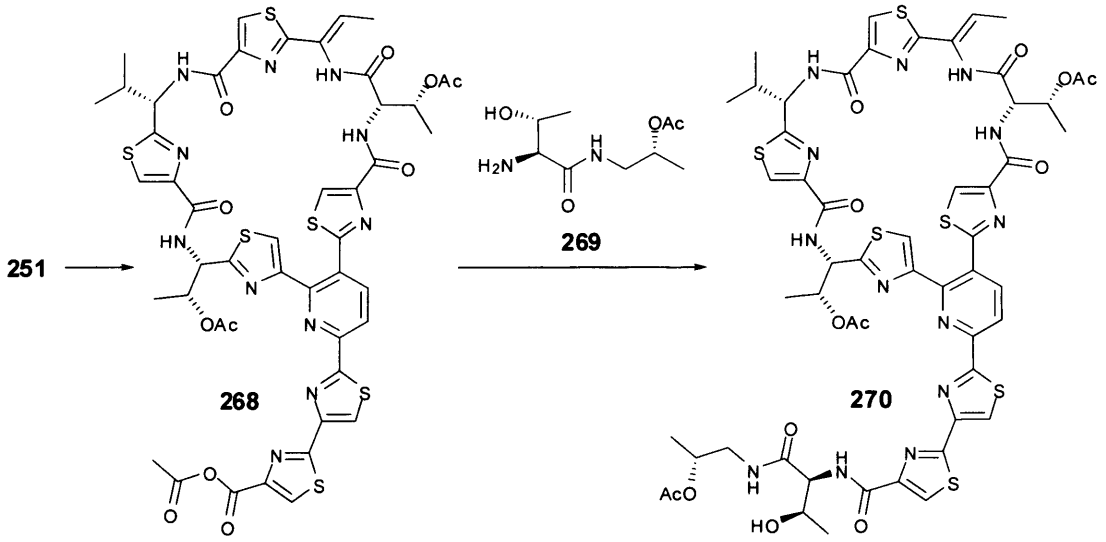
(*R*)-1-((2*S*,3*R*)-2-Amino-3-hydroxybutanamido)propan-2-yl acetate (269)



269

A saturated solution of hydrogen chloride in ethyl acetate (2 mL) was added to side chain dipeptide **256** (38 mg, 106 µmol) and the resulting solution stirred at room temperature for 1 hour. The solvent was removed *in vacuo* and the residue used immediately without further purification.

Acetate protection of macrocycle 251 [section 5.6.2]

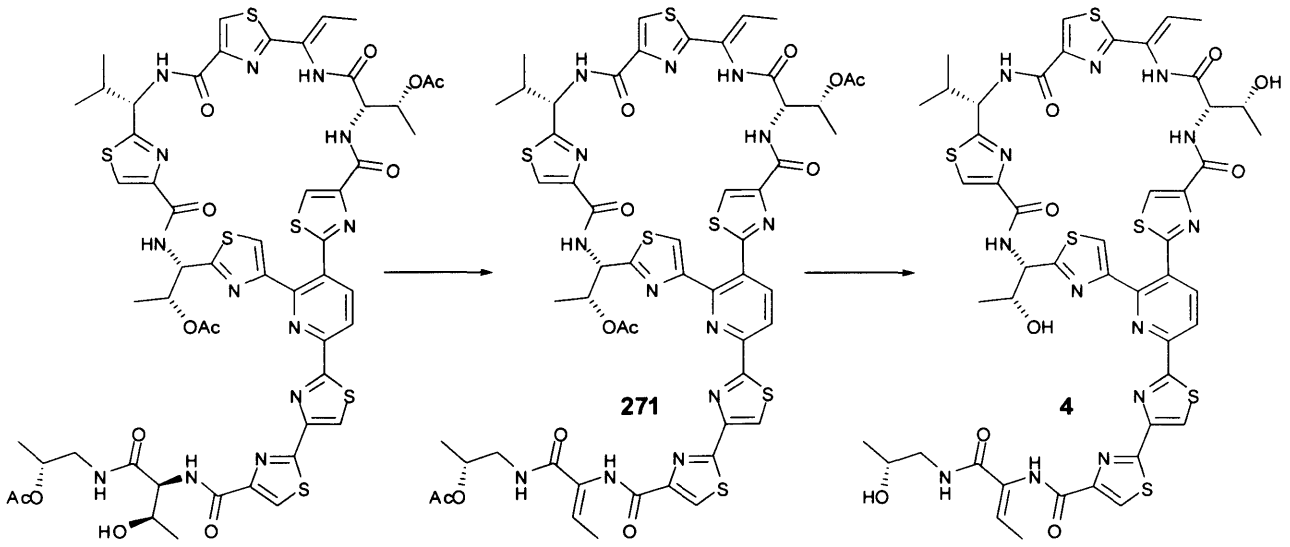


251 **268** **269** **270**

To a stirred solution of **251** (11 mg, 11 µmol) in dry pyridine (2 mL), acetic anhydride (100 µL, 108 mg, 106 µmol, 96 equiv) was added and the mixture stirred at room temperature for 24 h. The solution was evaporated *in vacuo* and the residue dissolved in DMF (2 mL) and HATU (16 mg, 52 µmol, 5 equiv) and diisopropylethylamine (18 µL, 14 mg, 106 µmol, 10 equiv) added. After 1 hour, a solution of amine **265** (~27 mg, 106 µmol, 10 equiv) and diisopropylethylamine

(37 µL, 27 mg, 212 µmol, 20 equiv) in DMF (1 mL) was added and the mixture stirred for a further 24 h at room temperature. The solvent was removed *in vacuo* and the residue purified by column chromatography on silica, eluting with chloroform-methanol (9:1). The fractions containing a blue-fluorescent spot, R_f 0.30 were mixed and evaporated *in vacuo* and the residue loaded onto silica and washed with ethyl acetate. Removal from silica by eluting with methanol furnished an off-white solid (10 mg).

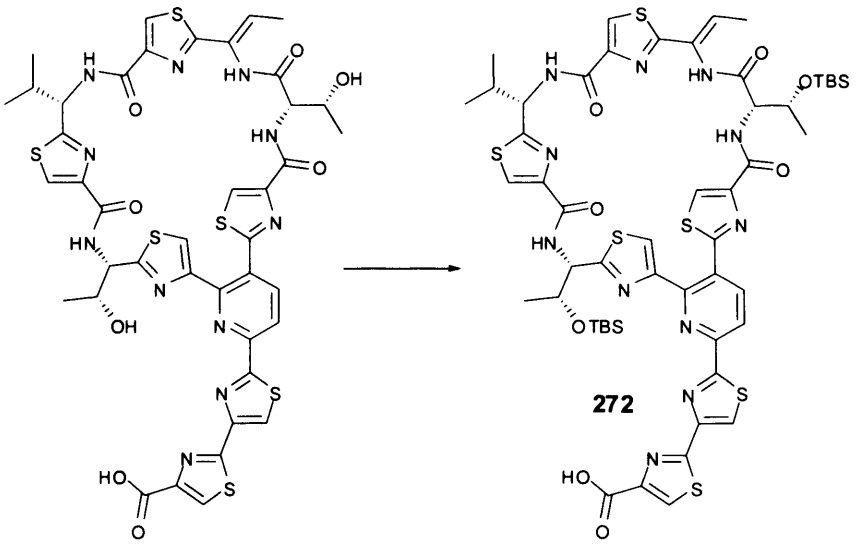
Attempted elaboration of 270 to MP1



MP1 skeleton **270** (~10 mg, 7.5 µmol) was dissolved in dichloromethane (2 mL) and triethylamine (50 µL, 36 mg, 359 µmol, 48 equiv) added, followed by dropwise addition of methanesulfonyl chloride (25 µL, 37 mg, 359 µmol, 43 equiv). The solution was stirred at room temperature for 1 hour, then partitioned between chloroform (5 mL) and water (5 mL). The organic layer was dried (Na_2SO_4) and evaporated *in vacuo*. The residue was dissolved in chloroform (2 mL), DBU (25 µL, 25 mg, 167 µmol, 22 equiv) was added and the solution stirred at room temperature for 2 hours, washed with 5% w/v potassium hydrogen sulfate, dried (Na_2SO_4) and evaporated *in vacuo*. Purification on a silica plug, eluting with ethyl acetate followed by removal with methanol furnished a chromatographically homogeneous off-white solid (4 mg).

MP1 skeleton **271** (~4 mg, 3 µmol) was dissolved in methanol-water (5:1, 3 mL) and lithium hydroxide monohydrate (4 mg, 95 µmol, 32 equiv) added. The solution was stirred at room temperature for 12 h then evaporated *in vacuo*. Water (2 mL) and 10% w/v potassium hydrogen sulfate (2 mL) were added and the acidic aqueous solution extracted with ethyl acetate (2 x 5 mL). The combined organic extracts were dried (Na_2SO_4) and evaporated *in vacuo* to afford an intractable mixture of unidentifiable products as a colourless solid (2 mg).

Silyl-protection of macrocycle 251 [section 5.6.3]



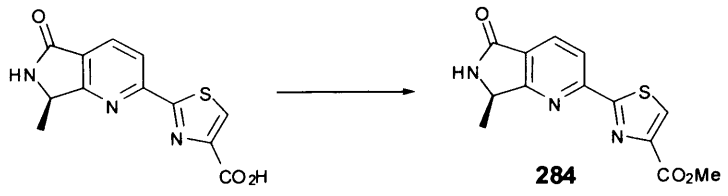
TBDMSOTf and Et_3N

Macrocycle **251** (9 mg, 9 μmol) was dissolved in DMF (1 mL) and triethylamine (50 μL, 36 mg, 359 μmol, 40 equiv) added. TBDMS-triflate (50 μL, 58 mg, 217 μmol, 24 equiv) was added and the solution stirred at room temperature for 18 hours, evaporated *in vacuo* and the residue stirred at room temperature in methanol (1 mL) and aqueous sodium hydrogen carbonate (1 M, 1 mL) for 2 hours. The solution was extracted with hexane (1 mL), acidified to pH 3 with 10% w/v potassium hydrogen carbonate, and extracted with chloroform (4 x 1 mL). A pale brown precipitate (6 mg) was formed on addition of the organic solvent, and was subsequently identified as unreacted macrocycle **251**.

TBDMSOTf and imidazole

Macrocycle **251** (~5 mg, 5 μmol) was dissolved in d_7-DMF (0.5 mL) and imidazole (17 mg, 250 μmol, 50 equiv) added. TBDMS-triflate (50 μL, 58 mg, 217 μmol, 24 equiv) was added and the solution stirred at 50 °C for 24 hours. No product or starting material could be identified in the reaction mixture.

(*R*)-Methyl 2-(7-methyl-5-oxo-6,7-dihydro-5H-pyrrolo[3,4-b]pyridin-2-yl)thiazole-4-carboxylate (284)



Cyclothiazomycin γ-lactam **276** (4.5 mg, 16 μmol) was dissolved in methanol (3 mL) and hydrochloric acid (6 M, 0.3 mL) added. The solution was heated at reflux for 3 hours, allowed to cool to room temperature, then evaporated *in vacuo* to furnish the title compound (4 mg, 85%) as an off-white solid, $[\alpha]_D^{24}$ +8.00 (*c* 0.2, chloroform) (Found: $[M+H]^+$, 290.0609. Calc. for $C_{13}H_{12}N_3O_3S$: 290.0599); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 2956, 2915, 2845, 1719, 1656, 1598, 1547, 1443, 1408, 1258, 1082, 1017, 805; δ_H(400 MHz; $CDCl_3$) 8.38 (1 H, d, *J* 8.0, pyr-4), 8.27 (1 H, s, thz-4), 8.16 (1 H, d, *J* 8.0, pyr-5), 6.35 (1 H, s, exch. D_2O, NH), 4.72 (1 H, q, *J* 7.0, CH_3CH), 3.94 (3 H, s, O*Me*), 1.57 (2 H, d, *J* 7.0, CH_3CH); *m/z* (APCI) 290 ($[M+H]^+$, 100%); spectroscopic properties identical to the natural material.

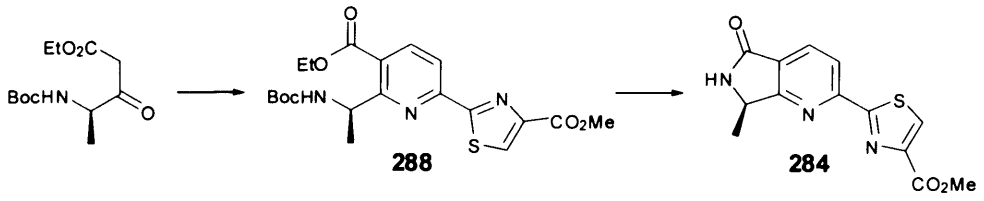
(*R*)-Ethyl 4-(*tert*-butoxycarbonylamino)-3-oxopentanoate (285)

285

i) *n*–Butyllithium in hexanes (2.5 M, 4.00 mL, 10.0 mmol, 2 equiv) was added dropwise to a stirred solution of diisopropylamine (1.40 mL, 10.0 mmol, 2 equiv) in dry THF (10 mL) at 0 °C and stirred for 30 minutes. The solution was cooled to –78 °C and dry ethyl acetate (0.49 mL, 5.01 mmol, 1 equiv) was added dropwise and stirred for 30 minutes.

ii) Ethyl chloroformate (0.52 mL, 5.5 mmol, 1.1 equiv) was added dropwise to a stirred solution of *N*-Boc-D-alanine (946 mg, 5.00 mmol, 1 equiv) and triethylamine (0.77 ml, 5.5 mmol, 1.1 equiv)) in dry THF (10 mL) at 0 °C and stirred for 30 minutes. The mixture was filtered and added dropwise to i.), stirred at –78 °C for 1 h, warmed to room temperature and quenched with saturated aqueous ammonium chloride (60 mL). The organic solvent was evaporated *in vacuo* and the resulting aqueous solution extracted with ethyl acetate (2 x 30 ml). The combined organic layers were washed with brine (40 ml), dried (Na_2SO_4) and evaporated *in vacuo*. Purification by column chromatography on silica, eluting with petroleum ether–ethyl acetate (2:1) gave the title compound (391 mg, 30%) as a pale yellow oil, $[\alpha]_D^{25}$ +17.2 (*c* 1.00, chloroform) {lit.,[143] +10.7 (*c* 2.50, chloroform)}; R_f 0.30 (petroleum ether–ethyl acetate, 2:1); δ_H(250 MHz; $CDCl_3$) 5.10 (1 H, br s, N*H*Boc), 4.54 (1 H, s, =CH), 4.32 (1 H, q, *J* 7.1, C*H*Me), 4.12 (2 H, q, *J* 7.2, CH_2CH_3), 1.37 (9 H, s, CMe_3), 1.29 (3 H, d, *J* 7.1, CH*Me*), 1.21 (3 H, t, *J* 7.2, CH_2CH_3); δ_C(67 MHz; $CDCl_3$) 172.9 (C), 166.9 (C), 155.2 (C), 80.1 (CH), 80.0 (C), 55.4 (CH_2), 45.8 (CH), 28.3 (CH_3), 17.1 (CH_3), 14.1 (CH_3).

(*R*)-Methyl 2-(7-methyl-5-oxo-6,7-dihydro-5H-pyrrolo[3,4-b]pyridin-2-yl)thiazole-4-carboxylate (284)



To a stirred solution of (*R*)-ethyl 4-(*tert*-butoxycarbonylamino)-3-oxopentanoate (30 mg, 116 µmol) in ethanol (10 mL) was added ammonium acetate (45 mg, 578 µmol, 5 equiv) and the solution stirred at 50 °C for 20 hours, allowed to cool to room temperature, and methyl 2-(propynoyl)thiazole-4-carboxylate (34 mg, 174 µmol, 1.5 equiv) added. The solution was stirred for 2.5 hours, cooled to 0 °C and *N*-iodosuccinimide (26 mg, 116 µmol, 1 equiv) added. The mixture was stirred for 30 minutes, evaporated *in vacuo* and partitioned between ethyl acetate (15 mL) and saturated aqueous sodium hydrogen carbonate (10 mL). The aqueous layer was further extracted with ethyl acetate (10 mL) and the combined organic extracts washed with water (10 mL) and brine (10 mL), dried (Na_2SO_4) and evaporated *in vacuo*. Purification by column chromatography on silica, eluting with petroleum ether-ethyl acetate (2:1) furnished compound **288** (18 mg, 36%) as a pale yellow solid, R_f 0.30 (petroleum ether–ethyl acetate, 2:1).

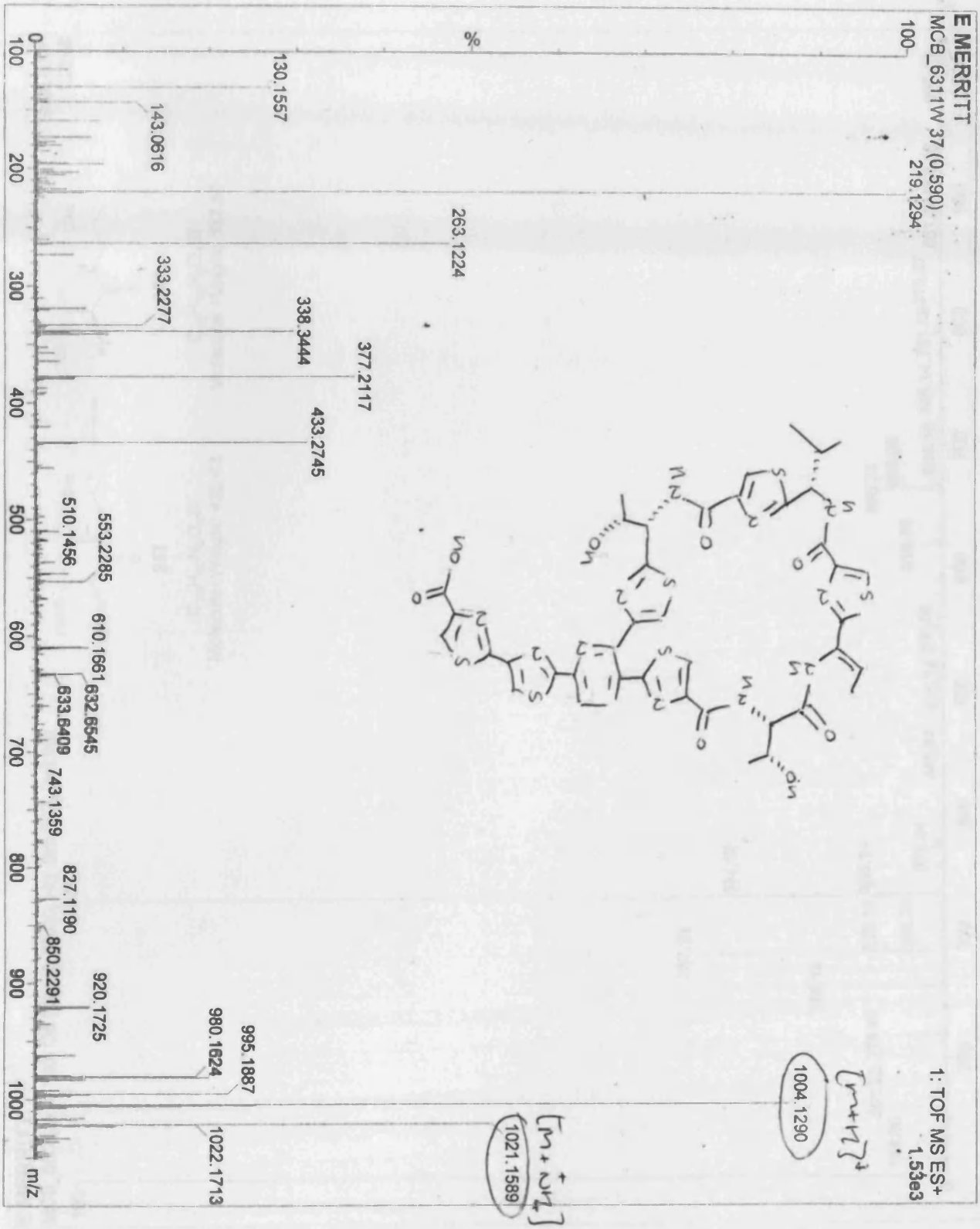
Pyridine **288** (5 mg, 12 µmol) was stirred in hydrochloric acid (6 M, 3 mL) at room temperature for 6 hours. The solution was neutralised by addition of aqueous sodium hydroxide (5 M) and extracted with dichloromethane (3 x 5 mL). The combine organic extracts were dried (Na_2SO_4) and evaporated *in vacuo* to furnish the title compound (4 mg, 85%) as an off-white solid, $[\alpha]_D^{22}$ +59.20 (*c* 0.20, chloroform); spectroscopic properties identical to the previously prepared synthetic material.

Appendices

Appendices

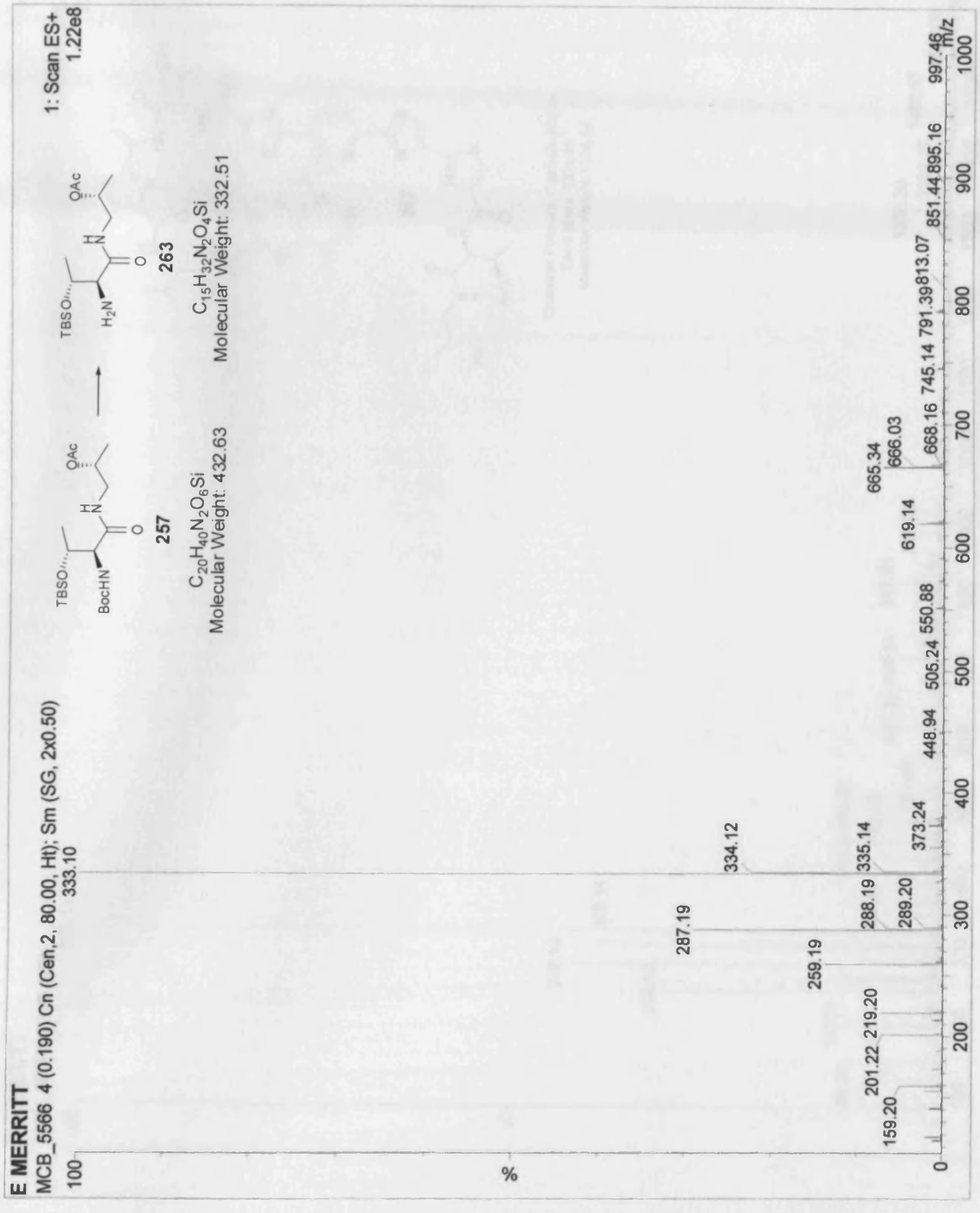
Appendix One

Mass spectrum of macrocycle **251**



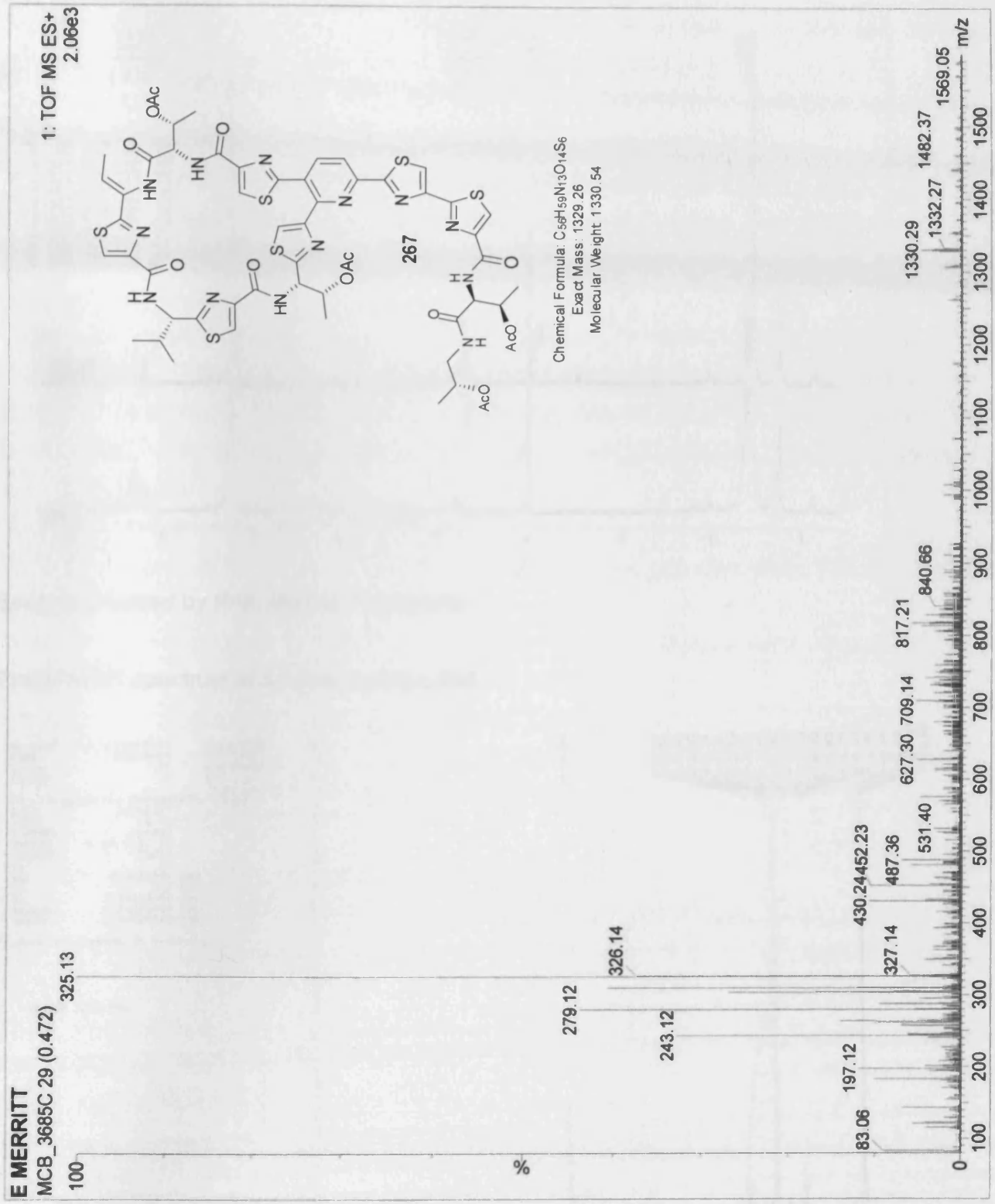
Appendix Two

Mass spectrum illustrating selective Boc-deprotection of side chain fragment **257**



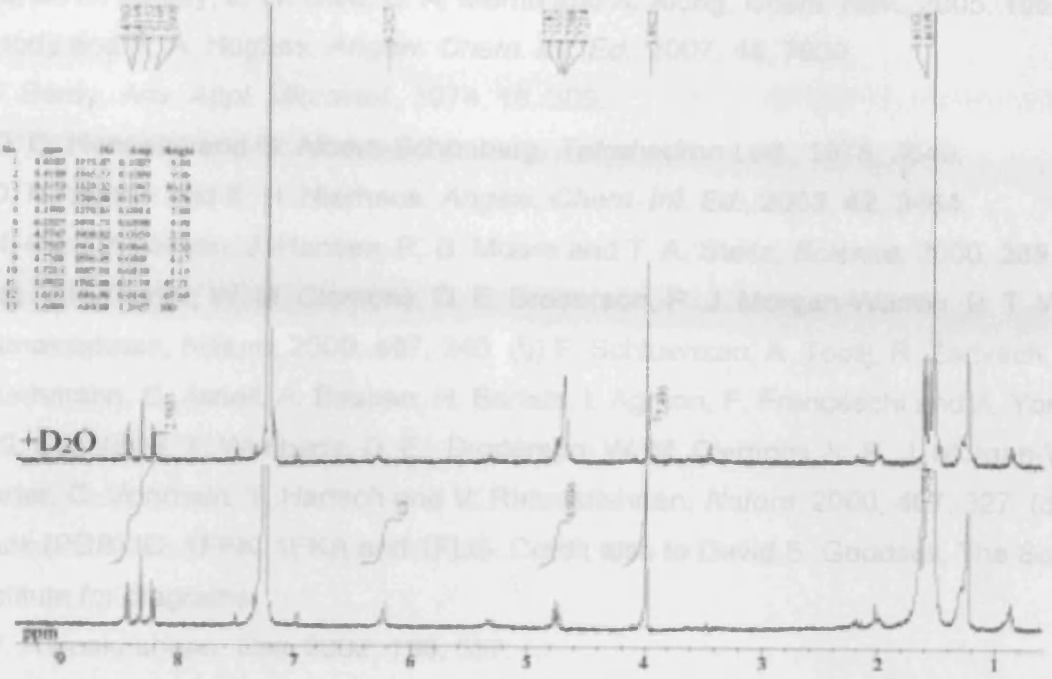
Appendix Three

Mass spectrum of acetate-protected macrocycle **267**



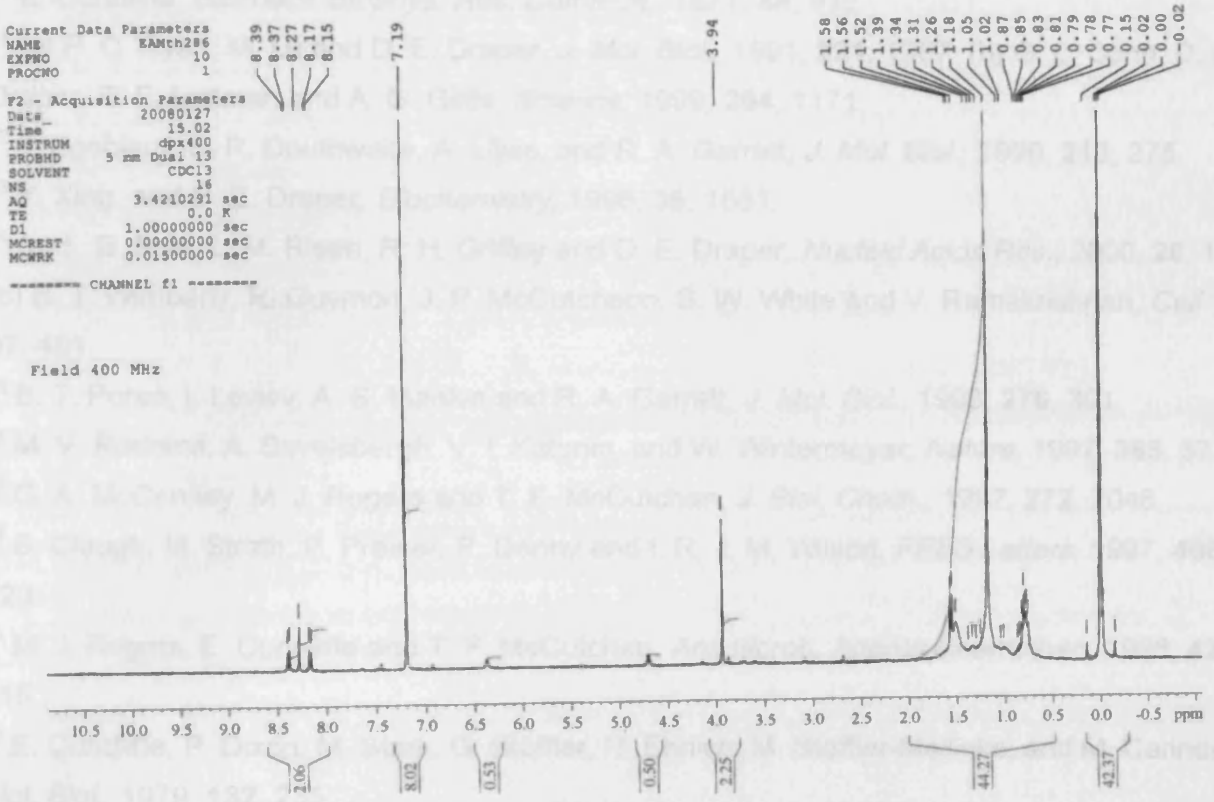
Appendix Four

Proton NMR spectra of lactam **284** derived from cyclothiazomycin B1



Spectra provided by Prof. Masaru Hashimoto

Proton NMR spectrum of synthetic lactam **284**



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