UNIVERSITY OF SOUTHAMPTON

SYNTHESIS OF LANTIBIOTIC BASED TEMPLATES FOR SOLID PHASE SYNTHESIS AND COMBINATORIAL CHEMISTRY

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ABSTRACT

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In an attempt to strive for environmental supremacy, many organisms biosynthesise and secrete peptide antibiotics which display activity against a variety of competitors. One such class of compounds is the lantibiotics, which are characterised by their unusually high proportion of non-proteinogenic residues, including the bridged amino acid lanthionine. These novel residues essentially govern the structure and function of these exciting peptides. It is our wish to exploit the medicinally relevant properties of the lantibiotics by ultimately using lanthionyl scaffolds to support combinatorial libraries.

We describe a variety of approaches to the lanthionine skeleton, and in particular focus our synthetic effort upon the iodoalanines. A stereoselective synthesis of lanthionines is presented, and the optical course of the reaction investigated by the method of Mosher. Finally, we apply our methodology to the synthesis of lanthionines suitable for solid phase applications, and demonstrate the utility of our approach by synthesising a lanthionyl peptide on a solid support.

Contents

Abbreviations

δ	chemical shift (ppm)
Ac	acetyl
ADDP	1,1'-(azodicarbonyl)dipiperidine
Ala	alanine
Ar	aryl
Arg	arginine
Asn	asparagine
Asp	aspartic acid
Bn	benzyl
Boc	*tert*-butyloxycarbonyl
br	broad
Bu	butyl
Cbz	carboxybenzyl
Cys	cysteine
d	doublet
DCM	dichloromethane
DEAD	diethyl azodicarboxylate
dec.	decomposes
Dha	didehydroalanine
Dhb	dehydrobutyrine
DIAD	diisopropyl azodicarboxylate
DIC	*N, N*-diisopropylcarbodiimide
DIPEA	*N, N*-diisopropylethylamine
DMAP	4-dimethylaminopyridine

DMF	*N, N*-dimethylformamide
DMSO	dimethylsulfoxide
DTNB	5,5'-dithiobis(2-nitrobenzoic acid)
ES	electrospray
Et	ethyl
FLEC	1-(9-fluorenyl)ethyl chloroformate
Fmoc	9*H*-fluorenylmethoxycarbonyl
FT	Fourier transform
Gln	glutamine
Glu	glutamic acid
His	histidine
HMPA	hexamethylphosphoric triamide
HOBt	1-hydroxybenzotriazole
HPLC	high performance liquid chromatography
HR	high resolution
i.d.	internal diameter
Ida	iodoalanine
Ile	isoleucine
Im	imidazolyl
^{i}Pr	isopropyl
IR	infra-red
J	coupling constant (Hz)
Lan	lanthionine
Leu	leucine
LR	low resolution
Lys	lysine

m	multiplet, medium
M. Pt.	melting point
Me	methyl
Met	methionine
MS	mass spectrometry
Ms	methanesulfonyl
NMM	*N*-methylmorpholine
NMR	nuclear magnetic resonance
Orn	ornithine
PCOR	peptide cyclisation on oxime resin
Ph	phenyl
Phe	phenylalanine
PLE	porcine liver esterase
PPL	porcine pancreatic lipase
Pro	proline
q	quartet
R. T.	retention time
r.t.	room temperature
R_f	retention factor
RP	reversed phase
s	singlet, strong
Ser	serine
spa	small peptide antibiotic
SPPS	solid phase peptide synthesis
Su	succinimidyl
t	triplet

^{t}Bu	*tert*-butyl
TFA	trifluoroacetic acid
TFE	trifluoroethanol
th.	theoretical
THF	tetrahydrofuran
Thr	threonine
TES	triethylsilane
TIS	triisopropylsilane
TLC	thin layer chromatography
TMS	trimethylsilyl
Trp	tryptophan
Trt	triphenylmethyl
Ts	4-toluenesulfonyl
Tyr	tyrosine
UV	ultra-violet
Val	valine
w	weak

1. The Lantibiotics

1.1 Introduction

The considerable success of the pharmaceutical industry in combating bacterial infection has instilled an unwarranted sense of complacency in society.[1] Factors such as over-prescription, patient non-compliance and bacterial exchange of genetic material have rendered antibiotics largely ineffective in many cases due to the increasingly prevalent phenomenon of bacterial resistance (Table 1).

The situation has been exacerbated by the discovery of multiple-drug resistant strains.[2] To this end, much effort has been directed at curbing the further spread of bacterial resistance. Improved hygiene programmes in care facilities coupled with a more conservative approach to antibiotic dispensation have moderated the problem, though there remains a pressing need for a new generation of efficacious anti-bacterial agents.

The laborious technique of rational drug design has been greatly complemented by recent advances in combinatorial chemistry and high-throughput screening. However, the requirement for lead compounds continues to act as the major stumbling block in the process. Inevitably, the medicinal chemist has sought inspiration from nature's vast repertoire of pharmacophores. In particular, naturally expressed peptides have received substantial attention from the pharmaceutical community.[3, 4]

1.2 Naturally Occurring Peptide Antibiotics

In an attempt to strive for environmental supremacy, many organisms biosynthesise and secrete peptide antibiotics which display activity against a variety of competitors. Examples include the crecopins[5] from insects, the magainins[6] from amphibians, the tachyplesins[7] from crabs and the well-documented family of α-defensins[8] isolated from a variety of mammalian sources, including humans.

Antibiotic	Mechanism of Resistance	Resistant Pathogens
β-Lactams: Penicillins Cephalosporins Monobactams Carbapenems	Altered penicillin-binding proteins	*Staphylococcus aureus* *Staphylococcus epidermidis* *Staphylococcus pneumoniae* *Staphylococcus sanguis* *Haemophilus influenzae* *Neisseria gonorrhoeae* *Neisseria meningitidis* *Escherichia coli* *Pseudomonas aeruginosa*
	Reduced uptake	*Pseudomonas aeruginosa* *Enterobacteriaceae cloacae* *Kiebsiella pneumoniae* *Kiebsiella oxytoca*
	β-Lactamase	*Staphylococcus aureus* *Staphylococcus epidermidis* *Enterococcus sp.* *Pseudomonas aeruginosa* *Enterobacteriaceae sp.* *Neisseria gonorrhoeae* *Neisseria meningitidis* *Moraxella sp.* *Bacteroides sp.* *Acinetobacter sp.*
	Reduced permeability	*Enterobacteriaceae sp.* *Pseudomonas aeruginosa*
Aminoglycosides: Gentamicin Tobramycin	Reduced uptake	*Pseudomonas sp.* *Bacteroides sp.* *Enterobacteriaceae sp.*
	Decreased ribosomal binding	*Streptococcus sp.*
	Modifying enzymes	*Staphylococcus sp.* *Pseudomonas sp.* *Streptococcus sp.* *Enterococcus sp.* *Enterobacteriaceae sp.*

Antibiotic	Mechanism of Resistance	Resistant Pathogens
Macrolides: Erythromycin Clindamycin	Methylating enzymes	*Streptococcus sp.* *Staphylococcus pneumoniae* *Enterococcus sp.*
Chloramphenicol	Acetyl transferase	*Streptococcus sp.* *Staphylococcus pneumoniae* *Enterobacteriaceae sp.* *Neisseria sp.*
Tetracyclines: Tetracycline Minocycline Doxycycline	Efflux	*Staphylococcus sp.* *Streptococcus sp.* *Enterococcus sp.* *Enterobacteriaceae sp.* *Neisseria gonorrhoeae* *Bacteroides sp.*
	Ribosomal protein modification	*Neisseria gonorrhoeae*
Rifampin	Reduced DNA polymerase binding	*Staphylococcus sp.* *Streptococcus sp.* *Enterococcus sp.* *Enterobacteriaceae sp.* *Pseudomonas sp.*
Folate inhibitors: Trimethoprim Sulfamethoxazole	Altered targets	*Staphylococcus sp.* *Streptococcus sp.* *Enterobacteriaceae sp.* *Neisseria sp.*
	Reduced permeability	*Pseudomonas sp.* *Camphylobacter sp.*
Glycopeptides: Vancomycin Teicoplanin	Altered target	*Enterococcus sp.* *Leuconostac sp.* *Lactococcus sp.* *Pediococcus sp.* *Lactobacillus sp.*

Table 1: Examples and mechanisms of bacterial resistance towards antibiotics.

Host defence peptides have also been isolated in flora e.g. the thionins[9] from monocotyledonous plants.

The prolific nature of bacteria results in their interaction with almost every cell line at some point in time. It is therefore unsurprising to discover that antibiosis is widespread amongst the prokaryotes. Such peptide antibiotics may be classified into two broad sub-groups, depending on their method of biosynthesis.

The first class comprises peptides which are not encoded for by a specific gene, but are constructed by the action of multi-enzyme complexes[10] e.g. gramicidin S[11], alamethicin[12], bacitracin[13], valinomycin[14] and polymixin B[15]. Since the genetic code does not dictate their composition, such antibiotics can often contain novel amino acid residues. The synthetic process often utilises exceptionally large enzymes, each accommodating many domains capable of effecting one or more biosynthetic transformations. After each step, the nascent peptide is passed to the next domain for further modification.[16] Due to the low affinities associated with the synthetic domains, these antibiotics commonly manifest themselves as families of related peptides rather than single homogeneous entities.

In contrast, the second category of peptide antibiotics is characterised by their ribosomal origins. They are specifically coded for at a genetic level and expressed ribosomally as precursor peptides containing only the twenty proteinogenic amino acids. However, many of these pre-peptides are then subjected to post-translational enzymatic modifications, allowing for the inclusion of residues beyond the scope of the triplet genetic code, such as stereo-inverted, unsaturated and bridged amino acids.

Examples of peptide antibiotics within this division include the glycine rich microcin B17[17] from *Escherichia coli*, the lantibiotics[18] and pediocin PA-1[19], a bacteriocin that displays activity against a range of food-borne pathogens including *Listeria monocytogenes*. In many instances, the cellular targets of these antibiotics are quite distinct from those of conventional therapeutics. It is therefore conceivable that structural motifs from these peptides may be adopted by medicinal chemists as templates for future anti-bacterial agents. Of particular interest to us are the lantibiotics, a family of polycyclic, thioether containing peptides, which will be discussed presently.

1.3 Discovery and Structure of the Lantibiotics

Although the fortuitous discovery of penicillin by Fleming in 1928 was a significant milestone in the fight against bacterial infection, it was not the first reported instance of an anti-microbial agent. Literature accounts of potent anti-bacterial substances[20, 21] derived from lactic acid bacteria marginally pre-date Fleming's work, though the causative agents were not elucidated for another forty years. In hindsight, the bactericidal activity could most probably be attributed to a class of peptides we now refer to as the lantibiotics.

The structural determination of the lantibiotics was greatly hampered by their high content of unusual amino acids such as 2,3-didehydroalanine **1**, (Z)-2,3-didehydrobutyrine **2**, *erythro*-3-hydroxyaspartic acid **3**, (2*S*, 6*R*)-lanthionine **4**, (2*S*, 3*S*, 6*R*)-methyllanthionine **5**, (2*S*, 9*S*)-lysinoalanine **6** and *S*-[(Z)-2-aminovinyl]-cysteine **7** (Fig. 1).

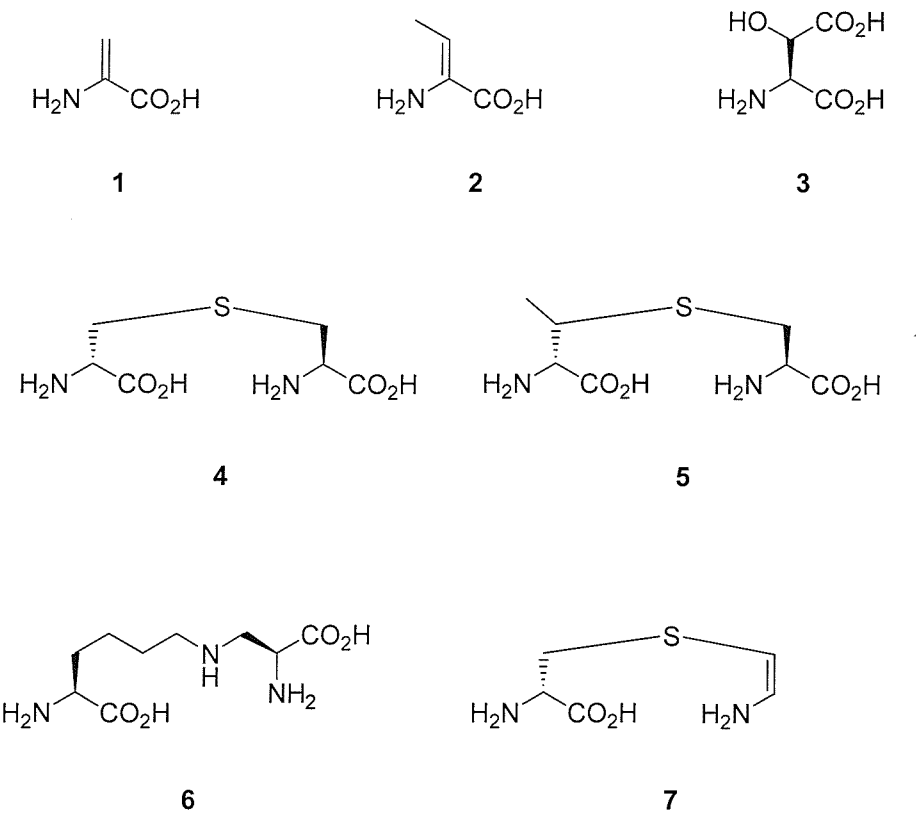


Fig. 1: Non-proteinogenic amino acids which are found incorporated in lantibiotics.

In fact, the name "lantibiotic" was coined by Schnell[22] in 1988 to reflect the fact that this remarkable family of peptide antibiotics all contain derivatives of the amino acid lanthionine **1**.

The unique structural features of the lantibiotics were revealed for the first time following pioneering work by Gross and Morell in the late sixties.[23, 24] Their correct elucidation of the structure of nisin **8** in 1971 was a major accomplishment, considering both the complexity of the peptide and the unavailability of now routine analytical methodology such as high field NMR and HPLC. This achievement was soon followed by the publication of the primary structure of subtilin **9**, a lantibiotic produced by *Bacillus subtilis*. The sequencing of the structural genes of nisin and subtilin some twenty years later confirmed their structural assignments to be correct.[25, 26]

The past two decades have seen the number of newly discovered lantibiotics rise sharply (Figs. 2, 3). The fascinating structural and biological properties of these molecules have attracted much research interest, and ongoing investigations promise to unveil more members of the lantibiotic family. Over thirty lantibiotics originating from a wide variety of bacterial strains are now known. All of these peptides have been shown to contain lanthionine as well as having an extremely high ratio of modified amino acids (Tables 2, 3).

The majority of these non-proteinogenic residues arise from the post-translational processing of serine, threonine and cysteine residues. Such enzyme mediated modifications mainly result in the formation of dehydroamino acids and lanthionyl residues. In addition, a variety of other structural embellishments to the basic peptide structure can be found. The precursor peptides of epidermin and gallidermin contain C-terminal cysteines, which are subsequently oxidised and decarboxylated, ultimately affording *S*-[(Z)-2-aminovinyl]-cysteine residues, **7**.

α, β-unsaturated amino acids exposed at the amino terminus of the lantibiotic following scission of the leader sequence are highly unstable. They rapidly undergo hydrolytic deamination to give 2-oxopropionyl and 2-oxobutyryl residues as found in lactocin S and Pep5 respectively. In epilancin K7 and epicidin 280, the oxacyl functions are further reduced to give the *N*-terminal 2-hydroxypropionyl peptides.

Other non-ribosomally expressed residues found in lantibiotics include lysinoalanine, D-alanine, *erythro*-3-hydroxyaspartic acid and *allo*-isoleucine. The biosynthetic origins of these novel amino acids will be discussed in a later section.

Lantibiotic	Producer Organism	Molecular Mass (Da)	% Modified Residues
Type-A Lantibiotics			
Nisin A	*Lactococcus lactis*	3353	38
Nisin Z	*Lactococcus lactis*	3353	38
Subtilin	*Bacillus subtilis*	3317	40
Epidermin	*Staphylococcus epidermis*	2164	41
[V_1, L_6]-Epidermin	*Staphylococcus epidermis*	2151	41
Gallidermin	*Staphylococcus gallinarium*	2164	41
Mutacin B-Ny266	*Streptococcus mutans*	2270	41
Pep5	*Staphylococcus epidermis*	3488	26
Epicidin 280	*Staphylococcus epidermis*	3133	27
Epilancin K7	*Staphylococcus epidermis*	3032	32
Lactocin S	*Lactobacillus sake*	3764	24
SA-FF22	*Streptococcus pyogenes*	2795	27
Lacticin 481	*Lactococcus lactis*	2901	26
Salivaricin A	*Streptococcus salivarius*	2315	27
[K_2, F_7]-Salivaricin A	*Streptococcus salivarius*	2321	27
Variacin	*Micrococcus varians*	2658	28
Cypemycin	*Streptomyces sp.*	2094	41

Table 2 : The properties of some type-A lantibiotics.

Lantibiotic	Producer Organism	Molecular Mass (Da)	% Modified Residues[b]
Type-B Lantibiotics			
Cinnamycin	*Streptomyces cinnamoneus*	2042	47
Duramycin	*Streptomyces cinnamoneus*	2014	47
Duramycin B	*Streptoverticillium sp.*	1951	47
Duramycin C	*Streptomyces griseoluteus*	2008	47
Ancovenin	*Streptomyces sp.*	1959	37
Mersacidin	*Bacillus sp.*	1825	42
Actagardine	*Actinoplanes sp.*	1890	45
Structure incomplete			
Cytolysin LL[a]	*Enterococcus faecalis*	4164	ND
		2631	ND
Lacticin 3147[a]	*Lactococcus lactis*	2852	ND
		3323	ND
Streptococcin C55[a]	*Staphylococcus aureus*	3339	ND
		2993	ND
Carnocin U149	*Carnobacterium piscicola*	4635	ND
Salivaricin G-32	*Streptococcus salivarius*	2667	ND
Sublancin 168	*Bacillus subtilis*	3877	ND
Mutacin T8	*Streptococcus mutans*	3245	ND

[a] Two-component lantibiotics.

[b] ND, not determined.

Table 3 : The properties of some type-B and structurally incomplete lantibiotics.

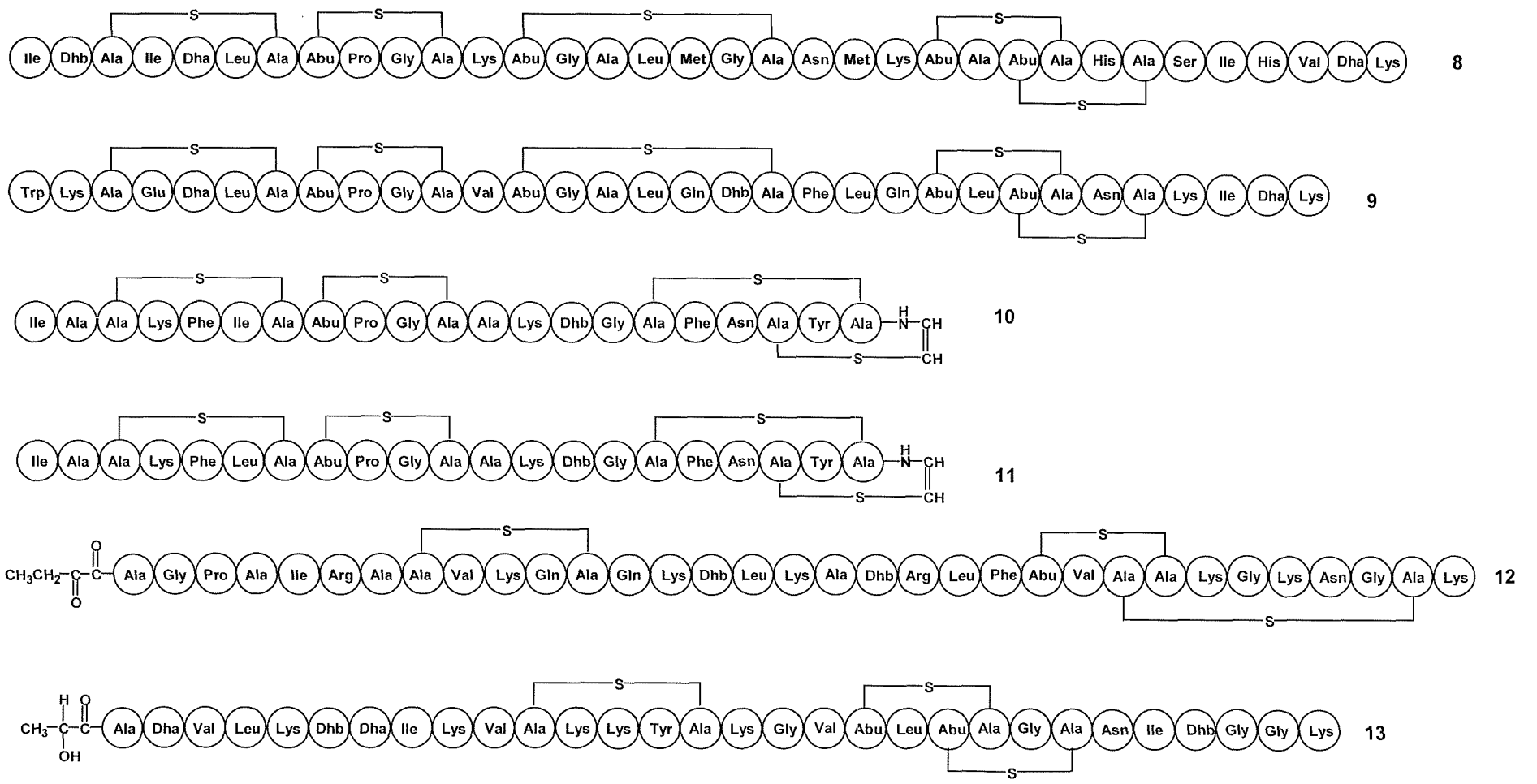


Fig. 2 : The primary structure of some type-A lantibiotics: nisin **8**, subtilin **9**, epidermin **10**, gallidermin **11**, Pep5 **12** and epilancin K7 **13**. Abu-S-Ala: β-methyllanthionine, Dha: dehydroalanine, Dhb: dehydrobutyrine. By convention, rings are labelled alphabetically from the amino terminus.

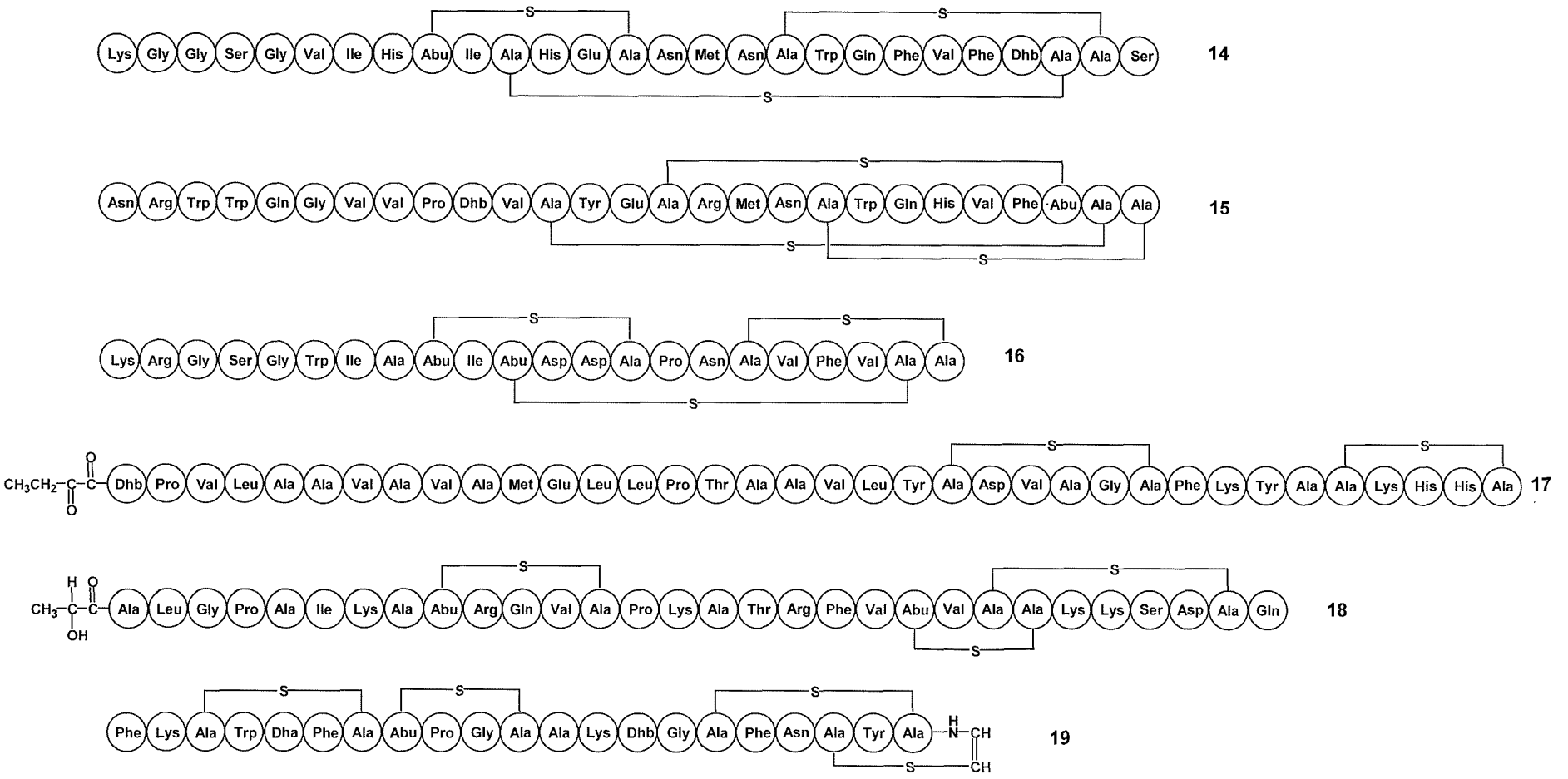


Fig. 3 : The primary structure of some type-A lantibiotics: lacticin 481 **14**, mutacin II **15**, salivaricin A **16**, lactocin S **17**, epicidin 280 **18** and mutacin B-Ny266 **19**. Abu-S-Ala: β-methyllanthionine, Dha: dehydroalanine, Dhb: dehydrobutyrine. By convention, rings are labelled alphabetically from the amino terminus.

1.4 Classification of Lantibiotics

According to a proposal by Jung[27], the lantibiotics may be classified by virtue of their structural and biological properties. The type-A lantibiotics (Table 2, Figs. 2, 3) such as nisin **8** and subtilin **9** are strongly cationic, corkscrew shaped peptides, having molecular masses in excess of 2100 Da. Despite differences in their sizes, these peptides display a marked structural relationship, in that they share significant sequence and ring homology. The anti-microbial activity of the peptides in this sub-group stems from their ability to permeabilise the cytoplasmic membrane of their target cells.

In contrast, type B lantibiotics (Table 3, Fig. 4) such as duramycin and cinnamycin are almost neutral, globular structures with molecular masses below 2100 Da. Difficulties in determining the structures of these complex peptides, and several reisolations under different names caused considerable confusion until the situation was clarified by Fredenhagen[28] in 1990. The residues within their ring systems are usually highly conserved, and they exert their bactericidal activity by inhibiting specific enzymes.

The assignment of lantibiotics into these two groupings is becoming increasingly difficult as novel peptides with intermediate structures are discovered. Of considerable interest are the relatively new dual-component lantibiotics cytolysin[29], lacticin 3147[30] and streptococcin C55[31] which require the presence of both factors to effect their anti-bacterial properties (Table 3). Investigation of these lantibiotics at a pre-ribosomal level indicates the presence of two structural genes. Both encode for products that are post-translationally modified and secreted independently.

1.5 Conformational Analysis of Lantibiotics

Difficulties associated with obtaining crystals and X-ray structures of lantibiotics have forced researchers to adopt techniques such as 2D-NMR[32], molecular dynamics simulations and circular dichromism to probe the conformation of the lantibiotics. In solution, the type A-lantibiotics were found to be more flexible than once thought.

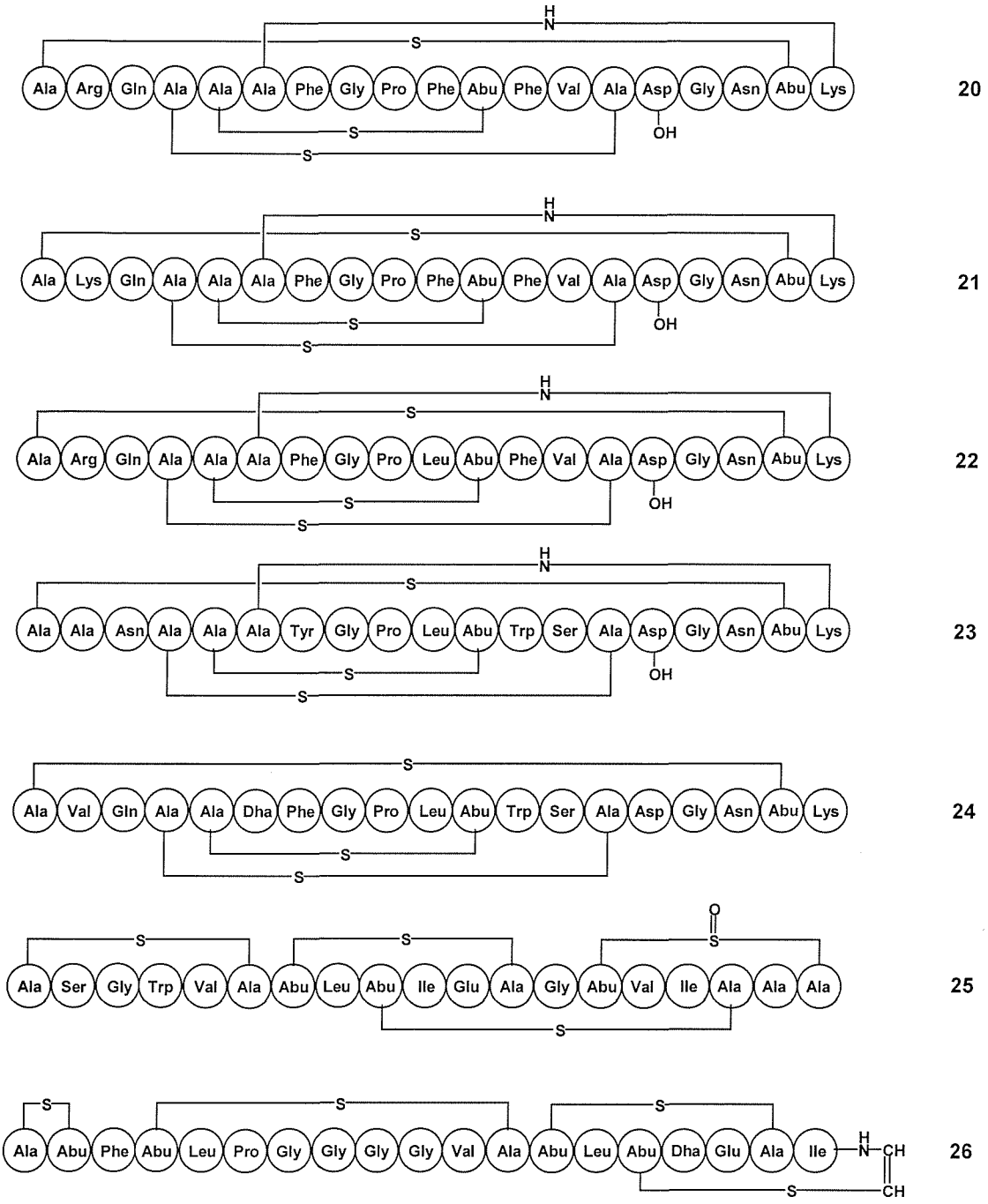


Fig. 4 : The primary structure of some type-B lantibiotics: cinnamycin **20**, duramycin **21**, duramycin B **22**, duramycin C **23**, ancovenin **24**, actagardine **25** and mersacidin **26**. Ala-S-Ala: lanthionine, Abu-S-Ala: β-methyllanthionine, Dha: dehydroalanine, Asp-OH: 3-hydroxyaspartic acid, Ala-NH-Lys: lysinoalanine. By convention, rings are labelled alphabetically from the amino terminus.

Defining structural motifs were only identified in small rings, such as the interlocked D/E ring systems of subtilin and nisin.

However, in membrane-mimicking solvents such as DMSO and trifluoroethanol, the peptides display definite amphiphilic behaviour and assume aligned, rod-shaped structures, with polar and hydrophobic residues occupying distal locations on the molecule.[33] This type of conformation is deemed a necessity for the membrane-depolarising activity of the lantibiotics.

The structural determination of nisin in SDS and DOPC micelles confirmed the amphiphilic nature of the peptide and provided valuable models for the study of lantibiotic interactions with biomembranes.[34] Further NMR studies identified that the B, D and E rings of nisin act essentially as β-turns, rigidified by the thioether bonds. A less conformationally demanding region was revealed between the C and D rings; it is believed that the flexibility of this region is pivotal for the membrane insertion properties of the peptide.[35]

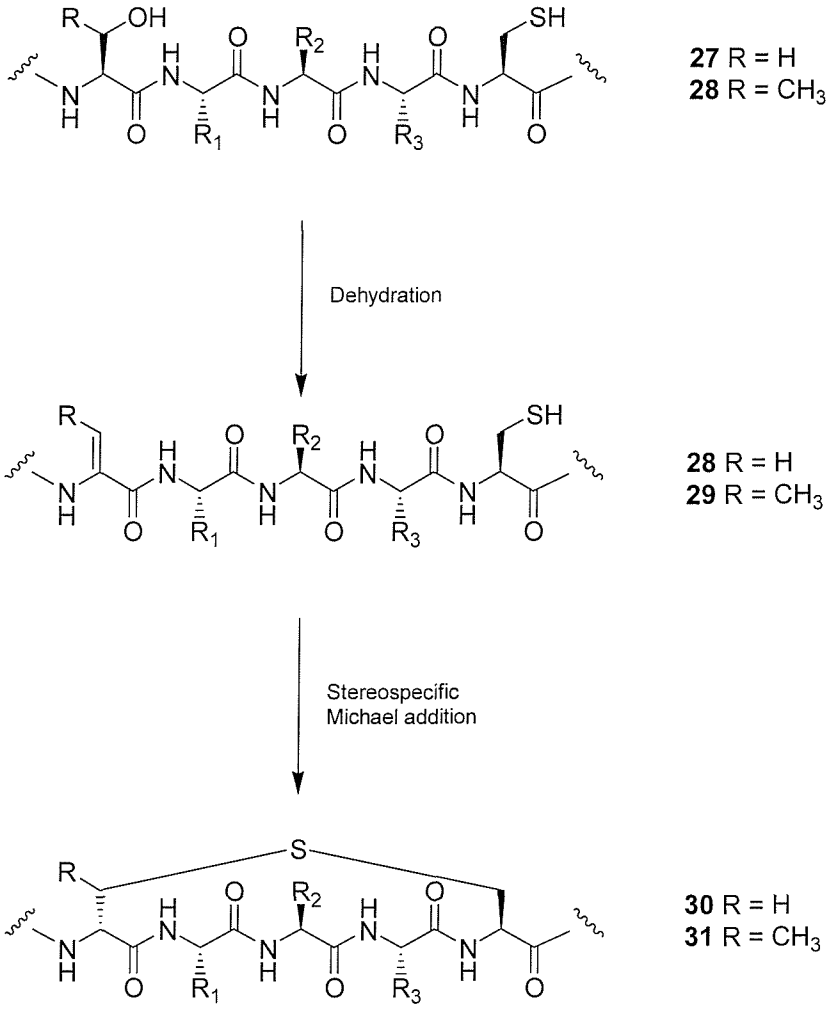
The type-B lantibiotics also exhibit strong amphiphilic character, but due to their high degree of intramolecular cross bridging they lack the flexibility of their type-A counterparts. NMR studies performed on duramycin[36], mersacidin[37] and actagardine[38] show these peptides to adopt rigid, globular conformations. This is unsurprising, considering that virtually all the residues in these peptides are part of at least one cyclic system. However, the conserved ring in actagardine and mersacidin has been demonstrated to form a U-shaped cleft in the molecule. It is possible that this feature is important to the biological function of these peptides.

1.6 Biosynthesis of the Lantibiotics

The publication of the gene-encoded peptide sequences of nisin[23] and subtilin[24] by Gross *et al.* inspired a great deal of speculation concerning the mode of synthesis of these intriguing peptides. The unprecedented content of non-proteinogenic amino acids in the lantibiotics prompted Bodanszky *et al.* to suggest that these molecules were biosynthesised by non-ribosomal means.[39] They proposed that an invariant genetic code did not allow for the synthesis of peptides containing unusual residues.

However, in 1966 Hurst demonstrated that inhibitors of protein biosynthesis, such as chloramphenicol, blocked nisin production, forcing researchers to reassess the manner by which lantibiotics are generated.[40]

A major development occurred in 1969, when Ingram proved that radio-label from [^{14}C]-serine, [^{14}C]-threonine and [^{35}S]-cysteine was incorporated into mature nisin. These observations led Ingram to propose that nisin was synthesised from a ribosomally derived precursor peptide in which the unusal amino acids were replaced by serine, threonine and cysteine.[41, 42] Following translation, the β-hydroxylated amino acids are dehydrated to dehydroalanine and dehydrobutyrine residues. Subsequently, the enzyme-mediated Michael addition of proximal Cys residues to the dehydroamino acids results in the formation of lanthionine bridges (Scheme 1).



27 R = H
28 R = CH_3

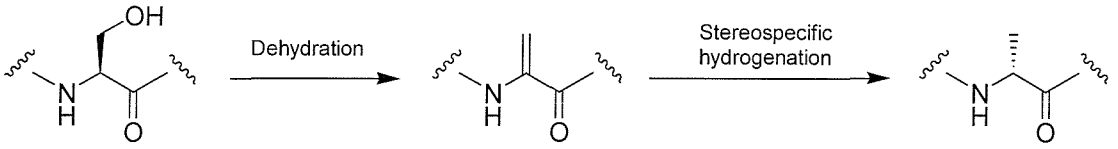
28 R = H
29 R = CH_3

30 R = H
31 R = CH_3

Scheme 1 : The formation of Dha, Dhb, Lan and MeLan from serine, threonine and cysteine.

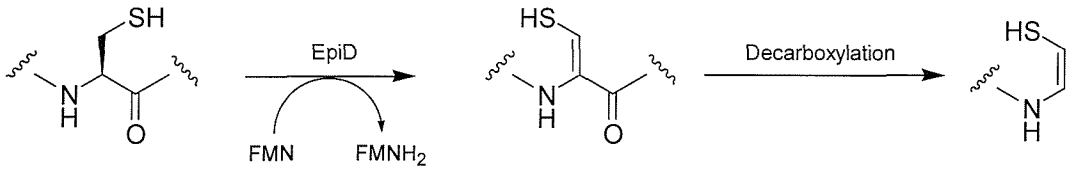
The cloning and sequencing of a variety of lantibiotic genes in the late eighties confirmed Ingram's hypothesis.[22, 25, 26] In all cases, the structural gene sequences contain serine, threonine and cysteine residues in the precise locations predicted by Ingram. Further complimentary evidence was provided by Nishio *et al.* who incubated a pre-subtilin peptide with cellular extracts from *Bacillus subtilis,* and obtained a product with identical electrophoretic mobility to subtilin.[43]

The mechanisms by which Dha, Dhb, Lan and MeLan were synthesised were subsequently extrapolated to encompass the biosynthesis of D-alanine (found in lactocin S, **17**) and *S*-[(Z)-2-aminovinyl]-cysteine (Schemes 2, 3).[44, 45]



32 **33** **34**

Scheme 2 : Proposed mechanism for the synthesis of D-Ala residues in lantibiotics. It is likely that both steps are enzyme mediated.



35 **36** **37**

Scheme 3 : Proposed mechanism for the synthesis of 2-AviCys residues in lantibiotics. Oxidation of cysteinyl residues by the protein EpiD in the presence of the cofactor FMN gives the dehydrocysteine residue **36**, which is decarboxylated to afford **37**. The highly reactive 2-AviCys residues then usually add stereospecifically to Dha or Dhb residues in the pre-lantibiotic to form cyclic thiothers.

Further investigation of the gene clusters revealed open reading frames for a variety of other proteins which have now been implicated in lantibiotic biosynthesis. Modifications to the proteinogenic precursor peptides are effected by one (LanM) or two (LanB and LanC) specific enzymes. The modified peptide is then transported and further processed by the action of dedicated LanT and LanP proteins.

Throughout the synthetic process, the producing strain protects itself from the anti-microbial properties of the lantibiotic with specialised immunity proteins (LanI, LanE, LanF and LanG). Recently it has been discovered that in some cases, gene expression is autoregulated by the fully competent lantibiotics *via* the regulatory proteins LanR and LanK. An overview of the biosynthesis of nisin is shown in Fig. 5.

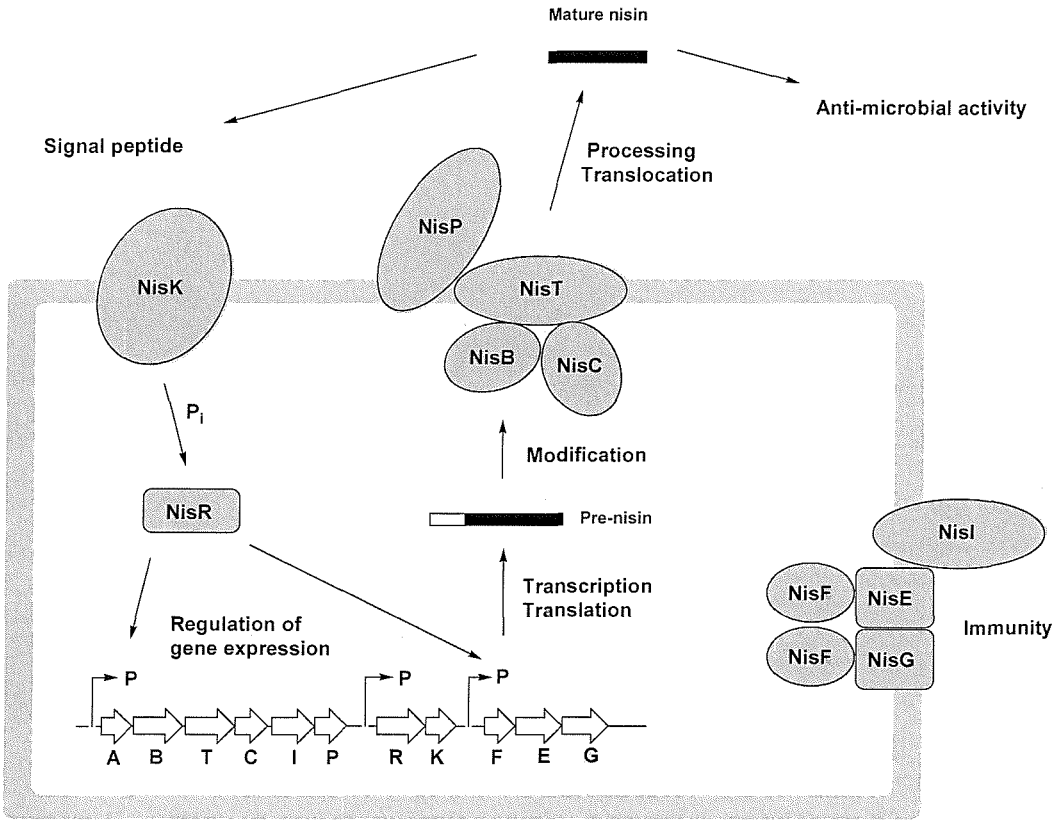


Fig. 5 : Biosynthesis of the lantibiotic nisin. Expression of *nisA* results in a precursor nisin peptide, containing only proteinogenic amino acids. NisB and NisC install dehydroamino acids and thioether bridged rings. The peptide is then translocated across the cellular membrane by NisT, and processed to give mature nisin which has anti-microbial competence. Nisin levels are sensed by NisK which autophosphorylates and activates NisR the regulator which controls the level of gene expression *via* promoter sequences, P. NisI, NisE, NisF and NisG offer the cell immunity from its own peptide.

1.7 The Genetics of Lantibiotic Biosynthesis

The wide-ranging applications touted for the lantibiotics have greatly fuelled research into the complex biosynthesis of these molecules. In particular, the elucidation of the genetic mechanisms involved in lantibiotic biosynthesis is of paramount importance if the anti-microbial properties of these peptides are to be fully exploited.

Of the type-A lantibiotics, nisin has been studied the most extensively at a genetic level.[46] The genes involved in nisin biosynthesis reside on a 70kb transposon that also contains genetic material for sucrose metabolism (Fig. 6).

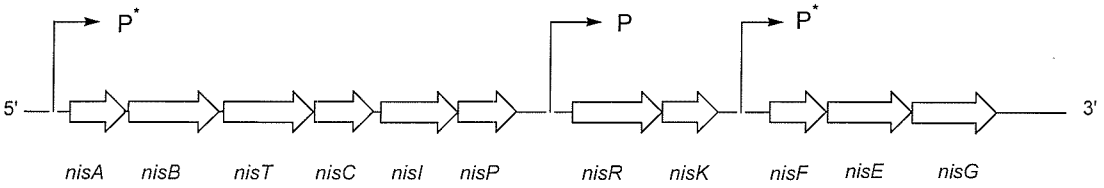


Fig. 6: The nisin operon. P: promoter, P*: nisin-regulated promoter. Lantibiotic genes are generically referred to as *lan* and their gene products as Lan. In the case of specific lantibiotics, both genes and their protein products take their name from the peptide e.g. *nisB* expresses NisB in nsin biosynthesis. The exception is subtilin, where the *spa*/Spa (small peptide antibiotic) nomenclature is used.

The 11-gene cluster is headed by *nisA*, the structural gene which codes for a 57-residue precursor peptide. The ten downstream genes *nisBTCIPRKFEG* code for regulatory proteins, proteases, transporters and immunity proteins.[47] Significant homology can be found between these proteins and the gene products from other lantibiotic operons e.g. subtilin, epidermin and Pep5. However, not all of the afore-mentioned genes are present in all of the lantibiotic gene clusters. Moreover, the organisation of the genes within these operons is subject to variation.

1.8 Lantibiotic Processing and Transport

The operons for nisin[48], subtilin[49], epidermin[50], Pep5[51] and epicidin280[52] all contain the genes *lanB* and *lanC*, which do not display any marked similarity to characterised genes in sequence databases. While the function of these genes is not completely understood, it is known that their products are vital to the biosynthetic process, since their disruption culminates in the termination of lantibiotic production.[48, 49, 53]

LanB and LanC are purported to be involved in the post-translational modification of pre-propeptides, which involves dehydration of Ser and Thr residues, and the stereo-controlled formation of Lan and MeLan residues. The LanB proteins are about 100kDa in size, and most probably function as serine and threonine dehydratase.

In contrast, the LanC proteins are much smaller, typically 400 amino acids in length. Mutants of *Staphylococcus epidermis* with *pepC* deleted have been shown to produce incorrectly modified Pep5, lacking two of the three lanthionine rings. This evidence implicates LanC in the thioether formation process.[50]

Studies on SpaB[51] and EpiB[54] found these proteins to associate with the cytoplasmic membrane, while co-immunoprecipation techniques have shown that pre-prosubtilin is manipulated by a membrane subtilin synthetase complex[55] comprising of the LanB, LanC and LanT proteins.

The gene clusters of several lantibiotics including cytolysin, mutacin II and the lacticins are deficient in *lanB*, but instead house *lanM*, which shares some homology with *lanC*. Presumably, LanM[56] is capable of catalysing both reactions involved in lantibiotic post-translational modification.

The epidermin operon is unusual in that it contains the gene *epiD*, which codes for a 181 residue flavoenzyme.[57] Subsequent studies have shown EpiD to catalyse the oxidative decarboxylation of the *C*-terminal Cys in epidermin, thus paving the way for the synthesis of an AviCys residue. Epicidin 280 contains an *epiO* gene within its cluster; since this bears a marked resemblance to a family of oxidoreductases, it is most likely that EpiO is involved in the processing of the *N*-terminal dehydro residue.[52]

Since the lantibiotics generally exert their bactericidal properties outside their producer cell, they must be exported across the cellular membrane following biosynthesis. This task is accomplished by the LanT family of transporters.

Preliminary studies in this area focused on the subtilin operon, where it was found that knock-out of *spaT* led to a rise in the intracellualar subtilin concentration.[49] Genes coding for transporter proteins have now been identified for nisin[58], lacticin 481[56], lactocin S[52] and lacticin 3147.[30] In all cases, LanT is a 60kDa membrane spanning protein belonging to the ATP-binding cassette (ABC) superfamily of transporters.[59]

Epidermin biosynthesis is unique in that host transporter proteins are most likely responsible for translocation of the lantibiotic.[60] This apparent anomaly is not so surprising when one considers that ABC transporters are known to have low substrate specificities.

The final step in lantibiotic biosynthesis is the scission of the leader peptide, which liberates the mature antibiotic. The gene clusters of many lantibiotics have been shown to contain *lanP*, which codes for a subtilisin-like serine protease. In the case of nisin biosynthesis, NisP is found anchored to the outside of the cellular membrane. However, PepP[51], ElkP[61] and LasP[62] all act cytoplasmically, probably in association with LanB, LanC and LanT. LanP deficient cultures have been shown to accumulate large amounts of pre-lantibiotic peptide.[63] Analysis of the *Bacillus subtilis* genome shows that it lacks a *spaP* gene. It is thus assumed that in the case of subtilin, cleavage of the leader sequence is facilitated by general host serine proteases.[64]

The role of the lantibiotic leader sequences has yet to be conclusively ascertained. It has been suggested that the leader peptide moderates the anti-microbial action of the lantibiotic until it is excreted.[27] However, this theory is undermined by the fact that several lantibiotics are processed cytoplasmically.

Alternatively, it has been proposed that the leader peptide acts as a signal to target the precursor peptide to the appropriate protein machinery. Site directed mutagenesis of residues within the leader sequence has been shown to have detrimental consequences upon lantibiotic production.[65] This fact strongly suggests that a specific pre-peptide sequence is a pre-requisite for the accurate biosynthesis and subsequent export of lantibiotics.

1.9 Lantibiotic Producer Strain Immunity

The production of an anti-microbial agent is an inherently risky operation for a bacterial cell. In the absence of a mechanism to protect the producer organism, the membrane disrupting capabilities of the lantibiotic would be fatal. To date, all the lantibiotic gene clusters that have been studied contain the gene *lanI*, which codes for a hydrophobic protein of variable size, LanI (50-250 amino acids).[66, 67]

The role of LanI in conferring immunity to the producer is poorly understood, but it is possible that this protein acts as a sensor for the presence of extracellular lantibiotic. Disruption of *nisI* does not lead to a cessation of nisin biosynthesis; lantibiotic production continues, but at a diminished rate.[68] These findings suggest that there are other factors involved in providing immunity for lantibiotic producers.

A variety of lantibiotic gene clusters[69] express LanE, LanF and LanG, which belong to the ABC superfamily of translocators. LanF acts as an intracellular ATP-binding domain, while LanE and LanG are trans-membraneous. It is concievable that these proteins protect the cell by causing the efflux of lantibiotics that are initially detected by LanI. Other proteins may also be implicated in the immunity process. The LanB, LanC and LanT proteins are known to form a membrane-spanning complex with translocase ability.

The elucidation of the immunity mechanism remains a major challenge for investigators in the lantibiotic field. A detailed understanding of this process could enable molecular biologists to increase production levels of lantibiotics. Moreover, a comprehensive knowledge of bacterial defence strategies could prove invaluable in designing a new generation of therapeutic agents.

1.10 Regulation of Lantibiotic Biosynthesis

Virtually all biological anabolic processes are regulated to ensure that cellular energy reserves are not unnecessarily depleted. The biosynthesis of lantibiotics is no exception. In 1995, it was shown that nisin is capable of regulating its own biosynthesis.[70] This autoregulation mechanism relies on the proteins NisK and NisR, that are coded for by two proteins at the 3' end of the nisin regulon.[71]

NisK, a histidine kinase senses for extracellular nisin and autophosphorylates if it detects the lantibiotic. The phosphate function is subsequently transferred to NisR, the response regulator. This action triggers the binding of NisR to two inducible promoters in the operon upstream of *nisA* and *nisF*, thus activating transcription of the genes *nisABTCIP* and *nisFEG*.

Despite the limited understanding of these processes, it is evident that nisin autoregulation bears a marked similarity to the quorum-sensing abilities of several Gram negative bacteria.[72, 73] This 'peptide-pheromone' paradigm has been shown to be a feasible option for the regulation of a number of other lantibiotics. However, epidermin biosynthesis appears to be regulated by only protein, EpiQ, a transcriptional activator.[74] It has been postulated that the phosphorylase activity required for signal transduction is supplied by an indigenous host component.

Once again these genetic processes have come under close scrutiny, as it is strongly believed that they have considerable future industrial applications e.g: biosensing, super-producing strains and improved gene expression systems.[75]

1.11 Mode of Action of the Lantibiotics

The type-A lantibiotics are capable of killing a range of Gram positive bacterial strains including, *Streptococcus sp.*, *Bacillus sp.*, *Listeria sp.*, *Clostridia sp.* and *Staphylococcus sp.* at nM concentrations. However, eukaryotic cells are insensitive to the lantibiotics, even at mM levels.[76]

Gram negative bacteria also exhibit resistance to these peptides; this is probably due to the outer membrane acting as a barrier to the lantibiotics. It has been shown that disruption of the outer membrane in *Escherichia coli* or *Salmonella sp.* by osmotic shock or EDTA treatment dramatically increases their susceptibility to the lantibiotics.[76, 77]

The initial cellular target for lantibiotics appears to be the cytoplasmic membrane. Following treatment with the lantibiotic, sensitive cells are observed to release ions, small molecules and ATP, thus disrupting the membrane potential and leading to cell death.[78] Higher molecular weight compounds (> 500 Da) were not detected,

suggesting that a degree of membrane integrity remains during the permeabilisation process.[79]

Secondary effects of the lantibiotics include activation of autolytic enzymes[80] and inhibition of cell wall synthesis.[81] However, these processes are relatively slow in comparison to membrane disruption and require higher concentrations of the lantibiotic.

The binding of nisin to the cytoplasmic membrane is believed to occur *via* electrostatic interactions between the negatively charged phospholipids (probably phosphatidylglycerol) and the cationic *C*-terminal region of the lantibiotic (Fig. 7a). Lys-22, His-27, His-31 and Lys-34, four of the six positively charged residues in nisin, are considered to be involved in binding.[82] Incorporation of a negatively charged Glu residue into this region interfered with binding, and comprised the bactericidal activity of nisin.[83]

The majority of explanations for the membrane-activity of nisin are based on the 'barrel-stave' model, first proposed by Sahl *et al.*[84, 85] Following the binding of nisin to anionic lipids, the peptides insert into the membrane. Tryptophan fluorescence spectroscopy has shown that these peptides initially adopt an orientation which is almost parallel to the lipid bilayer surface[86] (Fig. 7b).

In this conformation, the non-polar *N*-terminal peptide residues are associated with fatty acyl chains of the membrane lipids, while the polar *C*-terminal is exposed to water at the membrane surface. Subsequently, it is believed that the peptides re-orientate to span the membrane.[87] Since lantibiotics have a length of *ca.* 5nm, they can only bridge the membrane once; it is therefore assumed that a trans-membraneous pore cannot be formed by a single peptide.

According to the barrel-stave model, the membrane spanning peptides associate to form a water filled channel, resulting in efflux of essential cellular material (Fig. 7c). Pore formation is probably a transient process, with a lifetime of a few ms.[88]

An alternative theory, the 'wedge-model' suggests that the bound anionic lipids co-insert with the peptide, causing a bending of the lipid bilayer and the creation of wedge-shaped pores.[82]

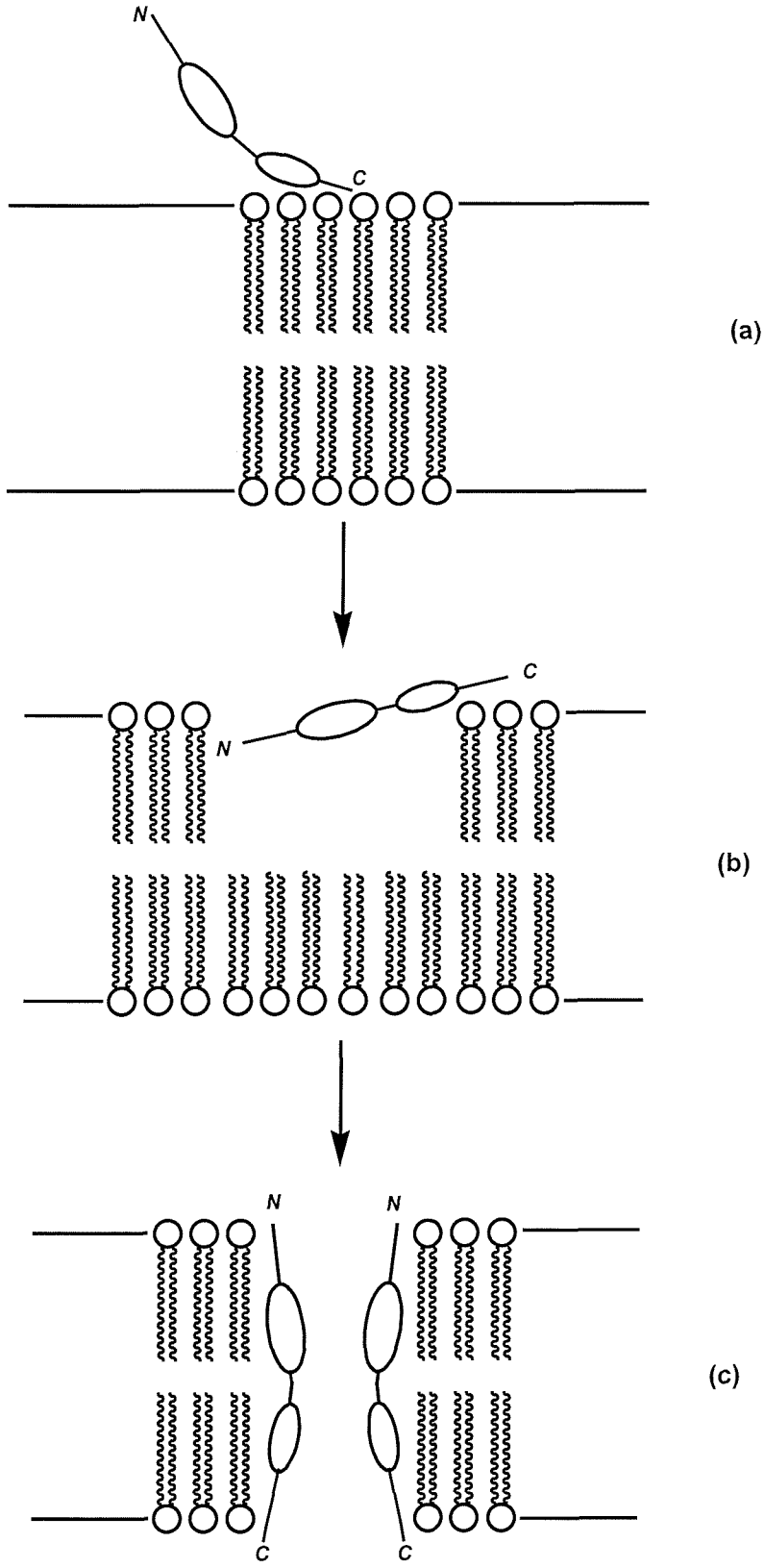


Fig. 7: The interaction of nisin with membranes: (a) binding to the membrane *via* electrostatic interactions (b) insertion parallel to the membrane (c) pore formation, causing small molecule efflux.

1.12 Applications of the Lantibiotics

Since the discovery of the first lantibiotic, nisin A, the range of applications for both the type-A and B lantibiotics has grown considerably. Currently, lantibiotics are utilised in agriculture, veterinary medicine and personal care products. However, the intense research activity in this field promises to extend the scope of these present applications.

The initial application of nisin in clinical and veterinary medicine[89] was short-lived due to its low stability at physiological pH. However, several genetically modified variants of nisin Z exhibit improved pharmacokinetics, and could find medical uses in the future.[90]

Mersacidin has been shown to inhibit the cell wall synthesis of methicillin resistant *Staphylococcus aureus* (MRSA) with comparable efficiency to vancomycin.[91] More importantly, since these antibiotics operate by different mechanisms, it is possible that they could be co-administered in prospective therapeutics.[92]

The lantibiotics epidermin and gallidermin both show activity against *Propionibacterium acnei*, the causative agent of juvenile acne.[27] They are also capable of antagonising *Staphylococcus sp.* and *Streptomyces sp.* which have been isolated from human skin; as such it has been proposed that these lantibiotics may find applications in deodorants and skin care products.

With regard to the type-B lantibiotics, the duramycins and cinnamycin have been demonstrated to inhibit phospholipase A_2, a pivotal enzyme in the inflammatory and allergenic response pathways.[93] Ancovenin has been shown to inhibit angiotensin-converting enzyme (ACE), and could well be utilised as an anti-hypertensive in future therapies.[27]

Nisin, by far the most widely applied lantibiotic, has been used as a food preservative (E 234) for over thirty years.[94] It was initially employed in processed cheese products to counter the spoilage bacteria *Clostridium tyrobutyricum, Clostridium butyricum and Clostridium sporogenes*.[95] Subsequently, food technologists have exploited the fact that nisin is expressed from a transposon, by engineering nisin-producing starter cultures for the dairy industry.[96] Nisin containing cheese has been shown to exhibit immunity against infection from *Clostridium*

tyrobutyricum and *Staphylococcus aureus* throughout the whole ripening process. Nisin can also be found as an additive in pasteurised milk, especially in countries with high ambient temperatures or inadequate refrigeration facilities.[94]

Since the solubility of nisin[97] increases dramatically at low pH (3 mg/mL at pH 5, 56 mg/mL at pH 2), it has been widely utilised in acidified foods e.g. salad dressings. Nisin is also commonly added to alcoholic drinks, canned foods and meat products,[98] typically providing protection against infection from *Listeria sp.*[99] and *Salmonella sp.*[100] as well as other food-borne pathogens.[98]

A major development that has resulted in the increased use of nisin is the discovery that its anti-bacterial properties can be improved by a variety of additives. Divalent ion chelators such as EDTA or citrate extend the anti-microbial spectrum of nisin to encompass Gram negative bacteria.[77] Synergistic activity is also exhibited by magainins, fatty esters and the lantibiotic lactocin 481.[101]

Despite the ubiquitous presence of nisin in the food industry, it is highly probable that the future applications of nisin will be subject to political pressures. Public concerns over genetically-modified foods and the use of anti-microbials in agriculture will inevitably influence the future usage of nisin and other lantibiotics. However, the progressive nature of this high activity field suggests that the lantibiotics will continue to attract considerable attention for some time yet.

1.13 Aims for the Project

It has long been recognised that peptides have enormous potential in the field of medicinal chemistry. However, the rendering of peptides into pharmaceutically efficacious drugs has been hampered by their poor oral activity and metabolic instability.[102] To some extent, the poor pharmacokinetics of peptides are a consequence of their conformational flexibility. In physiological solution, peptides can adopt multiple conformations, while they are also susceptible to proteolytic digestion by host proteases.

These problems have led to the design of peptidomimetics,[103] compounds which retain the ability to present ordered chemical and spatial information to the biological target, but are no longer based on the peptide polyamide backbone. Considerable

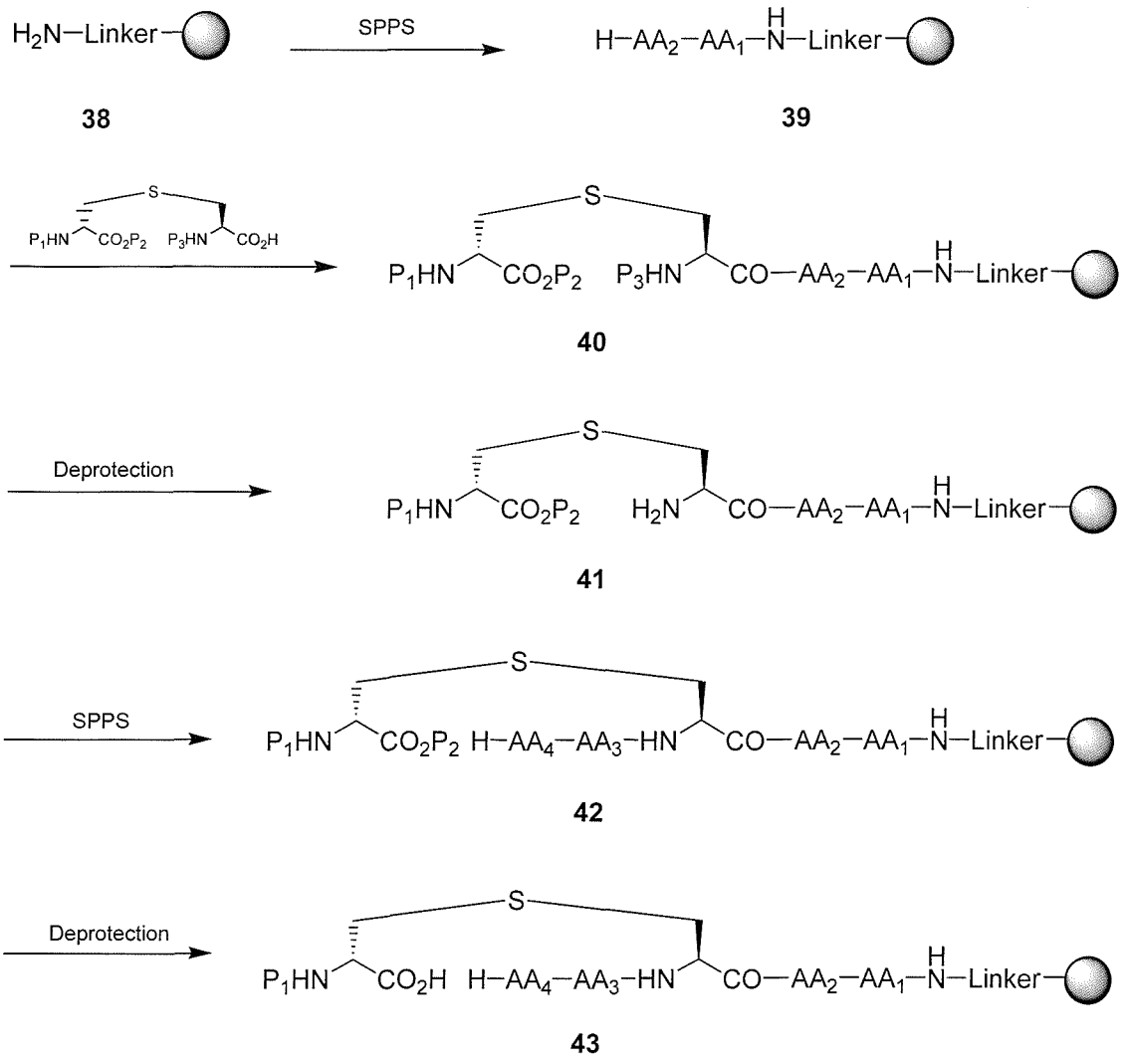
research effort in this field has yielded a diverse array of peptide analogues,[104] such as the peptoids.[105]

An alternative approach has been to restrict the conformational freedom of the peptide. The increase in structural rigidity of a peptide should result in greater specificity, affinity and augmented oral activity.[106] One strategy for reducing conformational mobility involves the use of a rigid moiety as a scaffold or template upon which amino acid side chains may be attached. Examples of template molecules utilised in peptidomimetic synthesis are numerous and include steroids,[107, 108] carbohydrates,[109] β-turn mimetics[110] and aromatic rings.[111, 112] Cyclisation is another common method for restraining peptides,[113] and has been used in conjunction with rigid linkers to prepare a variety of compounds, such as anti-thrombotics.[114]

Recently, Ellman has proposed several criteria for the selection of an appropriate scaffold for peptidomimetic construction.[115] Ideally, the template should be based upon an existing pharmacophoric structure, since information on the toxicology, activity, metabolism and biological targeting of the scaffold may be known. The molecular weight of the fully functionalised molecule should be below 700Da to improve bioavailability. The ability to functionalise a scaffold molecule with a high degree of diverse structure is a highly desirable attribute, particularly if the powerful techniques of combinatorial chemistry[116, 117] are to be invoked.

Much effort has been directed towards the *de novo* design and synthesis of template molecules which satisfy the above requirements. However, it has already been demonstrated that natural products can provide an excellent source of scaffolds. We wish to exploit the conformational properties of selected structural fragments derived from the lantibiotics, to yield templates for library synthesis. It is hoped that this methodology will identify lead compounds for rational drug design, and ultimately therapeutic agents.

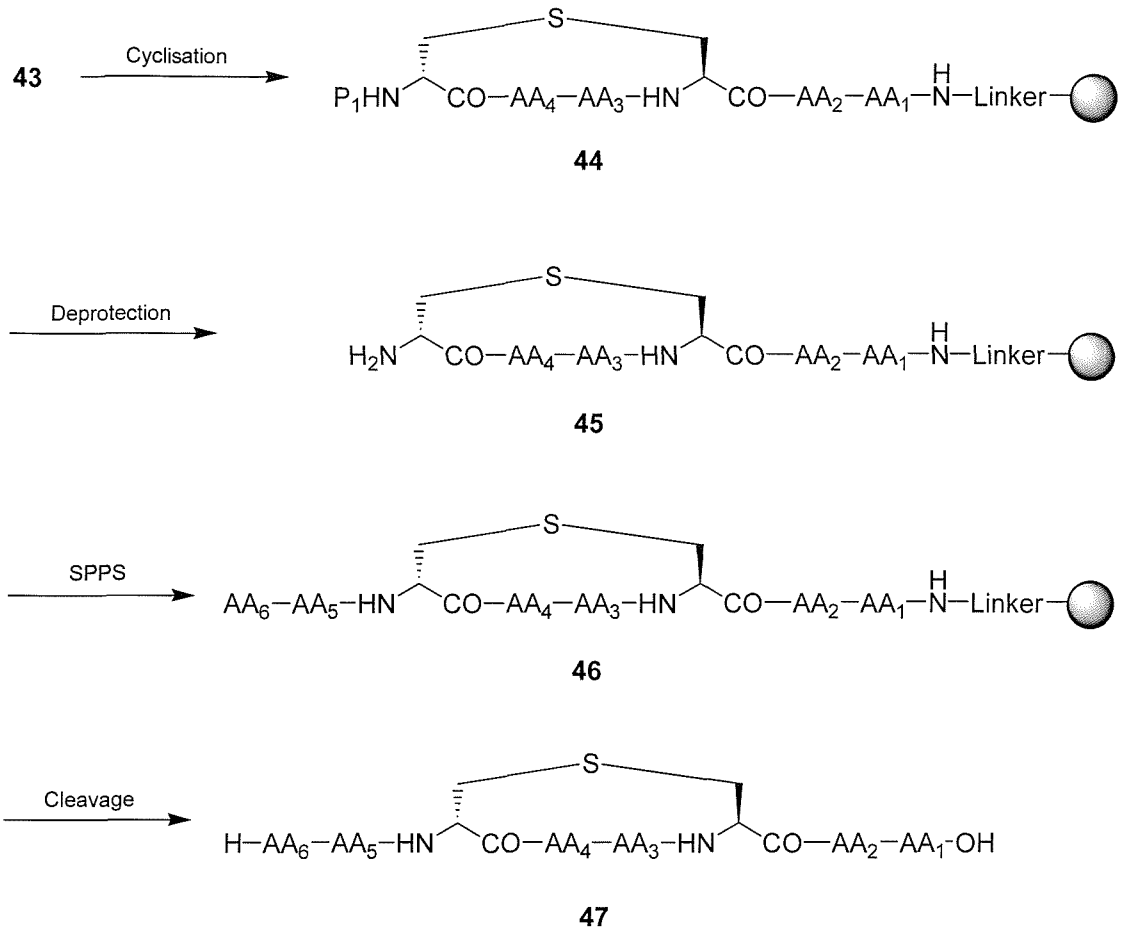
Our initial aim is to establish methodology which will allow us to synthesise model cyclic lantibiotic fragments on the solid phase. It is envisaged that a novel lanthionine monomer, suitably protected for solid phase peptide synthesis, will be required (Scheme 4).



Scheme 4: Synthesis of lanthionine containing peptides on the solid phase.

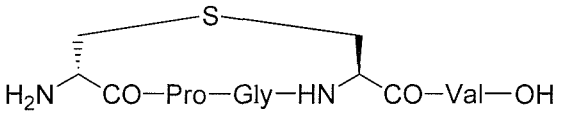
Following the assembly of a linear peptide sequence on the solid phase **39**, it is anticipated that a tri-protected lanthionine derivative will be coupled to the nascent peptide to give the thioether **40**. The deprotection of one of the lanthionyl amino terminii will then allow for the incorporation of endocyclic residues. The unmasking of the remaining acid function should then afford **43**, the cyclisation precursor.

After the linear peptide has been cyclised to give resin bound **44** (Scheme 5) it should then be possible to continue solid phase peptide synthesis to furnish the fragment **46**. Liberation of the peptide from the linker would afford the cyclic lantibiotic segment **47.**



Scheme 5: Synthesis of cyclic lantibiotic fragments utilising solid phase chemistry.

A preliminary target will be the thioether **48**, an analogue of the subtilin B-ring containing a Lan residue in place of MeLan to simplify the stereochemical analysis. An appealing feature of this model cyclic system is the Pro-Gly motif, which should greatly facilitate ring closure. Subsequently, we intend to extend this technology to the generation of combinatorial libraries based on lanthionyl templates. However, the realisation of these goals is dependent on the development of a suitably protected lanthionine residue with defined stereochemistry. Such issues will be discussed in the following chapter.



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2. Stereospecific Synthesis of Lanthionines

2.1 Lanthionine

Lanthionine **4**, is a non-proteinogenic amino acid comprised of two alanyl residues bridged by a thioether linkage. This unusual amino acid was first detected in 1941 in alkaline hydrolysates of wool[118], and was subsequently found in human hair[119], feathers[120], chick embryo[121] and epidermal keratin.[122] Biosynthetically, lanthionine is derived from the Michael addition of cysteine onto dehydroalanine.[123] Both lanthionine and its 3-methyl homologue **5** have been the subject of much interest since their discovery in the lantibiotic family of peptides.

Lanthionine has also been found in cataractous human lenses.[124] It is believed that the photo-oxidative degradation of cystine residues results in the formation of lanthionine; the subsequent increase in the extent of protein cross-bridging leads to increased tissue rigidity and hardening of the eye lens nucleus. Other bridged amino acids such as lysinoalanine **6** have also been implicated in the ageing process.[125]

The processing of many protein containing foods involves treatment with heat or alkali at some stage. These treatments are known to give rise to novel amino acids such as histidinoalanine, lysinoalanine and lanthionine,[126] which in turn could have consequences for nutrition, food safety and health.[125] Lanthionine is also of interest in the silk[127] and leather[128] industries.

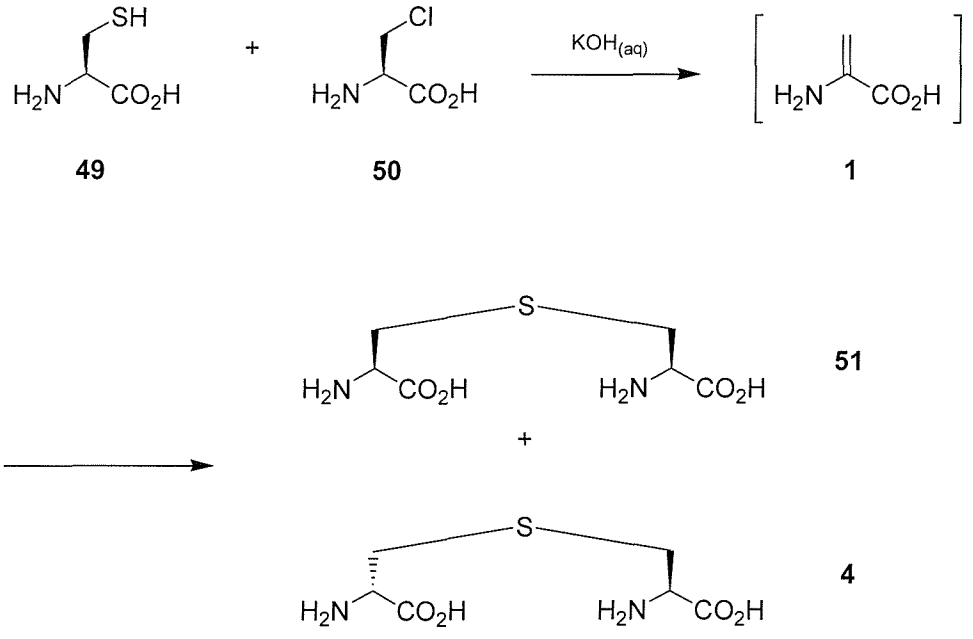
In contrast to the labile disulfide bond of cystine, the monosulfur bridge of lanthionine is chemically far more robust, and offers greater stability towards protease attack. Cyclic structures based on lanthionines also display a greater degree of conformational rigidity relative to cystines. For these reasons, lanthionine is an attractive monomer for the design of novel conformationally constrained peptidomimetics.

However, the synthesis of lanthionine residues for use in peptide synthesis is non-trivial for two main reasons. Often, the reactive synthons employed in the construction of the lanthionine skeleton can undergo eliminative side reactions. The maintainance of stereochemical integrity throughout the reaction course is frequently a problem.

A second issue is the discrimination of the two amine and two acid functions of the lanthionine residue. The selection of orthogonal protecting groups can be pivotal to the success of lanthionine forming reactions.

2.2 Literature Review: Solution Phase Synthesis of Lanthionines

The first synthesis of lanthionine was performed by du Vigneaud and Brown[129, 130] in 1941 (Scheme 6). Their strategy involved the *S*-alkylation of L-cysteine with L-β-chloroalanine **50**. However, the strongly basic conditions required for the reaction resulted in the formation of dehydroalanine **1** from the chloroalanine. The subsequent 1,4-addition of the thiolate anion to **1** afforded a mixture of diastereomers, which could not be easily separated, in poor yield. This reaction has also been effected with serine in place of chloroalanine with similar results.[123]

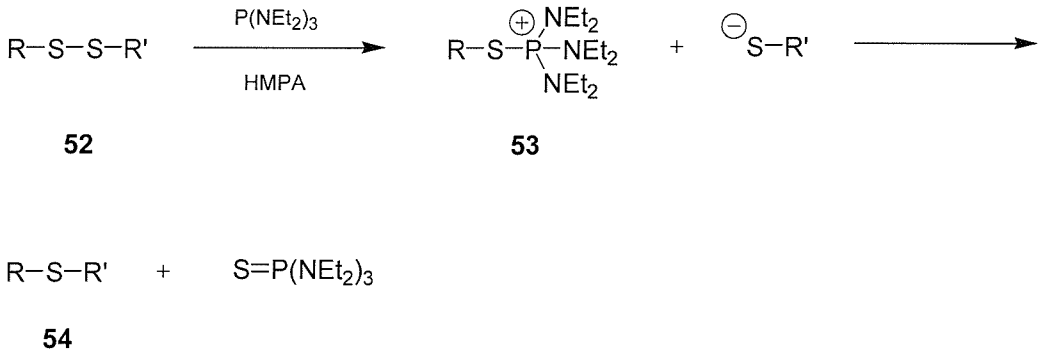


Scheme 6: The first synthesis of lanthionine.

A variety of other serine β-cation synthons have been partnered with cysteines in similar alkylation reactions, including β-haloalanines, serine and threonine *O*-tosylates, amino acrylates and aziridines.[131, 132, 133] However, these multi-step

syntheses displayed limited utility due to low yields, difficult purifications or poor stereoselectivities.

An attractive approach to the synthesis of lanthionines involves the extrusion of sulfur from cystine residues, methodology which was first proposed by Harpp and Gleason.[134, 135, 136] (Scheme 7). The extension of this strategy to cystine containing peptides should allow access to cyclolanthionines, and obviate the need for orthogonal protection.

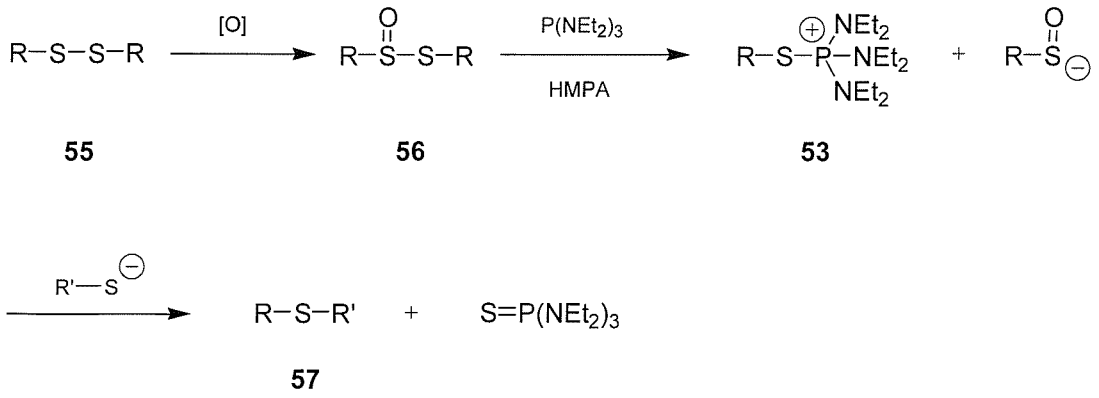


Scheme 7: Mono-desulfurisation of disulfides.

However the application of this method to lanthionine synthesis has been problematic for several groups.[137, 138] With symmetric disulfides, desulfurisation takes place *via* a reversible reaction, yielding a mixture of lanthionines and cystines.[136, 139] In the case of mixed disulfides, recombination of the four possible ionic intermediates gives rise to a mixture of three lanthionines and three cystine derivatives.[135, 136]

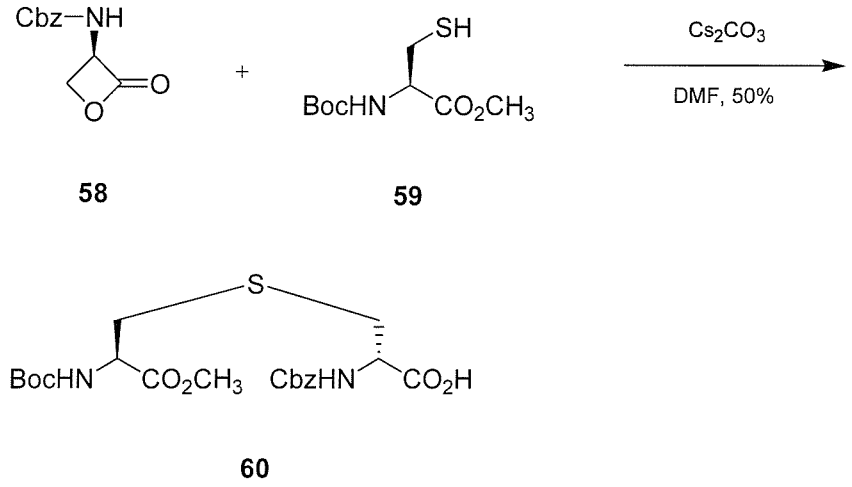
Kini attempted to solve the problem by changing the oxidation state of one of the sulfur atoms in the starting disulfide, thus differentiating the termini of the cystine **56** (Scheme 8). Unfortunately, the reversible nature of the reaction prevailed, again furnishing a mixture of thioethers and disulfides.

Despite these problems, Shiba *et al.* accomplished the total synthesis of the lantibiotic nisin using the sulfur extrusion method to generate lanthionine ring fragments which were duly condensed.[140]



Scheme 8: Mono-desulfurisation of oxidised disulfides.

Another route to lanthionines involves the opening of a serine β-lactone[141] with protected cysteine residues. This method was utilised by Goodman and co-workers to prepare a series of tri-protected lanthionine residues (Scheme 9).[142]

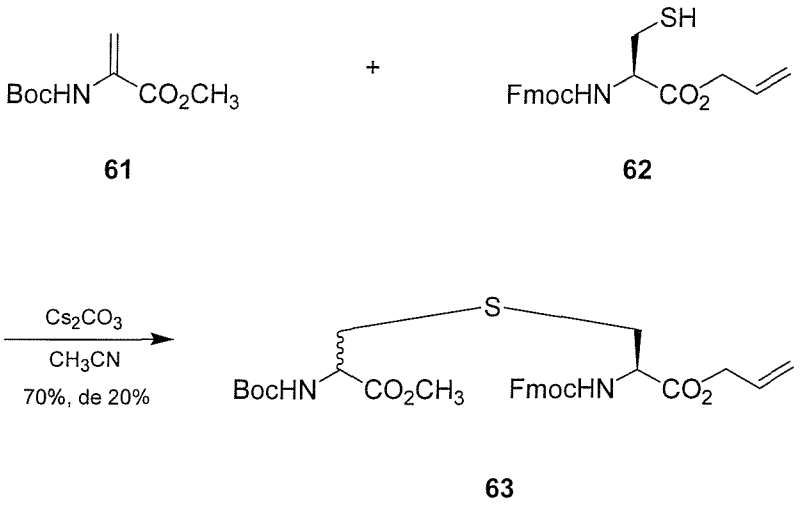


Scheme 9: Synthesis of a lanthionine **60**, by ring opening of the serine β-lactone **58**.

The moderate yields for the cysteine series can be explained by the competing O-acyl fission reaction which generates a thioester. Yields were drastically improved by employing bulkier penicillamine derivatives in place of **59**. This methodology was used to generate lanthionine derivatives for the solid phase synthesis of

peptidomimetics.[143, 144] However, the use of protecting groups such as Cbz severely limits the applications of this synthesis. Other researchers have tried to employ protecting groups which are more compatible with the solid phase without success.[137]

Bradley *et al.* have utilised a biomimetic synthesis to allow the preparation of a fully protected lanthionine derivative suitable for solid phase synthesis (Scheme 10) Thus the Michael reaction between Boc-Dha-OMe **61** and Fmoc-Cys-OAll **62** afforded the desired lanthionine **63** in 70% yield.[137] The two diastereoisomers were easily separable by RP-HPLC. Drawbacks to this approach include the low overall yield of the desired *meso* isomer, and the limitations imposed by the methyl ester upon choice of solid support.

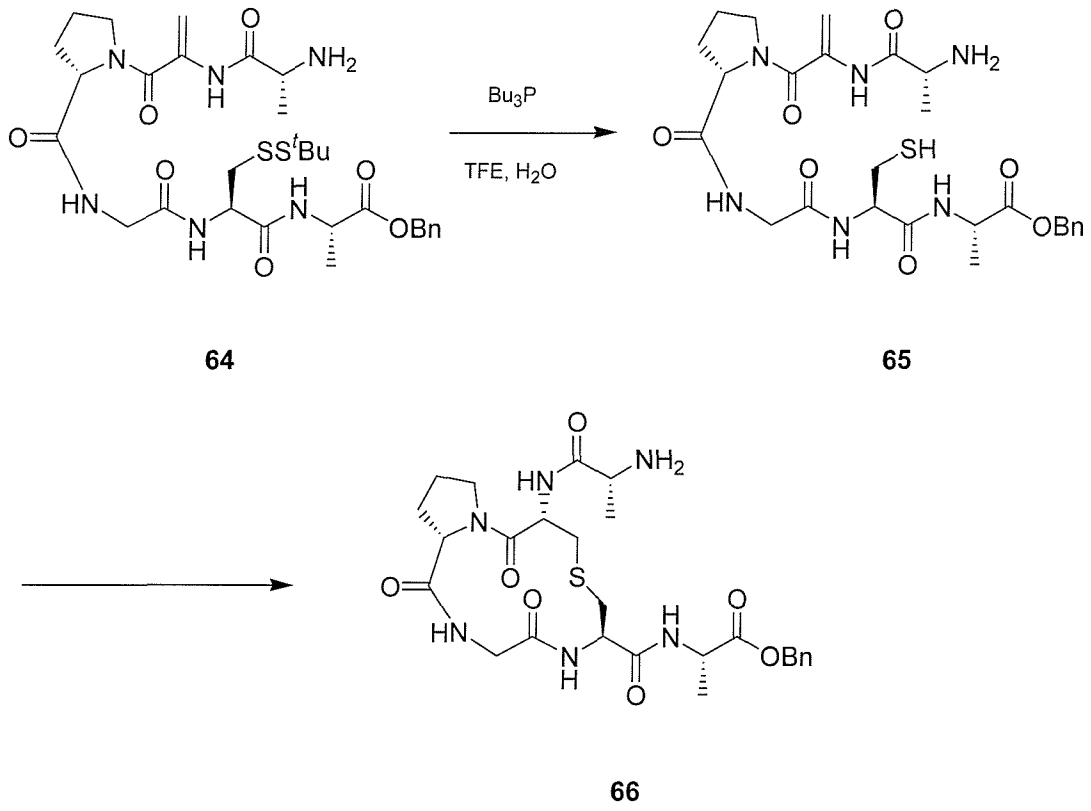


Scheme 10: A biomimetic approach to lanthionines for solid phase chemistry.

A similar approach was employed by Toogood to synthesise a B-ring analogue of epidermin.[145] Dehydration[146] of a serine containing tripeptide gave a protected Dha-containing 3-mer, which was subsequently elongated in solution to give the cyclisation precursor **64** (Scheme 11). Deprotection of the cysteine with tributylphosphine afforded the cyclic peptide in 77% yield as a single diastereomer **66**.

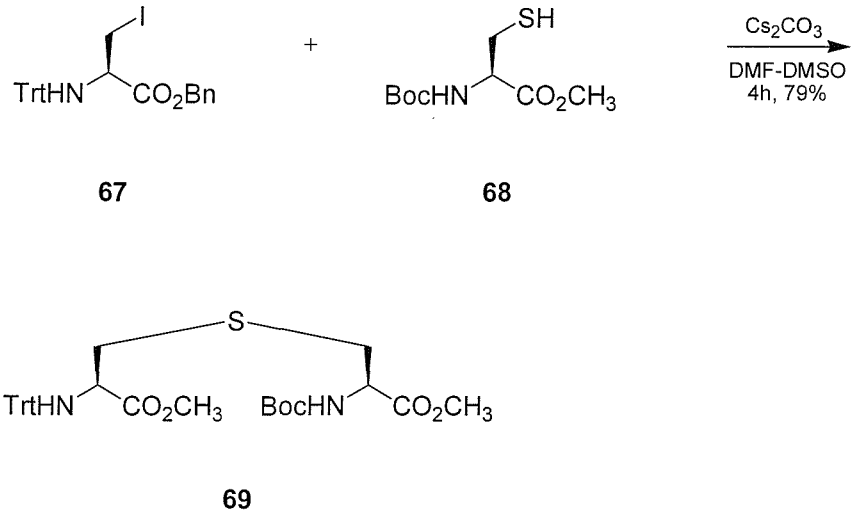
With regard to the remarkable stereoselectivity of the reaction, it is presumed that the chiral content of **64** has a dramatic influence on the stereochemical course of the reaction. These findings have also led to suggestions that the formation of lanthionine

residues *in vivo* could occur spontaneously once the required dehydroamino acids have been installed.



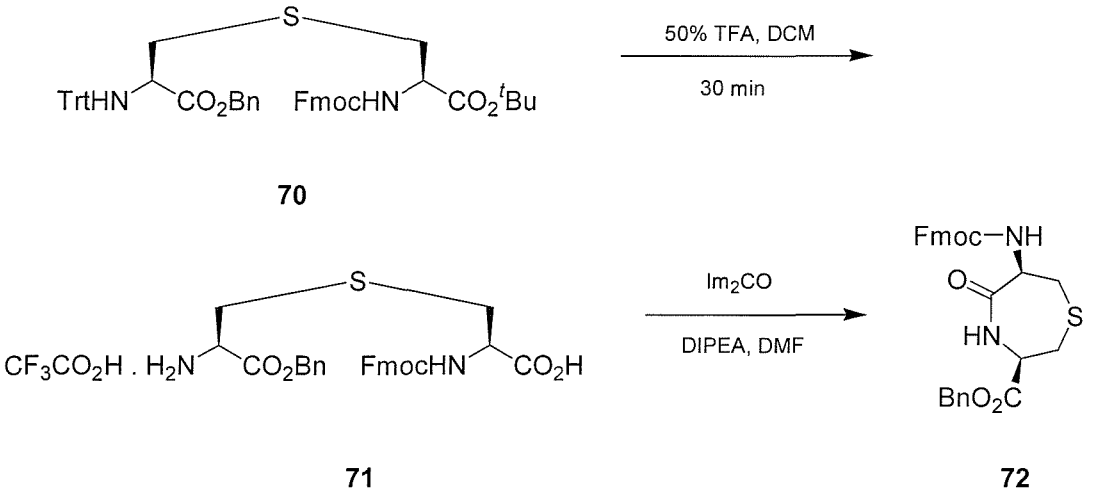
Scheme 11: Synthesis of an epidermin B-ring analogue.

Recently, Dugave and Menez have detailed a solution phase synthesis of lanthionines.[175] Their approach involved the alkylation of cysteinyl derivatives with *N*-triphenylmethyl (trityl) protected iodoalanines (Scheme 12). It was found that the steric buttressing properties of the trityl group sufficiently protected the iodoalanine residue from β-elimination, though small amounts of the aziridine were isolated. FLEC derivatisation and HPLC analysis of the lanthionines showed the stereochemistry of the iodoalanine chiral centre to be retained during the substitution reaction.



Scheme 12: The Dugave-Menez approach to lanthionines utilising β-iodoalanines.

However, the scope of their thioalkylation methodology was limited largely to solution phase applications due to the protecting groups utilised. Nevertheless, it was demonstrated that a di-protected lanthionine could be easily cyclised[175] (Scheme 13), affording a 7-membered ring, that could feasibly serve as a rigid template molecule.

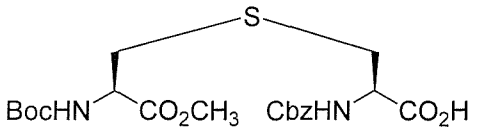


Scheme 13: Synthesis of a cyclic lanthionine.

2.3 Literature Review: Solid Phase Synthesis of Lanthionines

Despite the interest surrounding the lantibiotic family of peptides, relatively little has been reported concerning the solid phase incorporation of lanthionine into synthetic peptides. The reasons for this have been discussed in earlier sections of this text; the formation of an optically homogeneous lanthionine skeleton is non-trivial, and even if this is accomplished, chemo-differentiation of the two acid and two amino functions is highly problematic.

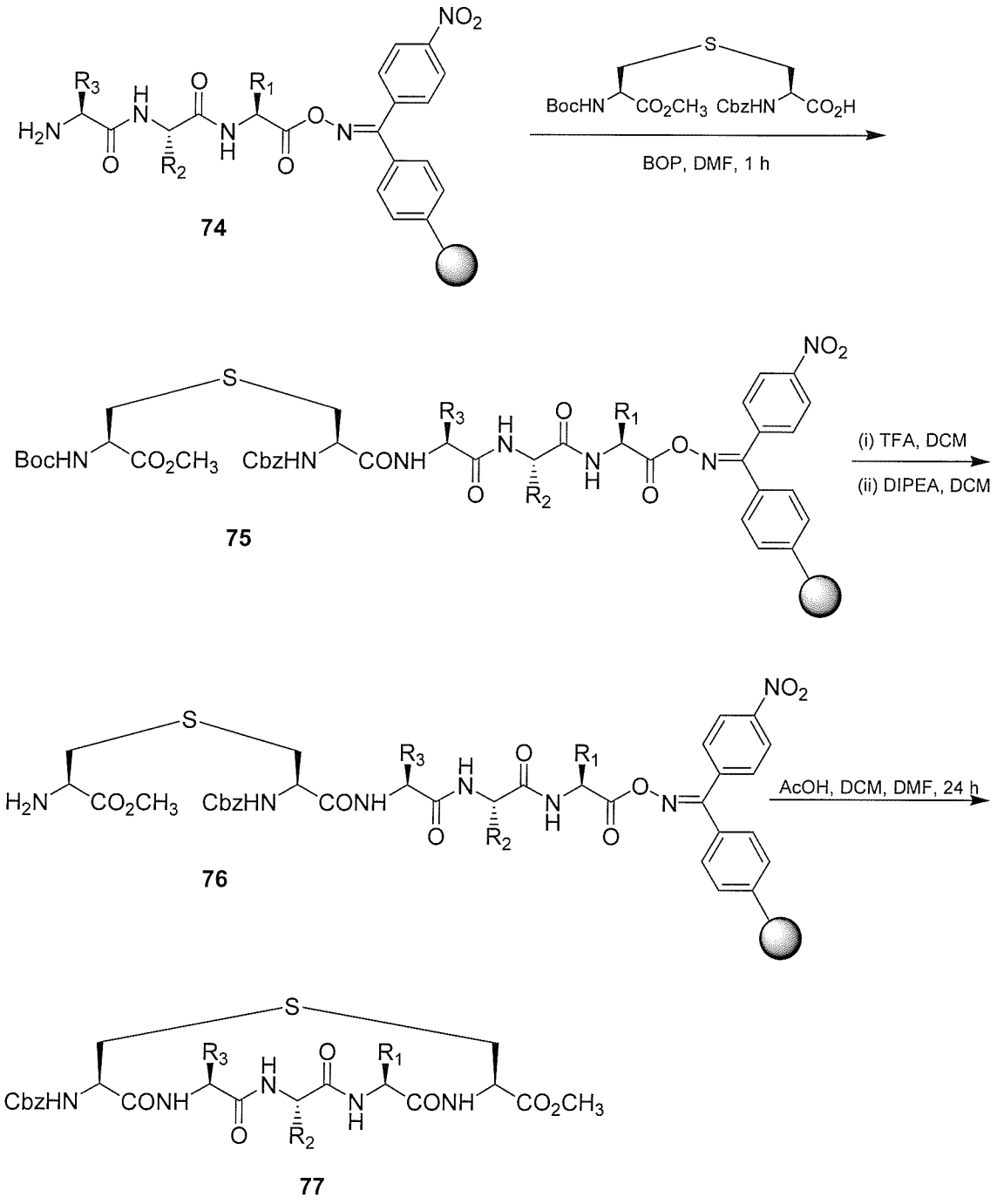
Goodman *et al.* have recently reported methodology which allows for the solid phase synthesis of cyclolanthionines. Their technique, known as 'peptide cyclisation on an oxime resin' (PCOR) utilises lanthionyl monomers such as **73**, which are derived from serine β-lactones.



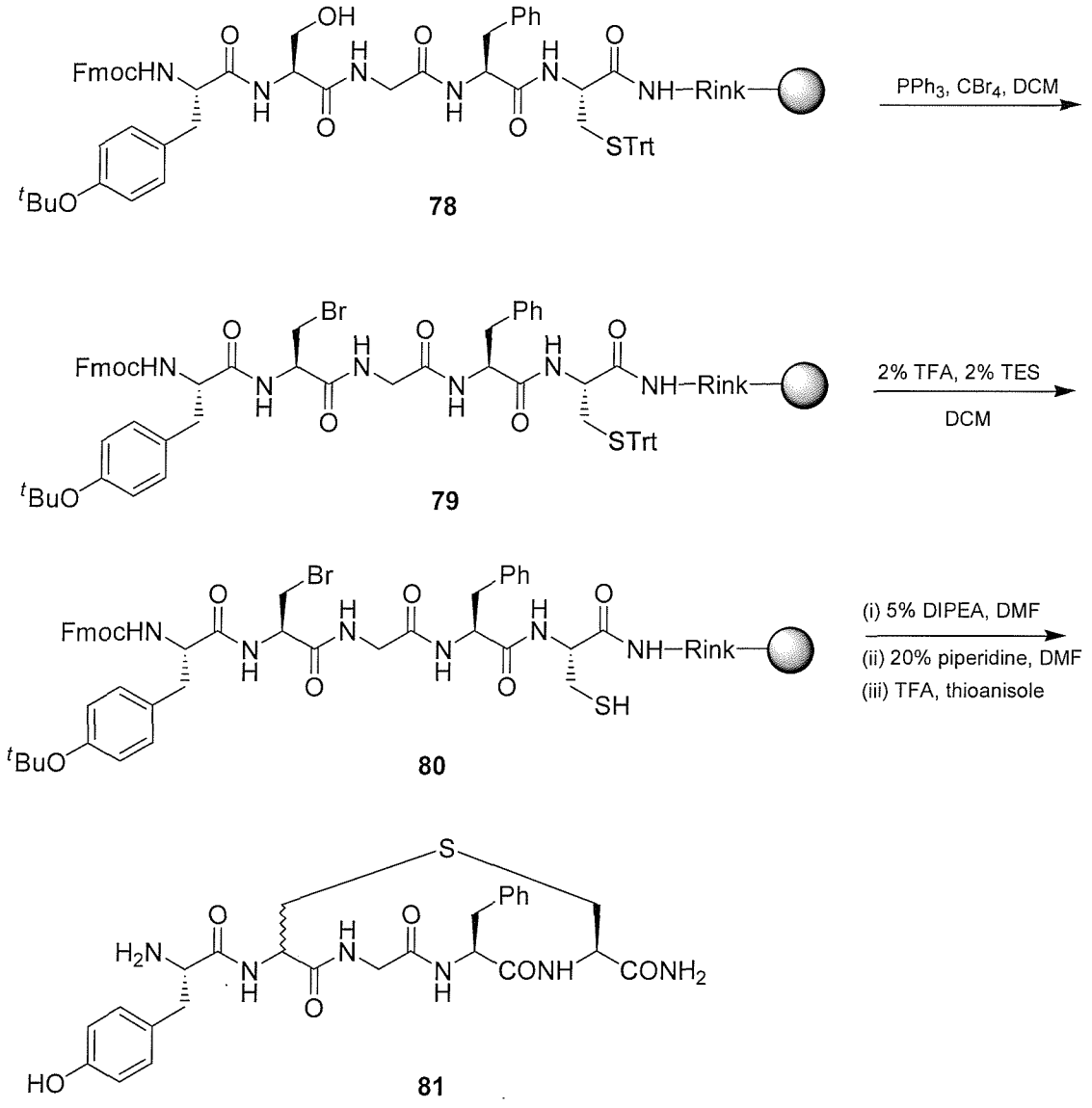
73

Their synthetic strategy required the orthogonally protected lanthionine unit **73** to be coupled to the terminus of a peptide anchored to an oxime resin[147] (Scheme 14). Once the oxime resin is end-capped with **73,** the Boc group is removed by acidolysis, which facilitates the cyclo-release of the lanthionyl peptide. A problem with this approach is the poor compatibility of the Cbz group with solid phase applications. The Goodman group has also reported a solid phase biomimetic protocol for the generation of lanthionine peptides.[148]

Mayer and co-workers have detailed a procedure for the *in situ* synthesis of lanthionines on the solid phase.[149] Their approach involves the conversion of a serine unit within the peptide sequence into bromoalanine (Scheme 15). This residue is then used to alkylate a cysteinyl monomer, also contained within the peptide. However, the stereochemical integrity of these conversions remains questionable; it is highly probable that the reaction proceeds *via* a dehydroalanine residue, therefore leading to a scrambling of optical information.



Scheme 14: Synthesis of a cyclic lanthionyl containing peptide by the PCOR method.

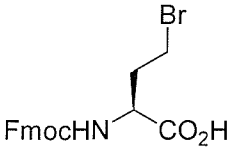


Scheme 15: Synthesis of a cyclic lanthionyl containing peptide on the solid phase.

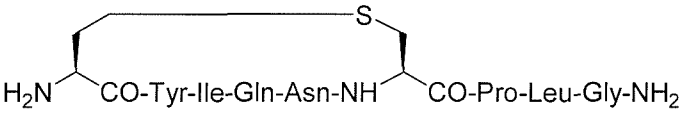
It is thus evident that despite much activity in this area, there is still a requirement for a high yielding synthesis of a lanthionine derivative, which is appropriately protected for solid phase initiatives. Furthermore, it is essential that the reaction proceeds without epimerisation of either chiral centre, and that the desired lanthionine product can be isolated without difficulty. The remainder of this chapter is concerned with our attempts to synthesise a model lanthionine derivative, and to evaluate the stereochemistry of the reaction.

2.4 Solid Phase Synthesis of Cyclic Thioethers

Due to the complexities involved in the synthesis of lanthionine containing cyclopeptides, much attention has been directed at the generation of alternative thioether templates. For example, Mayer *et al.* have utilised the SPPS monomer **82**, to achieve the synthesis of the oxytocin surrogate **83**.[150] Jones and co-workers have used similar methodology to synthesise a cyclic thioether peptide that binds anti-cardiolipin antibodies.[151]



82

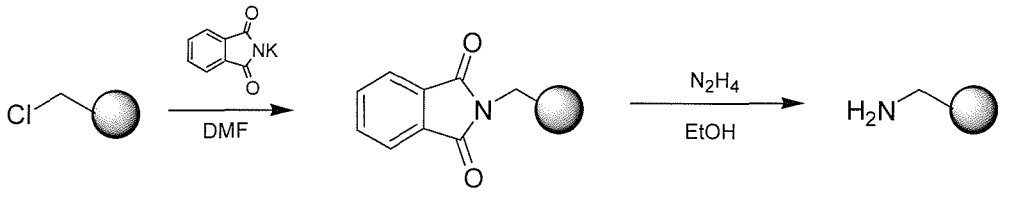


83

Other groups have reported similar approaches to cyclic thioether containing peptides using bromoalkanoic acids.[152, 153, 154]

2.5 Solid Phase Synthesis of Cyclic Lanthionyl Peptides

We first wished to investigate the formation of cyclic thioethers on the solid phase. To simplify the synthesis with regards to orthogonal protection and stereochemistry, it was decided to adopt the bromoacetic acid capping methodology described above. Although this approach would not furnish lanthionines, it was felt that this methodology would serve as an important model for future elaborations.



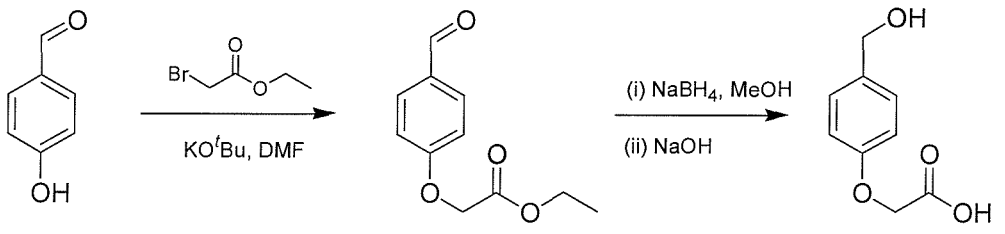
84 **85** **86**

Scheme 16: Synthesis of aminomethyl resin **86.**

Our synthesis began with the preparation of the solid support, which was synthesised from Merrifield resin by standard chemistry. Chloromethyl resin **84** was converted to the synthetically more useful aminomethyl resin **86** using a common phthalidation-hydrazinolysis protocol (Scheme 16).

We next attempted the synthesis of the acid-labile Wang linker[155], a commonly used handle in peptide synthesis. Not only is this linker compatible with Fmoc/^{t}Bu peptide synthesis, but it is also orthogonal to the *S*-trityl group, a pivotal part of our strategy.

Thus 4-hydroxybenzaldehyde **87** was alkylated with ethyl bromoactetate to give the intermediate **88**, which was subsequently reduced and saponified in a one-pot reaction to give the desired acid-labile Wang linker[155] **89** in good overall yield (Scheme 17).

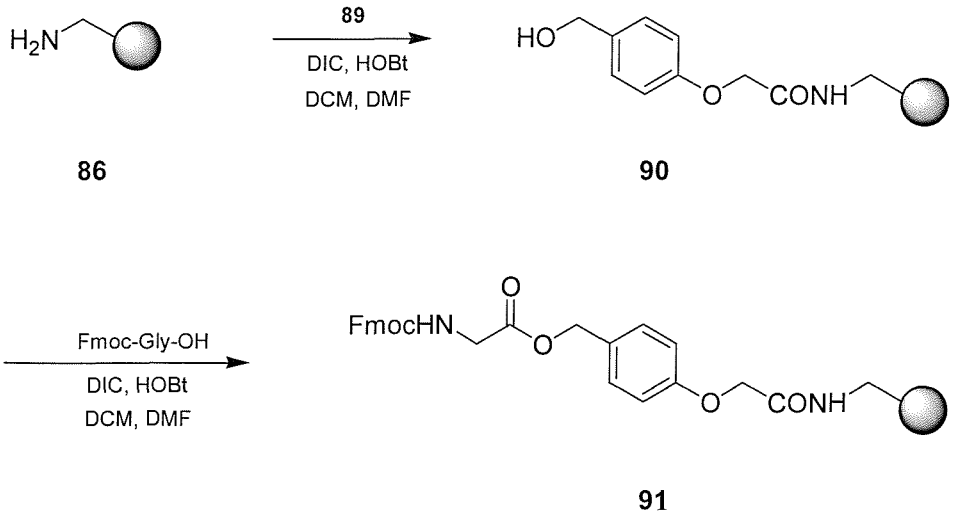


87 **88** **89**

Scheme 17: Synthesis of the acid labile Wang linker **89**.

The amidation of **89** with aminomethyl polystyrene resin proceeded quantitatively, as evidenced by a ninhydrin assay for free amines.[156] Commerically available Fmoc-Gly-OH was then esterified onto the linker by using the benzotriazole ester method of activation in 86% yield, as shown by a quantitative Fmoc test (Scheme 18).

We next assembled the sequence Fmoc-Pro-Gly-Cys(Trt)-Gly-Wang Linker-Resin using standard conditions (Scheme 19). Following the deprotection of the *N*-terminal Fmoc group, the resin was capped with a bromoacetyl group.[153] This peptide was intended to serve as a model for the B-rings of subtilin and nisin.



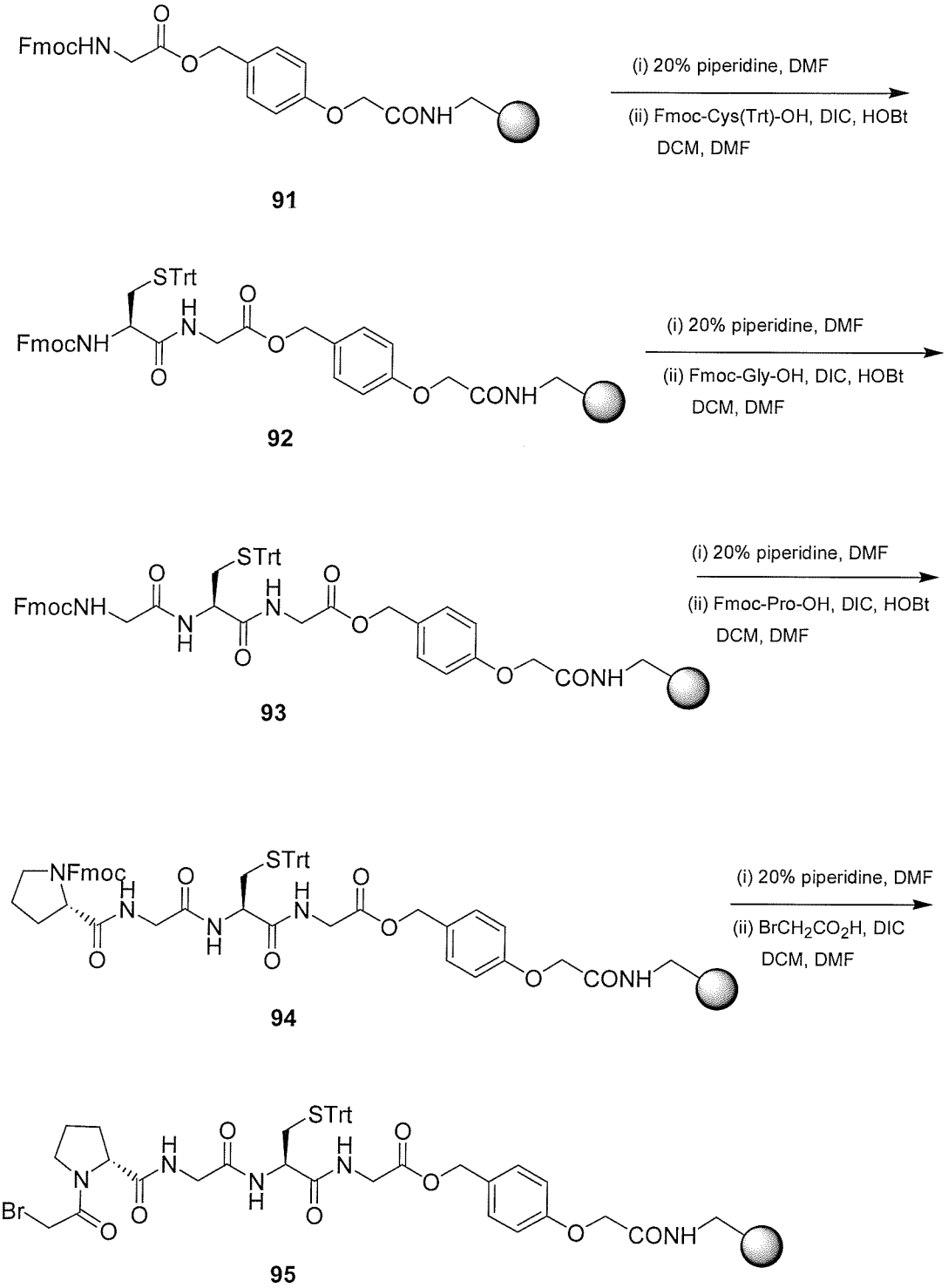
Scheme 18: Attachment of the acid labile Wang linker **89** and the first amino acid.

Before any cyclisation work was attempted on the bromoacetylated peptide, we sought methodology for the quantification of thiols on the solid phase. This type of assay would allow us the monitor the detritylation reaction to completion, and also enable us to ascertain the degree of cyclisation.

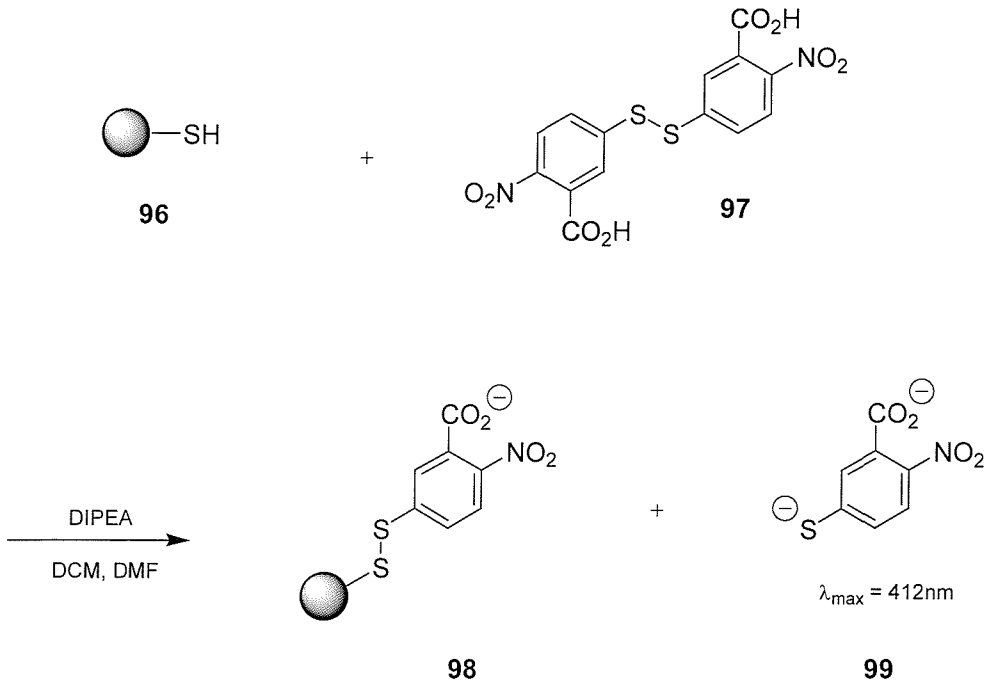
In particular, we wished to adapt the Ellman test[157] for thiols, such that it would be suited to solid phase applications. Although instances of this assay utilising solid supports have been reported,[158] no attempt has been made to quantify the concentration of resin bound thiols.

The test involves the reaction of a known mass of sample with 5,5'-dithiobis(2-nitrobenzoic acid), also know as DTNB or Ellman's reagent (Scheme 20), leading to the liberation of the highly coloured anion **99**, which can then be assayed spectrophotometrically to give the free thiol concentration.

Our initial efforts at transferring this methodology to the solid phase were unsuccessful, due to the aqueous nature of the assay conditions, which were considered to be incompatible with the polystyrene resins used in this work. Attempts to promote the reaction between a resin bound model thiol and DTNB by heating resulted in the rapid degradation of Ellman's reagent.



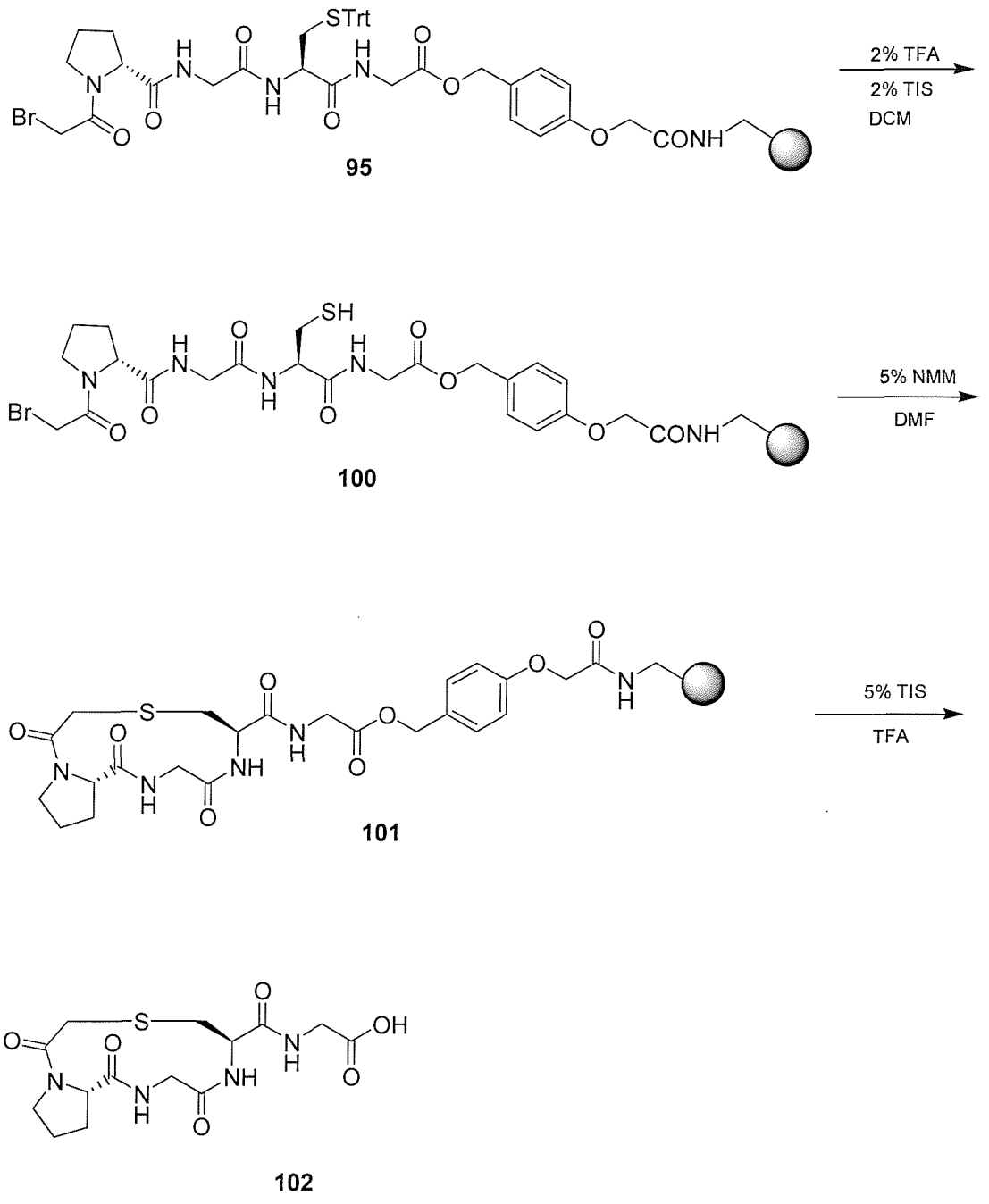
Scheme 19: Synthesis of linear peptide based on the nisin and subtilin B-rings.



Scheme 20: Application of the Ellman test for free thiols to the solid phase.

Eventually, an optimised set of assay conditions was found which gave accurate and reproducible thiol substitution values for resin bound cysteines. Our methodology involved performing the initial disulfide exchange reaction in a DCM-DMF co-mixture to swell the resin, followed by extraction of the chromophore **99** into phosphate buffer for the UV assay. The extinction coefficient of **99** was not determined experimentally; instead a literature value was used.[157] These conditions gave close agreement (5-10%) with the well-documented Fmoc and ninhydrin tests when our assay was performed on resin bound amino thiols.

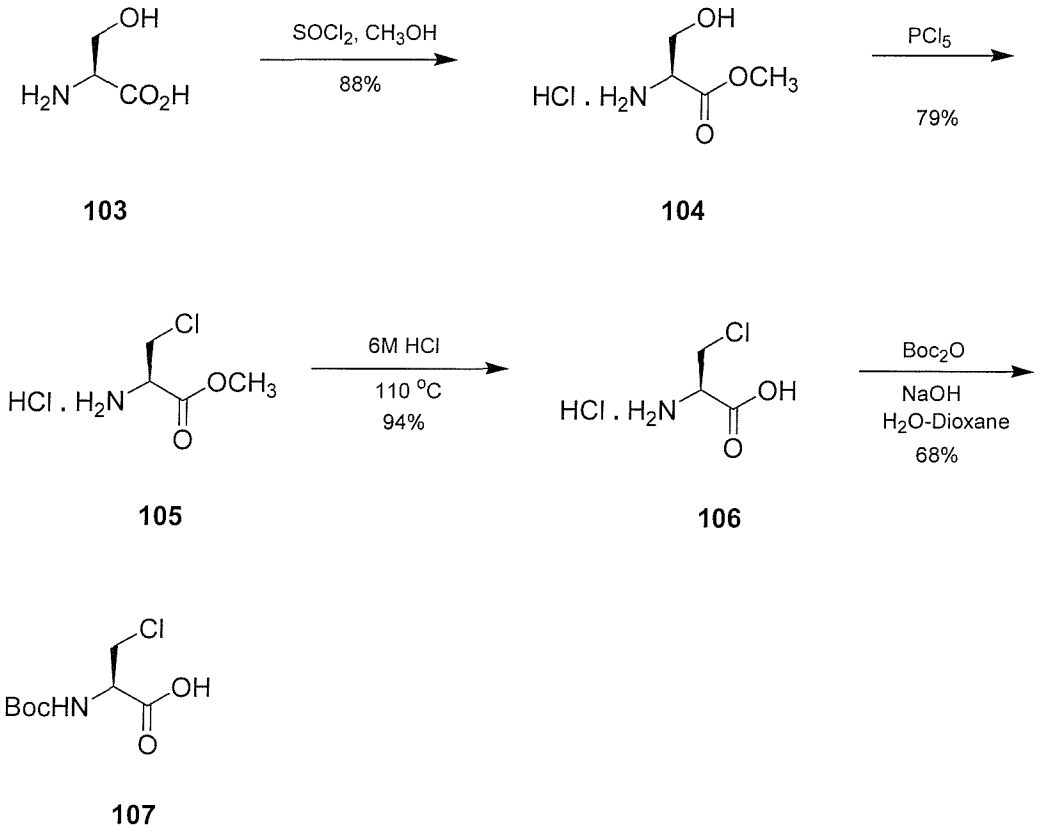
The deprotection of resin bound peptide **95** was effected by exhaustive treatment of the resin with 2% TFA, 2% TIS, DCM (Scheme 21). An Ellman test at this stage indicated that detritylation had transpired near quantitatively. Cyclisation of the peptide was then attempted using an overnight treatment of 5% NMM in DMF. The resin was washed, and then subjected to a second Ellman assay, which indicated >95% loss of sulfhydryl groups. Acidolytic cleavage of the resin **101** followed by RP-HPLC purification afforded the desired thioether peptide as a mixture of conformers. NMR and MS analyses were consistent with the structure of **102**.



Scheme 21: Synthesis of a cyclic thioether on the solid phase.

Following the success of this approach, we wished to elaborate on the complexity of the thiether. Of particular interest to us, was the ability to introduce an exocyclic amino function, which would allow for the continuation of peptide synthesis, following a cyclisation. We reasoned that the capping of a peptide with a suitably protected β-chloroalanine, would allow entry into polycyclic lanthionine systems.

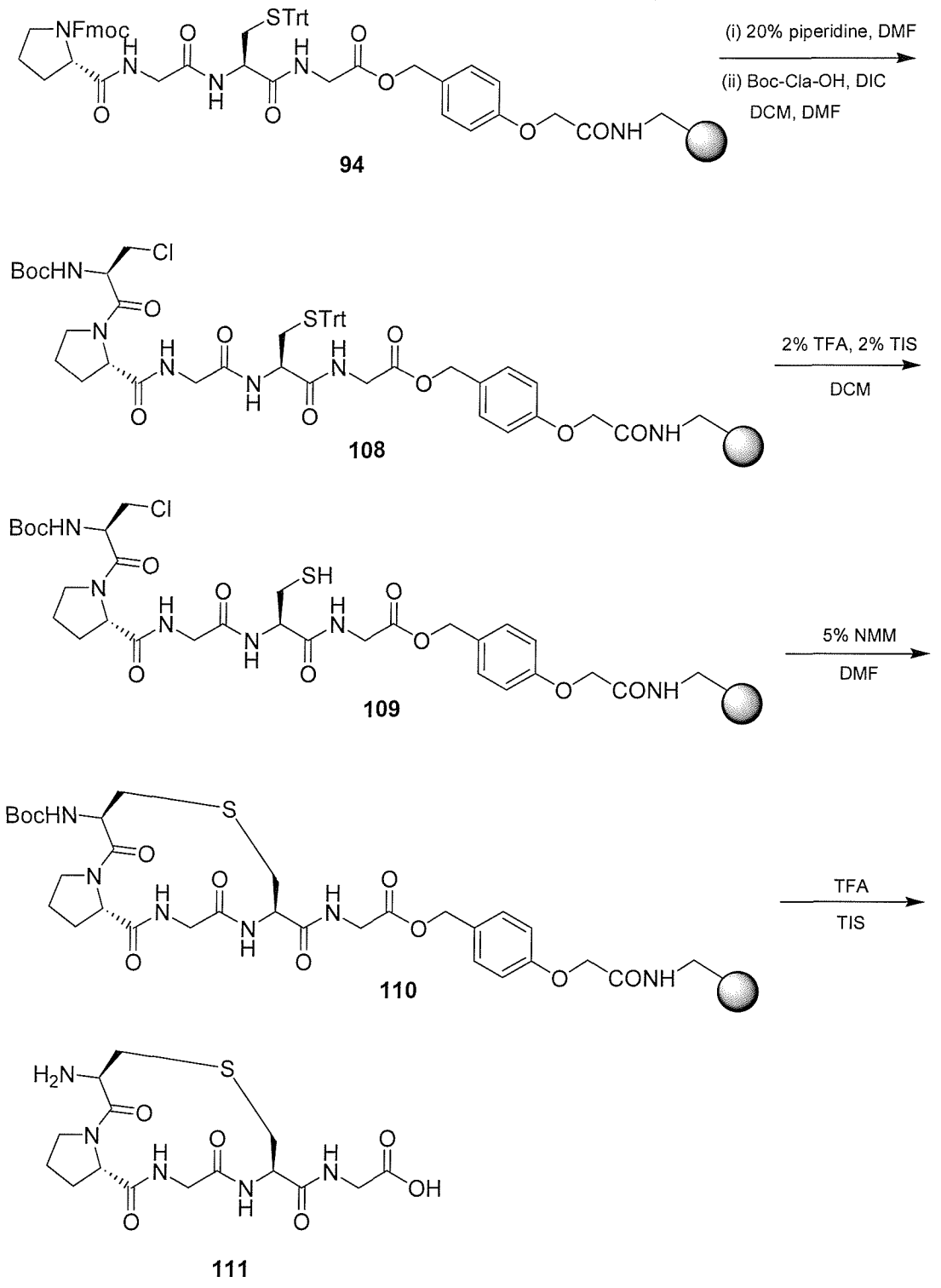
Our route to chloroalanines commenced with the protection of commercially available L-serine (Scheme 22). Esterification with thionyl chloride proceeded to give **104** in 88% yield. Deoxychlorination was achieved with phosphorus pentachloride in acetyl chloride. Following recrystallisation, **105** was isolated in 79% yield.



Scheme 22: Synthesis of a protected chloroalanine for solid phase peptide synthesis.

Concerns that **105** could undergo β-elimination during saponification, led us to remove the methyl ester function by acidolysis. The chloroalanine **106** was then protected according to a literature procedure, to give the Boc derivative **107**.

The linear peptide **94** was deprotected with 20% piperidine in DMF, and capped with **107** using standard peptide coupling conditions (Scheme 23). A qualititative ninhydrin test was negative, following washing of the resin. After detritylation of **108**, the intramolecular thioalkyation reaction was attempted, again using NMM-DMF. An Ellman test after 24 h, indicated 11% of sulfhydryl groups were still present.



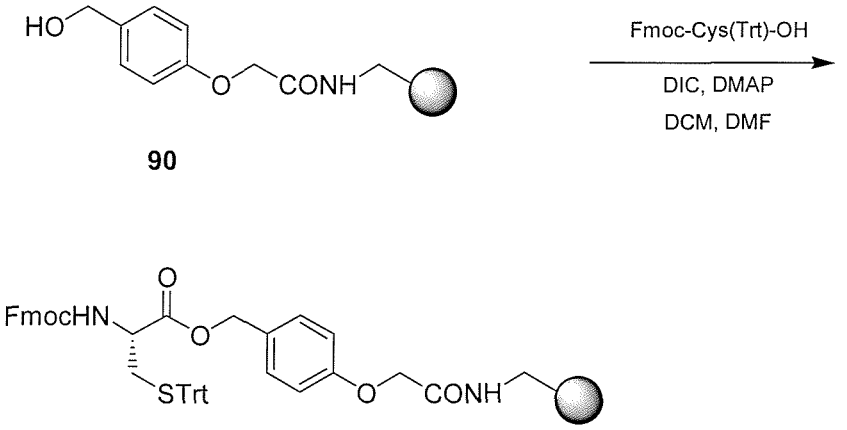
Scheme 23: Synthesis of a protected chloroalanine for solid phase peptide synthesis.

Materials were cleaved from the resin and analysed by HPLC-MS, which indicated that the desired cyclic peptide had been formed. However, the presence of two peaks in the HPLC chromatogram with similar retention times, both giving rise to

m/z = 402 $(M + H)^+$, suggested that the product had been formed as a pair of diastereoisomers, possibly *via* a dehydroalanine pathway. No attempt was made to further characterise or purify the sample. The use of cesium carbonate or tetramethylguanidine in the cyclisation step gave similar results. At this point we abandoned the chloroalanine approach to lanthionines, and sought an alternative method.

We next considered a solid phase approach to lanthionines involving the capture of *N*-trityl protected iodoalanines with resin bound cysteinyl residues. This resin capture method has the advantage that any decomposition products of the iodide such as the corresponding aziridine remain in solution and can be removed by exhaustive washing at the end of the reaction.

Commerically available Fmoc-Cys(Trt)-OH was first esterified onto the Wang linker **90** by using the symmetrical anhydride method of activation in 80% yield, as shown by a quantitative Fmoc test (Scheme 24).

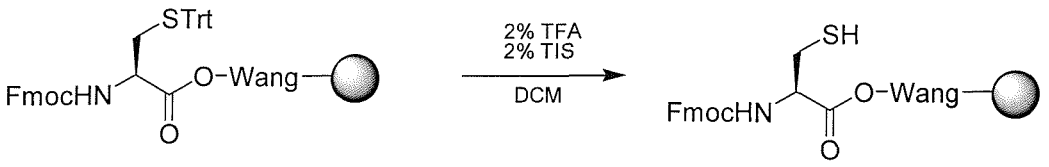


90

112

Scheme 24: Attachment of a protected cysteine to the Wang linker on the solid support.

Detritylation of **112** was achieved by repeatedly washing the resin with 2% TFA and 2% TIS in DCM, until an aliquot of resin beads from the reaction remained colourless when treated with neat TFA (Scheme 25).

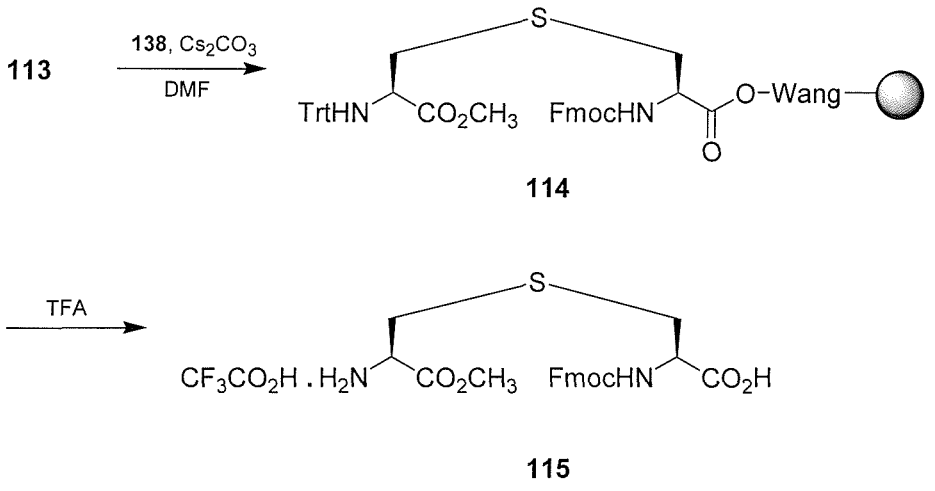


112 **113**

Scheme 25: Detritylation of a protected cysteine on the solid support.

An Ellman test indicated that the removal of the trityl group occurred in 95% yield. The resin capture reaction of the iodide **138** was attempted in DMF with Cs_2CO_3 (Scheme 26).

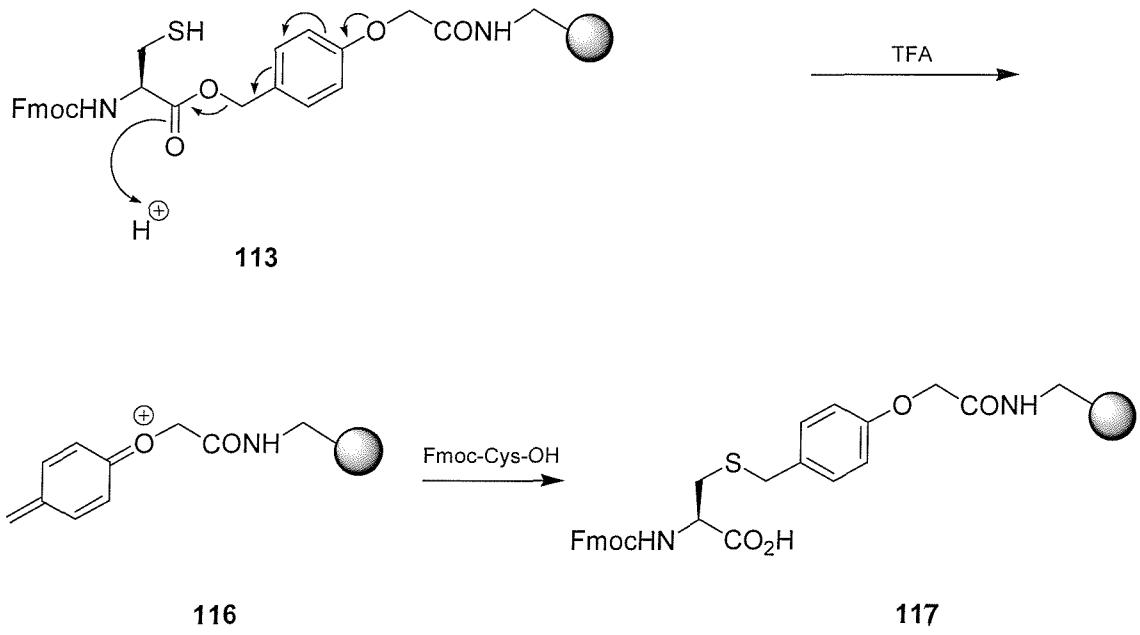
However, an Ellman test performed after the reaction indicated that a large proportion of sulfhydryl groups had not been alkylated. A second coupling reaction failed to improve the situation.



Scheme 26: Resin capture of iodide **138** by a thiol immobilised on the solid phase.

Cleavage of the resin **114** in TFA furnished the lanthionine **115** as the sole product, but in extremely low yield. The results of the Ellman test and the low mass recovery from the acidolysis of **114** led us to believe that residual unalkylated

cysteine **113** was being scavenged by the quinone methide **116** during TFA cleavage (Scheme 27).

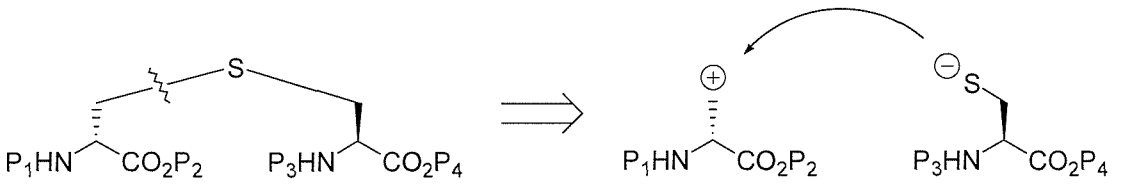


Scheme 27: Linker alkylation by cysteine from incomplete thioalkylation of **113**.

We attempted to resolve the problem by using a Gly spacer residue between the linker and the cysteine. In this case, cleavage of the peptide from the solid phase resulted in greater mass recovery. Again, the desired lanthionine was isolated in poor yield. The remainder of the mass balance was accounted for by the unalkylated dipeptide, Fmoc-Cys-Gly-OH. A variety of solvents and bases were tested in the alkylation reaction, but no significant improvements were made over the Cs_2CO_3/DMF system, and this approach to lanthionine synthesis was abandoned.

2.6 Use of the Mitsunobu Reaction in Lanthionine Synthesis

Our early efforts at synthesising lanthionine derivatives were based on the condensation of β-alanyl cation equivalents[159] with protected cysteine derivatives (Scheme 28).



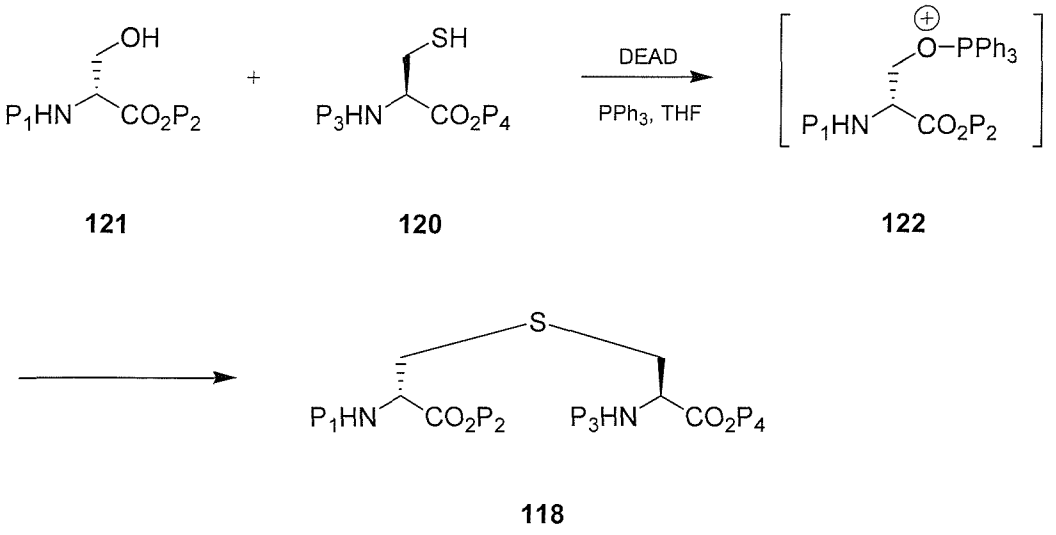
118 **119** **120**

Scheme 28: Disconnection of a fully protected lanthionine derivative.

The use of β-haloalanine derivatives in solid phase approaches to lanthionines proved unsuccessful. It was believed that the thioalkylation process was proceeding *via* the dehydroalanine derivative, formed by the base-mediated elimination of the haloalanine.

This reaction is a recurrent problem in the synthesis of lanthionines and is due to the use of highly activated serine derivatives in the presence of bases. We reasoned that the use of weakly acidic[160] or neutral reaction conditions could suppress the β-elimination of the electrophilic component.

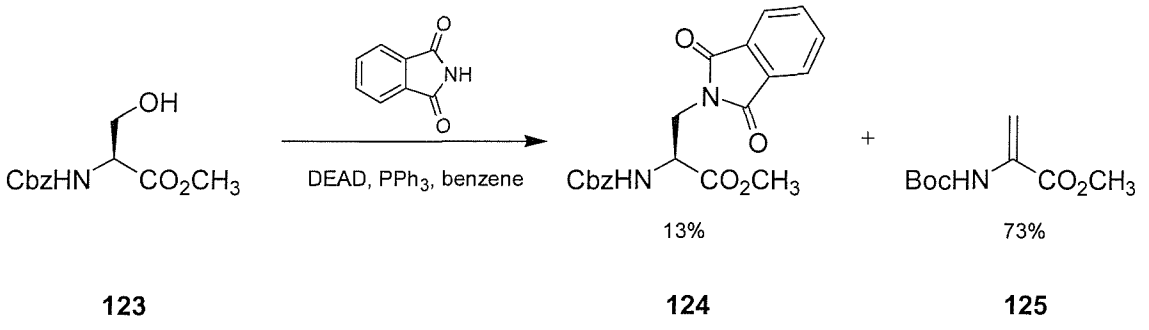
In particular, the application of the Mitsunobu reaction[161] to lanthionine synthesis was of great interest to us (Scheme 29). As well as proceeding under neutral conditions, the reaction is well documented for having a defined stereochemical course. A variety of aromatic thiols and mercaptoacids have been utilised as the acid component in the reaction.[161, 162] However, a literature search revealed only one example of a Mitsunobu reaction performed on an aliphatic thiol.



Scheme 29: A Mitsunobu approach to lanthionines.

A major consideration in Mitsunobu reactions performed on serine is the fate of the activated alcohol **122**. In the case of mono-protected serines (**121**, P_2 = H), the activated intermediate decomposes intramolecularly, yielding lactones analogous to **58**. Bis-protected derivatives of **122** are highly prone to elimination, forming the dehydroalanine, even in the absence of base.

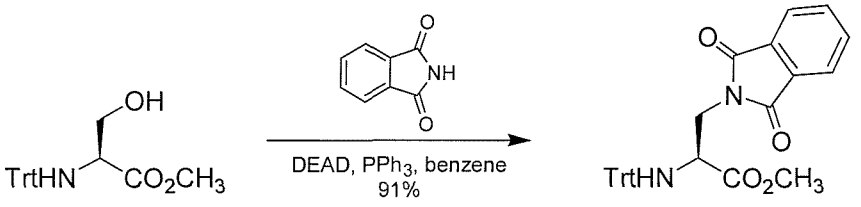
These issues were recently addressed by Cherney and Wang.[163] Their attempts at the Mitsunobu reaction of Boc and Cbz-protected serine derivatives were generally unsuccessful, and resulted in the formation of dehydroalanine **125** as the major product (Scheme 30).



Scheme 30: Attempted Mitsunobu reaction of a Cbz-protected derivative.

In order to circumvent this problem, they utilised triphenylmethyl (trityl, Trt) as an amino protecting group in their serine derivative. The steric bulk of this function has been shown to shield the α-carbon centre of amino acids, thus protecting them from base promoted racemisation.[164] *N*-Trityl amino acid esters have also been shown to withstand saponification, again due to the spatial properties of the trityl group.

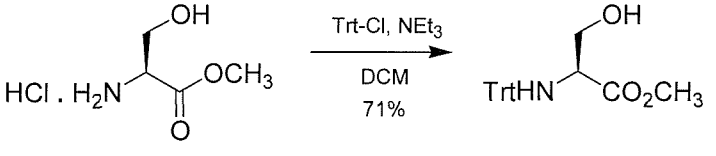
It was found that the replacement of the urethane protecting group with the more sterically demanding trityl function (**126**) prevented the β-elimination completely, and resulted in isolation of the desired imide product **127** in 91% yield (Scheme 31).



126 **127**

Scheme 31: Use of the trityl group to provide steric protection during a Mitsunobu reaction.

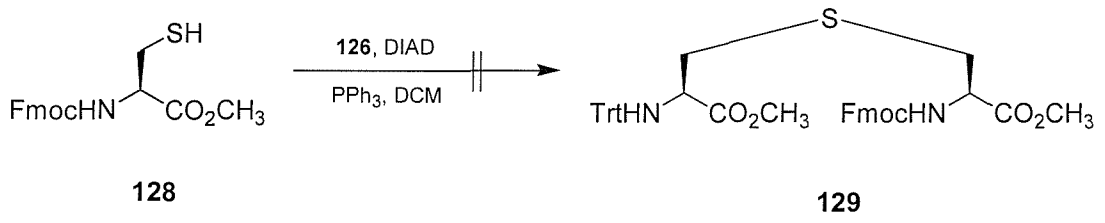
In the light of these results, we attempted the synthesis of lanthionine derivatives *via* the Mitsunobu reaction. The desired serine derivative was synthesised by the method of Baldwin *et al.* (Scheme 32).[159] Thus, *N*-protection of serine methyl ester **104** with trityl chloride gave the required alcohol in 71% yield following recrystallisation.



104 **126**

Scheme 32: Protection of serine for subsequent Mitsunobu reactions.

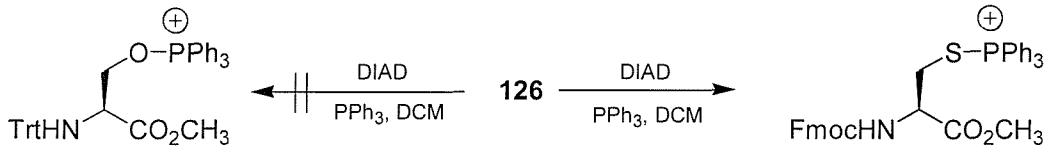
The attempted model Mitsunobu reaction of **126** with ethanethiol under standard conditions (DEAD, PPh_3, DCM) was unsuccessful and only starting alcohol was recovered. Problems associated with drying the thiol led us to employ the solid cysteine derivative **128** (Scheme 33).



128 **129**

Scheme 33: Attempted Mitsunobu reaction of a protected cysteine with Trt-Ser-OMe **126**.

However, this reaction again failed to generate the desired thioether **129**. TLC analysis of the reaction mixture indicated consumption of DIAD, PPh_3 and the thiol **128**, and the presence of the starting serine **126**. It is believed that following the initial activation of the triphenylphosphine by DIAD, the more nucleophlilic thiol component probably reacts at the phosphorus centre instead of the required *O*-attack (Scheme 34).

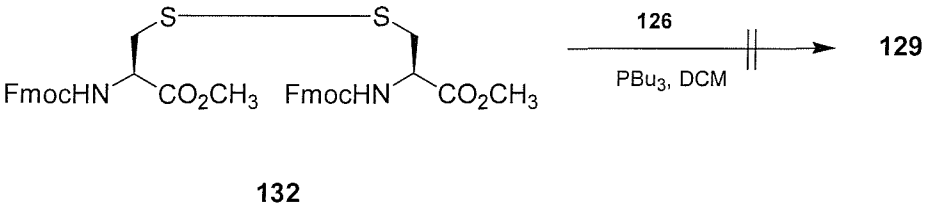


130 **131**

Scheme 34: Mechanism of failed Mitsunobu thioalkylation reaction.

The reaction was repeated with a variety of redox carriers (DEAD, DIAD, TMAD, PPh_3, PBu_3) without success. A variation on the Mitsunobu reaction whereby the phosphine is activated by a disulfide[165] also failed to furnish **129** (Scheme 35); the

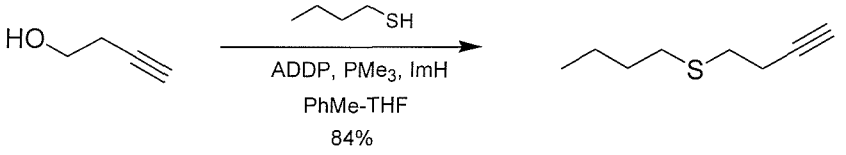
probable cause for this is again the high nucleophilicity of the thiol compared to the alcohol, both of which compete for the activated phosphine.



132

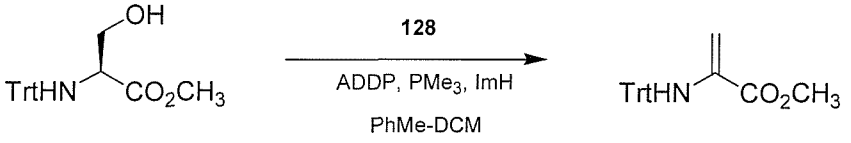
Scheme 35: Attempted synthesis of lanthionine **129** from disulfide **132** with a phosphine redox carrier.

At this stage, a timely report on the synthesis of thioethers using modified Mitsunobu chemistry caused us to change our strategy. Falck and co-workers demonstrated that a variety of aliphatic thiols could undergo Mitsunobu reactions with primary alcohols under the more forcing conditions of ADDP/PMe_3 in the presence of imidazole (Scheme 36).[166]



133 **134**

Scheme 36: Synthesis of a thioether by modified Mitsunobu conditions.



126 **135**

Scheme 37: Failed synthesis of **129** by modified Mitsunobu chemistry.

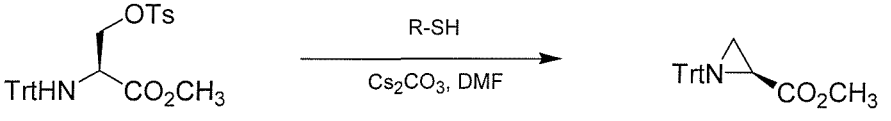
We therefore attempted the coupling of **126** and **128** under these conditions, but were dismayed to isolate the aminoacrylate **135** as the major product (Scheme 37). The subsequent Michael addition of **128** to **135** to give **129** and its diastereomer is probably precluded by the enamine character of the dehydroalanine. **135** was found to decompose rapidly on standing. The order of addition of the reagents was found to be unimportant to the result of the reaction.

We attribute the failure of this reaction to a combination of the forcing activating conditions and the steric congestion of the β-carbon centre of **126**. Falck *et al.* have reported that secondary alcohols give very poor yields in this reaction.[166] Nevertheless, the isolation of **135** instead of the aziridine carboxylate was still surprising. At this point, it was considered that the Mitsunobu reaction was inapplicable to the synthesis of lanthionines, and other avenues were investigated.

2.7 The Application of Iodoalanines to Lanthionine Synthesis

Although the Mitsunobu approach to lanthionine synthesis proved unsuccessful, we were encouraged to find that the trityl group prevented the β-elimination of activated serines in a variety of cases. As such it was decided to utilise other *N*-trityl protected alanine β-cation equivalents in subsequent attempts at synthesising lanthonines.

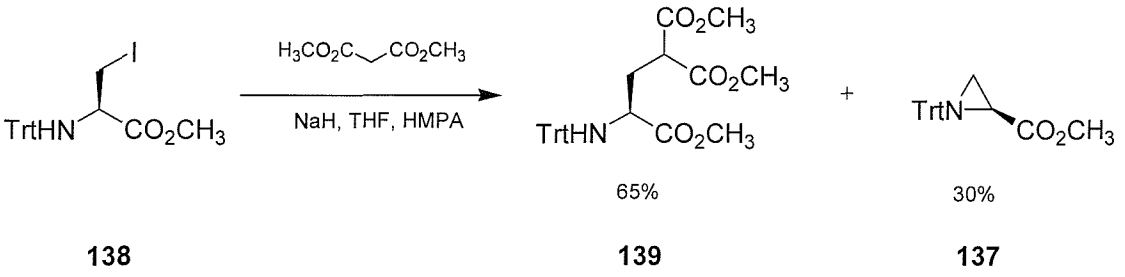
Our attempts to use sulfonate esters of serine in thioalkylations were fruitless and resulted in formation of the aziridine **137** (Scheme 38).



136 **137**

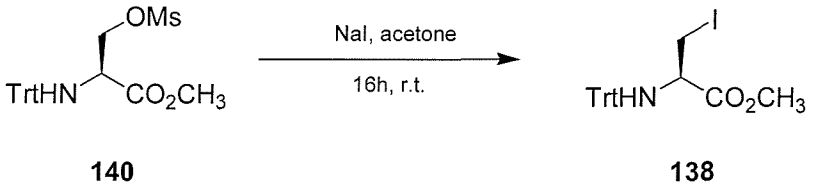
Scheme 38: Attempted thioalkylations with serine *O*-tosylates.

Similar findings were reported by Dugave and Menez who attempted to react the mesylate analogue of **136** with malonate nucleophiles.[167] However, conversion of the mesylate to the iodide **138** enabled the isolation of the 4-carboxyglutamate derivative **139** with considerable success (Scheme 39).



Scheme 39: Novel amino acid synthesis from iodide **138**.

We envisaged that the iodide **138** was a potentially useful intermediate in the synthesis of lanthionines. As such we prepared the iodide **138** according to a literature procedure (Scheme 40).

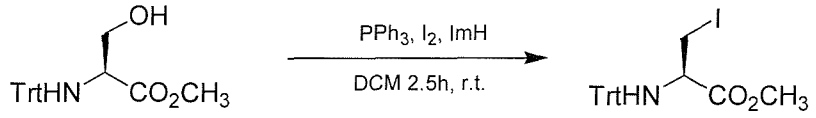


Scheme 40: Preparation of the iodide **138**.

However, we found the reaction to be highly capricious; iodide **138** was often accompanied by significant amounts of aziridine **137**. This approach was abandoned, and the one-step phosphine mediated iodination[168] of serine **126** was utilised instead (Scheme 41). A later publication by Dugave and Menez revealed that the displacement of the serine mesylate **140** by sodium iodide is conducted at 35 °C, possibly explaining our limited success with the reaction.

The deoxyiodination proceeded without problem to afford the desired iodide **138** as a photosensitive paste. NMR analysis of **138** indicated the presence of two

rotamers arising from the restricted rotation of the C_α-C_β bond. Variable temperature NMR experiments showed no coalescence of rotameric signals below 323K. Above this temperature, the sample decomposed to the aziridine **137**.



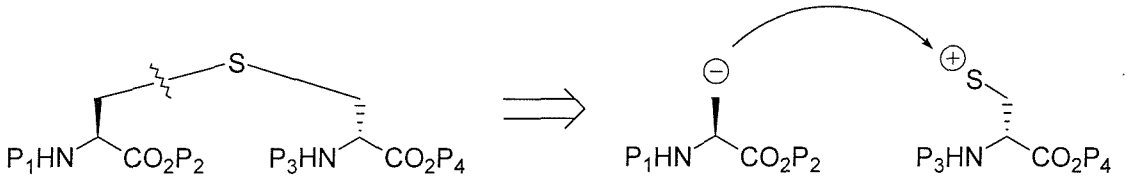
126 **138**

Scheme 41: An alternative synthesis of iodide **138**.

The iodide was found to be highly photosensitive and thermally unstable. Samples of iodide were found to decompose overnight, even in the absence of light. However, it was found that the iodide could be stored for up to a month at –20 °C without appreciable degradation. The utility of such iodides in the synthesis of lanthionines will now be discussed.

2.8 An Organozinc Approach to the Lanthionines

Following our limited success with the β-alanyl cation equivalents, we considered an alternative disconnection of protected lanthionine (Scheme 42).

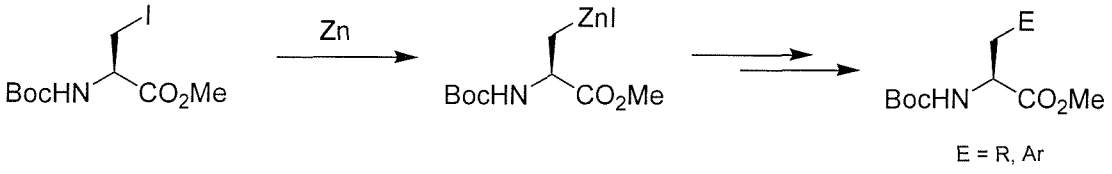


141 **142** **143**

Scheme 42: An alternative disconnection of fully protected lanthionine.

It was envisaged that the cysteine cation synthon **143** would be derived from a sulfenyl halide or a disulfide, while the anionic synthon would be realised from the metallation of iodide **138**. The high reactivity of organolithium species and Grignard reagents towards esters and acidic protons precluded their use in this case.

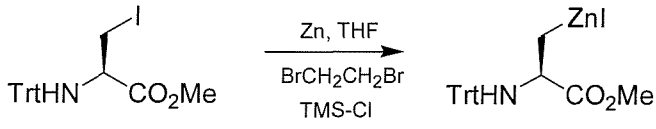
In contrast, the carbon-metal bond in zincates has been shown to have low organic character (*ca.* 15%).[169] As such, organozinc compounds are compatible with a variety of functional groups, including esters. This approach has been used with great success by Jackson *et al.* in the synthesis of novel amino acids (Scheme 43).[170, 171]



144 **145** **146**

Scheme 43: Use of iodoalanines in the synthesis of novel amino acids.

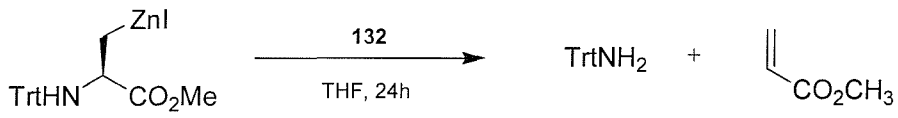
The insertion of zinc into iodide **138** was attempted using Knochel-type activation[172, 173, 174] conditions (Scheme 44). Concerns about the stability of iodide **138** with respect to aziridine formation led us to use slightly reduced temperatures. We found zinc insertion to occur slowly (4-6 h, *cf.* 30-40 min for **144**) as judged by disappearance of **138** by TLC.



138 **147**

Scheme 44: Metallation of iodide **138** with zinc under Knochel activating conditions.

Following the addition of disulfide **132** in THF, the reaction (Scheme 45) was monitored by RP-HPLC. Little consumption of **132** was seen during the reaction, though the distinctive smell of methyl acrylate could be detected. Work-up of the reaction mixture after 24 h afforded the disulfide and tritylamine as major products.



147 **148** **149**

Scheme 45: Attempted thioalkylation of zincate **147** with disulfide **132.**

Presumably, the slow insertion of the zinc into **138** (possibly due to the steric encumberance of the trityl group) led to the β-elimination of the zincate **147**. A further insight into the stability of iodoalanine derived organozinc complexes can be gleaned from the chelation model proposed by Jackson and co-workers (Fig. 8).

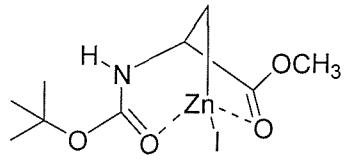


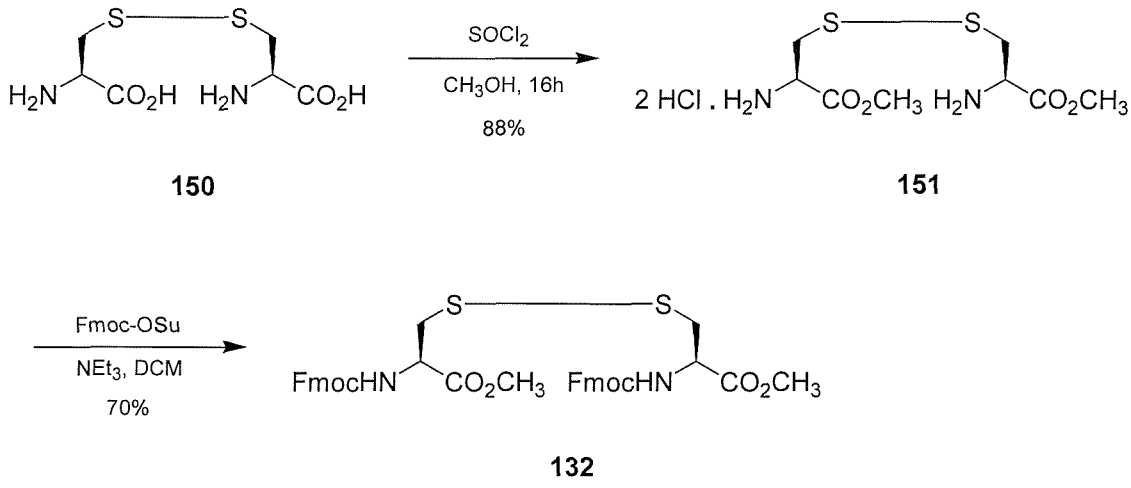
Fig. 8: Stabilisation of zinc by urethane and ester carbonyls in zincate **145**.

^{13}C NMR analysis of the zincate **145** has shown that both the ester and the carbamate carbonyls exhibit downfield shifts on insertion of Zn into the C-I bond. These changes in chemical shift are reflective of zinc chelation by the carbamate and ester carbonyls. The low rate of β-elimination of this complex can thus be explained by the fact that the required conformation for elimination cannot be achieved.

However, in the case of the trityl protected zincate **147**, it can be seen that the lack of urethane protection allows for greater rotational freedom about the C_α-C_β bond, facilitating β-elimination.

2.9 Thioalkylations of Protected Cysteines in Solution

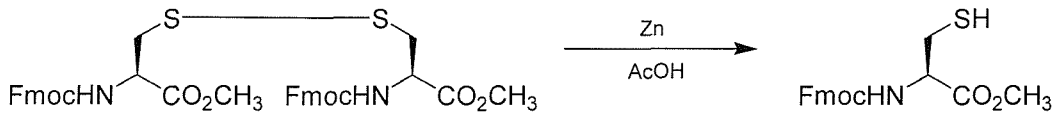
We next attempted the synthesis of lanthionines by utilising iodide **138** in solution phase thioalkylation reactions. It was hoped that dehydroalanine formation would be suppressed by the steric protection of the trityl group, while aziridine formation could be minimised by the careful selection of reaction conditions. Model reactions with simple thiols proved promising, and led us to synthesise a fully protected cysteine synthon.



Scheme 46: Synthesis of a fully protected cystine derivative.

Our synthetic strategy involved manipulating cysteine at the oxidation level of the disulfide whilst amine and acid protection was effected (Scheme 46). Thus esterification of L,L-cystine in a similar fashion to serine gave the diester **151** in 88% yield as the dihydrochloride salt. Double amino protection with Fmoc-OSu proceeded cleanly to give the fully protected cystine in 70% yield.

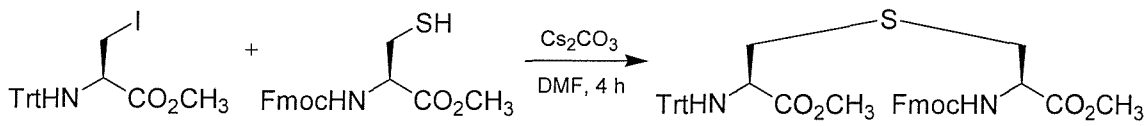
Conversion of **132** to the cysteine **128** was achieved by reduction with zinc in acetic acid (Scheme 37). HPLC analysis of the reaction indicated complete conversion of **132** to a more polar species in 16 h. Subsequent characterisation of the product by MS and NMR confirmed the isolation of the desired thiol. In addition, a small sample of **128** gave a positive Ellman test.



132 **128**

Scheme 47: Reduction of cystine **132** to give the free thiol.

The coupling of iodide **138** with the cysteine **128** was performed in DMF with 1 eq of Cs_2CO_3 (Scheme 48). Work-up of the reaction after 4 h and purification by column chromatography afforded the desired lanthionine in 71% yield. A small quantity (12%) of aziridine **137** was also isolated.

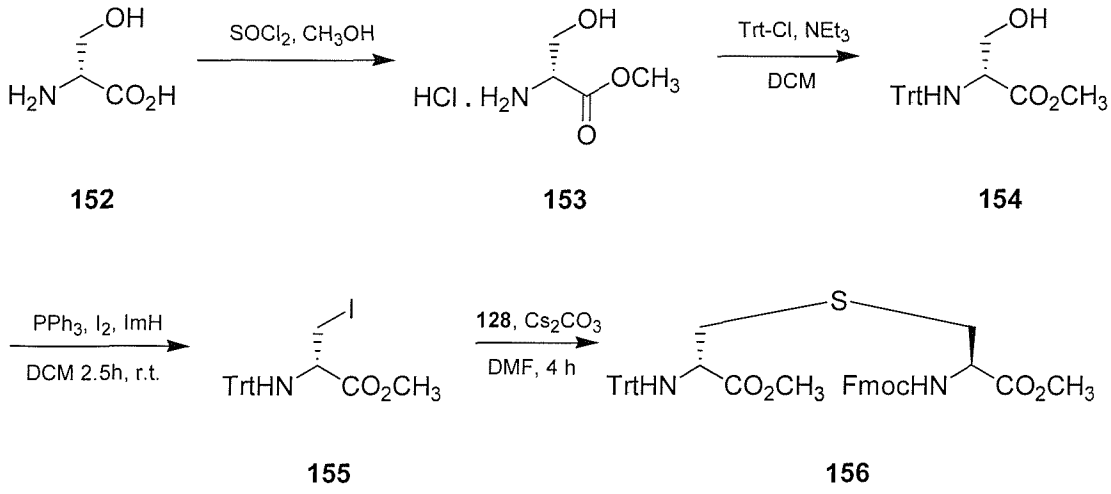


138 **128** **129**

Scheme 48: Successful synthesis of lanthionine **129** by thioalkylation of **138** with **128**.

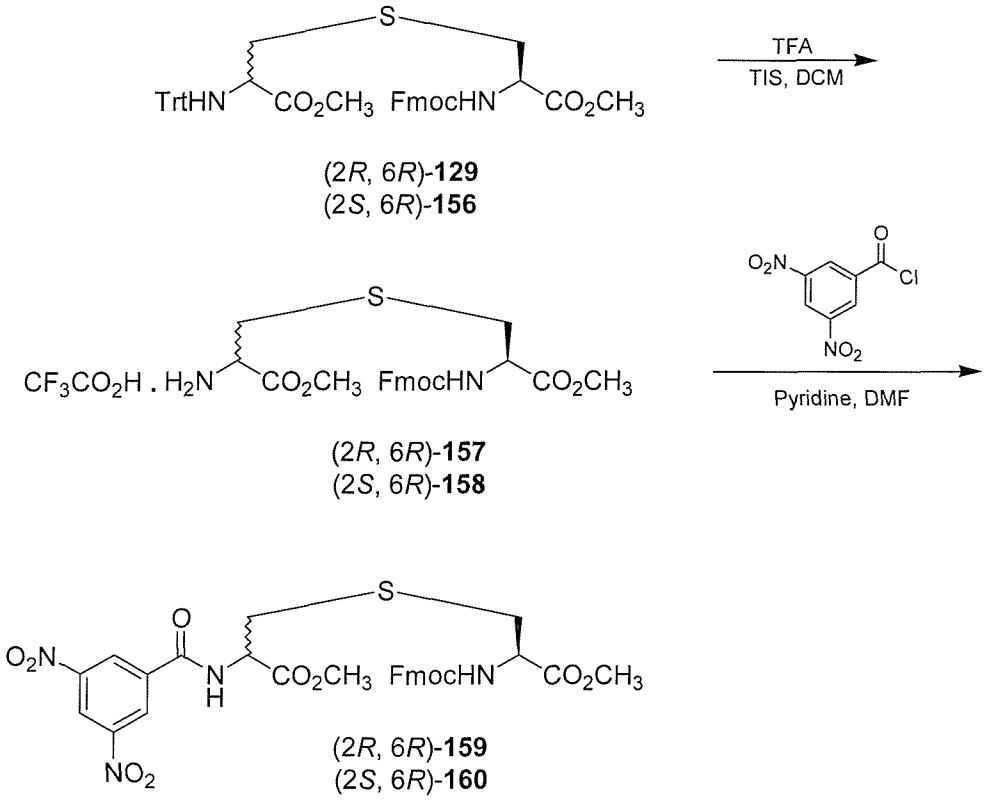
2.10 Determination of Stereochemical Course of Lanthionine Formation

Concerns about the optical integrity of **138** during the thioalkylation reaction prompted us to fully investigate the stereochemistry of lanthionine formation by chemical correlation. Thus, the diastereomer of **129** was prepared in similar fashion (Scheme 49). Optical rotations for the intermediates **153**, **154** and **155** correlated well with the corresponding compounds from the L-series and also with literature data.



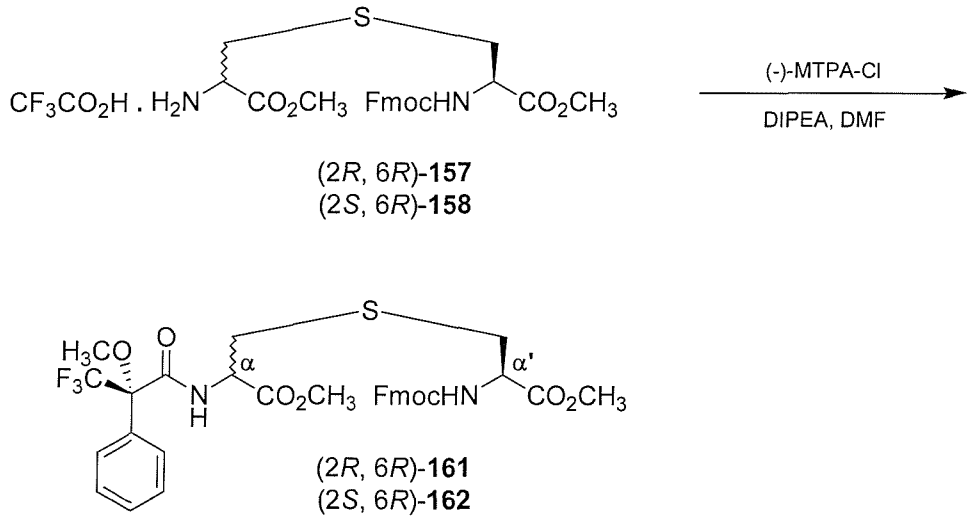
Scheme 39: Synthesis of the diastereoisomer of lanthionine **80**.

NMR (^{1}H and ^{13}C) and HPLC (normal and reversed phase) methods failed to discriminate between the lanthionine diastereomers **129** and **156**. As such, we attempted to functionalise both isomers with the 3,5-dinitrobenzoyl group (Scheme 50), making them amenable to chiral HPLC analysis.



Scheme 50: Synthesis of *N*-3,5-dinitrobenoyl derivatives of lanthionine.

Detritylation of the lanthionines **129** and **156** proceeded without incident to give the TFA salts **157** and **158**; again these diastereomers could not be discriminated by NMR or HPLC analysis. The subsequent acylation reaction afforded the 3,5-dinitrobenzoyl derivatives **159**, **160** which were found to co-elute under a wide variety of analytical conditions on a Pirkle-type chiral HPLC column. At this juncture we abandoned the HPLC analysis of the diastereomeric lanthionines in favour of a NMR method. To this end, lanthionines **157** and **158** were converted to the Mosher amides **161** and **162** (Scheme 51).



Scheme 51: Synthesis of Mosher amides of diastereomeric lanthionines for NMR analysis.

^{1}H NMR analysis of **161** and **162** confirmed that the lanthionine synthesis had transpired stereoselectively. In particular, the resonance of the Mosher's acid methoxy group was found to vary significantly according to the lanthionine stereochemistry (Fig. 9, Table 4).

By recording the ^{1}H NMR of a mixed sample of **161** and **162** (*ca.* equimolar) we were able to demonstrate the optical purity of the individual samples, and thus the stero-integrity of the thioalkyation step.

In the light of these encouraging findings, we wished to manipulate the tetra-functionalised lanthionines **129** and **156** such that they became amenable to solid

phase, and ultimately combinatorial chemistry. This work is described in the next chapter.

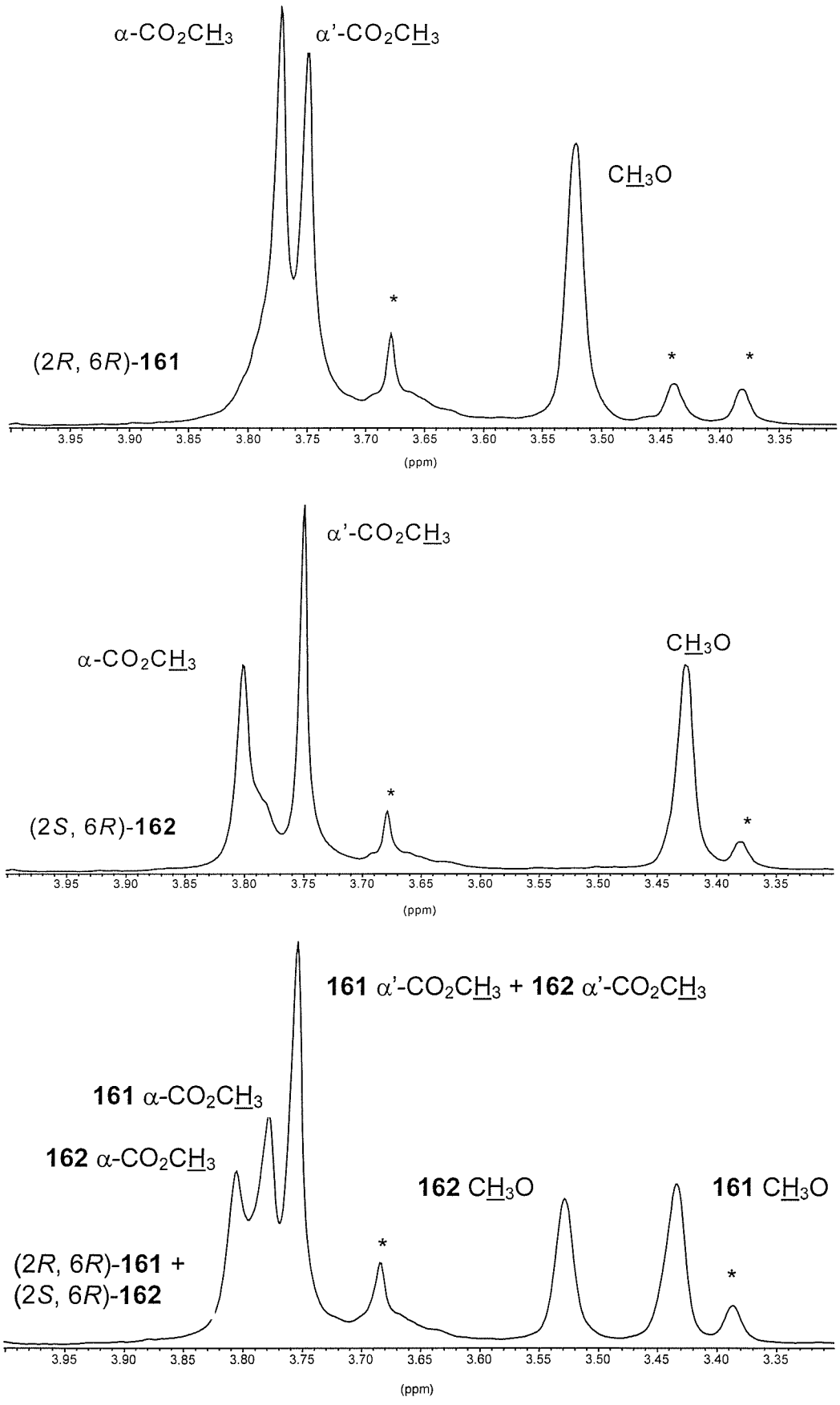


Fig. 9: NMR analysis of Mosher amides **161** and **162**, * indicates impurities.

	δ_H (2*R*, 6*R*)-**161**	δ_H (2*S*, 6*R*)-**162**	$\Delta\delta_H$ [a]
$C\underline{H}_2SC\underline{H}_2$	2.79 - 3.21	2.98 – 3.21	
$OC\underline{H}_3$	3.52	3.43	0.09
α'-$CO_2C\underline{H}_3$ [b]	3.75	3.75	0.00
α-$CO_2C\underline{H}_3$ [b]	3.77	3.80	0.03
Fmoc $\underline{H}$-9	4.24	4.25	0.01
Fmoc $C\underline{H}_2$	4.46	4.42	0.04
$C\underline{H}_\alpha$	4.51	4.64	0.13
$C\underline{H}_{\alpha'}$	4.89	4.86	0.03
Fmoc$N\underline{H}$ [c]	5.60	5.82	0.22
$Ar\underline{H}$	7.28 – 7.78	7.28 – 7.78	

[a] $\Delta\delta_H$ is the difference in chemical shift (ppm) between corresponding resonances for **161** and **162**. Differences are not calculated for resonances which appear as multiplets.

[b] See diagrams of **161** and **162** for designation of α and α'.

[c] The amide $N\underline{H}$ is not clearly visible in the ^1H NMR spectra of either isomer.

Table 4: Comparison of ^1H NMR data for lanthionines derivatised with Mosher's acid chloride.

3. Synthesis of Lanthionines for Solid Phase Applications

3.1 Protecting Group Strategy

Following our successful stereospecific synthesis of lanthionines **161** and **162**, we wished to extend our methodology to solid phase applications. In particular, we envisaged that the use of a Trt/Allyl/Fmoc strategy would facilitate the entry to cyclolanthionyl systems.

It was anticipated that the peptide synthesis monomer **163** would initially be immobilised on a solid support *via* esterification or amidation of the unprotected *C*-terminus (Figure 10). Subsequently, standard Fmoc/^{t}Bu chemistry could then be invoked to introduce endocyclic residues. Deblocking of the allyl function would then enable on-resin cyclisation. Finally, mild acidolysis of the trityl group would unveil the remaining *N*-terminus, allowing peptide chemistry to be continued.

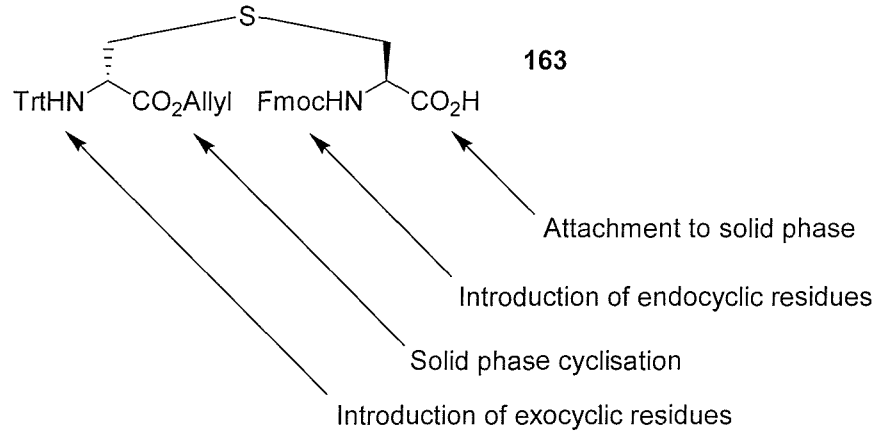
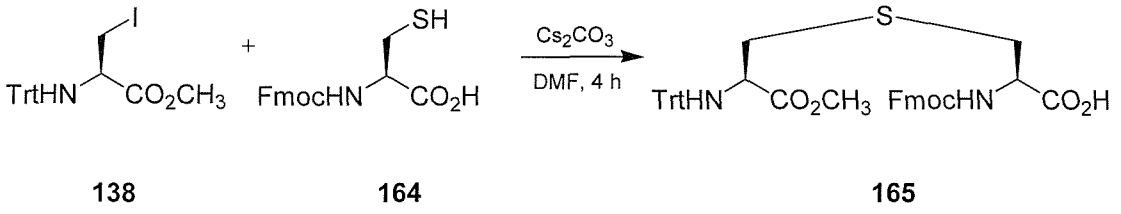


Fig. 10: Protecting group strategy for synthesis of cyclic lanthionine fragments.

Our initial attempts at generating such tri-protected lanthionines utilised Fmoc-Cys-OH **164** as the nucleophilic synthon, again partnered with an iodoalanine (Scheme 52). However, this approach furnished poor yields of the required lanthionine **165**; the mass balance was accounted for by aziridine from decomposition of the iodoalanine.

The failure of this reaction can probably be attributed to the high charge density of the mono-protected thiol component under the basic reaction conditions. This phenomenon has also been described by others attempting to synthesise lanthionine acids.[175]

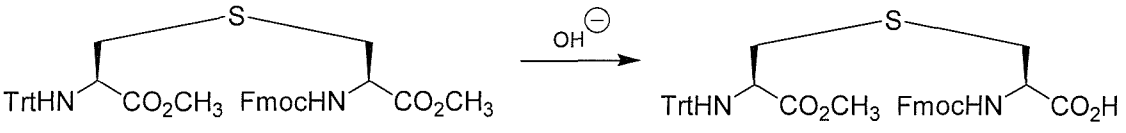


138 **164** **165**

Scheme 52: Attempted synthesis of a tri-protected lanthionine containing a free acid for solid phase applications.

3.2 Selective Saponification of Tetra-Protected Lanthionines

The difficulties encountered with the synthesis of lanthionine monomer **165** prompted us to adopt a different strategy. Instead of installing the acid function directly into the lanthionine unit, it was decided to subject the fully protected model residue **129** to a regioselective saponification in an attempt to obtain **165**, a suitable monomer for attachment to the solid phase (Scheme 53)

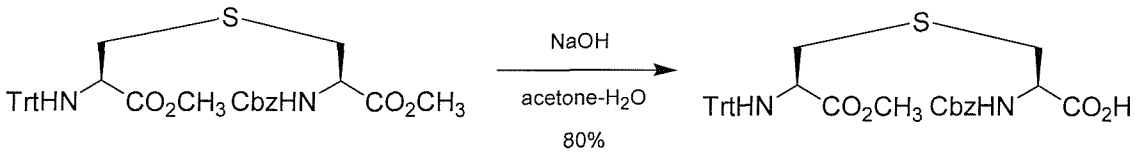


129 **165**

Scheme 53: Discrimination of the two ester functions in a tetra-protected lanthionine residue.

Although the lanthionine **129** contains three potentially base labile functions, two carboxylic esters and the Fmoc group, we were heartened to find literature accounts which suggested that, in this instance, the three moieties were orthogonal. In particular, Zervas *et al.* have demonstrated that the lanthionine diester **166** may be

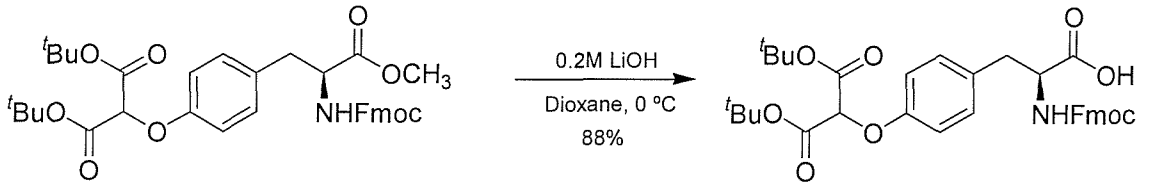
partially saponified to **167** by virtue of the steric properties of the *N*-Trt group (Scheme 54).



166 **167**

Scheme 54: Partial saponification of a fully protected lanthionine residue. The steric encumbrance of the trityl group prevents its proximal ester function from hydrolysis. Only the distal methyl ester is saponified.

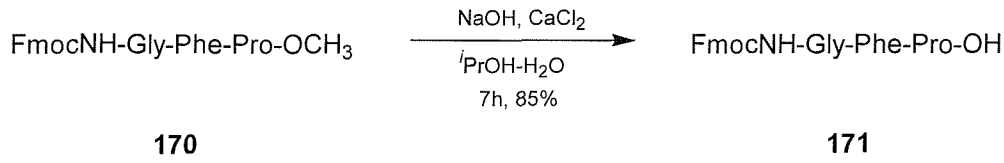
Two groups have reported the selective removal of a methyl ester in the presence of an Fmoc group. Burke and co-workers saponified the tyrosine derivative **168** without significant loss of the Fmoc group by using ice-cold 0.2M LiOH in dioxane (Scheme 55).[176]



168 **169**

Scheme 55: Removal of a methyl ester in the presence of an Fmoc group.

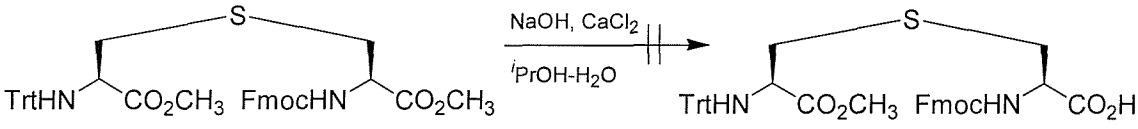
Also of note is the selective saponification of Fmoc protected peptidyl esters by Pascal and Sola.[177] The authors observed that the addition of calcium chloride to the reaction media dramatically increased the lifetime of the Fmoc group in basic solution (Scheme 56). They attributed this phenomenon to the reduction of free hydroxide ion concentration, due to the formation of calcium hydroxide.



170 **171**

Scheme 56: Removal of a methyl ester in the presence of an Fmoc group.

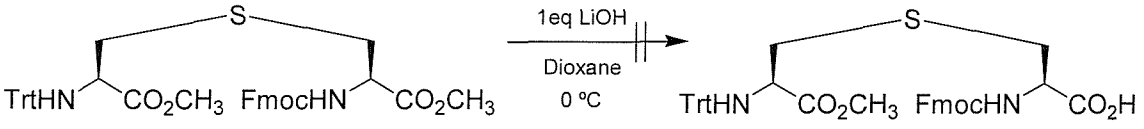
We therefore attempted the regioselective saponification of **129** in alkaline solution containing calcium chloride (Scheme 57). Unfortunately, work-up of the reaction furnished only starting material and not the required acid **165**. It was believed that the failure of this reaction was due to the limited solubility of the substrate under the reaction conditions (^{i}PrOH:H_2O, 7:3). Attempts at using other mixed solvent systems were also unsuccessful.



129 **165**

Scheme 57: Attempted selective hydrolysis of a lanthionine diester.

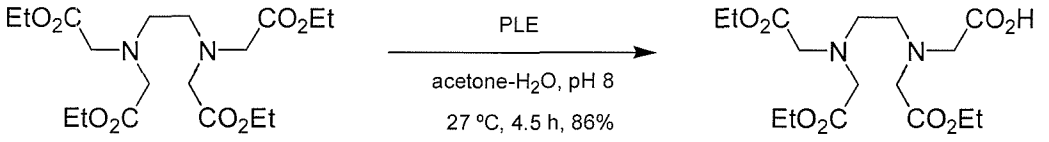
Our attempts at performing the selective hydrolysis with lithium hydroxide in dioxane were equally fruitless (Scheme 58). A single equivalent of base failed to afford the tri-protected monomer **165**, while a molar excess of LiOH led to deprotection of the Fmoc group.



129 **165**

Scheme 58: Attempted saponification of lanthionine **129** in ice-cold dioxane.

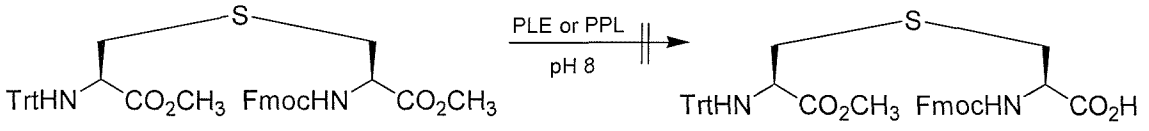
We next considered a chemoenzymatic approach to lanthionyl acids. The use of hydrolytic enzymes, especially esterases and lipases, in organic synthesis has been well documented.[178] In particular, porcine liver esterase (PLE) has found considerable use in a variety of stereo and regioselective tranformations.[179, 180, 181] Gopolan and co-workers have achieved the selective monohydrolysis of polyaminocarboxylic esters in excellent yield by using PLE (Scheme 59).[182]



172 **173**

Scheme 59: Selective monohydrolysis of a tetraester with porcine liver esterase.

To this end, we attempted the enzymatic monohydrolysis of lanthionine **129** with PLE (Scheme 60). Despite using a variety of reaction conditions, we failed to isolate the requisite acid **165**. It was considered that the lack of hydrolysis could be ascribed to either the poor solubility of **129** in acetone-water mixtures, or the bulky nature of the substrate. Attempts to circumvent the solubility problems by using porcine pancreatic lipase (PPL) were also unsuccessful in furnishing **165**.

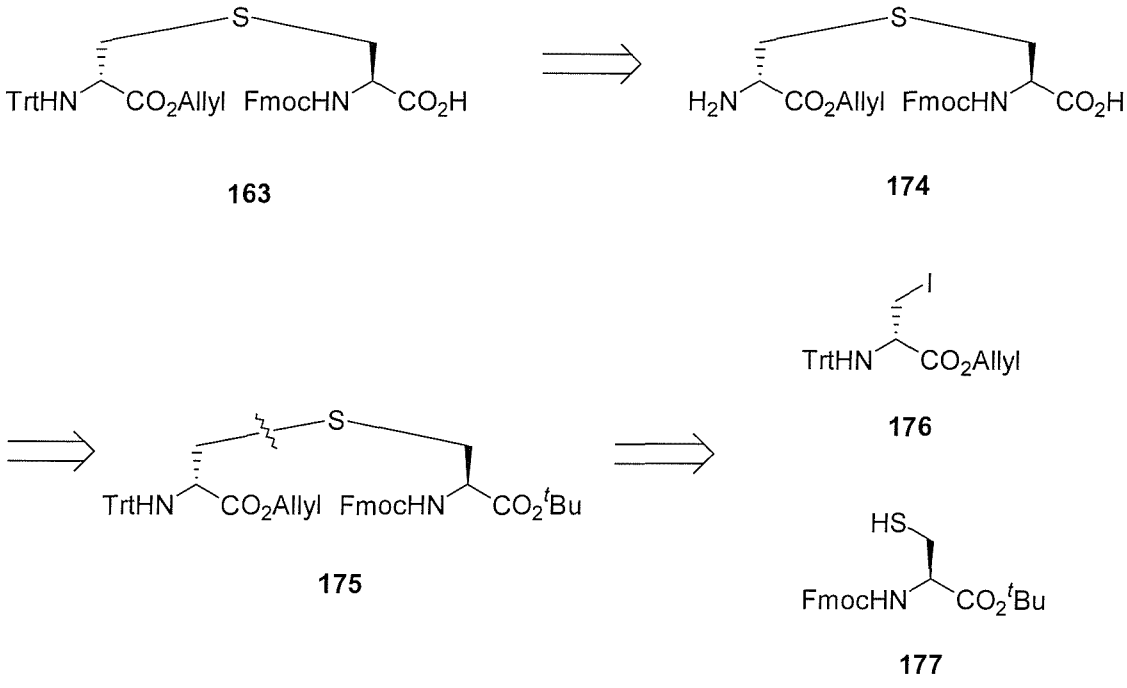


129 **165**

Scheme 60: Attempted enzymatic hydrolysis of tetra-protected lanthionine **129**.

3.3 Chemo-Differentiation of Lanthionine Diesters

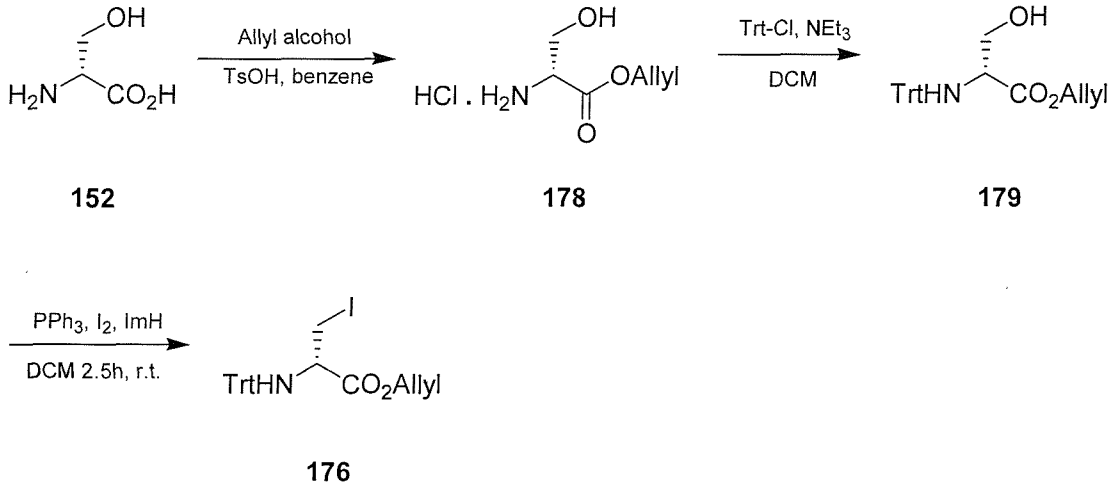
The problems associated with the regioselective monohydrolysis of lanthionyl diesters led us to reassess our route to lanthionine acids. We considered that the implementation a Trt/Allyl/Fmoc/^{t}Bu strategy would facilitate the discrimination of the two lanthionyl *C*-termini,[183] and ultimately yield lanthionine **163** (Scheme 61).



Scheme 51: Revised protecting group strategy for synthesis of lanthionine monomer **163.**

It was anticipated that the differential protection of the acid moieties in **176** and **177** would eventually afford **175**, a tetra-functionalised lanthionine unit. Acidolysis of this molecule would furnish **174**, which could be re-protected to **163** for solid phase applications.

Our synthesis of the novel iodoalanine **176** began with the esterification of D-serine **152** under Dean-Stark conditions (Scheme 52).[184] Although ^{1}H NMR analysis of the product showed it to contain an excess of *p*-toluenesulfonic acid, **178** was carried through to the next reaction without further purification.

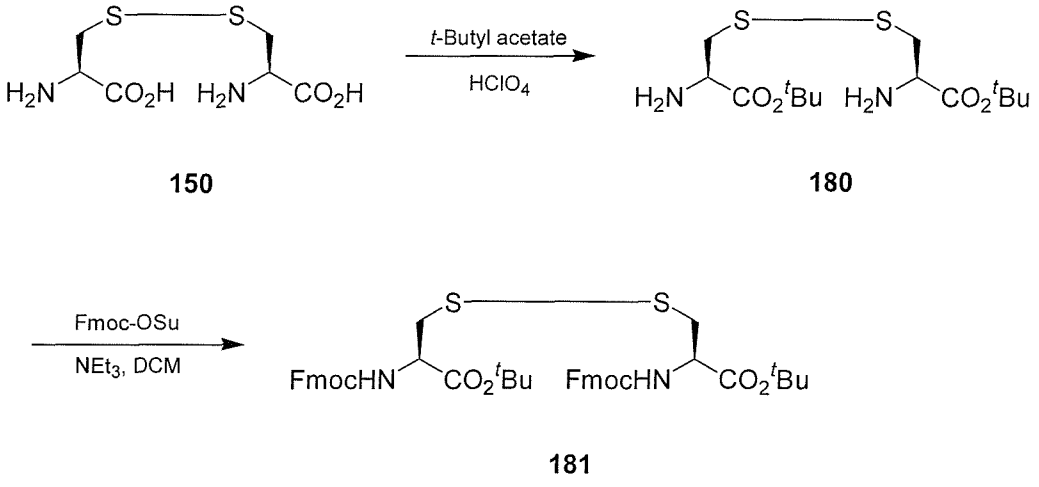


152 **178** **179**

176

Scheme 62: Synthesis of Trt-Ida-OAll **176**, a precursor to a lanthionine monomer for SPPS.

N-Tritylation of ester **178** was achieved in similar fashion to its methyl analogue **126**, affording the di-protected serine **179** as a clear oil following chromatography. Transformation of **179** to the desired iodide was once again effected under redox conditions to give the **176** as a photosensitive foam. As expected, **176** exhibited rotational isomerism by NMR, and degraded to the corresponding aziridine at elevated temperatures. The thiol component **177** was again realised from cystine **150**, thus obviating the need for transient *S*-protection (Scheme 63).



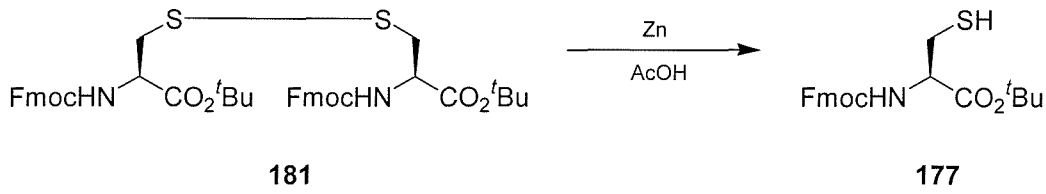
150 **180**

181

Scheme 63: Protection of cystine to give **181**, a precursor to a lanthionine monomer for SPPS.

The protection of both acid functions in **150** with *tert*-butyl actetate in perchloric acid proceeded without problem in 64% yield to give the diester **180**.[185] Double *N*-protection of **180** under standard conditions (Fmoc-OSu, NEt_3, DCM) furnished the symmetric cystine derivative **181** in 77% yield following purification by flash column chromatography.[186, 187]

Reduction of **181** was accomplished with the zinc-acetic acid acid system[188] (Scheme 64). HPLC-MS analysis of the reaction showed clean conversion of **181** to the thiol **177** in 16 h (Fig. 11). The thiol **177** was isolated in 88% yield, and was found to possess far greater aerial stability than its methyl counterpart.



181 **177**

Scheme 64: Reduction of **181** to give the thiol synthon for lanthionine synthesis.

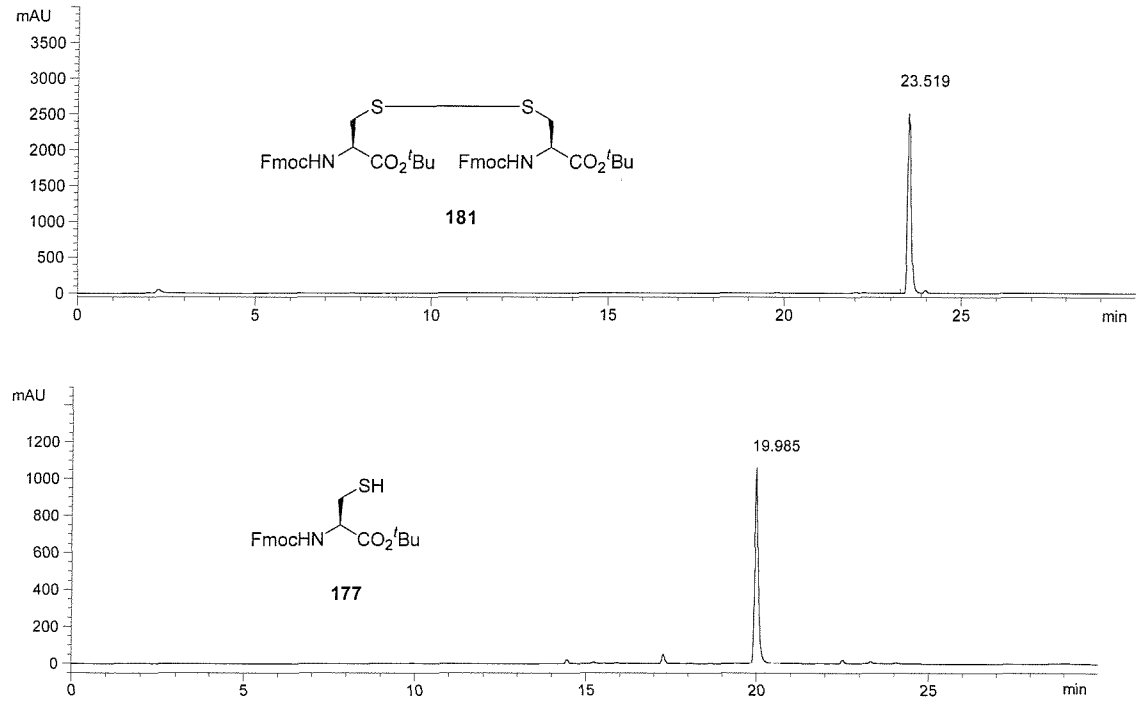
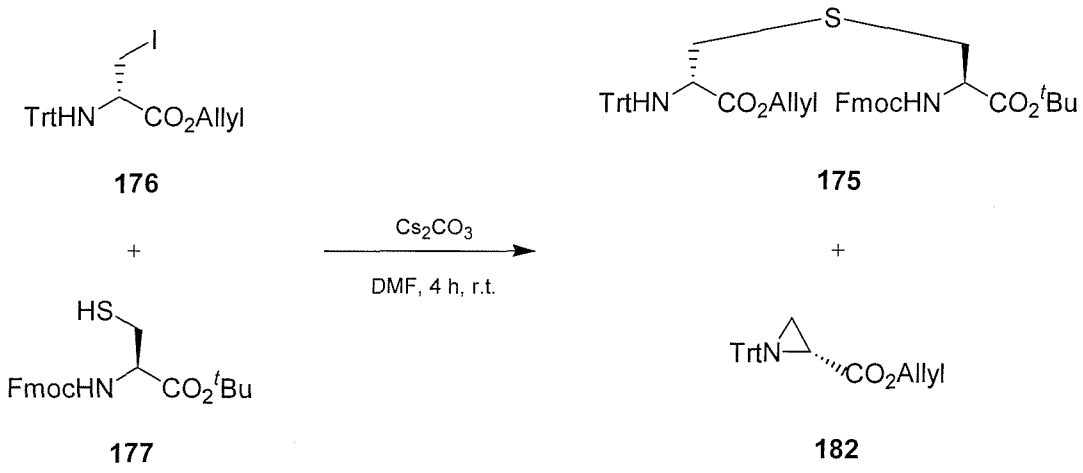


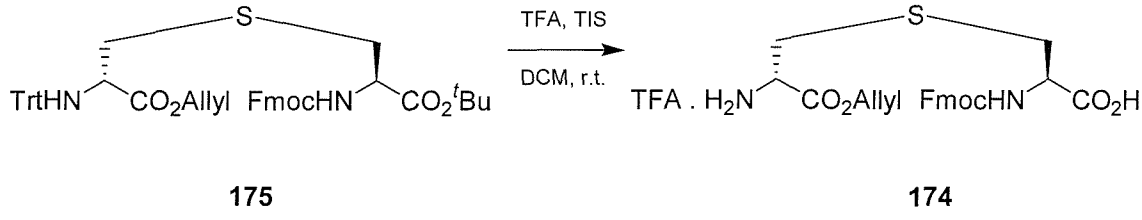
Fig. 11: HPLC analysis of disulfide **181** and thiol **177**.

Following the successful synthesis of the lanthionine precursor synthons **176** and **177,** we next attempted their coupling (Scheme 65).[175] The cesium salt of thiol **177** was alkylated with the β-iodoalanine **176** in DMF solution to afford the desired tetra-functional lanthionine **175** as a white foam (74%). The aziridine **182**, formed by the intra-molecular degradation of **176**, was also obtained (16%).



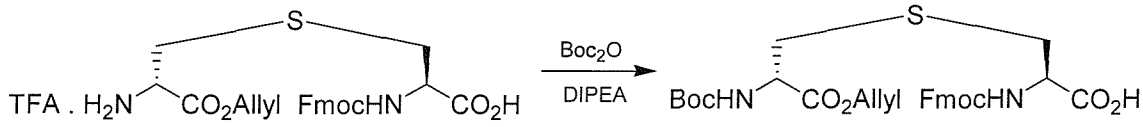
Scheme 65: Formation of tetra-protected lanthionine **175**.

Acidolytic deprotection of **175** was effected with TFA in DCM (Scheme 66). Our fears that the TIS/TFA system could reduce the allyl olefin[189] were assuaged by ES-MS and NMR analysis of amino acid **174**.



Scheme 66: Acidolytic cleavage of the Trt and ^{t}Bu functions in lanthionine **175**.

The *N*-protection of **174** with Boc_2O in dioxane proved facile, affording the peptide synthesis monomer **183** in 68% yield (Scheme 67).



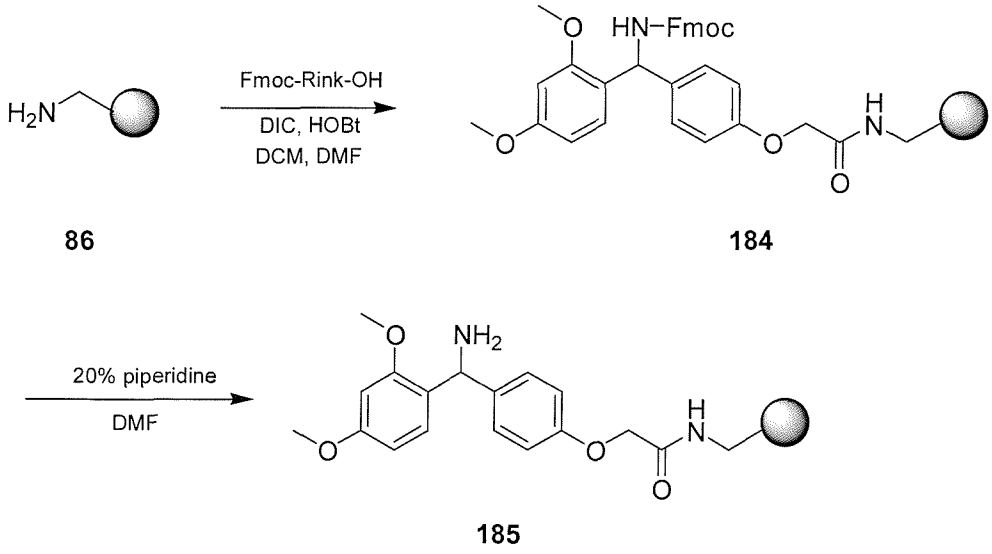
174 **183**

Scheme 67: Synthesis of a lanthionine monomer for solid phase peptide synthesis.

It should be noted that the *N*-Boc protected lanthionine **183** will only allow entry into monocyclic systems; the conditions used to remove the Boc group following cyclisation are incompatible with most practical linkers. However, the re-protection of **174** with Trt-Cl would afford the lanthionine **163,** which could be utilised to furnish polycyclic lanthionyl peptides.

3.4 Solid Phase Lanthionyl Peptide Synthesis

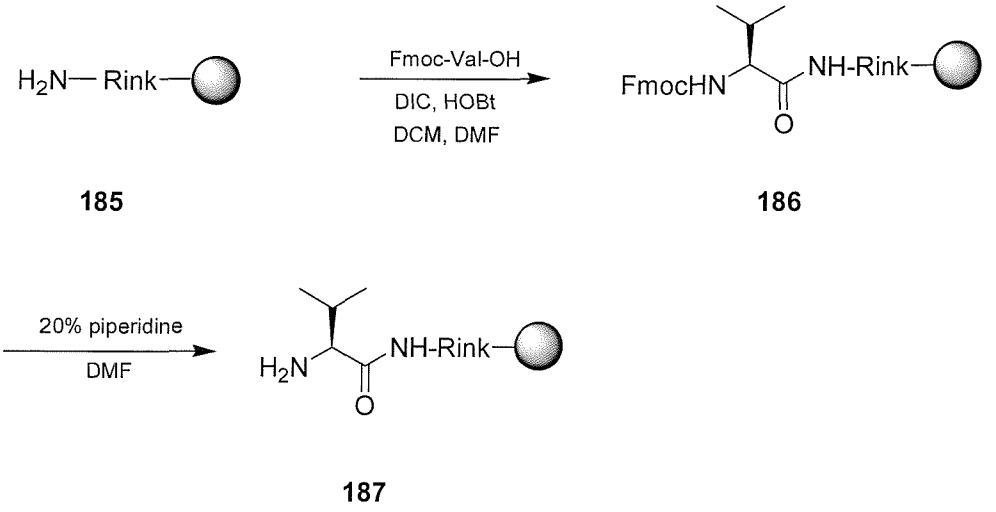
It is evident that there remains a niche within the field of peptide chemistry for a high yielding, optically pure synthesis of lanthionine containing peptides. Following our successful synthesis of the lanthionine monomer **183**, we wished to test its utility in solid phase peptide synthesis.



Scheme 68: Attachment of the Rink amide linker to the solid phase.

Resin **86** was amidated with Fmoc-Rink-OH under standard coupling conditions to give **184** (Scheme 68). Deprotection of this resin with 20% piperidine in DMF afforded resin **185**, suitable for the synthesis of peptide carboxamides.

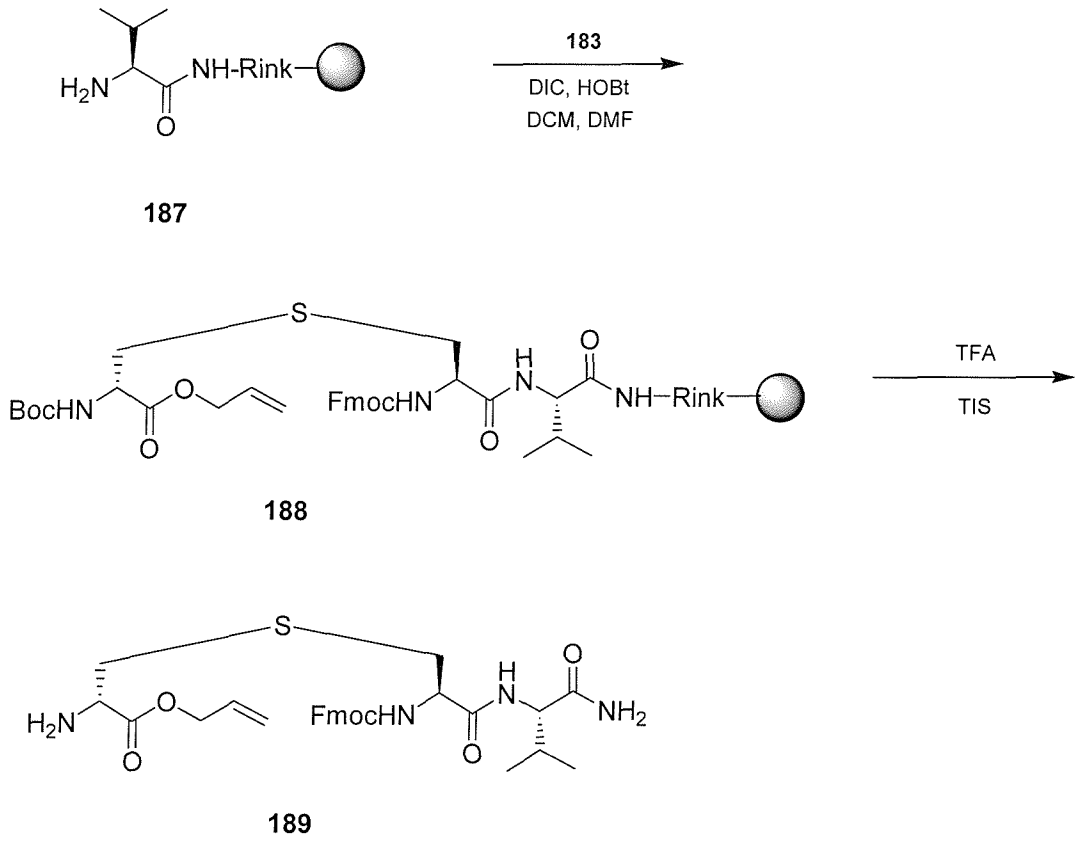
Peptide synthesis began with the coupling of Fmoc-Val-OH to **185** (Scheme 69). The reaction was monitored using the qualitative ninhydrin test for amines and was adjudged to be complete after 2 h. Once again Fmoc removal was effected with 20% piperidine in DMF, revealing the resin bound amine **187**.



Scheme 69: Coupling of the first residue onto the Rink amide linker.

We next attempted the coupling of our lanthionine monomer **183** onto the solid support **187** (Scheme 70) using standard conditions (DIC, HOBt, 2 h). A qualititative ninhydrin test performed at the end of the reaction suggested the coupling had gone to completion. To verify if this was the case, the resin **188** was treated with TFA/TIS to liberate the lanthionyl peptide **189**.

Following acidolytic cleavage, the liberated product was subjected to column chromatography, and was ultimately shown by NMR and ES-MS to be consistent with the structure of **189**. We were encouraged to note that the allyl group was resistant to prolonged exposure to TFA/TIS, a well documented reducing system.



Scheme 70: Solid phase synthesis of a lanthionine dipeptide

3.5 Conclusions for the Project

It has been seen that the lantibiotics are an intriguing family of peptide antibiotics that have much to offer the field of medicinal chemistry. Their structures and functions are heavily influenced by the non-proteinogenic bridged amino acid, lanthionine. Despite the simplicity of its structure, the synthesis of lanthionine derivatives which have utility as peptidomimetics is by no means trivial. A major issue which surrounds the formation of the Lan thioether bridge is that of optical integrity. Of equal importance is the ability to chemically differentiate between the symmetric termini of lanthionine.

We have demonstrated the utility of iodoalanines in the generation of synthetically valuable lanthionines. Such iodide synthons may be rapidly accessed from the chiral pool, though their instability should not be forgotten. Furthermore, it has been shown

by chemical correlation and the method of Mosher that the formation of lanthionines by thioalkylation of iodides is a sterospecific process.

By utilising a Trt/Allyl/Fmoc strategy, we have demonstrated a versatile lanthionyl monomer which is amenable to solid phase applications. It is believed that these findings will greatly accelerate progress in the field of lantibiotic synthesis.

4. Experimental

4.1 General

NMR spectra were obtained at 298 K using a Bruker AC-300 spectrometer (300 MHz for ^{1}H NMR, 75 MHz for ^{13}C NMR and 282 MHz for ^{19}F NMR) and a Bruker DPX-400 spectrometer (400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR). Chemical shifts (δ) were referenced to the residual signals of solvents used with exception of ^{19}F NMR spectra which were referenced to an internal TFA standard (δ_F = 0.00 ppm). Coupling constants (*J*) are given in Hz. Spectra were solved using DEPT, COSY and HMQC experiments as required.

ES-MS was performed on a VG Platform quadrupole electrospray ionisation mass spectrometer. FAB mass spectra were obtained on a VG analytical 70-250-SE normal geometry double focusing mass spectrometer, using argon as a bombarding gas in a *m*-nitrobenzyl alcohol (*m-NBA*) matrix. High resolution accurate mass measurements were recorded at 10,000 resolution using mixtures of polyethylene glycol and/or polyethylene glycol methyl ethers as mass calibrants for FAB. FT-MS was performed by Pfizer UK.

Infra-red spectra were recorded on a Bio-Rad Golden Gate FTS 135 spectrophotometer. Spectra were obtained from neat solids or solutions.

UV-VIS spectra were recorded using a Hewlett-Packard 8452A diode array spectrophotometer.

Optical rotations were measured using an AA-100 polarimeter from Optical Activity Ltd using a path length of 5 cm.

Melting Points were determined using open capillaries on Gallenkamp apparatus and remain uncorrected.

TLC was performed using Alugram® silica gel 60 F_{254} (0.25 mm) plates. Spots were detected using UV, phosphomolybdic acid, ninhydrin and bromocresol green.

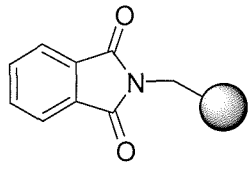
RP-HPLC was effected using a Hewlett-Packard HP1100 Chemstation, using a Phenomenex Prodigy C_{18} 5μ column (150 mm x 3.0 mm i.d.) eluting from 0.1%

TFA/H_2O to 0.042% TFA/CH_3CN over 20 min. UV detection was carried out at 220 nm, 254 nm and 270 nm.

DCM, DIPEA, pyridine, and triethylamine were distilled freshly from CaH_2 under N_2 prior to use. THF was distilled freshly from sodium benzephenone ketyl under N_2. Other laboratory reagents were purified where necessary by conventional means.[190] DMF was of peptide synthesis grade and was purchased from Rathburn UK. Amino acids and their derivatives were purchased from Novabiochem.

4.2 Experimental to Chapter 2

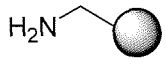
Phthalimidomethyl polystyrene resin[191] (85)



The title compound was prepared by the method of Merrifield *et al.*[191] Thus, Merrifield resin (10.00 g, 0.70 mmol/g, 7.0 mmol) and potassium phthalimide (3.88 g, 2.1 mmol, 3 eq) were slowly stirred together in DMF (200 mL) at 120 °C overnight. The reaction was filtered and the resin was washed with hot DMF (2 x 200 mL), hot DMF-water (1:1 v:v, 2 x 200 mL), hot water (2 x 200 mL), water-dioxane (1:1 v:v, 2 x 200 mL), dioxane (2 x 200 mL) and ether (2 x 200 mL). The resin was dried *in vacuo* to give 11.71g of the title product.

IR (neat) ν/cm^{-1}: 1712 (s); 1390 (m); 1119 (w); 1083 (w); 937 (w); 873 (w); 718 (m); 698 (m)

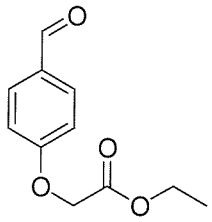
Aminomethyl polystyrene resin[191] (86)



The title compound was prepared by the method of Merrifield *et al.*[191] Thus, phthalimidomethyl polystyrene resin (11.71g, 7 mmol) and hydrazine hydrate (5.2 mL, 105 mmol, 15 eq) were refluxed in ethanol (200 mL) for 3 h. The reaction mixture was filtered and the resin washed with hot DMF (3 x 200 mL), hot DMF-water (1:1 v:v, 3 x 200 mL), hot water (3 x 200 mL), water-dioxane (1:1 v:v, 3 x 200 mL), dioxane (3 x 200 mL) and ether (3 x 200 mL). The resin was dried *in vacuo* to

give 9.76 g (quantitative, 2 steps) of the title compound. A quantitative ninhydrin assay gave a substitution of 0.56 mmol/g NH_2 (80%, 2 steps).

(4-Formylphenoxy)acetic acid ethyl ester[192] (88)



The title compound was prepared using a modification of the Robertson procedure.[192] Thus potassium *tert*-butoxide (24.7 g, 0.22 mol, 1.1 eq) was slowly added to a solution of 4-hydroxybenzaldehyde (25.0 g, 0.20 mol 1 eq) in dry DMF (100 mL) at r.t. with stirring. The solution was allowed to stir at r.t. for 10 min at which point ethyl bromoacetate (25 mL, 0.22 mol, 1.1 eq) was added and stirring continued at 110 °C overnight. Ethyl acetate was added and the reaction mixture filtered. The filtrate was washed with water (100 mL), 5% sodium carbonate solution (3 x 100 mL) and brine (2 x 100 mL). The organic phase was dried over Na_2SO_4 and solvent removed *in vacuo* to afford the title product as a yellow-brown solid (20.3 g, 56%) which was used in the subsequent step without further purification.

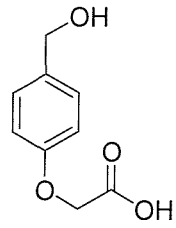
R_f (ethyl acetate:hexane, 1:3 v/v): 0.29

M. Pt.: 40 - 42 °C [lit.[192] 42 – 42.5 °C]

δ_H (300 MHz; $CDCl_3$): 1.30 (t, J = 7 Hz, 3H, $CH_2C\underline{H}_3$); 4.27 (q, J = 7 Hz, 2H, $C\underline{H}_2CH_3$); 4.69 (s, 2H, $ArOC\underline{H}_2$); 7.01 (d, J = 9 Hz, 2H, *o*-Ar$\underline{H}$); 7.84 (d, J = 9 Hz, 2H, *m*-Ar$\underline{H}$); 9.89 (s, 1H, C$\underline{H}$O)

δ_C (75 MHz; $CDCl_3$): 14.3 ($CH_2\underline{C}H_3$); 61.8 ($\underline{C}H_2CH_3$); 65.4 ($ArO\underline{C}H_2$); 115.1 (*o*-Ar$\underline{C}$H); 130.1 (*p*-Ar$\underline{C}$); 132.1 (*m*-Ar$\underline{C}$H); 162.8 (*ipso*-Ar$\underline{C}$); 168.2 ($\underline{C}OOCH_2$); 190.9 ($\underline{C}$HO)

(4-Hydroxymethylphenoxy)acetic acid[193] (89)



The title compound was prepared by using a modification of the Sheppard procedure.[194] Thus, (4-formylphenoxy)acetic acid ethyl ester **(93)** (20.3 g, 97 mmol, 1 eq) was dissolved in 1M NaOH (75 mL), H_2O (75 mL) and methanol (75 mL). Sodium borohydride (3.78 g, 100 mmol, 1.03 eq) was added and the reaction mixture stirred overnight at r.t. Solid sodium hydroxide (33.0 g, 83 mmol) was added and the solution stirred for a further 1 h at r.t. The mixture was cooled with ice and water (400 mL) was added, and the pH adjusted to 2 with 4M HCl. The solution was extracted with ethyl acetate (2 x 200 mL). The combined organics were washed with brine (3 x 300 mL), dried over Na_2SO_4 and reduced *in vacuo* to give a brown solid. Recrystallisation from ethyl acetate-hexane furnished the title compound as a white solid (11.73g, 66%).

R_f (ethyl acetate:hexane, 1:1 v/v): 0.13

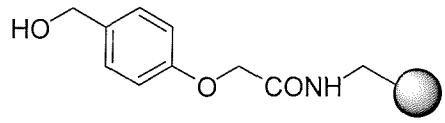
M. Pt.: 109 - 111 °C [lit.[193] 110.5 – 112 °C]

LRMS (ESI-) m/z: 181 $(M - H)^-$

δ_H (300 MHz; DMSO-d_6): 4.41 (s, 2H, ArC$\underline{H}_2$OH); 4.63 (s, 2H, ArOC$\underline{H}_2$); 6.85 (d, J = 9 Hz, 2H, Ar$\underline{H}$); 7.22 (d, J = 9 Hz, 2H, *m*-Ar$\underline{H}$)

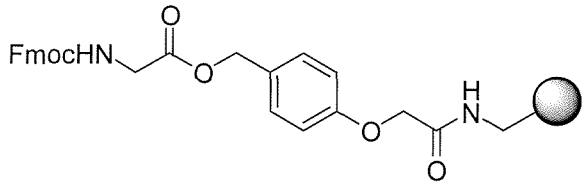
δ_C (75 MHz; DMSO-d_6): 62.6 ($\underline{C}H_2$); 64.6 ($\underline{C}H_2$); 114.1 (*o*-Ar$\underline{C}$H); 128.0 (*m*-Ar$\underline{C}$H); 135.1 (*p*-Ar$\underline{C}$); 156.7 (*ipso*-Ar$\underline{C}$); 170.4 ($\underline{C}$OOH)

(4-Hydroxymethylphenoxy)acetamidomethyl resin (90)



The title compound was synthesised using the procedure of Fields and Noble.[195] (4-Hydroxymethylphenoxy)acetic acid **(94)** (2.00 g, 10.93 mmol, 2 eq) and HOBt (1.48g, 10.93 mmol) were dissolved in DCM (90 mL) and DMF (10 mL). The solution was stirred at r.t. for 5 min and DIC (1.71 mL, 1.38 g, 10.93 mmol, 2 eq) added. The solution was stirred at r.t. for a further 5 min and then added to aminomethyl polystyrene resin (9.76 g, 0.56 mmol/g, 5.47 mmol, 1 eq). The reaction mixture was shaken overnight at r.t. and the resin filtered. The resin was washed with DMF (2 x 100 mL x 1 min), DCM (2 x 100 mL x 1 min) and ether (2 x 100 mL x 1 min) and dried *in vacuo* to give the title compound (10.86 g, quantitative). A small aliquot of resin gave a negative qualitative ninhydrin test.

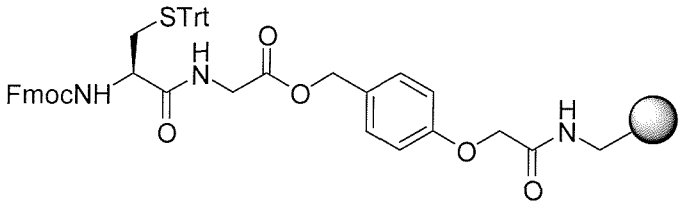
***N*-9H-Fluorenylmethoxycarbonyl-L-glycinyl-Wang Linker-Resin (91)**



(4-Hydroxymethylphenoxy)acetamidomethyl resin (1.00 g, 0.7 mmol), Fmoc-Gly-OH (0.42 g, 1.4 mmol), DIC (0.18 g, 0.22 mL, 1.4 mmol) and DMAP (0.04 g, 0.35 mmol) were shaken together in DCM (25 mL) for 3 h at r.t. the resin was filtered and washed with DMF (2 x 10 mL x 1 min), DCM (2 x 10 mL x 1 min), ether (2 x 10 mL x 1 min) and dried *in vacuo* to give 1.15 g (96%) of resin. A quantitative Fmoc assay gave a loading of 0.58 mmol/g (quantitative).

IR (neat) ν/cm^{-1}: 2932 (w); 1664 (br, s); 1500 (m); 1423 (m); 1224 (m); 1162 (s); 1100 (m); 818 (m); 741 (s); 701 (m)

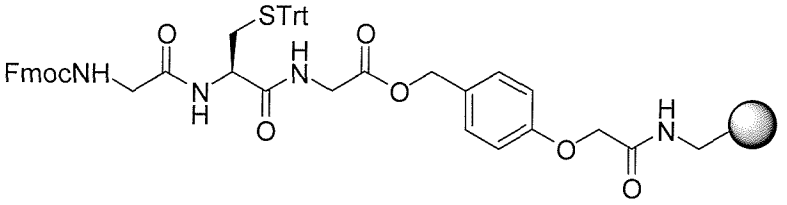
_N_-9H-Fluorenylmethoxycarbonyl-(_S_-triphenylmethyl-L-cysteinyl)-L-glycinyl-Wang Linker-Resin (92)



The title compound was synthesised using the procedure of Fields and Noble.[195] *N*-9H-Fluorenylmethoxycarbonyl-L-glycinyl-Wang Linker-Resin (1.15 g, 0.58 mmol/g, 0.67 mmol) was shaken with 20% piperidine in DMF (20mL) at r.t. for 5 min. The resin was filtered and again shaken with 20% piperidine in DMF at r.t for 15 min. The resin was filtered and washed with DMF (2 x 10 mL x 1 min), DCM (2 x 10 mL x 1 min) and ether (2 x 10 mL x 1 min). A small aliquot of resin gave a positive ninhydrin test. Fmoc-Cys(Trt)-OH (0.78 g, 1.34 mmol) and HOBt (0.18 g, 1.34 mmol) were dissolved in DCM (18 mL) and DMF (2 mL) with stirring at r.t. for 5 min. DIC (0.21 mL, 1.34 mmol) was added and stirring continued at r.t for a further 5 min. The coupling mixture was added to the resin and the reaction mixture shaken for 2 h. The resin was filtered and washed with DMF (2 x 20 mL x 1 min), DCM (2 x 20 mL x 1 min), ether (2 x 20 mL x 1 min) and dried *in vacuo* to give 1.51 g (99%) resin. A small aliquot of resin gave a negative ninhydrin test. A quantitative Fmoc assay gave a substitution of 0.43 mmol/g (90%).

IR (neat) ν/cm^{-1}: 1671 (br, s); 1499 (s); 1443 (m); 1301 (w); 1219 (m); 1167 (s); 1130 (m); 818 (w); 738 (s)

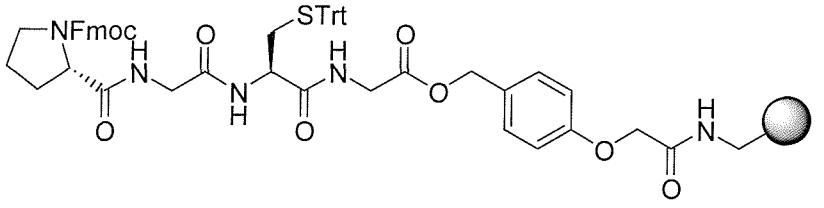
***N*-9*H*-Fluorenylmethoxycarbonyl-L-glycinyl-(*S*-triphenylmethyl-L-cysteinyl)-L-glycinyl-Wang Linker-Resin (93)**



The title compound was synthesised using the procedure of Fields and Noble.[195] *N*-9H-Fluorenylmethoxycarbonyl-(*S*-trityl-L-cysteinyl)-L-glycinyl-Wang Linker-Resin (1.51 g, 0.43 mmol/g, 0.65 mmol) was shaken with 20% piperidine in DMF (20mL) at r.t. for 5 min. The resin was filtered and again shaken with 20% piperidine in DMF at r.t for 15 min. The resin was filtered and washed with DMF (2 x 20 mL x 1 min), DCM (2 x 20 mL x 1 min) and ether (2 x 20 mL x 1 min). A small aliquot of resin gave a positive ninhydrin test. Fmoc-Gly-OH (0.39 g, 1.30 mmol) and HOBt (0.17 g, 1.30 mmol) were dissolved in DCM (9 mL) and DMF (1 mL) with stirring at r.t. for 5 min. DIC (0.20 mL, 1.30 mmol) was added and stirring continued at r.t for a further 5 min. The coupling mixture was added to the resin and the reaction mixture shaken for 2 h. The resin was filtered and washed with DMF (2 x 20 mL x 1 min), DCM (2 x 20 mL x 1 min), ether (2 x 20 mL x 1 min) and dried *in vacuo* to give 1.73 g (quantitative) resin. A small aliquot of resin gave a negative ninhydrin test. A quantitative Fmoc assay gave a substitution of 0.35 mmol/g (92%).

IR (neat) ν/cm^{-1}: 2919 (w); 1660 (br, s); 1507 (s); 1440 (m); 1224 (m); 1167 (s); 1110 (m); 818 (w); 743 (s)

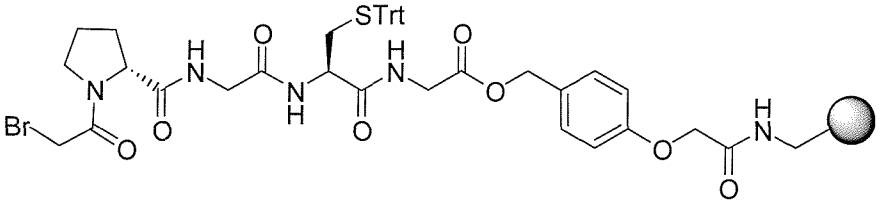
***N*-9*H*-Fluorenylmethoxycarbonyl-L-prolinyl-L-glycinyl-(*S*-triphenylmethyl-L-cysteinyl)-L-glycinyl-Wang Linker-Resin (94)**



The title compound was synthesised using the procedure of Fields and Noble.[195] *N*-9H-Fluorenylmethoxycarbonyl-(*S*-triphenylmethyl-L-glycinyl-L-cysteinyl)-L-glycinyl-Wang Linker-Resin (1.70 g, 0.35 mmol/g, 0.60 mmol) was shaken with 20% piperidine in DMF (20mL) at r.t. for 5 min. The resin was filtered and again shaken with 20% piperidine in DMF at r.t for 15 min. The resin was filtered and washed with DMF (2 x 20 mL x 1 min), DCM (2 x 20 mL x 1 min) and ether (2 x 20 mL x 1 min). A small aliquot of resin gave a positive ninhydrin test. Fmoc-Pro-OH (0.41 g, 1.20 mmol) and HOBt (0.16 g, 1.20 mmol) were dissolved in DCM (18 mL) and DMF (2 mL) with stirring at r.t. for 5 min. DIC (0.19 mL, 1.20 mmol) was added and stirring continued at r.t for a further 5 min. The coupling mixture was added to the resin and the reaction mixture shaken for 2 h. The resin was filtered and washed with DMF (2 x 20 mL x 1 min), DCM (2 x 20 mL x 1 min), ether (2 x 20 mL x 1 min) and dried *in vacuo* to give 2.21 g (quantitative) resin. A small aliquot of resin gave a negative ninhydrin test. A quantitative Fmoc assay gave a substitution of 0.31 mmol/g (quantitative).

IR (neat) ν/cm^{-1}: 2976 (m); 2926 (w); 2864 (m); 1742 (w); 1665 (br, s); 1510 (m); 1492 (m); 1445 (m); 1172 (m); 1116 (s); 822 (m); 741 (s)

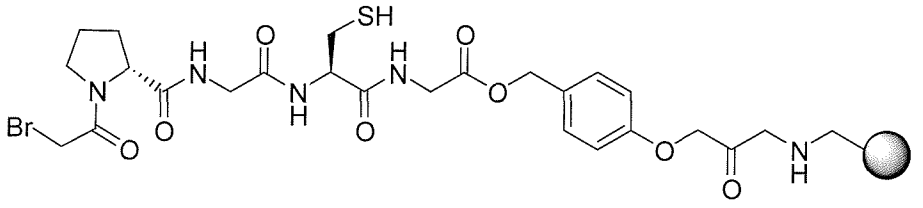
***N*-Bromoacetyl-L-prolinyl-L-glycinyl-(*S*-triphenylmethyl-L-cysteinyl)-L-glycinyl-Wang Linker-Resin (95)**



The title compound was prepared using the method of Robey and Fields.[153] Thus, *N*-9*H*-Fluorenylmethoxycarbonyl-L-prolinyl-L-glycinyl-(*S*-triphenylmethyl-L-cysteinyl)-L-glycinyl-Wang Linker-Resin (2.00 g, 0.31 mmol/g, 0.62 mmol) was shaken with 20% piperidine in DMF (20mL) at r.t. for 5 min. The resin was filtered and again shaken with 20% piperidine in DMF at r.t for 15 min. The resin was filtered and washed with DMF (2 x 20 mL x 1 min), DCM (2 x 20 mL x 1 min) and ether (2 x 20 mL x 1 min). A small aliquot of resin gave a positive ninhydrin test. Bromoacetic acid (0.86 g, 6.2 mmol) was dissolved in DCM (18 mL) and DMF (2 mL) with stirring at r.t. for 5 min. DIC (0.49 mL, 3.10 mmol) was added and stirring continued at r.t for a further 5 min. The coupling mixture was added to the resin and the reaction mixture shaken for 2 h. The resin was filtered, washed with DMF (2 x 20 mL x 1 min), DCM (2 x 20 mL x 1 min), ether (2 x 20 mL x 1 min) and dried *in vacuo* to give 2.14 g (quantitative) resin.

IR (neat) ν/cm^{-1}: 2913 (w); 1740 (w); 1656 (br, s); 1508 (s); 1444 (s); 1222 (m); 1172 (s); 1104 (m); 818 (w); 748 (s)

_N_-Bromoacetyl-L-prolinyl-L-glycinyl-L-cysteinyl-L-glycinyl-Wang Linker-Resin (100)



_N_-Bromoacetyl-L-prolinyl-L-glycinyl-(_S_-trityl-L-cysteinyl)-L-glycinyl-Wang Linker-Resin (2.14 g, 0.29 mmol/g, 0.62 mmol) was shaken with TFA:TIS:DCM (2:2:96 v/v, 5 mL) for 5 min at r.t. The solution was filtered and the process repeated until a small aliquot of beads remained colourless in neat TFA. The resin was washed with DMF (2 x 10 mL x 1 min), DCM (2 x 10 mL x 1 min) and ether (2 x 10 mL x 1 min) and dried *in vacuo* to give the title compound (1.90 g, 96%). A quantitative Ellman test gave the loading of the resin as 0.29 mmol/g SH (94%).

IR (neat) ν/cm^{-1}: 3004 (w); 2911 (w); 1736 (w); 1671 (br, s); 1501 (m); 1421 (w); 1400 (w); 1211 (m); 1173 (s); 1093 (w); 800 (w); 706 (m)

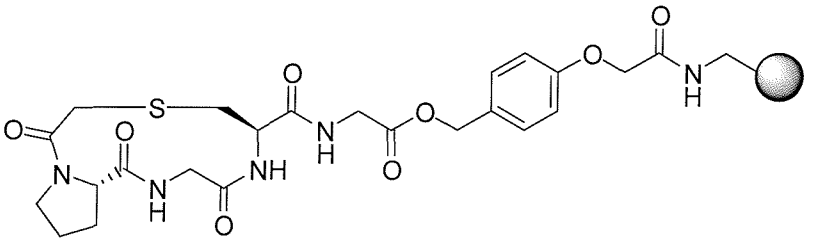
Solid phase Ellman test[157] for free thiols

To an accurately known mass of resin (*ca.* 5 mg) was added DCM (4 mL), DTNB (2 mL, 10 mM in DMF) and DIPEA (50 μL). The solution was allowed to stand at r.t. for 5 min and extracted with pH 8 phosphate buffer (3 x 5 mL). The combined aqueous extracts were diluted to 25 mL with pH 8 phosphate buffer, and assayed against a blank at 412 nm. Free thiol concentration was calculated as:

$$\text{mmol/g [SH]} = (A_{412} \times V_{mL} \times 1000) / (W_{mg} \times \varepsilon)$$

where A_{412} = Absorbance (sample) – Absorbance (blank) at λ = 412 nm, W_{mg} = mass of resin used, V_{mL} = final dilution volume and ε = TNB^{2-} extinction coefficient in phosphate buffer[157] = 13, 600 $M^{-1}cm^{-1}$.

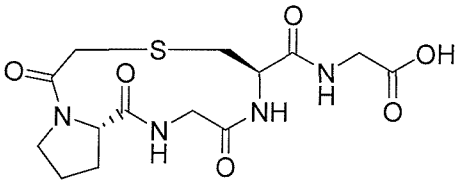
Cyclo(_S_-acetyl-L-prolinyl-L-glycinyl-L-cysteinyl)-L-glycinyl-Wang Linker-Resin (101)



_N_-Bromoacetyl-L-prolinyl-L-glycinyl-L-cysteinyl-L-glycinyl-Wang Linker-Resin (1.90g, 0.33mmol/g, 0.62mmol) was suspended in DMF (9.5 mL) and NMM (0.5 mL) added. The mixture was shaken overnight at r.t. and the resin was filtered, washed with DMF (2 x 20 mL x 1 min), DCM (2 x 20 mL x 1 min), ether (2 x 20 mL x 1 min) and dried *in vacuo* to give 1.96 g (quantitative) resin. An Ellman test gave a thiol substitution of 0.01 mmol/g (96%).

IR (neat) ν/cm^{-1}: 2901 (w); 1741 (w); 1660 (br, s); 1504 (m); 1418 (m); 1402 (w); 1168 (s); 1099 (w); 743 (w); 702 (m)

Cyclo(_S_-acetyl-L-prolinyl-L-glycinyl-L-cysteinyl)-L-glycine (102)



Cyclo(*S*-acetyl-L-prolinyl-L-glycinyl-L-cysteinyl)-L-glycinyl-Wang Linker-Resin (200mg) was shaken with TFA (1.9 mL) and TIS (0.1 mL) for 3 h at r.t. The mixture was filtered, and the resin was washed with TFA (0.5 mL). The organic fractions were pooled and reduced *in vacuo*. The residue was dissolved in TFA (0.2 mL) and cold ether (5 mL) added dropwise. The supernatant was decanted and the procedure repeated again. The resultant white solid was purified by RP-HPLC to afford 10 mg (41%) of the title compound as a white foam.

LRMS (ESI+) m/z: 373 $(M + H)^+$; 395 $(M + Na)^+$

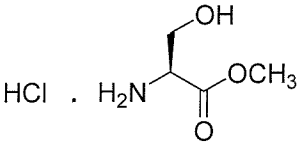
δ_H (400 MHz; DMSO-d_6, major conformer):1.93 – 2.19 (m, 2H, γ-C$\underline{H}_2$); 2.28 – 2.50 (m, 2H, β-C$\underline{H}_2$); 3.08 – 3.40 (m, 4H, NCOC$\underline{H}_2$S + SC$\underline{H}_2$CH); 3.52 – 3.92 (m, 6H, Pro δ-C$\underline{H}_2$ + Gly C$\underline{H}_2$ + Gly' C$\underline{H}_2$); 4.14 (ddd, J = 3, 7, 10, 1H, Cys C$\underline{H}_\alpha$); 4.56 (dd, J = 4, 8, 1H, Pro C$\underline{H}_2$); 7.48 (d, J = 7, 1H, Cys N$\underline{H}$); 8.33 (t, J = 6, 1H, Gly N$\underline{H}$); 8.52 (t, J = 6, 1H, Gly N$\underline{H}$)

δ_C (100 MHz;DMSO-d_6, major conformer): 22.0 (Pro $\underline{C}_\gamma$); 24.0 (Pro $\underline{C}_\beta$); 33.8 (S$\underline{C}$H$_2$CH); 34.3 (NCO$\underline{C}$H$_2$S); 40.0 (Pro $\underline{C}_\delta$); 42.9 (Gly $\underline{C}_\alpha$); 46.0 (Gly' $\underline{C}_\alpha$); 53.8 (Cys $\underline{C}_\alpha$); 59.2 (Pro $\underline{C}_\alpha$); 169.6 ($\underline{C}$=O); 169.8 ($\underline{C}$=O); 169.9 ($\underline{C}$=O);170.3 ($\underline{C}$=O); 170.4 ($\underline{C}$=O);

IR (neat) ν/cm^{-1}: 2917 (br, w); 1743 (w); 1665 (br, s); 1611 (m); 1511 (s); 1445 (m); 1170 (s); 1111 (s); 826 (w); 758 (m)

HPLC (λ_{220}) R.T.: 5.7 min

(2*S*)-Serine methyl ester hydrochloride[196] (104)



The title compound was prepared using a modification of the Falorni procedure.[196] To a stirred suspension of (2*S*)-serine (10.00 g, 95.2 mmol) in methanol (150 mL) was

added thionyl chloride (7.64 mL, 104.3 mmol, 1.1 eq) dropwise at 0 °C. The solution was refluxed for 16 h and solvent removed *in vacuo*. The white residue was recrystallised from methanol-ether to give the title compound as a white crystalline solid (14.03 g, 90%).

R_f ($CHCl_3$:MeOH:AcOH, 31:6:3 v/v): 0.29

M. Pt.: 163 °C (dec.) [lit.[196] 165 – 168 °C (dec.)]

LRMS (ESI+) m/z: 120 $(M + H)^+$; 161 $(M + CH_3CN + H)^+$

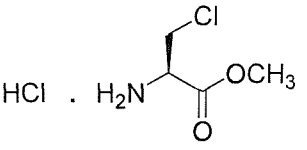
δ_H (400 MHz; D_2O): 3.83 (s, 3H, $CO_2C\underline{H}_3$); 3.97 (dd, J = 4, 13 Hz, 1H, $\underline{H}_\beta$); 4.07 (dd, J = 4, 13 Hz, 1H, $\underline{H}_{\beta'}$); 4.26 (dd, J = 4, 4 Hz, 1H, $\underline{H}_\alpha$)

δ_C (100 MHz; D_2O): 54.1 ($CO_2\underline{C}H_3$); 55.1 ($\underline{C}H_\alpha$); 59.6 ($\underline{C}H_\beta H_{\beta'}$); 169.3 ($\underline{C}O_2CH_3$)

IR (neat) ν/cm^{-1}: 3343 (s); 2915 (br, s); 1746 (s, C=O); 1592 (w); 1510 (s); 1472 (w); 1442 (m); 1381 (w); 1344 (w); 1297 (w); 1244 (br, s); 1159 (w); 1128 (w); 1092 (s); 1037 (s); 966 (m); 900 (w); 844 (w); 794 (w)

$[\alpha]_D^{20}$: +4.1° (c=2, CH_3OH) [lit.[197] $[\alpha]_D^{20}$: +3.5° (c=2, CH_3OH)

(2*R*)-3-Chloroalanine methyl ester hydrochloride[198] (105)



The title compound was prepared by the method of Walsh *et al.*[199] Thus, (2*S*)-serine methyl ester hydrochloride (5.00 g, 32.2 mmol) was dissolved in acetyl chloride (100 mL) with stirring, and the solution cooled to 0 °C. Phosphorus pentachloride (9.91 g, 47.6 mmol, 1.5 eq) was added slowly with stirring. The solution was stirred at r.t. for 2 h, and the white precipitate was filtered and washed with acetyl chloride (100 mL) and ether (100 mL). Recrystallisation from methanol-ether gave the title compound as a white crystalline solid (4.42 g, 79%).

M. Pt.: 153 – 155 °C (dec.) [lit.[200] 156 °C (dec.)]

LRMS (ESI+) m/z: 138 $(M + H)^+$; 179 $(M + CH_3CN + H)^+$

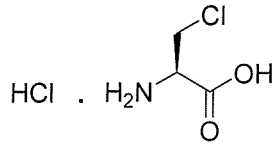
δ_H (300 MHz; D_2O): 3.82 (s, 3H, $CO_2C\underline{H}_3$); 4.02 (dd, J = 3, 13 Hz, 1H, $\underline{H}_\beta$); 4.14 (dd, J = 4, 13 Hz, 1H, $\underline{H}_{\beta'}$); 4.64 (dd, J = 3, 4 Hz, 1H, $\underline{H}_\alpha$) [in accordance with lit.[198]]

δ_C (75 MHz; D_2O): 44.4 ($\underline{C}H_\beta$); 56.5 ($\underline{C}H_\alpha$), 56.7 ($CO_2\underline{C}H_3$); 170.3 ($\underline{C}O_2CH_3$)

IR (neat) ν/cm^{-1}: 2800 (br, m); 1746 (s); 1579 (w); 1514 (s); 1444 (m); 1390 (w); 1335 (m); 1291 (w); 1243 (s); 1202 (m); 1151 (w); 1109 (w); 1070 (m); 1006 (w); 949 (w); 898 (w); 864 (m); 791 (w)

$[\alpha]_D^{20}$: -7.6° (c=1, H_2O) [lit.[198] $[\alpha]_D^{20}$: -6.1° (c=0.95, H_2O)]

(2*R*)-3-Chloroalanine hydrochloride[201] (106)



The title compound was prepared by the method of Walsh *et al.*[199] Thus, (2*R*)-3-chloroalanine methyl ester hydrochloride (4.00 g, 23.1 mmol) was dissolved in 6M HCl (50 mL) and heated at reflux for 3 h. Water (50 mL) was added, and the solvent removed *in vacuo*. The residue was recrystallised from methanol-ether to give the title compound as a white crystalline solid (3.45 g, 94%).

M. Pt.: 190 – 192 °C (dec.) [lit.[202] 193 - 194 °C (dec.)]

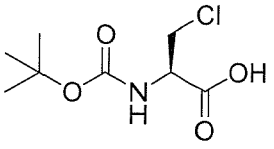
LRMS (ESI+) m/z: 124 $(M + H)^+$; 165 $(M + CH_3CN + H)^+$

δ_H (300 MHz; D_2O): 3.95 (dd, J = 4, 13, 1H, $C\underline{H}_\beta$); 3.99 (dd, J = 4, 13, 1H, $C\underline{H}_{\beta'}$); 4.45 (t, J = 4, 1H, $C\underline{H}_\alpha$)

δ_C (75 MHz; D_2O): 44.6 ($\underline{C}H_\beta$); 56.6 ($\underline{C}H_\alpha$); ($\underline{C}O_2H$)

$[\alpha]_D^{20}$: +1.4° (c=1, H_2O) [lit.[201] $[\alpha]_D^{20}$: +0.8° (c=1, H_2O)]

(2*R*)-*N*-*tert*-Butoxycarbonyl-3-chloroalanine[203] (107)



The title compound was prepared by the method of Miller *et al.*[204] Thus, di-*tert*-butyl dicarbonate (2.00 g, 9.2 mmol) and triethylamine (2.54 mL, 18.4 mmol) were added to a solution of (2*R*)-3-chloroalanine hydrochloride (1.47 g , 9.2 mmol in DMF (25 mL) at r.t. A precipitate formed immediately. The mixture was stirred overnight at r.t and then poured into ethyl acetate (50 mL). The suspension was extracted with water (2 x 25 mL) and 5% $NaHCO_3$ (2 x 25 mL). The combined aqueous layers were carefully acidified to pH 3 with solid citric acid and then extracted with ethyl acetate (3 x 30 mL). The combined organics were washed with brine (30 mL), dried over $MgSO_4$, reduced *in vacuo* to give a thick oil which crystallised on standing. Recrystallisation from ethyl acetate-hexane gave the title compound as a white solid (0.83 g, 43%).

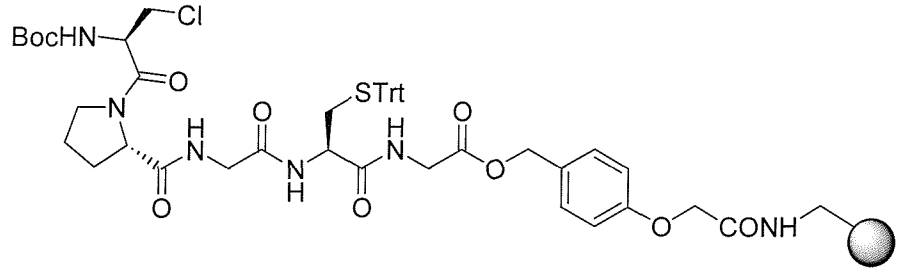
M. Pt.: 123 – 124 °C (dec.) [lit.[204] 123 - 125 °C (dec.)]

LRMS (ESI+) m/z: 224 $(M + H)^+$; 246 $(M + Na)^+$; 265 $(M + CH_3CN + H)^+$

δ_H (300 MHz; D_2O): 1.46 (s, 9H, $(C\underline{H}_3)_3C$); 4.03 (m, 2H, $C\underline{H}_\beta$); 4.79 (m, 1H, $C\underline{H}_\alpha$); 5.88 (d, $J = 7$, 1H, $N\underline{H}$) [in accordance with lit.[204]]

$[\alpha]_D^{20}$: +25.1° (c=2, CH_3OH) [lit.[204] $[\alpha]_D^{20}$: +22.9° (c=2, CH_3OH)]

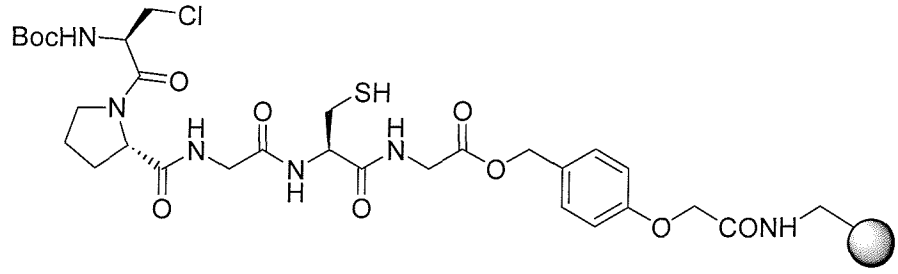
***N-tert*-Butoxycarbonyl-3-chloro-L-alanyl-L-prolinyl-L-glycinyl-(*S*-trityl-L-cysteinyl)-L-glycinyl-Wang Linker-Resin (108)**



The title compound was prepared using the method of Noble and Fields.[195] Thus, *N*-9*H*-Fluorenylmethoxycarbonyl-L-prolinyl-L-glycinyl-(*S*-triphenylmethyl-L-cysteinyl)-L-glycinyl-Wang Linker-Resin (200 mg, 0.31 mmol/g, 0.06 mmol) was shaken with 20% piperidine in DMF (10mL) at r.t. for 5 min. The resin was filtered and again shaken with 20% piperidine in DMF at r.t for 15 min. The resin was filtered and washed with DMF (2 x 10 mL x 1 min), DCM (2 x 10 mL x 1 min) and ether (2 x 10 mL x 1 min). A small aliquot of resin gave a positive ninhydrin test. (2*R*)-*N*-*tert*-Butoxycarbonyl-3-chloroalanine (27 mg, 0.12 mmol) and HOBt (16 mg, 0.12 mmol) was dissolved in DCM (9 mL) and DMF (1 mL) with stirring at r.t. for 5 min. DIC (19 μL, 0.12 mmol) was added and stirring continued at r.t for a further 5 min. The coupling mixture was added to the resin and the reaction mixture shaken for 2 h. The resin was filtered, washed with DMF (2 x 10 mL x 1 min), DCM (2 x 10 mL x 1 min), ether (2 x 10 mL x 1 min) and dried *in vacuo* to give 219 mg (quantitative) resin.

IR (neat) ν/cm^{-1}: 2103 (w); 1740 (m); 1670 (br, s); 1512 (m); 1414 (m); 1161 (m); 1073 (m); 741 (m); 702 (m)

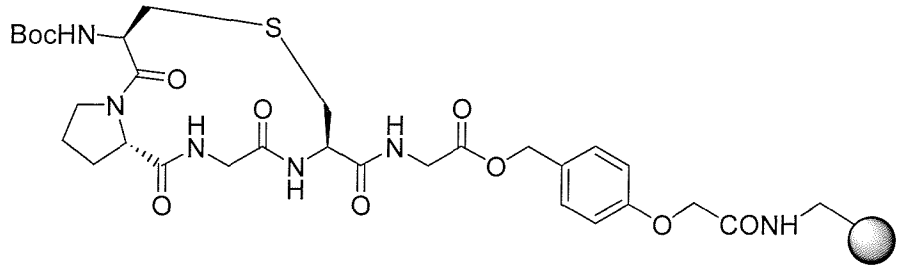
***N-tert*-Butoxycarbonyl-3-chloro-L-alanyl-L-prolinyl-L-glycinyl-L-cysteinyl-L-glycinyl-Wang Linker-Resin (109)**



N-tert-Butoxycarbonyl-3-chloro-L-alanyl-L-prolinyl-L-glycinyl-(*S*-trityl-L-cysteinyl)-L-glycinyl-Wang Linker-Resin (200 mg, 0.29 mmol/g, 0.06 mmol) was shaken with TFA:TIS:DCM (2:2:96 v/v, 5 mL) for 5 min at r.t. The solution was filtered and the process repeated until a small aliquot of beads remained colourless in neat TFA. The resin was washed with DMF (2 x 10 mL x 1 min), DCM (2 x 10 mL x 1 min) and ether (2 x 10 mL x 1 min) and dried *in vacuo* to give the title compound (180 mg, 97%).

IR (neat) ν/cm^{-1}: 1736 (w); 1664 (br, s); 1511 (w); 1421 (m); 1411 (m); 1160 (s); 1091 (m); 743 (m); 700 (m)

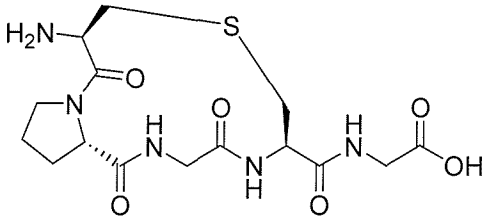
***N-tert*-Butoxycarbonyl-*cyclo*(*S*-alanyl-L-prolinyl-L-glycinyl-L-cysteinyl)-L-glycinyl-Wang Linker-Resin (110)**



N-tert-Butoxycarbonyl-3-chloro-L-alanyl-L-prolinyl-L-glycinyl-L-cysteinyl)-L-glycinyl-Wang Linker-Resin (100 mg, 0.32 mmol/g, 0.03 mmol) was suspended in DMF (4.75mL) and NMM (0.25 mL) added. The mixture was shaken overnight at r.t. and the resin was filtered, washed with DMF (2 x 10 mL x 1 min), DCM (2 x 10 mL x 1 min), ether (2 x 10 mL x 1 min) and dried *in vacuo* to give 107 mg (quantitative) resin.

IR (neat) ν/cm^{-1}: 2911 (w); 1744 (w); 1673 (br, s); 1507 (m); 1419 (w); 1402 (m); 1171 (s); 1093 (w); 741 (w); 703 (m)

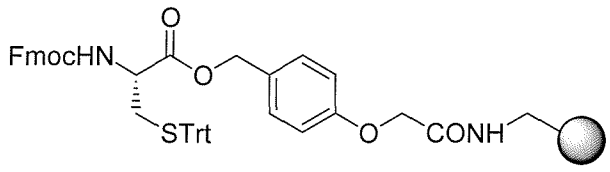
***Cyclo*(*S*-alanyl-L-prolinyl-L-glycinyl-L-cysteinyl)-L-glycine (111)**



N-tert-Butoxycarbonyl-*cyclo*(*S*-alanyl-L-prolinyl-L-glycinyl-L-cysteinyl)-L-glycinyl-Wang Linker-Resin (100 mg, 0.33 mmol/g, 0.03 mmol) was shaken with TFA (1.9 mL) and TIS (0.1 mL) for 3 h at r.t. The mixture was filtered, and the resin was washed with TFA (0.5 mL). The organic fractions were pooled and reduced *in vacuo*. The residue was dissolved in TFA (0.2 mL) and cold ether (5 mL) added dropwise. The supernatant was decanted and the procedure repeated again. The resultant white solid was purified by RP-HPLC to afford 8 mg of a white solid, which was not purified further.

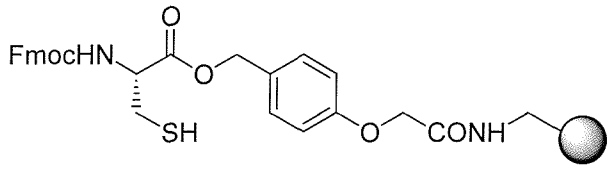
LRMS (ESI+) m/z: 402 $(M + H)^+$; 424 $(M + Na)^+$

Fmoc-Cys(Trt)-Wang Linker-Resin (112)



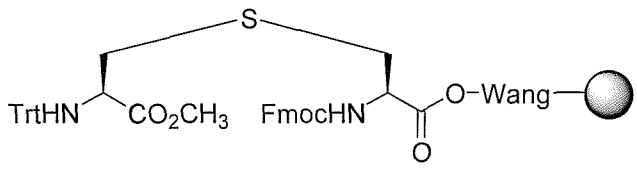
Fmoc-Cys(Trt)-OH (1.23 g, 2.1 mmol, 3 eq) was dissolved in DCM (9 mL) and DMF (1 mL) with stirring at r.t. DIC (164 μL, 133 mg, 1.05 mmol, 1.5 eq) was added, and the solution stirred for 10 min at which point it was added to (4-hydroxymethylphenoxy)acetamidomethyl resin (1.00 g, 0.7 mmol/g, 0.7 mmol, 1 eq). DMAP (26 mg, 0.21 mmol, 0.3 eq) was added and the reaction shaken for 3 h at r.t. The reaction mixture was shaken overnight at r.t. and the resin filtered. The resin was washed with DMF (2 x 10 mL x 1 min), DCM (2 x 10 mL x 1 min) and ether (2 x 10 mL x 1 min) and dried *in vacuo* to give the title compound (1.36 g). A quantitative Fmoc test gave the loading of the resin as 0.40 mmol/g Fmoc (87%).

Fmoc-Cys(SH)-Wang Linker-Resin (113)



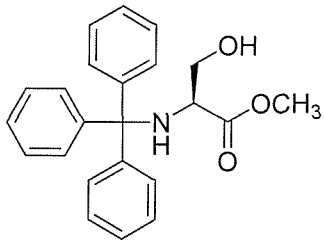
Fmoc-Cys(Trt)-Wang Linker-Resin (100 mg, 0.40 mmol/g, 0.04 mmol) was shaken with TFA:TIS:DCM (2:2:96 v/v, 5 mL) for 5 min at r.t. The solution was filtered and the process repeated until a small aliquot of beads remained colourless in neat TFA. The resin was washed with DMF (2 x 10 mL x 1 min), DCM (2 x 10 mL x 1 min) and ether (2 x 10 mL x 1 min) and dried *in vacuo* to give the title compound (87 mg). A quantitative Ellman test gave the loading of the resin as 0.38 mmol/g SH (86%).

(2*R*, 6*R*)-N^2-Triphenylmethyl-N^6-(9*H*-fluorenylmethoxycarbonyl)-O^1-methyl-O^7-Wang linker-resin lanthionine (114)



To Fmoc-Cys(SH)-Wang Linker-Resin (100 mg, 0.38 mmol/g, 0.04 mmol, 1 eq) was added (2*R*)-*N*-triphenylmethyl-3-iodoalanine methyl ester (75 mg, 0.16 mmol, 5 eq) and cesium carbonate (65 mg, 0.16 mmol, 5 eq) in DMF (10 mL). The reaction mixture was shaken under a nitrogen atmosphere for 5 h at r.t. The resin was filtered and washed with DMF (2 x 10 mL x 1 min), DCM (2 x 10 mL x 1 min) and ether (2 x 10 mL x 1 min) and dried *in vacuo* to give the title compound (136 mg). A quantitative Ellman test gave a thiol concentration of 0.32 mmol/g SH.

(2*S*)-*N*-Triphenylmethyl serine methyl ester[159] (126)



The title compound was synthesised by the procedure of Baldwin *et al.*[159] Thus, (2*S*)-serine methyl ester hydrochloride (10.00 g, 64.3 mmol) was suspended in dry DCM (50 mL) and stirred on ice for 10 min. Triethylamine (12.99 g, 17.90 mL, 128.6 mmol, 2 eq) was added and stirring continued at 0 °C for a further 5 min. A solution of triphenylmethyl chloride (19.70 g, 70.7 mmol, 1.1 eq) in dry DCM (50 mL) was added dropwise, and the solution stirred on ice overnight. The solution was filtered to remove triethylamine hydrochloride and solvent removed *in vacuo*. The residue was taken up in ethyl acetate (100 mL) and washed with 2M $KHSO_4$ (2 x 100 mL), 1M

$NaHCO_3$ (2 x 100 mL) and water (100 mL). The solution was dried over $MgSO_4$ and solvent removed *in vacuo*. The residue was recrystallised from ethyl acetate-hexane to afford the title compound as a white solid (16.48 g, 71%).

R_f (DCM): 0.11

M. Pt.: 148-151 °C [lit.[159] 77 – 78 °C]

LRMS (ESI+) m/z: 243 (Trt^+); 362 $(M + H)^+$

δ_H (400 MHz; $CDCl_3$): 2.45 (br s, 1H, OH or NH); 3.00 (br s, 1H, OH or NH); 3.36 (s, 3H, CO_2CH_3); 3.60 (dd, J = 4, 10 Hz, 1H, CH_β); 3.64 (dd, J = 5, 5 Hz, 1H, CH_α); 3.78 (dd, J = 4, 10 Hz, 1H, $CH_{\beta'}$); 7.25 (t, J = 7 Hz, 3H, Ar *p*-H); 7.33 (dd, J = 7, 7 Hz, 6H Ar *m*-H), 7.56 (d, J = 7 Hz, 6H, Ar *o*-H) [in accordance with lit.[159]]

δ_C (100 MHz; $CDCl_3$): 46.2 (CO_2CH_3); 58.3 (CH_α); 65.4 (CH_β); 71.4 (Ph_3C); 127.4 (Ar *p*-CH); 128.4 (Ar *o/m*-CH); 129.2 (Ar *o/m*-CH); 146.1 (*ipso* Ar-C); 174.4 (CO_2CH_3) [in accordance with lit.[159]]

IR (neat) ν/cm^{-1}: 3447 (w); 1700 (s, C=O); 1480 (m); 1443 (w); 1422 (w); 1207 (m); 1055 (w); 963 (w); 893 (w); 753 (w); 697 (s)

UV (DCM) λ_{max}: 232 nm

$[\alpha]_D^{20}$: +28.7° (c=1, CH_3OH) [lit.[159] $[\alpha]_D^{20}$: +29.9° (c=1.62 CH_3OH)]

Attempted synthesis of (129) by Mitusnobu reaction of (126) and (128)

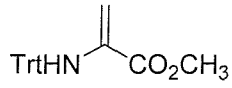
Method 1: Use of DIAD/PPh_3 as a redox system.

The procedure of Hughes was employed.[162] To a solution of (2*S*)-*N*-triphenylmethyl serine methyl ester (360 mg, 1 mmol, 1 eq), triphenylphosphine (262 mg, 1 mmol, 1 eq) and (2*R*)-*N*-(9*H*-fluorenylmethoxycarbonyl) cysteine methyl ester (357 mg, 1 mmol, 1 eq) in DCM (10 mL) was added DIAD (222 mg, 216 µL, 1.1 mmol, 1.1 eq) with stirring at r.t. The solution was stirred at r.t overnight and solvent removed *in vacuo*. The residue was purified by column chromatography (SiO_2, DCM) affording only starting materials and degradation products.

Method 2: Use of ADDP/PMe_3 as a redox system.

The procedure of Falck *et al.* was employed.[166] Thus, 1.0 M trimethylphosphine in toluene (1 mL, 1 mmol) was added dropwise under a nitrogen atmosphere to a stirred solution of (2*S*)-*N*-triphenylmethyl serine methyl ester (180 mg, 0.5 mmol, 0.5 eq), (2*R*)-*N*-(9*H*-fluorenylmethoxycarbonyl) cysteine methyl ester (536 mg, 1.5 mmol, 3 eq), ADDP (252 mg, 1 mmol, 1 eq) and imidazole (68 mg, 1 mmol, 1 eq) in DCM (5 mL) at r.t. The solution was stirred at r.t overnight and solvent removed *in vacuo*. The residue was purified by column chromatography (SiO_2, DCM) affording the highly unstable dehydroalanine **(135)** as a clear oil in 43% yield.

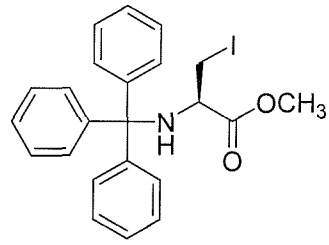
_N_-Triphenylmethyl-2,3-didehydroalanine methyl ester (135)



δ_H (400 MHz; $CDCl_3$): 1.49 (s, 1H, NH); 3.68 (s, 3H, CO_2CH_3); 4.72 (s, 1H, CH_β); 5.74 (s, 1H, $CH_{\beta'}$); 7.09 – 7.27 (m, 15H, Trt ArH)

δ_C (75 MHz; $CDCl_3$): 52.9 (CO_2CH_3); 71.6 (Ph_3C); 95.5 ($CH_\beta H_{\beta'}$); 127.5 (Ar *p*-CH); 128.3 (Ar *o*/*m*-CH); 129.6 (Ar *o*/*m*-CH); 137.1 (C_α); 144.9 (*ipso* Ar-C); 166.8 (CO_2CH_3)

(2*R*)-*N*-Triphenylmethyl-3-iodoalanine methyl ester (138)[167]



The title compound was prepared by a modification to the Garegg procedure.[168] Thus (2*S*)-*N*-triphenylmethyl serine methyl ester (2.00 g, 5.54 mmol), triphenylphosphine (1.45 g, 5.54 mmol, 1 eq) and imidazole (0.38 g, 5.54 mmol, 1 eq) were dissolved in dry DCM (50 mL) with stirring. The solution was cooled on ice to 0 °C and stirring continued for 5 min. Iodine (1.41 g, 5.54 mmol, 1 eq) was added portionwise over 2 min and stirring continued at r.t for 2.5 h in the dark. Solvent was removed *in vacuo* at r.t. and the residue was purified by column chromatography to afford the title compound as a photosensitive amorphous solid (2.19 g, 84%).

R_f (ether:hexane, 1:9 v/v): 0.41

LRMS (ESI+) m/z: 243 $(Trt)^+$; 472 $(M + H)^+$

δ_H (300 MHz; $CDCl_3$; * denotes minor rotamer): 2.20 (bs, 1H, NH); 2.51* (dd, J = 6, 13 Hz) + 3.14 (dd, J = 7, 10 Hz) (1H, CH_β); 2.68* (dd, J = 9, 13 Hz) + 3.27 (dd, J = 4, 10 Hz) (1H, $CH_{\beta'}$); 3.23 (s) + 3.69* (s) (3H, CO_2CH_3); 3.39 (m) + 4.39* (m) (1H, CH_α); 7.09 – 7.28 (m, 9H, Trt ArH); 7.41 – 7.56 (m, 6H, Trt ArH) [in accordance with lit.[167]]

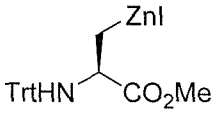
δ_C (75 MHz; $CDCl_3$; * denotes minor rotamer): 9.8* + 48.5 (CH_β); 20.3 + 56.4* (CH_α); 52.1* + 53.0 (CO_2CH_3); 71.2* + 77.4 (Ph_3CNH); 126.7 – 129.4 (complex, Ar CH); 145.6 + 145.8* (*ipso* Ar-C); 171.3 + 172.9* (CO_2CH_3) [in accordance with lit.[167]]

IR (neat) ν/cm^{-1}: 3100-2800 (br; w); 1733 (m; C=O); 1595 (w); 1489 (w); 1447 (w); 1208 (m); 1158 (m); 1028 (w); 900 (w); 743 (s); 701 (s)

UV (DCM) λ_{max}: 234 nm

$[\alpha]_D^{20}$: +23.2° (c=1, $CHCl_3$) [lit.[167] $[\alpha]_D^{25}$: +21° (c=1, $CHCl_3$)]

Insertion of zinc (138) into iodide to give zincate (147)

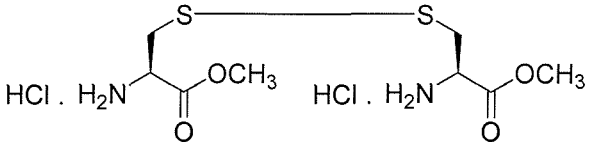


The title compound was prepared using a modification of the Jackson procedure.[174] A suspension of zinc dust (300 mg, 4.5 mmol) in dry THF (0.5 mL) and 1,2-dibromoethane (19.4 μL, 0.23 mmol) was heated under nitrogen to 60 °C for 5 min. The mixture was cooled to 35 °C and trimethylsilyl chloride (6 μL, 0.05 mmol) was added. The mixture was sonicated for 30 min and (2*R*)-*N*-triphenylmethyl-3-iodoalanine methyl ester (361 mg, 0.10 mmol) in THF (2 mL) was added. The solution was stirred for a further 5 h and used immediately in the subsequent reaction.

Attempted reaction of (147) with disulfide (132)

To a solution of zincate (111) (428 mg, 0.1 mmol th.) in THF (2.5 mL) was added (2*R*, 7*R*)-*N*, *N*-bis-(9*H*-fluorenylmethoxycarbonyl) cystine dimethyl ester (798 mg, 0.1 mmol, 1 eq) in THF (2.5 mL). The reaction was stirred at r.t. overnight, at which time the distinctive odour of acrylates could be detected. RP-HPLC analysis of the reaction mixture indicated the solution to contain tritylamine as well as starting disulfide. HPLC-MS failed to detect the presence of the desired lanthionine.

(2*R*, 7*R*)-Cystine dimethyl ester dihydrochloride[205] (151)



The title compound was prepared by using a modification of the Zervas procedure.[205] Thus, to a stirred suspension of (2*R*, 7*R*)-cystine (10.00 g, 41.7 mmol) in methanol (150 mL) was added thionyl chloride (6.69 mL, 91.7 mmol, 2.2 eq) dropwise at 0 °C. The solution was refluxed for 16 h and solvent removed *in vacuo*. The white residue was recrystallised from methanol-ether to give the title compound as a white crystalline solid (12.50 g, 88%).

R_f ($CHCl_3$:MeOH:AcOH, 31:6:3 v/v): 0.20

M. Pt.: 181 °C (dec.)

LRMS (ESI+) m/z: 269 $(M + H)^+$; 291 $(M + Na)^+$

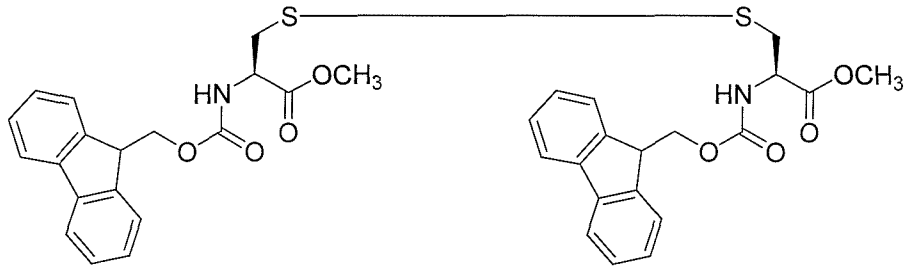
δ_H (400 MHz; D_2O): 3.32 (dd, J = 7, 15 Hz, 2H, $\underline{H}_\beta$); 3.39 (dd, J = 5, 15 Hz, 2H, $\underline{H}_{\beta'}$); 3.84 (s, 6H, $CO_2C\underline{H}_3$); 4.54 (dd, J = 5, 7 Hz, 2H, $\underline{H}_\alpha$)

δ_C (100 MHz; D_2O): 36.0 ($\underline{C}H_\beta H_{\beta'}$); 51.9 ($\underline{C}H_\alpha$); 54.3 ($CO_2\underline{C}H_3$); 169.5 ($\underline{C}O_2CH_3$)

IR (neat) ν/cm^{-1}: 2815 (br; s); 1742 (s); 1537 (m); 1495 (m); 1198 (s); 1074 (s); 882 (m)

$[\alpha]_D^{20}$: -31.2° (c=4, CH_3OH)

(2*R*, 7*R*)-*N*, *N*-Bis-(9*H*-fluorenylmethoxycarbonyl) cystine dimethyl ester[206] (132)



The title compound was prepared by using a modification of the Paquet procedure.[207] (2*R*, 7*R*)-Cystine dimethyl ester dihydrochloride (7.00 g, 20.5 mmol) was suspended in DCM (100 mL) and triethylamine (8.61 mL, 6.20 g, 61.6 mmol, 3 eq) added dropwise. *N*-(9*H*-fluorenylmethoxycarbonyloxy)succinimide (13.16 g, 41.1 mmol, 2 eq) in DCM (100 mL) was added and the solution stirred at r.t. for 2 h and filtered. The filtrate was washed with 2M HCl (200 mL) and water (200 mL), dried over $MgSO_4$ and concentrated *in vacuo*. Recrystallisation from chloroform-hexane furnished the title compound as white crystals (4.92 g, 70%).

R_f (DCM): 0.71

LRMS (ESI+) m/z: 713 $(M + H)^+$

δ_H (400 MHz; $CDCl_3$): 3.24 (d, J = 4 Hz, 4H, $\underline{H}_\beta$); 3.81 (s, 6H, $CO_2C\underline{H}_3$); 4.27 (t, J = 7 Hz, 2H, Fmoc $\underline{H}$-9); 4.46 (d, J = 6 Hz, 4H, Fmoc $C\underline{H}_2O$); 4.74 (m, 2H, $\underline{H}_\alpha$); 5.79 (d, J = 7 Hz, 2H, N$\underline{H}$); 7.35 (ddd, J = 1, 7, 7 Hz, 4H, Fmoc $\underline{H}$-2 + $\underline{H}$-7); 7.44 (dd, J = 7, 7 Hz, 4H, Fmoc $\underline{H}$-3 + $\underline{H}$-6); 7.65 (br dd, J = 2, 7 Hz, 4H, Fmoc $\underline{H}$-1 + $\underline{H}$-8); 7.81 (d, J = 7 Hz, 4H, Fmoc $\underline{H}$-4 + $\underline{H}$-5) [in accordance with lit.[206]]

δ_C (100 MHz; $CDCl_3$): 41.6 ($\underline{C}H_\beta$); 48.8 (Fmoc $\underline{C}$H); 53.2 ($CO_2\underline{C}H_3$); 53.7 ($\underline{C}H_\alpha$); 67.8 (Fmoc $\underline{C}H_2$); 121.7, 126.8, 128.8, 129.4 (Fmoc Ar $\underline{C}$H); 143.0, 145.4, 145.5 (Fmoc Ar-$\underline{C}$); 157.4 (Fmoc O$\underline{C}$ONH); 172.5 ($\underline{C}O_2CH_3$) [in accordance with lit.[206]]

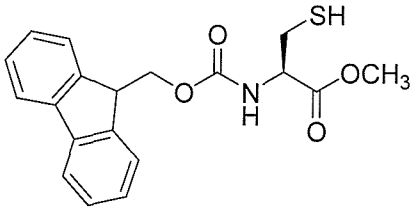
IR (neat) ν/cm^{-1}: 3272 (br; w); 1739 (s); 1674 (s); 1539 (s); 1434 (m); 1273 (s); 1213 (s); 1048 (m); 1003 (m); 748 (w); 728 (s)

UV (DCM) λ_{max}: 268 nm

HPLC (λ_{270}) R.T.: 21.3 min

$[\alpha]_D^{20}$: +25.2° (c=1, CH_3CN) [lit.[206] $[\alpha]_D^{20}$: +23.1 (c=1, CH_3CN)]

(2*R*)-*N*-(9*H*-Fluorenylmethoxycarbonyl) cysteine methyl ester (128)

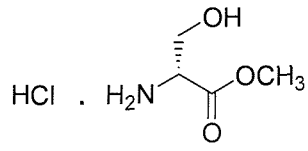


The title compound was prepared using a modification of the Kihlberg procedure.[188] To a solution of (2*R*, 7*R*)-*N*, *N*-bis-(9*H*-fluorenylmethoxycarbonyl) cystine dimethyl ester (5.00 g, 7.0 mmol) in acetic acid (50 mL) and DCM (25 mL) was added zinc dust (4.56 g, 70.2 mmol, 10 eq) portionwise. The mixture was stirred under nitrogen at r.t. overnight and the solvent removed *in vacuo*. 2M HCl (250 mL) was added to the residue and the mixture extracted with DCM (2 x 250 mL). The combined

organics were washed with water, dried over $MgSO_4$ and concentrated *in vacuo* to afford the title compound as a white solid (4.71 g, 94%) which was used immediately.

HPLC (λ_{270}) R.T.: 18.1 min

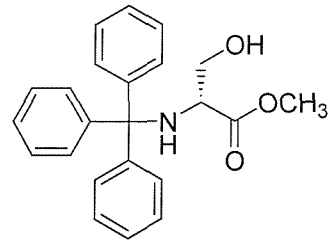
(2*R*)-Serine methyl ester hydrochloride (153)



Repetition of the methodology used to synthesise **(104)** afforded the title compound in 88% yield. Data as above except for:

$[\alpha]_D^{20}$: -4.1° (c=2, CH_3OH) [lit.[197] $[\alpha]_D^{20}$: -3.3° (c=2, CH_3OH)]

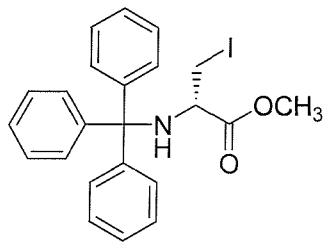
(2*R*)-*N*-Triphenylmethyl serine methyl ester (154)



Repetition of the methodology used to synthesise **(126)** afforded the title compound in 73% yield. Data as above except for:

$[\alpha]_D^{20}$: -33.2° (c=1, CH_3OH)

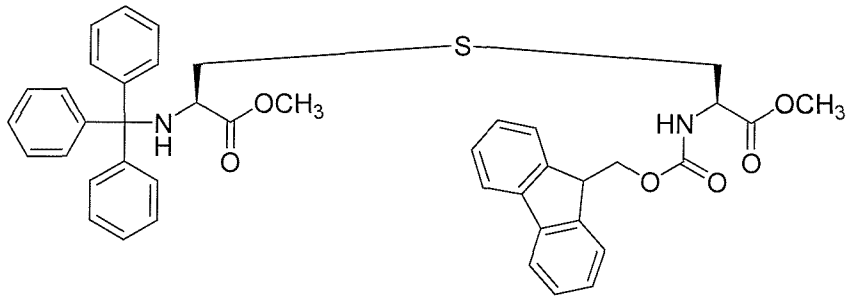
(2*S*)-*N*-Triphenylmethyl-3-iodoalanine methyl ester (155)



Repetition of the methodology used to synthesise **(138)** afforded the title compound in 85% yield. Data as above except for:

$[\alpha]_D^{20}$: -21.0° (c=1, $CHCl_3$)

(2*R*, 6*R*)-N^2-Triphenylmethyl-N^6-(9*H*-fluorenylmethoxycarbonyl)-O^1,O^7-dimethyl lanthionine (129)



The title compound was prepared by using a modification of the Dugave and Menez procedure.[175] Thus, (2*R*)-*N*-triphenylmethyl-3-iodoalanine methyl ester (1.70 g, 3.61 mmol) and (2*R*)-*N*-(9*H*-fluorenylmethoxycarbonyl) cysteine methyl ester (1.29 g, 3.61 mmol, 1 eq) were dissolved in dry DMF (20 mL). Cesium carbonate (1.18 g, 3.61 mmol, 1 eq) was added and the reaction mixture stirred under nitrogen in the dark for 4 h. 10% Citric acid solution (100 mL) was added and the solution extracted with ether (2 x 50 mL). The combined organics were washed with brine, dried over

$MgSO_4$ and solvent removed *in vacuo*. The residue was purified by column chromatography to afford the title compound as a white foam (1.80 g, 71%).

R_f (ethyl acetate:hexane, 3:17 v/v): 0.12

LRMS (ESI+) m/z: 243 $(Trt)^+$; 701 $(M + H)^+$; 723 $(M + Na)^+$; 739 $(M + K)^+$

HRMS (FT-MS) Calc'd for $C_{42}H_{41}O_6N_2S$ 701.2680. Found 701.2682.

δ_H (400 MHz; $CDCl_3$): 2.27 (br s, 1H, Trt NH); 2.40 – 3.01 (m, 4H, CH_2SCH_2); 3.14 (s, 3H, $TrtNHCH(R)CO_2CH_3$); 3.39 – 3.51 (m, 1H, CH_α); 3.81 (s, 3H, $FmocNHCH(R)CO_2CH_3$); 4.15 (t, J = 7 Hz, 1H, Fmoc H-9); 4.21 – 4.43 (m, 2H, Fmoc CH_2O); 4.52 (m, 1H, $CH_{\alpha'}$); 5.70 (d, J = 7 Hz, 1H, Fmoc NH); 7.24 (t, J = 7 Hz, 3H, Trt Ar *p*-H); 6.95 – 7.51 (m, 19H, Trt Ar H + Fmoc H); 7.68 (br d, J = 7 Hz, 2H, Fmoc H-1 + H-8); 7.83 (d, J = 7 Hz, 2H, Fmoc H-4 + H-5)

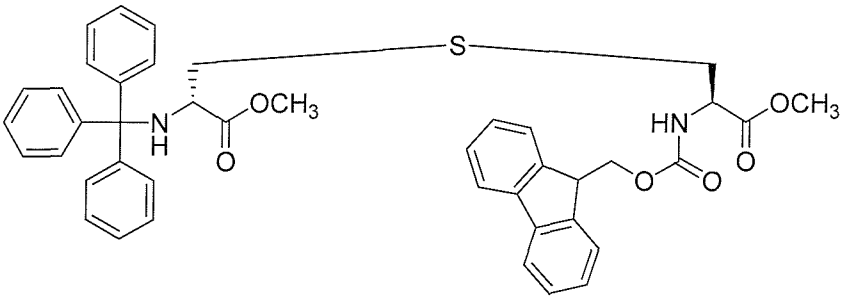
δ_C (100 MHz; $CDCl_3$): 34.5 (CH_2SCH_2); 35.8 (CH_2SCH_2); 47.5 (Fmoc CH); 52.3 (CO_2CH_3); 53.2 (CO_2CH_3); 54.2 (CH_α); 56.9 ($CH_{\alpha'}$); 67.6 (Fmoc CH_2); 71.6 (Ph_3C); 120.4, 125.6, 127.0, 127.5, 128.2, 128.4, 129.2 (Fmoc Ar CH + Trt Ar CH); 141.7, 144.2 (Fmoc Ar-C); 146.0 (Trt *ipso*-Ar-C); 156.2 (Fmoc OCONH); 171.4 (CO_2CH_3); 174.3 (CO_2CH_3)

IR (neat) ν/cm^{-1}: 1722 (br; s); 1493 (m); 1446 (m); 1205 (br; s); 1028 (br; m); 739 (s); 704 (s)

UV (DCM) λ_{max}: 230, 268, 302 nm

$[\alpha]_D^{20}$: +48.8° (c=0.5, ethyl acetate)

(2*S*, 6*R*)-N^2-Triphenylmethyl-N^6-(9*H*-fluorenylmethoxycarbonyl)-O^1,O^7-dimethyl lanthionine (156)



R_f (ethyl acetate:hexane, 3:17 v/v): 0.14

LRMS (ESI+) m/z: 243 $(Trt)^+$; 701 $(M + H)^+$; 723 $(M + Na)^+$; 739 $(M + K)^+$

δ_H (400 MHz; $CDCl_3$): 1.95 (br s, 1H, Trt NH); 2.80 (dd, J = 8, 13 Hz, 1H, $CH_\beta H_{\beta'}S$); 2.94 – 3.03 (m, 2H, $CH_\beta H_{\beta'}S$ + $SCH_\beta H_{\beta'}$); 3.09 (dd, J = 5, 10 Hz, 1H, $SCH_\beta H_{\beta'}$); 3.30 (s, 3H, $TrtNHCH(R)CO_2CH_3$); 3.59 (dd, J = 5, 7 Hz, 1H, CH_α); 3.81 (s, 3H, $FmocNHCH(R)CO_2CH_3$); 4.30 (t, J = 7 Hz, 1H, Fmoc H-9); 4.48 (d, J = 7 Hz, 2H, Fmoc CH_2O); 4.69 (dd, J = 5, 13 Hz, 1H, $CH_{\alpha'}$); 5.74 (d, J = 8 Hz, 1H, Fmoc NH); 7.24 (t, J = 7 Hz, 3H, Trt Ar *p*-H); 7.32 (dd, J = 7, 7 Hz, 6H, Trt Ar *m*-H); 7.35 – 7.40 (m, 2H, Fmoc H-2 + H-7); 7.46 (dd, J = 7, 7 Hz, 2H, Fmoc H-3 + H-6); 7.56 (d, J = 7 Hz, 6H, Trt Ar *o*-H); 7.68 (br d, J = 7 Hz, 2H, Fmoc H-1 + H-8); 7.83 (d, J = 7 Hz, 2H, Fmoc H-4 + H-5)

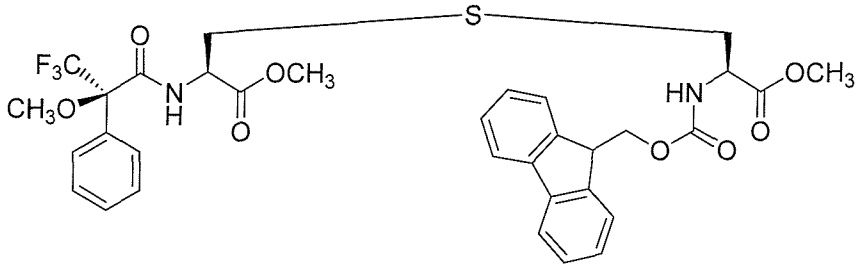
δ_C (100 MHz; $CDCl_3$): 34.2 (CH_2SCH_2); 35.0 (CH_2SCH_2); 47.2 (Fmoc CH); 52.0 (CO_2CH_3); 52.6 (CO_2CH_3); 53.7 (CH_α); 56.3 ($CH_{\alpha'}$); 67.4 (Fmoc CH_2); 71.3 (Ph_3C); 120.1, 125.3, 126.7, 127.2, 127.9, 128.1, 128.9 (Fmoc Ar CH + Trt Ar CH); 141.4, 143.9 (Fmoc Ar-C); 145.7 (Trt *ipso*-Ar-C); 155.9 (Fmoc OCONH); 171.2 (CO_2CH_3); 174.1 (CO_2CH_3)

IR (DCM) ν/cm^{-1}: 1721 (br; s); 1495 (m); 1444 (m); 1205 (br; s); 1027 (br; m); 738 (s); 699 (s)

UV (DCM) λ_{max}: 230, 268, 302 nm

$[\alpha]_D^{20}$: -51.2° (c=0.5, ethyl acetate)

(2*R*, 6*R*)-N^2-[(2*R*)-(2-Methoxy-2-phenyl-3,3,3-trifluoropropionoyl]-N^6-(9*H*-fluorenylmethoxycarbonyl)-O^1,O^7-dimethyl lanthionine (161)



The title compound was prepared by using a modification of the Mosher procedure.[208] Thus, (2*R*, 6*R*)- N^6-(9*H*-fluorenylmethoxycarbonyl)-O^1,O^7-dimethyl lanthionine (50 mg, 109 μmol) in DMF (5 mL) was treated with (2*R*)-(2-methoxy-2-phenyl-3,3,3-trifluoroacetyl chloride 36 mg, 141 μmol) and DIPEA (57 μL, 328 μmol). The reaction mixture was stirred overnight at r.t. and ethyl acetate (15 mL) added. The solution was extracted with 10% citric acid (3 x 15mL), 10% $NaHCO_3$ (3 x 15 mL), brine (2 x 15 mL) and dried over $MgSO_4$. Solvent was removed *in vacuo* to afford a white foam (41 mg).

R_f (ethyl acetate:hexane, 7:13 v/v): 0.27

LRMS (ESI+) m/z: 675 $(M + H)^+$; 697 $(M + Na)^+$; 713 $(M + K)^+$; 1371 $(2M + H)^+$

HRMS (FT-MS) Calc'd for $C_{33}H_{34}F_3O_8N_2S$ 675.1988. Found 675.1998.

δ_H (300 MHz; $CDCl_3$): 2.79 – 3.21 (m, 4H, $C\underline{H}_2S$ + $SC\underline{H}_2$); 3.52 (br s, 3H, $OC\underline{H}_3$); 3.75 (s, 3H, FmocNHCH(R)$CO_2C\underline{H}_3$); 3.77 (s, 3H, CONHCH(R)$CO_2C\underline{H}_3$); 4.24 (t, J = 7 Hz, 1H, Fmoc $\underline{H}$-9); 4.46 (d, J = 7 Hz, 2H, Fmoc $C\underline{H}_2O$); 4.51 (dd, J = 5, 13 Hz, 1H, $C\underline{H}_\alpha$); 4.89 (dd, J = 6, 12 Hz, 1H, $C\underline{H}_{\alpha'}$); 5.60 (d, J = 8 Hz, 1H, Fmoc N$\underline{H}$); 7.28 – 7.70 (m, 11H, $C_6\underline{H}_5$ + Fmoc Ar$\underline{H}$); 7.78 (d, J = 7 Hz, 2H, Fmoc $\underline{H}$-4 + $\underline{H}$-5)

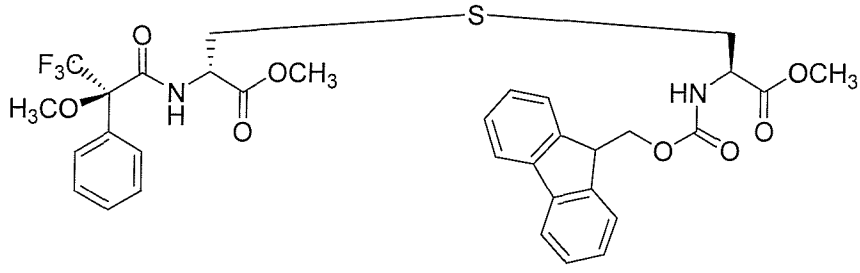
δ_C (75 MHz; $CDCl_3$): 34.9 ($\underline{C}H_2SCH_2$); 35.0 ($CH_2S\underline{C}H_2$); 47.2 (Fmoc $\underline{C}$H); 52.0 ($\underline{C}H_\alpha$); 53.0 ($CO_2\underline{C}H_3$); 53.1 ($CO_2\underline{C}H_3$); 53.8 ($\underline{C}H_{\alpha'}$); 55.4 ($O\underline{C}H_3$); 67.3 (Fmoc $\underline{C}H_2$); 77.4 ($\underline{C}F_3$); 120.2, 125.2, 127.3, 127.8, 127.9, 128.7, 129.8, 132.6 (Ph $\underline{C}$H + Fmoc Ar $\underline{C}$H); 155.8 (Fmoc O$\underline{C}$ONH); 166.7 ($\underline{C}$ONH); 170.5 ($\underline{C}O_2CH_3$); 171.0 ($\underline{C}O_2CH_3$)

δ_F (MHz; $CDCl_3$): 8.65 (s, 3F, $C\underline{F}_3$)

IR (neat) ν/cm^{-1}: 3346 (br, w); 1742 (s); 1721 (s); 1692 (s); 1511 (s); 1448 (m); 1440 (m); 1342(m); 1317 (w); 1264 (m); 1213 (s); 1164 (s); 1105 (m); 911 (m); 734 (s); 697 (m)

UV (DCM) λ_{max}: 268nm

(2*S*, 6*R*)-N^2-[(2*R*)-(2-Methoxy-2-phenyl-3,3,3-trifluoropropionoyl]-N^6-(9*H*-fluorenylmethoxycarbonyl)-O^1,O^7-dimethyl lanthionine (162)



The title compound was prepared by using a modification of the Mosher procedure.[209] Thus, (2*S*, 6*R*)- N^6-(9*H*-fluorenylmethoxycarbonyl)-O^1,O^7-dimethyl lanthionine (50 mg, 109 μmol) in DMF (5 mL) was treated with (2*R*)-(2-methoxy-2-phenyl-3,3,3-trifluoroacetyl chloride 36 mg, 141 μmol) and DIPEA (57 μL, 328 μmol). The reaction mixture was stirred overnight at r.t. and ethyl acetate (15 mL) added. The solution was extracted with 10% citric acid (3 x 15mL), 10% $NaHCO_3$ (3 x 15 mL), brine (2 x 15 mL) and dried over $MgSO_4$. Solvent was removed *in vacuo* to afford a white foam (33 mg).

R_f (ethyl acetate:hexane, 7:13 v/v): 0.27

LRMS (ESI+) m/z: 675 $(M + H)^+$; 697 $(M + Na)^+$; 713 $(M + K)^+$; 1371 $(2M + H)^+$

HRMS (FAB) Calc'd for $C_{33}H_{34}F_3O_8N_2S$ 675.1988. Found 675.1988.

δ_H (300 MHz; $CDCl_3$): 2.98 – 3.21 (m, 4H, $C\underline{H}_2S$ + $SC\underline{H}_2$); 3.43 (br s, 3H, $OC\underline{H}_3$); 3.75 (s, 3H, FmocNHCH(R)$CO_2C\underline{H}_3$); 3.80 (s, 3H, CONHCH(R)$CO_2C\underline{H}_3$); 4.25 (t, *J*

= 7 Hz, 1H, Fmoc H-9); 4.42 (d, J = 7 Hz, 2H, Fmoc CH_2O); 4.64 (dd, J = 4, 7 Hz, 1H, CH_α); 4.86 (dd, J = 7, 13 Hz, 1H, $CH_{\alpha'}$); 5.82 (d, J = 8 Hz, 1H, Fmoc NH); 7.28 – 7.71 (m, 11H, C_6H_5 + Fmoc ArH); 7.78 (d, J = 7 Hz, 2H, Fmoc H-4 + H-5)

δ_C (75 MHz; $CDCl_3$): 34.9 (CH_2SCH_2); 35.0 (CH_2SCH_2); 47.2 (Fmoc CH); 52.0 (CH_α); 53.0 (CO_2CH_3); 53.1 (CO_2CH_3); 53.8 ($CH_{\alpha'}$); 55.4 (OCH_3); 67.3 (Fmoc CH_2); 77.4 (CF_3); 120.2, 125.2, 127.3, 127.8, 127.9, 128.7, 129.8, 132.6 (Ph CH + Fmoc Ar CH); 155.8 (Fmoc OCONH); 166.7 (CONH); 170.5 (CO_2CH_3); 171.0 (CO_2CH_3)

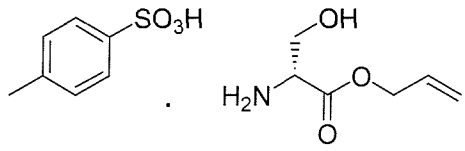
δ_F (MHz; $CDCl_3$): 8.38 (s, 3F, CF_3)

IR (neat) ν/cm^{-1}: 3346 (br, w); 1742 (s); 1721 (s); 1692 (s); 1511 (s); 1448 (m); 1440 (m); 1342(m); 1317 (w); 1264 (m); 1213 (s); 1164 (s); 1105 (m); 911 (m); 734 (s); 697 (m)

UV (DCM) λ_{max}: 268nm

4.3 Experimental to Chapter 3

(2*R*)-Serine allyl ester hydrotoluenesulfonate[184] (178)



The title compound was prepared by the method of Kunz and Waldmann.[184] To a suspension of (2*R*)-serine (10.50 g, 0.10 mol) in benzene (200 mL) was added allyl alcohol (58.00 g, 68.17 mL, 10 eq) and *para*-toluenesulfonic acid monohydrate (22.83 g, 0.12 mmol, 1.2 eq). The solution was refluxed under Dean-Stark conditions for 3 days, and solvent removed *in vacuo* to give the crude title compound as a red-brown oil (33.70 g), which was used without further purification.

LRMS (ESI+) m/z: 146 $(M + H)^+$; 187 $(M + CH_3CN + H)^+$

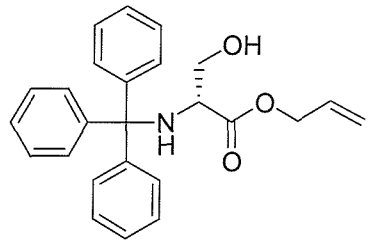
δ_H (300 MHz; $CDCl_3$): 2.32 (s, 3H, TsOH C<u>H</u>$_3$); 3.89 (dd, J = 4, 17 Hz, 1H, C<u>H</u>$_\beta$); 4.01 (dd, J = 2, 17 Hz, 1H, C<u>H</u>$_\beta$); 4.07 – 4.20 (br m, 1H, C<u>H</u>$_\alpha$); 4.51 (dd, J = 5, 5 Hz, 2H, COOC<u>H</u>$_2$); 5.13 (d, J = 10 Hz, allyl $CH_2CHC\underline{H}H'$); 5.19 (d, J = 17 Hz, allyl $CH_2CHCH\underline{H}'$); 5.29 – 5.40 (br m, 1H, O<u>H</u>); 5.73 (ddt, J = 6, 11, 16 Hz, allyl $CH_2C\underline{H}CHH'$); 7.07 (d, J = 7 Hz, TsOH Ar<u>H</u>); 7.68 (d, J = 7 Hz, TsOH Ar<u>H</u>); 8.00 (br d, J = 4 Hz, 3H, N<u>H</u>$_3^+$) [in accordance with lit.[184]]

δ_C (75 MHz; $CDCl_3$): 21.5 (TsOH <u>C</u>H$_3$); 55.5 (<u>C</u>H$_\alpha$); 59.9 (<u>C</u>H$_2$OH); 67.1 (COO<u>C</u>H$_2$); 119.1 (allyl $CO_2CH_2CH=\underline{C}H_2$); 126.1, 129.1, 131.2 (Ar <u>C</u>H); 140.8 (allyl $CO_2CH_2\underline{C}H=CH_2$); 140.9 (*ipso* Ar-<u>C</u>); 167.7 (<u>C</u>O$_2$CH$_2$)

IR (neat) ν/cm^{-1}: 1747 (s); 1513 (w); 1121 (s); 1032 (s); 1007 (s); 815 (m); 752 (w); 681 (s)

UV (DCM) λ_{max}: 252 nm

(2*R*)-*N*-Triphenylmethyl serine allyl ester (179)



The title compound was prepared by using a modification of the Baldwin procedure.[159] (2*R*)-Serine allyl ester hydrotoluenesulfonate (5.60 g, 16.7 mmol) was dissolved in dry DCM (100 mL) with stirring and cooled to 0 °C. Triethylamine (3.38 g, 4.66 mL, 2 eq) was added dropwise, followed by triphenylmethyl chloride (5.12 g, 18.4 mmol, 1.1 eq) in DCM (50 mL). The solution was stirred at 0 °C overnight and filtered. The filtrate was washed with 1M citric acid (2 x 100 mL), water (2 x100 mL), dried over $MgSO_4$ and solvent removed *in vacuo*. The residue was purified by flash column chromatography (SiO_2, DCM) to afford the title compound as a clear oil (4.39 g, 68%).

R_f (DCM): 0.20

LRMS (ESI+) m/z: 243 (Trt^+); 388 $(M + H)^+$; 410 $(M + Na)^+$

HRMS (FAB) Calc'd for $C_{25}H_{26}O_3N$ 388.1913. Found 388.1907.

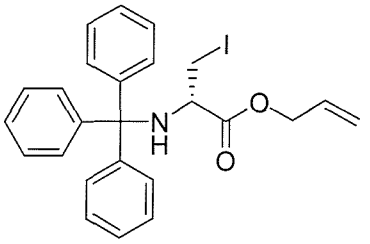
δ_H (400 MHz; $CDCl_3$): 2.42 (br s, 1H, O<u>H</u> or N<u>H</u>); 3.07 (br s, 1H, O<u>H</u> or N<u>H</u>); 3.62 (dd, J = 5, 11 Hz, 2H, C<u>H</u>$_2$OH); 3.77 (dd, J = 7, 13 Hz, 2H, C<u>H</u>$_\alpha$); 4.14 (dd, J = 6, 13 Hz, 1H, allyl C<u>H</u>H'CH=CH$_2$); 4.26 (dd, J = 4, 13 Hz, 1H, allyl CH<u>H</u>'CH=CH$_2$); 5.23 (dd, J = 1, 11 Hz, 1H, allyl CH$_2$CH=C<u>H</u>H'); 5.24 (dd, J = 2, 17 Hz, 1H, allyl CH$_2$CH=CH<u>H</u>'); 5.76 (ddt, J = 6, 12, 17 Hz, 1H, allyl CH$_2$C<u>H</u>=CHH'); 7.21 – 7.43 (m, 9H, Trt Ar<u>H</u>); 7.45 – 7.62 (m, 6H, Trt Ar<u>H</u>)

δ_C (100 MHz; $CDCl_3$): 58.2 (<u>C</u>H$_\alpha$); 65.4 (<u>C</u>H$_2$OH or allyl <u>C</u>H$_2$CH=CH$_2$); 66.1 (<u>C</u>H$_2$OH or allyl <u>C</u>H$_2$CH=CH$_2$); 71.4 (Ph$_3$<u>C</u>); 119.0 (allyl CO$_2$CH$_2$CH=<u>C</u>H$_2$); 127.1, 128.4, 129.1 (Ar<u>C</u>H); 132.0 (allyl CO$_2$CH$_2$<u>C</u>H=CH$_2$); 146.0 (*ipso* Ar-<u>C</u>); 173.6 (<u>C</u>O$_2$CH$_2$)

IR (neat) ν/cm^{-1}: 3056 (br; w); 1731 (s); 1489 (w); 1447 (w); 1169 (s); 1154 (s); 984 (m); 933 (m); 745 (s); 697 (s);

UV (DCM) λ_{max}: 234 nm

(2*S*)-*N*-Triphenylmethyl-3-iodoalanine allyl ester (176)



The title compound was prepared by using a modification of the Garegg procedure.[168] (2*S*)-*N*-Triphenylmethyl serine allyl ester (1.19 g, 3.07 mmol), triphenylphosphine (0.80 g, 3.07 mmol, 1 eq) and imidazole (0.29 g, 3.07 mmol, 1 eq) were dissolved in dry DCM (50 mL) with stirring. The solution was cooled on to 0 °C and stirring continued for 5 min. Iodine (0.78 g, 3.07 mmol, 1 eq) was added portionwise over 2 min and stirring continued at r.t for 2.5 h in the dark. Solvent was removed *in vacuo* at r.t. and the residue was purified by flash column chromatography (SiO_2, ether-hexane, 1:15 v/v) to afford the title compound as a clear photosensitive gum (1.28 g, 84%).

R_f (ether:hexane, 1:15 v/v): 0.30

LRMS (ESI+) m/z: 243 $(Trt)^+$; 498 $(M + H)^+$

HRMS (FAB) Calc'd for $C_{25}H_{25}O_2NI$ 498.0930. Found 498.0925

δ_H (300 MHz; $CDCl_3$; rotamers 2:1; * denotes minor rotamer): 2.25 – 2.49* (br s) + 2.93 (d, J = 10 Hz) (1H, TrtNH); 2.54 – 2.65* (m) + 3.25 (dd, J = 7, 10 Hz) (1H, H_β); 2.70 – 2.82* (m) + 3.36 (dd, J = 4, 10 Hz) (1H, $H_{\beta'}$); 3.50 – 3.58 (m) + 4.45* (dd, J = 7, 9 Hz) (1H, H_α); 4.12 (dddd, J = 1, 1, 6, 13 Hz) + 4.27 (dddd, J = 1, 1, 6, 13 Hz) + 4.68* (ddd, J = 1, 1, 6 Hz) (2H, allyl $CH_2CH{=}CH_2$); 5.20 (dd, J = 1, 10 Hz) + 5.32* (dd, J = 1, 10 Hz) (1H, allyl $CH_2CH{=}CHH'$); 5.24 (dd, J = 1, 18) + 5.42* (dd, J = 1,

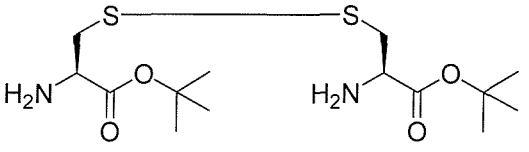
18) (1H, allyl $CH_2CH{=}CH\underline{H}$'); 5.77 (dddd, J = 6, 6, 10, 18 Hz) + 5.97* (dddd, J = 6, 6, 10, 18 Hz) (1H, allyl $CH_2C\underline{H}{=}CH_2$); 7.18 – 7.25 (m, 3H, Trt Ar$\underline{H}$); 7.26 – 7.36 (m, 6H, Trt Ar$\underline{H}$); 7.45 – 7.57 (m, 6H, Trt Ar$\underline{H}$)

δ_C (75 MHz; $CDCl_3$; * denotes minor rotamer): 10.1 + 48.6* ($\underline{C}H_\beta$); 20.6* + 56.3 ($\underline{C}H_\alpha$); 66.2 + 66.5* (allyl $CO_2\underline{C}H_2CH{=}CH_2$); 71.0* + 71.3 ($Ph_3\underline{C}NH$); 118.9 + 119.1* (allyl $CO_2CH_2CH{=}\underline{C}H_2$); 126.7, 126.8, 128.2, 128.6, 128.8 (Ar $\underline{C}H$); 131.5* + 131.8 (allyl $CO_2CH_2\underline{C}H{=}CH_2$); 145.7* + 145.8 (*ipso* Ar-$\underline{C}$); 170.7* + 172.1 ($\underline{C}O_2CH_2$)

IR (neat) ν/cm^{-1}: 3039 (br, w); 1730 (s); 1596 (w); 1490 (m); 1448 (m); 1415 (w); 1364 (w); 1323 (w); 1272 (w); 1206 (m); 1171 (s); 1115 (w); 1031 (w); 985 (w); 935 (w) 901 (w); 774 (w); 747 (m); 705 (s)

UV (DCM) λ_{max}: 234 nm

(2*R*, 7*R*)-Cystine di-*tert*-butyl ester[185] (180)



The title compound was synthesised by the method of Amaral *et al.*[185] Thus (2*R*, 7*R*)-cystine (1.92 g, 8.0 mmol) was dissolved in 60% perchloric acid (5.88 g, 35.2 mmol, 4.4 eq) with stirring. To the solution was added *tert*-butyl acetate (50 mL) and the mixture stirred for 2 h to give a homogeneous solution. The reaction mixture was maintained at r.t. for a further 48 h during which time a white solid crystallised out. The solution was cooled to 0 °C for 24 h and filtered. The white solid was dissolved in ether (50 mL) and 1M $NaHCO_3$ (25 mL) and the organic layer washed with 1M $NaHCO_3$ (2 x 25 mL) and saturated sodium chloride solution (3 x 25 mL). The organic phase was dried over $MgSO_4$ and reduced *in vacuo* to furnish the desired compound as a chromatagraphically homogeneous oil (1.81 g, 64%).

LRMS (ESI+) m/z: 353 $(M + H)^+$; 375 $(M + Na)^+$; 393 $(M + CH_3CN + H)^+$; 705 $(2M + H)^+$

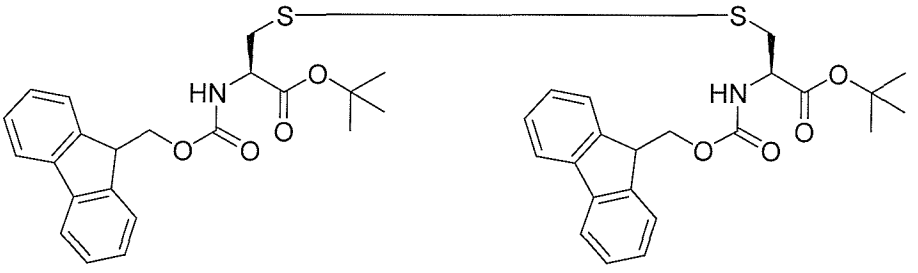
δ_H (300 MHz; $CDCl_3$): 1.50 (s, 18H, $C(C\underline{H}_3)_3$); 1.71 (s, 4H, N$\underline{H}$); 2.85 (dd, J = 7, 14 Hz, 2H, $\underline{H}_\beta$); 3.12 (dd, J = 4, 14 Hz, 2H, $\underline{H}_{\beta'}$); 3.67 (dd, J = 4, 7 Hz, 2H, $\underline{H}_\alpha$)

δ_C (75 MHz; $CDCl_3$): 28.2 ($C(\underline{C}H_3)_3$); 44.2 ($\underline{C}H_\beta H_{\beta'}$); 54.4 ($\underline{C}H_\alpha$); 81.2 ($\underline{C}(CH_3)_3$); 173.0 ($\underline{C}O_2{}^t$Bu)

IR (neat) ν/cm^{-1}: 2977 (w); 1725 (s); 1478 (w); 1459 (w); 1393 (w); 1368 (m); 1251 (m); 1152 (s); 991 (w); 845 (w); 752 (w)

$[\alpha]_D^{20}$: -7.8° (c=2, CH_3OH)

(2*R*, 7*R*)-*N*, *N*-Bis-(9*H*-fluorenylmethoxycarbonyl) cystine di-*tert*-butyl ester[186] (181)



The title compound was prepared according to the procedure of Jung *et* al.[186] Thus, *N*-(9*H*-fluorenylmethoxycarbonyloxy)succinimide (5.40 g, 16.0 mmol, 0.8 eq) and (2*R*, 7*R*)-cystine di-*tert*-butyl ester (3.52 g, 10.0 mmol) were dissolved in THF (10 mL). A solution of *N*-methylmorpholine (2.02 g, 2.20 mL, 20.0 mmol, 1.0 eq) in THF (5 mL) was added dropwise and the solution stirred at r.t. for 3 h. Solvent was removed *in vacuo* and the residue taken up in ethyl acetate (100 mL). The solution was washed with 2M $KHSO_4$ (3 x 100 mL) and water (2 x 100 mL), dried over $MgSO_4$ and reduced *in vacuo*. Further purification by flash column chromatography (SiO_2, chloroform) afforded the requisite compound as a white foam (6.11 g, 77%).

R_f ($CHCl_3$): 0.15

M. Pt.: 150 - 152 °C [lit.[187] 151.5 – 152 °C]

LRMS (ESI+) m/z: 797 $(M + H)^+$; 814 $(M + NH_4)^+$; 819 $(M + Na)^+$; 835 $(M + K)^+$

δ_H (300 MHz; $CDCl_3$): 1.50 (s, 18H, $C(C\underline{H}_3)_3$); 3.18 (dd, J = 6, 14 Hz, 2H, $\underline{H}_\beta$); 3.26 (dd, J = 5, 14 Hz, 2H, $\underline{H}_{\beta'}$); 4.22 (t, J = 7 Hz, 2H, Fmoc <u>H</u>-9); 4.46 (d, J = 7 Hz, 4H, Fmoc $C\underline{H}_2O$); 5.78 (d, J = 7, 2H, FmocN<u>H</u>); 7.30 (dd, J = 7, 7 Hz, 4H, Fmoc <u>H</u>-2 + <u>H</u>-7); 7.40 (dd, J = 7, 7 Hz, 4H, Fmoc <u>H</u>-3 + <u>H</u>-6); 7.60 (d, J = 7 Hz, 4H, Fmoc <u>H</u>-1 + <u>H</u>-8); 7.76 (d, J = 7 Hz, 4H, Fmoc <u>H</u>-4 + <u>H</u>-5) [in accordance with lit.[187]]

δ_C (75 MHz; $CDCl_3$): 28.2 ($C(\underline{C}H_3)_3$); 42.0 ($\underline{C}H_\beta H_{\beta'}$); 47.2 (Fmoc <u>C</u>H); 54.3 ($\underline{C}H_\alpha$); 67.3 (Fmoc $\underline{C}H_2$); 83.3 ($\underline{C}(CH_3)_3$); 120.1, 125.3, 127.2, 127.9 (Fmoc Ar <u>C</u>H); 141.4, 143.9 (Fmoc Ar-<u>C</u>); 155.9 (Fmoc O<u>C</u>ONH); 169.5 ($\underline{C}O_2{}^t$Bu) [in accordance with lit.[186]]

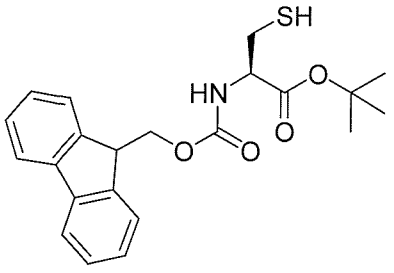
IR (neat) ν/cm^{-1}: 3341 (br, w); 2979 (br, w); 1708 (s); 1503 (m); 1449 (m); 1394 (w); 1368 (m); 1341 (m); 1221 (s); 1149 (s); 1045 (m); 842 (m); 756 (m); 736 (s)

UV (DCM) λ_{max}: 268 nm

HPLC (λ_{270}) R.T.: 23.5 min

$[\alpha]_D^{20}$: -5.1° (c=2, $CHCl_3$) [lit.[187] $[\alpha]_D^{23}$: -6.4° (c=0.56, $CHCl_3$)]

(2*R*)-*N*-(9*H*-Fluorenylmethoxycarbonyl) cysteine *tert*-butyl ester[186] (177)



The title compound was prepared by using a modification of the Kihlberg procedure.[188] To a solution of (2*R*, 7*R*)-*N*, *N*-bis-(9*H*-fluorenylmethoxycarbonyl) cystine di-*tert*-butyl ester (3.00 g, 3.9 mmol) in acetic acid (30 mL) was added zinc dust (2.53 g, 39.0 mmol, 10 eq) portionwise. The mixture was stirred under nitrogen

at r.t. overnight and the solvent removed *in vacuo*. 2M $KHSO_4$ (100 mL) was added to the residue and the mixture extracted with DCM (2 x 50 mL). The combined organics were washed with water (2 x 50 mL), dried over $MgSO_4$ and concentrated *in vacuo*. Purification by flash column chromatography (SiO_2, ether:hexane, 1:4 v/v) afforded the title compound as a white foam (2.64 g, 88%).

R_f (ether:hexane, 1:4 v/v): 0.35

LRMS (ESI+) m/z: 417 $(M + NH_4)^+$; 438 $(M + K)^+$; 799 $(2M + H)^+$; 816 $(2M + NH_4)^+$; 821 $(2M + Na)^+$; 837 $(2M + K)^+$

HRMS (FAB) Calc'd for $C_{22}H_{25}O_4NS$ 400.1583. Found 400.1584.

δ_H (400 MHz; $CDCl_3$): 1.26 (dd, J = 9, 9 Hz, 1H, $CH_2S\underline{H}$); 1.42 (s, 9H, $C(C\underline{H}_3)_3$); 2.86 – 2.98 (m, 2H, $C\underline{H}_\beta\underline{H}_{\beta'}$); 4.15 (t, J = 7 Hz, 1H, Fmoc H-9); 4.28 – 4.40 (m, 2H, Fmoc $C\underline{H}_2O$); 4.43 – 4.49 (m, 1H, $C\underline{H}_\alpha$); 5.61 (d, J = 7, 2H, FmocN$\underline{H}$); 7.24 (ddd, J = 8, 8, 2 Hz, 2H, Fmoc H-2 + H-7); 7.33 (dd, J = 8, 8 Hz, 2H, Fmoc H-3 + H-6); 7.53 (d, J = 8 Hz, 2H, Fmoc H-1 + H-8); 7.68 (d, J = 8 Hz, 2H, Fmoc H-4 + H-5)

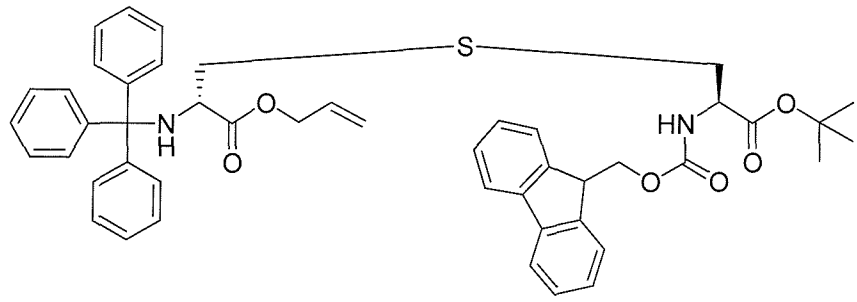
δ_C (100 MHz; $CDCl_3$): 27.2 ($\underline{C}H_\beta H_{\beta'}$); 28.4 ($C(\underline{C}H_3)_3$); 47.6 (Fmoc $\underline{C}$H); 55.9 ($\underline{C}H_\alpha$); 67.5 (Fmoc $\underline{C}H_2$); 83.5 ($\underline{C}(CH_3)_3$); 120.4, 125.5, 127.5, 128.2 (Fmoc Ar $\underline{C}$H); 141.8, 144.1, 144.3 (Fmoc Ar-$\underline{C}$); 156.1 (Fmoc O$\underline{C}$ONH); 169.4 ($\underline{C}O_2{}^t$Bu)

IR (neat) ν/cm^{-1}: 2978 (br, w); 2360 (br, w); 1721 (br, s); 1511 (m), 1345 (m); 1249 (w); 1220 (w); 1155 (s); 1060 (w); 913 (w); 844 (w); 740 (m)

UV (DCM) λ_{max}: 268 nm

HPLC (λ_{270}) R.T.: 20.0 min

(2*S*, 6*R*)-N^2-Triphenylmethyl-N^6-(9*H*-fluorenylmethoxycarbonyl)-O^1-allyl-O^7-*tert*-butyl lanthionine (175)



The title compound was prepared by using a modification of the Dugave and Menez procedure.[175] Thus, (2*S*)-*N*-triphenylmethyl-3-iodoalanine allyl ester (497 mg, 1 mmol, 1eq) and (2*R*)-*N*-(9*H*-fluorenylmethoxycarbonyl) cysteine *tert*-butyl ester **(142)** (399 mg, 1 mmol, 1 eq) were dissolved in dry DMF (10 mL). Cesium carbonate (326 mg, 1 mmol, 1 eq) was added and the reaction mixture stirred under nitrogen in the dark for 4 h. 10% Citric acid solution (100 mL) was added and the solution extracted with ether (2 x 50 mL). The combined organics were washed with brine, dried over $MgSO_4$ and solvent removed *in vacuo*. The residue was purified by column chromatography (SiO_2, ethyl acetate:hexane, 3:17 v:v) to afford the title compound as a white foam (568 mg, 74%) and also the aziridine **182** (59 mg, 16%).

R_f (ethyl acetate:hexane, 3:17 v:v): 0.13

LRMS (ESI+) m/z: 769 $(M + H)^+$; 791 $(M + Na)^+$; 807 $(M + K)^+$; 1537 $(2M + H)^+$

HRMS (FAB) Calc'd for $C_{47}H_{48}O_6N_2S$ 768.3233. Found 768.3311.

δ_H (300 MHz; $CDCl_3$): 1.49 (s) + 1.50 (s) (9H, $C(C\underline{H}_3)_3$); 2.25 (br s, 1H, N$\underline{H}$); 2.42 – 2.73 (m, 2H, $C\underline{H}_2SCH_2$); 2.83 – 3.19 (m, 2H, $CH_2SC\underline{H}_2$); 3.58 (ddd, J = 7, 7, 24 Hz, 1H, C$\underline{H}_\alpha$); 4.24 (dd, J = 7, 15 Hz, 1H, Fmoc $\underline{H}$-9); 4.32 – 4.44 (m, 2H, Fmoc $C\underline{H}_2O$); 4.48 – 4.60 (m, 1H, C$\underline{H}_{\alpha'}$); 4.68 (dd, J = 4, 6 Hz, 2H, allyl $CO_2C\underline{H}_2CH{=}CH_2$); 5.27 (dd, J = 1, 10 Hz, 1H, allyl $CO_2CH_2CH{=}C\underline{H}H'$); 5.36 (ddd, J = 1, 1, 18 Hz, 1H, allyl $CO_2CH_2CH{=}CH\underline{H}'$); 5.63 (dd, J = 8 Hz) + 5.74 (dd, J = 8 Hz) (1H, N$\underline{H}$); 5.95 (dddd,

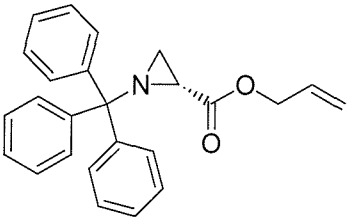
J = 3, 5, 11, 23 Hz, 1H, allyl $CO_2CH_2C\underline{H}$=CHH'); 7.14 – 7.83 (m, 23H, Fmoc Ar$C\underline{H}$ + Trt $C\underline{H}$)

δ_H (75 MHz; $CDCl_3$): 28.2 ($C(\underline{C}H_3)_3$); 34.1 + 34.6 ($\underline{C}H_2SCH_2$); 44.4 + 44.7 ($CH_2S\underline{C}H_2$); 47.3 (Fmoc $\underline{C}$H-9); 47.8 + 48.6 ($\underline{C}H_\alpha$); 54.1 + 54.4 ($\underline{C}H_{\alpha'}$); 66.1 + 66.2 (allyl $CO_2\underline{C}H_2CH=CH_2$); 67.4 (Fmoc $\underline{C}H_2O$); 80.0 ($\underline{C}(CH_3)_3$); 83.1 ($Ph_3\underline{C}$); 119.0 + 119.1 (allyl $CO_2CH_2CH=\underline{C}H_2$); 120.1, 125.3, 126.6, 127.3, 128.12, 128.7 (Fmoc Ar$C\underline{H}$ + Trt $C\underline{H}$); 131.8 + 131.9 (allyl $CO_2CH_2\underline{C}H=CH_2$); 141.4, 144.0, 145.8 (Fmoc Ar$C\underline{H}$ + Trt $C\underline{H}$); 155.9 (Fmoc O$\underline{C}$ONH);169.6, 171.6 ($\underline{C}O_2R$)

IR (neat) ν/cm^{-1}: 3308 (w), 2974 (w); 1720 (s); 1492 (w); 1448 (w); 1216 (m); 1148 (s); 1032 (m); 739 (s); 704 (s)

UV (DCM) λ_{max}: 230, 268, 300 nm

(2*S*)-*N*-Triphenylmethylaziridine-2-carboxylic acid allyl ester (182)



R_f (ethyl acetate:hexane, 3:17 v:v): 0.67

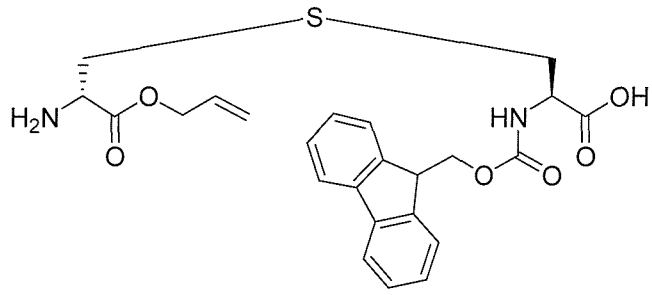
δ_H (400 MHz; $CDCl_3$): 1.27 (dd, J = 1, 6 Hz, 2H, TrtNC$\underline{H}_2$); 1.76 (dd, J = 3, 6 Hz, 1H, TrtNC$\underline{H}$); 2.12 (t, J = 2 Hz, 1H, N$\underline{H}$); 4.52 (d, J = 5 Hz, 2H, allyl $CO_2C\underline{H}_2CH=CH_2$); 5.10 (dd, J = 1, 11 Hz, 1H, allyl $CO_2CH_2CH=C\underline{H}$H'); 5.18 (dd, J = 1, 17 Hz, 1H, allyl $CO_2CH_2CH=CH\underline{H}$'); 5.79 (ddt, J = 5, 11, 17 Hz, allyl $CO_2CH_2C\underline{H}=CH_2$); 7.00 – 7.15 (m, 9H, Trt Ar$\underline{H}$); 7.26 – 7.38 (m, 6H, Trt Ar$\underline{H}$)

δ_C (75 MHz; $CDCl_3$): 28.9 (TrtN$\underline{C}H_2$); 31.9 (TrtN$\underline{C}$H); 65.7 (allyl $CO_2\underline{C}H_2CH=CH_2$); 74.6 ($Ph_3\underline{C}$); 118.7 (allyl $CO_2CH_2CH=\underline{C}H_2$); 127.1, 127.8, 129.5 (Fmoc Ar$\underline{C}$H); 132.1 (allyl $CO_2CH_2\underline{C}H=CH_2$); 143.8 (*ipso* Ar-$\underline{C}$); 171.3 ($\underline{C}O_2CH_2CH=CH_2$)

IR (neat) ν/cm^{-1}: 1740 (s); 1488 (m); 1446 (m); 1400 (w); 1275 (m); 1237 (m); 1176 (s); 1078 (w); 745 (s); 698 (s)

UV (DCM) λ_{max}: 230 nm

(2*S*, 6*R*)-N^6-(9*H*-Fluorenylmethoxycarbonyl)-O^1-allyl lanthionine (174)



(2*S*, 6*R*)-N^2-Triphenylmethyl-N^6-(9*H*-fluorenylmethoxycarbonyl)-O^1-allyl-O^7-*tert*-butyl lanthionine **(140)** (2.00g, 2.71 mmol) was stirred with TFA (4.9 mL), DCM (4.9 mL) and TIS (0.2 mL) for 1 h at r.t. Solvent was removed *in vacuo* and the residue purified by column chromatography (SiO_2, MeOH:$CHCl_3$, 1:9 v:v) to afford the title compound as a white solid (1.16 g, 91%).

LRMS (ESI+) m/z: 471 $(M + H)^+$; 493 $(M + Na)^+$

HRMS (FAB) Calc'd for $C_{24}H_{27}O_6N_2S$ 471.1590. Found 471.1571.

δ_H (400 MHz; $CDCl_3$): 2.71 – 3.40 (br m, 4H, $C\underline{H}_2SC\underline{H}_2$); 4.01 – 4.47 (br m, 5H, Fmoc C<u>H</u>-9 + Fmoc $OC\underline{H}_2$ + $C\underline{H}_\alpha$ + $C\underline{H}_{\alpha'}$); 4.50 – 4.71 (br m, 2H, allyl $CO_2C\underline{H}_2CH{=}CH_2$); 5.00 – 5.47 (br m, 2H, allyl $CO_2CH_2CH{=}C\underline{H}H'$ + allyl $CO_2CH_2CH{=}CH\underline{H}'$); 5.61 – 5.91 (br m, 1H, allyl $CO_2CH_2C\underline{H}{=}CH_2$); 6.13 – 6.35 (br s, 1H, N<u>H</u>); 7.10 – 7.84 (m, 8H, Fmoc Ar<u>H</u>)

δ_C (100 MHz; $CDCl_3$): 33.1 ($\underline{C}H_2SCH_2$); 36.2 ($CH_2S\underline{C}H_2$); 47.3 (Fmoc <u>C</u>H-9); 53.4 ($\underline{C}H_\alpha$); 54.7 ($\underline{C}H_{\alpha'}$); 67.1 + 67. 9 (allyl $CO_2\underline{C}H_2CH{=}CH_2$ + Fmoc $\underline{C}H_2O$); 120.3 (allyl $CO_2CH_2\underline{C}H{=}CH_2$); 120.6 (allyl $CO_2CH_2CH{=}\underline{C}H_2$); 125.5, 127.5, 128.2, 130.6 (Fmoc

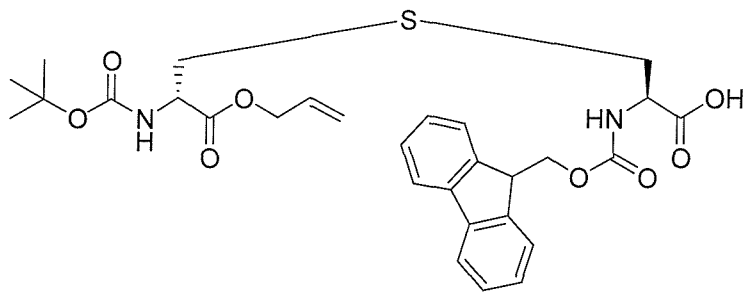
Ar CH); 141.6, 143.9 (Fmoc Ar C); 157.1 (Fmoc OCON); 167.9 (allyl $CO_2CH_2CH=CH_2$); 174.2 (CO_2H)

IR (neat) ν/cm^{-1}: 1673 (br; s); 1512 (br; m); 1181 (br; s); 1133 (br; s); 721 (br; m); 739 (br; m)

UV (DCM) λ_{max}: 268 nm

HPLC (λ_{270}) R.T.: 12.5 min

(2*S*, 6*R*)-N^2-*tert*-Butoxycarbonyl-N^6-(9*H*-fluorenylmethoxycarbonyl)-O^1-allyl lanthionine (183)



The title compound was prepared by using a modification of the procedure of Yamamoto *et al.*[210] To a solution of (2*S*, 6*R*)-N^6-(9*H*-fluorenylmethoxycarbonyl)-O^1-allyl lanthionine (1.35 g, 2.87 mmol, 1 eq) in dioxane at 0 °C was added di-*tert*-butyl dicarbonate (626 mg, 1 eq) and DIPEA (0.50 mL, 372 mg, 1 eq). The solution was stirred at r.t. for 3 h and solvent removed *in vacuo*. The residue was purified by column chromatography (SiO_2, methanol:chloroform, 1:19 v:v) to yield the title compound as a white solid (1.12 g, 68%)

LRMS (ESI+) m/z: 571 $(M + H)^+$; 593 $(M + Na)^+$; 609 $(M + Na)^+$; 1163 $(2M + Na)^+$

HRMS (FAB) Calc'd for $C_{29}H_{35}O_8N_2S$ 571.2114. Found 571.2090.

δ_H (400 MHz; $CDCl_3$): 1.38 (br s, 9H, $C(CH_3)_3$); 2.61 – 3.20 (br m, 4H, CH_2SCH_2); 3.81 – 4.63 (br m, 7H, Fmoc CH-9 + Fmoc OCH_2 + CH_α + $CH_{\alpha'}$ + allyl $CO_2CH_2CH=CH_2$); 4.89 – 5.31 (br m, 2H, allyl $CO_2CH_2CH=CHH'$ + allyl

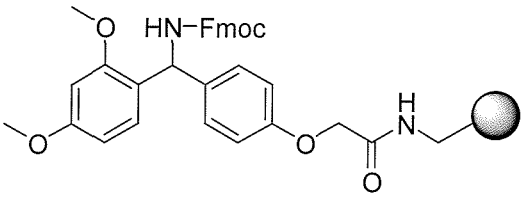
$CO_2CH_2CH{=}CH\underline{H}$'); 5.40 – 5.91 (br m, 1H, allyl $CO_2CH_2C\underline{H}{=}CH_2$); 7.00 – 7.71 (m, 8H, Fmoc Ar$\underline{H}$)

δ_C (75MHz; $CDCl_3$): 28.3 ($C(\underline{C}H_3)_3$); 35.1 ($\underline{C}H_2SCH_2$); 41.2 ($CH_2S\underline{C}H_2$); 47.1 (Fmoc $\underline{C}$H-9); 53.7 ($\underline{C}H_\alpha$); 56.3 ($\underline{C}H_{\alpha'}$); 66.3 + 67. 3 (allyl $CO_2\underline{C}H_2CH{=}CH_2$ + Fmoc $\underline{C}H_2O$); 80.4 ($\underline{C}(CH_3)_3$); 119.1 (allyl $CO_2CH_2\underline{C}H{=}CH_2$); 119.9 (allyl $CO_2CH_2CH{=}\underline{C}H_2$); 125.3, 127.2, 127.7, 131.5 (Fmoc Ar $\underline{C}$H); 141.2, 144.1 (Fmoc Ar $\underline{C}$); 156.1 (Fmoc O$\underline{C}$ON); 157.5 (Boc O$\underline{C}$ON); 171.3 (allyl $\underline{C}O_2CH_2CH{=}CH_2$); 177.2 ($CO_2H$)

UV (DCM) λ_{max}: 270 nm

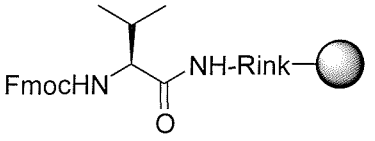
HPLC (λ_{270}) R.T.: 18.1 min

9*H*-Fluorenylmethoxycarbonyl -Rink Linker-Polystyrene Resin (184)



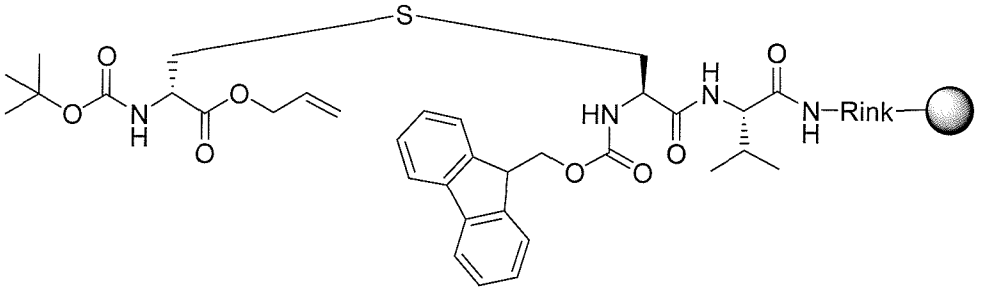
To a solution of HOBt (1.15 g, 7.5 mmol, 2 eq) and *p*-[(*R*, *S*)-α-[1-(9*H*-fluoren-9-yl)-methoxyformamido]-2,4-dimethoxybenzyl]-phenoxyacetic acid (4.05 g, 7.5 mmol, 2 eq) in DCM (9 mL) and DMF (1 mL) was added DIC (1.16 mL, 7.5 mmol, 1eq). The solution was stirred at r.t. for 5 min and added to aminomethyl polystyrene resin (3.00 g, 1.25 mmol/g, 3.75 mmol, 1 eq). The reaction mixture was shaken for 2 h at r.t. and filtered. The resin was washed with DMF (2 x 10 mL x 1 min), DCM (2 x 10 mL x 1 min) and ether (2 x 10 mL x 1 min). A small aliquot of resin gave a negative ninhydrin test. The resin was dried *in vacuo* to give 4.32 g (85%) of the title compound.

9*H*-Fluorenylmethoxycarbonyl-valinyl-Rink Linker-Polystyrene Resin (187)



Fmoc-Rink Linker-Polystyrene Resin (3.11 g, 3.75 mmol) was shaken with 20% piperidine in DMF (20mL) at r.t. for 5 min. The resin was filtered and again shaken with 20% piperidine in DMF at r.t for 15 min. The resin was filtered and washed with DMF (2 x 10 mL x 1 min), DCM (2 x 10 mL x 1 min) and ether (2 x 10 mL x 1 min). A small aliquot of resin gave a positive ninhydrin test. Fmoc-Val-OH (2.84 g, 7.5 mmol) and HOBt (1.15 g, 7.5 mmol) were dissolved in DCM (9 mL) and DMF (1 mL) with stirring at r.t. for 5 min. DIC (1.16 mL, 7.5 mmol) was added and stirring continued at r.t for a further 5 min. The coupling mixture was added to the resin and the reaction mixture shaken for 2 h. The resin was filtered and washed with DMF (2 x 10 mL x 1 min), DCM (2 x 10 mL x 1 min) and ether (2 x 10 mL x 1 min). A small aliquot of resin gave a negative ninhydrin test.

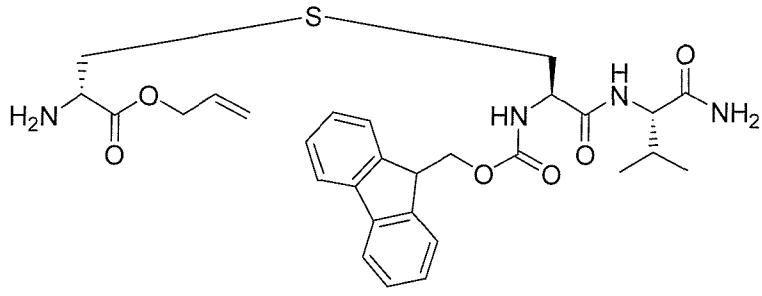
(2*S*, 6*R*)-N^2-*tert*-Butoxycarbonyl-N^6-(9*H*-fluorenylmethoxycarbonyl)-O^1-allyl lanthionyl-valinyl-Rink Linker-Polystyrene Resin (188)



Fmoc-Val-Rink Linker-Polystyrene Resin (1.20 g, 0.99 mmol) was shaken with 20% piperidine in DMF (20mL) at r.t. for 5 min. The resin was filtered and again shaken with 20% piperidine in DMF at r.t for 15 min. The resin was filtered and washed with

DMF (2 x 10 mL x 1 min), DCM (2 x 10 mL x 1 min) and ether (2 x 10 mL x 1 min). A small aliquot of resin gave a positive ninhydrin test. (2*S*, 6*R*)-N^2-*tert*-Butoxycarbonyl-N^6-(9*H*-fluorenylmethoxycarbonyl)-O^1-allyl lanthionine (1.13 g, 1. 98 mmol) and HOBt (0.30 g, 1.98 mmol) were dissolved in DCM (9 mL) and DMF (1 mL) with stirring at r.t. for 5 min. DIC (0.31 mL, 1.98 mmol) was added and stirring continued at r.t for a further 5 min. The coupling mixture was added to the resin and the reaction mixture shaken for 2 h. The resin was filtered and washed with DMF (2 x 10 mL x 1 min), DCM (2 x 10 mL x 1 min) and ether (2 x 10 mL x 1 min). A small aliquot of resin gave a negative ninhydrin test.

(2*S*, 6*R*)- N^6-(9*H*-Fluorenylmethoxycarbonyl)-O^1-allyl lanthionylvalinamide (189)



(2*S*, 6*R*)-N^2-*tert*-Butoxycarbonyl-N^6-(9*H*-fluorenylmethoxycarbonyl)-O^1-allyl lanthionyl-valinyl-Rink Linker-Polystyrene Resin (200mg) was shaken with TFA (1.9 mL) and TIS (0.1 mL) for 3 h at r.t. The mixture was filtered, and the resin was washed with TFA (0.5 mL). The organic fractions were pooled and reduced *in vacuo*. The residue was dissolved in TFA (0.2 mL) and cold ether (5 mL) added dropwise. The supernatant was decanted and the procedure repeated again. The resultant white solid was purified by column chromatography (SiO_2, methanol:chloroform, 1:19 v:v) to give the title compound as a white foam (15 mg).

LRMS (ESI+) m/z: 569 $(M + H)^+$; 591 $(M + Na)^+$

HRMS (FAB) Calc'd for $C_{29}H_{37}O_6N_4S$ 569.2434. Found 569.2447.

δ_H (400 MHz; CD_3OD): 0.87 (d, J = 6 Hz, 3H, Val $C\underline{H}_3$); 0.89 (d, J = 6 Hz, 3H, Val $C\underline{H}_3$); 1.91 – 2.10 (m, 1H, Val $C\underline{H}_\beta$); 2.71 – 2.91 (m, 4H, $C\underline{H}_2SC\underline{H}_2$); 3.60 – 3.71 (m, 1H, Lan $C\underline{H}_\alpha$); 4.09 – 4.17 (m, 2H, Val $C\underline{H}_\alpha$ + Fmoc C<u>H</u>-9); 4.21 – 4.38 (m, 3H, Lan $C\underline{H}_{\alpha'}$ + Fmoc $C\underline{H}_2O$); 4.59 (d, J = 5 Hz, 2H, allyl $CO_2C\underline{H}_2CH{=}CH_2$); 5.10- 5.32 (m, 2H, allyl $CO_2CH_2CH{=}C\underline{H}_2$); 5.78 – 5.92 (m, 1H, allyl $CO_2CH_2C\underline{H}{=}CH_2$); 7.14 – 7.73 (m, 8H, Fmoc Ar<u>H</u>)

δ_C (100 MHz; CD_3OD): 18.7 + 20.2 (2 x Val $\underline{C}H_3$); 32.3 (Val $\underline{C}H_\beta$); 36.0 + 37.5 ($\underline{C}H_2S\underline{C}H_2$); 48.7 (Fmoc C<u>H</u>-9); 55.2 + 56.5 + 60.1 (2 x Lan $\underline{C}H_\alpha$ + Val $\underline{C}H_\alpha$); 67.6 (allyl $CO_2\underline{C}H_2CH{=}CH_2$); 68.6 (Fmoc $\underline{C}H_2O$); 119.6 (allyl $CO_2CH_2CH{=}\underline{C}H_2$); 121.4 allyl $CO_2CH_2\underline{C}H{=}CH_2$); 126.6, 128.6, 129.2, 133.5 (Fmoc Ar <u>C</u>H); 142.9, 145.5 (Fmoc Ar <u>C</u>); 158.9 (Fmoc O<u>C</u>ON); 163.9, 173.3, 176.3 (2 x <u>C</u>ONH + $\underline{C}O_2R$)

IR (neat) ν/cm^{-1}: 3352 (br, w); 1732 (br s); 1727 (s); 1687 (s); 1521 (s); 1432 (m); 1324 (m); 1326 (w); 1254 (m); 1161 (s); 1105 (w); 914 (m); 704 (m); 697 (m)

HPLC (λ_{270}) R.T.: 10.3 min

UV (DCM) λ_{max}: 220, 270 nm

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