**SYNTHESIS OF LANTHIONINE-CONTAINING
PEPTIDES ON SOLID PHASE *VIA* AN ORTHOGONAL
PROTECTING GROUP STRATEGY**

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A thesis presented in partial fulfillment of the requirements for the
Doctor of Philosophy degree of the University of London

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*“Life is like a box of chocolate – you never know what
you’re gonna get”*

Tom Hanks as “Forest Gump”

Declaration

I, Mohd Firouz Mohd Mustapa, hereby state that the following is entirely my own work and has not been for any other degree or examination.

Mohd Firouz MOHD MUSTAPA

August 2002

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Abstract

Lanthionine (Lan), a monosulfide analogue of cystine, is a naturally occurring amino acid found in a group of polycyclic peptide antibiotics called lantibiotics. Nisin is so far the only lantibiotic that has been produced synthetically. Biological studies of these peptides are of interest, due to their activity against Gram-positive bacteria, especially in the search for new groups of antibiotics. With the difficulty in obtaining lantibiotics in respectable quantities for biological studies it was therefore the aim of this Ph.D. project to establish the fundamentals of the total synthesis of lantibiotics solely on solid phase, by investigating the possibility of a rapid and high yielding method for synthesizing smaller Lan-containing peptides.

Following literature procedures of the formation of Lan residues, the synthesis of Lan derivative **1** was attempted. Using a triply orthogonal protecting group strategy the syntheses of several Lan-containing cyclic peptides were attempted; only the 21-membered nisin ring C analogue **2** yielded enough material for extensive NMR studies (TOCSY, HSQC, HMQC, HMBC) to be carried out. The two major products were found to be diastereoisomers **6** and contained nor-Lan, a regioisomer of the desired product **1**. nor-Lan **3** was formed from an enantiomeric mixture of α -iodo- β -alanine derivatives **4**, originally identified in literature as a locked conformer of the desired β -iodo- α -alanine derivative **5**.

Despite the lack of success in terms of the target peptides and amino acids, it has been shown that the use of a differentially-protected double-headed amino acid monomer could be used in the synthesis of cyclic peptides on solid phase utilizing an orthogonal protecting group strategy.

CONTENTS

	Page
Abbreviations	11
List of Figures	15
 Chapter 1	
Introduction I	23
1.1. Lanthionine	23
1.2. Lantibiotics	24
1.3. Nisin	31
1.4. Synthetic lanthionine-containing peptides	32
1.5. Synthesis of lanthionine residues	34
1.5.1. Sulfur extrusion	34
1.5.2. Biomimetic approach	37
1.5.3. Serine β -lactones	38
1.5.4. β -Haloalanine derivatives	39
1.5.5. Ring opening of aziridine-2-carboxylates	41
 Chapter 2	
Introduction II	42
2.1. Solid phase peptide synthesis	42
2.2. Resins for Fmoc solid phase peptide synthesis	45
2.2.1. Cleavage conditions	46
2.2.1.1. Acid labile resins yielding peptide acids	46
2.2.1.2. Acid labile resins yielding peptide amides	47
2.2.1.3. Resins cleaved <i>via</i> nucleophilic displacement and photolysis	48
2.3. Coupling reagents and cyclisation reagents (<i>via</i> amide bond formation)	50
2.4. Orthogonal protecting groups	55
2.4.1. Allylic protecting groups	55
2.4.2. Dimedone-based protecting groups	60
2.5. Synthesis of cyclic and polycyclic peptides	67

2.5.1. Multiply-orthogonal protecting group strategies for SPPS	68
2.6. Synthesis of lanthionine-containing peptides	72
2.6.1. Sulfur extrusion	72
2.6.2. Biomimetic approach	73
2.6.3. Serine β -lactones	74
2.6.4. β -Haloalanine derivatives	76

Chapter 3

Introduction III	80
3.1. Nucleophilic ring opening of aziridine-2-carboxylates	80
3.2. Aims	92

Chapter 4

Results and Discussion I	97
4.1. Synthesis of protected cysteine 9	99
4.2. Synthesis of protected (<i>R</i>)-iodoalanine (<i>R</i>)- 6a	101
4.3. Synthesis of protected lanthionine (<i>R,R</i>)- 10	105
4.4 Synthesis of protected (<i>S</i>)-iodoalanine (<i>S</i>)- 6a	107
4.5. Synthesis of protected lanthionine (<i>S,R</i>)- 10	109
4.6. Synthesis of ivDde/Dmab protected (<i>S,R</i>)-lanthionine 2	111

Chapter 5

Results and Discussion II	116
5.1. Investigation of the conformational isomer issue	116
5.1.1. HPLC analyses	117
5.1.2. NMR analyses	119
5.1.3. Test reactions	128
5.1.4. Synthesis of (<i>R,R</i>) and (<i>S,R</i>)-lanthionine derivatives 14 and 15 (<i>via</i> the Mitsunobu reaction)	131

Chapter 6

Results and Discussion III	133
6.1. Synthesis of a lanthionine analogue of an enkephalin opioid peptide	133
6.2. Synthesis of a lanthionine analogue of ring C of nisin	143
6.3. Synthesis of an analogue of rings A and B of nisin	146
6.4. Further NMR analyses of peptide 25	149

Chapter 7

Experimental	153
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Appendix A	203
Appendix B	204
Appendix C	205
Appendix D	206
Appendix E	207
Appendix F	208
Appendix G	209
Appendix H	210
Appendix I	211
Appendix J	212
Appendix K	213
Appendix L	214
Appendix M	215
Appendix N	216
Appendix O	217
Appendix P	218
Appendix Q	219
Appendix R	220
Appendix S	221
Appendix T	222
Appendix U	223

Appendix V	224
Appendix W	225
Appendix X	226
Appendix X-a	227
Appendix Y	228
Appendix Z	229
 <i>References</i>	 230

ABBREVIATIONS

ADDP	: 1,1'-(azodicarbonyl)dipiperidine
All	: allyl
Ala	: alanine
Aloc	: allyloxycarbonyl
AOP	: (7-azabenzotriazol-1-yloxy)-tris-(dimethylamino)-phosphonium hexafluorophosphate
APCI	: atmospheric pressure chemical ionisation
Asn	: asparagine
Asp	: aspartic acid
Boc	: tertiary butoxycarbonyl
BOP	: (benzotriazol-1-yloxy)-tris-(dimethylamino)-phosphonium hexafluorophosphate
^t Bu	: tertiary butyl
Bz	: benzyl
Clz	: 2-chlorobenzyloxycarbonyl
COSY	: correlation spectroscopy
Cys	: cysteine
Da	: Daltons
Dab	: <i>N</i> -Dde-4-aminobenzyl
DBU	: 1,8-diazabicyclo[5,4,0]-undec-7-ene
DCC	: <i>N,N'</i> -dicyclohexylcarbodiimide
Dcm	: <i>N</i> -4,4-dimethyl-2,6-dioxocyclohexylidenemethyl
DCM	: dichloromethane
DCU	: <i>N,N'</i> -dicyclohexyl urea
Dde	: <i>N</i> -1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl
DEPT	: distortionless enhancement by polarisation transfer
Dha	: 2,3-dehydroalanine
Dhb	: 2,3-dehydrobutyrine
DIC	: <i>N,N'</i> -diisopropylcarbodiimide
DIPEA	: <i>N,N</i> -diisopropylamine

Dmab : 4-[*N*-{1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)-3-methylbutyl}amino]
 benzyl
 DMAD: dimethylazodicarboxylate
 DMF : *N,N'*-dimethylformamide
 DMSO: dimethylsulfoxide
 DPPA : diphenylphosphoryl azide
 EI : electron ionisation
 equiv. : (molar) equivalents
 ES+ : electrospray positive
 FAB : fast atom bombardment
 FLEC : 1-(9-fluorenyl)ethyloxycarbonyl
 Fm : fluorenylmethyl
 Fmoc : 9-fluorenylmethoxycarbonyl
 Glu : glutamic acid
 Gly : glycine
 HATU : *N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]-pyridin-1-yl-methylene]-*N*-
 methylmethanaminium hexafluorophosphate *N*-oxide
 HAPyU: 1-[1-pyrrolidinyl-1*H*-1,2,3-triazolo[4,5-*b*]-pyridin-1-yl-methylene]-pyrroli-
 dinium hexafluorophosphate *N*-oxide
 HBTU : *N*-[1*H*-benzotriazol-1-yl]-(dimethylamino)-methylene]-*N*-methylmethan-
 aminium hexafluorophosphate *N*-oxide
 His : histidine
 HMPA: hexamethylphosphoric triamide
 HMPB: 4-(4-hydroxymethyl-3-methoxyphenoxy)butyryl
 HOAt : 1-hydroxy-7-azabenzotriazole
 HOBt : 1-hydroxybenzotriazole
 HPLC : high performance liquid chromatography
 Ile : isoleucine
 I.R. : infra-red
 ivDde : *N*-1-(4,4-dimethyl-2,6-dioxocyclohexylidene) isovaleryl
 LCMS : liquid chromatography-mass spectrometry
 Lan : lanthionine

Leu : leucine
 Lys : lysine
 Me : methyl
 MeLan: methyl lanthionine
 Met : methionine
 Ms : methanesulfonyl
 NMA : *N*-methylaniline
 NMM : *N*-methylmorpholine
 NMR : nuclear magnetic resonance
 NOESY: nuclear overhauser effect spectroscopy
 OPfp : pentafluorophenyl esters
 PAL : tris(alkoxy)benzylamide linkage
 PCOR : peptide cyclisation on an oxime resin
 PEG-PS: poly(ethylene glycol)-polystyrene
 Phe : phenylalanine
 Pro : proline
 PyAOP: (7-azabenzotriazol-1-yl)-tris(pyrrolidino)-phosphonium hexafluoro-phosphate
 PyBOP: (benzotriazol-1-yl)-tris(pyrrolidino)-phosphonium hexafluorophosphate
 Ser : serine
 SPPS : solid phase peptide synthesis
 TBDMS: tertiary butyldimethylsilyl
 TBTU : 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate
 TEA : triethylamine
 TES : triethylsilane
 TFA : trifluoroacetic acid
 THF : tetrahydrofuran
 Thr : threonine
 TIPS : triisopropylsilane
 TMS : trimethylsilyl
 tlc : thin layer chromatography
 Trt : triphenylmethyl

Tyr : tyrosine
U.V. : ultra-violet
Val : valine
VT : variable temperature
Z : benzyloxycarbonyl

List of Figures and Tables

	Page
Figure 1.1. (a) <i>meso</i> -Lanthionine and (b) cystine	24
Figure 1.2. Unusual amino acids in lantibiotics	25
Figure 1.3. Unusual amino acids in lantibiotics	25
Figure 1.4. Biosynthesis of lantibiotics	26
Figure 1.5. Mode of action of mersacidin	28
Figure 1.6. Structures of the two-component lantibiotic, Plantaricin W	31
Figure 1.7. Mode of action of nisin	32
Figure 1.8. A lanthionine enkephalin opioid analogue	33
Figure 1.9. (a) Lanthionine-somatostatin analogue; (b) proposed bioactive conformation	33
Figure 1.10. Structures of (a) Lan-7; (b) TT-232	33
Figure 1.11. (a) Monosulfide and (b) disulfide analogues of an integrin antagonist	34
Figure 1.12. A thioether cyclic peptide	34
Figure 1.13. Formation of a (<i>R,R</i>)-lanthionine derivative <i>via</i> sulfur extrusion method	34
Figure 1.14. Mechanism of sulfur extrusion from a disulfide; stereochemical inversion of a β -substituted disulfide	35
Figure 1.15. Cross-coupling in sulfur extrusion reaction	36
Figure 1.16. <i>meso</i> -Lanthionine formation <i>via</i> a cyclic cystine derivative	36
Figure 1.17. Retrosynthetic analysis of <i>meso</i> -lanthionine	37
Figure 1.18. Michael addition of cysteine to dehydroalanine	38
Figure 1.19. Competing reactions; (a) thioester formation, (b) thioether formation	38
Figure 1.20. Lanthionine formation using a β -iodo-(<i>R</i>)-alanine derivative with a cysteine derivative	39
Figure 1.21. Determination of chiral integrity of a (<i>R,R</i>)-lanthionine derivative	40
Figure 2.1. Different degrees of amino acid protection, allowing for repeated amide bond formation	43
Figure 2.2. General scheme for Fmoc SPPS	44
Figure 2.3. Cleavage mechanism for Wang and NovaSyn [®] TGA resins	46

Figure 2.4. Cleavage mechanism for Rink acid, 2-chlorotrityl, SASRIN and HMPB resins	47
Figure 2.5. Cleavage mechanism for Rink amide and PAL (5-(4- <i>N</i> -fluorenyl-methoxycarbonyl-aminomethyl-3,5-dimethoxyphenoxy)valeryl) resin	48
Figure 2.6. Cleavage mechanism for Sieber amide resin	48
Figure 2.7. Cleavage mechanism for HMBA resin	48
Figure 2.8. Cleavage mechanism for 3-nitro-4-hydroxymethyl benzoyl and α -methyl-6-nitro-veratrylamine alcohol resins	49
Figure 2.9. Formation of oxazolone (b) and its enantiomer (d) from an activated amino acid (a) <i>via</i> enolisation of the oxazolone (c) resulting in racemisation: (e)	50
Figure 2.10. Coupling <i>via</i> the azide method using DPPA	51
Figure 2.11. A Pfp-activated amino acid	51
Figure 2.12. Formation of the <i>O</i> -acylisourea intermediate (a) followed by nucleophilic attack of an amino group	51
Figure 2.13. Formation of the symmetrical anhydride (a) followed by nucleophilic attack of the amino group yielding the desired amide bond	52
Figure 2.14. Formation of an amide bond <i>via in situ</i> activation of the <i>O</i> -acylisourea intermediate (b) with HOBt (a)	52
Figure 2.15. Formation of the unreactive <i>N</i> -acylisourea (a)	53
Figure 2.16. Formation of the carcinogenic HMPA during BOP activation	53
Figure 2.17. Various coupling and/or cyclisation reagents for SPPS	54
Figure 2.18. Stabilising effect of the pyridinium nitrogen on HOAt	54
Figure 2.19. Allylic protection of various functional groups	56
Figure 2.20. Incorporation of allyl and Alloc onto alcohols, carboxyls and amines	56
Figure 2.21. Mechanism for allylic deprotection	57
Figure 2.22. Competing allyl amine formation reaction <i>via</i> routes (a) and (b)	58
Figure 2.23. Scavengers used for palladium-mediated allylic deprotection	59
Figure 2.24. Structures of (a) 4-bromocrotonic acid, (b) 4-trityloxybut-(<i>Z</i>)-2-enyloxyacetic acid and (c) 4-(4'-methoxytrityloxy)-but-2-enyloxyhexanoic acid	59
Figure 2.25. Synthesis of Dcm and its incorporation into amino acids	60
Figure 2.26. Mechanism for the deprotection of Dcm	61
Figure 2.27. Synthesis of Dde, incorporation onto Fmoc-Lys-OH and deblock	62

Figure 2.28. Structure of ivDde	63
Figure 2.29. Synthesis of Dab and its incorporation onto amino acids	64
Figure 2.30. Synthesis of Dmab, its protection of and deblock from amino acids	65
Figure 2.31. Formation of the pyroglutamyl ring on Glu	65
Figure 2.32. Unsuccessful cyclisation due to incomplete removal of Dmab	66
Figure 2.33. Peptide cyclisation mechanism on an oxime resin	67
Figure 2.34. On-resin peptide cyclisation: (a) head-to-tail (b) tail-to-side chain (c) side chain-to-head and (d) side chain-to-side chain	68
Figure 2.35. On-resin head-to-tail cyclisation <i>via</i> Lys side chain anchoring	69
Figure 2.36. On-resin head-to-tail cyclisation <i>via</i> Asp side chain anchoring	69
Figure 2.37. Solid phase synthesis of a monocyclic peptide	70
Figure 2.38. Synthesis of a branched cyclic peptide <i>via</i> a three-dimensional orthogonal protecting group strategy	71
Figure 2.39. Synthesis of an overlapping bi-cyclic peptide	71
Figure 2.40. Schematics for the synthesis of nisin	72
Figure 2.41. Synthesis of a lanthionine analogue of an enkephalin opioid peptide	73
Figure 2.42. Formation of ring B of nisin <i>via</i> a stereospecific Michael addition	74
Figure 2.43. Synthesis of Lan-7 in solution	75
Figure 2.44. Synthesis of a lanthionine-somatostatin analogue	76
Figure 2.45. Synthesis of a β,β -dimethylanthionine analogue of an enkephalin opioid peptide	77
Figure 2.46. Lanthionine macrocyclisation by <i>in situ</i> activation of serine	78
Figure 2.47. Synthesis of a thioether cyclic peptide	79
Figure 3.1. Nucleophilic ring opening at C_2 or C_3 of aziridine-2-carboxylates	81
Figure 3.2. Reaction of an aziridine-2-carboxylate with $^n\text{BuCu}(\text{CN})\text{Li}_2$	81
Figure 3.3. Reaction of an aziridine-2-carboxylic acid with organocuprates	82
Figure 3.4. Reaction of an aziridine-2-carboxylate with Grignard reagents in the presence of copper(I) bromide-dimethylsulfide	82
Figure 3.5. Reaction of an aziridine-2-carboxylates with a Wittig reagent	83
Figure 3.6. Aziridine-2-carboxylate reaction with tetrabutylammonium [^{11}C]cyanide	83
Figure 3.7. Hydration of aziridine-2-carboxylates	84

Figure 3.8. Reaction of aziridine-2-carboxylates with various alcohols	84
Figure 3.9. Reaction of aziridine-2-carboxylates with methanol	85
Figure 3.10. Reaction of a C_3 -substituted aziridine-2-carboxyl amide with NH_3	85
Figure 3.11. Reaction of an aziridine-containing peptide with diethylamine	85
Figure 3.12. Reaction of an aziridine-2-carboxylate with the α -amino function of L-His	86
Figure 3.13. Reaction of an aziridine-2-carboxylate with trimethylsilyl azide	86
Figure 3.14. Reaction of an aziridine-2-carboxylate with methylmercaptan	86
Figure 3.15. Reaction of aziridine-2-carboxylates with various thiols	87
Figure 3.16. Formation of lanthionine and 3-methylanthionine derivatives	88
Figure 3.17. Reaction of an aziridine-2-carboxylate with a racemic thiol	88
Figure 3.18. Formation of lanthionine and 3-methylanthionine derivatives	89
Figure 3.19. Reaction of a trifluoromethyl C_3 -substituted aziridine-2-carboxylate with various thiols	89
Figure 3.20. Reaction of an aziridine-2-carboxylate with hydrogen fluoride	90
Figure 3.21. Reaction of aziridine-2-carboxylates with metal halides	90
Figure 3.22. Reaction of an aziridine-2-carboxylate with hydrogen chloride	91
Figure 3.23. Reduction of an aziridine-2-carboxylate	91
Figure 3.24. Aziridine-2-carboxylate system	92
Figure 3.25. Proposed mechanism for Lewis acid-mediated C_3 ring opening of aziridine-2-carboxylates	92
Figure 3.26. Target molecule 1 for SPPS	93
Figure 3.27. General scheme for proposed synthesis of lanthionine containing peptides, using a linker yielding peptide amides	94
Figure 3.28. Target molecule 2 for SPPS	95
Figure 3.29. General scheme for the proposed synthesis of lanthionine containing peptides with overlapping thioether linkages, using a linker yielding peptide acids	96
Figure 4.1. General scheme for the synthesis of lanthionine derivatives	98
Figure 4.2. Structure of (<i>R/S</i>)-2-iodo-3-(triphenylmethylamino)-propionic acid allyl ester, an α -iodo- β -alanine derivative	99
Figure 4.3. Trans-esterification of (<i>R,R</i>)-cystine with ^t butyl acetate	99
Figure 4.4. Fmoc protection of (<i>R,R</i>)-cystine <i>bis</i> - ^t butyl ester	100

Figure 4.5. Disulfide reduction of <i>N,N'</i> -bis(fluorenylmethoxycarbonyl)-(<i>R,R</i>)-cystine- <i>bis</i> - ^t butyl ester	100
Figure 4.6. <i>N</i> -Triphenylmethylation of (<i>S</i>)-serine	101
Figure 4.7. Allylation of <i>N</i> -triphenylmethyl-(<i>S</i>)-serine	102
Figure 4.8. Mesylation of <i>N</i> -triphenylmethyl-(<i>S</i>)-serine allyl ester	103
Figure 4.9. Iodination of <i>N</i> -triphenylmethyl-(<i>S</i>)-serine(<i>O</i> -mesyl) allyl ester	103
Figure 4.10. (<i>S</i>)-1-Triphenylmethyl-aziridine-2-carboxylic acid allyl ester	104
Figure 4.11. Fisher projections displaying three conformations of <i>N</i> -trityl-3-iodo-(<i>R</i>)-alanine 6-nitroveratryl, ⁷⁸ benzyl, ⁷⁶ methyl ⁷⁶ and allyl ester	105
Figure 4.12. Formation of the triphenylmethyl-derived (<i>R,R</i>)-lanthionine	105
Figure 4.13. Replacement of triphenylmethyl with allyloxycarbonyl on (<i>R,R</i>)-lanthionine (<i>R,R</i>-10)	107
Figure 4.14. <i>N</i> -Triphenylmethylation, allylation and mesylation of (<i>R</i>)-serine followed by conversion to (<i>S</i>)-iodoalanine derivative (<i>S</i>-6a)	108
Figure 4.15. (<i>R</i>)-1-Triphenylmethyl-aziridine-2-carboxylic acid allyl ester	108
Figure 4.16. Formation of the lanthionine derivative (<i>S,R</i>-10)	109
Figure 4.17. Replacement of triphenylmethyl with allyloxycarbonyl on lanthionine (<i>S,R</i>-10)	110
Figure 4.18. Removal of ^t butyl from lanthionine (<i>S,R</i>-12)	110
Figure 4.19. ivDde/Dmab derivative of (<i>S,R</i>)-lanthionine	
Figure 4.20. Unsuccessful attempts at esterification of <i>N</i> -triphenylmethyl-(<i>R</i>)-serine (<i>R</i>-3) with Dmab-OH	111
Figure 4.21. Scheme for carboxyl protection of <i>N</i> -triphenylmethyl-(<i>R</i>)-serine (<i>R</i>-3) with Dmab-OH <i>via</i> TBDMS β-OH protection	112
Figure 4.22. Bromination of Dmab-OH	113
Figure 4.23. Replacement of triphenylmethyl protecting group with ivDde on compound (<i>S,R</i>-10)	113
Figure 4.24. Replacement of allyl with Dmab protecting group on ivDde-derived (<i>S,R</i>)-lanthionine 16	113
Figure 4.25. Removal of ^t butyl from ivDde-derived (<i>S,R</i>)-lanthionine 17	115
Figure 5.1. Iodoalanine and lanthionine targets	117
Figure 5.2. Structures of (<i>R,R</i>-10) and (<i>S,R</i>-10)	118

Figure 5.3. Mechanism for the formation of aziridine (S)- 11 from <i>N</i> -triphenylmethyl- α -iodo-(<i>R</i>)-alanine allyl ester (R)- 6a	120
Figure 5.4. Regioisomeric iodination product, (<i>R/S</i>)-2-iodo-3-(triphenylmethylamino)-propionic acid allyl ester, an α -substituted β -alanine derivative	121
Figure 5.5. Indication of α_1 -C, α_2 -C, β_1 -C and β_2 -C for (R,R)- 10 and (S,R)- 10	122
Figure 5.6. Expected HMBC 3J correlations for (S,R)- 10 across the thioether linkage	123
Figure 5.7. Structure of nor-lanthionine 18 , as proposed by NMR	123
Figure 5.8. Scheme indicating how (S)- 6 gave (S,R)- 10 and hence (S,R)- 12 ; and 6b gave 18 and hence 19 . Similarly, (R)- 6 gave (R,R)- 10 and hence (R,R)- 12 ; and 6b gave 18 and hence 19 . The loss of stereochemical integrity is discussed in Chapter 6	124
Figure 5.9. Structure of nor-lanthionine 20 , a regioisomer of 1	125
Figure 5.10. Structure of nor-lanthionine 21 , a regioisomer of 16	126
Figure 5.11. Lanthionine derivatives 17 and 2 and their regioisomers 22 and 23 , respectively	126
Figure 5.12. Proposed mechanism for the formation of regioisomers of <i>N</i> -trityl- α -iodo-(<i>S</i>)-alanine allyl ester (S)- 6a via the aziridine-2-carboxylate (R)- 11	127
Figure 5.13. Structures of all nor-lanthionine derivatives synthesised	128
Figure 5.14. Partial conversion of <i>N</i> -triphenylmethyl-(<i>R</i>)-serine(<i>O</i> -mesyl) allyl ester (R)- 5 into aziridine (R)- 11	128
Figure 5.15. Ring opening of aziridine (R)- 11 by sodium iodide	129
Figure 5.16. Equilibrium between the mesylate serine (R)- 5 , aziridine derivative (R)- 11 , the correct iodoalanine (S)- 6a and its regioisomers 6b	130
Figure 5.17. Unsuccessful ring-opening reaction of aziridine derivative (R)- 11 with cysteine derivative 9	131
Figure 5.18. Formation of lanthionine via Mitsunobu reaction conditions	132
Figure 6.1. nor-Lanthionine derivatives 20 and 23 for SPSS	134
Figure 6.2. Lanthionine opioid enkephalin peptide analogue (a) and the analogue synthesised 24	134
Figure 6.3. Synthesis of a sulfur-bridged opioid enkephalin peptide analogue 24	135

Figure 6.4. Sieber amide resin	135
Figure 6.5. NovaSyn® TG Sieber resin	138
Figure 6.6. Structure of the doubly oxidised peptide 24a	140
Figure 6.7. (a) Ring C of nisin and an analogue of ring C of nisin 25	143
Figure 6.8. NovaSyn® TGT alcohol resin pre-loaded with Asn(Trt)	144
Figure 6.9. Synthesis of a lanthionine analogue of ring C of nisin 25	145
Figure 6.10. Lanthionine derivative (S,R)- 10	146
Figure 6.11. Rings A and B of nisin	146
Figure 6.12. nor-Lanthionine analogue of rings A and B of nisin 26	147
Figure 6.13. Synthesis of an analogue of rings A and B of nisin 26	148
Figure 6.14. Structure of the nor-Lan analogue of ring C of nisin 25 across the thioether linkage as proposed by NMR	149
Figure 6.15. Structures of 25a and 25b , indicating the stereochemistry at the β -amino acid moiety	151
Figure 6.16. Formation of lanthionine <i>via</i> Mitsunobu reaction conditions	152
Figure 6.17. Application of Mitsunobu conditions to the synthesis of MeLan	152
Figure 7.1. Major product: (<i>R/S</i>)-2-iodo-3-(triphenylmethylamino)-propionic acid allyl ester	167
Figure 7.2. Major product: (<i>R/S</i>)-2-iodo-3-(triphenylmethylamino)-propionic acid allyl ester	169
Figure 7.3. Major product: (<i>R/S</i>)-2-[(<i>R</i>)-2- ^t butoxycarbonyl-2-(fluoren-9-ylmethoxycarbonylamino)-ethylsulfanyl]-3-(triphenylmethylamino)-propionic acid allyl ester	171
Figure 7.4. Major product: (<i>R/S</i>)-2-[(<i>R</i>)-2- ^t butoxycarbonyl-2-(fluoren-9-ylmethoxycarbonylamino)-ethylsulfanyl]-3-(triphenylmethylamino)-propionic acid allyl ester	174
Figure 7.5. Major product: (<i>R/S</i>)-2[(<i>R</i>)-2- ^t butoxycarbonyl-2-(fluoren-9-ylmethoxycarbonylamino)-ethylsulfanyl]-3-(allyloxycarbonylamino)-propionic acid allyl ester	176
Figure 7.6. Major product: (<i>R/S</i>)-2-[(<i>R</i>)-2- ^t butoxycarbonyl-2-(fluoren-9-ylmethoxycarbonylamino)-ethylsulfanyl]-3-(allyloxycarbonylamino)-propionic acid allyl ester	177

Figure 7.7. Intended product: 3-[(<i>S</i>)-2-allyloxycarbonyl-2-(allyloxycarbonylamino)-ethylsulfanyl]-(<i>R</i>)-2-(fluoren-9-ylmethoxycarbonylamino)-propionic acid	180
Figure 7.8. Intended product: 3-[(<i>R</i>)-2- <i>t</i> -butoxycarbonyl-2-(fluoren-9-ylmethoxycarbonylamino)-ethylsulfanyl]-(<i>S</i>)-2-{1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)isovaleryl-amino}-propionic acid allyl ester	182
Figure 7.9. Intended product: 3-[(<i>R</i>)-2- <i>t</i> -butoxycarbonyl-2-(fluoren-9-ylmethoxycarbonylamino)-ethylsulfanyl]-(<i>S</i>)-2-{1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)isovaleryl-amino}-propionic acid	184
Figure 7.10. Intended product: 3-[(<i>R</i>)-2- <i>t</i> -butoxycarbonyl-2-(fluoren-9-ylmethoxycarbonylamino)-ethylsulfanyl]-(<i>S</i>)-2-{1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)isovaleryl-amino}-propionic acid 4-{ <i>N</i> -[1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)-3-methylbutyl]amino}benzyl ester	186
Figure 7.11. Intended product: 3-[(<i>S</i>)-2-{4-[<i>N</i> -(1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)-3-methylbutyl)amino]benzyloxycarbonyl}-2-{1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)isovaleryl-amino}-ethylsulfanyl]-(<i>R</i>)-2-(fluoren-9-ylmethoxycarbonylamino)-propionic acid	188
Figure 7.12. Major product: (<i>R/S</i>)-2-iodo-3-(triphenylmethylamino) propionic acid allyl ester	195
Table 1. Type A lantibiotics, structures and applications	29
Table 2. Type B lantibiotics, structures and applications	30
Table 3. Product of Cys-Ala ((<i>R</i>)-Ala) couplings with cesium carbonate in DMF	39
Table 4. Optical rotation values for (<i>R</i>)- 6 and (<i>S</i>)- 6	108
Table 5. Optical rotation values for (<i>R,R</i>)- 10 and (<i>S,R</i>)- 10	110
Table 6. ¹ H and ¹³ C chemical shifts for peptide 25a	200
Table 7. ¹ H and ¹³ C chemical shifts for peptide 25b	201

Chapter 1

Introduction I

1.1. Lanthionine

Lanthionine (Lan) is an unusual amino acid which was first isolated from wool which had been treated with sodium carbonate in 1941¹ and has since been found in human hair, chicken feathers and lactalbumin.^{2, 3} It is a double-headed or bi-functional amino acid (Figure 1.1) which is analogous to cystine, having two alanine residues linked by a thioether bridge instead of a disulfide bridge. It occurs in nature as the (*S,R*)-diastereoisomeric form (*meso*-lanthionine, *meso*-Lan)⁴ whereas cystine exists naturally as a (*R,R*)-diastereoisomer.

Figure 1.1. (a) *meso*-Lanthionine and (b) cystine

The presence of lanthionine in peptides plays a very important role. When two alanine residues on a peptide are connected *via* a thioether bridge (i.e. lanthionine), the degree of conformational freedom of the now cyclic peptide is considerably reduced. The peptide becomes more rigid⁵ and behaves differently compared to its linear parent. The presence of more cyclic units (i.e. more lanthionine) would reduce the degrees of conformational freedom even more. Polycyclic lanthionine-containing peptides have been shown to have high specificity against Gram-positive bacteria. Studies have shown that peptides containing thioether bridges have greater stability towards enzymatic degradation compared to their disulfide analogues.^{5, 6} The synthesis of these naturally-occurring lanthionine-containing peptides would therefore lead to further studies of conformationally restrained peptides, their applications in the food and pharmaceutical industry and the complete understanding on their extensive biological activities, as well as the development of analogues to improve solubility and bioactivity.

1.2. Lantibiotics

Polycyclic *lanthionine*-containing peptides are known as *lantibiotics*. They have been shown to exhibit *antibiotic* properties. To date over thirty lantibiotics have been identified and characterised including nisin,⁷ subtilin,⁸ epidermin,^{9, 10, 11} gallidermin,¹² ancovenin,¹³ duramycin,¹⁴ duramycin B,¹⁵ duramycin C,¹⁵ cinnamycin,¹⁶ Pep5,¹⁷ actagardine,¹⁸ mersacidin,¹⁹ streptococcin A-FF22,²⁰ epilancin K7,²¹ lactocin S,²² salivaricin A,²³ variacin,²⁴ lacticin 481,²⁵ bacteriocin ISK-1,²⁶ plantaricin C,²⁷ staphylococcin T,²⁸ along with other lantibiotics whose structures have not been completely assigned such as mutacin II,²⁹ sublancin 168,³⁰ carnocin UI 49³¹ and nukacin ISK-1.³²

These lantibiotics consist of 19 – 34 amino acid residues with up to five thioether bridges. Lanthionine is present in all lantibiotics, and is only one of four such thioether-containing residues. Other unusual amino acids also contribute to the polycyclic structure of these lantibiotics such as 3-methylanthionine (*threo*-MeLan), *S*-aminovinyl-D-cysteine, *S*-aminovinyl-D-methylcysteine and lysinoalanine (Figure 1.2).

Figure 1.2. Unusual amino acids in lantibiotics; (a) *(2S, 6R)*-Lanthionine (*meso*-Lan), (b) *(2S, 3S, 6R)*-3-Methylanthionine (*threo*-MeLan), (c) *(2S, 3S)*-[(*Z*)-2-aminovinyl]-D-methylcysteine (MeCys(Avi)), (d) *S*-[(*Z*)-2-aminovinyl]-D-cysteine (Cys(Avi)), (e) *(2S, 9S)*-Lysinoalanine (Lys-*N'*-Ala)

Lantibiotics also contain other unusual amino acids, which include 2,3-dehydroalanine (Dha), (*Z*)-2,3-dehydrobutyrine (Dhb) and 3-hydroxyaspartic acid (Figure 1.3).

Figure 1.3. Unusual amino acids in lantibiotics; (a) 2,3-dehydroalanine (Dha), (b) (*Z*)-2,3-dehydrobutyrine (Dhb), (c) *erythro*-3-hydroxy-L-aspartic acid (HyAsp)

The occurrence of *meso*-Lan, *threo*-MeLan, Cys(Avi), MeCys(Avi), Lys-*N'*-Ala, Dha and Dhb can be attributed to the biosynthetic mechanism of the formation of lantibiotics (Figure 1.4).¹¹

Figure 1.4. Biosynthesis of lantibiotics

A precursor peptide is initially formed in the ribosome through processes of transcription and translation. These “prelantibiotics” are each made up of a leader peptide and a “prolantibiotic”. These “prelantibiotics” are then enzymatically modified resulting in dehydration of β -hydroxy amino acids such as serine and threonine to give Dha and Dhb respectively, allowing regio- and stereospecific Michael addition of cysteine residues forming Lan and MeLan, and lysine residues forming Lys-*N'*-Ala.^{11, 14, 17} The number of cysteine residues does not necessarily match the number of dehydroamino acids hence the presence of the latter in lantibiotics. The occurrence of Cys(Avi) and MeCys(Avi) is a result of *C*-terminal cysteine residues in lantibiotics such as epidermin and mersacidin which undergo oxidation and decarboxylation by modifying enzymes before addition to

dehydroamino acids forming the corresponding thioether linkages.^{11, 14} Proteolytic cleavage of the modified “prolantibiotics” from the leader peptides and liberation from the cell yield the antimicrobial lantibiotics. These post-translational side-chain modifications of lantibiotics are unique and not known to arise in any other peptide antibiotics.

Structural elucidation of these lantibiotics proved to be difficult due to the presence of the dehydroamino acids. By Edman degradation, proteinogenic amino acids on a peptide are converted to their phenylthiohydantoin derivatives and analysed by HPLC, as part of an automated Edman sequencer, with comparisons of retention times with standard values. Lan, MeLan, Dha, Dhb and other unusual amino acids in lantibiotics give blanks in the HPLC trace. *N*-terminal Dha or Dhb on the peptides are extremely unstable and “de-amine” after spontaneous addition of water during the sequencing reaction hence resulting in a halt in the process.^{33, 34} Meyer *et al.* converted these α,β -unsaturated amino acids into their cysteinyl-*S*-alkyl derivatives by reaction with ethanethiol.³³ These derivatives do not block the sequencing. Interestingly, Lan and MeLan thioether bridges are cleaved under the reaction condition and form stable, sequenceable cysteine derivatives. Jung *et al.* have also incorporated the use of electrospray mass spectrometry in series with the automated Edman sequencer to improve amino acid identification in peptides.³⁴

Lantibiotics are generally divided into two subtypes; Type A and Type B.¹⁴ Type A lantibiotics are in excess of 2000 Da in molecular weight, adopt corkscrew-like structures and are positively charged. There have been studies on the structure-activity relationship of nisin, a Type A lantibiotic, where its flexible and elongated structure is utilised for creating transient pores in cytoplasmic membranes of Gram-positive bacteria, allowing smaller molecules from the cytoplasm to exit resulting in cell death.³⁵ It has been shown that an interaction with lipid II is required for nisin to create these pores, however Pep5 – also a type A lantibiotic – has no affinity for it.³⁶ It is not yet known which membrane molecule Pep5 targets for the purpose of pore formation.

Type B lantibiotics adapt more rigid and globular structures due to their head-to-tail cross-linkages and are either negatively charged or neutral.¹⁴ Despite the lack of head-to-tail cross-linkages mersacidin and actagardine (Table 2) are regarded as Type B lantibiotics³² due to the overall similarity of their precursor peptides although this view was not shared by Jung¹⁴ who initially suggested the classification of lantibiotics.

Little is known about these lantibiotics except for the fact that some of them are enzyme inhibitors.¹⁴ Both mersacidin and actagardine potently inhibit the biosynthesis of bacterial cell walls by inhibiting transglycosylation although the biosynthesis of RNA, DNA and proteins are not affected (Figure 1.5).³²

Figure 1.5. Mode of action of mersacidin. Cell wall precursor lipid II is transferred to the outer membrane during peptidoglycan synthesis. This accessibility to mersacidin allows complexation with the disaccharide moiety hence inhibiting its incorporation into the growing peptidoglycan chain. Diagram taken from ref.³²

Tables 1 and 2 list some of the better known Type A and B lantibiotics, respectively.

Lantibiotic Type A	Bacteria	Residues	Cyclic structure	Application/Effect
Nisin	<i>Lactococcus lactis</i>	34	5; 1 Lan, 4 MeLan	Food preservative
Subtilin	<i>Bacillus subtilis</i>	32	5; 1 Lan, 4 MeLan	Inhibits peptidoglycan biosynthesis
Epidermin	<i>Staphylococcus epidermidis</i>	21	4; 2 Lan, 1 MeLan, 1 Cys(Avi)	Juvenile acne, eczema
Gallidermin	<i>Staphylococcus epidermidis</i>	21	4; 2 Lan, 1 MeLan, 1 Cys(Avi)	Juvenile acne, eczema
Pep5	<i>Staphylococcus epidermidis</i>	33	3; 2 Lan, 1 MeLan	Bacteriocin

Table 1. Type A lantibiotics, structures and applications

<i>Lantibiotic Type B</i>	<i>Bacteria</i>	<i>Residues</i>	<i>Cyclic structure</i>	<i>Application</i>
Ancovenin	<i>Streptomyces sp.</i>	19	3; 1 Lan, 2 MeLan	Inhibits angiotensin-converting enzyme
Actagardine	<i>Actinoplanes liguriae</i>	19	4; 1 Lan, 3 MeLan	Inhibits peptidoglycan biosynthesis
Mersacidin	<i>Bacillus Y-85, 54728</i>	19	4; 3 MeLan, 1 MeCys(Avi)	Immunosuppressor
Cinnamycin (Lanthiopeptin)	<i>Streptomyces cinnamoneus</i>	19	4; 1 Lan, 2 MeLan, 1 Lys-N'-Ala	Inhibits phospholipase A ₂ and angiotensin-converting enzyme

Table 2. Type B lantibiotics, structures and applications

The classification of lantibiotics into subtypes A and B is limited to the classic definition of these peptide antibiotics. Plantaricin W,³⁷ lacticin 3147,³⁸ cytolysin³⁹ and staphylococcin C55⁴⁰ are all unique two-component lantibiotics which do not possess antimicrobial properties when the two lanthionine-containing peptidic components are separated. Figure 1.6 illustrates the structure for Plantaricin W as an example.

Figure 1.6. Structures of the two-component lantibiotic, Plantaricin W

1.3. Nisin

Nisin is perhaps the most well known lantibiotic. It was the first to be discovered in 1928 from a culture broth of *Lactococcus lactis*.^{41, 42} However it was not isolated until 1947 from a culture broth of *Streptococcus lactis*⁴³ and it was only in 1971 that Gross and Morrell proposed the complex structure of this lantibiotic.⁷ It contains 5 cyclic structures made up of one Lan and four MeLan residues together with two Dha and one Dhb residues (Table 1). Since 1951 nisin has been an important preservative^{44, 45} in the food industry due to its remarkable anti-growth effect on Gram-positive microorganisms.⁴⁶ It has also been found that it exhibits antimalarial properties,⁴⁷ however, due to its poor solubility in water and low stability at physiological pH nisin has not been developed into a chemotherapeutic drug.⁴⁸ Already wild-type nisin has been modified through site-directed mutagenesis to give nisin Z, substituting the histidine at position 27 or 31 with lysine, which showed increased solubility in water without affecting its antimicrobial activity.⁴⁹

Classified as a Type A lantibiotic, nisin creates pores in bacterial cell walls resulting in cell death. Its *N*-terminal ionic interaction with lipid II is a vital docking mechanism for targeted pore formation and simultaneously blocks peptidoglycan synthesis, much like mersacidin and actagardine (Figure 1.7).³²

Figure 1.7. Mode of action of nisin. Nisin binds to the cell wall precursor, using it as a docking molecule for pore formation and inhibits peptidoglycan synthesis. Diagram taken from ref.³²

1.4. Synthetic lanthionine-containing peptides

Some naturally occurring opioids show marked receptor selectivity⁵⁰ but their linearity and high flexibility hinders any conformational analyses. Their cyclic analogues, useful in structure-activity studies due to their constrained structures, are studied in the hope of elucidating the critical portions of the molecule for opiate receptor binding. There have been several reports on the incorporation of lanthionine, as analogues, in these natural peptides.

Enkephalins are important in a variety of physiological functions such as the modulation of pain and regulation of the immune system.⁵¹ Since their discovery, several classes of opioids have been identified and a lanthionine analogue (Figure 1.8) has been synthesised *via* several different methods.^{50, 52, 53, 54} Although lanthionine analogues of these peptides are more constrained than disulfide analogues, it was shown that this did not result in increased activity.⁵⁰

Figure 1.8. A lanthionine enkephalin opioid analogue

Somatostatin is a 14-residue peptide, which plays important physiological roles such as being an inhibitor of the release of several growth hormones.⁵⁵ Several highly bioactive somatostatin analogues have been synthesised. Sandostatin is a cyclic peptide somatostatin analogue with eight residues containing a disulfide bridge.⁵⁶ It was suggested that the disulfide bridge might not be sufficient to constrain the cyclic backbone in the proposed bioactive conformation (Figure 1.9).⁵⁷ Therefore a lanthionine analogue was developed; this showed similar activity to sandostatin but with increased stability against enzymatic degradation.^{6, 58}

Figure 1.9. (a) Lanthionine-somatostatin analogue; (b) proposed bioactive conformation

Lan-7 is a monocyclic lanthionine-containing peptide, which has been shown to have a remarkable potential as a cancer chemotherapeutic agent. It is a monosulfide analogue of the less stable TT-232, a sandostatin analogue, which has been shown to exhibit strong anti-tumour effects both *in vitro* and *in vivo* by inhibiting growth on a number of human tumour cell lines.⁵⁹ Its potency has been shown to be two to three times that of the already-clinically-used octreotide.^{59, 60} The reduction of the ring size, from TT-232 to Lan-7, did not affect its anti-tumour activity but Lan-7 showed no toxicity to normal human cells.

Figure 1.10. Structures of (a) Lan-7; (b) TT-232

Initial studies on small sulfur-containing molecules indicated that *tris*(diethylamino)phosphine was most effective for selective extrusion of one sulfur atom from a disulfide.^{67, 68} Any carboxyl function present required protection, otherwise a competing amide formation occurred instead of the intended sulfur extrusion reaction.⁶⁷

The mechanism of the sulfur extrusion involves a fragmentation of the disulfide, followed by an S_N2 step. For C_β -substituted cystine, therefore, this results in stereochemical inversion at the C_β -position (Figure 1.14).⁶⁸

Figure 1.14. Mechanism of sulfur extrusion from a disulfide; stereochemical inversion of a β -substituted disulfide

Problems, however, occur in asymmetrical or acyclic disulfides where cross-coupling could take place (Figure 1.15).⁶⁶ As a result, *meso*-lanthionine, which has one *R* and one *S* chiral centre, cannot be synthesised *via* this method. Similarly, it is impossible to produce differentially protected lanthionine derivatives. This problem can be solved by tethering the *R* and *S* cysteine precursors together (Figure 1.16) and forming the cyclic disulfide.⁶⁹ Sulfur extrusion can then be successfully carried out, to give the desired lanthionine, at high dilution to minimise the formation of intermolecular thioethers.

Figure 1.15. Cross-coupling in sulfur extrusion reaction

Figure 1.16. *meso*-Lanthionine formation via a cyclic cystine derivative

However, in an acyclic structure much like the synthesis of a derivative of a lanthionine monomer – instead of being part of a peptide – this procedure would still be faced with the cross-coupling problem described above. Additionally, the use of *tris*(diethylamino)phosphine might not be compatible with the proceedings of solid phase peptide synthesis. Besides, the method described in Figure 1.16 is such that the two carboxyl functions of the *meso*-lanthionine are unprotected. This removes the element of a discriminative peptide coupling as well as the possibility of the use of a triply orthogonal protecting group strategy in solid phase peptide synthesis.

As a result of these limitations other synthetic strategies have been sought. Retrosynthetic analysis of lanthionine (Figure 1.17) produces synthons, which suggest three possible approaches; cysteine and dehydroalanine, cysteine and serine β -lactone, and cysteine and β -halo-alanine. All the abovementioned approaches have been reported and are discussed below.

Figure 1.17. Retrosynthetic analysis of *meso*-lanthionine

1.5.2. Biomimetic approach

As described above, serine and threonine residues on prelantibiotic peptides undergo dehydration to give dehydroalanine and dehydrobutyrine respectively. Subsequent Michael addition by cysteine –SH results in cyclic thioether-bridged lantibiotics, in which the regio- and stereospecificity is enzymatically controlled. This biosynthetic mechanism was mimicked by Bradley *et al.* by reacting a dehydroalanine derivative with a cysteine derivative (Figure 1.18).⁷⁰

Figure 1.18. Michael addition of cysteine to dehydroalanine

The reaction proceeded to form the lanthionine derivative monomer. Unsurprisingly, the reaction was non-stereospecific and gave both diastereoisomers with thiolate attack of the cysteine taking place on both faces of the dehydroalanine derivative. The two diastereoisomers were separable by HPLC. Monteiro *et al.* have also reported on the same approach of synthesising lanthionine derivatives.⁷¹

1.5.3. Serine β -lactones

(*S*)-Serine and (*R*)-serine β -lactones were synthesised (76 – 81 %) ⁷² by treating *N*-protected (*Z* or Boc) serine with triphenylphosphine and dimethyl azodicarboxylate in THF at $-78\text{ }^{\circ}\text{C}$.⁷³ These lactones were then treated with (*S*)-cysteine and (*R*)-cysteine derivatives in the presence of a base to form lanthionine.⁵ However a competing reaction can also take place forming a thioester instead of the desired lanthionine (Figure 1.19).⁵ Although the reaction was stereospecific, the yields of the desired lanthionine residues were only 50 % due to the competing reaction.

Figure 1.19. Competing reactions; (a) thioester formation, (b) thioether formation

Vederas *et al.* discovered the ring-opening of serine β -lactone derivatives by silylamines yields more of the *O*-acyl cleavage product when the reactions were

carried out in chlorinated solvents, and the regiospecificity is changed when acetonitrile is used as solvent, yielding 95 % of the *O*-alkyl cleaved product.⁷⁴ Shirahama on the other hand reacted *N*-Boc-serine- β -lactone with sodium phenylselenide in THF and yielded the selenide ether exclusively.⁷⁵ A more reliable method is still needed to produce lanthionine derivatives.

1.5.4. β -Haloalanine derivatives

The use of β -bromo- and β -iodoalanine derivatives for the β -cationic alanine synthon in the formation of lanthionine have been reported. In each case the β -haloalanines were derived from serine.

Dugave and Menez reported the successful synthesis of the natural (*S,R*)- and unnatural (*R,R*)-lanthionine derivatives using *N*-triphenylmethylated (*R/S*) serine benzyl/methyl esters which were mesylated at the β -hydroxy position, followed by iodination to form the β -iodoalanine derivatives.⁷⁶ These were then treated with cysteine and β -substituted cysteine derivatives in the presence of caesium carbonate to yield Lan and MeLan derivatives in high yields (Figure 1.20, Table 3).

Figure 1.20. Lanthionine formation using a β -iodo-(*R*)-alanine derivative with a cysteine derivative

R^1	R^2	R'	R''	Yield / %
H	H	Fmoc	^t Bu	88
H	H	Boc	Me	79
H	Me	Boc	Me	83
Me	Me	Boc	Me	81

Table 3. Product of Cys-Ala ((*R*)-Ala) couplings with cesium carbonate in DMF

The iodoalanine derivatives were reported to be present as two conformers, not inter-converting due to the bulkiness of the triphenylmethyl group and the size of the iodine atom. Restricted rotation, resulting in two conformers at ambient temperature, has been reported in triphenylmethylated homoserine lactones.⁷⁷

The lanthionine derivatives produced in Table 2 also gave ^1H NMR signals with similar patterns to the iodoalanine derivatives, indicating the presence of conformers. The chiral integrity of the first two entries was investigated, to ascertain that the two species observed by NMR were not diastereoisomers. Removal of the triphenylmethyl group and desulfurisation was followed by acidic hydrolysis and (+)-FLEC derivatisation of the resulting alanine residues. These were analysed by chiral HPLC, where elution of the (+)-FLEC-derivatised alanine with genuine samples of (+)-FLEC-(D,L)-alanine and (+)-FLEC-(L)-alanine indicated that the chiral integrity of the lanthionine derivatives was retained (Figure 1.21). The enantiomeric excess of the alanine derivatives were in excess of 98 %, which clearly dismissed the hypothesis of a significant degree of racemisation during the coupling.

Figure 1.21. Determination of chiral integrity of a (R,R) -lanthionine derivative

Other tests were carried out to confirm the presence of conformers. VT ^1H NMR experiments on the iodoalanine derivatives did not confirm the theory, as no coalescence of signals was observed due to the formation of aziridine at temperatures higher than 50 °C. VT experiments of the lanthionine derivatives, however, were not carried out. Since there was no indication of diastereoisomer formation at the lanthionine stage, as determined by the experiments described above which yielded only (+)-FLEC-derivatised L-alanine residues for (*R,R*)-lanthionine, Dugave and Menez concluded that the two conformers produced at the iodoalanine stage reacted with the cysteine derivatives to give two lanthionine conformers. Previous work in the Tabor group, in which the nitroveratryl group and its oxycarbonyl derivative were used as photolabile protecting groups, appeared to corroborate these observations: two conformers were present at both the iodoalanine and lanthionine stages.⁷⁸

1.5.5. Ring opening of aziridine-2-carboxylates

Lanthionine derivatives can also be formed from nucleophilic ring opening reactions of aziridine-2-carboxylates by cysteine derivatives.^{79, 80} This, and other ring opening reactions of aziridine-2-carboxylates are discussed in Chapter 3.

Chapter 2

Introduction II

2.1 Solid phase peptide synthesis

Merrifield was awarded a Nobel Prize in 1984 for his seminal work on solid phase peptide synthesis (SPPS).⁸¹ The principal of solid phase synthesis is that an insoluble polymeric matrix in the form of resin beads, appropriately functionalised with a linker or handle, can be used to host reactions forming amide or ester bonds with the linker (Figure 2.1). The functional groups on the amino acids need to be suitably protected. The α -N protection needs to be temporary, being easily removed without affecting any other protecting groups, to allow for the formation of repeated amide bonds to form the peptide. The side chain protecting groups, on the other hand, are

semi-permanent as they need to be able to withstand the conditions applied throughout the peptide synthesis and are only removed at the end of the synthesis, often concurrently with peptidyl-resin cleavage to yield the fully deprotected peptide.

Figure 2.1. Different degrees of amino acid protection, allowing for repeated amide bond formation

Due to the insolubility of the resins, which could be placed on sinters, excess reagents, solvents and by-products for the removal of the temporary protection and amide bond formation could simply be filtered through once the reactions have taken place, avoiding tedious aqueous work-up procedures required after each amino acid coupling carried out in solution.

The Boc methodology employed by Merrifield involves the use of a copolymer of styrene and 2 % divinylbenzene as the insoluble support, with chloromethylation to provide the active site for first residue coupling. The temporary Boc *N*-protection used is cleaved in relatively mild acidic conditions (HCl/AcOH) whereas the peptidyl-resin cleavage is carried out using hydrogen fluoride, which also cleaves semi-permanent side chain protections such as benzyl or Z. The serious hazards of the use of hydrogen fluoride in the cleavage of peptides from Merrifield-type resins led Sheppard in the 1970s to develop a different approach to SPPS.^{82a, 82b} Instead of the acid-based Boc methodology employed by Merrifield, a new methodology was developed: Fmoc-based *C*→*N* methodology used in conjunction with acid labile semi-permanent side chain protections and peptide cleavage from the linker-resin system. Figure 2.2 shows the general scheme for an Fmoc SPPS using a resin with an amino-linker yielding a *C*-terminal peptide amide upon cleavage.

Figure 2.2. General scheme for Fmoc SPPS

In step (i) the temporary Fmoc protecting group is removed with a mild base, typically with 20 % piperidine solution in DMF. In step (ii) the first amino acid, pre-activated to form an active ester, is coupled onto the resin. This is followed by the *N*-acetylation step (iii), carried out to “cap” any unreacted amino group, minimising the amount of by-products - in the form of peptides with varying lengths – formed during the synthesis. This sequence is then repeated as necessary to form the required peptide sequence. The manual repeated cycle of SPPS has been adapted for automation in the development of the solid phase peptide synthesiser.

Following each Fmoc deprotection and amino acid coupling steps in a manual synthesis, a small amount of resin is normally removed, washed and treated with solutions of Kaiser test.⁸³ The Kaiser test is a mixture of solutions which essentially

is a ninhydrin test for free amines, which indicates a dark blue to purple colouration. A positive Kaiser test should be observed after an Fmoc deprotection step and a negative one (colour of resulting solution remains unchanged) after an amino acid coupling step. In automated SPPS – either batch (automated delivery of reagents to sintered column containing resin and agitation during reaction) or continuous flow (automated delivery to column followed by continuous flow of reagents in a loop) - each Fmoc deprotection and amino acid coupling is monitored by U.V. instead. Finally, in step (iv) the peptide is cleaved from the linker-resin.

2.2. Resins for Fmoc solid phase peptide synthesis

The original resin developed by Merrifield was polystyrene-based. Atherton and Sheppard developed a polyamide resin which overcame the problems of aggregation of the growing peptide observed during SPPS with Merrifield's resin and possessed greater swelling properties in DMF.⁸² Both types of resins were found to be unable to withstand high pressures, and hence were unsuitable for continuous flow peptide synthesis. A new type of resin was then developed in 1985 by Bayer and Rapp where poly(ethylene glycol) was grafted to polystyrene (PEG-PS).⁸⁴ This resin is stable to the increased flow rates and hence increased pressure in continuous flow peptide synthesis. Its swelling properties have also been shown to be superior and have been described as tentacle-like; these resins are known as Tentagel resins.⁸⁵

An important factor in the choice of resins used for SPPS is the linker. The sensitivity of the linker to acids, bases, nucleophiles, metal catalysts and U.V. radiation plays an important role in the success of a synthesis. For Fmoc SPPS, a wide variety of functionalised resins are currently commercially available. Rink,⁸⁶ Sieber,⁸⁷ 2-chlorotrityl,⁸⁸ Wang,⁸⁹ TGT^{90, 91} and PAL⁹² resins and their derivatives and refinements are widely used. These are chosen based on whether peptide acids or amides are required, and whether fully protected or deprotected peptides are desired.

2.2.1. Cleavage conditions

The linkers in Fmoc SPPS have to be stable to the reaction conditions carried out during the synthesis. Fmoc is removed by base and therefore most of the linkers are base-stable and acid-labile. The resins mentioned above are of varying degrees of sensitivity to acids.

2.2.1.1. Acid labile resins yielding peptide acids

Resins such as the Wang resin⁸⁹ and NovaSyn[®] TGA resin⁹³ are cleaved under highly acidic concentrations, typically with 90 – 95 % TFA solutions in the presence of scavengers such as triethylsilane or triisopropylsilane for up to 2 hr (Figure 2.3). These conditions allow for the production of fully deprotected peptides by simultaneous deprotection of acid labile side chain protecting groups such as ^tBu and Boc.

Figure 2.3. Cleavage mechanism for Wang and NovaSyn[®] TGA resins

Resins such as the Rink acid resin,⁸⁶ 2-chlorotrityl resin,⁸⁸ SASRIN resin⁹⁴ and HMPB resin⁹⁵ contain hyperacid labile linkers. Peptide cleavage is carried out with 0.1 – 5 % TFA solutions in DCM between 1 min and 1 hr (Figure 2.4). Under these conditions the peptides produced are fully protected. The use of these resins is beneficial for monitoring the synthesis, where small amounts of the peptide could be

cleaved from the resin at different stages in the synthesis, with all side chain protecting groups intact for LCMS analyses. Another advantage of using such hyperacid labile linkers is the possibility of synthesising protected peptide fragments, which could then be coupled *via* segment condensation in solution.

Figure 2.4. Cleavage mechanism for Rink acid, 2-chlorotrityl, SASRIN and HMPB resins

2.2.1.2. Acid labile resins yielding peptide amides

Resins such as the Rink amide⁸⁶ and PAL resins⁹² are cleaved under high acidic concentrations, typically with 50 – 95 % TFA solutions in the presence of scavengers such as triethylsilane or triisopropylsilane for up to 2 hr (Figure 2.5). These conditions also allow for the production of fully deprotected peptides by simultaneous deprotection of acid labile protecting groups such as ^tBu and Boc.

Resins such as the Sieber amide resin⁸⁷ contain hyperacid labile linkers with peptide cleavage carried out with 1 – 5 % TFA solutions in DCM for up to 15 min (Figure

2.6). Similar to hyperacid labile linkers yielding peptide acids, the use of this resin is beneficial for monitoring the peptide synthesis at various stages by LCMS.

Figure 2.5. Cleavage mechanism for Rink amide and PAL (5-(4-*N*-fluorenylmethoxycarbonyl-aminomethyl-3,5-dimethoxyphenoxy)valeryl) resin

Figure 2.6. Cleavage mechanism for Sieber amide resin

2.2.1.3. Resins cleaved via nucleophilic displacement and photolysis

Ammonia has been used to cleave peptides from HMBA resins yielding peptide amides.⁹⁶ Its use does not interfere with the Fmoc protecting group as cleavage is carried out at the end of the synthesis, once the base labile group has been removed (Figure 2.7).

Figure 2.7. Cleavage mechanism for HMBA resin

Figure 2.8. Cleavage mechanism for 3-nitro-4-hydroxymethyl benzoyl and α -methyl-6-nitro-veratrylamine alcohol resins

There have also been reports of linkers that undergo photolytic cleavage, yielding both peptide acids and amides. The α -methyl-6-nitro-veratrylamine based resin⁹⁷ is cleaved by irradiating with U.V. light of a specific wavelength, typically 350 nm, over periods of 1 – 3 hr (Figure 2.8).

2.3. Coupling reagents and cyclisation reagents (via amide bond formation)

In coupling a carboxylic acid group with an amino group to form an amide bond, the reaction requires harsh conditions of strong acid or base. This less than ideal method for peptide synthesis could be avoided by increasing the electrophilicity of the carboxylic acid group, making it more susceptible to the amino nucleophilic attack.

For peptide bond formation, two factors are important: high yields and retention of stereochemical integrity. Amino acids can undergo racemisation should the carboxyl group be “over-activated”. This could take place *via* the formation of an oxazolone (Figure 2.9).

Figure 2.9. Formation of an oxazolone (b) and its enantiomer (d) from an activated amino acid (a) *via* enolisation of the oxazolone (c) which results in racemisation: (e)

In solution peptide synthesis, the azide method is perhaps the earliest means of amide bond formation.⁹⁸ In SPPS, diphenylphosphoryl azide (DPPA) is still used for amide bond formation due to its efficiency at forming activated peptide azides *in situ* (Figure 2.10).^{99, 100}

Figure 2.10. Coupling *via* the azide method using DPPA

Pentafluorophenyl esters (OPfp) of amino acids (Figure 2.11) have found wide application in Fmoc SPPS. These active esters react to form amide bonds rather slowly, but the addition of HOBt increases the rate of reaction.¹⁰¹

Figure 2.11. A Pfp-activated amino acid

Merrifield originally used dicyclohexylcarbodiimide (DCC) as coupling reagent in SPPS.⁸¹ The formation of the *O*-acylisourea intermediate leads to nucleophilic attack of the amino group, forming the required amide bond (Figure 2.12).

Figure 2.12. Formation of the *O*-acylisourea intermediate (a) followed by nucleophilic attack of an amino group

The formation of a symmetrical anhydride is possible *via* reaction of the *O*-acylisourea intermediate with a second, non-activated carboxylic acid (Figure 2.13). This symmetrical anhydride can also react with an amino group to form the desired

amide bond. Pre-formed symmetrical anhydrides are generated by reaction of the Fmoc amino acid with 0.5 equivalents of carbodiimide. However, this means of amide bond formation is, among other things, wasteful as only one amino acid from the anhydride is used for amide bond formation.

Figure 2.13. Formation of the symmetrical anhydride (a) followed by nucleophilic attack of the amino group yielding the desired amide bond.

In solution peptide chemistry, the urea by-product is simply filtered off due to its sparing solubility in most organic solvents. However, repeated washing is a requirement in SPPS. The introduction of diisopropylcarbodiimide (DIC) eliminates this problem.¹⁰² It is often used in conjunction with a hydroxylamine such as 1-hydroxybenzotriazole (HOBt),¹⁰³ forming the active ester *in situ*, to avoid possible racemisation *via* the symmetrical anhydride formation (Figure 2.14).

Figure 2.14. Formation of an amide bond *via in situ* activation of the *O*-acylisourea intermediate (b) with HOBt (a)

HOBt also prevents the formation of *N*-acylisourea *via* a competing intramolecular rearrangement of the *O*-acylisourea, which occurs when the latter is not immediately reacted with the nucleophilic amino group (Figure 2.15).¹⁰⁴

Figure 2.15. Formation of the unreactive *N*-acylisourea (a)

Phosphonium derivatives of HOBt – BOP reagents – were developed by Castro *et al.* and proved to be efficient coupling reagents for peptide synthesis (Figure 2.16).^{105a} A major problem, however, is the formation of hexamethylphosphoric triamide (HMPA) by-product which is a carcinogen.

Figure 2.16. Formation of the carcinogenic HMPA during BOP activation

Over the years various non-toxic coupling reagents have been developed, including PyBOP^{105b} and HBTU,^{105c} mainly by varying the derivatives on the phosphorous (Figure 2.17).

Figure 2.17. Various coupling and/or cyclisation reagents for SPPS

The 1-hydroxy-7-azabenzotriazole (HOAt) derived reagents have been shown to be superior to HOBt with increased coupling efficiencies and lower racemisation.^{106, 107}

These are due to the nitrogen on the 7-position, which stabilises the overall negative charge on the leaving group, as well as having a stabilising effect *via* a hydrogen bond formed with the amino group on the incoming amino acid (Figure 2.18).¹⁰⁸

Figure 2.18. Stabilising effect of the pyridinium nitrogen on HOAt

In fact, the use of phosphonium and “so-called” uronium (aminium) salts of HOAt – PyAOP and HATU/HAPyU, respectively - has been shown to be superior to coupling methods *via* carbodiimides and HOBt. Albericio *et al.* demonstrated that for a simple coupling of Z-Gly-Phe-OH and H-Pro-NH₂, the use of PyAOP-HOAt and HAPyU-HOAt yielded negligible racemisation compared to the more traditional DCC-HOBt and BOP systems.¹⁰⁹ Various other examples have been reported to

Figure 2.19. Allylic protection of various functional groups

In amino acid chemistry, the two main allylic groups (allyl and Aloc) are used for protecting carboxyl functions as allyl esters, alcohol functions as allyl ethers or allyl carbonates and amino functions as allyl carbamates. These have recently been extensively reviewed.^{118, 119} Incorporation of the allyl group onto carboxyl functions is generally achieved by one of three means: acid catalysed esterification with allyl alcohol, activated carboxyl with allyl alcohol or nucleophilic displacement of allyl halides with carboxylates. Allyl carbonates and carbamates are generally generated from treatment of the alcohol and amine, respectively, with allyl chloroformate in the presence of base (Figure 2.20). There has also been a report on the use of diallyl dicarbonate for the synthesis of allyl carbamates.¹²⁰

Figure 2.20. Incorporation of allyl and Aloc onto alcohols, carboxyls and amines

The main challenge in the use of allylic protecting groups for amino acids and peptides has been their deprotection. The well known and widespread method involves the cleavage of allyl esters or allyl carbamates using palladium(0) catalysts in the presence of nucleophiles under near-neutral conditions. The mechanism for allyl and Aloc deprotection is shown in Figure 2.21. The palladium(0) catalyst is coordinated to the allylic double bond and is then oxidised, liberating the carboxyl group (or amino group and carbon dioxide for Aloc-protection) which results in a cationic palladium(II)-allyl species. A nucleophilic scavenger then attacks the allylic cation, resulting in a reductive elimination of the palladium(II).

Figure 2.21. Mechanism for allylic deprotection

The problem often encountered in Aloc deprotection is the nucleophilicity of the liberated free amine towards the palladium(II)-allyl species, which can compete with the nucleophilicity of the allyl group scavenger (route (a) in Figure 2.22). This would result in the formation of a secondary allyl amine on an amino acid or peptide, which would put a halt to the synthesis. The use of an excess amount of scavenger would also be advantageous as it eliminates the formation of this secondary allyl amine *via*

the routes (a) and (b) described in Figure 2.22. The use of a weakly basic nucleophile as a scavenger is also essential as it avoids Fmoc deprotection; this consolidates the orthogonality of the allyl group with the Fmoc/^tBu system.

Figure 2.22. Competing allyl amine formation reaction *via* routes (a) and (b)

Scavengers that have been used include oxygen nucleophiles such as potassium 2-ethylhexanoate,¹²¹ nitrogen nucleophiles such as morpholine,^{122, 123} *N*-methylaniline,^{124, 125} diethylamine,^{126, 127} and *N*-methylmorpholine^{128, 129} carbon nucleophiles (acidic β -dicarbonyls) such as dimedone¹³⁰ and *N,N*-dimethylbarbituric acid,¹¹⁹ sulfur nucleophiles such as 2-thiobenzoic acid,¹³¹ silylated derivatives of nitrogen nucleophiles such as trimethylsilylmorpholine¹³² and trimethylsilyldimethylamine¹³² and hydride donors such as formic acid,¹³³ tributyltin hydride,¹³⁴ borohydrides¹³⁵ and silicon hydrides^{136, 137} (Figure 2.23).

In SPPS, allylic groups were initially used as resin linkers. Kunz *et al.* were the pioneers in using 4-bromocrotonic acid (Figure 2.24), coupled to the aminomethyl polystyrene resin (DCC/HOBt) forming an allylic anchor.¹³⁸ This and other allylic handles such as 4-trityloxybut-(*Z*)-2-enoxyacetic acid¹³⁹ and 4-(4'-methoxytrityloxy)-but-2-enoxyhexanoic acid¹⁴⁰ are removed by palladium(0) species in the presence of various nucleophiles.^{119, 141}

Figure 2.23. Scavengers used for palladium-mediated allylic deprotection

Figure 2.24. Structures of (a) 4-bromocrotonic acid, (b) 4-trityloxybut-(Z)-2-enyloxyacetic acid and (c) 4-(4'-methoxytrityloxy)-but-2-enyloxyhexanoic acid

The high selectivity, mild and almost neutral conditions of the cleavage of allylic groups together with their compatibility with sulfur derivatives¹¹⁹ have made them an excellent choice for three-dimensional orthogonal protecting group strategy in SPPS. They have also been proven to be compatible with the traditional Fmoc/^tBu methodology.^{129, 141, 142, 143, 144, 145, 146}

There have also been successful cases of simultaneous allyl and Aloc deprotection. Hayakawa *et al.* carried out the cleavage of the phosphate-bound allyl and amine-

bound Aloc groups in the solution and solid phase synthesis of nucleic acids simultaneously, using tris(dibenzylideneacetone)dipalladium(0), PPh_3 , butylamine and formic acid in THF.¹⁴⁷ Schmidt *et al.* have reported simultaneous cleavage of the amine-bound Aloc and the carboxyl-bound allyl groups of a linear peptide using $\text{Pd}(\text{PPh}_3)_4$ /morpholine/THF¹²² as have Arnould *et al.* who cleaved an allyl ester and a doubly Aloc protected amino function of a carbapenem antibiotic using $\text{Pd}(\text{PPh}_3)_4$ /dimedone/THF.¹⁴⁸

2.4.2. Dimedone-based protecting groups

In 1993 Bycroft *et al.* introduced Dcm (*N*-4,4-dimethyl-2,6-dioxocyclohexylidene-methyl), the prototype of a series of novel temporary non-urethane based *N*-protecting groups.¹⁴⁹ Its precursor was formed by reacting dimedone with *N,N*-dimethylformamide dimethylacetal¹⁵⁰ and the resulting 5,5-dimethyl-2-(dimethylaminomethylene)cyclohexane-1,3-dione was treated with amino acids in ethanol at room temperature yielding stable crystalline derivatives (Figure 2.25).

Figure 2.25. Synthesis of Dcm and its incorporation into amino acids

The removal of the Dcm protecting group was successfully carried out with 1 % and 0.1 % hydrazine hydrate solutions (v/v) in DMF over 10 and 20 minutes, respectively. The reaction is driven by the formation of the 1,2-diazole (Figure 2.26). It was discovered that Dcm could be removed under Fmoc deprotection conditions

making it incompatible with Fmoc SPPS, although the piperidine-aided deprotection of Dcm required treatment for 20 minutes, which is longer than that required for the removal of Fmoc.

Figure 2.26. Mechanism for the deprotection of Dcm

Bycroft *et al.* also introduced the Dde group (*N*-1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl),¹⁵¹ synthesised from dimedone and acetic acid with DCC and DMAP.¹⁵² Its reactivity with amino acids was readily shown by the side chain derivatisation of Fmoc-Lys-OH in ethanol. The deprotection of the Dde group was carried out by treatment with a 2 % hydrazine hydrate solution in DMF under continuous flow conditions for 3 minutes (Figure 2.27). The base stability of the Dde

group was found to be superior to that of its predecessor. A peptidyl-resin containing a Lys residue with its side chain protected with Dde was treated with a 20 % piperidine solution in DMF for 2 and 4 hours and showed a mere 3 % and 6.5 % loss of the protecting group, respectively. It was argued that a total exposure time of 2 hours with 20 % piperidine represents 17 x 7 minutes of Fmoc deprotection cycles on a peptide synthesiser; this would allow the synthesis of medium-sized peptides with insignificant loss of the Dde group.

Figure 2.27. Synthesis of Dde, its incorporation onto Fmoc-Lys-OH and its deprotection

The isovaleryl derivative of Dde (*N*-1-(4,4-dimethyl-2,6-dioxocyclohexylidene)isovaleryl), previously known as Ddiv and currently known as ivDde (Figure 2.28), was developed mainly due to the recurring problem of Dde migration in peptides with unprotected Lys side chains during Fmoc deprotection.¹⁵³ It was observed that neat DMF displaces the Dde group and the presence of piperidine during Fmoc deprotection creates an unstable piperidine-Dde adduct which in turn undergoes

nucleophilic attack by an adjacent unprotected Lys side chain. Although the problem could be alleviated by using DBU for Fmoc removal, the introduction of the more hindered ivDde protecting group eliminates the problem almost completely.¹⁵⁴

Figure 2.28. Structure of ivDde

Dcm, Dde and ivDde are primary amino protecting groups. The use of a pair of protecting groups in SPPS – one each for amino and carboxyl functions – could be useful for simultaneous deprotection, as seen for the allyl/Aloc, Bz/Z, Fm/Fmoc and ^tBu/Boc pairs. The novel Dab (*N*-Dde-4-aminobenzyl) carboxylic acid protecting group was therefore developed, based on the safety-catch principle, utilising the lability of the *N*-Dde and 4-aminobenzyl ester functions.¹⁵⁵ It was synthesised from 4-aminobenzyl alcohol and Dde-OH, and the carboxylic acid moiety was subsequently esterified with the benzyl alcohol derivative *via* activation as the pivaloyl mixed anhydride in very good yields (Figure 2.29).

The deprotection of Dab is a two-step mechanism involving hydrazinolysis removal of the Dde moiety followed by spontaneous collapse of the 4-aminobenzyl ester group *via* a 1,6-elimination.^{155, 156} This, combined with the knowledge that the Dde group is mildly unstable to 20 % piperidine in DMF suggested that the Dab group, also, would be unstable to Fmoc deprotection condition. This was certainly the case as Bycroft *et al.* discovered a 13 % loss of the Dab group after exposure to 20 % piperidine for 1 hour.¹⁵⁶ The Dmab (4-[*N*-(1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)-3-methylbutyl)amino]benzyl) protecting group was developed mainly due to the relative instability of the Dab group to Fmoc deprotection conditions.¹⁵⁶ Due to the superiority of the ivDde group compared to its Dde predecessor, the Dmab group was developed by synthesising 4-aminobenzyl alcohol with 2-(3-methylbutyryl)dimedone in THF under reflux. Dmab protection of the carboxylic

acid of Fmoc-Glu(O^tBu)-OH using the resulting benzyl alcohol derivative was successfully carried out with DCC, HOBT and DIPEA in DCM at room temperature for 18 hours. Its deprotection mechanism is identical to that of Dab (Figure 2.30).

Figure 2.29. Synthesis of Dab and its incorporation onto amino acids

All the dimedone based protecting groups – Dcm, Dde, ivDde, Dab, Dmab – have been used in Fmoc SPPS as part of a triply orthogonal protecting group system, protecting α -amino and α -carboxyl groups, as well as side chains.^{149, 151, 153, 155, 156, 157, 158, 159, 160, 161}

The use of Dmab in SPPS as a side chain protection to Glu is not without problems. Johnson *et al.* attempted to use it as an orthogonal side chain protecting group for the synthesis of side chain to side chain lactam bridged peptides.¹⁶¹ The use of Dmab resulted in the formation of the pyroglutamyl ring *via* an intramolecular nucleophilic attack of the secondary *N*-terminus to the activated γ -carboxyl of Glu in the presence of base (Figure 2.31). This shortens the peptide and contaminates the product, where the truncated by-product was found to be 15 % of the final product.

Figure 2.30. Synthesis of Dmab and its protection of and deprotection from amino acids

Figure 2.31. Formation of the pyroglutamyl ring on Glu

There were also by-products that corresponded to the uncyclised peptide with an excess mass of 105. The limiting step for the Dmab deprotection is the disintegration of the 4-aminobenzyl ester group. Despite its ephemeral existence, the species was isolated by HPLC. This prevents the sufficient activation needed for the γ -carboxyl of Glu hence hindering cyclisation with the Lys side chain (Figure 2.32).¹⁶¹

Figure 2.32. Unsuccessful cyclisation due to incomplete removal of Dmab

Another problem discovered with the use of dimedone based protecting groups was their partial incompatibility with allylic protecting groups. It was shown that the deprotection of Dde with hydrazine hydrate in the presence of Aloc, even for 5 minutes, led to some reduction of the Aloc double bond.¹⁵⁹ This then, in turn, led to the failure of the subsequent Aloc deprotection step. It was thought that the cause of the problem was the presence of a small amount of diimide impurity in hydrazine hydrate, often formed *in situ*, arising from traces of oxidising agents or base. This problem, however, can be solved by means of degassing the solutions used and shortening deprotection times. However, the most advantageous method involved the use of 200 equivalents of allyl alcohol, acting as scavengers to the diimides. This made the dimedone based protecting groups completely orthogonal to the Fmoc/^tBu/allyl system.

2.5. Synthesis of cyclic and polycyclic peptides

Numerous advances and improvements have been made in SPPS over the last 3 decades since Merrifield first realised the use of insoluble resin beads for the assembly of amino acids to synthesise peptides. With the discovery of the lantibiotics, and the biological importance of conformationally restrained peptides, the synthesis of polycyclic peptides has been and remains a challenging undertaking for synthetic chemists in the peptide field. Nisin was synthesised *via* fragment condensation of individually prepared monocyclic structures in solution to produce the target peptide (see section 2.6.1).¹⁶² This is the more common method of synthesising polycyclic peptides.

The oxime resin¹⁶³ has been widely used for the synthesis of monocyclic peptides. The nature of the utilisation of the oxime resin for the synthesis of cyclic peptides does not allow for the synthesis of polycyclic peptides. Its mechanism for an on-resin peptide cyclisation leads to a concomitant detachment of the peptide from the resin (Figure 2.33). The incorporation of additional cyclic structures would have to be carried out *via* segment condensation in solution.

Figure 2.33. Peptide cyclisation mechanism on an oxime resin

Lee¹⁶⁴ synthesised an octadepsipeptide using the oxime resin in high yields. Although not eventually part of a bigger peptide structure, the synthesis of the

peptide clearly highlights the limiting use of the oxime resin. In fact, Kaiser *et al.*¹⁶³ and Ösapay and Taylor^{165a} demonstrated that fully protected peptide fragments may be synthesised using the oxime resin, which are then combined *via* segment condensation to produce the final target peptides.

2.5.1. Multiply-orthogonal protecting group strategies for SPPS

An orthogonal protecting group system is one where the protecting groups may be removed independently of each other. The use of two orthogonal protecting groups is known as a two-dimensional orthogonal protecting group system and theoretically this can be as high as a six-dimensional orthogonal system with the wide availability of resins and linkers with varying degrees of sensitivities to acids, bases, metal catalysts, nucleophiles and U.V. radiation.

There have been numerous reports on the on-resin synthesis of mono- and polycyclic peptides utilising the orthogonality of various protecting groups. These cyclic peptides (Figure 2.34) are formed either with head-to-tail (a), tail-to-side chain (b), side chain-to-head (c) or side chain-to-side chain (d) cyclisation. These cyclisation strategies have recently been comprehensively reviewed^{165b} and the following section therefore gives a brief overview of examples for each pathway.

Figure 2.34. On-resin peptide cyclisation: (a) head-to-tail (b) tail-to-side chain (c) side chain-to-head and (d) side chain-to-side chain

Albericio *et al.* reported the synthesis of monocyclic peptides utilising a three-dimensional orthogonal protecting group strategy with side chain attachment to the resin and a head-to-tail cyclisation (Figure 2.35).¹⁴² Cyclisation was achieved using BOP in the presence of HOAt and a base. The use of an Fmoc/^tBu/allyl system is perhaps the most commonly used three-dimensional orthogonal protecting group strategy.

Albericio and Carpino *et al.* synthesised a monocyclic peptide, again utilising the orthogonal properties of Fmoc/^tBu/allyl with head-to-tail cyclisation carried out using the superior coupling reagents PyAOP-HOAt (Figure 2.36).¹¹⁴

Figure 2.35. On-resin head-to-tail cyclisation *via* Lys side chain anchoring

Figure 2.36. On-resin head-to-tail cyclisation *via* Asp side chain anchoring

Sharma *et al.* also reported the synthesis of monocyclic peptides utilising a three-dimensional orthogonal protecting group strategy, but with a side chain-to-head cyclisation (Figure 2.37).¹⁰⁰ The synthesis was carried out utilising the orthogonality of the Fmoc, Boc/^tBu and Dde protecting groups.

Figure 2.37. Solid phase synthesis of a monocyclic peptide

Bloomberg *et al.* reported the use of a four-dimensional protecting group strategy with a “tail-to-side chain” peptide cyclisation (Figure 2.38).¹²⁹ The synthesis was carried out utilising the orthogonality of the Fmoc, Boc/^tBu, allyl and Dde protecting groups and cyclisation was achieved with the use of HBTU, HOBt and base.

Taylor and Zhang reported the use of a four-dimensional protecting group strategy with double side chain-to-side chain cyclisation steps, creating a bi-cyclic peptide (Figure 2.39).¹⁶⁶ The ingenious manipulation of protecting groups – Boc/allyl and

Aloc/Fm and Fmoc/Bzl and ClZ – made way for the synthesis of this overlapping bi-cyclic peptide. Cyclisations *via* lactam bridge formation were carried out using BOP.

Figure 2.38. Synthesis of a branched cyclic peptide *via* a three-dimensional orthogonal protecting group strategy

Figure 2.39. Synthesis of an overlapping bi-cyclic peptide

2.6. Synthesis of lanthionine-containing peptides

There have been numerous reports on the synthesis of lanthionine-containing analogues of biologically active peptides, as described in section 1.4. and one report of the total synthesis of a complete lantibiotic, nisin.

2.6.1. Sulfur extrusion

This sulfur extrusion strategy was successfully employed by Shiba *et al.*,¹⁶² in the presence of amino acids “under” the thioether bridge instead of the ethylene glycol tether in Figure 1.16, in their total synthesis of nisin. So far nisin is the only lantibiotic that has been successfully synthesised. All five ring structures of nisin were individually synthesised (with the exception of rings D and E which were synthesised as a bi-cyclic peptide) *via* solution peptide synthesis, employing Boc methodology, in the form of disulfide cyclic peptides. One sulfur atom from each cyclic structure was then extruded, using *tris*(diethylamino)phosphine, forming the desired thioether linkage and the rings were assembled in solution *via* segment condensation forming the target lantibiotic (Figure 2.40).

Figure 2.40. Schematics for the synthesis of nisin

2.6.2. Biomimetic approach

The enkephalin opioid analogue shown in Figure 2.41 was synthesised on the solid phase with cyclisation achieved *via* side-chain Michael addition as described in section 1.5.2.⁵⁰

Similarly, Bradley *et al.* applied the strategy on peptides (subtilin ring B) containing dehydroalanine and cysteine residues and achieved regio- and stereospecific cyclisation, mimicking the biosynthesis of lantibiotics (Figure 2.42).¹⁶⁷ It seems that in a synthesis resulting in a constrained, cyclic environment the formation of the thioether bridge may favour the correct stereochemistry, forming the desired *meso*-lanthionine moiety, which was also observed in the synthesis of ring E of subtilin.

Figure 2.41. Synthesis of a lanthionine analogue of an enkephalin opioid peptide

Figure 2.42. Formation of ring B of subtilin/nisin *via* a stereospecific Michael addition

However, the same methodology applied to the synthesis of ring A of subtilin produced a diastereoisomeric mixture of peptides, although the Michael addition was completely regiospecific in the presence of two dehydroalanine residues.

Despite the potential of this approach it is difficult to envisage its application to the synthesis of overlapping lanthionine- or 3-methylanthionine-bridged peptides, which are widespread and prominent in most lantibiotics.

2.6.3. Serine β -lactones

The use of a lanthionine monomer, synthesised from serine- β -lactone derivatives, in the synthesis of cyclic peptides has also been reported.^{52, 59, 61, 62} In each case the amino acid was synthesised along with its thioester by-product, as described in section 1.5.3. Lan-7 was synthesised in solution from lanthionine prepared *via* this method (Figure 2.43).⁵⁹

Figure 2.43. Synthesis of Lan-7 in solution

The lanthionine-somatostatin analogue in Figure 2.44 was also prepared from lanthionine synthesised from a serine- β -lactone derivative.⁶

Figure 2.44. Synthesis of a lanthionine-somatostatin analogue

2.6.4. β -Haloalanine derivatives

The approach with the use of a cysteine-haloalanine coupling has also been used to synthesise lanthionine-containing cyclic peptides. In the synthesis of a β,β -dimethylanthionine analogue of an opioid enkephalin,¹⁶⁸ an iodoalanine derivative was treated with a peptide containing a β,β -dimethylcysteine in the presence of cesium carbonate to form a lanthionine analogue, within a short peptide chain. The cyclic structure was formed by cleavage of the peptide from the resin followed by cyclisation (Figure 2.45). There was no indication, however, on the presence of

conformers at the iodoalanine derivative stage, which might have led to conformers in the peptide.

Figure 2.45. Synthesis of a β,β -dimethylanthionine analogue of an enkephalin opioid

In theory, this particular route could be applied to synthesise polycyclic lanthionine-containing peptides, especially ones that are more globular in conformation much like Type B lantibiotics, with some modifications to the choice of protecting groups.

In addition, there has also been a report of the use of a bromoalanine derivative in a peptidic on-resin macrocyclisation.^{53, 54} Both enantiomers of serine were used in the

assembly of the linear peptide with triphenylmethyl-protected cysteine residues. The serine residues were then converted to β -bromoalanine residues, allowing thiolate attack of the cysteine (Figure 2.46). The peptide is a cyclic analogue of an opioid enkephalin.

The use of both (*S*)- and (*R*)-serine, resulting in (*R*)- and (*S*)-bromoalanine, respectively, produced the same peptide with the correct lanthionine stereochemistry as seen in lantibiotics. It was concluded that the cyclisation *via* thioether formation went through a dehydroalanine intermediate resulting in a stereospecific Michael addition as reported by Bradley *et al.*¹⁶⁷

Figure 2.46. Lanthionine macrocyclisation by *in situ* activation of serine

The lanthionine-type cyclic peptide shown in Figure 1.12 was synthesised, on solid phase *via* Fmoc methodology, from a TBDMS-protected homoserine which was converted into a chloro-homoalanine with subsequent cyclisation with cysteine thiolate in the presence of base (Figure 2.47).⁶⁵

Figure 2.47. Synthesis of a thioether cyclic peptide

Chapter 3

Introduction III

3.1. Nucleophilic ring opening of aziridine-2-carboxylates

Aziridine-2-carboxylates – either activated or not - can serve as key synthetic intermediates, to various unusual amino acids. This is due to their strained ring structure, analogous to other 3-membered cyclic ring structures such as cyclopropane and epoxides. Their susceptibility to various nucleophilic attacks has been reported.

Both soft and hard nucleophiles have been shown to ring-open aziridine-2-carboxylates with varying results. The nucleophilic attack could occur at one of two

positions on the ring – C_2 or C_3 – resulting in a C_2 -N or C_3 -N scission, respectively (Figure 3.1). Hard nucleophiles include amines, alcohols, azides and carbon nucleophiles and soft nucleophiles that have been used in the ring opening reactions of aziridine-2-carboxylates include thiols and halogens.

Figure 3.1. Nucleophilic ring opening at C_2 or C_3 of aziridine-2-carboxylates

One of the most common type of carbon nucleophiles is from organometallic reagents.^{169, 170, 171} Baldwin *et al.* demonstrated that (*R/S*)-1-benzyl-aziridine-2-carboxylic acid ^tbutyl ester undergoes nucleophilic ring opening by the organocuprate reagent ⁿBuCu(CN)Li₂ in the presence of boron trifluoride etherate in modest yields (Figure 3.2). (*S*)-1-Tosyl-aziridine-2-carboxylic acid ^tbutyl ester can undergo ring opening by various Grignard reagents in the presence of a catalytic amount of CuBr.SMe₂. This suppressed the competing nucleophilic attack at the ester group on C_1 .¹⁷⁰ Both types of reactions yielded a mixture of products from nucleophilic attack at C_2 (α -amino acid) and C_3 (β -amino acid) in moderate yields.

Figure 3.2. Reaction of an aziridine-2-carboxylate with ⁿBuCu(CN)Li₂

Young and Church, on the other hand, treated (*S*)-1-tosyl-aziridine-2-carboxylic acid with various organocuprate reagents in the absence of Lewis acids, yielding only the α -amino acid products (Figure 3.3).¹⁶⁹

Figure 3.3. Reaction of an aziridine-2-carboxylic acid with various organocuprates

Baldwin *et al.* also demonstrated that the proportion of products from C_2 and C_3 attacks from (*S*)-1-tosyl-aziridine-2-carboxylic acid ^tbutyl ester varies with temperature and with the Grignard reagent (Figure 3.4).¹⁷¹ The bulky ⁿbutyl Grignard reagent prefers attack at C_3 whereas the methyl prefers attack at C_2 , at $-25\text{ }^{\circ}\text{C}$. It also seems that there is an optimal temperature for maximisation of yields.

Figure 3.4. Reaction of an aziridine-2-carboxylate with Grignard reagents in the presence of copper(I) bromide-dimethylsulfide

Phosphonium ylides may also act as carbon nucleophiles. Baldwin *et al.* treated (*S*)-aziridine-2-carboxylic acid methyl ester derivatives with (triphenylphosphoranylidene)acetic acid ethyl ester in toluene and yielded ylide products from C_2 -*N* and C_3 -*N* scissions of the ring.¹⁷² It was also observed that *N*-amide ($\text{COC}_6\text{H}_4\text{NO}_2$ -*p*) and *N*-sulphonyl ($\text{SO}_2\text{C}_6\text{H}_4\text{Me}$ -*p*) aziridine derivatives produced more of the C_3 -*N* cleavage product (yielding α -amino acids) relative to the C_2 -*N* cleavage product and with higher combined yields, whilst *N*-urethane ($\text{CO}_2\text{CH}_2\text{C}_6\text{H}_4\text{NO}_2$ -*p* and $\text{CO}_2\text{CH}_2\text{Ph}$) aziridine derivatives yielded exclusively the C_3 -*N* cleavage product but with much lower yields (Figure 3.5). This is presumably due to the different electron withdrawing capacity of the two different types of protecting groups.

Figure 3.5. Reaction of an aziridine-2-carboxylates with a Wittig reagent

The ^{11}C -labelled cyanide nucleophilic ring opening of (*R/S*)-1-*t*-butoxycarbonyl-aziridine-2-carboxylic acid methyl ester has also been reported (Figure 3.6).¹⁷³

Figure 3.6. Aziridine-2-carboxylate reaction with tetrabutylammonium [^{11}C]cyanide

Oxygen nucleophiles (R-OH or H_2O) have also been used to ring open aziridine-2-carboxylates. Harada and Nakamura^{174, 175} and Young *et al.*¹⁷⁶ demonstrated that

(2*S*,3*S*)-1-[(*S*)-1-phenylethyl]-aziridine-3-methyl-2-carboxylic acid methyl ester and (*R*)-1-triphenylmethyl-aziridine-2-carboxylic acid methyl ester, respectively, could be converted to β -hydroxy- α -amino acids (Ser or Thr) by treatment with water in the presence of acid (Figure 3.7). In each case, the β -amino acids - resulting from nucleophilic attack at C_2 - were not observed.

Figure 3.7. Hydration of aziridine-2-carboxylates

The nucleophilic ring opening of aziridine-2-carboxylates by alcohols is also highly regiospecific. Okawa *et al.*¹⁷⁷ and Vessiere *et al.*¹⁷⁸ have all reported on the attack of alcohols on aziridine-2-carboxylates – successful only in the presence of boron trifluoride etherate - leading exclusively to C_3 - N cleavage (Figure 3.8).

Figure 3.8. Reaction of aziridine-2-carboxylates with various alcohols

Ben-Ishai *et al.* highlighted the same mode of nucleophilic attack of methanol towards several derivatives of (*R/S*)-aziridine-2-carboxylic acid methyl ester but in

the presence of a catalytic amount of sulphuric acid instead of boron trifluoride etherate (Figure 3.9).¹⁷⁹

Figure 3.9. Reaction of aziridine-2-carboxylates with methanol

Nitrogen nucleophiles that have been used for the ring opening of aziridine-2-carboxylates include amines and azides. Shiba *et al.* demonstrated the nucleophilic ring opening of (*R*)-1-tosyl-aziridine-3-(*N*-^tbutoxycarbonyl)ethyleneamino-2-carboxyl amide *via* ammonolysis only at C₃ on the ring (Figure 3.10).^{180, 181}

Figure 3.10. Reaction of a C₃-substituted aziridine-2-carboxyl amide with ammonia

Okawa *et al.* theorised that the concept could be extended to the reaction of primary amines (such as benzylamine) and secondary amines (such as diethylamine) on the peptide Z-Gly-Azi-Gly-OBz where Azi denotes an aziridine-2-carboxyl amide moiety.¹⁸² However only diethylamine reacted at the C₃ position to give the substituted peptide without any by-products (Figure 3.11).

Figure 3.11. Reaction of an aziridine-containing peptide with diethylamine

However, Hashimoto *et al.* successfully utilised the free amino group on L-His and reacted it with (2*S*,3*S*)-1-tosyl-aziridine-3-methyl-2-carboxylic acid methyl ester yielding both *C*₂ and *C*₃ cleavage products (Figure 3.12).¹⁸³

Figure 3.12. Reaction of an aziridine-2-carboxylate with the amino function of L-His

Ha *et al.* demonstrated that azides could also be used as nitrogen nucleophiles in ring opening reactions of (2*S*,3*S*)-1-[(*S*)-1-phenylethyl]-aziridine-3-methyl-2-carboxylic acid methyl ester (Figure 3.13).¹⁸⁴

Figure 3.13. Reaction of an aziridine-2-carboxylate with trimethylsilyl azide

Soft nucleophiles that have been used in the ring opening reactions of aziridine-2-carboxylates include thiols and halogens. *S*-methyl cysteine derivatives were synthesised by reaction of various (*R/S*)-aziridine-2-carboxylic acid methyl ester derivatives with methylmercaptan in the presence of boron trifluoride etherate exclusively *via* attack at *C*₃ of the aziridine ring (Figure 3.14).¹⁷⁹

Figure 3.14. Reaction of an aziridine-2-carboxylate with methylmercaptan

Okawa *et al.* have used various thiol nucleophiles for ring opening reactions of aziridine-2-carboxylates. The use of thiobenzoic acid in the presence of boron trifluoride etherate yielded only the α -amino acid derivatives.⁸⁰ The use of alkyl and aromatic thiols on Z-protected aziridine-2-carboxylic acid benzyl ester derivatives also produced the S-substituted cysteine derivatives in very good yields (Figure 3.15).¹⁸⁵

Figure 3.15. Reaction of aziridine-2-carboxylates with various thiols

Interestingly the use of Z-L-Cys-OBz as nucleophile in the presence of boron trifluoride etherate was less successful – the lanthionine products were isolated in yields ranging from 12 – 37 % only (Figure 3.16). There was no indication if the products resulting from C₂ thiol attack were isolated or even present.

Parry and Naidu¹⁸⁶ and Baldwin *et al.*¹⁸⁷ have all demonstrated that thiol nucleophilic attacks on aziridine-2-carboxylates yielded the α -amino acid derivatives with no indication of the products for C₂ attack (Figure 3.17).

Figure 3.16. Formation of lanthionine and 3-methylanthionine derivatives

Figure 3.17. Reaction of an aziridine-2-carboxylate with a racemic thiol

Hata and Watanabe treated both (*R*)- and (*S*)-aziridine-2-carboxylic acids and (2*R*,3*R*)- and (2*S*,3*S*)-aziridine-3-methyl-2-carboxylic acids with thiol nucleophiles such as thiophenol and cysteine in a phosphate buffer and isolated both α - and β -amino acids with the latter being the predominant product.⁷⁹ However, when the reactions with thiophenol were carried out in 95 % acetonitrile in water and in 99 % DMF in water the results were reversed, yielding 1.1:1 and 2:1 proportions, respectively, for the (α : β) amino acids. In the phosphate buffer, the lanthionine and 3-methylanthionine derivatives produced were present in only between 20 – 29 % of the total Cys-Azi coupling products (Figure 3.18).

Figure 3.18. Formation of lanthionine and 3-methylanthionine derivatives

Crousse *et al.* utilised a C_3 -substituted (2*S*,3*S*)-1-benzyl-aziridine-3-trifluoromethyl-2-carboxylic acid ethyl ester and various thiols in the presence of acid to produce exclusively the α -thio β -amino acid derivatives (Figure 3.19).¹⁸⁸

Figure 3.19. Reaction of a trifluoromethyl C_3 -substituted aziridine-2-carboxylate with various thiols

Halogens have been used as nucleophiles in ring opening reactions of aziridine-2-carboxylates. Wade *et al.* treated (2*R*/*S*,3*R*/*S*)-aziridine-3-phenyl-2-carboxylic acid isopropyl ester and (2*R*/*S*,3*R*/*S*)-aziridine-3-methyl-2-carboxylic acid ^tbutyl ester with hydrogen fluoride in the presence of base. This yielded the β -fluorinated alanine derivatives, with no indication of the presence of regioisomers (Figure 3.20).^{189, 190}

Figure 3.20. Reaction of an aziridine-2-carboxylate with hydrogen fluoride

Righi and D'Achille demonstrated that C_3 -substituted (2*R*,3*R*)-1-ethoxycarbonyl-aziridine-2-carboxylic acid methyl/ethyl esters undergo nucleophilic ring opening by metal halides at room temperature.¹⁹¹ Magnesium bromide was shown to ring open the rings at the C_3 position ($C_3:C_2$ 99:1). Similar results were obtained with magnesium iodide; however, the use of sodium iodide and sodium bromide with Amberlyst 15 in acetone at $-40\text{ }^\circ\text{C}$ gave a mixture of products, predominantly the β -amino acids, where up to 1:1 proportion of $C_2:C_3$ attack products were observed. Substitution of C_3 with bulky groups (isopropyl, cyclohexyl) yielded almost exclusively β -amino acids (Figure 3.21).

R	R'	MX _n	A:B
ⁿ Pr	Me	MgBr ₂	99:1
c-Hex	Me	MgBr ₂	99:1
Ph	Me	MgBr ₂	99:1
c-Hex	Et	NaI	1:99
ⁱ Pr	Et	NaI	1:99
ⁿ Pr	Et	NaI	1:3
ⁿ Pr	Et	NaBr	22:73
Ph	Et	NaI	1:1

Figure 3.21. Reaction of aziridine-2-carboxylates with metal halides

Crousse *et al.* utilised trifluoromethyl C_3 -substituted (2*S*,3*S*)-1-benzyl-aziridine-2-carboxylic acid ethyl ester to produce the α -chloro- β -amino acid derivative (Figure 3.22).¹⁸⁸

Figure 3.22. Reaction of an aziridine-2-carboxylate with hydrogen chloride

C_3 -substituted (ethyl/phenyl) and C_2 -substituted (methyl) (2*R*,3*S*)-1-tosyl-aziridine-2-carboxylic acid methyl ester derivatives have also been subjected to nucleophilic ring opening as a subsequent reaction following reduction with lithium aluminium hydride.¹⁹² The products obtained were then oxidised to form the α,β -substituted β -amino acids (Figure 3.23).

Figure 3.23. Reduction of an aziridine-2-carboxylate

In all of the examples given above retention of stereochemical integrity was observed, except for reactions using racemic mixtures of aziridine-2-carboxylates where retention or inversion would be difficult to predict.

From all the above examples it seemed that X (Figure 3.24) needs to be an electron-withdrawing group to avoid nucleophilic attack at C_1 (when $Y \neq H$) by making C_2 and C_3 more electrophilic. It also seemed that the electrophilicity of C_2 is decreased relative to C_3 when Y is a hydrogen (i.e. the acid rather than the ester). Bulky substituents on either C_2 or C_3 increase the susceptibility of C_3 or C_2 , respectively, to nucleophiles. The different types of nucleophiles also contribute to the manner of ring opening reactions of aziridine-2-carboxylates.

Figure 3.24. Aziridine-2-carboxylate system

The use of Lewis acids such as boron trifluoride etherate, CuBr.SMe and perchloric acid promotes ring opening reactions, presumably *via* the mechanism illustrated in Figure 3.25.

Figure 3.25. Proposed mechanism for Lewis acid-mediated C_3 ring opening of aziridine-2-carboxylates

3.2. Aims

The ultimate aim of this project was to lay the foundation for the synthesis of polycyclic lanthionine-containing peptides on the solid phase. With the dawn of methicillin-resistant staphylococcus aureus (MRSA) and staphylococcus epidermidis (MRSE) and vancomycin-resistant enterococci (VRE) the need is even greater, to find the next class of antibiotics for the future.

The total synthesis of nisin¹⁶² has not been optimised for the solid phase, although there have been reports of the synthesis of smaller lanthionine-containing analogues of biologically active peptides^{6, 50, 52, 53, 54, 59, 61, 62, 168} and monocyclic fragments of lantibiotics¹⁶⁷ on solid phase. As discussed in Chapter 1, previous syntheses of

lanthionine-containing peptides were carried out either *via* a lanthionine residue incorporation into a peptide, often with concomitant cleavage of peptide upon cyclisation or on-resin thioether bond formation between two side chains, some with inefficient stereochemical control.

This project aimed to synthesise differentially protected lanthionine residues for solid phase synthesis utilising Fmoc methodology and to incorporate them into cyclic and polycyclic lanthionine-containing peptides. It was also of interest to synthesise these peptides solely *via* continuous flow peptide synthesis. The orthogonality of Fmoc, ^tBu or Boc and allylic protecting groups proved to be an attractive choice due to its precedence and widespread use in SPPS.^{129, 141, 142, 143, 144, 145, 146} In this project, its usage is highlighted in Figure 3.26 where target molecule **1** is used as a double-headed amino acid residue incorporated into peptides.

Figure 3.26. Target molecule **1** for SPPS

The proposed approach for synthesising lanthionine containing peptides on solid phase for this project is outlined in Figure 3.27.

Figure 3.27. General scheme for proposed synthesis of lanthionine containing peptides, using a linker yielding peptide amides

The orthogonality of the allyl ester and Aloc groups to Fmoc and $t\text{Bu}$ allows their selective removal from the residue's second amino acid functionality. Due to the liberation of the *N*-terminal amine (Fmoc removal) and carboxyl function (allyl deprotection), cyclisation of the peptide could be attempted in the presence of a suitable coupling reagent. With the availability of the other *N*-terminal amine (Aloc removal), the peptide could be further extended. This novel methodology for lanthionine-containing peptides would allow for an unprecedented, solely on-resin synthesis of polycyclic lanthionine-containing peptides.

For the synthesis of overlapping bi-cyclic lanthionine/3-methylanthionine structures encountered in lantibiotics (such as the rings D and E of nisin), a second, orthogonally protected lanthionine was required. It was envisaged that target molecule **2** (Figure 3.28) would be used as a second, differentially protected

lanthionine structure to be incorporated into a linear peptide, prior to cyclisation of the first lanthionine (Figure 3.29).

2

Figure 3.28. Target molecule **2** for SPPS

The unique combination of protecting groups for **1** and **2** would facilitate the synthesis of lanthionine containing peptides with overlapping thioether bridges, and hence lantibiotics.

Figure 3.29. General scheme for the proposed synthesis of lanthionine containing peptides with overlapping thioether linkages, using a linker yielding peptide acids

Chapter 4

Results and Discussion I

The aim of this project was to synthesise lanthionine-containing peptides, from orthogonally protected lanthionine residues, on the solid phase. The high yielding strategy of coupling an iodoalanine derivative with a cysteine derivative⁷⁶ was chosen as the method to synthesise the orthogonally protected lanthionine. As discussed in Chapter 1, this approach appeared to be the most direct and reproducible, and had already been investigated with a range of protected iodoalanine derivatives. The observations that two conformers were present in a number of key compounds gave rise for concern, as it was far from clear why derivatives such as *N*-triphenylmethyl- β -iodoalanine methyl ester⁷⁶ should exist in

two locked conformations, despite the bulky triphenylmethyl group and the large iodine atom. However, in the original paper, extensive evidence supporting the chiral integrity of the lanthionine residues had been presented, as discussed in Chapter 1.

The original work of Dugave and Menez was followed closely initially, but with an allyl group on the iodoalanine, providing a lanthionine template for further protecting group manipulations. To compare structural and spectral properties, syntheses of both the unnatural and natural lanthionine derivatives **(R,R)-10** and **(S,R)-10** were attempted. The general scheme for the synthesis is outlined in Figure 4.1.

Figure 4.1. General scheme for the synthesis of lanthionine derivatives

A critical discovery was made at later stages of the peptide synthesis, which completely invalidated the concept of locked conformations in the acyclic system of *N*-triphenylmethyl- β -iodoalanine derivatives reported by Dugave and Menez. Extensive NMR analyses have proven that the two conformers reported were in fact a mixture of the desired compound and its regioisomer (α -iodo- β -amino acid derivative, Figure 4.2) – present as a mixture of enantiomers – with the regioisomer being the predominant product. In this chapter the initial synthetic work is presented, with preliminary findings, thoughts and ideas. In Chapter 5, a complete analysis of the problem is discussed, with NMR and other analyses that led to the above conclusion.

Figure 4.2. Structure of (*R/S*)-2-iodo-3-(triphenylmethylamino)-propionic acid allyl ester, an α -iodo- β -alanine derivative

4.1 Synthesis of protected cysteine 9

Protection of the carboxyl groups of cystine as their ^tbutyl esters (Figure 4.3) was first carried out following a literature procedure.¹⁹³ Initial low yields of the desired product arose from the inefficiency of the reported work-up procedure, which was simplified to yield the doubly esterified compound, in excellent yields. Work carried out within the group indicated that only the use of the potentially explosive perchloric acid led to the solvation of cystine.⁷⁸

Figure 4.3. Trans-esterification of (*R,R*)-cystine with ^tbutyl acetate

The amino groups on **7** were then protected with the Fmoc group and the product purified by recrystallisation (Figure 4.4).¹⁹⁴ The use of exactly two equivalents of

Fmoc chloride was imperative as the Fm-OH by-product - which would have resulted from quenching of excess reagent - was precipitated together with the desired compound. This required an additional purification step by flash column chromatography. Additionally, the precautionary use of base in excess of two equivalents was also necessary to prevent the formation of HCl, which would cleave the ^tbutyl group.

Figure 4.4. Fmoc protection of (*R,R*)-cystine *bis*-^tbutyl ester

The final step for preparing the first half of the lanthionine residues (*R,R*)-**10** and (*S,R*)-**10** was the disulfide bond cleavage of **8** with tri-ⁿbutylphosphine (Figure 4.5). Dugave and Menez⁷⁶ reported the use of wet THF for this reaction. In our hands the use of at least one equivalent of water in this reaction resulted in the production of the desired compound in high yields. This is due to the fact that the water molecule is required to drive the reaction forward, forming the thermodynamically stable P=O bond of the tri-ⁿbutylphosphine oxide by-product.

Figure 4.5. Disulfide reduction of *N,N'*-bis(fluorenylmethoxycarbonyl)-(*R,R*)-cystine-*bis*-^tbutyl ester

The reactions described above were reproducible at larger scales of up to 21 mmol.

4.2 Synthesis of protected (*R*)-iodoalanine (*R*)-6a

(*S*)-Serine provided the template for the second half of the lanthionine derivative (*R,R*)-**10**. The *N*-triphenylmethylation of serine was carried out following a procedure described by Theodoropoulos *et al.*¹⁹⁵ where a one-pot synthesis was

achieved (Figure 4.6). Silylation of all three functional groups of serine was followed by selective removal of the most labile trimethylsilyl protection with anhydrous methanol – at the amino function - thus enabling selective triphenylmethylation, forming **(S)-3**. The use of this temporary *N*-triphenylmethyl protection was to prevent abstraction of the α -H on the serine by a non-nucleophilic base,^{196a, 196b} which would lead to dehydration and hence the formation of a dehydroalanine derivative. Once the thioether linkage is formed the triphenylmethyl group is removed, as the risk of elimination by a base at this stage is almost non-existent.

The use of dry trimethylsilyl chloride was imperative as it affected the yield. In our hands, the use of exactly one equivalent of dry methanol in the selective removal of the amino-trimethylsilyl group instead of 1.5 equivalents as reported originally, increased the overall yield by over 5 %.

Figure 4.6. *N*-Triphenylmethylation of *(S)*-serine

The carboxyl function of the serine was then protected with an allyl group. The first attempt at allylation was carried out in dichloromethane with triethylamine as the base. This however produced the desired compound in a maximum 50 % yield.

Another method was then attempted, using phase-transfer catalysis with Aliquat 336, sodium hydrogen carbonate and allyl bromide in dichloromethane.¹⁹⁷ The reaction was quantitative by t.l.c., however the crude material proved to be impossible to purify.

Using a known allylation procedure,¹⁹⁸ involving initial treatment with caesium carbonate to form the carboxylate salt, followed by addition of allyl bromide, carboxyl protection as an allyl ester was achieved (Figure 4.7).

Figure 4.7. Allylation of *N*-triphenylmethyl-(*S*)-serine

With the successful allylation reaction, it was imperative that the use of caesium carbonate be kept to 0.5 equivalents and the allyl bromide to one equivalent relative to the amino acid, or else the doubly allyl-protected species could be formed, even with a mere 0.51 equivalents of the base and 1.1 equivalents of the bromide.

Treatment of the *N*-triphenylmethyl-(*S*)-serine allyl ester (**S**)-4 with methanesulfonyl chloride under standard conditions⁷⁶ (Figure 4.8) afforded the mesylated derivative (**S**)-5.

Compound (**S**)-5 was then treated with 10 equivalents of sodium iodide (Figure 4.9) to displace the methanesulfonyl group giving the iodoalanine derivative (**R**)-6a.

Figure 4.8. Mesylation of *N*-triphenylmethyl-(*S*)-serine allyl ester

Figure 4.9. Iodination of *N*-triphenylmethyl-(*S*)-serine(*O*-methanesulfonyl) allyl ester

The results obtained, by ^1H and ^1H -COSY NMR, concurred with the observations made by Dugave and Menez⁷⁶ with their *N*-triphenylmethyl- β -iodoalanine benzyl and methyl esters, where two isomers and an aziridine-2-carboxylate by-product (approximately 10 %) were present (Appendix A). It was noticed that the proportion of these two isomers were dependent upon the temperature at which the iodination reaction was carried out. At 15 °C over 3 days the proportion – isomers **(R)-6a** and **6b** - was 3:2 (60:40) compared to 1:2 (33:67) at 25 °C.

The aziridine-2-carboxylate derivative **(S)-11** (Figure 4.10) has an almost identical R_f to iodoalanine **(R)-6a** and its regioisomer **6b** by t.l.c., which led to difficulties in purification. After chromatographic purification to remove traces of starting material, the products – including the aziridine **(S)-11** - were dissolved in warm dichloromethane then methanol (1:5) followed by gradual cooling to room temperature, then to 4 °C and finally –20 °C when the viscous oily mixture precipitated out. The ^1H NMR spectrum of the precipitated product confirmed the absence of the aziridine by-product. However, the proportion of the isomers had changed from 1:2 to approximately 1:4 (**(R)-6a:6b**).

^1H NMR revealed that the filtrate consisted of the two isomers, (*R*)-**6a** and **6b**, and the aziridine derivative (*S*)-**11** in a 2:1:1 mixture. Further precipitation attempts failed. Attempts were also made to separate out the two isomers *via* flash column chromatography with very slow gradients but these were unsuccessful.

A hypothesis was previously proposed,⁷⁸ that restricted rotation about the $\text{C}_\alpha\text{-C}_\beta$ bond led to the formation of the two spectroscopically distinct rotational isomers. Therefore, although three different conformations were possible (Figure 4.11), two were thought to appear similar by NMR leading to the observation of only two apparent sets of signals. The same hypothesis was initially applied to (*R*)-**6a** and **6b**.

The formation of two locked conformers in this reaction, although preceded in the work of Dugave and Menez, appeared unlikely: apart from this work, restricted rotation in acyclic compounds of such a size has not, to our knowledge, been widely reported elsewhere. Careful analysis of the purified products, however, did not clarify the situation; microanalysis, high resolution mass spectrometry, ^{13}C and ^1H -COSY NMR and HPLC experiments proved to be insufficient. The hypothesis that the second isomer might have arisen from triphenylmethyl cleavage by acidic deuterated chloroform in the NMR tube was also rejected, as the ^1H NMR spectrum of the sample recorded with the use of alumina-treated deuterated chloroform produced identical results.

Figure 4.11. Fisher projections displaying three conformations of *N*-triphenylmethyl-3-iodo-(*R*)-alanine 6-nitroveratryl,⁷⁸ benzyl,⁷⁶ methyl⁷⁶ and allyl ester

Both the crude iodoalanine derivative (in the presence of the aziridine-2-carboxylate) and the precipitated product were used in the next coupling step with cysteine derivative **9**.

The reactions described above were reproducible at larger scales of up to 42 mmol.

4.3 Synthesis of protected lanthionine (*R,R*)-**10**

With the apparent success of the synthetic route to the *N*-triphenylmethyl- β -iodo-(*R*)-alanine allyl ester (*R*)-**6a** and *N*-fluorenylmethoxycarbonyl-(*R*)-cysteine ^tbutyl ester **9**, the two compounds were treated with caesium carbonate in an attempt to form the lanthionine derivative (*R,R*)-**10** (Figure 4.12).

Figure 4.12. Formation of the triphenylmethyl-derived (*R,R*)-lanthionine

The order in which the reagents were added was significant to the success of the reaction. Although caesium carbonate was needed to promote the formation of the cysteine thiolate anion, it could also, to a certain extent, remove the Fmoc group if left solely in solution with the cysteine derivative for as little as 5 minutes. It was therefore imperative to have both residues in solution before adding the caesium carbonate.

The reaction seemed to produce the desired lanthionine in respectable yields as what appeared to be two rotational isomers as determined by ¹H NMR. The proportion of the isomers was identical (1:2) to that observed for the crude iodoalanine starting material. Again, the products were carefully analysed by microanalysis, high resolution mass spectrometry, ¹³C and ¹H-COSY NMR and HPLC experiments to clarify the issue of conformational isomers, but without success.

In previous work, Goodman *et al.*¹⁹⁹ produced all four diastereoisomeric lanthionine residues (*via* serine- β -lactone route), albeit with different protecting groups, and concluded that the difference in chemical shifts between them were small but detectable by 1-D ^1H NMR (500 MHz, 300 K). The fact that the proportion of isomers in the reaction above was retained from the proportion of the iodoalanine isomers, and that there was an absence of any additional ^1H signals adjacent to the expected signals, would suggest retention of stereochemistry at both stereogenic centres as suggested by Dugave and Menez.⁷⁶ However, when the precipitated iodoalanine derivative with isomer ratio of 1:4 ((*R,R*)-6a:6b) was treated with the cysteine derivative **9** the reaction yielded the major isomer as the sole product, enabling the ^1H NMR spectrum of the previous 1:2 mixture of isomers to be unambiguously assigned (Appendix B). The reason for this observation is discussed in Chapter 5, section 5.1.2.

The protecting groups on (*R,R*)-**10** were then adjusted, to afford the target molecule. The bulky triphenylmethyl group was cleaved with 5 % trifluoroacetic acid in DCM liberating the free amine, which was not isolated but was immediately treated with allyloxycarbonyl chloride to give (*R,R*)-**12** (Figure 4.13). The low acid concentration of the first step was not strong enough to cleave the t butyl group.

Figure 4.13. Replacement of triphenylmethyl with allyloxycarbonyl on lanthionine (*R,R*)-**10**

Initial analysis of the ^1H NMR of the compound appeared to indicate that there were no longer two different isomers present, as expected from the removal of the triphenylmethyl group, which was originally thought to be responsible for the restricted bond rotations resulting in two conformers. However, careful observation revealed that the integrations for the ^1H NMR did not add up to the correct number of protons and in some cases gave integrations of less than one ^1H . Further analyses were carried out, discussed in Chapter 5, which clearly support the evidence

suggesting that the iodoalanine (**(R)**-6 in fact consisted of a mixture of regioisomers, (**(R)**-6a and (**(R/S)**-6b).

The reactions described above were reproducible at larger scales of up to 2 mmol.

Believing that the lanthionine derivative (**(R,R)**-12 had been synthesised, efforts then turned to producing the protected *meso*-lanthionine (**(S,R)**-10).

4.4 Synthesis of protected (*S*)-iodoalanine (**(S)**-6a

The synthesis of the lanthionine derivative (**(S,R)**-10 was then attempted, starting from (*R*)-serine. The *N*-triphenylmethylation of serine was carried out following the method described above. This was then followed by esterification of the carboxyl functionality with an allyl group, followed by the mesylation step as described above with the reaction carried out in THF. The next step was the iodination reaction, which produced similar results to (**(R)**-6a (Figure 4.14).

- i) $\text{Me}_3\text{Si-Cl}$, TEA, DCM, ii) MeOH (dry), 0°C , iii) $\text{Ph}_3\text{C-Cl}$, TEA, iv) MeOH, TEA, v) Cs_2CO_3 , MeOH, vi) Allyl bromide, DMF, viii) Methanesulfonyl chloride, TEA, THF, viii) NaI, acetone

Figure 4.14. Preparation of (*S*)-iodoalanine derivative (**(S)**-6a

The proportion of the isomers, (*S*)-**6a** and **6b**, prior to precipitation was 1:3 (with approximately 10 % aziridine derivative (*R*)-**11** by-product, (Figure 4.15)) and 1:5 after precipitation (Appendix C).

Figure 4.15. (*R*)-1-Triphenylmethyl-aziridine-2-carboxylic acid allyl ester

In an attempt to clarify the isomer issue, optical rotation experiments were carried out on both (*R*)- and (*S*)-iodoalanine derivatives (Table 4):

	(<i>R</i>)- 6	(<i>S</i>)- 6
$[\alpha]_D^{25} / ^\circ$	+8.9 (15 °C), 1:4 isomers	-3.3 (17 °C), 1:5 isomers

Table 4. Optical rotation values for (*R*)-**6** and (*S*)-**6** as mixtures of isomers

It should be noted that even though the two enantiomers deflect the plane of polarised light oppositely, two conformationally locked conformers would have different optical rotation values. It was therefore not surprising that the modulus of the optical rotation values ($|[\alpha]_D^{25}|$) for the two enantiomers were not identical, initially thought to be due to the difference in conformer proportions for each enantiomer. However, evidence discussed in Chapter 5 clearly demonstrates that the difference in optical rotation values between (*R*)-**6** and (*S*)-**6** (both as mixtures of isomers) was caused by a mixture of regioisomers for each enantiomer.

4.5 Synthesis of protected lanthionine (*S,R*)-**10**

With the synthetic route to the fully protected lanthionine (*R,R*)-**10** apparently resolved, the same methodology was applied in an attempt to synthesise the corresponding derivative of *meso*-lanthionine. With the same conditions as above the reaction proceeded in high yields (Figure 4.16).

Figure 4.16. Formation of the lanthionine derivative **(S,R)-10**

Again, the proportion of isomers in **(S,R)-10** was retained (1:3) from the crude iodoalanine **(S)-6**. Interestingly, the proportion of the isomers in **(S,R)-10** was also retained (1:5) from the precipitated iodoalanine derivative **(S)-6**. Small differences were observed in the ^1H NMR chemical shifts of this derivative (Appendix D) compared to its diastereoisomer **(R,R)-10**, which would suggest that the two are diastereoisomers, confirming the observations made by Goodman *et al.* concerning their very similar spectra of diastereoisomeric lanthionine derivatives differing by only one stereogenic centre.¹⁹⁹

With the difference in isomer proportions it was difficult to establish what the optical rotation for one diastereoisomer should be compared to the other (Table 5). Goodman *et al.*¹⁹⁹ reported that two identical derivatives of lanthionine diastereoisomers, which differ by only one chiral centre, possessed optical rotation values differing only in magnitude but not necessarily in sign. However, the difference in protecting groups used here would probably account for this. Further discussion in Chapter 5 examines the nature of the isomerism of the two species in each diastereoisomer.

Optical rotations were also carried out on both residues (Table 5):

	(R,R)-10	(S,R)-10
$[\alpha]_{\text{D}}^{25} / ^\circ$	+12.1 (16 °C), 1:2 isomers	-4.1 (18 °C), 1:3 isomers

Table 5. Optical rotation values for **(R,R)-10** and **(S,R)-10**

Replacement of the triphenylmethyl group with an allyloxycarbonyl protecting group then followed, as before (Figure 4.17). The ^1H NMR for this compound was virtually

identical to that of the (*R,R*)-diastereoisomer (*R,R*)-**12**. Further discussion follows, in Chapter 5.

Figure 4.17. Replacement of triphenylmethyl with allyloxycarbonyl on lanthionine (*S,R*)-**10**

The final step was deprotection of the *t*-butyl protecting group with trifluoroacetic acid (Figure 4.18).

Figure 4.18. Removal of *t*-butyl from allyloxycarbonyl-derived lanthionine (*S,R*)-**12**

The ^1H NMR of this final residue, which was ready for incorporation onto solid phase resin, produced very broad signals in deuterated chloroform and relatively broad signals in methanol- d_4 due to the free carboxyl group. It was therefore difficult to assign (Appendix E). However, high-resolution mass spectrometry and elemental analysis apparently demonstrated that the desired compound was present in high purity.

4.6 Synthesis of ivDde/Dmab protected (*S,R*)-lanthionine **2**

With the ultimate aim of this research being the synthesis of peptides with overlapping thioether bridges, as found in lantibiotics such as nisin,⁷ subtilin⁸ and plantaricin W,³⁷ the synthesis of (*S,R*)-lanthionine having protecting groups orthogonal to Fmoc, *t*Bu/Boc and allyl/Aloc was required. Due to the proven orthogonality of the dimedone-based protecting groups to Fmoc, *t*Bu/Boc and allyl/Aloc systems (Chapter 2, section 2.4.2.), the synthesis of the ivDde/Dmab-protected (*S,R*)-lanthionine **2** (Figure 4.19) was therefore attempted.

Figure 4.19. ivDde/Dmab derivative of (*S,R*)-lanthionine

The proposed strategy for synthesising ivDde/Dmab (*S,R*)-lanthionine initially involved incorporation of the Dmab group at the *N*-triphenylmethyl-(*R*)-serine (**R**)-3 stage to give **13**. The first attempt at esterification, with diisopropylcarbodiimide, 1-hydroxybenzotriazole and DIPEA in dichloromethane, was carried out using the method described by Chan *et al.*¹⁵⁶ (Figure 4.20). The attempt was unsuccessful, with recovery of the Dmab-OH and an unidentified triphenylmethyl-containing compound. It was noted that 1-hydroxybenzotriazole did not dissolve in dichloromethane. Hence a second attempt was carried out omitting this reagent, and using dicyclohexyl – instead of diisopropyl - carbodiimide where the urea by-product could now be easily removed through filtration. This attempt also proved to be unsuccessful. A third attempt was carried out using 4-dimethylaminopyridine, which, apart from being a base, is also a powerful acylation catalyst, but this also failed. It seemed that all efforts were futile. In each case only the Dmab-OH starting material was isolated (Figure 4.20).

Figure 4.20. Unsuccessful attempts at esterification of *N*-triphenylmethyl-(*R*)-serine **(R)-3** with Dmab-OH

The reason for the lack of success of the coupling of the serine with the Dmab-OH was thought to be due to the availability of the β -OH on serine. This could have led to intra- and/or intermolecular β -hydroxyl displacement of the -ODCC group. However, there is no hard evidence to explain why these reactions failed.

As an alternative, the β -OH was protected as the *t*-butyldimethylsilyl ether **14** (Figure 4.21). Esterification of **14** with Dmab-OH was then attempted using various conditions but without success.

Conversion of the Dmab-OH to the bromo analogue **15**, to allow a similar reaction with the caesium salt of *N*-triphenylmethyl-(*R*)-serine **(R)-3** to that used in the synthesis of *N*-triphenylmethyl-(*R*)-serine allyl ester **(R)-4**, was then attempted (Figure 4.22). Although the reaction produced the desired Dmab-Br, as confirmed by $[\text{M}+^{79}\text{Br}]^+$ and $[\text{M}+^{81}\text{Br}]^+$ in mass spectrometry, purification of the compound proved to be extremely problematic. After numerous flash column chromatographic attempts, this approach was abandoned.

Figure 4.21. Scheme for carboxyl protection of *N*-triphenylmethyl-(*R*)-serine **(R)-3** with Dmab-OH via TBDMS β -OH protection

Figure 4.22. Bromination of Dmab-OH

A different approach, in which the protecting groups were manipulated once at the lanthionine stage, was therefore attempted. This has the added advantage of using common intermediates up to the thioether bridge formation step.

Compound (*S,R*)-**10** (as a mixture of isomers - see Chapter 5) was treated with 5 % trifluoroacetic acid in DCM to selectively cleave the triphenylmethyl group, liberating the free amine. The resulting product was then treated, without further purification, with a solution of ivDde-OH in ethanol at 40 °C (Figure 4.23).¹⁵⁴

Figure 4.23. Replacement of triphenylmethyl protecting group with ivDde on compound (*S,R*)-**10**

The ¹H NMR of the product initially suggested the presence of one single isomer as a result of the removal of the triphenylmethyl group. However, repeated attempts at this reaction produced the desired compound in a maximum yield of 60 % only, for a batch of starting material containing isomers in a 1:3 ratio. This suggested that only one isomer had reacted with the ivDde-OH.

The search for the other isomer, either already protected with the ivDde group or not, led to the discovery of a polar species that was eluted last from the flash column. It was thought to be the other isomer present with some impurities, but without the ivDde group attached. Mass spectrometry and the colourimetric ninhydrin test

confirmed this. This crude material was then treated with the ivDde-OH as before, however the amino acid did not dissolve in the solvent used. This resulted in the failure of the ivDde-OH to couple with the free amine. Various solvents were tested for solubility properties, including water, but none dissolved the other conformer. It did, however, dissolve in deuterated dimethylsulfoxide and this confirmed, by ^1H NMR, that the crude material did contain the other isomer, de-triphenylmethylated, along with other unidentified impurities which were not separable *via* flash column chromatography. The peculiarity of one isomer reacting with the ivDde-OH but not the other was difficult to explain at the time. However, it was decided that the available ivDde-protected species should be pushed forward, prior to dealing with the solubility issue of the other isomer. Further discussion, explaining these observations, follows in Chapter 5.

With one isomer of the ivDde-protected species in hand, the allyl group was cleaved with tetrakis(triphenylphosphine) palladium(0) using the published procedure (Figure 4.24).¹²⁸ The resulting product was carried through to the next step without purification, and reacted with Dmab-OH to apparently give the desired ester (Figure 4.24).

The reaction conditions for coupling the Dmab group onto the de-allylated species **16** were identical to earlier trial reactions which had been carried out with *N*-triphenylmethyl-(*R*)-serine(*O*-*t*-butyldimethylsilyl) but had failed. The reason for this was unclear. Compound **17** was carried through to the next step without purification as several attempts to purify *via* normal phase flash column chromatography proved unsuccessful. This may be due to the difficulty of separating tetrakis(triphenylphosphine) palladium(0) debris.

Figure 4.24. Replacement of allyl with Dmab protecting group on ivDde-derived (*S,R*)-lanthionine **16**

With the ivDde/Dmab product **17** thought to be in hand, the crude material was treated with 50 % trifluoroacetic acid in DCM to cleave the ^tbutyl group (Figure 4.25).

Figure 4.25. Removal of ^tbutyl from ivDde-derived (*S,R*)-lanthionine **17**

A small amount of **2** was purified *via* preparative reverse phase HPLC for full characterisation and ready for solid phase peptide synthesis.

Chapter 5

Results and Discussion II

5.1. Investigation of the conformational isomer issue

The anomalies observed by NMR for the iodoalanine derivatives (*R*)-**6** and (*S*)-**6** and the thioether amino acid derivatives (*R,R*)-**10**, (*S,R*)-**10**, (*R,R*)-**12**, (*S,R*)-**12**, **16**, and **17** (Figure 5.1) raised concerns with regard to the stereochemical integrity of these molecules, despite the apparently proven hypothesis that these anomalies are caused by sterically locked conformational isomers.⁷⁶ With the apparent success of the synthesis of the target lanthionine derivatives **1** and **2**, an in-depth investigation on the stereochemistry of these and related compounds commenced. HPLC analyses,

further NMR experiments, test reactions and attempts at recrystallisation for x-ray structural determination were carried out, yielding unexpected results.

Figure 5.1. Iodoalanine and lanthionine targets

5.1.1. HPLC analyses

The fact that the two isomers of each iodoalanine enantiomer **(S)-6** and **(R)-6** were partially separable *via* precipitation led to HPLC studies of these compounds. Preliminary analytical normal phase HPLC suggested that separation of the two isomers of **(S)-6** and **(R)-6** could be achieved. Separation of the isomers of **(S)-6** by preparative normal phase HPLC was attempted. A gradient of 2 – 3 % ethyl acetate in hexane over 40 min gave only partial separation [samples contained approximately 9:1 of **(S)-6a:6b** and **6b:(S)-6a**] of the two species (Appendix F, Appendix G). It was noticed that the sample containing predominantly the major isomer was a solid and the one containing predominantly the minor isomer was a liquid.

Recrystallisation of the sample containing predominantly the major isomer was unsuccessful, preventing structure determination by x-ray crystallography.

Further analyses were carried out on the precipitated (major isomer enriched) **(S)-6**. Elemental analysis indicated a high accuracy to the calculated theoretical mass and mass spectrometry indicated the presence of only one species where $M^+ = 497$, with all the expected fragmentations. Although these data indicated the absence of an impurity of a different elemental constitution or mass, they did not rule out an impurity of the same mass, i.e. some form of isomer.

Compound **(S,R)-10** was also analysed by HPLC. Analytical reverse phase HPLC under a range of conditions showed only a single peak. Under the same conditions, a single peak was also observed for compound **(R,R)-10**, with a very marginal difference in the retention times. Each analytical normal phase HPLC of **(R,R)-10** and **(S,R)-10**, on the other hand, produced a broad peak.

Recrystallisation of **(R,R)-10** and **(S,R)-10** (Figure 5.2) was also unsuccessful, again preventing structure determination by x-ray crystallography.

Figure 5.2. Structures of **(R,R)-10** and **(S,R)-10**

Analytical normal phase HPLC analysis of compound **(S,R)-12** revealed that it contained two separable components. The two components were successfully separated by preparative normal phase HPLC (Appendix H). It was thought that ^1H NMR indicated that the two components were the major and minor isomers. The spectra of the isolated isomers were more readily interpretable (Appendix I) than that of the mixture (with a marked difference in chemical shift values between their corresponding α -H signals near the Alloc moiety), enabling complete interpretation of the initially ambiguous integrations described in Chapter 4.

These results made the conformational isomer hypothesis increasingly unlikely and therefore detailed NMR analyses were carried out.

5.1.2. NMR analyses

¹H-COSY NMR analysis of both enantiomers of the iodoalanine (**R**)-**6** and (**S**)-**6** confirmed that there was no overlap between the off-diagonal cross peaks of the major and minor isomers. This observation was also true for compounds (**R,R**)-**10** and (**S,R**)-**10**, and for compounds (**R,R**)-**12** and (**S,R**)-**12**. Each isomer's COSY pattern was independent of that of the other.

If the two species observed by ¹H NMR for iodoalanine and supposed lanthionine derivatives were conformers as originally reported,⁷⁶ a VT experiment should confirm this by coalescence of the signals at higher temperatures. As a general example, the two singlets observed by ¹H NMR for the *N*-methyl groups of DMF are caused by restricted rotation about the N-C(O) bond. On warming, the two lines broaden and coalesce with a coalescence temperature of 64°C (60 MHz), as the molecules are given sufficient energy to overcome the barrier to rotation about that bond, indicating that the time averaged environment of the two methyl groups is now identical.²⁰⁰ This concept was applied to compound (**R**)-**6**, as it had been hypothesised that it existed as two spectroscopically different conformers.⁷⁶ VT ¹H NMR experiments were carried out with T_{max} = 50 °C. Unfortunately the precipitated iodoalanine derivative (**R**)-**6** started to degrade at higher temperatures in solution. It was also noticed that once (**R**)-**6** was dissolved in deuterated DMSO, the aziridine derivative (**S**)-**11** immediately started to form (the precipitated iodoalanine derivative (**R**)-**6** did not show the presence of the aziridine by-product in deuterated chloroform). As the temperature was increased the amount of the aziridine-2-carboxylate also increased (Figure 5.3), along with other unidentified degradation product(s) (Appendix J). In fact, Kato *et al.* conveniently prepared optically active (*S*)-1-triphenylmethyl-aziridine-2-carboxylates from β-halo amino acids with heating, in the presence of potassium hydrogen sulfate.²⁰¹ It was therefore not

possible to verify that (*R*)-**6a** and **6b** were conformationally restricted conformers using this technique.

Figure 5.3. Mechanism for the formation of aziridine (*S*)-**11** from *N*-triphenylmethyl- α -iodo-(*R*)-alanine allyl ester (*R*)-**6a**

VT ^1H NMR experiments were also carried out on the presumed lanthionine derivative (*R,R*)-**10**. However, even at temperatures over 100 °C, no coalescence of peaks was observed. Again, only unidentified degradation products were observed (Appendix K).

Assignment of the ^{13}C spectra for the iodoalanine and lanthionine derivatives proved to be difficult due to their being mixtures. The isomers of the iodoalanine (*S*)-**6** (as well as (*R*)-**6**) each gave a ^{13}C signal between 9 – 21 ppm and another between 48 – 56 ppm, with the former being the expected upfield signals for a carbon atom attached to iodine (shielding effect) and the latter representing carbon atoms attached to nitrogen; they had been assumed to be the β - and α -C, respectively. A ^{13}C DEPT experiment confirmed that, for the minor product, the peak at 9.6 ppm was a β -CH₂-I signal and the peak at 55.8 ppm was an α -CH-N signal, which is consistent with the desired product. However, the DEPT experiment also clearly indicated that, for the major product, the peak at 20.1 ppm was a CH instead of the expected H₂C-I signal and the peak at 48.2 ppm was a CH₂ signal. The implication was that, for the major isomer, the iodine was attached to a tertiary carbon instead of a secondary one, with the structure proposed in Figure 5.4. It was therefore decided that ^{13}C - ^1H correlation experiments (HSQC/HMQC/HMBC) should be carried out in order to clarify the assignments and to further investigate the structures of the isomers.

Figure 5.4. $(S)\text{-}6a$ and the regioisomeric iodination product, $(R/S)\text{-}2\text{-iodo-3-(triphenylmethylamino)propionic acid allyl ester}$, an α -substituted β -alanine derivative

A $^{13}\text{C}\text{-}^1\text{H}$ HSQC experiment confirmed the observation made above (Appendix L). As expected for iodoalanine $(S)\text{-}6a$, the β -Hs on the minor isomer correlated to the ^{13}C signal at 9.6 ppm and the α -H correlated to the ^{13}C signal at 55.8 ppm. As for the major isomer **6b**, however, the β -Hs correlated to the ^{13}C signal at 48.2 ppm and the α -H correlated to the ^{13}C signal at 20.1 ppm. This is consistent with the structure proposed in Figure 5.4 – an upfield ^{13}C chemical shift for a carbon attached to iodine. A $^{13}\text{C}\text{-}^1\text{H}$ HMBC experiment consolidated the theory, indicating an absence of a 3J correlation between the NH proton and the carbonyl carbon for the major isomer. It was extrapolated that the regioisomeric product then reacted with cysteine **9** to afford regioisomers of lanthionine. This indicates that not only had the wrong compound been predominantly synthesised at the iodoalanine and all subsequent lanthionine stages, but also that Dugave and Menez⁷⁶ had wrongly characterised their iodoalanine and hence lanthionine derivatives. As discussed in Chapter 1, Dugave and Menez probed the chiral integrity of lanthionine derivatives synthesised *via* the iodoalanine route. Only the FLEC[®] derivatives of L-alanine were detected for a (R,R) -lanthionine derivative, and only traces of D-alanine derivatives were found, indicating negligible racemisation during the thioether bridge formation. Since the chiral HPLC results are compared against chromatographic traces for true samples of FLEC[®]-L-alanine and FLEC[®]-D-alanine, any other peak in the chromatogram would have been considered impurities, including a FLEC[®]- β -alanine derivative peak. Work carried out within the group²⁰² showed that the *N*-triphenylmethyl- β -iodoalanine benzyl ester claimed by Dugave and Menez to be present as a mixture of locked conformers, produced under identical conditions, undoubtedly gave similar $^{13}\text{C}\text{-}^1\text{H}$ correlation patterns in an HSQC experiment.

The regioisomer **6b** of the iodoalanine derivative (**S**)-**6a** was thought to have been formed *via* the ring opening of the aziridine-2-carboxylate derivative (**R**)-**11** by iodide (which was present in abundance during the iodination reaction of (**R**)-**5**), at the more hindered, but perhaps more electronically favoured, α -position (Figure 5.5).

Figure 5.5. Proposed mechanism for the formation of **6b** *via* the aziridine-2-carboxylate (**R**)-**11**

As discussed in Chapter 3, aziridine-2-carboxylate ring opening by a nucleophile has been reported to take place at either α - or β - or both positions depending on various different factors. Nucleophilic attack by iodide at the β -position of (**R**)-**11**, formed from (**S**)-**6a**, would straightforwardly return the original starting material. However, attack by an iodide at the α -position of (**R**)-**11** would be more complicated. Due to the chirality at the α -C, an $\text{S}_{\text{N}}2$ attack by iodide would initially result in stereo-inversion; indeed, there have been reports of this after nucleophilic ring opening reactions at C_2 of aziridine-2-carboxylates.^{170, 171, 183, 188} However, due to the lability of the iodine substituent and the abundance of sodium iodide (10 equivalents) in the reaction, a second inversion by another iodide would then be possible. The result would be a mixture of enantiomers: (**R**)-**6b** and (**S**)-**6b** as a mixture (Figure 5.5), represented as **6b**. Notably, this potential loss of stereochemical integrity was not apparently significant in the work of Righi and D'Achille who produced predominantly α -iodo- β -amino acid derivatives by reacting different derivatives of aziridine-2-carboxylates with sodium iodide, presumably due partly to the fact that only one equivalent of sodium iodide was used.¹⁹¹

In light of these results, NMR studies were carried out on the compounds **(R,R)-10** and **(S,R)-10**. The HSQC results indicated a big difference in ^{13}C chemical shifts for the α_1 -Cs of the two species present in each sample, as well as the β_1 -Cs (Figure 5.6).

Figure 5.6. Indication of α_1 -C, α_2 -C, β_1 -C and β_2 -C for **(R,R)-10** and **(S,R)-10**

The α_1 -C on the major product has a lower chemical shift by 9 ppm compared to the corresponding ^{13}C signal for the minor product. The β_1 -C has a 6-ppm higher chemical shift compared to the corresponding ^{13}C signal for the minor product. Such large differences in chemical shifts could not be caused by a difference in conformation.

An HMBC experiment was carried out on **(S,R)-10** to explore the connectivity of these molecules. In the experiment, β_2 -Hs of the lanthionine should have a 3J correlation with the β_1 -C, across the thioether linkage (Figure 5.7). The same pattern should be observed between β_1 -Hs and the β_2 -C (Figure 5.7).

Figure 5.7. Expected HMBC 3J correlations for **(S,R)-10** across the thioether linkage

However, according to the HMBC experiment carried out, this is not the case for the major product. The β_2 -Hs have a 3J correlation with the α_1 -C instead, which is unlikely for lanthionines, due to the fact that the α_1 -C is four bonds away. The same observation was made with the α_1 -H, which had a 3J correlation with the β_2 -C

(Appendix M). This is consistent with the proposed regioisomeric structure **18**, henceforth referred to as nor-lanthionine (nor-Lan), not that of the desired lanthionine (Figure 5.8). As for the minor component, which was thought to be the correct lanthionine derivative, the ^{13}C signals were relatively weak compared to the major component (1:5 isomer proportion). Therefore, the HMBC correlations were not strong enough to unambiguously deduce the structure of the minor isomer.

Figure 5.8. Structure of nor-lanthionine **18**, as proposed by NMR

NMR studies were also carried out on the lanthionine (**S,R**)-**12**, as a mixture of two species. Apart from the large difference in ^1H chemical shifts for the α_1 -Hs between the two species, it was noticed that the ^{13}C signal for the α_1 -C for the major component was too different (according to the HMQC experiment) to be a conformer of the minor component. It is now thought that the major component is the allyloxycarbonyl-derived nor-lanthionine **19** (Figure 5.9). Due to the low proportion of the minor component relative to the major one, its ^{13}C - ^1H correlation was weak. It was thought that, based on the HSQC experiments carried out on iodoalanine (**S**)-**6**, the minor component was the desired lanthionine derivative (Figure 5.9).

NMR studies were then carried out on compound **1**, which had been thought to contain a mixture of the correct lanthionine and its regioisomer, nor-lanthionine **20** (Figure 5.10). HSQC experiments clearly indicated a similar pattern for α_1 -C and β_1 -C and ^1H chemical shifts to that observed for **18** and **19**. The correct lanthionine, with the correct thioether connectivity, was not detected. It was either absent from the sample, or in such small quantity it was undetected by the HSQC experiment.

Figure 5.9. Scheme indicating how (S)-6 gave (S,R)-10 and hence (S,R)-12; and 6b gave 18 and hence 19. Similarly, (R)-6 gave (R,R)-10 and hence (R,R)-12; and 6b gave 18 and hence 19. The loss of stereochemical integrity is discussed later in this chapter and in Chapter 6.

Figure 5.10. Structure of nor-lanthionine **20**, a regioisomer of **1**

NMR studies were also carried out on the supposed ivDde-derivative **16**. ^{13}C - ^1H correlation experiments identified compound **21**, a regioisomer of lanthionine, as the only product (Figure 5.11). This explained the observation, discussed in Chapter 4, that only the major isomer reacted with the ivDde-OH. This does not mean that the correct lanthionine will not react at all to form its ivDde derivative. There could simply be competition between the correct lanthionine, which has a primary amine attached to a tertiary carbon, and its regioisomer, which has a primary amine connected to a less sterically hindered secondary carbon. Related to this, it has been reported that Dde can migrate between unprotected lysine side chains. However, no Dde migration onto an unprotected $\alpha\text{-NH}_2$ was detected.¹⁵³ Unfortunately further studies could not be carried out due to the poor solubility of the triphenylmethyl-cleaved lanthionine. The use of the ivDde and Dmab protecting groups, as part of an orthogonal protecting group system should therefore not be dismissed until further studies can be carried out using a genuine lanthionine derivative with ivDde-OH.

Figure 5.11. Structure of nor-lanthionine **21**, a regioisomer of **16**

As a result of the above, the lanthionine derivatives **17** and **2** were not present. Their regioisomers **22** and **23** (Figure 5.12) had instead been synthesised by the method described in Chapter 4.

Figure 5.12. Lanthionine derivatives **17** and **2** and their regioisomers **22** and **23**, respectively

The fact that the ¹³C NMR spectrum for compound (*R,R*)-**10** (in the presence of its regioisomer **18**) produced three peaks for many of the carbon environments indicated the presence of three species. One carbon signal was for the desired lanthionine (*R,R*)-**10** and two for the diastereoisomeric nor-lanthionine **18**. This hypothesis was further supported by the ¹³C NMR spectrum of **18** prepared free of (*R,R*)-**10** from the precipitated iodoalanine (1:4 for (*R*)-**6a**:**6b**, see Chapter 4, section 4.3): two signals were present for many of the carbon environments with the experiment carried out at 125 MHz. Although enantiomers cannot be distinguished by ¹³C NMR, two diastereoisomers can. It was therefore hypothesised that compounds **18** and **19** are present as diastereoisomers, as are compounds **20**, **21**, **22**, **23** because of the partial racemisation of **6b** (Figure 5.13). It was thought that **6b** was an unequal mixture of enantiomers and not completely racemised mixture because the intensities of ¹³C signals for the diastereoisomeric nor-lanthionine derivatives were not equal. This is further supported by the outcome of one of the peptide syntheses, discussed in Chapter 6, sections 6.2 and 6.4.

With regards to the exclusive formation of **18** when a precipitated batch of iododalanine (1:4 for (*R*)-**6a**:**6b**) was used in an attempt at forming (*R,R*)-**10** (Chapter 4, section 4.3), it was thought that this was due to the cysteine **9** being contaminated with the oxidised product (cystine **8**) hence resulting in a reduced amount of thiol species, with thiolate attack favouring **6b** over (*R*)-**6a**.

Figure 5.13. Diastereoisomeric nor-lanthionine derivatives inadvertently synthesised

5.1.3. Test reactions

Several tests were carried out to determine the mechanism of ring opening of aziridine-2-carboxylate (**R**)-**11** by nucleophiles; sodium iodide and cysteine derivative **9** were used.

The hypothesis for the mechanism of formation of the incorrect iodoalanine regioisomer was tested. The aziridine-2-carboxylate (**R**)-**11** was isolated by treating *N*-triphenylmethyl-(*R*)-serine(*O*-methanesulfonyl) allyl ester (**R**)-**5** with base at room temperature for 18 hr (Figure 5.14).

Figure 5.14. Partial conversion of *N*-triphenylmethyl-(*R*)-serine(*O*-methanesulfonyl) allyl ester (**R**)-**5** into aziridine (**R**)-**11**

Under these conditions the reaction was not complete, however the aziridine-2-carboxylate could be separated out from the mesylate (**R**)-**5** and purified *via* flash column chromatography. Aziridine-2-carboxylate (**R**)-**11** was then treated with 10 equivalents of sodium iodide for 3 days at 20 °C (Figure 5.15).

Figure 5.15. Ring opening of aziridine (**R**)-**11** by sodium iodide

The ^1H NMR of the crude material clearly indicated the presence of the regioisomer **6b** in a 3:1 ratio to the desired β-iodoalanine (**S**)-**6a** (despite still containing 75 % of the starting aziridine (**R**)-**11**) whereas the iodination of mesylate (**R**)-**5** at 20 °C yielded the iodoalanine isomers in a ratio of 2:1. The increase in the proportion of (**S**)-**6a**, from the reaction of (**R**)-**11** → (**S**)-**6** over the reaction of (**R**)-**5** → (**S**)-**6** suggested that the formation of the correct β-iodoalanine (**S**)-**6a** goes *via* both an $\text{S}_{\text{N}}2$ mechanism (displacement of the methanesulfonyl group by an iodide) and *via* the ring opening of (**R**)-**11**. It was thought that iodoalanine regioisomer **6b** is in fact present as a mixture of enantiomers due to stereo-inversion of an $\text{S}_{\text{N}}2$ nucleophilic attack (see Figure 5.5). Also, there is probably an equilibrium between (**S**)-**6a** and (**R**)-**11** during the iodination reaction (Figure 5.16). The same hypothesis could be made for the corresponding compounds originating from (*R*)-serine.

Figure 5.16. Equilibrium between the correct iodoalanine **(S)-6a** and its regioisomer **6b** via **(R)-11**

Since the formation of the aziridine derivative was also seen during the thioether bridge formation step, another test reaction was carried out. The ring opening of the aziridine derivative **(R)-11** by the cysteine **9** was investigated. Dugave and Menez⁷⁶ reported that a coupling between cysteine **9** and serine mesylates in the presence of caesium carbonate did not produce the desired lanthionine. Instead, starting materials and the aziridine derivative were isolated, implying that no ring opening took place by the cysteine. The test reaction, where the aziridine-2-carboxylate **(R)-11** was treated with the cysteine derivative **9** in DMF with caesium carbonate for 4 hr at room temperature, was therefore carried out (Figure 5.17). Neither the desired lanthionine derivative **(S,R)-10** nor its diastereoisomeric nor-lanthionine regioisomer **18** was formed under these conditions. Since the role of the caesium carbonate was to generate thiolate anions but the reaction did not work, its presence led to the basic cleavage of the Fmoc group on cysteine instead (Figure 5.17). However, Hata and Watanabe⁷⁹ succeeded in ring opening unprotected aziridine-2-carboxylates with unprotected cysteines, without the addition of any Lewis acid, which is often required for aziridine-2-carboxylate ring opening reactions, in the presence of a phosphate buffer. This difference in observations remains unclear. Okawa *et al.* observed that boron trifluoride etherate was necessary for coupling a cysteine derivative with aziridine-2-carboxylates to yield lanthionines.¹⁸⁵ Other contributing

factors could be the ineffectiveness of the triphenylmethyl group on the nitrogen as an electron-withdrawing group, to increase the susceptibility of C₂ and C₃ on the aziridine ring to thiolate nucleophilic attack, and the bulkiness of the triphenylmethyl group. It has been shown on numerous occasions that the triphenylmethyl group is often used in the synthesis of aziridine-2-carboxylates, but it is more often than not removed and/or substituted during aziridine-2-carboxylate ring opening reactions.^{80, 171, 176, 172, 203}

Figure 5.17. Unsuccessful ring-opening reaction of **(R)-11** with cysteine derivative **9**

The formation of the aziridine-2-carboxylate during the thioether bridge formation step was caused by intramolecular iodide displacement by the nitrogen on the iodoalanine derivatives, presumably assisted by the presence of a polar solvent: DMF. Since the cysteine **9** does not ring open the aziridine, this was thought to be the reason for the presence of the aziridine-2-carboxylates **(S)-11** and **(R)-11** as by-products in the thioether bridge formation reactions.

5.1.4. Synthesis of (R,R) and (S,R)-lanthionine derivatives **14** and **15** (via the Mitsunobu reaction)

Work carried out within the group²⁰⁴ indicated that lanthionine derivatives could be directly synthesised *via* a modified version of the Mitsunobu reaction, in which the hydroxyl groups on serine derivatives **(S)-4** and **(R)-4** are activated with trimethylphosphine and 1,1'-(azodicarbonyl)dipiperidine (ADDP)^{205a, 205b} and this

mixture was treated with the cysteine derivative **9** in the presence of a zinc(II)^{205c} catalyst (Figure 5.18).

Figure 5.18. Formation of lanthionine *via* Mitsunobu reaction conditions

Although work is in progress to improve the mass recovery and yield of this reaction, coupling **(S)-4** with **9** yielded only one isomer. The use of the serine derivative **(R)-4** also yielded only one isomer, with small differences in the ¹H NMR chemical shift values (Appendix N). HSQC experiments carried out on both diastereoisomers confirmed the correct thioether bridge connectivity with similar ¹³C chemical shift values between the α- and β-Hs, and α- and β-Cs on both sides of the thioether bridge. HMBC experiments confirmed this with the presence of ³J couplings between β-Hs and β-Cs across the bridge (see Figure 5.7). Not surprisingly, each diastereoisomer produced *via* this method had identical ¹H and ¹³C chemical shifts and coupling patterns to the minor component of the corresponding lanthionine derivatives **(R,R)-10** and **(S,R)-10** produced *via* the iodoalanine route.

Chapter 6

Results and Discussion III

6.1. Synthesis of a lanthionine analogue of an enkephalin opioid peptide

With orthogonally protected nor-lanthionine derivatives in hand, an investigation of the SPPS of cyclic peptides containing thioether bridges using the proposed methodology commenced. During the course of these investigations the NMR studies described in Chapter 5 proved that only compounds **20** and **23** had been synthesised, instead of the desired **1** and **2**, respectively (Figure 6.1). However, the SPPS studies were continued, in order to test the methodology. It was also suspected that the resulting novel cyclic peptides could possess unusual conformational properties, as the nor-lanthionine bridge was previously unknown.

Figure 6.1. nor-Lanthionine derivatives **20** and **23** for SPSS

Although the lanthionine analogue of the enkephalin opioid peptide (Figure 6.2) is no longer of interest for further biological studies,⁵⁰ it was chosen as the first target for the peptide synthesis due to the small size of the peptide and hence short synthesis. A number of attempts at the synthesis were made; in each case the synthesis was carried out either manually in a Merrifield bubbler (Appendix O) or in an automated fashion (continuous flow) on a peptide synthesiser.

Figure 6.2. Lanthionine opioid enkephalin peptide analogue (a) and the analogue synthesised **24**

First attempt

The resin used for the synthesis was the highly acid labile Sieber amide resin⁸⁷ (Figure 6.3) with a loading of 0.47 mmol/g and the synthesis is outlined in Figure 6.4. The Fmoc protecting group at each stage of the peptide synthesis was removed by a 20 % piperidine solution in DMF. A small amount of resin was washed after each Fmoc deprotection step and treated with the Kaiser test solution.⁸³

Figure 6.3. Sieber amide resin

- i) HOBt/DMF, DIC/Dioxane, ii) Ac₂O, HOBt, DIPEA, DMF, iii) Piperidine/DMF
 iv) Fmoc-Phe, i, ii, iii, v) Fmoc-Gly, i, ii, vi) Pd(PPh₃)₄, CHCl₃/AcOH/NMM, iii,
 vii) PyAOP, HOAt/DIPEA/DMF, viii) Fmoc-Tyr, i, ii, iii, ix) TFA/H₂O, Et₃SiH

Figure 6.4. Synthesis of a thioether-bridged opioid enkephalin peptide analogue **24**

Incorporation of the amino acids (nor-Lan, Phe, Gly) was carried out by pre-activation of these residues with *N,N'*-diisopropylcarbodiimide (DIC) and 1-hydroxybenzotriazole (HOBt) for 15-20 minutes before being added to the resin. Each coupling step was carried out twice and the resin subsequently washed in preparation for the colorimetric Kaiser test. No problems were encountered during incorporation of the first residue, nor-Lan **20**, onto the resin as confirmed by Kaiser test.

Each amino acid coupling step was also followed by a capping wash. The capping wash consisted of an acetic anhydride solution in DMF. Its role was to ensure that all unreacted amino groups are capped, leading to a truncated sequence. This is to prevent the formation of peptides with sequences lacking one or more (random) residues, which are more difficult to remove in the purification step than shorter, truncated sequences.

After assembling the linear peptide, the allylic protecting groups on the nor-lanthionine were removed with tetrakis(triphenylphosphine) palladium(0) in a mixture of $\text{CHCl}_3/\text{AcOH}/\text{NMM}$ (37:2:1)¹²⁸ or $\text{DMF}/\text{CHCl}_3/\text{AcOH}/\text{NMM}$ (18.5:18.5:2:1). Following that were several washes with solutions of diisopropylethylamine (DIPEA) and sodium diethyldithiocarbamate - to remove traces of acetic acid and organometallic by-products - and the Fmoc deprotection step. The results from the Kaiser test after the allylic deprotection was a positive blue and an intense purple was observed after the Fmoc deprotection step. This was due to the presence of firstly one then two amino groups on the resin. A small amount of the resin was taken at the allylic deprotection and Fmoc deprotection stages and treated with a 1 % solution of TFA in dichloromethane and the expected peptide ion searched for by electrospray mass spectrometry. Each of the two stages in the synthesis above gave dubious parent ion peaks at very low relative abundances. This is perhaps not surprising as the presence of trifluoroacetic acid, or its salts, tends to suppress signals in mass spectrometry.

Cyclisation of the peptide was carried out using the highly reactive mixture of PyAOP, HOAt and DIPEA.¹⁰⁹ This mixture has been shown to be a potent peptide coupling reagent, especially for cyclisation of peptides. The Kaiser test following the cyclisation step was a positive blue (i.e. not purple), which suggested that the amine intended for cyclisation was no longer present, hence, the success of the cyclisation step was indicated. A small amount of the resin was again set aside for cleavage and examination by mass spectrometry. The result was similar to the previous test where the parent ion, 18 mass units less than the previous stage due to loss of H₂O was observed but gave a very low relative abundance.

As a test of the methodology, the final amino acid on the sequence, Tyr, was coupled to the remaining amino group and its Fmoc group then removed. Removal of the peptide from the resin would then release a C-terminal peptide amide. The peptide was repeatedly washed with dichloromethane to remove traces of DMF, which could hinder the cleavage reaction²⁰⁶ and treated with an 85 % trifluoroacetic acid solution, plus water (10 %) and triethylsilane (TES, 5 %) portion-wise on the bubbler with collection of the aliquots in a round-bottom flask. The high concentration of the acid was to ensure that the ^tbutyl side chain protection on Tyr was completely removed. After stirring for 4 hours the mixture was concentrated *in vacuo* and the peptide was precipitated with cold ether. Removal of the solvent followed by lyophilisation from acetic acid solution yielded the peptide in crude form.

The mass spectrum of the crude peptide gave the expected parent ion as the base peak (554, Appendix P), despite the fact that the crude weight was 240 mg when the maximum possible yield was only 55 mg. Analytical HPLC, however, showed that the peptide contained a host of peaks especially at higher concentrations of acetonitrile (Appendix Q).

Purification *via* preparative HPLC proved difficult due to impurities with retention times close to that of the peptide. Eventually isolation of the peptide was achieved but in a disappointing 0.8 % yield. Two HPLC peaks adjacent to the desired peptide displayed, among others, the parent ion but at less than 30 % relative abundance, and

consist mainly of unidentified contaminants. Insufficient peptide was isolated to obtain full ^1H NMR characterisation.

Second attempt

A second attempt at the synthesis was then carried out. This time, the linear peptide was synthesised *via* continuous flow solid phase peptide synthesis (MilliGen 9050 peptide synthesiser) since it was part of the aim of this project to apply the methodology to continuous flow solid phase peptide synthesis eventually, with the automation of the allyl deprotection and cyclisation steps as described by Albericio *et al.*¹²⁸

A PEG version of the Sieber amide resin was used (NovaSyn[®] TG Sieber resin)⁸⁷ (Figure 6.5) for continuous flow synthesis with a lower loading of 0.16 mmol/g, to avoid inter-chain coupling at the cyclisation stage.

Figure 6.5. NovaSyn[®] TG Sieber resin

Each Fmoc deprotection step and amino acid coupling was monitored by U.V. at 365 nm. The U.V. traces were integrated to indicate the degree and comparative success of each Fmoc deprotection and amino acid coupling step (Appendix R). All the reagents used were the same as in the batch synthesis described above with the exception of 1-acetylimidazole, which was used as the capping reagent.

The Fmoc deprotection step for the incorporated nor-lanthionine was inefficient and thought to be caused by a dilution factor in the sequence of the synthesiser protocol. This resulted in poor Phe coupling, inefficient Fmoc deprotection and low Gly coupling. Despite all these, it was decided to continue the synthesis offline, with the

allylic deprotection, Fmoc deprotection on Gly, cyclisation and Tyr coupling as described above. The Kaiser tests at these stages were ambiguous.

The cleavage of the peptide was carried out as before. It was perhaps not surprising that the parent ion was absent by mass spectrometry, considering the results of the U.V. integrations. Analytical HPLC showed a peptide that was at least 70 % pure with a different R_T compared to the first peptide synthesised. There was still a host of peaks present on the HPLC trace at higher acetonitrile concentrations.

Isolation of the peptide gave a mass of 222 as the base peak and ^1H NMR suggested that the peptide that was synthesised was in fact a $\text{H}_2\text{N-Tyr-Phe-CONH}_2$ dipeptide. This in turn suggested that the incorporation of nor-lanthionine onto the resin was not as successful as it should have been, which explained the weak U.V. trace and integration for the following Fmoc deprotection step (Appendix R). It was then decided that the concentration of piperidine needed to be increased to 30 % due to the dilution factor in the sequence of the synthesiser protocol to maximise Fmoc deprotection. However, it could not be explained how the capping step immediately after the incorporation of nor-lanthionine in the synthesis failed to prevent the formation of this dipeptide side product.

Third attempt

The third attempt to synthesise peptide **24** was carried out in a similar manner to the second attempt with the linear peptide synthesised on the MilliGen 9050. However, the nor-lanthionine residue was incorporated onto the resin offline, as a precautionary measure. A Kaiser test gave a negative result indicating a successful coupling of the first residue and the synthesis was continued as before, online, followed by offline allylic deprotection, cyclisation and Tyr coupling as described above.

The cleavage of the peptide from the resin and precipitation were carried out as before. The analytical HPLC trace indicated one major product, apart from the

amount of this was present in the sample. Data from the ^1H -COSY experiment suggested that the major product was a $\text{H}_2\text{N-Tyr-Gly-Gly-Phe-Phe-CONH}_2$ pentapeptide, which coincidentally has a molecular mass identical to that of the doubly-oxidised **24a**. Again it could not be explained how the capping steps in the synthesis failed to prevent the formation of this predominant peptide by-product.

The repeating trend observed by HPLC, where a cluster of peaks was observed at higher concentrations of acetonitrile, gave rise to concern. The hypothesis was advanced that the conditions for the peptide cleavage were so highly acidic that they led to degradation of the linker. It was therefore thought that a two-step cleavage procedure could improve product purity. The first step involved a mild acid cleavage in the bubbler with repeated washes of a 5 % TFA, 2.5 % H_2O and 2.5 % TES (as the scavenger for t -butyl or trityl species) solution in DCM, followed by a methanol wash and collection of the filtrate in a flask containing trifluoroacetic acid. Stirring the mixture for 2 - 3 hours then followed.

Fourth attempt

The next attempt at synthesising peptide **24** was carried out solely on the peptide synthesiser. This involved some manual intervention with changes in solvents and reagents required for the allylic deprotection and cyclisation steps. The original settings subsequently needed to be restored before the Tyr was coupled to the peptide.

In order to monitor the progress of the first few steps of the synthesis, a small amount of resin was removed from the column following Fmoc deprotection, and also following nor-lanthionine coupling. Each batch of resin was checked by I.R. and U.V. The I.R. spectrum of the resin following Fmoc deprotection showed the absence of a carbamate C=O stretch: this was visible again, although more weakly, after nor-lanthionine coupling. U.V. measurements were carried out to determine the amount of the Fmoc group present at each of these two stages. This was carried out by taking a known quantity of the resin and cleaving the Fmoc group with a 30 %

piperidine solution on DMF and measuring the absorbance of the aliquot at 300 nm, which is the absorbance of the piperidine-dibenzofulvene adduct (Appendix S).

The absorbance for the resin after Fmoc removal gave a near-zero value, which was expected. The loading was quantitatively assigned by using the absorbance value for the nor-lanthionine-incorporated resin in the calculation in Appendix S (0.16 mmol/g, 0.16 mmol/g claimed by manufacturer).

The synthesis was then carried through to the end, despite the apparent lack of increase in carbamate C=O stretch intensity in I.R. as the synthesis progressed, and low integrations on the peptide synthesiser.

Once the synthesis was completed, the peptide was cleaved from the resin following the two-step method and precipitated as previously described to give 70 mg of crude material. Mass spectrometry indicated that the material contained the desired peptide. The analytical HPLC trace showed that the impurity peaks previously detected by U.V. at higher concentrations of acetonitrile were no longer present, confirming the hypothesis that the Sieber amide linker was not stable to the highly acidic cleavage conditions previously employed.

Purification of the peptide was carried out *via* preparative HPLC. As previously observed the parent ion of the peptide was the base peak. Two other adjacent peaks gave the parent ion with much smaller relative abundances. These were thought to be impurities that contained an extremely small amount of the peptide. A very poor yield (2.7 %) was obtained.

12-membered cyclic tetrapeptides are known to be difficult to synthesise *via* lactam bridge formation. This is mainly due to the elongated conformation of the linear precursor, maximising the distance between the two termini, which affects the degree of success during cyclisation.^{207, 208} Cyclisation is favoured by the presence of Pro or Gly or D-amino acids²⁰⁹ and the absence of these leads to slow cyclisation resulting in dimerisation and racemisation.²¹⁰ These factors perhaps account for the difficulty

in obtaining a reasonable yield from the synthesis of peptide **24**, which is a 12-membered cyclic tetrapeptide formed *via* ring closure to give an amide bond, containing a sulfur bridge and a β -amino acid.

To test the above explanation for a poor yield of cyclic tetrapeptide, the synthesis of a bigger cyclic peptide was considered. The best candidate was thought to be an analogue of ring C of nisin, which is a 21-membered ring with no dehydro-amino acids (Figure 6.7).

6.2. Synthesis of a nor-lanthionine analogue of ring C of nisin

Figure 6.7. (a) Ring C of nisin and an analogue of ring C of nisin **25**

Since the C-terminus of the peptide is an acid, the resin used for the synthesis was the highly acid labile NovaSyn[®] TGT alcohol resin pre-loaded with Asn(Trt) (Figure 6.8).^{90, 91, 211}

The whole synthesis was carried out offline on the Merrifield bubbler. The Fmoc deprotection steps, amino acid couplings, capping washes, allylic deprotection,

cyclisation and Kaiser tests were all carried out as described above. The loading was quantitatively assigned to be 0.21 mmol/g (0.20 mmol/g determined by the manufacturer) by U.V. measurements (Appendix T).

Figure 6.8. NovaSyn[®] TGT alcohol resin pre-loaded with Asn(Trt)

The synthesis (Figure 6.9) proceeded well, as monitored by Kaiser test, and the peptide cleavage was carried out *via* the two-step procedure described above. Precipitation of the peptide with cold ether followed by lyophilisation with aqueous acetic acid yielded the crude material as a pale yellow powder (85 mg).

The crude material gave a mass spectrum with the parent ion as a 50 % relative abundance peak and $[M + 2]^{2+}$ as the base peak. Analytical LCMS showed that there were two peaks on the HPLC trace, which gave identical mass spectra – the parent ion of the peptide and $[M + 2]^{2+}$ (Appendix U). Purification of the peptide by preparative HPLC confirmed the presence of two peptidic species, **25a** and **25b**, with the same mass with a ratio of approximately 3:1, giving a total yield of 55 %. This implied an average coupling efficiency of 94 % over 10 steps, assuming the Fmoc deprotection steps proceeded quantitatively. The ratio of the two peptidic species was the same as the proportion of what was originally thought to be lanthionine conformers (**S,R**)-**10** (Figure 6.10). However, due to the disproval of the conformer theory no correlation could be made. Further purification was carried out under a different gradient by HPLC. It was observed that there was a small shoulder on the broad HPLC trace of the major product, which did not separate out even with two other gradient systems on HPLC.

- i) HOBt/DMF, DIC/Dioxane, ii) Ac₂O, HOBt, DIPEA, Dioxane, iii) Piperidine/DMF, iv) Fmoc-Gly, i, ii, iii, v) Fmoc-Met, i, ii, iii, vi) Fmoc-Leu, i, ii, iii, vii) Fmoc-Ala, i, ii, iii, viii) Fmoc-Gly, i, ii, ix) Pd(PPh₃)₄, CHCl₃/AcOH/NMM, iii, x) PyAOP, HOAt/DIPEA/DMF, xi) Fmoc-Lys, i, ii, iii, xii) TFA/H₂O, Et₃SiH

Figure 6.9. Synthesis of a lanthionine analogue of ring C of nisin **25**

(*S,R*)-10**Figure 6.10.** Lanthionine derivative (*S,R*)-10

The ^1H NMR of the two peptides in deuterated methanol at 283 K confirmed that only the minor product was pure. The major product produced a ^1H spectrum with doubling of the methyl peaks for Met, Ala and Leu at approximately 2.0, 1.4 and 0.9 ppm respectively and increased breadth of some aliphatic signals for Lys, Leu and Met. At this stage the impurity in the major product was thought to be a deletion sequence with common residues such as Lys, Leu and Met. In the meantime another peptide was synthesised, an analogue of rings A and B of nisin, a sequential bi-cyclic thioether-bridged peptide.

6.3. Synthesis of an analogue of rings A and B of nisin

Figure 6.11. Rings A and B of nisin

One of the advantageous features of this method for the synthesis of cyclic side-chain bridged peptides is the possibility of making several successive rings on resin. It was decided that synthesising an analogue of rings A and B of nisin, the non-

overlapping bi-cyclic peptide **26**, should test the strategy. In this case, the synthesis was carried out entirely on the Merrifield bubbler and all reagents used were identical to the synthesis of ring C of nisin. The loading was determined by U.V. measurements as previously described (0.22 mmol/g, 0.20 mmol/g specified by manufacturer) (Appendix V).

Figure 6.12. nor-Lanthionine analogue of rings A and B of nisin **26**

Despite the fact that the analogue of ring B is a 12-membered ring, it was envisaged that the proline residue would encourage cyclisation by twisting the linear peptide. A small amount of the resin was therefore treated with a 5 % trifluoroacetic acid solution in dichloromethane after the cyclisation step and checked by mass spectrometry. Indeed, the crude material gave a relative abundance of 83 % for the parent ion for the ring B analogue.

With this information the synthesis was carried through, with the incorporation of a second nor-lanthionine and subsequent residues, to form the second ring (ring A of nisin, Figure 6.13).

Cleavage of the peptide from the resin was carried out as previously described. Mass spectrometric analysis of the crude product gave the parent ion peak with a relative

abundance of 18 % and $[M + 2]^{2+}$ as the base peak. Purification *via* preparative HPLC however indicated that only a small amount of the peptide with the correct mass was present (< 1 %). The presence of proline in the sequence was thought not to have increased the yield significantly during cyclisation of the analogue of ring B of nisin.

Figure 6.13. Synthesis of an analogue of rings A and B of nisin **26**

6.4. Further NMR analyses of peptide 25

Although all other contaminants seemed to have been removed, the ^1H NMR for the minor product of **25a** was difficult to assign despite the availability of ^1H COSY and TOCSY spectra. The major product, **25b**, on the other hand, produced signals at lower fields in the α -H region with integrations less than that for one proton and which did not correlate to any other signals in the TOCSY spectrum. In light of these observations and following the high yielding synthesis of peptide **25**, extensive NMR studies were carried out in collaboration with Dr. Richard Harris (Department of Biochemistry, UCL).

^1H - ^{13}C HSQC (Appendix W, Appendix X) and HMBC (Appendix X-a) correlation experiments were first carried out on the two peptides isolated in the synthesis of **25**. The results obtained confirmed earlier findings: both peptidic species showed what would have been unlikely 3J correlations in a lanthionine moiety between β_2 -Hs and the α_1 -C across the sulfur bridge, hence giving a structure illustrated in Figure 6.14. The same observation was made with the α_1 -H, which had a 3J correlation with the β_2 -C on the across the thioether bridge. At the time of these findings the nature of the isomerism of the lanthionine precursors was not known. The determination of the thioether connectivity at the peptide stage provided further proof to the conclusion made in Chapter 5.

Figure 6.14. Structure of the nor-Lan analogue of ring C of nisin **25** across the thioether linkage as proposed by NMR

Structural determination was carried out by Dr. Richard Harris, involving NOESY experiments, where structure calculations were carried out using the

PARALLHDGv5.1 (parameter all hydrogens version 5.1) parameter, with non-bonded energy function of PROLSQ, within the CNS (Crystallography and NMR System) program. 175 and 63 inter- ^1H distance restraints were applied in restrained molecular dynamics calculations for **25a** and **25b**, respectively. Each calculation was run with both *R*- and *S*-stereochemistry at the α -C position of the β -amino acid moiety of the peptide. The superimposition of the 20 lowest energy structures for the calculations indicated that the minor isomer **25a** displayed a distinct conformation for the *R*-diastereoisomer (Appendix Y). This strongly suggests that **25a** is the *R*-diastereoisomer. However, the superimposition of the 20 lowest energy structures calculated for an *S*-diastereoisomer was less defined (Appendix Y). Both *S*- and *R*-models for the major component **25b** were vague (Appendix Z) due to the low number of inter- ^1H distance restraints obtained from NOESY, as well as ROESY experiments. This could be due to the fact that **25b** is inherently more flexible, therefore giving rise to fewer NOEs. Since it has been proven that the major product does not contain a lanthionine moiety but a nor-lanthionine one instead, and its mass and ^1H NMR spectra are almost identical to those of **25a**, it could be deduced that it is in fact the *S*-diastereoisomer **25b** (Figure 6.15).

Although the hypothesis was made, that partial racemisation occurred at the iodoalanine stage (for regioisomer **6b**), it could not be confirmed due to the inefficiency of separation throughout the synthesis. The peptide synthesis, finally provided concrete evidence that the stereochemistry of the α -iodo- β -alanine derivative **6b** was compromised during the ring-opening reaction of aziridine derivatives (*S*)-**11** and (*R*)-**11**. This resulted in a mixture of diastereoisomers for the subsequent nor-lanthionine derivatives **18**, **19**, **20**, **21**, **22** and **23** as discussed in Chapter 5, section 5.1.3.

Figure 6.15. Structures of **25a** and **25b**, indicating the stereochemistry at the β -amino acid moiety

6.5. Future work

The use of a double-headed amino acid in the synthesis of cyclic peptides on solid phase has now been proven.^{212a, 212b} Although the wrong regioisomer of lanthionine was used, forming an isomeric cyclic peptide, it is highly probable that the methodology will work on the correct lanthionine residue. Future work will need to be concentrated on the synthesis of suitably protected lanthionines.

As mentioned in Chapter 5 (section 5.1.4), both lanthionine derivatives **(R,R)-10** and **(S,R)-10** could be synthesised utilising new Mitsunobu reaction conditions (Figure 6.16). It was discovered that the use of trimethylphosphine with ADDP^{205a, 205b} is more powerful than the original Mitsunobu reactants triphenylphosphine and diethylazodicarboxylate.²¹³ Recent reports have also indicated that the use of Zn

catalysts could be employed in Mitsunobu reactions involving nitrogen nucleophiles.^{205c} The use of trimethylphosphine and ADDP in the presence of zinc(II) tartarate produced the desired lanthionine successfully if in only moderate yields.^{202, 204} Attempts to optimise the yield are still continuing.

Figure 6.16. Formation of lanthionine *via* Mitsunobu reaction conditions

These preliminary findings provided invaluable information with regards to the ¹H NMR of the two diastereoisomeric lanthionine residues. Crucially, this produced even further confirmation of the regioisomer theory, which was proved by ¹H-¹³C NMR correlation studies.

Once the synthesis of the lanthionine residue has been optimised, the use of ivDde as part of an orthogonal protecting group strategy must be carried out, as discussed in Chapter 5, section 5.1.2.

Effort will also need to be focused on the synthesis of 3-methylanthionine, whose occurrence is widespread in lantibiotics. Theoretically the Mitsunobu conditions could also be applied in this instance (Figure 6.17).

Figure 6.17. Application of Mitsunobu conditions to the synthesis of MeLan

Work will also need to be carried out on introducing dehydro-amino acids into the synthesis, either during the on-resin synthesis or after peptide-resin cleavage.

Chapter 7

Experimental

Unless otherwise indicated, all reagents were obtained from commercial suppliers and used without further purification. THF was distilled using sodium and benzophenone. TEA was distilled over CaH_2 and DCM was distilled over P_2O_5 . MeOH was distilled using magnesium and iodine, and stored over 4 Å molecular sieves. DMF, NMM, piperidine, acetic anhydride and dioxane were distilled from CaH_2 and DIEA was distilled from KOH: all were stored over 4 Å molecular sieves. Hexane is described as the fraction boiling between 67 – 70 °C. Ether refers to diethyl ether and brine refers to a saturated solution of sodium chloride.

NMR spectra were recorded using Bruker AC 300, Varian VXR400, AMX400, AVANCE500 and Varian UNITYplus 500 and 600 MHz machines and all samples were dissolved in deuterated chloroform, unless otherwise stated. The chemical shifts (δ) are given in units ppm relative to tetramethylsilane. Coupling constants (J) are measured in Hertz (Hz). Multiplicities for ^1H are shown as s (singlet), d (doublet), t (triplet), dd (doublet of doublets), dt (doublet of triplets), m (complex multiplet), bs (broad singlet), bd (broad doublet), bt (broad triplet) and bm (broad multiplet). ^{13}C chemical shifts are listed where characterisation of peaks were carried out from experiments described in parentheses following the “ δ_{C} ” sign. The term “*ca.*” in front of a ^{13}C chemical shift indicates an approximate value for two or three signals for the same carbon that are indistinguishable (regioisomers and/or diastereoisomers) and ^{13}C chemical shifts expressed as ($X + Y$) indicate two distinct diastereoisomeric carbon signals.

Mass spectra were recorded using a VG ZAB 2SE mass spectrometer for FAB and Micromass Quattro for ES and APCI (MassLynx software). High resolution mass spectra were recorded using a FAB VG ZAB SE machine.

I.R. spectra were recorded on Perkin-Elmer 1600 FTIR or Shimadzu FTIR-8700 spectrophotometers for solutions in chloroform (with KBr cell) unless otherwise stated, and Nicolet Protégé for solid I.R.

U.V. spectra were recorded on Shimadzu UV-2401C and are measured against absolute ethanol.

Optical rotations were recorded on Optical Activity:polAAR2000 polarimeter in alumina-treated chloroform solutions (unless otherwise indicated) quoted in 10^{-1} deg $\text{cm}^2 \text{g}^{-1}$ with concentration (c.) in g/100 ml.

Melting points were determined using a Gallenkemp instrument and are uncorrected.

Microanalyses were determined, where possible, using a Perkin Elmer 2400 Elemental Analyser (CHN) and Perkin Elmer 240 Elemental Analyser (S, halogen). However a number of compounds prepared in this work were extremely viscous liquids and their purity was determined by accurate mass spectrometry and ^1H and ^{13}C NMR spectroscopy.

Thin layer chromatography (t.l.c.) was performed on pre-coated aluminium/glass-backed Merck 60 F₂₅₄ silica plates with 0.25-mm thickness. Visualisation was done by absorption of U.V. light and by dipping with permanganate solution or bromocresol green solution where applicable. Normal phase flash column chromatography was carried out using silica gel with particle size 40 – 63 μm and reverse phase flash column chromatography was carried out using silicagel 60 silanized (63 – 200 μm), both supplied by Merck.

Analytical reverse phase HPLC was carried out using a Waters 600E quaternary gradient system pump, Rheodyne 7125 valve injector, Gilson 115 variable-wavelength U.V. detector and Hewlett-Packard HP3396A integrator with a Vydac™ C₁₈ column, 4.6 mm x 25 cm. Preparative reverse phase HPLC was carried out using a Waters 600E quaternary gradient system pump, Rheodyne 7125 valve injector, Gilson Holochrome variable-wavelength U.V. detector and Waters 745B Data Module with a Vydac™ C₁₈ column, 21.4 mm x 25 cm. Analytical normal phase HPLC was carried out using a Gilson binary gradient system pump, Rheodyne 7725 valve injector, spectroMonitor 3320 Holochrome variable-wavelength U.V. detector and Hewlett-Packard HP3390A integrator with an Alphasil5SILICA column, 4.6 mm x 25 cm. Preparative normal phase HPLC was carried out using a Waters 600E quaternary gradient system pump, Rheodyne 7125 valve injector, Gilson Holochrome variable-wavelength U.V. detector and Waters 745B Data Module with a Techsil5SILICA column, 22 mm x 25 cm. LC-MS was carried out using a Hewlett-Packard HP1100 for HPLC with a Phenomenex® LUNA 3 μ C18(2) column, 2 x 50 mm and Micromass Quattro LC (MassLynx software) or Finnigan A Q A (XCALIBUR software) for mass spectrometry. Retention times are given in min.

Kaiser tests were carried out with a small amount of peptidyl-resin using 4 drops each of ethanolic solutions of phenol (40 g / 10 ml), ninhydrin (0.5 g / 10 ml) and a pyridine (49 ml) solution of 1 mM aqueous potassium cyanide (1 ml) and the combined solution was heated at $T \sim 100^\circ\text{C}$ for approximately 4 min). Solutions containing potassium cyanide were treated with a 1M solution of iron (II) sulfate before being disposed off in the normal fashion for organic materials.

1. (R,R)-Cystine bis-^tbutyl ester¹⁹³

^tButyl acetate (50 ml) was added to a stirred solution of L-cystine (5.0 g, 21 mmol) in perchloric acid (70 % aq. v/v, 7.8 ml, 84 mmol, 4 equiv.) at room temperature resulting in two immiscible layers, which dispersed on stirring after approximately 3 hr. The reaction mixture was left to stir for a further 72 hr after which it was cooled in an ice bath for 30 min. Sodium hydroxide (2M aq.) was slowly added until the solution reached basic pH (11). The product was extracted with ether (6 x 100 ml) and ethyl acetate (6 x 100 ml) and the combined organic layers were dried over anhydrous sodium sulfate. Removal of the solvents *in vacuo* yielded the product as a yellow liquid.

Yield: 6.6 g, 19 mmol, 90 % (lit.¹⁹³ 67 %)

NMR: δ_{H} (400 MHz) 3.63 (2H, dd, J 7.9, 4.5, 2 x CHCH_2S), 3.08 (2H, dd, J 13.4, 4.6, 2 x CHCHHS), 2.82 (2H, dd, J 13.4, 7.9, 2 x CHCHHS), 1.42 (18H, s, 2x $\text{CO}_2\text{C}(\text{CH}_3)_3$)

Mass spectrum: m/z (APCI+) 353 ($[\text{M} + \text{H}]^+$, 100 %), 375 ($[\text{M} + \text{Na}]^+$, 0.4 %), 297 ($[\text{M} - ^t\text{Bu} + 2]^+$, 39 %), 241 ($[\text{M} - 2^t\text{Bu} + 3]^+$, 18 %)

2. *N,N'*-Bis(9-fluorenylmethoxycarbonyl)-(*R,R*)-cystine-bis-^tbutyl ester¹⁹⁴

(*R,R*)-Cystine bis-^tbutyl ester **7** (6.6 g, 18 mmol) and fluorenylmethoxycarbonyl chloride (10 g, 36 mmol, 2 equiv.) were dissolved in THF under inert conditions. The reaction was cooled in an ice bath and NMM (4.5 ml, 36 mmol, 2 equiv.) was slowly added to the solution and the resulting mixture was stirred for 3 hr. Ethyl acetate (200 ml) was added to the mixture, which was then washed with potassium hydrogen sulfate (5 % aq. w/v, 4 x 50 ml) and water (3 x 50 ml). The organic layer was dried over anhydrous sodium sulfate and the solvent was removed *in vacuo* yielding the crude product as a yellow liquid. The product was precipitated with DCM:MeOH, 1:5 at -20°C . ^{13}C and ^1H NMR data were consistent with literature.^{194, 215, 216}

Yield: 12 g, 15 mmol, 81 %

R_f: 0.25 (hexane:ether, 2:1)

M.p.: 146 – 148 $^{\circ}\text{C}$ (lit.²¹⁶ 151.5 - 152 $^{\circ}\text{C}$)

Elemental analysis: Found C, 66.59; H, 6.06; N, 3.38; S, 7.97 %. $\text{C}_{44}\text{H}_{48}\text{N}_2\text{O}_8\text{S}_2$ requires C, 66.31; H, 6.07; N, 3.51; S, 8.05 %.

NMR: δ_{H} (400 MHz) 7.78 (4H, bd, J 7.5, $H_{\text{a}'}$), 7.62 (4H, bd, J 7.4, H_{a}), 7.41 (4H, bt, J 7.2, $H_{\text{b}'}$), 7.33 (4H, bt, J 7.3, H_{b}), 5.74 (2H, d, J 7.0, NH), 4.56 (2H, bm, CHCH_2S), 4.41 (4H, bm, $\text{CH}_2\text{CH}_2\text{O}$), 4.23 (2H, bm, H_{c}), 2.99 (4H, bm, CHCH_2S), 1.51 (18H, s, $\text{CO}_2\text{C}(\text{CH}_3)_3$); δ_{C} (75.4 MHz, DEPT135) 169.7, 156.0, 144.3, 144.2, 141.7, 128.1, 127.5, 125.5, 120.3, 83.5, 67.6, 54.6, 47.5, 28.4

Mass spectrum: m/z (FAB) 819.2750 ($[\text{M} + \text{Na}]^+$. $\text{C}_{44}\text{H}_{48}\text{N}_2\text{O}_8\text{S}_2\text{Na}$ requires 819.2725), 797 (M^+ , 30 %), 685 ($[\text{M} - 2^t\text{Bu} + 3]^+$, 100 %), 463 ($[\text{M} - 2^t\text{Bu} - \text{Fmoc} + 4]^+$, 54 %), 237 ($[\text{M} - 2^t\text{Bu} - 2\text{Fmoc} + 5]^+$, 49 %)

3. N-9-Fluorenylmethoxycarbonyl-(R)-cysteine ^tbutyl ester⁷⁶

A solution of *N,N'*-bis(fluorenylmethoxycarbonyl)-(*R,R*)-cystine-bis- ^tbutyl ester **8** (7.5 g, 9.4 mmol) in dry THF (50 ml) was treated with tributylphosphine (2.4 ml, 9.4 mmol). The mixture was stirred for 2 minutes after which water (distilled, 0.94 ml, 10 % of THF volume, 5 equiv.) was added and the reaction was stirred for a further 2 hr. The mixture was then concentrated *in vacuo* and dissolved in ethyl acetate (200 ml). The solution was then washed with citric acid (10 % aq. w/v, 150 ml) then with brine (100 ml). The organic layer was dried over anhydrous sodium sulfate and the solvent was removed under reduced pressure yielding a pale yellow liquid. Purification *via* flash column chromatography (hexane:ether 2:1, 6 cm width, 15 cm height) yielded the product as an extremely viscous colourless liquid.

Yield: 6.8 g, 17 mmol, 85 %

R_f: 0.30 (hexane:ether, 2:1)

Elemental analysis: Found C, 65.96; H, 6.40; N, 3.42; S, 7.83 %. C₂₂H₂₅NO₄S requires C, 66.14; H, 6.31; N, 3.51; S, 8.02 %.

NMR: δ_{H} (400 MHz) 7.78 (2H, d, *J* 7.5, *H*_{a'}), 7.62 (2H, d, *J* 7.4, *H*_a), 7.41 (2H, t, *J* 7.4, *H*_{b'}), 7.33 (2H, dt, *J* 7.5, 1.2, *H*_b), 5.74 (1H, d, *J* 7.0, *NH*), 4.56 (1H, m, *CHCH*₂SH), 4.41 (2H, m, *CH*_c*CH*₂O), 4.23 (1H, m, *H*_c), 3.00 (2H, m, *CHCH*₂SH), 1.51 (9H, s, CO₂C(CH₃)₃); δ_{C} (75.4 MHz, DEPT135) 169.4, 156.1, 144.1, 141.7, 128.2, 127.5, 125.5, 120.4, 83.5, 67.5, 55.8, 47.6, 28.4, 27.8

Mass spectrum: *m/z* (FAB) 422.1402 ([*M* + Na]⁺. C₂₂H₂₅NO₄SNa requires 422.1391), 400 ([*M* + H]⁺, 0.3 %), 344 ([*M* - ^tBu]⁺, 3.8 %), 220 ([*M* - Fmoc]⁺, 0.2 %), 179 (Fmoc⁺, 100 %), 163 ([*M* - Fmoc - ^tBu]⁺, 6.9 %)

4. *N*-Triphenylmethyl-(*S*)-serine¹⁹⁵

To a suspension of (*S*)-serine (4.4 g, 42 mmol) in DCM (65 ml) under nitrogen was added trimethylsilyl chloride (17 ml, 0.13 mol, 3.1 equiv.). The mixture was allowed to reflux for 20 minutes. After cooling the mixture to room temperature, triethylamine (18 ml, 130 mmol, 3.1 equiv.) in DCM (20 ml) was added and the reaction was heated under reflux for a further 45 minutes. After cooling in an ice bath anhydrous MeOH (2.5 ml, 42 mmol) was added dropwise to the mixture, which was then allowed to warm to room temperature. Triethylamine (6 ml, 42 mmol) was added followed by triphenylmethyl chloride (12 g, 42 mmol) and the mixture was stirred for 20 hr. An excess of triethylamine (20 ml) and MeOH (200 ml) was then added until the white solids present in the mixture dissolved. The solvents were removed *in vacuo* yielding a mixture of yellow and white solids, which was partitioned between ethyl acetate (200 ml) and citric acid pre-cooled to 4 °C (5 % aq. w/v, 150 ml). The organic layer was washed with a solution of sodium hydroxide (1M, 2 x 50 ml) followed by water (2 x 30 ml). The aqueous layers were combined, washed with ethyl acetate (50 ml) and neutralised with glacial acetic acid at 0 °C. The precipitated product was extracted with ethyl acetate (3 x 50 ml) and the organic layer was washed with water (2 x 20 ml). Removal of the solvent under reduced pressure produced a white solid.

Yield: 5.8 g, 17 mmol, 40 %

R_f: 0.33 (DCM:MeOH, 9:1)

M.p.: 107 – 108 °C (dec.) (lit.²¹⁴ 111 - 112 °C, racemic)

Elemental analysis: Found C, 75.93; H, 6.21; N, 3.29 %. C₂₂H₂₁NO₃ requires C, 76.06; H, 6.09; N, 4.03 %.

NMR: δ_H (400 MHz) 7.41 (6H, m, Trt), 7.24 (9H, m, Trt), 3.71 (1H, dd, *J* 11.2, 3.1, CHCH₂OH), 3.49 (1H, dd, *J* 4.2, 3.5, CHCH₂OH), 2.88 (1H, dd, *J* 11.2, 4.5, CHCH₂OH)

Mass spectrum: m/z (FAB) 370.1419 ($[M + Na]^+$, $C_{22}H_{21}NO_3Na$ requires 370.1430), 370 ($[M + Na]^+$, 1.5 %), 348 ($[M + H]^+$, 0.6 %), 243 (Trt^+ , 100 %); m/z (APCI+) 243 (Trt^+ , 100 %), 106 ($[M - Trt + 2]^+$, 0.7 %)

5. *N*-Triphenylmethyl-(*R*)-serine²¹⁴

To a suspension of (*R*)-serine (15 g, 0.14 mol) in DCM (200 ml) under nitrogen was added trimethylsilyl chloride (56 ml, 0.43 mol, 3.1 equiv.). The mixture was allowed to reflux for 20 minutes. After cooling the mixture to room temperature, triethylamine (62 ml, 0.43 mol, 3.1 equiv.) in DCM (200 ml) was added and the reaction was heated under reflux for a further 45 minutes. After cooling in an ice bath anhydrous MeOH (7 ml, 0.14 mol) was added dropwise to the mixture, which was then allowed to warm to room temperature. Triethylamine (20 ml, 0.14 mol) was added followed by triphenylmethyl chloride (30 g, 0.14 mol) and the mixture was stirred for 20 hr. An excess of triethylamine (100 ml) and MeOH (700 ml) was then added until the white solids present in the mixture dissolved. The solvents were removed *in vacuo* yielding a mixture of yellow and white solids, which was partitioned between ethyl acetate (500 ml) and citric acid pre-cooled to 4 °C (5 % aq. w/v, 300 ml). The organic layer was washed with a solution of sodium hydroxide (2M, 2 x 150 ml) followed by water (3 x 100 ml). The aqueous layers were combined, washed with ethyl acetate (100 ml) and neutralised with glacial acetic acid at 0 °C. The precipitated product was extracted with ethyl acetate (6 x 200 ml) and the organic layer was dried over anhydrous sodium sulfate. Removal of the solvent under reduced pressure yielded the product as a white solid.

Yield: 7.2 g, 21 mmol, 85 %

R_f: 0.33 (DCM:MeOH, 9:1)

M.p.: 109 – 110 °C (dec.) (lit.²¹⁴ 111 - 112 °C, racemic)

NMR: δ_H (500 MHz) 7.45 (6H, m, Trt), 7.28 (9H, m, Trt), 3.72 (1H, dd, J 11.3, 3.0, $CHCH_2OH$), 3.52 (1H, m, $CHCH_2OH$), 2.88 (1H, dd, J 11.3, 4.3, $CHCH_2OH$); δ_C (75.4 MHz, DEPT135) 175.9, 144.4, 128.7 128.2, 127.1, 72.3, 62.9, 59.3

Mass spectrum: m/z (FAB) 370.1419 ($[M + Na]^+$. $C_{22}H_{21}NO_3Na$ requires 370.1430), 370 ($[M + Na]^+$, 4 %), 243 (Trt^+ , 100 %)

6. N-Triphenylmethyl-(S)-serine allyl ester

A suspension of *N*-triphenylmethyl-(*S*)-serine (**S**)-3 (0.51 g, 1.5 mmol) in a solution of sodium hydrogen carbonate (0.13 g, 1.6 mmol) in water (5 ml) was treated with a solution of Aliquat 336 (0.70 ml, 1.5 mmol) and allyl bromide (0.14 ml, 1.6 mmol) in DCM (2.2 ml). After 10 – 15 seconds of vigorous stirring the starting compound dissolved and the mixture was left stirring for 95 hr. The mixture was extracted with DCM (3 x 15 ml) and the combined organic layers were dried over anhydrous sodium sulfate. Removal of the solvent *in vacuo* yielded a yellow liquid whose NMR spectrum contained the desired product and the Aliquat 336. This proved impossible to purify by chromatography.

7. N-Triphenylmethyl-(S)-serine allyl ester

A solution of *N*-triphenylmethyl-(*S*)-serine (**S**)-3 (0.31 g, 0.89 mmol) in DCM (2 ml) was prepared under argon and treated with triethylamine (0.18 ml, 1.3 mmol, 1.5 equiv.). A solution of allyl bromide (0.08 ml, 0.92 mmol, 1.04 equiv.) in DCM was added to the mixture and the reaction was stirred for 42 hr. The mixture was treated with citric acid (5 % aq. w/v, 2 ml) and extraction with DCM (3 x 10 ml) was performed. The combined organic layers were washed with water (3 x 10 ml) and dried over anhydrous sodium sulfate. A yellow liquid was produced and purified *via* flash column chromatography (DCM:MeOH, 9:1, 5 cm width, 8 cm height) yielding the desired product as a viscous pale yellow liquid, and the starting material

Yield: 0.17 g, 0.44 mmol, 50 %

R_f: 0.64 (DCM:MeOH, 9:1)

8. N-Triphenylmethyl-(S)-serine allyl ester

(S)-4

N-triphenylmethyl-(*S*)-serine (**S**)-3 (2.0 g, 5.7 mmol) was converted to its cesium salt by dissolving in dry MeOH (40 ml) with cesium carbonate (1.0 g, 2.9 mmol, 0.51 equiv.) under argon. After removing the solvent *in vacuo*, the salt produced was taken up in DMF (40 ml) and the resulting mixture was treated with allyl bromide (0.50 ml, 5.8 mmol, 1.01 equiv.). The mixture was left stirring for a further 18 hr, after which it was partitioned between ethyl acetate (200 ml) and citric acid (5 % aq. w/v, 50 ml). The aqueous layer was washed with further ethyl acetate (2 x 50 ml) and the organic layers were combined, washed with water (15 x 50 ml) and then dried over anhydrous sodium sulfate. Removal of the solvent *in vacuo* yielded a very pale yellow (almost colourless) liquid, which was purified by flash column-chromatography (DCM:MeOH, 40:1, 5 cm width) to give an extremely viscous colourless liquid.

Yield: 1.8 g, 4.6 mmol, 81 %

R_f: 0.33 (DCM:MeOH, 40:1)

[α]_D¹⁹: + 3.7 (c 0.98)

I.R.: $\nu_{\max}$ (cm⁻¹) 3581 (OH), 3325 (NH), 1732 (C=O ester), 1672 (C=C Ar), 1596 (C=C Ar). Absence of i) a broad H-bonded OH (acid) signal and ii) C=O (acid) signal between 1700-1725 cm⁻¹

NMR: δ_{H} (400 MHz) 7.48 (6H, m, Trt), 7.22 (9H, m, Trt), 5.70 (1H, m, CH=CH₂), 5.17 (2H, m, CH=CH₂), 4.20 (1H, dd, *J* 13.1, 5.8, OCH₂CH), 4.08 (1H, dd, *J* 13.1, 5.8, OCH₂CH), 3.70 (1H, m, HNCHCO₂), 3.56 (2H, m, CHCH₂OH); δ_{C} (75.4 MHz, DEPT135) 173.1, 145.6, 131.6, 128.5, 127.8, 126.5, 118.3, 70.9, 65.6, 64.9, 57.9

Mass spectrum: *m/z* (FAB) 410.1732 ([M + Na]⁺. C₂₂H₂₅NO₃Na requires 410.1750), 410 ([M + Na]⁺, 0.3 %), 388 ([M + H]⁺, 0.1 %), 387 (M⁺, 0.3 %), 243 (Trt⁺, 100 %), 146 ([M - Trt + 2]⁺, 1.8 %)

9. N-Triphenylmethyl-(R)-serine allyl ester

(R)-4

N-triphenylmethyl-(*R*)-serine (**(R)-3**) (20 g, 58 mmol) was converted to its cesium salt by dissolving in dry MeOH (200 ml) with cesium carbonate (1.0 g, 2.9 mmol, 0.51 equiv.) under argon. After removing the solvent *in vacuo*, the salt produced was treated with DMF (40 ml) and the resulting mixture was treated with allyl bromide (0.52 ml, 6.0 mmol, 1.01 equiv.). The mixture was left stirring for a further 18 hr, after which the DMF was removed under high vacuum. The mixture was then re-dissolved in ethyl acetate (200 ml) and washed with citric acid (5 % aq. w/v, 50 ml). The aqueous layer was washed with further ethyl acetate (2 x 50 ml) and the organic layers were combined, washed with water (12 x 50 ml) and then dried over anhydrous sodium sulfate. Removal of the solvent *in vacuo* yielded the product as an extremely viscous colourless liquid.

Yield: 19 g, 50 mmol, 87 %

R_f: 0.33 (DCM:MeOH, 40:1)

[α]_D¹⁹: - 2.0 (c 0.75)

I.R.: $\nu_{\max}$ (cm⁻¹) 3580 (OH), 3320 (NH), 1732 (C=O ester), 1493 (C=C Ar). Absence of i) a broad H-bonded OH (acid) signal and ii) C=O (acid) signal between 1700-1725 cm⁻¹

Elemental analysis: Found C, 75.65; H, 6.41; N, 3.49 %. C₂₅H₂₅NO₃ requires C, 77.49; H, 6.50; N, 3.61 %.

NMR: δ_{H} (500 MHz) 7.51 (6H, m, Trt), 7.25 (9H, m, Trt), 5.71 (1H, m, CH=CH₂), 5.17 (2H, m, CH=CH₂), 4.22 (1H, dd, *J* 13.1, 5.8, OCH₂CH), 4.11 (1H, dd, *J* 13.1, 5.8, OCH₂CH), 3.72 (1H, m, HNCHCO₂), 3.58 (2H, m, CHCH₂OH); δ_{C} (75.4 MHz, DEPT135) 173.6, 146.1, 132.1, 129.2, 128.3, 127.0, 118.8, 71.5, 66.1, 65.3, 58.3

Mass spectrum: *m/z* (FAB) 410.1732 ([M + Na]⁺. C₂₂H₂₅NO₃Na requires 410.1750), 388 ([M + H]⁺, 3 %), 387 (M⁺, 0.7 %), 243 (Trt⁺, 100 %), 146 ([M - Trt + 2]⁺, 2 %)

10. **N-Triphenylmethyl-(S)-serine(O-methanesulfonyl) allyl ester**

A solution of *N*-triphenylmethyl-(*S*)-serine allyl ester (**S**)-4 (0.092 g, 0.23 mmol) in DCM (3 ml) was treated with triethylamine (0.10 ml, 0.70 mmol, 3 equiv.) and methanesulfonyl chloride (0.04 ml, 0.47 mmol, 2 equiv.) at 0 °C. The reaction mixture was stirred for 2 hr at room temperature after which it was diluted with DCM (10 ml). The mixture was washed with a saturated solution of ammonium chloride (8 ml) and then with a saturated solution of sodium hydrogen carbonate (2 x 10 ml). The organic layer was dried over anhydrous sodium sulfate and removal of the solvent *in vacuo* gave a yellow-orange liquid. Flash column chromatography was used to purify the crude compound (hexane:ethyl acetate, 2:1, 1 cm width, 15 cm height) yielding a colourless liquid, which crystallised slowly. 1-D and 2-D ¹H NMR confirmed the correct compound, however a better procedure - described below - was followed.

Yield: 0.09 g, 0.18 mmol, 80 %

R_f: 0.3 (hexane:ethyl acetate, 2:1)

11. **N-Triphenylmethyl-(S)-serine(O-methanesulfonyl) allyl ester**

(S)-5

N-Triphenylmethyl-(*S*)-serine allyl ester (**S**)-4 (1.8 g, 4.5 mmol) was dissolved in dry THF (22 ml) under argon and the solution was cooled to 0 °C. Triethylamine (0.66 ml, 4.5 mmol) and methanesulfonyl chloride (0.70 ml, 9.0 mmol, 2 equiv.) were added, the ice bath removed and the mixture stirred for 4 hr. The reaction mixture was diluted with ether (150 ml) and washed with ice-cold water (5 x 50 ml) and then brine (3 x 40 ml). The organic layer was dried over anhydrous sodium sulfate and removal of the solvent yielded a pale yellow liquid that crystallised slowly. This material was recrystallised from DCM and MeOH (1:4) yielding white crystals.

Yield: 1.9 g, 4.0 mmol, 88 %

R_f: 0.3 (hexane:ethyl acetate, 2:1)

M.p.: 115 - 118 °C (dec.)

[α]_D¹⁵: + 27.2 (c 1.11)

I.R.: ν_{max} (cm⁻¹) 3018 (CH₃), 1736 (C=O), 1544 (C=C Ar), 1346 + 1178 (SO₂-O)

Elemental analysis: Found C, 66.74; H, 5.76; N, 2.91; S, 6.55 %. C₂₆H₂₇NO₅S requires C, 67.08; H, 5.85; N, 3.01; S, 6.89 %.

NMR: δ_H (400 MHz) 7.49 (6H, m, Trt), 7.27 (6H, m, Trt), 7.20 (3H, m, Trt), 5.72 (1H, m, CH=CH₂), 5.21 (2H, m, CH=CH₂), 4.46 (1H, dd, *J* 10.1, 4.2, CHCH₂OMs), 4.28 (1H, dd, *J* 10.1, 6.1, CHCH₂OMs), 4.25 (1H, dd, *J* 13.0, 5.9, OCH₂CH), 4.08 (1H, dd, *J* 13.0, 5.9, OCH₂CH), 3.73 (1H, m, HNCHCO₂), 2.97 (3H, s, OS(O)₂CH₃); δ_C (75.4 MHz, DEPT135) 171.3, 145.3, 131.4, 128.6, 128.1, 126.7, 118.8, 71.1, 70.7, 66.1, 55.7, 37.7

Mass spectrum: *m/z* (FAB) 466.1688 ([M + H]⁺. C₂₆H₂₈NO₅S requires 466.1664), 488 ([M + Na]⁺, 0.4 %), 243 (Trt⁺, 100 %), 224 ([M - Trt + 2]⁺, 0.2 %)

12. N-Triphenylmethyl-(R)-serine (O-methanesulfonyl) allyl ester

(R)-5

N-Triphenylmethyl-(*R*)-serine allyl ester (**(R)-4**) (19 g, 49 mmol) was dissolved in dry THF (200 ml) under argon and the solution was cooled to 0 °C. Triethylamine (7.0 ml, 49 mmol) and methanesulfonyl chloride (8.3 ml, 98 mmol, 2 equiv.) were added, the ice bath removed and the mixture stirred for 4 hr. The reaction mixture was diluted with ether (400 ml) and washed with ice-cold water (6 x 100 ml) and then brine (4 x 100 ml). The organic layer was dried over anhydrous sodium sulfate and removal of the solvent yielded a pale yellow liquid. Recrystallisation of the crude product was carried out in DCM and MeOH (1:4) yielding white crystals.

Yield: 21 g, 44 mmol, 91 %

R_f: 0.3 (hexane:ethyl acetate, 2:1)

M.p.: 116 - 118 °C (dec.)

[α]_D²⁰: -32.7 (c 0.46)

I.R.: $\nu_{\max}$ (cm⁻¹) 1736 (C=O), 1489 (C=C Ar), 1346 + 1177 (SO₂-O)

NMR: **δ_H** (400 MHz) 7.50 (6H, m, Trt), 7.29 (6H, m, Trt), 7.21 (3H, m, Trt), 5.70 (1H, m, CH=CH₂), 5.20 (2H, m, CH=CH₂), 4.45 (1H, dd, *J* 10.0, 4.2, CHCH₂OMs), 4.25 (1H, dd, *J* 10.0, 6.1, CHCH₂OMs), 4.21 (1H, dd, *J* 13.1, 6.0, OCH₂CH), 4.05 (1H, dd, *J* 13.1, 5.9, OCH₂CH), 3.69 (1H, m, HNCHCO₂), 2.99 (3H, s, OS(O)₂CH₃); **δ_C** (75.4 MHz, DEPT135) 171.3, 145.3, 131.5, 128.6, 128.1, 126.7, 118.8, 71.2, 70.7, 66.1, 55.8, 37.7

Mass spectrum: *m/z* (FAB) 488.1508 ([M + Na]⁺. C₂₆H₂₇NO₅SNa requires 488.1520), 488 ([M + Na]⁺, 1 %), 243 (Trt⁺, 100 %)

13. N-Triphenylmethyl-β-iodo-(R)-alanine allyl ester

A solution of *N*-triphenylmethyl-(*S*)-serine(*O*-methanesulfonyl) allyl ester (**S**)-**5** (1.1 g, 2.5 mmol) in acetone (6 ml) was added to a solution of sodium iodide (4.0 g, 29 mmol, 11 equiv.) in dry acetone (6 ml) under argon. The solution was stirred for 96 hr at 25 °C, and the resulting yellow-brown slurry was then concentrated *in vacuo*. Ether (100 ml) then sodium thiosulfate (10 % aq. w/v, 4 ml approx.) was then slowly added, dissolving the insoluble solids and substantially decolourising the organic layer to a pale yellow solution. The organic layer was separated and dried over anhydrous sodium sulfate and the solvent was removed *in vacuo*. The crude product was separated from the starting material (flash column chromatography, hexane:ethyl acetate, 2:1, 4 cm width) giving a pale yellow liquid. Further purification by precipitation (DCM:MeOH, 1:4) afforded the desired product, along with the regioisomeric by-product, (*R/S*)-2-iodo-3-(triphenylmethylamino)-propionic acid allyl ester, in 1:4 proportion as an inseparable mixture.

Yield: 1.02 g, 2.0 mmol, 82 % (overall); 0.20 g, 0.41 mmol, 16 % (desired product, based on NMR)

R_f: 0.60 (hexane:ethyl acetate. 2:1)

I.R.: $\nu_{\max}$ (cm⁻¹) 1730 (C=O), 1489 (C=C Ar). Absence of signals at 1346 (SO₂-O) and 1177 (SO₂-O) cm⁻¹

Elemental analysis: Found C, 60.15; H, 4.77; N, 2.72; I, 25.59 % on mixture. C₂₅H₂₄NO₂I requires C, 60.37; H, 4.86; N, 2.82; I, 25.51 %.

NMR: δ_{H} (400 MHz) 7.47 (6H, m, Trt), 7.30 (6H, m, Trt), 7.21 (3H, m, Trt), 5.74 (1H, m, CH=CH₂), 5.24 (1H, m, CH=CH₂), 5.21 (1H, m, CH=CH₂), 4.25 (1H, dd, *J* 13.0, 5.8, OCH₂CH), 4.10 (1H, dd, *J* 13.0, 5.7, OCH₂CH), 3.52 (1H, m, HNCHCO₂), 3.33 (1H, dd, *J* 9.7, 3.1, CHCH₂I), 3.22 (1H, dd, *J* 9.5, 7.0, CHCH₂I), 2.91 (1H, d, *J* 9.6, TrtNHCH); δ_{C} (75.4 MHz, DEPT135) 172.0, 145.6, 131.2, 128.6, 128.0, 126.5, 118.8, 70.8, 66.0, 56.4, 9.7

(R/S)-2-iodo-3-(triphenylmethylenamino)-propionic acid allyl ester (mixture of enantiomers): δ_{H} (400 MHz) 7.52 (6H, m, Trt), 7.30 (6H, m, Trt), 7.21 (3H, m, Trt), 5.94 (1H, m, CH=CH₂), 5.40 (1H, dd, *J* 17.2, 1.4, CH=CH₂), 5.30 (1H, dd, *J* 10.5, 1.1, CH=CH₂), 4.66 (2H, d, *J* 5.7, OCH₂CH), 4.42 (1H, m, CHICO₂), 2.74 (1H, dd, *J* 12.8, 8.6, HNCH₂CHI), 2.57 (1H, dd, *J* 12.9, 5.8, HNCH₂CHI), 2.27 (TrtNHCH₂); δ_{C} (75.4 MHz, DEPT135) 170.4, 145.4, 131.1, 128.3, 128.0, 126.5, 118.8, 70.8, 66.1, 48.2, 20.1

Mass spectrum: *m/z* (FAB) 498.0930 ([M + H]⁺. C₂₅H₂₅NO₂I requires 498.0954), 520 ([M + Na]⁺, 3.7 %), 498 ([M + H]⁺, 2.7 %), 497 (M⁺, 0.4 %); *m/z* (APCI+) 520 ([M + Na]⁺, < 0.1 %), 243 (Trt⁺, 100 %), 256 ([M - Trt + 2]⁺, 6 %)

Figure 7.1. Major product: (R/S)-2-iodo-3-(triphenylmethylenamino)-propionic acid allyl ester

14. **N-Triphenylmethyl-β-iodo-(S)-alanine allyl ester**

A solution of *N*-triphenylmethyl-(*R*)-serine(*O*-methanesulfonyl) allyl ester (**R**)-**5** (22 g, 46 mmol) in acetone (80 ml) was added to a solution of sodium iodide (70 g, 0.46 mol, 10 equiv.) in dry acetone (100 ml) under argon. The mixture was stirred for 72 hr at 25 °C and the resulting yellow-brown slurry was then concentrated *in vacuo*. Ether (400 ml) was added and sodium thiosulfate (10 % aq. w/v, 25 ml approx.) was then slowly added, dissolving the insoluble solids and substantially decolourising the organic layer to a pale yellow solution. The organic layer was separated and dried over anhydrous sodium sulfate and the solvent was removed *in vacuo*. The crude product was purified (flash column chromatography, hexane:ethyl acetate, 2:1, 5 cm width) to remove a small amount of starting material, giving a pale yellow liquid. Further purification by precipitation (DCM:MeOH, 1:5) afforded the desired product, along with the regioisomeric by-product, (*R/S*)-2-iodo-3-(triphenylmethylamino)-propionic acid allyl ester in a ratio of 1:5. These two products were not separable by HPLC, however, incomplete separation of the two species was attempted using preparative normal phase HPLC (2 – 3 % ethyl acetate in hexane over 10 minutes, 15 ml/min with DCM loading, R_T 12.1, 12.8 (regioisomer)).

Yield: 19.9 g, 40 mmol, 87 % (overall); 3.3 g, 6.7 mmol, 15 % (desired product, based on NMR)

R_f: 0.60 (hexane:ethyl acetate. 2:1)

I.R.: $\nu_{\max}$ (cm⁻¹) 1730 (C=O), 1489 (C=C Ar). Absence of signals at 1346 (SO₂-O) and 1177 (SO₂-O) cm⁻¹

NMR: δ_H (600 MHz) 7.51 (6H, m, Trt), 7.29 (6H, m, Trt), 7.20 (3H, m, Trt), 5.73 (1H, m, CH=CH₂), 5.22 (1H, dd, *J* 17.3, 1.4, CH=CH₂), 5.19 (1H, dd, *J* 10.2, 1.1, CH=CH₂), 4.22 (1H, dd, *J* 13.1, 6.1, OCH₂CH), 4.09 (1H, dd, *J* 13.1, 6.1, OCH₂CH), 3.50 (1H, m, HNCHCO₂), 3.30 (1H, dd, *J* 9.8, 3.4, CHCH₂I), 3.23 (1H, dd, *J* 9.8,

6.9, CHCH₂I), 2.88 (1H, d, *J* 9.8, TrtNHCH); δ_c (150.8 MHz, DEPT135, HSQC, HMBC) 171.9, 145.6, 131.3, 128.4, 127.9, 126.4, 118.6, 70.9, 65.8, 55.8, 9.55

(R/S)-2-iodo-3-(triphenylmethylamino)-propionic acid allyl ester (mixture of enantiomers): δ_H (600 MHz) 7.52 (6H, m, Trt), 7.30 (6H, m, Trt), 7.21 (3H, m, Trt), 5.94 (1H, m, CH=CH₂), 5.40 (1H, dd, *J* 17.2, 1.4, CH=CH₂), 5.30 (1H, dd, *J* 10.5, 1.1, CH=CH₂), 4.67 (2H, m, OCH₂CH), 4.42 (1H, m, CHICO₂), 2.73 (1H, dd, *J* 12.9, 8.5, HNCH₂CHI), 2.57 (1H, dd, *J* 12.9, 5.9, HNCH₂CHI), 2.27 (TrtNHCH₂); δ_c (150.8 MHz, DEPT135, HSQC, HMBC) 170.4, 145.4, 131.1, 128.3, 128.0, 126.5, 118.8, 70.8, 66.1, 48.2, 20.1

Mass spectrum: *m/z* (FAB) 520.0750 ([M + Na]⁺. C₂₅H₂₄NO₂INa requires 520.0732), 520 ([M + Na]⁺, 0.2 %), 243 (Trt⁺, 100 %); *m/z* (APCI+) 498 ([M + H]⁺, 2 %), 243 (Trt⁺, 100 %), 256 ([M – Trt + 2]⁺, 2 %)

6b

Figure 7.2. Major product: (R/S)-2-iodo-3-(triphenylmethylamino)-propionic acid allyl ester

15. 3-[(R)-2-'Butoxycarbonyl-2-(fluoren-9-ylmethoxycarbonylamino)-ethylsulfanyl]-(R)-2-(triphenylmethylamino)-propionic acid allyl ester

(R,R)-10

N-9-Fluorenylmethoxycarbonyl-(*R*)-cysteine ^tbutyl ester **9** (0.29 g, 0.73 mmol) was dissolved in DMF (3.2 ml) under inert conditions. To it a solution of *N*-

triphenylmethyl-3-iodo-(*R*)-alanine allyl ester (***R***-6a (mixture with regioisomer, 0.41 g, 0.83 mmol, 1.1 equiv.) in DMF (3.2 ml) was added. Cesium carbonate (0.24 g, 0.73 mmol) was then added and the mixture was stirred for 4 hr at 25 °C. The solvent was removed *in vacuo* and the residue was partly dissolved in ethyl acetate (75 ml). The organic layer was washed with citric acid (5 % aq. w/v, 25 ml), dissolving the remainder of the residue. The organic layer was then washed with water (10 x 30 ml) and dried over anhydrous sodium sulfate. Removal of the solvent *in vacuo* yielded a pale yellow oil which was purified *via* flash column chromatography (hexane:ethyl acetate, 4:1, 5.5 cm width) and produced the desired product along with its regioisomer in diastereoisomeric mixture (1:2 product:regioisomers), as a very pale yellow oil which foamed and solidified under highly reduced pressure.

Yield: 0.44 g, 0.57 mmol, 78 % (overall); 0.15 g, 0.19 mmol, 26 % (desired product, based on NMR)

R_f: 0.28 (hexane:ethyl acetate, 4:1)

I.R.: $\nu_{\max}$ (cm⁻¹) 1724 (C=O), 1506 (C=C Ar), 1450 (C=C Ar), 1215 + 1155 (C-S-C)

Found: C, 72.92; H, 6.45; N, 3.60; S, 3.98 %. C₄₇H₄₈N₂O₆S requires C, 73.41; H, 6.29; N, 3.64; S, 4.17 %.

NMR: δ_{H} (400 MHz) 7.73 (2H, m, *H*_a'), 7.64 (2H, m, *H*_a), 7.53 (6H, m, Trt), 7.36 (2H, m, *H*_b'), 7.20 – 7.31 (2H, m, *H*_b + 6H, m, Trt), 7.16 (3H, m, Fmoc (Ar)), 5.68 (1H, m, CH=CH₂), 5.62 (1H, bd, FmocNH), 5.22 (1H, m, CH=CH₂), 5.13 (1H, m, CH=CH₂), 4.47 (1H, m, FmocNHCH), 4.35 (2H, m, CH₂OCONH), 4.20 (1H, m, *H*_c), 4.05 (2H, m, OCH₂CH=CH₂), 3.58 (1H, m, TrtNHCH), 2.98 (2H, m, CHCH₂S), 2.72 – 2.90 (2H, m, TrtNHCHCH₂), 2.20 (1H, bs, TrtNH), 1.49 (9H, s, C(CH₃)₃); δ_{C} (75.4 MHz, DEPT135) 173.4, *ca.* 170, 156.1, *ca.* 146, 144.1, 141.6, *ca.* 132, *ca.* 129, *ca.* 128, 128.1, 127.5, *ca.* 127, 125.5, 120.3, *ca.* 119, *ca.* 83, *ca.* 71, 67.6, *ca.* 66, 56.9, *ca.* 54, 47.5, 38.6, 36.3, 28.3

(*R/S*)-2-[(*R*)-2-*t*Butoxycarbonyl-2-(fluoren-9-ylmethoxycarbonylamino)-ethylsulfanyl]-3-(triphenylmethylamino)-propionic acid allyl ester (diastereoisomeric mixture): δ_{H} (400 MHz) 7.73 (2H, m, *H*_a'), 7.56 (2H, m, *H*_a), 7.43 (6H, m, Trt), 7.36 (2H, m, *H*_b'), 7.20 – 7.31 (2H, m, *H*_b + 6H, m, Trt), 7.16 (3H, m, Fmoc (Ar)), 5.91 (1H, m, CH=CH₂), 5.58 + 5.69 (1H, bd, FmocNH), 5.33 (1H, m,

CH=CH₂), 5.23 (1H, m, CH=CH₂), 4.66 (2H, m, OCH₂CH=CH₂), 4.50 (1H, m, FmocNHCH), 4.29 – 4.41 (2H, m, CH₂OCONH), 4.18 – 4.25 (1H, m, H_c), 3.49 + 3.56 (1H, t, *J* 6.4 + 6.7, TrtNHCH₂CH), 2.81 – 3.12 (2H, m, CHCH₂S), 2.61 (1H, m, TrtNHCH₂), 2.45 (1H, m, TrtNHCH₂), 1.48 – 1.49 (9H, s, C(CH₃)₃); δ_c (75.4 MHz, DEPT135) 171.8, *ca.* 169, 156.1, *ca.* 146, 144.1, 141.6, *ca.* 132, *ca.* 129, *ca.* 128, 128.1, 127.5, *ca.* 127, 125.5, 120.3, *ca.* 119, *ca.* 83, *ca.* 71, 67.6, *ca.* 66, *ca.* 54, (48.8 + 48.0), 47.5, (44.9 + 44.6), (34.8 + 34.3), 28.3

Mass spectrum: *m/z* (FAB) 769.3311 ([M + H]⁺. C₄₇H₄₉N₂O₆S requires 769.3326), 769 (M⁺, 4.8 %), 527 ([M – Trt + 1]⁺, 1.9 %), 471 ([M – Trt – ^tBu + 2]⁺, 0.2 %), 243 (Trt⁺, 100 %), 208 ([M – Trt – Fmoc – ^tBu – All + 3]⁺, 0.3 %)

18

Figure 7.3. Major product: (*R/S*)-2-[(*R*)-2-^tbutoxycarbonyl-2-(fluoren-9-ylmethoxycarbonylamino)-ethylsulfanyl]-3-(triphenylmethylamino)-propionic acid allyl ester

16. 3-[/(R)-2-^tButoxycarbonyl-2-(fluoren-9-ylmethoxycarbonylamino)-ethylsulfanyl]-(S)-2-(triphenylmethylamino)-propionic acid allyl ester

N-9-Fluorenylmethoxycarbonyl-(*R*)-cysteine ^tbutyl ester **9** (5.6 g, 14 mmol) was dissolved in dry DMF (75 ml) under inert conditions. To it a solution of *N*-triphenylmethyl-3-iodo-(*S*)-alanine allyl ester (**S**)-**6a** (mixture with regioisomer, 7.7 g, 16 mmol, 1.1 equiv) in DMF (75 ml) was added. Cesium carbonate (4.6 g, 14 mmol) was then added and the mixture was stirred for 4 hr at 25 °C. The solvent was removed *in vacuo* and the residue was partly dissolved in ethyl acetate (250 ml). The organic layer was washed with citric acid (5 % aq. w/v, 100 ml), dissolving the remainder of the residue. The organic layer was then washed with water (8 x 100 ml) and dried over anhydrous sodium sulfate. Removal of the solvent *in vacuo* yielded a pale yellow oil which was purified *via* flash column chromatography (hexane:ethyl acetate, 4:1, 9 cm width) produced the desired product along with its regioisomer in diastereoisomeric mixture (1:5 product:regioisomers), as a very pale yellow oil which foamed and solidified under highly reduced pressure.

Yield: 9.7 g, 12.6 mmol, 90 % (overall); 1.6 g, 2.1 mmol, 15 % (desired product, based on NMR)

R_f: 0.28 (hexane:ethyl acetate, 4:1)

I.R.: $\nu_{\max}$ (cm⁻¹) 1724 (C=O), 1504 (C=C Ar), 1450 (C=C Ar), 1215 + 1153 (C-S-C)

NMR: δ_{H} (500 MHz) 7.74 (2H, m, Fmoc (Ar)), 7.60 (2H, m, Fmoc (Ar)), 7.49 (6H, m, Trt), 7.38 (2H, m, Fmoc (Ar)), 7.20 – 7.31 (2H, m, Fmoc (Ar) + 6H, m, Trt), 7.16 (3H, m, Fmoc (Ar)), 5.69 (1H, m, CH=CH₂), 5.63 (1H, bd, FmocNH), 5.18 (1H, m, CH=CH₂), 5.13 (1H, m, CH=CH₂), 4.49 (1H, m, FmocNHCH), 4.34 (2H, m,

CH_2OCONH), 4.18 (1H, m, $\text{CHCH}_2\text{OCONH}$), 4.04 (2H, m, $\text{OCH}_2\text{CH}=\text{CH}_2$), 3.56 (1H, m, TrtNHCH), 2.89 – 3.03 (2H, m, CHCH_2S), 2.70 – 2.89 (2H, m, TrtNHCHCH_2), 2.20 (1H, bs, TrtNH), 1.50 (9H, s, $\text{C}(\text{CH}_3)_3$); δ_{C} (125.8 MHz, DEPT135) 171.9, 169.7, 156.1, 146.0, 144.2, 141.7, 132.1, 129.2, 128.4, 128.1, 127.0, 127.5, 125.6, 120.4, 119.2, 83.4, 71.2, 67.6, 66.3, 56.2, *ca.* 54.3, 47.5, 28.4 (both β -Cs were too weak to be detected)

(R/S)-2-[(R)-2-^tButoxycarbonyl-2-(fluoren-9-ylmethoxycarbonylamino)-ethylsulfanyl]-3-(triphenylmethylamino)-propionic acid allyl ester (diastereoisomeric mixture): δ_{H} (400 MHz) 7.73 (2H, m, H_{a}), 7.56 (2H, m, H_{a}), 7.43 (6H, m, Trt), 7.36 (2H, m, H_{b}), 7.20 – 7.31 (2H, m, H_{b} + 6H, m, Trt), 7.16 (3H, m, Fmoc (Ar)), 5.91 (1H, m, $\text{CH}=\text{CH}_2$), 5.58 + 5.69 (1H, bd, FmocNH), 5.33 (1H, m, $\text{CH}=\text{CH}_2$), 5.23 (1H, m, $\text{CH}=\text{CH}_2$), 4.66 (2H, m, $\text{OCH}_2\text{CH}=\text{CH}_2$), 4.50 (1H, m, FmocNHCH), 4.29 – 4.41 (2H, m, CH_2OCONH), 4.18 – 4.25 (1H, m, H_{c}), 3.49 + 3.56 (1H, t, J 6.4 + 6.7, $\text{TrtNHCH}_2\text{CH}$), 2.81 – 3.12 (2H, m, CHCH_2S), 2.61 (1H, m, TrtNHCH_2), 2.45 (1H, m, TrtNHCH_2), 1.48 – 1.49 (9H, s, $\text{C}(\text{CH}_3)_3$); δ_{C} (75.4 MHz, DEPT135) 171.8, *ca.* 169, 156.1, *ca.* 146, 144.1, 141.6, *ca.* 132, *ca.* 129, *ca.* 128, 128.1, 127.5, *ca.* 127, 125.5, 120.3, *ca.* 119, *ca.* 83, *ca.* 71, 67.6, *ca.* 66, *ca.* 54, (48.8 + 48.0), 47.5, (44.9 + 44.6), (34.8 + 34.3), 28.3

Mass spectrum: m/z (FAB) 901.2287 ($[\text{M} + \text{Cs}]^+$. $\text{C}_{47}\text{H}_{48}\text{N}_2\text{O}_6\text{SCs}$ requires 901.2264), 527 ($[\text{M} - \text{Trt} + 1]^+$, 0.4 %), 471 ($[\text{M} - \text{Trt} - ^t\text{Bu} + 2]^+$, 0.3 %), 243 (Trt^+ , 100 %)

18

Figure 7.4. Major product: (*R* /*S*)-2-[(*R*)-2-^tbutoxycarbonyl-2-(fluoren-9-ylmethoxycarbonylamino)-ethylsulfanyl]-3-(triphenylmethylamino)-propionic acid allyl ester

17. 3-[(*R*)-2-^tButoxycarbonyl-2-(fluoren-9-ylmethoxycarbonylamino)-ethylsulfanyl]-(*R*)-2-(allyloxycarbonylamino)-propionic acid allyl ester

(*R,R*)-12

3-[(*R*)-2-^tButoxycarbonyl-2-(fluoren-9-ylmethoxycarbonylamino)-ethylsulfanyl]-(*R*)-2-(triphenylmethylamino)-propionic acid allyl ester (**(*R,R*)-10** (mixture with regioisomer, 0.69 g, 0.90 mmol) was treated with a 5 % solution of trifluoroacetic acid (0.30 ml, 3.8 mmol, 4.2 equiv.) in DCM (6.4 ml) under inert conditions for 4 h. The resulting solution was diluted with DCM (50 ml) and washed with sodium

hydrogen carbonate (5 % aq. w/v, 2 x 10 ml) and water (2 x 10 ml). The solvent was removed *in vacuo*. DCM (20 ml) and MeOH (20 ml) were then added and re-evaporation yielded a pale yellow liquid, which was then treated with sodium hydrogen carbonate (0.30 g, 3.6 mmol) in water (7.5 ml). The mixture was then cooled to 0 °C and a solution of allyl chloroformate (0.19 ml, 1.8 mmol) in dioxane (7.5 ml) was added. The resulting mixture was stirred at a temperature < 5 °C for 18 hr. The solvents were then removed *in vacuo* and the residue was re-dissolved in ethyl acetate (100 ml). The organic layer was washed with water (10 x 40 ml), dried over anhydrous sodium sulfate and concentrated yielding a viscous yellow liquid. Purification was carried out *via* flash column chromatography (hexane:ethyl acetate, 4:1 then 2:1, 4 cm width) yielding the title compound as a mixture of regioisomers and a yellow oil.

Yield: 0.44 g, 0.73 mmol, 81 % (overall); 0.15 g, 0.24 mmol, 27 % (desired product, based on starting material)

R_f: 0.29 (hexane:ethyl acetate, 2:1)

I.R.: $\nu_{\max}$ (cm⁻¹) 1720 (C=O), 1508 (C=C Ar), 1450 (C=C Ar), 1223 + 1153 (C-S-C)

NMR: δ_{H} (400 MHz) 7.77 (2H, m, H_{a}'), 7.62 (2H, m, H_{a}), 7.40 (2H, m, H_{b}'), 7.31 (2H, m, H_{b}), 5.80 – 5.96 (2H, m, CH=CH₂), 5.75 (1H, m, FmocNH), 5.66 (1H, m, AllocNH), 5.17 – 5.35 (4H, m, CH=CH₂), 4.56 – 4.65 (4H, m, OCH₂CH=CH₂), 4.61 (1H, m, AllocNHCH), 4.50 (1H, m, FmocNHCH), 4.39 (2H, m, CH₂CONH), 4.25 (1H, m, H_{C}), 2.94 – 3.10 (4H, m, CH₂SCH₂), 1.50 (9H, s, C(CH₃)₃); δ_{C} (125.8 MHz, DEPT135) 170.2, *ca.* 169.2, *ca.* 156.1, *ca.* 155.7, *ca.* 143.8, 141.3, *ca.* 132.5, *ca.* 131.3, *ca.* 127.8, 127.1, 125.1, 120.0, *ca.* 119.1, *ca.* 117.8, *ca.* 83.1, *ca.* 67.2, 66.4, *ca.* 66.1, *ca.* 54.0 (3C), *ca.* 47.1, *ca.* 35.5 (2C), 28.0 (δ_{H} values were extrapolated from the separated samples of 3-[(*R*)-2-^tbutoxycarbonyl-2-(fluoren-9-ylmethoxycarbonylamino)-ethylsulfanyl]-(*S*)-2- (allyloxycarbonylamino)-propionic acid allyl ester (*S,R*)-12)

(*R,S*)-2-[(*R*)-2-^tButoxycarbonyl-2-(fluoren-9-ylmethoxycarbonylamino)-ethylsulfanyl]-3-(allyloxycarbonylamino)-propionic acid allyl ester (diastereoisomeric mixture): δ_{H} (400 MHz) 7.76 (2H, m, H_{a}'), 7.62 (2H, m, H_{a}), 7.40 (2H, m, H_{b}'), 7.32 (2H, m, H_{b}), 5.90 (2H, m, CH=CH₂), 5.76 (1H, bm, FmocNH), 5.14 – 5.36 (4H, m, CH=CH₂), 5.33 (1H, m, AllocNH), 4.50 – 4.70 (4H, m,

OCH₂CH=CH₂), 4.54 (1H, m, FmocNHCH), 4.40 (2H, m, CH₂OCONH), 4.25 (1H, m, H_c), 3.45 – 3.66 (2H, m, SCHCH₂), 3.52 (1H, m, AllocNHCH), 2.97 – 3.25 (2H, m, SCH₂), 1.50 (9H, s, C(CH₃)₃); δ_c (125.8 MHz, DEPT135) 170.7, (169.2 + 169.1), *ca.* 156, *ca.* 157, 143.7, 141.2, 132.6, (131.4 + 131.3), 127.7, 127.0, 125.1, 119.9, (119.0 + 118.9), (117.72 + 117.65), *ca.* 83.1, (67.24 + 67.16), (66.1 + 66.0), 65.7, (54.3 + 53.9), 47.0, (46.7 + 46.0), (41.5 + 41.4), (34.34 + 34.26), 27.9

Mass spectrum: *m/z* (FAB) 611.2427 ([M + H]⁺. C₃₂H₃₉N₂O₈S requires 611.2440), 633 ([M + Na]⁺, 19 %), 611 ([M + H]⁺, 20 %), 555 ([M – ^tBu + 2]⁺, 55 %), 333 ([M – ^tBu – Fmoc + 3]⁺, 35 %); *m/z* (APCI+) 633 ([M + Na]⁺, 4.5 %), 611 ([M + H]⁺, 13 %), 555 ([M – ^tBu + 2]⁺, 100 %), 333 ([M – ^tBu – Fmoc + 3]⁺, 38 %)

19

Figure 7.5. Major product: (*R/S*)-2[(*R*)-2-^tbutoxycarbonyl-2-(fluoren-9-ylmethoxycarbonylamino)-ethylsulfanyl]-3-(allyloxycarbonylamino)-propionic acid allyl ester

18. 3-[(R)-2-^tButoxycarbonyl-2-(fluoren-9-ylmethoxycarbonylamino)-ethylsulfanyl]-(S)-2- (allyloxycarbonylamino)-propionic acid allyl ester

3-[(R)-2-^tButoxycarbonyl-2-(fluoren-9-ylmethoxycarbonylamino)-ethylsulfanyl]-(S)-2-(triphenylmethylamino)-propionic acid allyl ester **(S,R)-10** (mixture with regioisomer, 3.5 g, 4.6 mmol) was treated with a 5 % solution of trifluoroacetic acid (1.4 ml, 18 mmol, 4 equiv.) in DCM (27 ml) under inert conditions for 4 hr. The resulting solution was diluted with DCM (200 ml) and washed with sodium hydrogen carbonate (5 % aq. w/v, 2 x 75 ml) and water (2 x 50 ml). The solvent was removed *in vacuo*. DCM (20 ml) and MeOH (20 ml) were then added and re-evaporation yielded a pale yellow liquid, which was then treated with sodium hydrogen carbonate (1.5 g, 18 mmol, 4 equiv.) in water (30 ml). The mixture was then cooled to 0 °C and a solution of allyl chloroformate (0.80 ml, 9.2 mmol, 2 equiv.) in dioxane (30 ml) was added. The resulting mixture was stirred at a temperature < 5 °C for 18 hr. The solvents were then removed *in vacuo* and the residue was re-dissolved in ethyl acetate (250 ml). The organic layer was washed with water (8 x 50 ml), dried over anhydrous sodium sulfate and concentrated yielding a yellow liquid. Purification was carried out *via* flash column chromatography (hexane:ethyl acetate, 4:1 then 2:1, 7.5 cm width) yielding the title compound as a viscous yellow oil. Separation of the regioisomers was carried out *via* preparative normal phase HPLC (20 – 23 % ethyl acetate in hexane over 45 minutes, 15 ml/min, DCM loading, R_T 16.6, 20.1 (regioisomer)) yielding both products as viscous, pale yellow liquids.

Yield: 2.4 g, 4.0 mmol, 87 % (overall); 0.41 g, 0.67 mmol, 14 % (desired product, based on starting material)

R_f: 0.29 (hexane:ethyl acetate, 2:1)

I.R.: $\nu_{\max}$ (cm⁻¹) 3429 (NH), 1724 (C=O), 1512 (C=C Ar), 1450 (C=C Ar), 1226 + 1153 (C-S-C)

NMR: δ_{H} (400 MHz) 7.78 (2H, m, H_{a}), 7.63 (2H, m, H_{a}), 7.42 (2H, m, H_{b}), 7.33 (2H, m, H_{b}), 5.80 – 5.96 (2H, m, CH=CH₂), 5.75 (1H, m, FmocNH), 5.66 (1H, m, AlocNH), 5.17 – 5.35 (4H, m, CH=CH₂), 4.56 – 4.65 (4H, m, OCH₂CH=CH₂), 4.61 (1H, m, AlocNHCH), 4.50 (1H, m, FmocNHCH), 4.39 (2H, m, CH₂CONH), 4.25 (1H, m, H_{c}), 2.94 – 3.10 (4H, m, CH₂SCH₂), 1.50 (9H, s, C(CH₃)₃); δ_{C} (125.8 MHz, DEPT135) 170.2, *ca.* 169.2, *ca.* 156.1, *ca.* 155.7, 143.8, 141.3, *ca.* 132.5, *ca.* 131.3, 127.7, 127.1, 125.1, 120.0, *ca.* 119.1, *ca.* 117.7, *ca.* 83.1, *ca.* 67.2, 66.4, *ca.* 66.1, *ca.* 54.0 (3C), *ca.* 47.1, *ca.* 35.5 (2C), 28.0

(R/S)-2-[(R)-2-^tButoxycarbonyl-2-(fluoren-9-ylmethoxycarbonylamino)-ethylsulfanyl]-3-(allyloxycarbonylamino)-propionic acid allyl ester (diastereoisomeric mixture): δ_{H} (400 MHz) 7.76 (2H, m, H_{a}), 7.62 (2H, m, H_{a}), 7.40 (2H, m, H_{b}), 7.32 (2H, m, H_{b}), 5.90 (2H, m, CH=CH₂), 5.76 (1H, bm, FmocNH), 5.14 – 5.36 (4H, m, CH=CH₂), 5.31 (1H, m, AlocNH), 4.50 – 4.70 (4H, m, OCH₂CH=CH₂), 4.54 (1H, m, FmocNHCH), 4.40 (2H, m, CH₂CONH), 4.25 (1H, m, H_{c}), 3.45 – 3.66 (2H, m, SCHCH₂), 3.52 (1H, m, AlocNHCH), 2.97 – 3.25 (2H, m, SCH₂), 1.50 (9H, s, C(CH₃)₃); δ_{C} (125.8 MHz, DEPT135) 170.7, (169.2 + 169.1), *ca.* 156, *ca.* 157, 143.7, 141.2, 132.6, (131.4 + 131.3), 127.7, 127.0, 125.1, 119.9, (119.0 + 118.9), (117.72 + 117.65), *ca.* 83.1, (67.24 + 67.16), (66.1 + 66.0), 65.7, (54.3 + 53.9), 47.0, (46.7 + 46.0), (41.5 + 41.4), (34.34 + 34.26), 27.9

Mass spectrum: m/z (FAB) 633.2247 ([M + Na]⁺. C₃₂H₃₈N₂O₈SNa requires 633.2265), 633 ([M + Na]⁺, 31 %), 611 ([M + H]⁺, 38 %), 555 ([M - ^tBu + 2]⁺, 56 %), 333 ([M - ^tBu - Fmoc + 3]⁺, 100 %)

19

Figure 7.6. Major product: (*R/S*)-2-[(*R*)-2-^tbutoxycarbonyl-2-(fluoren-9-ylmethoxycarbonylamino)-ethylsulfanyl]-3-(allyloxycarbonylamino)-propionic acid allyl ester

19. 3-[(*R/S*)-1-Allyloxycarbonyl-2-(allyloxycarbonylamino)-ethylsulfanyl]-(*R*)-2-(fluoren-9-ylmethoxycarbonylamino)-propionic acid

20

3-[(*R*)-2-^tButoxycarbonyl-2-(fluoren-9-ylmethoxycarbonylamino)-ethylsulfanyl]-(*S*)-2-(allyloxycarbonylamino)-propionic acid allyl ester (***S,R***)-12 (mixture with regioisomer, 2.1 g, 3.4 mmol) was treated with a solution of TFA (5 ml, excess) in DCM (5 ml) and the mixture was stirred for 4 hr. The mixture was concentrated *in vacuo* and re-dissolved in DCM (100 ml) and MeOH (100 ml). The solvents were

then removed. The crude material was purified *via* reverse phase flash column chromatography (0 – 35 % MeCN, 20 % saturated NaHCO₃, H₂O) yielding a colourless liquid, which foamed and solidified under highly reduced pressure at –50 °C. Only the regioisomer, as a mixture of diastereoisomers, was isolated.

Yield: 1.4 g, 2.6 mmol, 76 %

R_f: 0.31 (MeCN:H₂O:sat. NaHCO₃, 4:5:1)

I.R.: $\nu_{\max}$ (cm⁻¹) 3433 (OH, negligible H-bonding), 3337 (NH), 1720 (C=O), 1512 (C=C Ar), 1450 (C=C Ar), 1223 + 1150 (C-S-C)

NMR: δ_{H} (500 MHz, CD₃OD) *Diastereoisomeric* 7.76 (2H, m, H_{a}), 7.62 (2H, m, H_{a}), 7.35 (2H, m, H_{b}), 7.27 (2H, m, H_{b}), 5.80 – 5.96 (2H, m, CH=CH₂ All + Aloc), 5.10 – 5.35 (4H, m, CH=CH₂ All + Aloc), 4.60 (2H, m, OCH₂CH=CH₂), 4.48 (2H, m, OCH₂CH=CH₂), 4.38 (1H, m, FmocNHCH), 4.28 – 4.41 (2H, m, CH₂OCONH), 4.22 (1H, m, H_{c}), 3.64 (1H, m, AlocNHCH₂CH), 3.44 (2H, m, AlocNHCH₂), 2.88 – 3.24 (2H, m, CHCH₂S); δ_{C} (125.8 MHz, HSQC) (172.5 + 172.4), 172.0, *ca.* 158 (2C), *ca.* 145.2, 142.5, (134.2 + 134.1), (133.2 + 133.0), 129.2, (128.7 + 128.6), 126.3, 120.6, 118.8, (117.7 + 117.6), (68.23 + 68.18), (66.99 + 66.96), (66.6 + 66.5), 55.6, 48.3, 47.2, (43.1 + 42.8), (34.7 + 34.2)

Elemental analysis: Found C, 61.06; H, 6.02; N, 4.68; S, 5.22 %. C₄₄H₄₈N₂O₈S₂ requires C, 60.64; H, 5.45; N, 5.05; S, 5.78 %.

Mass spectrum: m/z (ES+) 577 ([M + Na]⁺, 100 %), 555 ([M + H]⁺, 4 %), 333 ([M – Fmoc + 2]⁺, 4 %)

1

Figure 7.7. Intended product: 3-[(*S*)-2-allyloxycarbonyl-2-(allyloxycarbonylamino)-ethylsulfanyl]-(*R*)-2- (fluoren-9-ylmethoxycarbonylamino)-propionic acid

20. (R /S)-2-[*(R)*-2-^tButoxycarbonyl-2-(fluoren-9-ylmethoxycarbonylamino)-ethylsulfanyl]-3-{1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)isovalerylamino}-propionic acid allyl ester

21

3-[(*R*)-2-^tButoxycarbonyl-2-(fluoren-9-ylmethoxycarbonylamino)-ethylsulfanyl]-(*S*)-2-(triphenylmethylamino)-propionic acid allyl ester (*S,R*)-**10** (mixture with regioisomer, 3.5 g, 4.6 mmol) was treated with a 5 % solution of trifluoroacetic acid (1.4 ml, 18 mmol, 4 equiv.) in DCM (27 ml) under inert conditions for 4 hr. The resulting solution was diluted with DCM (100 ml) and washed with sodium hydrogen carbonate (5 % aq. w/v, 2 x 75 ml) and water (2 x 50 ml). The solvent was removed *in vacuo*. DCM (20 ml) and MeOH (20 ml) were then added and re-evaporation yielded a pale yellow liquid, which was then dissolved in ethanol (abs., 30 ml). ivDde-OH (0.99 ml, 4.6 mmol) was then added and the reaction mixture was stirred for 18 hr at 35 °C. The solvent was removed *in vacuo* and the resulting oil was re-dissolved in ethyl acetate (250 ml). The organic layer was washed with citric acid (5 % aq. w/v, 100 ml) and water (3 x 50 ml) and dried over anhydrous sodium sulfate. Removal of the solvent yielded a pale yellow liquid, which was purified *via* flash column chromatography (hexane:ethyl acetate, 2:1, 7.5 cm width). The product, which contained only the regioisomer as a mixture of diastereoisomers, was an extremely viscous colourless liquid.

Yield: 2.0 g, 2.7 mmol, 60 %

R_f: 0.35 (cyclohexane:ethyl acetate, 2:1)

I.R.: $\nu_{\max}$ (cm⁻¹) 1728 (C=O), 1566 (C=C Ar), 1450 (C=C Ar), 1224 + 1151 (C-S-C)

NMR: δ_{H} (500 MHz) *Diastereoisomeric* 7.77 (2H, m, H_{a}), 7.63 (2H, m, H_{a}), 7.40 (2H, m, H_{b}), 7.30 (2H, m, H_{b}), 6.15 + 6.24 (1H, bd, FmocNH), 5.94 (1H, m, CH=CH₂), 5.36 (1H, dd, J 18.4, 1.3, CH=CH₂), 5.21 (1H, dd, J 10.4, 1.1, CH=CH₂), 4.70 (2H, m, OCH₂CH), 4.44 – 4.52 (1H, m, FmocNHCH), 4.35 – 4.45 (2H, m, CH₂CONH), 4.24 (1H, m, H_{c}), 3.61 – 3.95 (2H, m, NCH₂), 3.60 (1H, m, NCH₂CH), 3.03 – 3.23 (2H, m, CH₂S), 3.02 (2H, m, C=CCH₂), 2.30 – 2.45 (4H, m, CH₂C=O), 1.94 (1H, m, CH(CH₃)₂), 1.50 (9H, s, C(CH₃)₃), 0.96 – 1.05 (12H, m, (CH₃)₂CCH₂, (CH₃)₂CH)); δ_{C} (125.8 MHz, DEPT135, HMQC) (199.7 + 196.1), (176.5 + 176.3), (169.9 + 169.8), (169.0 + 168.9), (155.9 + 155.8), (143.74 + 143.67), (141.18 + 141.17), (131.2 + 131.1), 127.6, 126.9, (125.1 + 125.0), 119.9, (119.3 + 119.1), (107.5 + 107.4), (83.04 + 82.98), (67.1 + 67.0), (66.5 + 66.4), (54.3 + 54.0), (53.7 + 52.2), 47.0, (46.4 + 45.9), (43.9 + 43.7), (37.1 + 37.0), (34.7 + 34.6), (29.74 + 29.71), (29.03 + 29.00), (28.1 + 28.0), (27.85 + 27.83), (22.42 + 22.37)

Mass spectrum: m/z (FAB) 733.3521 ([M + H]⁺. C₄₁H₅₃N₂O₈S requires 733.3583), m/z (ES⁺) 755 ([M + Na]⁺, 38 %), 733 ([M + H]⁺, 51 %)

16

Figure 7.8. Intended product: 3-[(*R*)-2-^tbutoxycarbonyl-2-(fluoren-9-ylmethoxycarbonylamino)-ethylsulfanyl]-(*S*)-2-{1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)isovaleryl-amino}-propionic acid allyl ester

21. (R / S)-2-[(R)-2-^tButoxycarbonyl-2-(fluoren-9-ylmethoxycarbonylamino)-ethylsulfanyl]-3-{1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)isovaleryl-amino}-propionic acid

(*R/S*)-2-[(*R*)-2-^tButoxycarbonyl-2-(fluoren-9-ylmethoxycarbonylamino)-ethylsulfanyl]-3-{1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)isovaleryl-amino}-propionic acid allyl ester **21** (1.7 g, 2.4 mmol) was treated with tetrakis(triphenylphosphine) palladium(0) (1.2 g, 1.0 mmol, 0.4 equiv.) in a cocktail solution of DMF:CHCl₃:AcOH:NMM (18.5:18.5:2:1 v/v/v/v, 9.3:9.3:1:0.5 ml). The solution was stirred at 25 °C for 3 hr. The mixture was then diluted with DCM (250 ml), washed with water (5 x 50 ml) and dried over anhydrous sodium sulfate. Removal of the solvent yielded a yellow liquid, which was used without further purification, in crude form. A small amount of the crude material was purified *via* preparative reverse phase HPLC for analyses (40 - 60 % MeCN in water over 30 min, 15 ml/min, R_T 13.2).

Yield: 100 % (crude)

R_f: 0.1 (DCM:MeOH, 19:1)

I.R.: ν_{max} (cm⁻¹) 3300 (OH, broad), 1717 (C=O), 1570 (C=C Ar), 1450 (C=C Ar), 1223 + 1153 (C-S-C)

NMR: δ_{H} (500 MHz, 323 K) *Diastereoisomeric* 7.68 (2H, bm, H_{a}), 7.54 (2H, bm, H_{a}), 7.30 (2H, bm, H_{b}), 7.20 (2H, bm, H_{b}), 6.30 + 6.13 (1H, bm, FmocNH), 4.34 – 4.59 (1H, bm, FmocNHCH), 4.30 (2H, bm, CH_2OCONH), 4.15 (1H, bm, H_{c}), 3.73 (2H, bm, NCH_2), 3.57 (1H, bm, NCH_2CH), 2.90 – 3.27 (2H, bm, CH_2S), 2.95 (2H, bm, $\text{C}=\text{CCH}_2$), 2.26 (4H, bs, $\text{CH}_2\text{C}=\text{O}$), 1.87 (1H, bm, $\text{CH}(\text{CH}_3)_2$), 1.40 (9H, bs, $\text{C}(\text{CH}_3)_3$), 0.91 (12H, bm, $(\text{CH}_3)_2\text{CCH}_2$, $(\text{CH}_3)_2\text{CH}$); δ_{C} (125.8 MHz, DEPT135, HMQC, 323 K) 197.0, 176.3, 169.7, (169.0 + 168.9), 157.2, 156.2, (143.9 + 143.8), 141.3, (128.6 + 128.5), (128.0 + 127.9), 125.2, 119.9, 107.7, 82.8, (67.7 + 67.5), (55.1 + 54.9), 52.9, 47.9, (47.12 + 47.08), 45.6, 37.0, 34.0, 29.7, 29.0, 28.1, 28.0, 22.5

Mass spectrum: m/z (FAB) 825.2186 ($[\text{M} + \text{Cs}]^+$, $\text{C}_{38}\text{H}_{48}\text{N}_2\text{O}_8\text{SCs}$ requires 825.2204), m/z (ES+) 693 ($[\text{M} + \text{H}]^+$, 72 %), 715 ($[\text{M} + \text{Na}]^+$, 4 %)

28

Figure 7.9. Intended product: 3-[(*R*)-2-^t-butoxycarbonyl-2-(fluoren-9-ylmethoxycarbonylamino)-ethylsulfanyl]-(*S*)-2-{1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)isovaleryl-amino}-propionic acid

22. (R/S)-2-[(R)-2-^tButoxycarbonyl-2-(fluoren-9-ylmethoxycarbonylamino)-ethylsulfanyl]-3-{1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)isovaleryl-amino}-propionic acid 4-{N-[1-(4,4-Dimethyl-2,6-dioxocyclohex-1-ylidene)-3-methylbutyllamino}benzyl ester

22

To a solution of crude (R/S)-2-[(R)-2-^tbutoxycarbonyl-2-(fluoren-9-ylmethoxycarbonylamino)-ethylsulfanyl]-3-{1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)isovaleryl-amino}-propionic acid **27** (1.5 g, crude) in DMF (20 ml) was added Dmab-OH (0.66 g, 2.0 mmol), dicyclohexylcarbodiimide (0.4 g, 2.0 mmol), 1-hydroxybenzotriazole (0.27 g, 2.0 mmol) and 4-dimethylaminopyridine (0.24 g, 2.0 mmol). The mixture was stirred for 18 hr. The precipitated urea by-product was filtered off and the mixture was diluted with ethyl acetate (200 ml). The organic layer was washed with citric acid (5 % aq. w/v, 50 ml) and water (6 x 50 ml) and dried over anhydrous sodium sulfate. Removal of the solvent yielded a yellow liquid, which foamed and solidified under highly reduced pressure at -50 °C and used without further purification, in crude form. A small amount of the crude material was purified *via* preparative reverse phase HPLC for analyses (70 – 90 % MeCN in water over 30 min, 15 ml/min, R_T 22.1).

Yield: 100 % (crude)

R_f: 0.45 (hexane:ethyl acetate, 1:1)

I.R.: $\nu_{\max}$ (cm^{-1}) 1728 (C=O), 1566 (C=C Ar), 1450 (C=C Ar), 1215 + 1152 (C-S-C)

NMR: δ_{H} (500 MHz) *Diastereoisomeric* 7.74 (2H, m, H_{a}), 7.58 (2H, m, H_{a}), 7.33 - 7.40 (2H, m, H_{b} + 2H, m, NHC=CH (Ar)), 7.24 (2H, m, H_{b}), 7.06 (2H, m, OCH₂C=CH (Ar)), 5.22 (2H, m, ArOCH₂), 4.55 (1H, m, FmocNHCH), 4.33 - 4.44 (2H, m, CH₂CONH), 4.22 (1H, m, H_{c}), 3.63 - 3.95 (2H, m, NCH₂), 3.60 (1H, m, NCH₂CH), 3.03 - 3.29 (2H, m, CH₂S), 2.99 (4H, bm, C=CCH₂), 2.31 - 2.49 (8H, bm, CH₂C=O), 1.92 (1H, m, CH(CH₃)₂), 1.81 (1H, m, CH(CH₃)₂), 1.45 (9H, s, C(CH₃)₃), 1.07 (6H, s, (CH₃)₂CCH₂), 0.96 - 0.99 (12H, m, (CH₃)₂CCH₂, (CH₃)₂CH), 0.75 (6H, d, J 6.7, (CH₃)₂CH); δ_{C} (125.8 MHz, DEPT135, HMQC) (200.2 + 199.9), 196.3, *ca.* 176.5, (170.1 + 169.9), (169.0 + 168.8), (155.9 + 155.8), (143.7 + 143.6), (141.27 + 141.25), (137.12 + 137.10), (134.6 + 134.5), 129.2, 127.7, 127.0, 126.7, (125.1 + 125.0), 120.0, 107.6, 107.5, (83.23 + 83.16), 67.1, (66.8 + 66.7), (54.2 + 54.1), 53.7, 52.3, 47.1, (46.5 + 46.0), (44.1 + 43.8), 38.3, (34.8 + 34.7), 30.0, (29.83 + 29.81), 29.5, (29.12 + 29.09), 28.22, 28.17, (27.92 + 27.91), *ca.* 22.5

Mass spectrum: m/z (FAB) 1004.5095 ($[\text{M} + \text{H}]^+$. C₅₈H₇₄N₃O₁₀S requires 1004.5020), m/z (ES+) 1026 ($[\text{M} + \text{Na}]^+$, 17 %), 1004 ($[\text{M} + \text{H}]^+$, 9 %)

17

Figure 7.10. Intended product: 3-[(*R*)-2-^tbutoxycarbonyl-2-(fluoren-9-ylmethoxycarbonylamino)-ethylsulfanyl]-(*S*)-2-{1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)isovalerylamino}-propionic acid 4-{*N*-[1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)-3-methylbutyl]amino}benzyl ester

23. 3 - / (R / S) - 1 - { 4 - [N - (1 - (4,4-Dimethyl-2,6-dioxocyclohex-1-ylidene) - 3 - methylbutyl) amino] benzyloxycarbonyl } - 2 - { 1 - (4,4-dimethyl-2,6-dioxocyclohex-1-ylidene) isovaleryl amino } - ethylsulfanyl } - (R) - 2 - (fluoren-9-ylmethoxycarbonylamino) - propionic acid

23

(*R/S*)-2-[(*R*)-2-^tButoxycarbonyl-2-(fluoren-9-ylmethoxycarbonylamino)-ethylsulfanyl]-3-{1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)isovaleryl-amino}-propionic acid 4-{*N*-[1-(4,4-Dimethyl-2,6-dioxocyclohex-1-ylidene)-3-methylbutyl]amino}benzyl ester **22** (1.4 g, crude) was treated with a solution of TFA (5 ml, excess) in DCM (5 ml) and the mixture was stirred for 4 hr. The mixture was concentrated *in vacuo* and re-dissolved in DCM (100 ml) and MeOH (100 ml). The solvents were then removed yielding a yellow liquid, which foamed and solidified *in vacuo*. A small amount of the sample was purified *via* preparative reverse phase HPLC (40-60 % MeCN in water over 30 min, 15 ml/min, R_T 12.6).

Yield: 100 % (crude)

R_f : 0.20 (DCM:MeOH, 19:1)

I.R.: ν_{max} (cm⁻¹) 3400 (OH, broad), 1717 (C=O), 1562 (C=C Ar), 1450 (C=C Ar), 1211 + 1151 (C-S-C)

NMR: δ_H (500 MHz) *Diastereoisomeric* 7.63 (2H, m, $H_{a'}$), 7.48 (2H, m, H_a), 7.23 (2H, m, $H_{b'}$ + 2H, m, NHC=CH (Ar)), 7.11 (2H, m, H_b), 6.92 (2H, m, OCH₂C=CH

(Ar)), 5.07 (2H, bm, ArOCH_2), 4.36 (1H, bm, FmocNHCH), 4.20 (2H, bm, CH_2OCONH), 4.09 (1H, bm, H_c), 3.60 – 3.90 (3H, bm, NCH_2 , NCH_2CH), 2.98 – 3.33 (2H, bm, CH_2S), 2.91 (4H, bm, $\text{C}=\text{CCH}_2$), 2.12 – 2.49 (8H, bm, $\text{CH}_2\text{C}=\text{O}$), 1.71 – 1.88 (2H, bm, $\text{CH}(\text{CH}_3)_2$), 1.04 (6H, s, $(\text{CH}_3)_2\text{CCH}_2$), 0.75 – 0.93 (12H, bm, $(\text{CH}_3)_2\text{CCH}_2$, $(\text{CH}_3)_2\text{CH}$), 0.68 (6H, bm, $(\text{CH}_3)_2\text{CH}$); δ_c (125.8 MHz, DEPT135, HMQC) 200.0, 196.2, 176.8, 176.2, 170.3, 156.6, (143.7 + 143.5), 141.0, 137.0, 134.2, 129.0, 127.7, 127.0, 126.5, 125.0, 119.9, 107.7, 67.2, 66.8, 55.0, 53.6, 52.1, 46.9, 45.9, 43.9, 38.2, 34.3, 30.0, 29.9, 29.7, 29.6, 29.4, *ca.* 28.1, 22.6, 22.5

Mass spectrum: m/z (FAB) 970.4288 ($[\text{M} + \text{H}]^+$, $\text{C}_{54}\text{H}_{66}\text{N}_3\text{O}_{10}\text{Na}$ requires 970.4310), m/z (ES+) 970 ($[\text{M} + \text{Na}]^+$, 10 %), 949 ($[\text{M} + \text{H}]^+$, 92 %), ($[\text{Dmab} + \text{H}]^+$, 100 %)

Figure 7.11. Intended product: 3-[(*S*)-2-{4-[*N*-(1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)-3-methylbutyl)amino]benzyloxycarbonyl}-2-{1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)isovaleryl-amino}-ethylsulfanyl]-(*R*)-2-(fluoren-9-ylmethoxy carbonylamino)-propionic acid

24. 2-(1-Hydroxy-3-methyl-butylidene)-5,5-dimethyl-cyclohexane-1,3-dione¹⁵⁴ **29**

To a stirred solution of isovaleric acid (0.11 ml, 1.0 mmol) in DMF (5 ml) was added dimedone (0.14 g, 1.0 mmol), DCC (0.40 g, 1.0 mmol) and DMAP (0.12 g, 1.0 mmol). The mixture was stirred for 48 hr, during which the urea by-product was precipitated. The solvent was removed *in vacuo* and re-dissolved in ethyl acetate (50 ml). The solid urea was filtered off and the organic layer was washed with KHSO₄ solution (1 M, 20 ml). Extraction was carried out with NaOH (5 % aq., 3 x 50 ml) and the aqueous layers were combined and neutralised with acetic acid. Further extraction was carried out using DCM (5 x 50 ml). The organic layer was dried with anhydrous sodium sulfate and removal of the solvent yielded the product as a yellow liquid.

Yield: 0.18 g, 0.8 mmol, 80 %

NMR: δ_H (300 MHz) 2.92 (2H, d, Me₂CHCH₂COH), 2.45 (4H, m, CH₂CMe₂CH₂), 2.13 (1H, m, Me₂CHCH₂COH), 1.08 (6H, s, CH₂CMe₂CH₂), 0.97 (6H, d, Me₂CHCH₂COH)

Mass spectrum: m/z (FAB) 225 ([M + H]⁺, 100 %)

25. Attempted synthesis of *N*-Triphenylmethyl-(*R*)-serine 4-[*N*-{1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)-3-methylbutyl}amino]benzyl ester **13**

N-triphenylmethyl-(*R*)-serine (**R**)-**3** (0.10 g, 0.29 mmol) was treated with Dmab-OH (0.099g, 0.29 mmol), diisopropylcarbodiimide (0.045 ml, 0.59 mmol, 2 equiv.), 1-

hydroxybenzotriazole (0.040 g, 0.29 mmol) and diisopropylethylamine (0.035 ml, 0.20 mmol, 0.7 equiv.) in dichloromethane. The mixture was stirred for 18 hours and then diluted with dichloromethane (20 ml) and washed with citric acid (5 %, aq., 10ml) and water (2 x 10 ml). The organic layer was dried over anhydrous sodium sulfate. The product was not formed, as indicated by mass spectrometry (ES+), t.l.c. and ^1H NMR.

26. Attempted synthesis of *N*-Triphenylmethyl-(*R*)-serine 4-[*N*-{1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)-3-methylbutyl}amino]benzyl ester

N-triphenylmethyl-(*R*)-serine (***R***)-3 (0.050 g, 0.14 mmol) was treated with Dmab-OH (0.047g, 0.14 mmol), dicyclohexylcarbodiimide (0.059 g, 0.29 mmol, 2 equiv.) and diisopropylethylamine (0.035 ml, 0.20 mmol, 0.7 equiv.) in dichloromethane. The mixture was stirred for 18 hours. The urea by-product was filtered and the mixture was then diluted with dichloromethane (10 ml) and washed with citric acid (5 %, aq., 5 ml) and water (2 x 10 ml). The organic layer was dried over anhydrous sodium sulfate. The product was not formed, as indicated by mass spectrometry (ES+), t.l.c. and ^1H NMR.

27. Attempted synthesis of *N*-Triphenylmethyl-(*R*)-serine 4-[*N*-{1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)-3-methylbutyl}amino]benzyl ester

N-triphenylmethyl-(*R*)-serine (***R***)-3 (0.050 g, 0.14 mmol) was treated with Dmab-OH (0.047g, 0.14 mmol), dicyclohexylcarbodiimide (0.059 g, 0.59 mmol, 2 equiv.) and 4-dimethylaminopyridine (0.018 g, 0.14 mmol) in dichloromethane. The mixture was stirred for 18 hours. The urea by-product was filtered and then diluted with dichloromethane (20 ml) and washed with citric acid (5 %, aq., 10ml) and water (2 x 10 ml). The organic layer was dried over anhydrous sodium sulfate. The product was not formed, as indicated by mass spectrometry (ES+), t.l.c. and ^1H NMR.

28. (R)-2-triphenylmethylamino-3-^tbutyldimethylsilyloxy-propionic acid

To a stirred solution of *N*-triphenylmethyl-(*R*)-serine (**R**)-**3** (0.10 g, 0.29 mmol) in DMF (0.2 ml) was added *tert*-butyldimethylsilyl chloride (0.052 g, 0.35 mmol, 1.2 equiv.) and imidazole (0.049 g, 0.72 mmol, 2.5 equiv.) and the mixture was stirred for 10 hours. The mixture was diluted with ethyl and then washed with water (3 x 10 ml) and brine (10 ml). The organic layer was then dried over anhydrous sodium sulfate and removal of the solvent yielded a viscous, colourless liquid. The compound was used without further purification in the presence of the *tert*-butyldimethylsilyl alcohol by-product.

Yield: 100 % (crude)

R_f: 0.55 (hexane:ethyl acetate, 2:1)

NMR: δ_{H} (400 MHz) 7.21 – 7.46 (15H, m, Trt (Ar)), 3.68 (1H, m, CH_2OSi), 3.42 (1H, m, TrtNHCH), 2.67 (1H, m, CH_2OSi), 0.93 (9H, s, $\text{Si}(\text{CCH}_3)_3$), 0.11 (6H, s, $\text{Si}(\text{CH}_3)_2$)

Mass spectrum: m/z (ES⁺) 466 ($[\text{M} + \text{H}]^+$, 0.1 %), 223 ($[\text{M} - \text{Trt} + \text{H}]^+$, 0.2 %), 243 (Trt⁺, 100 %)

29. Attempted synthesis of (R)-2-triphenylmethylamino-3-butyl
dimethylsilyloxy-propionic acid 4-[N-(1-{4,4-dimethyl-2,6-dioxocyclohex-1ylidene)-3-methylbutyl} amino]benzyl ester

30

2-triphenylmethylamino-3-butyl dimethylsilyloxy-propionic acid **37** (0.033g, 80 μ mol) in DMF (1 ml) was treated with Dmab-OH (0.025g, 80 μ mol), dicyclohexylcarbodiimide (0.016 g, 80 μ mol), 1-hydroxybenzotriazole (0.010 g, 80 μ mol) and 4-dimethylaminopyridine (0.0093 g, 80 μ mol) under inert conditions at room temperature. The reaction mixture was stirred for 18 hours. The mixture was then diluted with ethyl acetate (20 ml) and the urea by-product was filtered off. The organic layer was then washed with citric acid (5 %, aq., 10ml) and water (2 x 10 ml). The organic layer was dried over anhydrous sodium sulfate. Removal of the solvent yielded a mixture of viscous liquid and solids. T.l.c. and mass spectrometry indicated that the reaction had not worked, isolating starting materials only.

30 . 4 - [N-(1-{4,4-Dimethyl-2,6-dioxocyclohex-1ylidene)-3-
methylbutyl} amino]benzyl bromide

15

Bromine (0.01 ml, 0.2 mmol, 1.3 equiv.) was added to a solution of triphenylphosphine (0.040 g, 0.15 mmol) in dichloromethane (2.5 ml) at 0 °C. Upon warming to room temperature Dmab-OH (0.050 g, 0.15 mmol) in dichloromethane

(2.5 ml) was added and the reaction mixture was left stirring for 5 hours. The mixture was then diluted with dichloromethane (50 ml) and washed with sodium hydrogen carbonate (5 %, aq., 2 x 25 ml), water (2 x 25 ml) and finally brine (2 x 25 ml). Removal of the solvent yielded a viscous yellow-brown liquid. Purification attempts were unsuccessful.

Mass spectrum: m/z (ES⁺) 435 ($[M + ^{81}\text{Br} + K]^+$, 20 %), 433 ($[M + ^{81}\text{Br} + K]^+$, 19 %), 394 ($[M + ^{81}\text{Br}]^+$, 100 %), 392 ($[M + ^{79}\text{Br}]^+$, 98 %)

31. **(R)-1-Triphenylmethyl-aziridine-2-carboxylic acid allyl ester**

(R)-11

N-Triphenylmethyl-(*R*)-serine(*O*-methanesulfonyl) allyl ester **(R)-5** (0.10 g, 0.21 mmol) was dissolved in acetonitrile (2.5 ml) and treated with *N,N*-diisopropylethylamine (0.04 ml, 0.43 mmol, 2 equiv.). The reaction was stirred for 18 hours at room temperature. The reaction mixture was diluted with ethyl acetate (20 ml) and washed with citric acid (5 % aq., 10 ml) and water (2 x 10 ml) and dried over anhydrous sodium sulfate. Removal of the solvent yielded a mixture of colourless liquid and white solid. The aziridine derivative was purified via normal phase preparative HPLC (0-4 % ethyl acetate in hexane over 40 min, DCM loading, 15 ml/min, R_t 37.0) to give a colourless liquid.

Yield: 0.0096 g, 0.026 mmol, 12 %

R_f : 0.60 (hexane:ethyl acetate. 2:1)

NMR: δ_H (300 MHz) 7.43 (6H, m, Trt), 7.17 (9H, m, Trt), 5.87 (1H, m, $\text{CH}=\text{CH}_2$), 5.25 (1H, dd, J 17.2, 1.3, $\text{CH}=\text{CH}_2$), 5.20 (1H, dd, J 10.4, 1.4, $\text{CH}=\text{CH}_2$), 4.61 (1H, d, J 4.5, OCH_2CH), 4.60 (1H, d, J 4.5, OCH_2CH), 2.20 (1H, dd, J 2.6, 1.6, NCHCO_2), 1.84 (1H, dd, J 6.2, 2.6, NCH_2CH), 1.34 (1H, dd, J 6.2, 1.5, NCH_2CH); δ_C (75.4 MHz, DEPT135) 171.5, 144.0, 132.4, 129.7, 128.0, 127.3, 118.9, 65.9, 32.2, 29.1

Mass spectrum: m/z (FAB) 392.1626 ($[M + Na]^+$, $C_{25}H_{23}NO_2Na$ requires 392.1645), m/z (ES⁺) 370 ($[M + H]^+$, 9 %), 243 (Trt^+ , 100 %); (FAB) 393 ($[M + Na + H]^+$, 13 %), 243 (Trt^+ , 100 %)

32. **N-Triphenylmethyl-3-iodo-(S)-alanine allyl ester** (via aziridine ring-opening)

(*R*)-*N*-triphenylmethyl-aziridine-2-carboxylic acid allyl ester (**(R)-11**) (0.012 g, 33 μ mol) was treated with sodium iodide (0.049 g, 0.33 mmol, 10 equiv.) in acetone (0.5 ml) under argon and the mixture was stirred for 72 hr at 25 °C. The solvent was then removed *in vacuo*. Ether (10 ml) was added and sodium thiosulfate (10 % aq. w/v, 1 ml approx.) was then slowly added, decolourising the organic layer to a pale yellow solution. The organic layer was separated and dried over anhydrous sodium sulfate and the solvent was removed *in vacuo*. The crude product was purified by HPLC, however, incomplete separation of the product and its regioisomer (1:4 approx., respectively) was attempted using preparative normal phase HPLC (2 – 3 % ethyl acetate in hexane over 60 minutes, 15 ml/min with DCM loading, R_T 46.0, 47.1 (regioisomer)). The purification isolated the starting material at R_T 53.6 as the predominant species.

Yield: 0.0044 g, 8.8 μ mol, 27 % (overall); 0.0010 g, 1.9 μ mol, 6 % (desired product, based on NMR)

R_f : 0.60 (hexane:ethyl acetate. 2:1)

NMR: δ_H (600 MHz) 7.51 (6H, m, Trt), 7.29 (6H, m, Trt), 7.20 (3H, m, Trt), 5.73 (1H, m, $CH=CH_2$), 5.22 (1H, dd, J 17.3, 1.4, $CH=CH_2$), 5.19 (1H, dd, J 10.2, 1.1, $CH=CH_2$), 4.22 (1H, dd, J 13.1, 6.1, OCH_2CH), 4.09 (1H, dd, J 13.1, 6.1, OCH_2CH), 3.50 (1H, m, $HNCHCO_2$), 3.30 (1H, dd, J 9.8, 3.4, $CHCH_2I$), 3.23 (1H, dd, J 9.8, 6.9, $CHCH_2I$), 2.88 (1H, d, J 9.8, $TrtNHCH$)

(R/S)-2-iodo-3-(triphenylmethylamino) propionic acid allyl ester (mixture of enantiomers): δ_H (600 MHz) 7.52 (6H, m, Trt), 7.30 (6H, m, Trt), 7.21 (3H, m, Trt), 5.94 (1H, m, $CH=CH_2$), 5.40 (1H, dd, J 17.2, 1.4, $CH=CH_2$), 5.30 (1H, dd, J 10.5,

1.1, CH=CH₂), 4.67 (2H, m, OCH₂CH), 4.42 (1H, m, CHICO₂), 2.73 (1H, dd, *J* 12.9, 8.5, HNCH₂CHI), 2.57 (1H, dd, *J* 12.9, 5.9, HNCH₂CHI), 2.27 (TrtNHCH₂)

Mass spectrum: *m/z* (ES⁺) 520 ([M + Na]⁺, 7 %), 243 (Trt⁺, 100 %)

Figure 7.12. Major product: (*R/S*)-2-iodo-3-(triphenylmethylamino) propionic acid allyl ester

33. Attempted synthesis of 3-[(*R*)-2-^tButoxycarbonyl-2-(fluoren-9-ylmethoxycarbonylamino)-ethylsulfanyl]-(*S*)-2-(triphenylmethylamino)-propionic acid allyl ester (via aziridine ring-opening)

N-9-Fluorenylmethoxycarbonyl-(*R*)-cysteine ^tbutyl ester **9** (0.0088 g, 22 μmol) was dissolved in dry DMF (0.15 ml) under inert conditions. To it a solution of (*R*)-*N*-triphenylmethyl-aziridine-2-carboxylic acid allyl ester (**R**)-**11** (0.0081 g, 22 μmol) in DMF (0.15 ml) was added. Cesium carbonate (0.0072 g, 22 μmol) was then added and the mixture was stirred for 4 hr at 25 °C. The solvent was removed *in vacuo* and the residue was partly dissolved in ethyl acetate (10 ml). The organic layer was washed with citric acid (5 % aq. w/v, 1 ml), dissolving the remainder of the residue. The organic layer was then washed with water (4 x 5 ml) and dried over anhydrous sodium sulfate. Removal of the solvent *in vacuo* yielded a mixture of pale yellow oil and white solids. ¹H NMR of the crude material revealed that the reaction had not taken place, producing signals for the aziridine derivative and dibenzofulvene only.

General procedure for solid phase peptide synthesis with double-headed amino acids

Batch synthesis

All glassware is placed in the oven one day prior to synthesis. The resin used is suspended in DMF (5 ml) for 20 min in a vial and then transferred to the bubbler.

A. Removal of the solvent was followed by Fmoc deprotection of the resin, or, the pre-loaded amino acid on the resin using a piperidine solution in DMF (20 %, 4 ml) with vigorous argon bubbling for 20 minutes. The resin is then thoroughly washed with DMF (6 x 10 ml) after which the qualitative Kaiser test, to check the presence of free amines, is performed on a small amount of the resin with an anticipated positive result, yielding a blue-purple colouration.

B. The resin is then washed with DCM (3 x 10 ml). A small amount of the resin was taken (10 ± 0.1 mg approx.), dried, treated with a solution of piperidine in DMF (30 %, 0.5 ml) and left to stand for 30 min. Ethanol (abs., 19.5 ml) is then added and the mixture is left to settle. The aliquot is transferred into a U.V. cell and checked for a negative result for piperidyl-dibenzofulvene adduct (λ 300 nm).

C. 3-[(*R* / *S*)-1-Allyloxycarbonyl-2-(allyloxycarbonylamino)-ethylsulfanyl]-(*R*)-2-(fluoren-9-ylmethoxycarbonylamino)-propionic acid **20** (0.13 g, 0.25 mmol, 2.5 equiv.) is activated under inert conditions, in a separate vial, with a HOBt solution in DMF (0.033 g, 0.25 mmol, 2.5 equiv., 0.25 M, 1 ml) and a DIC solution in DMF (0.039 ml, 0.25 mmol, 2.5 equiv., 0.25 M, 1 ml) for 20 min. The activated amino acid solution is then added to the resin with bubbling for 30 min. After 15 min of the first coupling, another batch of 3-[(*R/S*)-1-allyloxycarbonyl-2-(allyloxycarbonylamino)-ethylsulfanyl]-(*R*)-2-(fluoren-9-ylmethoxycarbonylamino)-propionic acid **20** (0.13 g, 0.25 mmol, 2.5 equiv.) is activated in exactly the same manner. Removal of the first coupling solution is followed by addition of the second, with the same coupling duration. The resin is then washed with DMF (6 x 10 ml) after removal of

the second coupling solution. A Kaiser test is performed with an anticipated negative result.

D. The resin is then capped with a solution of 1-acetylimidazole in DMF (0.16 g, 1.5 mmol, 0.3 M, 4 ml). Steps A, B, C and D are repeated for the subsequent amino acid in the sequence and steps A, C and D only are repeated thereafter.

E. Simultaneous allylic deprotection is performed using tetrakis(triphenylphosphine) palladium(0) (0.23 g, 0.20 mmol, 2 equiv.), dissolved by gentle heating in a solution of DMF/ CHCl_3 /AcOH/NMM (18.5:18.5:2:1 volume, 1.85/1.85/0.2/0.1 ml, where the concentration of palladium(0) catalyst in the cocktail solution = 0.050 M). The mixture is bubbled for 2 hr in the dark. The resin is then washed with 0.5 % v/v DIEA in DMF (4 x 5 ml), DMF (6 x 10 ml), 0.5 % w/v sodium diethyldithiocarbamate trihydrate in DMF (4 x 5 ml) and more DMF (6 x 10 ml).

A Kaiser test is performed at this stage. This is followed by Fmoc deprotection as before (Kaiser test should yield a richer purple coloration, due to the presence of 2 free amines) and a HOBt/DMF acidic wash (0.4 % w/v, 5 ml) – to prevent any unwanted piperidyl-amide formation at the free carboxy end²¹⁷ – This is followed by further DMF washes (2 x 10 ml).

The cyclisation reagents are prepared during the Fmoc deprotection. PyAOP (0.26 g, 0.5 mmol, 5 equiv.) is dissolved in DMF (1 ml) and mixed with a solution of HOAt (0.068 g, 0.5 mmol, 5 equiv.) in DMF (1 ml). The mixture is transferred to the bubbler and a solution of DIEA (0.18 ml, 1.0 mmol, 10 equiv.) in DMF (0.5 ml) is added 1 min later. The mixture is bubbled for 1 hr followed by DMF washings (6 x 10 ml). Steps A, C and D are repeated for the appropriate amino acid.

F. Removal of the Fmoc group on the terminal residue is followed by DMF washes (6 x 10 ml) and DCM washes (6 x 10 ml). The cleavage of the peptide is carried out by treating the resin with a 5 % TFA, 2.5 % H_2O , 2.5 % TES solution in DCM (5 x 10 ml, 1 min each portion), collected in a flask containing TFA (50 ml) with

vigorous stirring for 3 hr. The mixture is then concentrated *in vacuo* to approximately 1 ml, after which cold ether is added (100 ml). The product is precipitated in the fridge overnight. The precipitate is collected by filtration, dissolved in a solution of 20 % AcOH/H₂O and lyophilised. Purification is carried out *via* preparative reverse phase HPLC and removal of the solvents followed by lyophilisation yielded the peptide as white solid.

Continuous flow synthesis

The peptide synthesiser used is a MilliGen 9050 synthesiser. The “notebook” is initially prepared, programming in detail the sequences, duration and reagents to be used for the whole synthesis, or parts of it.

The resin used is suspended in DMF (5 ml) for 20 min in a vial and then transferred to the synthesiser column (10 mm width). Fmoc deprotection is carried out using a 20 % piperidine solution in DMF for 5 minutes for standard Fmoc-protected amino acids and 10 minutes for the resin and the lanthionine residue.

The amino acid cycles included a 20 bubble-cycle activation of the residues with 0.6 M HOBt and 0.6 M DIC solutions, both in DMF, followed by 15-minute coupling cycles for standard amino acids which was carried out twice and a 30-minute coupling cycle for the lanthionine, also performed twice. Both the Fmoc deprotection and amino acid couplings were monitored quantitatively by measuring the U.V. absorbance (λ 365 nm). Each double-cycle was followed by a capping cycle using a 0.3 M solution of 1-acetylimidazole in DMF.

3-[(*R/S*)-1-Allyloxycarbonyl-2-(allyloxycarbonylamino)-ethylsulfanyl]-(*R*)-2-(fluoren-9-ylmethoxycarbonylamino)-propionic acid **20** (0.22 g, 0.4 mmol, 4 equiv.) is activated with the DIC solution (1.6 ml) and the HOBt solution (1.5 ml) and delivered to the column on the synthesiser for the coupling cycle. DMF washes, a capping cycle, further washes and the Fmoc deprotection of the newly attached residue follow the second cycle. Subsequent amino acid couplings then followed.

The simultaneous allylic deprotection, cyclisation and final amino acid coupling steps are then carried out manually on the Merrifield bubbler, as described previously (step E). Cleavage of the peptide from the resin and purification as described above in step F yielded the peptide as white solid.

Batch synthesis:

Resin: Sieber amide resin (0.47 mmol/g loading – manufacturer determination)

Yield: 0.5 mg, 0.8 μmol , 0.8 %

Mass spectrum: m/z (ES+) 579 ($[\text{M} + \text{Na}]^+$, 100 %), 557 ($[\text{M} + \text{H}]^+$, 37 %)

HPLC: R_T 20.2 (analytical, 0 – 50 % MeCN in 30 min, 50 – 90 % MeCN in 1 min, 90 – 90 % MeCN in 4 min, 90 – 0 % MeCN in 1 min)

Continuous flow:

Resin: NovaSyn[®] TG Sieber resin (0.16 mmol/g)

Yield: 1.1 mg, 2.0 μmol , 2.0 %

Mass spectrum: m/z (ES+) 758 ($[\text{M} + \text{H}]^+$, 20 %), 780 ($[\text{M} + \text{Na}]^+$, 100 %) for the linear tripeptide; 579 ($[\text{M} + \text{Na}]^+$, 37 %), 557 ($[\text{M} + \text{H}]^+$, 100 %)

HPLC: R_T 20.2 (analytical, 0 – 50 % MeCN in 30 min, 50 – 90 % MeCN in 1 min, 90 – 90 % MeCN in 4 min, 90 – 0 % MeCN in 1 min)

Batch synthesis:

Resin: NovaSyn[®] TGT resin, pre-loaded with Fmoc-Asn(Trt) (0.21 mmol/g calculated loading, 0.20 mmol/g manufacturer determination)

Yield: *Diastereoisomer 25a* 14.3 mg, 16.6 μmol , 16.6 %, *Diastereoisomer 25b* 33.1 mg, 38.4 μmol , 38.4 %, 55 % overall

NMR: δ_H (600MHz) see Table 6 and Table 7.

Mass spectrum: m/z (ES+) *Diastereoisomer 25a* 863 ($[M]^+$, 63 %), 432 ($[M + 2]^{2+}$, 100 %); *Diastereoisomer 25b* 863 ($[M]^+$, 100 %), 432 ($[M + 2]^{2+}$, 51 %)

*Diastereoisomer 25a*²¹⁸

Residue	Atom	δ_H (ppm)	δ_C (ppm)
Lys	α	3.88	53.8
	β	1.88	31.7
	γ	1.48	22.4
	δ	1.69	27.9
	ϵ	2.95	40.1
β -Ala _L	α	3.94	47.7
	β	3.61, 3.37	41.1
Gly	α	3.89	43.7
Ala	α	4.24	50.9
	β	1.38	16.8
Leu	α	4.27	53.5
	β	2.08, 1.55	40.2
	γ	1.87	25.6
	$\delta 1$	1.00	23.5
	$\delta 2$	0.94	20.9
Met	α	4.22	55.8
	β	2.04	31.5
	γ	2.63, 2.55	30.5
	ϵ	2.10	14.9
Gly	α	3.89, 3.85	44.2
Ala _L	α	4.58	55.0
	β	3.03	31.8
Asn	α	4.82	50.4
	β	2.76, 2.67	38.6

Table 6. 1H and ^{13}C chemical shifts for peptide **25a**

*Diastereoisomer 25b*²¹⁸

Residue	Atom	δ_H (ppm)	δ_C (ppm)
Lys	α	3.89	53.7
	β	1.90	31.6
	γ	1.49	22.4
	δ	1.69	27.9
	ϵ	2.91	40.1
β -Ala _L	α	3.75	46.5
	β	3.78, 3.56	41.1
Gly	α	4.18, 3.81	43.1
Ala	α	4.13	52.0
	β	1.40	17.0
Leu	α	4.30	54.4
	β	1.91, 1.60	40.4
	γ	1.68	25.8
	δ_1	0.96	23.1
	δ_2	0.91	21.1
Met	α	4.45	54.3
	β	2.13, 2.05	31.8
	γ	2.62, 2.49	30.6
	ϵ	2.08	14.8
Gly	α	4.13, 3.85	43.2
Ala _L	α	4.81	52.2
	β	3.47, 2.70	34.1
Asn	α	4.77	50.0
	β	2.84, 2.80	37.4

Table 7. 1H and ^{13}C chemical shifts for peptide **25b**

HPLC: Preliminary preparative HPLC of the crude material: 10 – 20 % MeCN in 30 min, 20 – 90 % MeCN in 15 min, 90 – 90 % MeCN in 5 min, 90 – 10 % in 1 min (**25a** R_T 17.0, **25b** R_T 20.1). *Diastereoisomer 25a* R_T 8.9 (15 – 18 % MeCN in 30 min, 18 – 50 % MeCN in 5 min, 50 – 90 % MeCN in 1 min, 90 – 90 % MeCN in 4

min, 90 – 15 % MeCN in 1 min); *Diastereoisomer 25b* R_T 11.7 (15 – 18 % MeCN in 30 min, 18 – 90 % MeCN in 5 min, 90 – 90 % MeCN in 5 min, 90 – 15 % MeCN in 1 min)

26

Batch synthesis:

Resin: NovaSyn[®] TGT resin, pre-loaded with Fmoc-Lys(Boc) (0.22 mmol/g loading, 0.20 mmol/g manufacturer determination)

Yield: Negligible

Mass spectrum: m/z (ES+) 473 ($[M + 1]^+$, 83 %) for ring B only; 1060 ($[M + 1]^+$, 18 %), 530 ($[M + 2]^{2+}$, 100 %)

Appendix A

^1H NMR spectrum of **(R)-6a** and **6b**

Appendix B

(a)

(b)

¹H NMR spectra for (a) (*R,R*)-**10** with **18** and (b) **18**

Appendix C

^1H NMR spectrum of (S)-6a and 6b

Appendix D

¹H NMR spectra for (a) (*S,R*)-**10** with **18** and (b) (*R,R*)-**10** with **18**

Appendix E

¹H NMR spectrum for **20**

Appendix F

(a)

(b)

(S)-6a

6b

^1H NMR of partially separated (a) **(S)-6a** (minor component) and (b) **6b** (major component)

Appendix G

Partial normal phase HPLC separation of (*S*)-**6a** (minor component) and **6b** (major component)

Appendix H

Normal phase HPLC separation of (S,R)-12 (minor component) and 19 (major component)

Appendix I

(a)

(b)

(c)

¹H NMR of (a) (S,R)-12 as a mixture and separated (b) (S,R)-12 and (c) 19

Appendix J

(R)-6a

Unsuccessful ^1H NMR VT experiment of what was originally thought to be
rotameric **(R)-6a**: (a) 25 °C K (b) 50 °C

Appendix K

(a)

(b)

(*R,R*)-10

Unsuccessful ¹H NMR VT experiment of what was originally thought to be rotameric (*R,R*)-10: (a) 25 °C K (b) 100 °C

Appendix L

(R)-6a

6b

^1H - ^{13}C HSQC NMR spectrum of (S)-6a and 6b

Appendix M

^1H - ^{13}C HMBC NMR spectrum of (*S,R*)-10

Appendix N

(a)

(b)

¹H NMR spectra for (a) (S,R)-10 and (b) (R,R)-10, both synthesised as a single isomer *via* the Mitsunobu route

Appendix O

Diagram of Merrifield bubbler

Appendix P

24

Crude mass spectrum of peptide **24**, clearly indicating the parent ion as the baseline peak

Appendix Q

24

Crude analytical reverse phase HPLC trace of peptide **24**

Appendix R

U.V. traces and integrations for Fmoc deprotection and amino acid coupling steps for continuous flow peptide synthesis of **24**

Appendix S

$$\begin{aligned}
 \text{Loading} &= (3.05 \times \text{Abs}_{300\text{nm}}) / m \\
 &= (3.05 \times 0.52841) / 10 \text{ mg} \\
 &= 0.16 \text{ mmol g}^{-1} \\
 &\text{where } m = \text{mass of peptidyl-resin, } 10 \pm 0.1 \text{ mg}
 \end{aligned}$$

UV spectrum for Fmoc deprotection of nor-lanthionine incorporated onto Sieber TG resin in the synthesis of **24**, for the quantitative determination of resin loading

Appendix T

$$\text{Loading} = (3.05 \times \text{Abs}_{300\text{nm}}) / m$$

$$= (3.05 \times 0.698) / 10.1 \text{ mg}$$

$$= 0.21 \text{ mmol g}^{-1}$$

where m = mass of peptidyl-resin, $10 \pm 0.1 \text{ mg}$

UV spectrum for Fmoc deprotection of nor-lanthionine incorporated onto Sieber TG resin in the synthesis of **25**, for the quantitative determination of resin loading

Appendix U

(a)

(b)

Mass spectra for (a) minor diastereoisomer and (b) major diastereoisomer of **25**

Appendix V

$$\text{Loading} = (3.05 \times \text{Abs}_{300\text{nm}}) / m$$

$$= (3.05 \times 0.715^*) / 10.0 \text{ mg}$$

$$= 0.22 \text{ mmol g}^{-1}$$

where m = mass of peptidyl-resin, $10 \pm 0.1 \text{ mg}$

* after baseline correction

UV spectrum for Fmoc deprotection of nor-lanthionine incorporated onto Sieber TG resin in the synthesis of **26**, for the quantitative determination of resin loading

Appendix W

¹H-¹³C HSQC spectrum of the minor diastereoisomer of **25a**, clearly indicating the difference in ¹³C chemical shifts between the α-Cs on both sides of the thioether bridge

Appendix X

^1H - ^{13}C HSQC spectrum of the major diastereoisomer of **25**, clearly indicating the difference in ^{13}C chemical shifts between the α -Cs on both sides of the thioether bridge. It also shows the presence of a small amount of unidentified impurities.

Appendix X-a

(a)

(b)

^1H - ^{13}C HMBC spectrum of (a) the minor diastereoisomer of **25a** and (b) the major diastereoisomer of **25b**, indicating the connectivity across the thioether bridge

Appendix Y

20 energy-minimised conformations for the minor diastereoisomer of peptide **25** in CD₃OD, with (a) *R*-stereochemistry at the β -amino acid moiety and (b) *S*-stereochemistry at the β -amino acid moiety

Appendix Z

(a)

(b)

20 energy-minimised conformations for the major diastereoisomer of peptide **25** in CD₃OD, with (a) *R*-stereochemistry at the β -amino acid moiety and (b) *S*-stereochemistry at the β -amino acid moiety

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