UNIVERSITY OF LONDON THESIS

Degree PhD Year 2007 Name of Author CHRISTOPHER GRAEME HOLFORD WHITE

Stabilisation of Peptide Secondary Structure By Incorporation of Side-Chain Linked Amino Acids

Christopher White

University College London

ProQuest LLC
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106-1346

I, Christopher White, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis

Abstract:

The aim of the project was to synthesise a novel small peptide containing an ether linkage between two side chains. Computer modelling had shown the structure was likely to form a stable β turn in solution and would therefore be a good candidate to study this class of protein secondary structure. To do this a differentially protected bis-amino acid containing the ether linkage was retrosynthesised to chiral pool synthons L-aspartic acid and L-methionine. Many attempts were then made to differentially protect and manipulate the respective acid and sulfide side chains into synthons that would participate in Williamson ether synthesis. Techniques explored included N-chlorosuccinimide hemithioacetal formation, sulfonium salt displacement, bismuth trichloride and silver salt induced etherifications.

Using molecules made during this research we then worked towards making a similarly constrained peptide containing a homolanthionine bridge, a thioether analogue of the anti-tryptic reactive site loop of Bowman Birk Inhibitor, a proteinase inhibitor protein.

Chapter 1 contains a review of peptides constrained through bridging of side chains and the effects that this has upon them.

Chapters 2 and 3 outline the synthetic steps that were used in the process of synthesising an ether linked bis-amino acid. Subchapter 2.2 shows the synthesis of

protected homoserine, a nucleophile for ether reactions. Subchapter 2.3 highlights the difficulty in making differentially protected electrophilic amino acids and coupling reactions between the two synthons. In chapter 3 the protected electrophilic homoserine is coupled with cysteine to make a homolanthionine bridge which is then integrated into a short peptide.

Chapter 6 contains the experimental procedures for the reactions carried out and the spectral data for isolated compounds.

I would like to thank the EPSRC for sponsoring this work.

UCL for giving me the time and space to perform the research.

My supervisor Dr. Tabor for giving me the opportunity to pursue a PhD and for all the help along the way to completing a challenging research project.

Prof. Marson my 2nd supervisor for sound advice on chemical synthesis.

The Tabor and Hailes groups past and present for encouragement, ideas and good times.

Table of Contents

List of Schemes

List of Figures

List of Tables

List of Abbreviations

All	Allyl
Aloc	Allyloxycarbonyl
AIBN	2,2'-Azobis(2-methylpropionitrile)
Bn	Benzyl
$(Boc)_2O$	Di-*tert*-butyl dicarbonate
Boc	*tert*-Butyloxycarbonyl
Cbz	Carboxybenzyl
DAST	(Diethylamino)sulfur trifluoride
DBN	1,5-Diazabicyclo[4.3.0]non-5-ene
DCC	*N,N'*-dicyclohexylcarbodiimide
DCHA	Dicyclohexylamine
DCU	*N,N'*-Dicyclohexylurea
DIBAL	Diisobutylaluminum hydride
DIC	*N,N'*-diisopropylcarbodiimide
DIEA	*N,N*-Diisopropylethylamine
DNA	Deoxyribonucleic acid
DMAP	4-(Dimethylamino)pyridine
DMF	*N,N*-Dimethylformamide
DMPU	3,4,5,6-Tetrahydro-2-(1H)-pyrimidinone
DMSO	Dimethyl sulfoxide
DMTSF	Dimethyl(methylthio)sulfonium fluoroborate

DPPA	Diphenylphosphoryl azide
DTBP	2,6-Di *tert*-butylpyridine
EDCI	1-Ethyl-3-(3'-dimethylaminopropyl)carbodiimide
EDTA	Ethylenediaminetetraacetic acid
Es	Electrospray
FAB	Fast atom bombardment
Fm	9-Fluorenylmethyl
Fmoc	9-Fluorenylmethyloxycarbonyl
HBTU	O-Benzotriazole-*N*,*N*,*N*',*N*'-tetramethyl-uronium-hexafluoro-phosphate
HOAt	1-Hydroxy-7-azabenzotriazole
HPLC	High performance liquid chromatography
HRMS	High resolution mass spectrometry
Hz	Herz
KHMDS	Potassium hexamethyldisilazide
LiHMDS	Lithium hexamethyldisilazide
MCPBA	3-Chloroperoxybenzoic acid
Ms	Mesyl
NCS	*N*-Chlorosuccinimide
NMR	Nuclear magnetic resonance
NMM	*N*-Methylmorpholine *N*-oxide
Phth	Phthaloyl
PMB	*para*-Methoxy benzyl

PyAOP	(7-Azabenzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate
RCAM	Ring closing alkyne metathesis
RCM	Ring closing metathesis
SPPS	Solid phase peptide synthesis
TBAF	Tetra-*N*-butylammonium fluoride
TBDMS	*tert*-Butyldimethylsilyl
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TMG	*N*,*N*,*N*',*N*'-Tetramethylguanidine
TPAP	Tetrapropylammonium perruthenate
TLC	Thin layer chromatography
Trt	Trityl
Ts	Tosyl
UV	Ultra violet

1.0 Introduction - Enforcing Protein Secondary Structure In Peptides Through Side-Chain Modifications

Protein secondary structure primarily consists of three different motifs, α helices, β sheets and reverse turns[1]. When they are found within the hydrophobic interiors of proteins they act as structure-stabilizing templates and aid in protein folding. Their presence within enzyme active sites imparts vast functionality and when present on the surfaces of proteins they play a key role in interacting with RNA, DNA and other proteins. The ubiquitous array of roles that these motifs play has sparked a great deal of interest in their specific nature[2]. Protein architecture is too complex to study motifs individually and therefore they must be expressed in small molecules and peptides[3]. New techniques for stabilizing short peptide motifs are valuable in drug therapy, studying protein folding, modeling proteins, creating artificial proteins, and may aid the design of inhibitors or mimics of protein function.

The helical structure is dependent on the backbone conformation of the polypeptide chain. The values of torsion angles ω, ψ and ϕ are affected by the types of amino acids that make up the sequence and the side chains that they possess (Figure 1)[4].

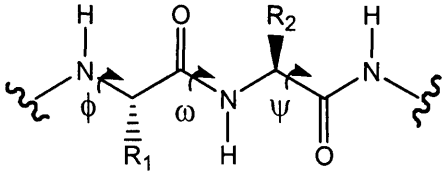


Figure 1: The torsion angles ω, ψ and ϕ of a peptide

Values of $\phi = -57^{\circ}$ and $\psi = -47^{\circ}$ result in the backbone adopting an α helical structure. Hydrogen bonds occur between the carbonyl groups and amide protons that are found between one turn of the helix, the i and (i+4) positions, representing 3.6 residues *per* complete turn which stabilise the structure (Figure 2).

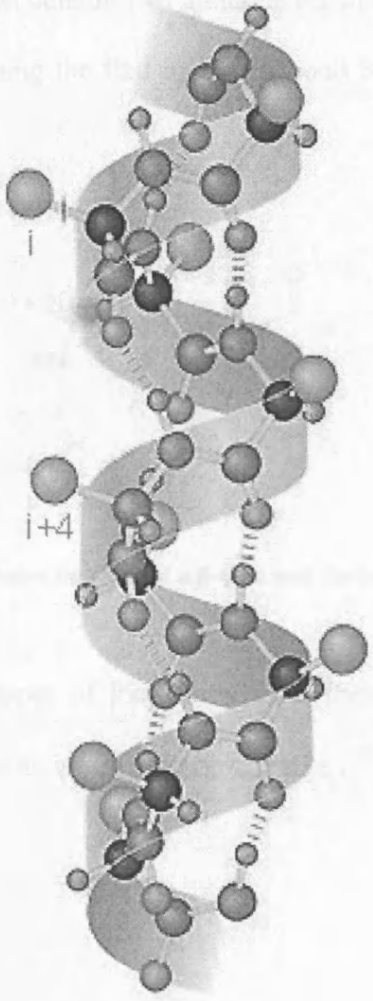


Figure 2: The i and (i+4) sites of an α-helix [5]

Natural α helices are right-handed because of the chirality of the amino acids and are not capable of forming left-handed types. The majority of helical structures found in

proteins are α helices but there are other types, of these the most notable is the 3_{10} helix. This is a more tightly wound helix with hydrogen bonds occurring between the i and (i+3) residues and torsion angles of $\phi = -70°$ and $\psi = -5°$.

β turns consist of a loop of two to six amino acid residues which link peptide strands together[6]. The most common contain two amino acids in the loop, i+1 and i+2, with the i and i+3 residues forming the first hydrogen bond between the strands (Figure 3)[7].

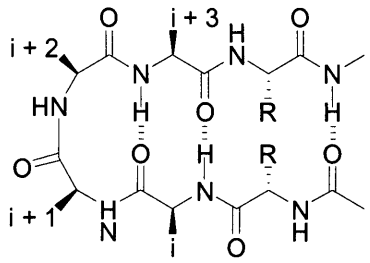


Figure 3: The hydrogen bonding of a β-turn and the i and (i+3) positions

There are many different types of loop known but there are six major categories classified by the bond angles ω, ψ and (Figure 4, Table 1)[8].

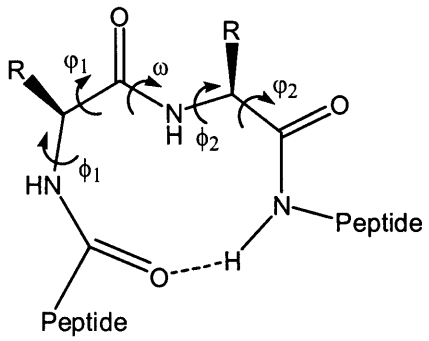


Figure 4: Diagram showing the torsion angles important to assign β-turn type

Table 1: The torsion angles important to assign β-turn type

	Turn Type					
Dihedral Angle	I	I'	II	II'	III	III'
ϕ_1	-60	60	-60	60	-60	60
ψ_1	-30	30	120	-120	-30	30
ϕ_2	-90	90	80	-80	-60	60
ψ_2	0	0	0	0	-30	-30

β turns are generally found within proteins as reverse turns for antiparallel β sheet propagation. Here they act to alternate the direction of a peptide strand and if found in series can produce antiparallel β sheets. The peptide chains in these motifs run in opposite directions a consequence of this is creation of a hydrogen bonding network between the carbonyl group and amide proton which are found opposite each other (Figure 5 & 6)[9].

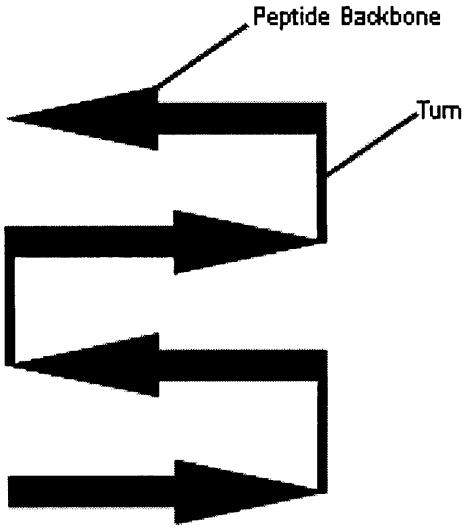


Figure 5: The build up of an antiparallel β-sheet by β-turns

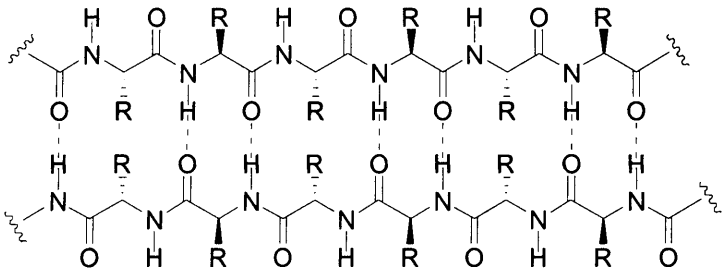


Figure 6: Hydrogen bonding found within antiparallel β-sheets

Parallel β sheets are less common, this is believed to be due to their folding motifs which involve bringing strands together from different parts of the peptide chain that are not close in sequence (crossover connections). Here the opposite strands run in the same direction to one another and create a non-planar hydrogen bonding network (Figure 7)[6].

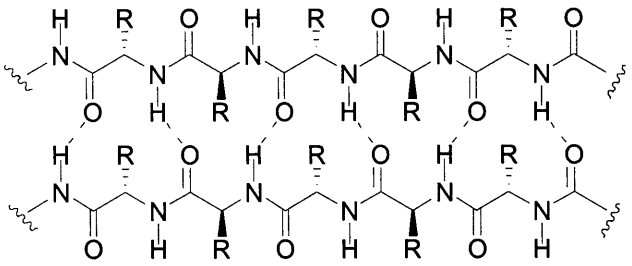


Figure 7: The hydrogen bonding found within parallel β-sheets

The study of secondary structure within small molecules has been wide ranging. Peptidomimetics have been targeted as specific drug molecules[10]. Molecules are made to copy the local topography about the amide bond by synthesis of amide bond isosteres, pyrrolinones or short portions of secondary structure. Generally these molecules such as **1**[11] and **2**[12] both factor Xa inhibitors look nothing like peptides but contain groups positioned in such a way that they either mimic the secondary structure of a peptide or the arrangement of the side-chains of the peptide being imitated (Figure 8).

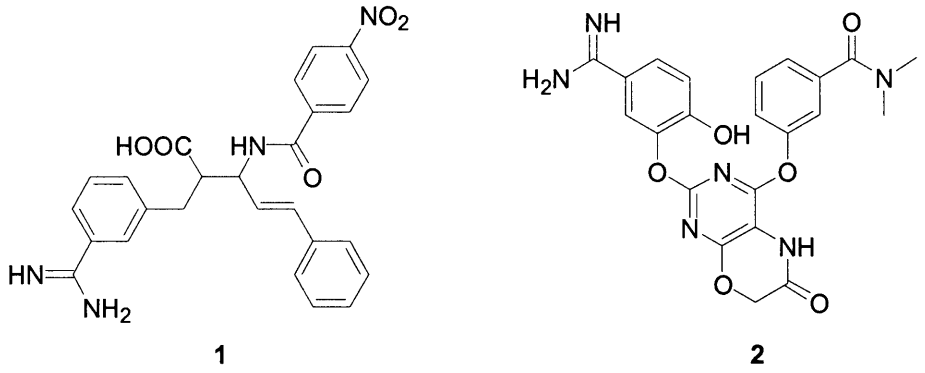


Figure 8: Two peptidomimetic drugs 1 and 2

These molecules tell us little about the shape of the secondary structure of proteins. To study the structure of proteins small peptides have been synthesized that adopt

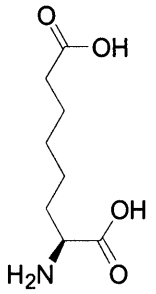
helices[13], turns[14] and sheets[15]. These peptides generally rely on either sequences that are specific to the motif[16] or artificial constraints being applied to the backbone or side chains[17]. This is extremely limiting as only certain amino acids can be included in the sequence[18]. This hinders the development of possible applications for example, synthesis of artificial enzymes, as the structure should be constant while amino acids are changed to achieve selectivity.

A route which is becoming more popular involves replacement of natural conformational constraints, such as disulfide bridges, by closely related mimics[19]. Performing this often leads to molecules with a defined three-dimensional shape and increased metabolic stability. Limiting the flexibility of short peptides in this way has also been found to increase the affinity for receptors by reducing the unfavorable entropy loss upon binding[20]. The relatively stable secondary structure formed by these molecules can be studied and give insights into protein folding, aggregation and bond angles[21]. This review aims to highlight the different ways in which this can be done using aliphatic bridges as well as covering syntheses of bridged bis amino acids.

1.1 Carbon Side-Chain Bridged Peptides

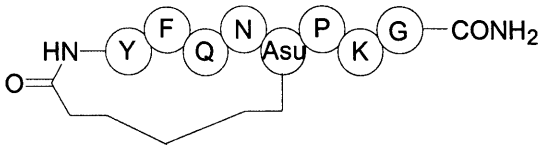
Carba analogues of several hormones were synthesized in the early 1970's[22, 23]. The first of these mimicked the disulfide bridge using a molecule of 1,6-aminosuberic acid **3** (Figure 9). This was incorporated into the peptide and then cyclised onto the *N* terminus to form an amide (Figure 10). Although not a linkage between two side

chains, the synthesized peptides **4** and **5** successfully mimicked and were denatured slower than the natural hormones although they did exhibit a drop in potency.

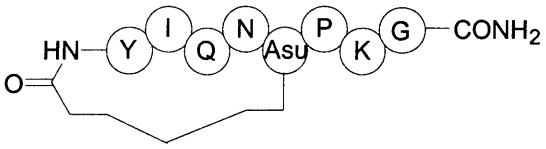


3

Figure 9: 1,6-aminosuberic acid 3



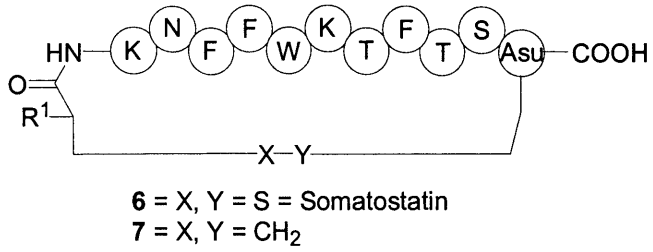
4 - [1,6-aminosuberic acid]-8-lysine-vasopressin



5 - [1,6-aminosuberic acid]-8-lysine-vasotocin

Figure 10: Synthetic mimics of vasopressin 4 and vasotocin 5

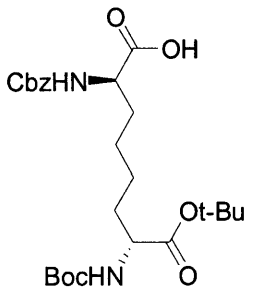
Similar work was completed on the peptidal release inhibiting factor somatostatin **6** (Figure 11). Again 1,6-diaminosuberic acid was used to cyclise the two ends of the peptide instead of a disulfide found in the natural peptide[24].



6 = X, Y = S = Somatostatin
7 = X, Y = CH_2

Figure 11: Natural somatostatin 5 and synthetic mimic 7

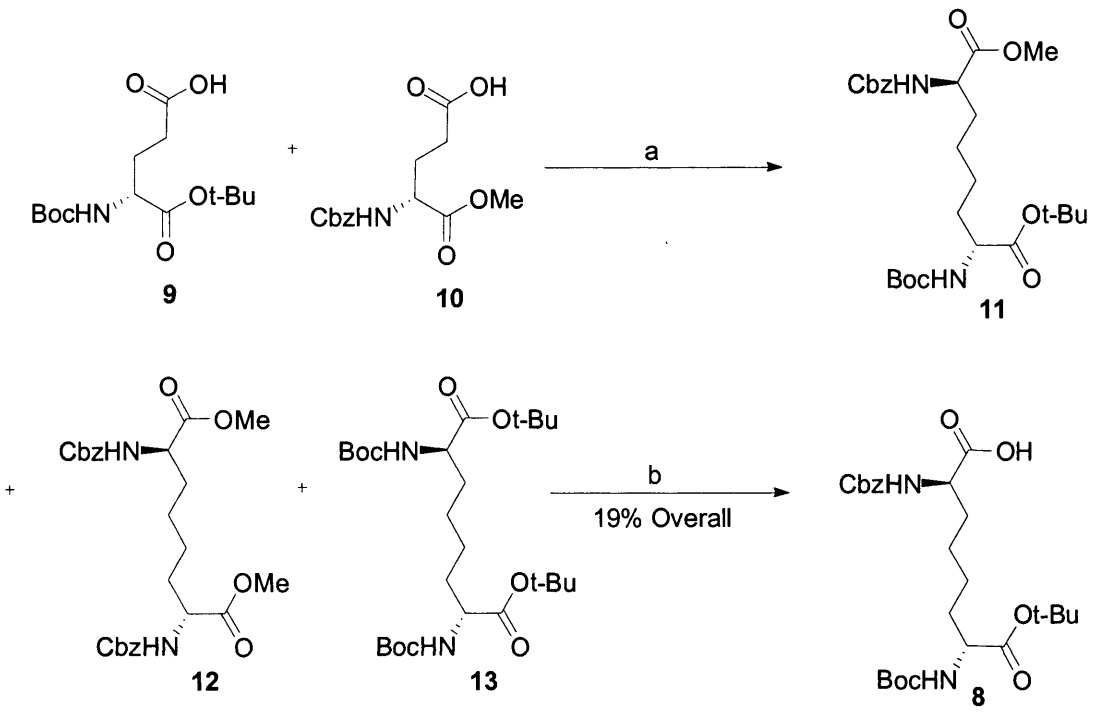
Synthesizing **7** was important to determine whether it was the dithiol linear form or the cyclised disulfide of the parent peptide which caused activity in vivo. Upon testing, **7** was found to show significant biological activity. In gastric secretion studies the authors found that **7** maintained the same inhibitory effect 30 minutes after the infusion had stopped, whereas somatostatin lost activity immediately. This highlights the increased stability that can result upon enforcing structural features found within natural peptides and shows that it is the dithiol form of the hormone which causes activity. Further analogues of somatostatin were synthesized some containing two carba bridges[25]. These molecules could not be synthesized using the standard peptide synthesis techniques used previously. An orthogonal strategy for the integration of the dicarba analogues into the peptide had to be used. First LL-α,α'-diaminosuberic acid **8** had to be synthesized in optically pure form containing orthogonal groups to those that were to be used in the peptide synthesis (Figure 12).



8

Figure 12: LL-α,α'-Diaminosuberic acid

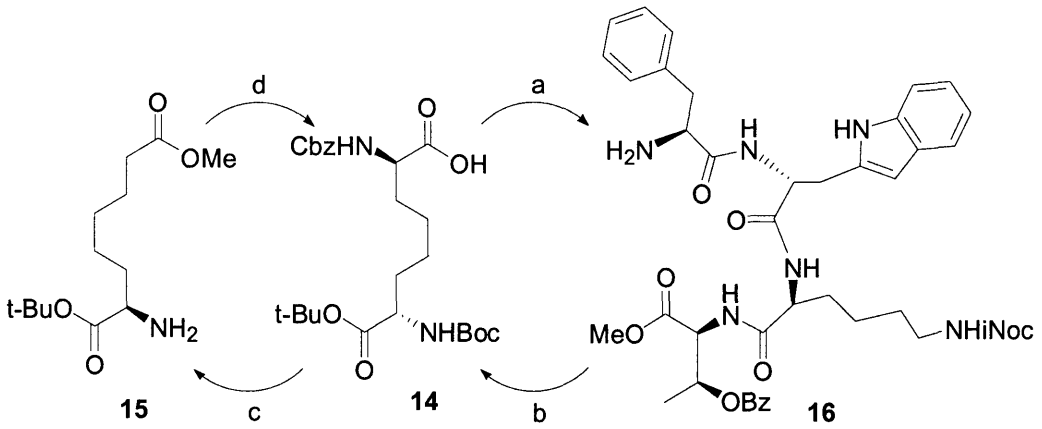
This was achieved by Kolbe electrolysis of two differentially protected glutamic acids[26]. Acids **9** and **10** were stirred together in a solution of sodium in methanol and pyridine and this was then treated electrolytically (Scheme 1). The close running fractions by flash column chromatography of homo coupled products **12** and **13** and desired bis amino acid **11** were separated by saponifying the methyl esters and resubjecting the diacid and monoacid to column chromatography.



a) Na, MeOH, pyridine, electrolysis, 20° - 25°C b) i) NaOH, dioxane ii) flash column chromatography

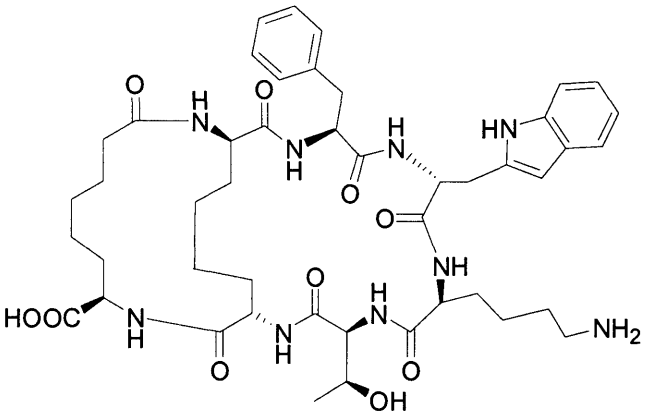
Scheme 1: Synthesis of protected LL-α,α'-diaminosuberic acid 8

The integrity of both the chiral centers remained throughout the synthesis and a 19 % yield was achieved. Although low yielding, the synthesis uses cheap and readily available starting materials producing chiral material without the need for asymmetric synthesis. The bis amino acid was then integrated into an analogue of somatostatin as shown in Scheme 2[27].



Scheme 2: Synthesis of butyl side-chain bridged cyclic peptide 17

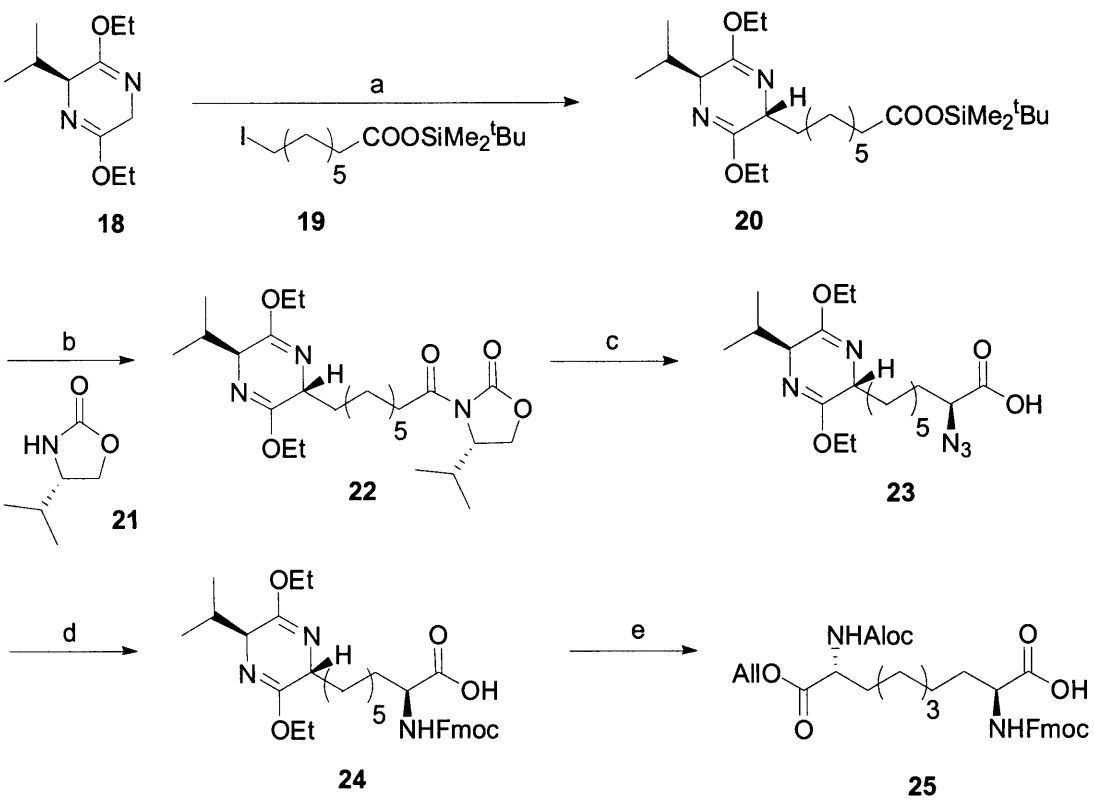
The free acid of **14** is coupled with the amine of previously synthesized tetrapeptide **16** (a). The Boc and ^{t}butyl groups were then removed and the methyl ester was converted to the acyl azide via the hydrazide before the peptide was cyclised (b). Condensation of protected aminosuberic acid amine **15** with the free acid of the cyclised peptide was achieved using diphenylphosphoryl azide (c). Cbz deprotection and azide cyclisation (d) followed by hydrogenation to remove side chain protecting groups gave analogue **17** (Figure 13). When tested *in vivo* analogue **17** was found to have an increased duration of action with respect to somatostatin but was not as effective at inhibiting the release of growth hormone. This highlighted the difficulty in designing structurally more stable analogues of natural peptides.



17

Figure 13: Synthetic mimic of somatostatin 17, with butyl side-chain bridge

A similar route was more recently employed by Tabor *et al*[28,29]. to study the shape of small peptides. Using chiral auxiliaries which differed in the conditions required to remove them it was possible to synthesize a differentially protected hexyl bridged bis amino acid (Scheme 3).

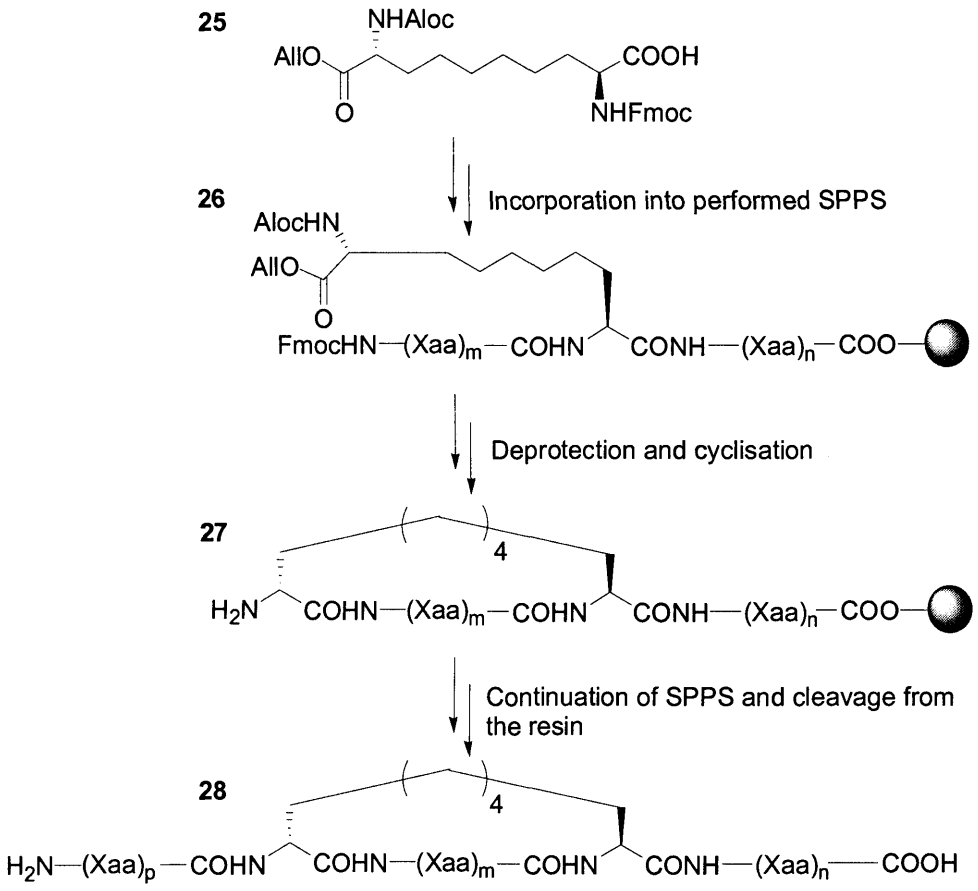


a) i) nBuLi ii) CuCN 3) **19** b) i) TBAF ii) Et_3N, ^{i}Butyl Chloroformate iii) **21** c) i) KHMDS ii) Trisyl Azide iii) KOAc, HOAc iv) LiOH, H_2O_2 d) i) PPh_3 ii) H_2O iii) Fmoc Chloroformate e) i) HCl ii) $NaHCO_3$ iii) Allyl Chloroformate iv) DBU, Allyl Bromide, LiBr.

Scheme 3: Synthesis of orthogonally protected hexyl bridged bis-amino acid 25

Alkylation of Schöllkopf's[30] bis lactam **18** by iodide **19** produced intermediate **20**. After acid deprotection the Evans[31] oxazolidinone chiral auxiliary **21** was coupled to the alkyl chain. Asymmetric amination was achieved using the electrophilic trisyl azide before the Evan's oxazolidinone was removed from **23** with LiOH and H_2O_2. Staudinger reduction of the azide **23** using triphenylphosphine produced amino acid **24**. Protection of the amine as an Fmoc carbamate allowed the bis lactam to be removed without producing equivalent amines. Allyl chloroformate protection of the

free amine and base catalysed transesterification of the ethyl ester for an allyl ester produced acid and orthogonally protected bis amino acid **25**.



Scheme 4: Synthesis of hexyl side-chain peptide 28

The differential protecting groups of the molecule meant that it could be incorporated into a peptide by SPPS in the following way (Scheme 4). Iterative SPPS is started on resin and after the required C-terminus amount of amino acids are added the acid moiety of **25** is coupled to the free amine terminus. The Fmoc group is then removed and SPPS continues. After addition of the requisite amount of protected amino acids the allyl groups are selectively deprotected and the Fmoc removed and the peptide

cyclised on resin (**27**). Amino acids are then added via SPPS and the peptide cleaved from the resin when complete to give **28**. Using this technique it is possible to insert any number of unnatural side-chain linkers between two groups in a peptide. The method is hugely advantageous as the whole synthesis can be performed on resin and therefore many purification steps can be removed. Creating linkers between amino acid side-chains often relies on post synthetic modifications of the peptide and therefore limits the presence of amino acids that may interfere with the reacting species. Using this technique allows for any amino acids to be used as it relies on protecting group deprotection and cyclisation, the side-chain linker being synthesized before the SPPS. The final peptide was found to exhibit a stabilized type II β turn (Figure 14).

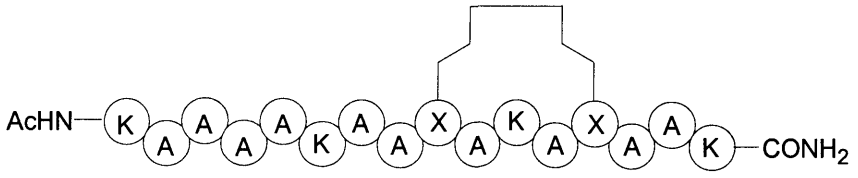
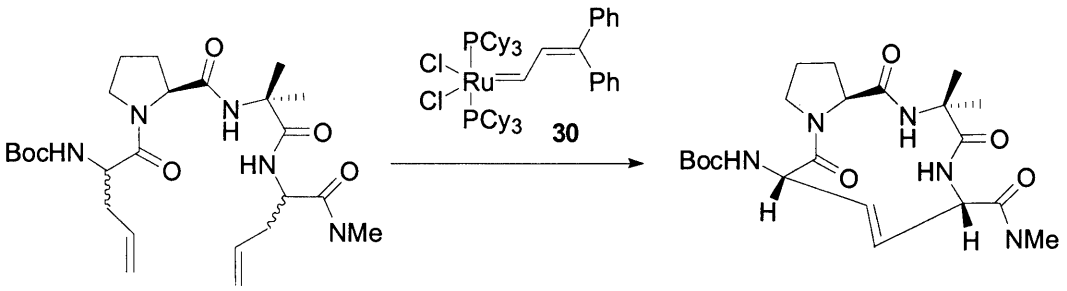


Figure 14: Hexyl side-chain bridged peptide

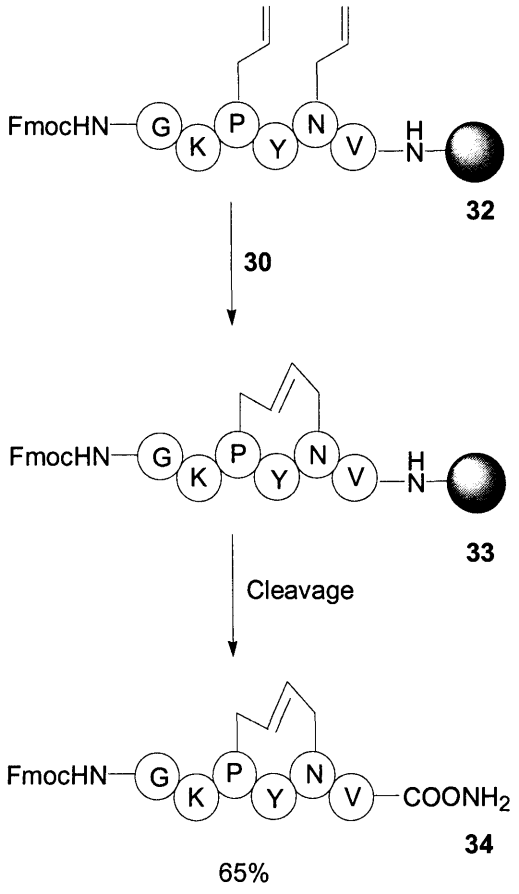
The most flexible method developed for introducing dicarba bridges to peptides within recent years is by RCM. Many groups have performed RCM both to create bis amino acids and to cross-link side-chains of synthesised peptides[32,33]. It has allowed for previously very difficult transformations to be carried out in one facile step and is highly amenable to create libraries of peptides. The group of Grubbs were first to perform RCM within a peptide scaffold[34]. A tetrapeptide previously shown to create a

β turn when two terminal cysteine residues formed a disulfide bridge was synthesised with the cysteine replaced by racemic allyl glycine units (Scheme 5).



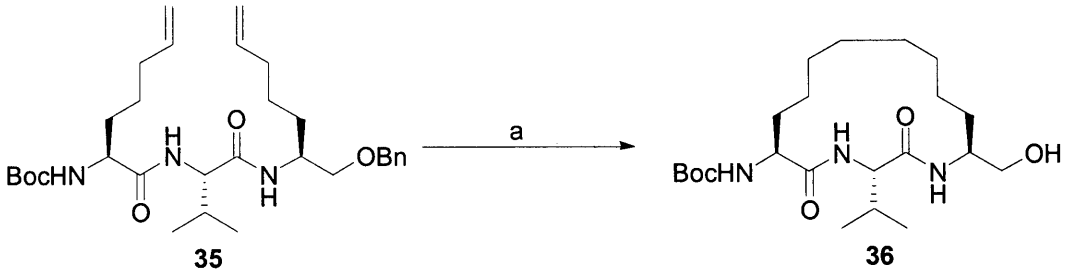
Scheme 5: Synthesis of alkene side-chain bridged tetrapeptide 31

Upon exposure of **29** to the ruthenium RCM catalyst **30** only the (*S,S*) isomer ring closed, thus creating diastereomerically pure product **31** which was found to replicate the hydrogen bonding network found within natural β turns. The group then went on to perform solid phase peptide synthesis incorporating allyl glycine residues which they subsequently ring closed on resin (Scheme 6)[35]. The resultant hexapeptide **34** was produced in 65 % overall yield and highlights the power of the method.



Scheme 6: Synthesis of alkene side-chain bridge peptide 34 via SPPS

A larger octyl side-chain linker was installed in a small tripeptide mimic protease inhibitor as a post synthetic modification using RCM[36] (Scheme 7). After asymmetric synthesis of the two alkene containing species they were coupled to either side of a valine residue to give **35**. The side-chains were then cyclised by RCM in good yield before the remaining double bond was hydrogenated (**36**).

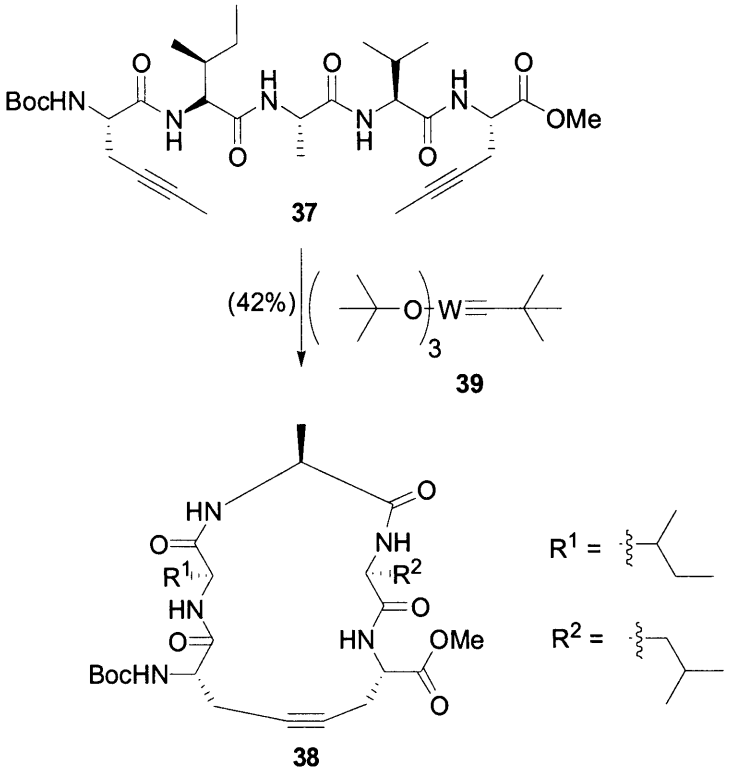


35 **36**

a) i) **30** ii) Na, NH_3 iii) Pd-C, H_2.

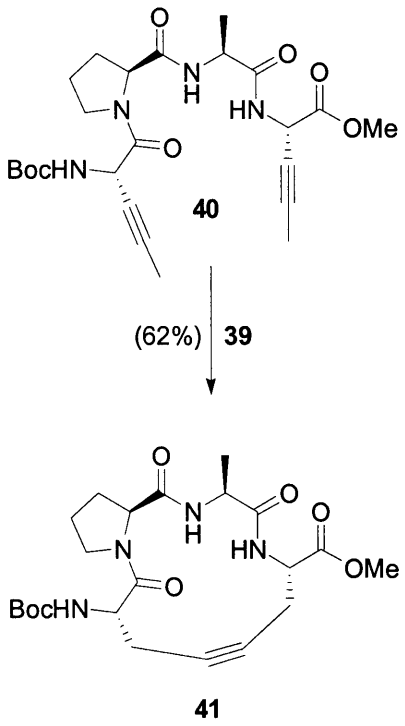
Scheme 7: Synthesis of octyl side-chain bridged tripeptide 36

Recently much progress has been achieved in the area of RCAM[37,38]. Alkyne amino acids[39] have been successfully synthesised and undergo RCAM in good yield[39]. This can be performed both to make bis amino acids[40] and also as a post synthetic modification[41]. Progress towards the synthesis of an alkyne carba analogue of antibiotic nisin was achieved by using RCAM to successfully synthesise a mimic of the A ring[41,42] (**38**) (Scheme 8).



Scheme 8: Synthesis of akyne side-chain bridged pentapeptide 38

The unnatural tetrapeptide **40** was synthesised and subjected to RCAM catalyst **39** to study the effects of alkyne analogues of disulfide bridges in β turns (Scheme 9)[43]. After cyclisation structural analysis by NMR indicated that the presence of the acetylene bridge in **41** rendered the cyclic peptide more rigid than a disulfide, showing stronger intrastrand proton-proton interactions than the natural linkage.

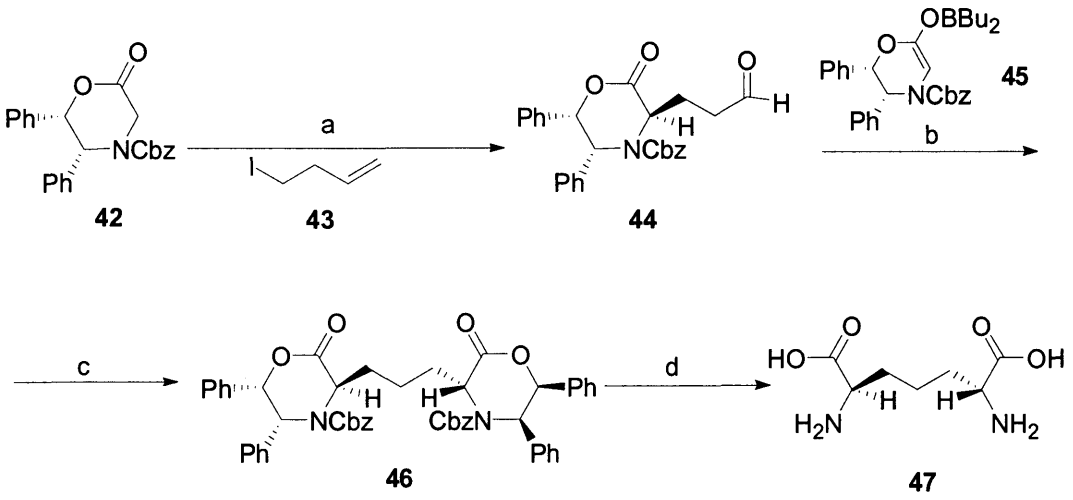


Scheme 9: Synthesis of akyne side-chain bridged tetrapeptide 41

1.1.1 Carbon Bridged Bis Amino Acids

Coverage in this section will be focused on a selection of syntheses that have used different methods to make carba bridged bis amino acids but for one reason or another the final molecules have not been incorporated into peptides.

Synthesis of propyl and butyl linked bis amino acids was reported by Williams *et al.*[44] (Scheme 10). Using commercially available lactone **42** as an asymmetric inducer they were able to prepare bis amino acids such as **47** enantiopure as either (*R*,*R*) or (*S*,*S*) diastereoisomers.

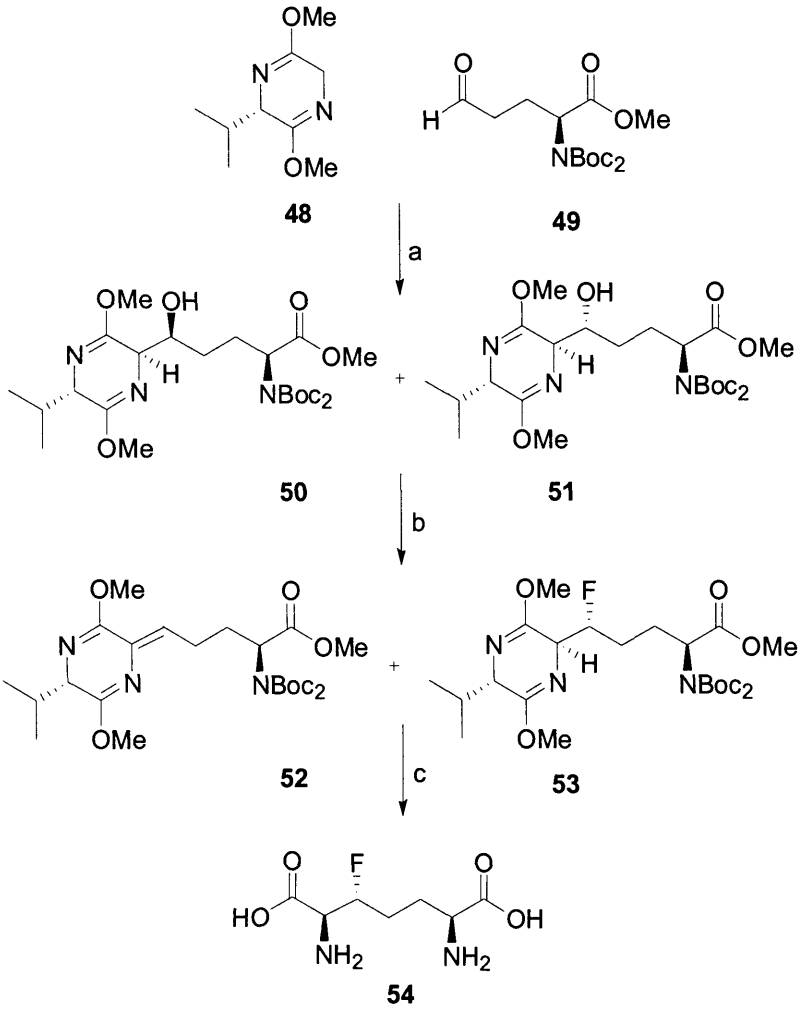


a) i) LiHMDS ii) O_3 iii) Me_2S c) radical deoxygenation d) H_2, $PdCl_2$

Scheme 10: Synthesis of propyl bridged bis-amino acid 47

The synthesized molecules were being prepared as antibiotics so no effort was made to make orthogonally protected derivatives.

Schöllkopf's bis lactam ether was employed by Vederas *et al*[45]. to synthesise an inhibitor of diaminopimelic acid epimerase (Scheme 11).

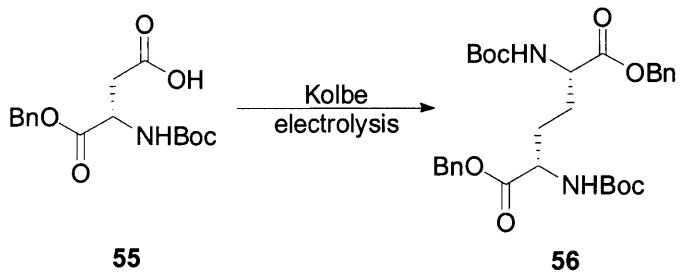


Scheme 11: Synthesis of 1-fluroro propyl bridged bis-amino acid 54

Protected glutamate semialdehyde **49** is reacted with the chiral glycine equivalent **48**. This gives the alcohols **50** and **51** in a 3:1 ratio respectively in favour of the desired product. The unwanted alcohol when subjected to fluorination with DAST eliminates to give alkene **52** and thus allows for easy separation of the alkyl fluoride **53**. The protecting groups are then cleaved with strong acid to yield **54**. The final material is required fully deprotected but if different protecting groups were used an

orthogonally protected derivative of this interesting class of bis amino acids could be produced.

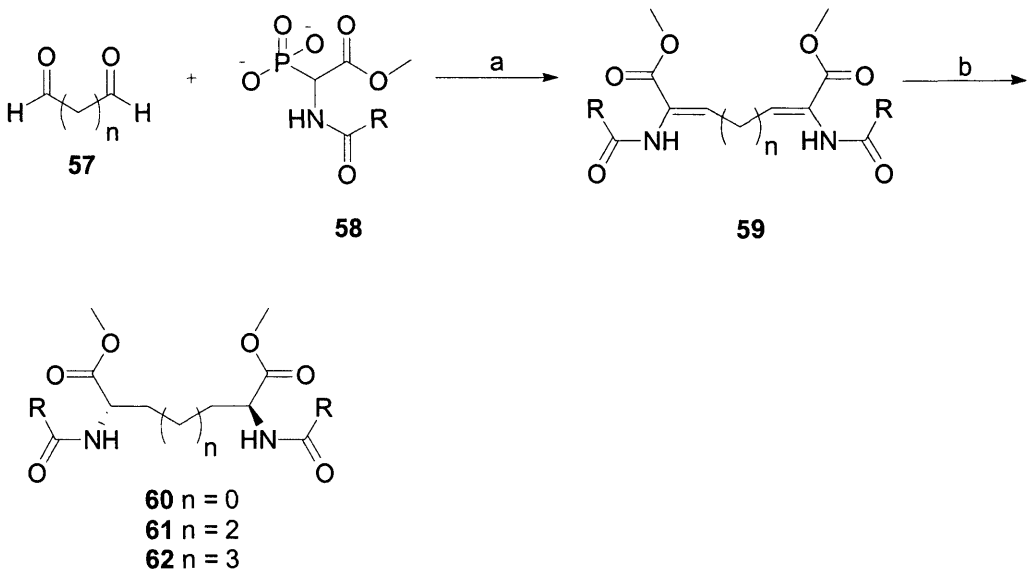
A thorough study of the Kolbe electrolysis reaction was performed by Hiebl *et al.*[46] (Scheme 12). Yields as high as 38% were reported for homo coupled glutamic acids. Aspartic acid was also coupled using this technique to give protected 2,5-diaminoadipic acid (**56**) in low 17 to 25 % yields depending on the protecting groups used.



55 **56**

Scheme 12: Synthesis of ethyl bridged bis-amino acid 56

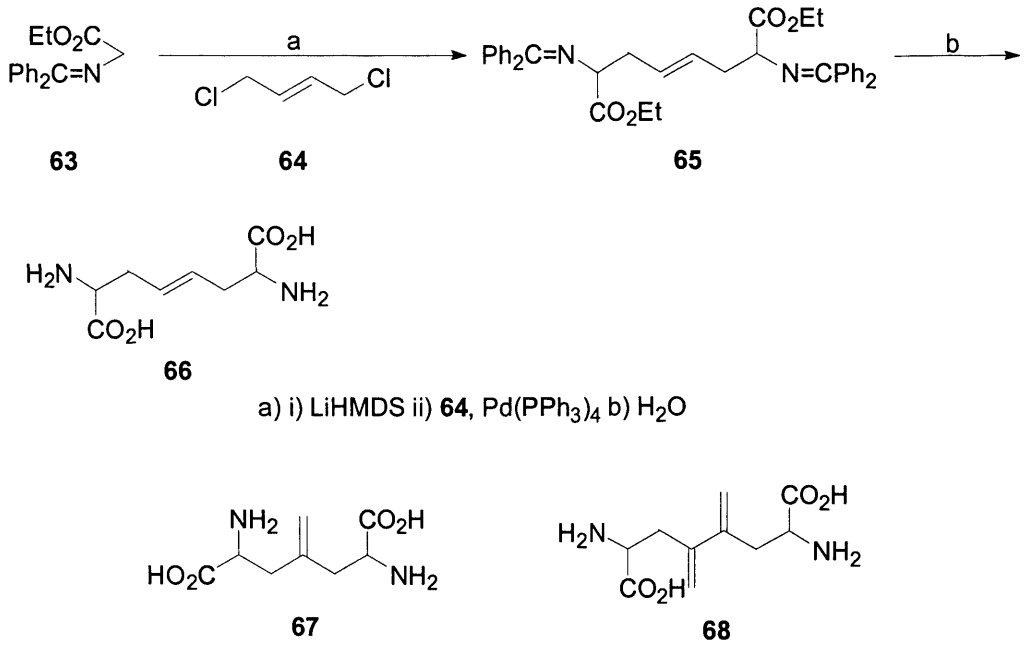
Although low yielding the reaction does show how complex bis amino acids can be synthesized using cheap starting materials, avoiding the high cost of metathesis catalysts.



Scheme 13: Synthesis of propyl, butyl and pentyl bis-amino acids 60, 61 and 62

Winkler *et al.*[47, 48]. more recently used asymmetric catalytic hydrogenation to install the stereocenters of bis amino acids (Scheme 13). This allowed them to produce a range of differing linkers between the protected amino acids such as **60**, **61** and **62**. Although these molecules were produced without orthogonal protecting groups the yields and ee's were good.

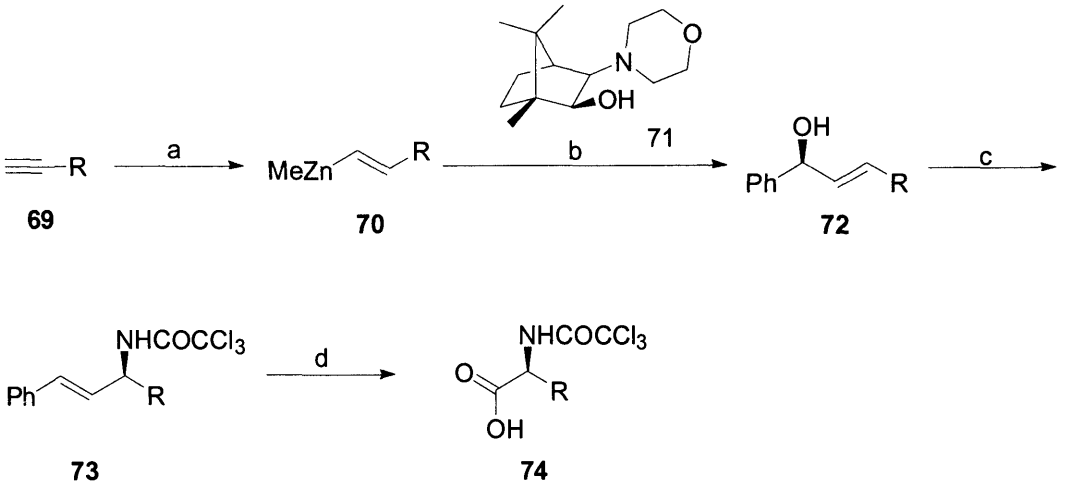
Using an approach based on the palladium mediated allylation of Schiff bases derived from glycine, Najera *et al.*[49]. were able to make some highly novel bis amino acids such as **67** and **68** (Scheme 14).



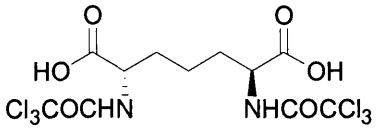
Scheme 14: Synthesis of novel alkene containing bis-amino acids 66, 67 and 68

Reaction of two equivalents of Schiff base (**63**) with aliphatic dihalide **64** in the presence of tetrakis(triphenylphosphine) palladium (0) and base produces the bis imino ester **65**. Upon hydrolysis of this intermediate the bis amino acid **66** was produced in good yields. The major drawback to this approach is the synthesis of a mixture of stereoisomers, as the alkylation reactions were not performed asymmetrically. Isolating the correct stereoisomer would be difficult and time consuming.

A very powerful method for the synthesis of unnatural amino acids was used to synthesise propyl linked bis amino acid **75** by Walsh *et al.*[50] (Scheme 15).



a) i) Cy_2BH ii) $ZnMe_2$ b) **71** cat (2 mol %), PhCHO c) i) cat. KH, Cl_3CCN ii) Toluene, reflux d) O_3

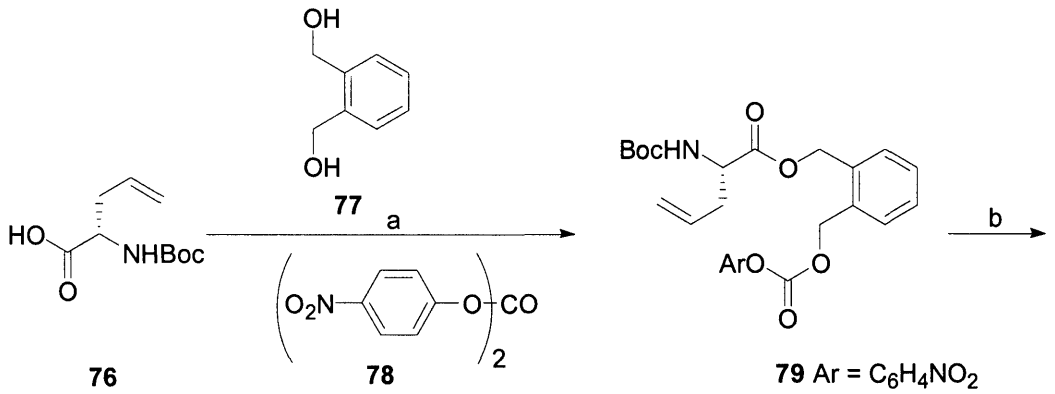


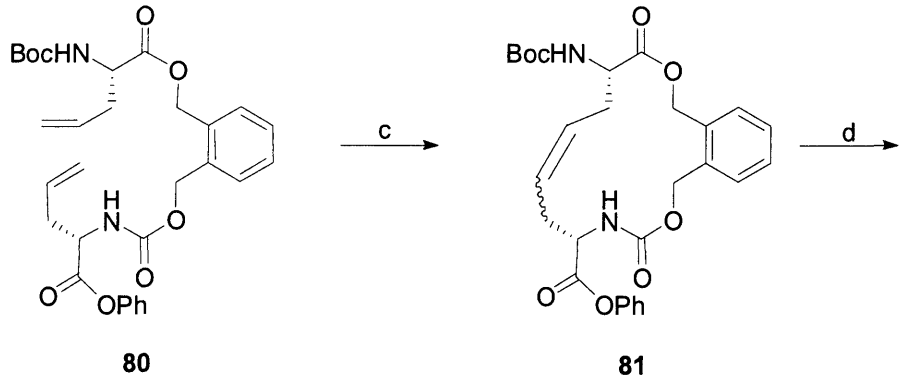
75

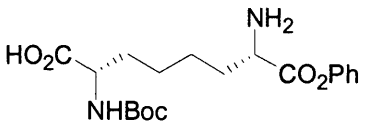
Scheme 15: Synthesis of propyl bridged bis-amino acid 75

An enantioselective and catalytic vinylation of benzaldehyde leads to allylic alcohol **72** that can then be transformed into allylic amine **73** via conversion to an imidate and subsequent [3,3]-sigmatropic rearrangement. Oxidative cleavage of allylic amine **73** furnishes amino acid **74** in good yields and excellent ee's. Using this approach bis amino acid **75** was produced in an excellent 89% yield from a bis allyl amine. However a drawback to this methodology would be the difficulty in installing orthogonal protecting groups.

The RCM methodology has also been used to prepare orthogonally protected bis amino acids[51]. Starting from allylglycine, 2,7-diaminosuberic acid derivative **82** was prepared using a dibenzyl ester protection strategy (Scheme 16).







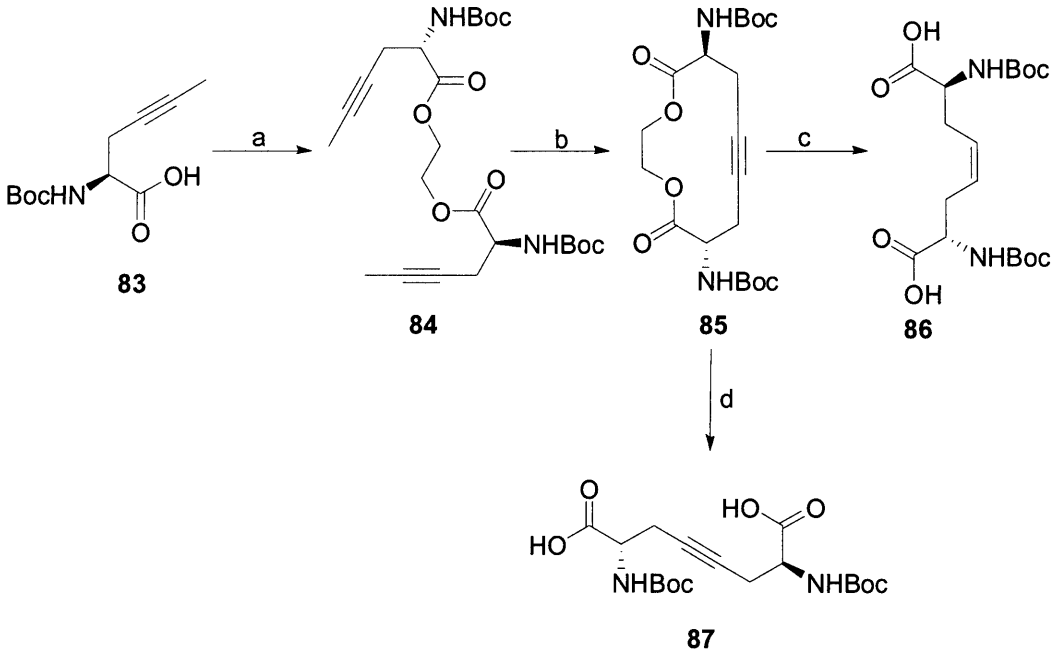
82

a) i) EDCI, DMAP, **77** ii) **78**, $^{i}Pr_2EtN$ b) 76, $^{i}Pr_2EtN$ c) Ru-cat d) H_2-Pd, *p*-TsOH.

Scheme 16: Synthesis of orthogonally protected butyl bridged bis-amino acid 82

The metathesis reaction provided the alkene **81** which was then hydrogenated to yield the final molecule **82** which could be easily incorporated into an amino acid via SPPS.

Synthesis of alkene and alkyne equivalents of diaminosuberic acid was performed using RCAM by first cyclising two equivalents of alkyne **84** tethered together with an ethyl ester using tungsten-alkylidyne **39** in a good 84 % yield[40] (Scheme 17).



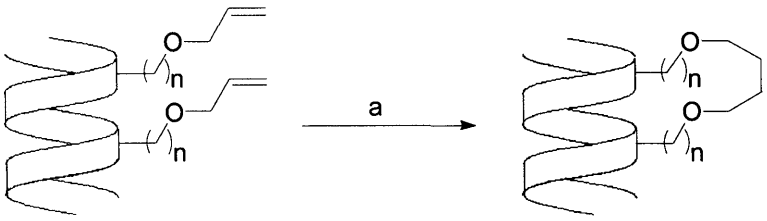
a) i) $HOCH_2CH_2OH$, DCC, DMAP ii) **92**, DCC, DMAP b) **39** c) i) H_2, Lindlar cat. ii) LiOH d) LiOH

Scheme 17: Synthesis of propyne bridged bis-amino acid 87

Removal of the ethyl ester protection produces alkyne bis amino acid **87**. Reduction of the alkyne before ethyl ester removal with Lindlar's catalyst gives a cis alkene which is then saponified to **86**.

1.2 Oxygen Side-Chain Bridged Peptides.

A post synthetic RCM was performed on a peptide that contained allyl ether residues and therefore produced a diether bridge[52] (Scheme 18).



88 **89** n = 1 **90** n = 2

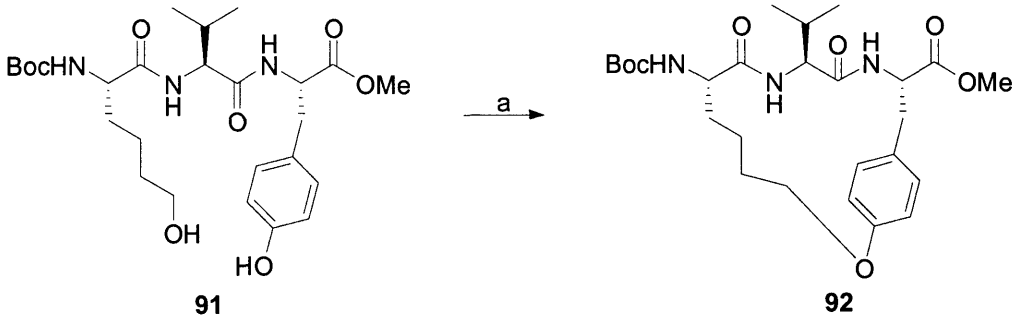
a) i) **30** ii) H_2 Pd-C

Scheme 18: Synthesis of diether side-chain bridged peptides 89 and 90

Using the sequence of a peptide that was shown to form a stable α helix in solution the bridge was formed between the i, (i+4) residues, sites that represent one complete turn of the helix. This was performed by allylating serine or homoserine side-chains found within the peptide. The allyl ethers were then subjected to RCM to yield the alkene. The double bond was then subjected to catalytic hydrogenation to give an unsaturated diether bridge. The bridge formed in **89** and **90** was shown to form a 3_{10} helix by both CD spectra and crystal structures.

A further side-chain linked ether was synthesised by Fairlie *et al*[53]. to mimic the shape of β strands (Scheme 19). After synthesis of tripeptide **91** the primary alcohol

was derivatized as an iodide. The introduction of a weak base then led to ring closure and ether **92**.



91 **92**

a) i) PPh_3, CBr_4 ii) NaI iii) K_2CO_3, DMF

Scheme 19: Synthesis of ether side-chain bridged tripeptide 92

The presence of orthogonal protecting groups gives the possibility of peptide chain extension.

1.3 Nitrogen Side-Chain Bridged Peptides.

Inouye *et al*[54]. used a post synthetic modification of the lysine residue of a peptide to stabilise α helical structures (Figure 15).

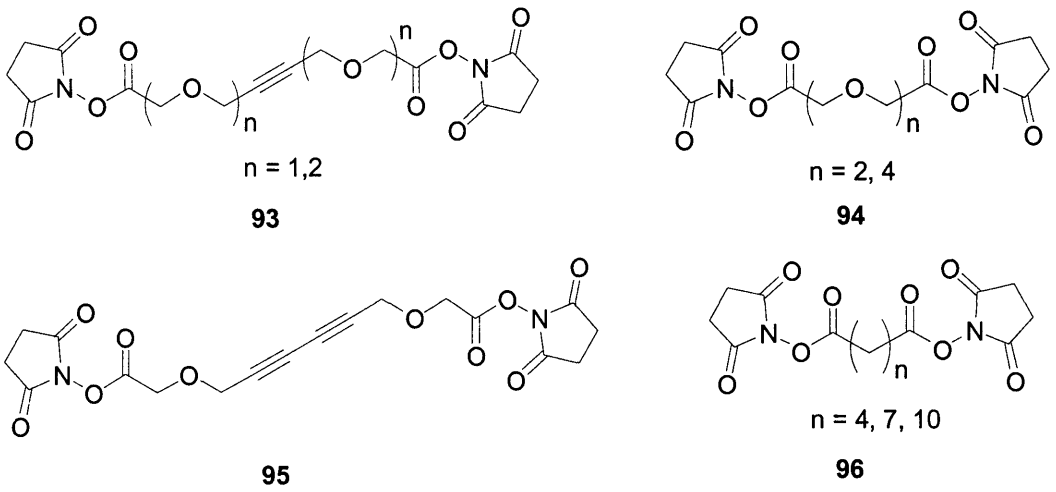
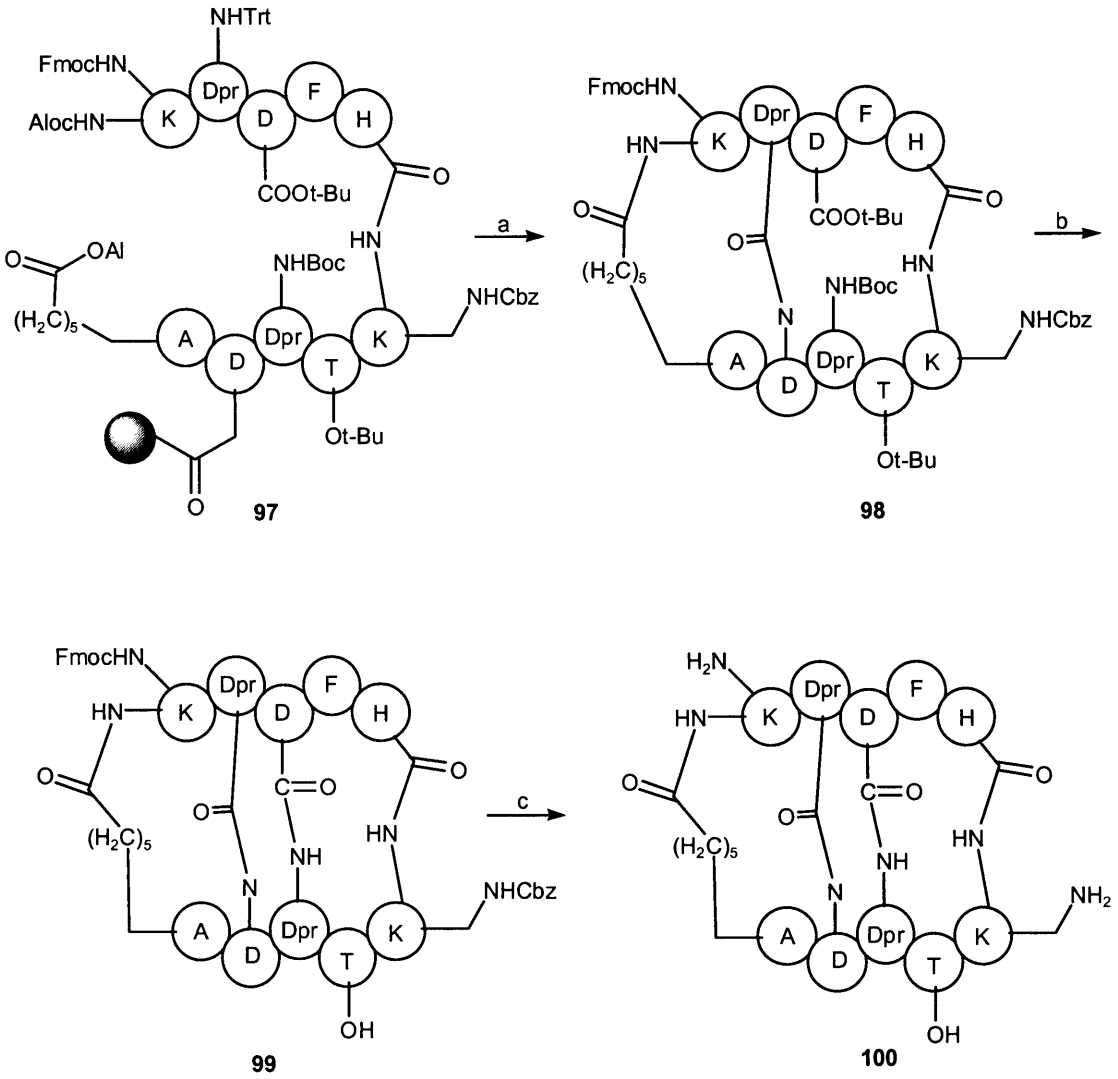


Figure 15: Succinimidyl esters used to bridge to lysine residues of a peptide

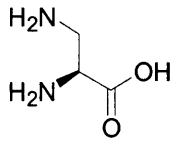
After each peptide was synthesised it was incubated with one of the cross-linking agent shown in Figure 15. The lysine residues were placed at the i and i+4 positions of one peptide and at the i and i+7 of the other chain to encourage a helical structure. The primary amine of the lysine residue reacted with the succinimide esters of the cross-linking agents to produce a bridged diamide. The reactions were performed in very dilute concentrations and no peptide dimers were observed. The propensity of the natural peptide to form α helical structures was found to greatly increase when acetylenic bridges **93** and **95** were introduced. The thermal stability of the bridged peptide was also very surprising with diacetylene **95** producing a 15% helical structure at 70°C. These molecules have possible applications as stable mimics of alpha helices for drug or artificial enzyme applications. The majordraw back is the requirement of the peptide backbone to contain no residues that are likely to be nucleophilic enough to react with the succinimidyl esters.

An ambitious mimic of somatostatin was synthesized by Smith *et al.* as an efficacious hormone inhibitor (Scheme 20)[55]. The synthesis relied on three pairs of triply orthogonal protecting groups to form three macrocycles by regiospecific side-chain cross-linking. After attachment of the aspartic acid side-chain to the solid phase the peptide **100** was synthesized via SPPS.



a) i) 0.75% TFA in CH_2Cl_2 ii) DPPA iii) $Pd(PPh_3)_4$ iv) DPPA b) i) 50% TFA in CH_2Cl_2 ii) DPPA c) i) Piperidine ii) H_2 Pd-C

Scheme 20: Synthesis of diamide side-chain bridged decapeptide 100



111

Figure 16: (S)-2,3-Diaminopropionic acid

Cleavage of **97** from the resin using a very low concentration of TFA resulted in concomitant deprotection of the previously trityl protected Dpr (Figure 16) residue (Scheme 23). The two free side-chains were then coupled using DPPA to give **99**. Next the allyl ester and carbamate were deprotected using $Pd(PPh_3)_4$ and the acid and amine coupled with DPPA to cyclise the termini of the peptide with a backbone linked octanoic acid. A more concentrated solution of TFA was then used to remove the t-butyl protecting groups and the Dpr and aspartic acid residues coupled with DPPA (**116**). The final two deprotection steps, removal of Fmoc from one lysine and Cbz from another, brought the total number of orthogonal protecting groups used to 5. The final molecule **100** was found to be only a moderately active mimic of somatostatin.

Simpler side chain amides have been widely reported. These tend to involve a post synthetic modification of lysine and either glutamic[56] or aspartic[57] acid to encourage stability of secondary structure (Figure 17).

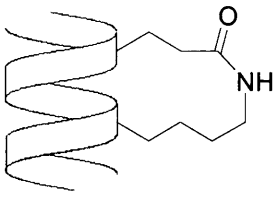
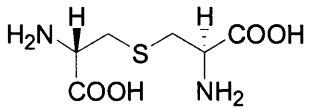


Figure 17: Example of an amide side-chain bridged peptide forming an α-helix

1.4 Sulfur Side-Chain Bridged Peptides.

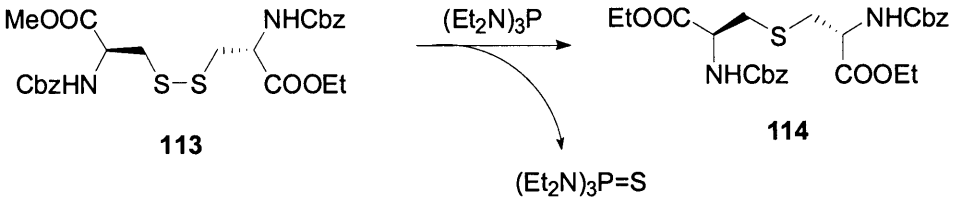
The use of sulfur as a linking moiety in side-chains as a stable means of mimicking disulfide bonds has seen much interest. Primarily this is due to research into a class of natural product antibiotics containing lanthionine bridges[58,59] (Figure 18).



112

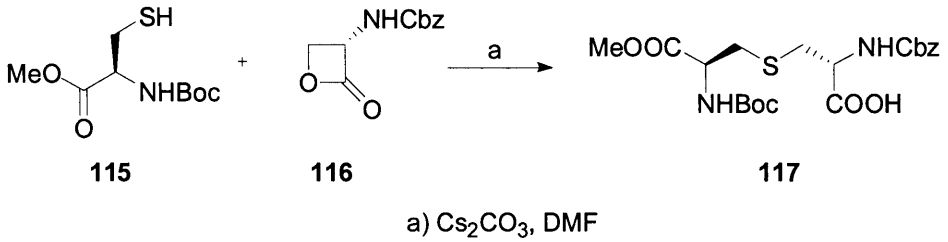
Figure 18: Lanthionine 112

There are many syntheses where these residues have been incorporated into peptides. Most have relied on either a post peptide synthesis modifications of the side-chains or synthesis of an orthogonally protected lanthionine derivative for iterative incorporation into a peptide. Notable syntheses include those by Harpp *et al*[60] where sulfur extrusion of a protected cysteine moiety such as **113** by $(Et_2N)_3P$ was used to produce non orthogonally protected lanthionine bridge **114** (Scheme 21). This technique was also used in the only total synthesis of the antibiotic nisin. Disulfide bridges were formed before sulfur extrusion to form lanthionine bridges occurred as a post synthetic modification[61].



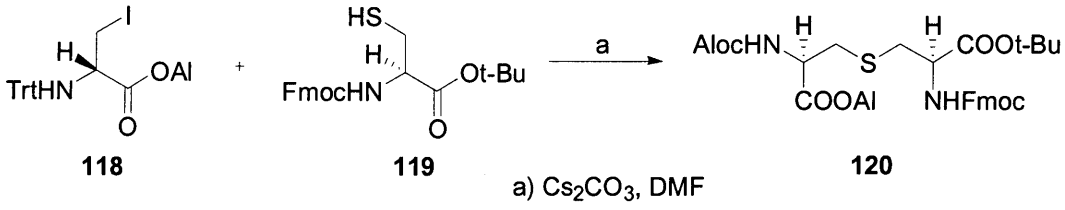
Scheme 21: Synthesis of lanthionine 114 via sulfur extrusion

Work by Goodman *et al.*[62] produced a differentially protected lanthionine bridge by opening β-lactone **116** derived from serine with cysteine derivative **115** (Scheme 22).



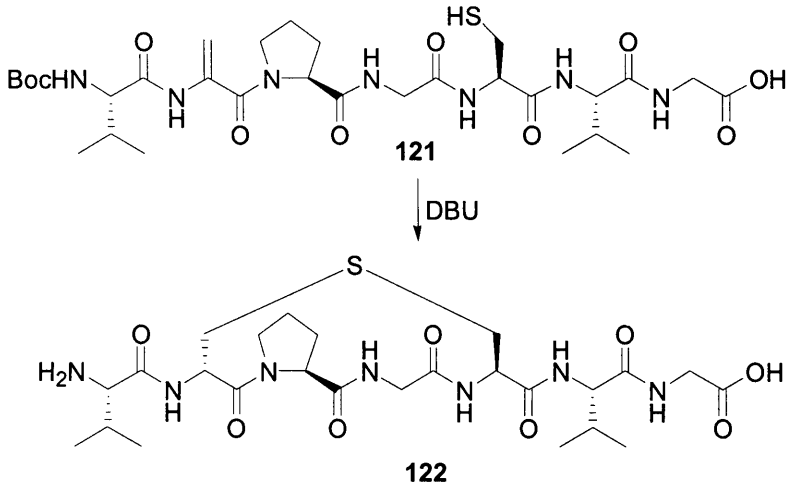
Scheme 22: Synthesis of orthogonally protected lanthionine 117 via β-lactone opening

Tabor *et al.* synthesised orthogonally protected lanthionine **120** by reaction of cysteine **119** with iodo alanine in the presence of Cs_2CO_3 (Scheme 23)[63,64]. The product **120** was then incorporated into a peptide in the same way as Scheme 4.



Scheme 23: Synthesis of orthogonally protected lanthionine 120 via iodo alanine and cysteine coupling

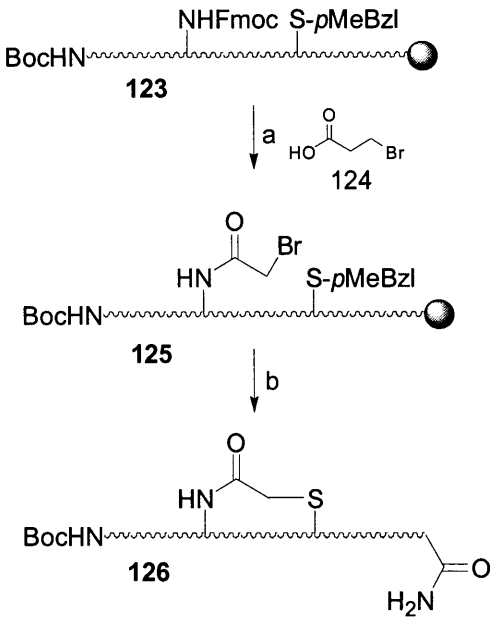
Bradley *et al.*[65] developed a method using a post synthetic modification of a synthesised peptide containing cysteine and dehydroalanine (Scheme 24). Exposure of peptide **121** to DBU resulted in a Michael reaction to generate the ring B of antibiotic nisin **122** with a single chiral centre being formed at the α carbon.



Scheme 24: Synthesis of ring B of nisin by intramolecular Michael reaction

Recently a side-chain sulfur coupling methodology was developed to stabilise helical peptides as an alternative to lactam bridges by Dawson *et al.*[66] (Scheme 25). Synthesis of peptide **123** was performed by SPPS, containing lysine and cysteine residues protected as their Fmoc carbamate and *para*-methoxybenzyl sulfide respectively. Deprotection of the Fmoc group by piperidine and reaction of the primary amine with bromoacetic acid **124** using DIC produces **125**. Concomitant deprotection of the sulfide protecting group and cleavage of the peptide from the resin is achieved with HF. The free thiol is then cyclised by reaction with the alkyl bromide

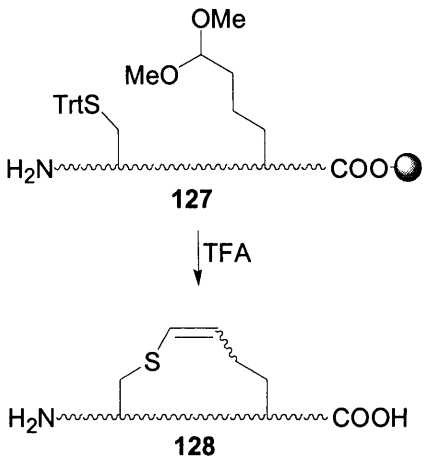
in a basic buffer to give cyclic sulfide **126**. In the peptides tested the presence of the sulfide bridge yielded greater helicity than those with lactam bridges and those without.



a) i) Piperidine ii) **124**, DIC b) i) HF ii) pH 8 Buffer

Scheme 25: Synthesis of amide and sulfide side-chain bridged peptide 126

Vinyl sulfide **128** was produced when cysteine was reacted in the presence of acid with a side-chain acetal by Hallberg *et al.*[67] in the synthesis towards angiotensin agonists (Scheme 26). These exhibited similar conformations and activity to the natural peptides.



Scheme 26: Synthesis of vinyl sulfide side-chain bridged peptide 128

In conclusion it has been shown that there are many different side-chain linkers that can be introduced into peptides to constrain and encourage secondary structure. The most common ways to incorporate these into peptides is either via an orthogonal protection strategy or synthesis of side-chains that can be reacted once the peptide is made. Another method involving linkage between two natural side-chains present in the peptide is also common but is limited by the amino acids that can be used in the peptide chain and the compatibility of the side chain-forming chemistry with the underlying peptide.

References

[1] Alberts, B.; Johnson, A.; Lewis, J.; Raff, M.; Roberts, K.; Walter, P. In *Molecular Biology of the Cell – Fourth Ed.* Garland, 2002.

[2] Lesk, A.M. In *Introduction to Protein Architecture : the Structural Biology of Proteins*, Oxford University Press, 2001.

[3] Ramirez-Alvarado, M.; Kortemme, T.; Blanco, F. J.; Serrano, L. *Bioorg. Med. Chem.* **1999**, *7*, 93-103.

[4] Andrews, M. J. I.; Tabor, A. B. *Tetrahedron* **1999**, *55*, 11711-11743.

[5] http://en.wikipedia.org/wiki/Image:AlphaHelixProtein.jpg, (last accessed 10/11/2006).

[6] Nelsony. C.L; Kelly, J.W. *Biorg. Med. Chem.* **1996**, *4*, 739.

[7] Sibanda, B.L.; Thornton J.M. *Nature*, **1986**, *316*, 170.

[8] Olson, G.L.; Voss, M.E.; Hill, D.E.; Kahn, M.; Madison, V.S.; Cook, C.M. *J. Am. Chem. Soc.* **1990**, *112*, 323.

[9] Blanco, F.; Ramirez-Alvarado, M.; Serrano, L. *Curr. Opin. Struct. Bio.* **1998**, *8*, 107-111.

[10] Ripka, A. S.; Rich, D. H. *Curr. Opin. Chem. Bio.* **1998**, *2*, 441-452.

[11] Klein, S. I.; Czekaj, M.; Gardner, C. J.; Guertin, K. R.; Cheney, D. L.; Spada, A. P.; Bolton, S. A.; Brown, K.; Colussi, D.; Heran, C. L.; Morgan, S. R.; Leadley, R. J.; Dunwiddie, C. T.; Perrone, M. H.; Chu, V. *J. Med. Chem.* **1998**, *41*, 437-450.

[12] Buckman, B. O.; Mohan, R.; Koovakkat, S.; Liang, A.; Trinh, L.; Morrissey, M. M. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2235-2240.

[13] Marqusee, S.; Baldwin, R.L. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 8898-8902.

[14] Blanco, F.J.; Jimenez, M.A.; Herranz, J.; Rico, M.; Santoro, J.; Nieto, J. *J. Am. Chem. Soc.* **1993**, *115*, 5887.

[15] Searle, M.S.; Griffths-Jones, S.R.; Maynard, A.J. *J. Mol. Biol.* **1999**, *292*, 1051.

[16] Searle, M.S. *J. Chem. Soc., Perkin Trans.* 2, **2001**, *1011*.

[17] Kemp, D.S.; Bowen, B.R. *Tetrahedron Lett.* **1988,** *29,* 5081.

[18] Balaram, P. *Journal of Peptide Research* **1999**, *54*, 195-199.

[19] Miles, S. M.; Leatherbarrow, R. J.; Marsden, S. P.; Coates, W. J. *Org. Biomol. Chem.* **2004**, *2*, 281-283.

[20] Reichwein, J. F.; Wels, B.; Kruijtzer, J. A. W.; Versluis, C.; Liskamp, R. M. J. *Angew. Chem., Intl. Ed. Engl.* **1999**, *38*, 3684-3687.

[21] Gellman, S. H. *Curr. Opin. Chem. Bio.* **1998**, *2*, 717-725.

[22] Hase, S.; Kirchber, M.; Schwartz, I. L.; Walter, R.; Wahrenbu, M.; Sakakiba, S. *J. Am. Chem. Soc.* **1972**, *94*, 3590-3600.

[23] Walter, R.; Yamanaka, T.; Sakakiba, S. *Proc. Natl. Acad. Sci. USA* **1974**, *71* , 1901-1905.

[24] Veber, D. F.; Strachan, R. G.; Bergstrand, S. J.; Holly, F. W.; Homnick, C. F.; Hirschmann, R.; Torchiana, M. L.; Saperstein, R. *J. Am. Chem. Soc.* **1976**, *98*, 2367-2369.

[25] Nutt, R. F.; Veber, D. F.; Saperstein, R. *J. Am. Chem. Soc.* **1980**, *102*, 6539-6545.

[26] Nutt, R. F.; Strachan, R. G.; Veber, D. F.; Holly, F. W. *J. Org. Chem.* **1980**, *45*, 3078-3080.

[27] Veber, D. F.; Holly, F. W.; Paleveda, W. J.; Nutt, R. F.; Bergstrand, S. J.; Torchiana, M.; Glitzer, M. S.; Saperstein, R.; Hirschmann, R. *Proc. Natl. Acad. Sci. USA* **1978**, *75*, 2636-2640.

[28] McNamara, L. M. A.; Andrews, M. J. I.; Mitzel, F.; Siligardi, G.; Tabor, A. B. *J. Org. Chem.* **2001**, *66*, 4585-4594.

[29] McNamara, L. M. A.; Andrews, M. J. I.; Mitzel, F.; Siligardi, G.; Tabor, A. B. *Tetrahedron Lett.* **2001**, *42*, 1591-1593.

[30] Schöllkopf, U.; Groth, U.; Deng, C. *Angew. Chem. Int. Ed. Engl.* **1981**, *20*, 798.

[31] Evans, D.A.; Britton, T.C. *J. Am. Chem. Soc.* **1987**, *109*, 6881.

[32] Kotha, S.; Lahiri, K. *Curr. Med. Chem.* **2005**, *12*, 849-875.

[33] Li, P.; Roller, P. P.; Xu, J. C. *Curr. Org. Chem.* **2002**, *6*, 411-440.

[34] Miller, S. J.; Grubbs, R. H. *J. Am. Chem. Soc.* **1995**, *117*, 5855-5856.

[35] Miller, S. J.; Blackwell, H. E.; Grubbs, R. H. *J. Am. Chem. Soc.* **1996**, *118*, 9606-9614.

[36] Ripka, A. S.; Bohacek, R. S.; Rich, D. H. *Bioorg. Med. Chem. Lett* **1998**, *8* , 357-360.

[37] Furstner, A.; Davies, P. W. *J. Chem. Soc., Chem. Commun.* **2005**, 2307-2320.

[38] Van de Weghe, P.; Eustache, J.; Cossy, J. *Curr. Top. Med. Chem.* **2005**, *5*, 1461-1472.

[39] IJsselstijn, M.; Kaiser, J.; van Delft, F. L.; Schoemaker, H. E.; Rutjes, F. P. J. T. *Amino Acids* **2003**, *24*, 263-266.

[40] Aguilera, B.; Wolf, L. B.; Nieczypor, P.; Rutjes, F. P. J. T.; Overkleeft, H. S.; van Hest, J. C. M.; Schoemaker, H. E.; Wang, B.; Mol, J. C.; Furstner, A.; Overhand, M.; van der Marel, G. A.; van Boom, J. H. *J. Org. Chem.* **2001**, *66*, 3584-3589.

[41] Ghalit, N.; Poot, A. J.; Furstner, A.; Rijkers, D. T. S.; Liskamp, R. M. J. *Org. Lett.* **2005**, *7*, 2961-2964.

[42] Ghalit, N.; Rijkers, D. T. S.; Kemmink, J.; Cees, V. B.; Liskamp, R. M. J. *J. Chem. Soc., Chem. Commun.* **2005**, 192-194.

[43] IJsselstijn, M.; Aguilera, B.; van der Marel, G. A.; van Boom, J. H.; van Delft, F. L.; Schoemaker, H. E.; Overkleeft, H. S.; Rutjes, F. P. J. T.; Overhand, M. *Tetrahedron Lett.* **2004**, *45*, 4379-4382.

[44] Williams, R. M.; Yuan, C. G. *J. Org. Chem.* **1992**, *57*, 6519-6527.

[45] Sutherland, A.; Vederas, J. C. *J. Chem. Soc., Chem. Commun.* **1999**, 1739-1740.

[46] Hiebl, J.; Blanka, M.; Guttman, A.; Kollmann, H.; Leitner, K.; Mayrhofer, G.; Rovenszky, F.; Winkler, K. *Tetrahedron* **1998**, *54*, 2059-2074.

[47] Hiebl, J.; Kollmann, H.; Rovenszky, F.; Winkler, K. *J. Org. Chem.* **1999**, *64*, 1947-1952.

[48] Hiebl, J.; Blanka, M.; Guttman, A.; Kollmann, H.; Leitner, K.; Mayrhofer, G.; Rovenszky, F.; Winkler, K. *Tetrahedron* **1998**, *54*, 2059-2074.

[49] Mazon, A.; Najera, C.; Ezquerra, J.; Pedregal, C. *Tetrahedron Lett.* **1995**, *36*, 7697-7700.

[50] Chen, Y.K; Lurain, A.E; Walsh, P.J. *J. Am. Chem. Soc.* **2002**, *124*, 12225-12231.

[51] Williams, R. M.; Liu, J. W. *J. Org. Chem.* **1998**, *63*, 2130-2132.

[52] Blackwell, H. E.; Grubbs, R. H. *Angew. Chem., Intl. Ed. Engl.* **1998**, *37*, 3281-3284.

[53] Glenn, M. P.; Pattenden, L. K.; Reid, R. C.; Tyssen, D. P.; Tyndall, J. D. A.; Birch, C. J.; Fairlie, D. P. *J. Med. Chem.* **2002**, *45*, 371-381.

[54] Fujimoto, K.; Oimoto, N.; Katsuno, K.; Inouye, M. *J. Chem. Soc., Chem. Commun.* **2004**, 1280-1281.

[55] Hirschmann, R.; Nicolaou, K. C.; Pietranico, S.; Salvino, J.; Leahy, E. M.; Sprengeler, P. A.; Furst, G.; Smith, A. B.; Strader, C. D.; Cascieri, M. A.; Candelore, M. R.; Donaldson, C.; Vale, W.; Maechler, L. *J. Am. Chem. Soc.* **1992**, *114*, 9217-9218.

[56] Chen, S.-T.; Chen, H.-J.; Yu, H.-M.; Wang, K.-T. *J Chem. Res. (S)* **1993**, 228-229.

[57] Osapay, G.; Taylor, J. W. *J. Am. Chem. Soc.* **1992**, *114*, 6966-6973.

[58] Jung, G. *Angew. Chem. Intl. Ed. Engl.* **1991**, *30*, 1051-1192

[59] Pag, U.; Sahl, H.-G. *Curr. Pharm. Des.* **2002**, *8*, 815-833.

[60] Harpp, D. N.; Gleason, J. G. *J. Org. Chem.* **1971**, *36*, 73.

[61] Fukase, K.; Kitazawa, M.; Sano, A.; Shimbo, K.; Horimoto, S.; Fujita, H.; Kubo, A.; Wakamiya, T.; Shiba, T. *Bull. Chem. Soc. Jpn.* **1992**, *65*, 2227-2240.

[62] Shao, H.; Wang, S. H. H.; Lee, C. W.; Osapay, G.; Goodman, M. *J. Org. Chem.* **1995**, *60*, 2956-2957.

[63] Mustapa, M. F. M.; Harris, R.; Bulic-Subanovic, N.; Elliott, S. L.; Bregant, S.; Groussier, M. F. A.; Mould, J.; Schultz, D.; Chubb, N. A. L.; Gaffney, P. R. J.; Driscoll, P. C.; Tabor, A. B. *J. Org. Chem.* **2003**, *68*, 8185-8192.

[64] Bregant, S.; Tabor, A. B. *J. Org. Chem.* **2005**, *70*, 2430-2438.

[65] Matteucci, M.; Bhalay, G.; Bradley, M. *Tetrahedron Lett.* **2004**, *45*, 1399-1401.

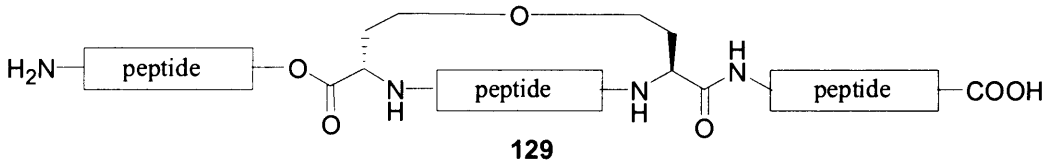
[66] Brunel, F. M.; Dawson, P. E. *J. Chem. Soc., Chem. Commun.* **2005**, 2552-2554.

[67] Johannesson, P.; Lindeberg, G.; Johansson, A.; Nikiforovich, G. V.; Gogoll, A.; Synnergren, B.; Le Greves, M.; Nyberg, F.; Karlen, A.; Hallberg, A. *J. Med. Chem.* **2002**, *45*, 1767-1777.

2.0 Results and Discussion

2.1 Introduction

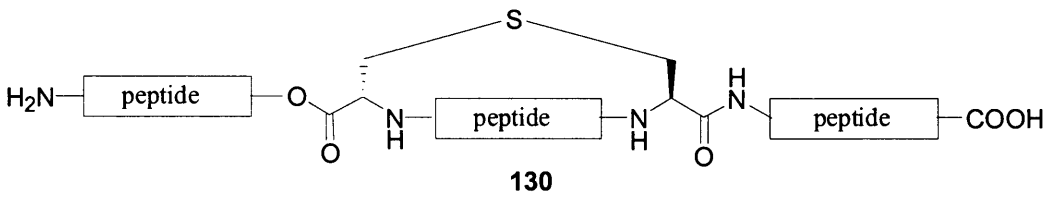
Computer modelling highlighted some small peptides that were envisaged to form stable β turns[1]. The modelled peptides were constrained by a variety of linkages between two side-chains of amino acids. The motif that appeared to be most promising in enforcing secondary structure was ether linkage **129** (Figure 19). It was decided to pursue a synthesis of these peptides to study the structures formed.



129

Figure 19: Ether side-chain bridged peptide 129

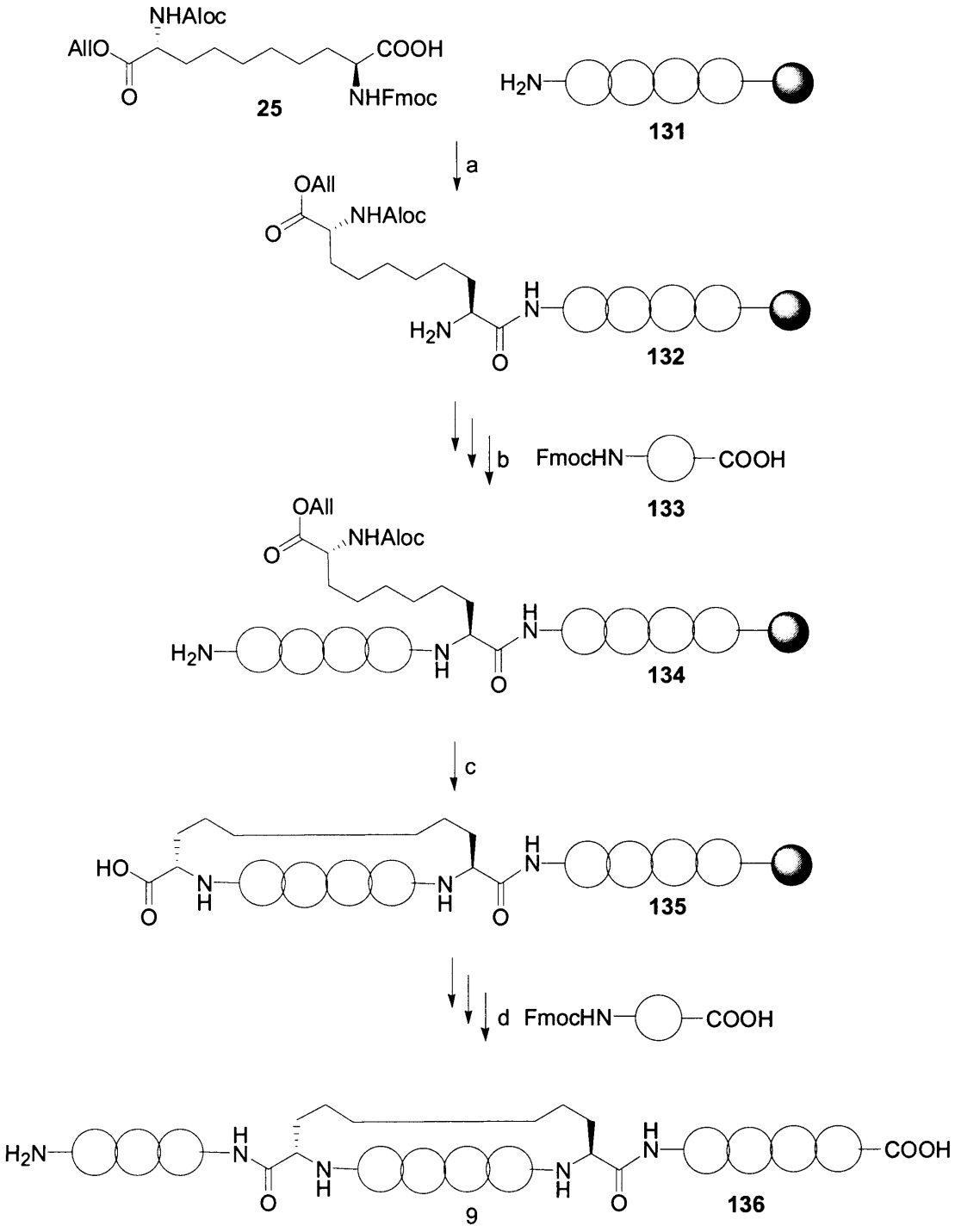
Synthesising peptides with unnatural side chains is a topic that has been well researched within the group[2,3,4]. Lanthionine bridged (**130**, Figure 20) and alkyl bridged (**9**) side chain linked peptides have been made using a proven methodology.



130

Figure 20: Lanthionine side chain bridged peptide 130

Initially a double headed amino acid **25** is synthesised with orthogonal protecting groups. This is then integrated into a peptide in the following way (Scheme 27).



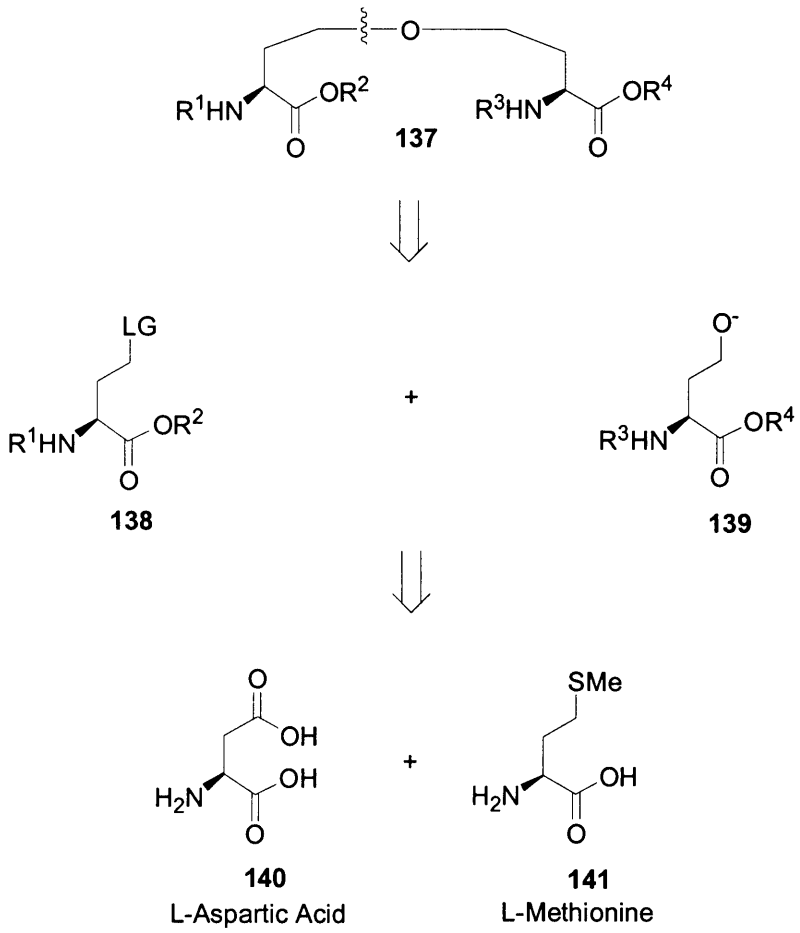
a) i) PyOAP, HOAt ii) Piperidine b) several rounds of synthesis c) i) $Pd(PPh_3)_4$ ii) PyOAP, HOAt d) i)several rounds of synthesis ii) TFA

○ = Amino Acid

Scheme 27: Synthesis of hexyl side-chain peptide 136

The C-terminus of the peptide is synthesised using standard Fmoc SPPS[5] up until the point where the first residue of the unnatural amino acid **25** is to be added. This is then coupled via the free carboxylic acid group to the amine of the resin-bound, protected peptide **131**. The Fmoc group is then deprotected and further amino acids are added via iterative SPPS until the second residue of the unnatural side chain is to be added to the linear peptide chain. The Aloc and Allyl groups are selectively deprotected with $Pd(PPh_3)_4$[6] and the peptide is cyclised on resin via coupling of the free acid with the amine terminus of the linear peptide. Amino acids are then added to the free carboxylic acid group of **134** until the peptide is complete. It is then cleaved from the resin to yield an unnatural side chain constrained peptide **136**.

The synthetic target for our synthesis was therefore a double headed amino acid (**137**) bearing a two carbon chain at either end bonded by an ether linkage (Scheme 28). It was also essential that the two amino acid groups were protected with orthogonal protecting groups. For peptide synthesis the Tabor group have found the combination of Fmoc and allyl groups ideal, as they can be deprotected with mild reagents that will not attack the peptide and can also be deprotected without removing the permanent acid labile side-chain protecting groups. Often these protecting groups are not compatible with the synthetic route to the final double headed amino acid. In these cases different orthogonal groups are used throughout the synthesis, these are then sequentially removed and replaced by the preferred Fmoc and allyl protecting groups before SPPS is commenced.



Scheme 28: Retrosyntheis of ether 137

The retrosynthetic step[7] that was favoured within **137** is at the oxygen carbon bond either side of the ether. Disconnection at this point yields an equivalent of nucleophilic alcohol **139** and an equivalent of electrophilic **138** containing a leaving group at the γ carbon. The synthetic step required to bring these two molecules together to form **137** would be a Williamson ether coupling reaction. To ensure the synthesis was scalable and repeatable **138** and **139** were further retrosynthesised to

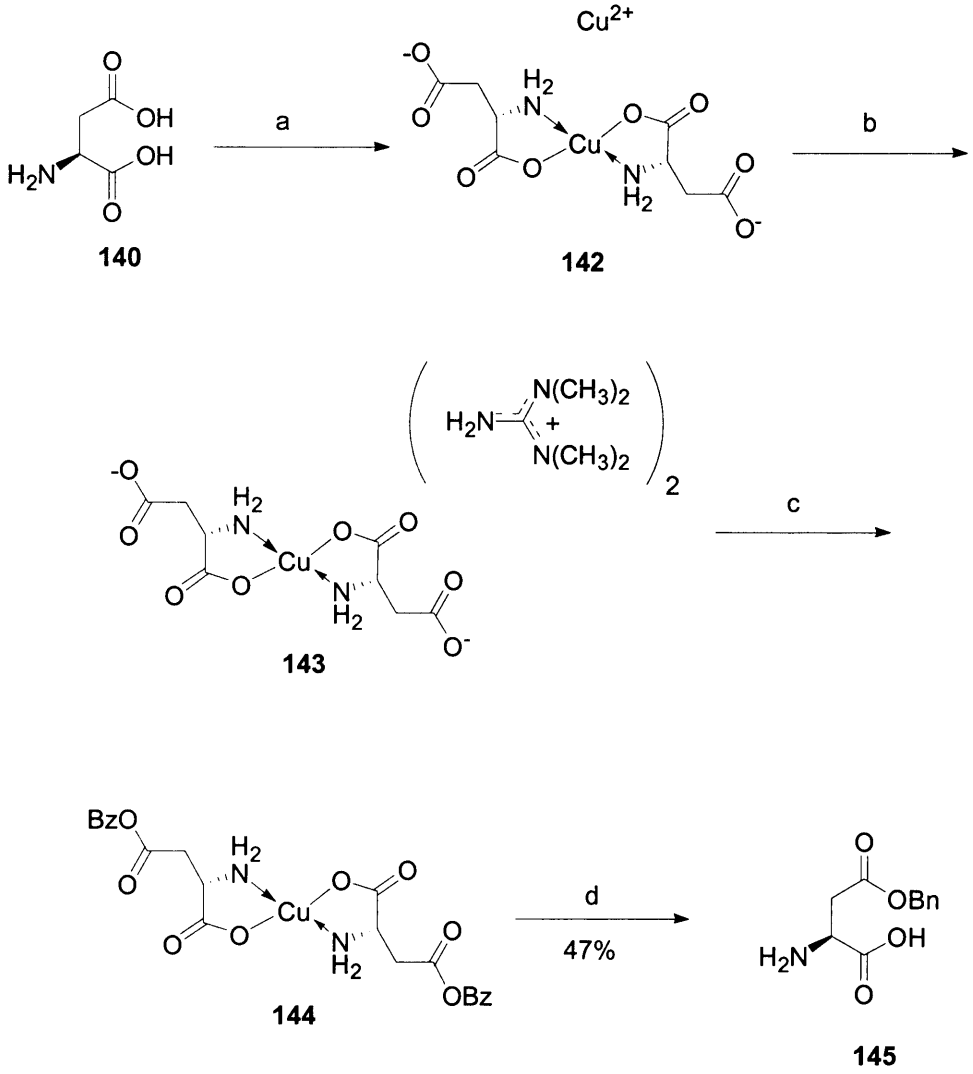
140 and **141** amino acids aspartic acid and methionine. Starting from the chiral pool of reagents eliminates the need for asymmetric creation of chiral centres, which can be both expensive and difficult to scale up successfully. It was important that the amino acids used contained the correct number of carbon atoms in the side chain. Making up for a deficit or excess would require extra steps and reactions that could damage the integrity of the chiral center. It was also imperative that the side chains contained functional groups that were synthetically malleable to allow for alcohol and leaving group formation.

2.2.1 Synthesis of the Nucleophile

It was anticipated that aspartic acid would yield the easiest route to the alcohol. It already contains an oxygenated functional group in the correct position. Reduction of protected aspartic acid would therefore yield **139**. The synthetic problem encountered with aspartic acid is that it contains two acid groups practically equivalent in reactivity. A review of the literature showed that it is possible to preferentially protect the β acid. This was not ideal as having the α acid preferentially protected it would be possible to reduce the other acid to the alcohol with treatment with *iso*-butyl chloroformate and $NaBH_4$. If both were protected it seemingly adds an extra step as the β acid would have to be deprotected. The lack of methods preferentially protecting at the α acid meant methods had to be found that would give orthogonal groups at each ester.

A widely used method for aspartic acid differentiation is complexation of the α acid and amine to a metal. The β acid can then be protected and the metal decomplexed to yield the protected amino acid. The most common elements used to perform this are copper and boron.

Boroxazolidones[8,9] are synthesised by reaction of the amino acid with alkyl boranes such as BPh_3[8] and BBu_3[9]. These species are then decomplexed with strong acid. A milder method uses copper chelation, this reaction has been more comprehensively studied[10,11,12]. Using this chemistry it was hypothesised that chelation of the α amino acid to the copper would allow for protection of the β acid with benzyl bromide. The presence of a benzyl ester deprotected by hydrogenation would allow for acid or base labile groups to be present at the α acid which would not be affected by the removal conditions to yield a β acid.

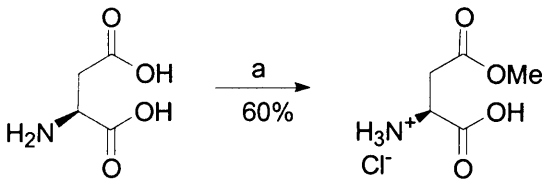


a) $CuCO_3$, H_2O, 70°C to rt, 24 h b) TMG, DMF, H_2O, rt, 3 h c) benzyl bromide, DMF, H_2O, rt, 20 h d) EDTA, $NaHCO_3$, H_2O, rt, 3 h

Scheme 29: Synthesis of β benzyl ester aspartic acid 145

After dissolving $CuCO_3$ in water at 70°C (Scheme 29), aspartic acid **140** was added and the solution was stirred for two days. The isolated bright blue solid **142** was then dried. The counterion was changed from Cu (II) to two equivalents of TMG. The literature encouraged this step as **143** was found to give far better yields in the following benzyl alkylation reaction than **142**. Addition of benzyl bromide to **143**

yielded **144** as a light blue powder after isolation, which was again dried. Decomplexation of the copper from the aspartic acid benzyl ester was achieved using EDTA. Isolation of **145** occurred by rapid crystallisation from a cold acetone:H_2O solution. This synthesis yielded **145** in 47% yield over 3 steps and the material produced was not in need of further purification. Taking into account the cheap and readily available starting materials 47% was an acceptable yield for the first step of a synthesis. The length of the pathway to a simple material was long and took some time to perfect and perform. Upon searching for new routes to differentiate the acids a synthesis involving just one step to pure material was discovered.



140 **146**

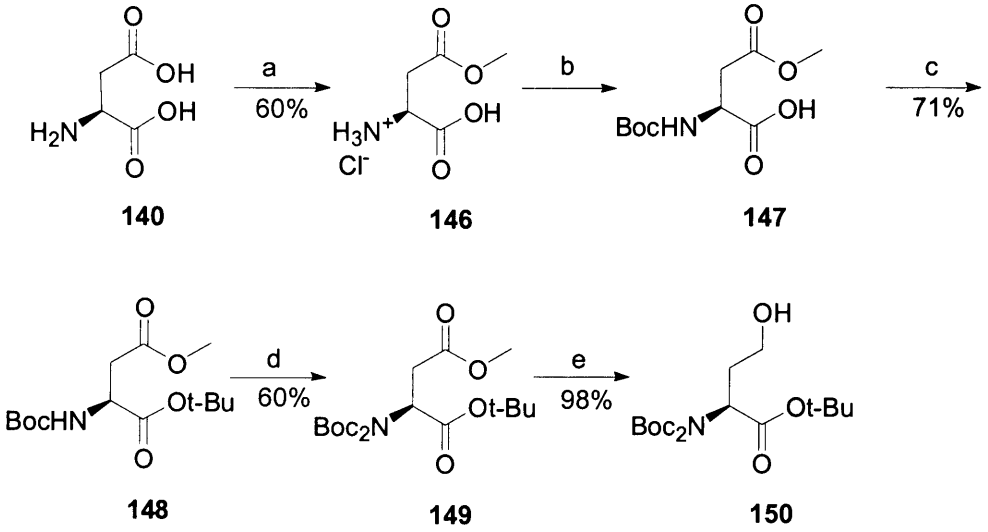
a) $SOCl_2$, MeOH, -10°C to rt, 20 min

Scheme 30: Synthesis of β methyl ester aspartic acid 146

Following well precedented procedures methyl esterification of the β acid was achieved by treatment of a solution of aspartic acid in methanol with thionyl chloride at –10°C in 60% yield[13] (Scheme 30). After warming to room temperature and adding cold ether, a solid was formed. This material was isolated by filtration and after two crystallizations was pure by melting point and NMR. This method although not high yielding uses cheap reagents and produces **146** pure within two hours. After

performing this reaction several times on a large 30 g scale it was found to be both scalable and repeatable.

We then sought a pathway that made use of this step as a means of differentiation to yield protected homoserine derivatives. A five step scheme to **150** (Scheme 31) was identified[14] and work commenced to test its feasibility.



a) $SOCl_2$, MeOH, -10°C to rt, 20 min b) $(Boc)_2O$, Na_2CO_3, THF/H_2O (2:1), rt, 48 h c) ^tBuOH, DCC, DMAP, CH_2Cl_2, 0°Cto rt, 48 h d) $(Boc)_2O$, DMAP, MeCN, rt, 24 h e) DIBAL, THF, -45°C, 1 h.

Scheme 31: Synthesis of protected homoserine 150

After selective β-esterification, protection of the amine with Boc anhydride and subsequent esterification of the free carboxyl group with ^{t}butanol, DCC and DMAP yielded protected aspartic acid derivative **148**. The amine was then protected with a further Boc group. This was initially performed by refluxing **148** in THF for two hours with sodium hydride and Boc anhydride. These conditions are very harsh and it

was thought that they may cause racemisation of the amino acid so a milder method was found. Using 4 equivalents of Boc anhydride with catalytic DMAP in acetonitrile at room temperature it was possible to add the second Boc group in 24 hours[15]. These conditions were slower and less atom efficient but did not risk racemisation of the amino acid. Following the literature, reduction of **149** with DIBAL in Et_2O gave two major products, the aldehyde and the desired product, alcohol **150**. Optimisations of the reaction involved changing the temperature, time, solvent, dilution and rate of addition of the DIBAL. Yields of 98% were realised using dilute THF solutions with a very slow dropwise addition at -45°C[16]. If the reaction was not very carefully judged, increasing amounts of aldehyde and **150** minus a Boc group were formed. Recently purchased DIBAL was also found to be important to ensure the fidelity of the protecting groups.

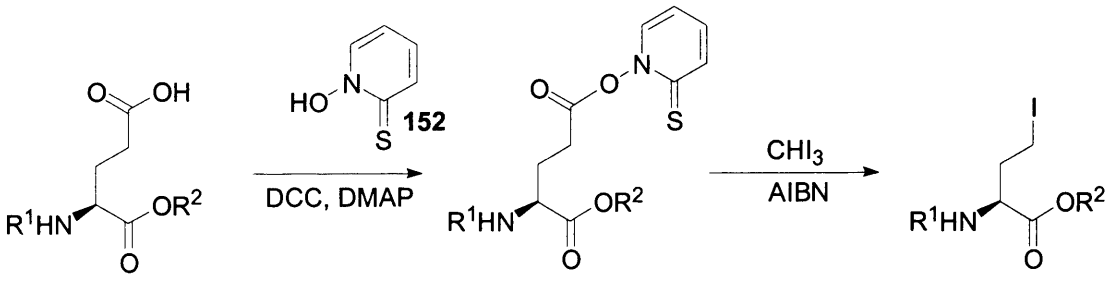
The importance of the di-Boc amine protecting group was studied. Reactions of **148** with DIBAL yielded a very dirty reaction. No purification was attempted as the TLC analysis performed showed the presence of over eight separate compounds with no clear major spot. It appears that an acidic proton in the molecule does not allow for a clean reduction with DIBAL. The presence of the two Boc protecting groups does not complicate further steps as they can removed together with standard deprotection conditions[17]. It is thought that the selectivity observed in this reduction is primarily due to steric interactions between the *iso*-butyl groups of DIBAL and the ^{t}butyl group of the α-carboxyl ester, although the susceptibility of methyl esters to DIBAL

reduction over larger esters may also have helped in the discrimination of the two esters.

With a successful synthesis of nucleophile **150** completed attention was turned to making electrophile **138**.

2.3.1 Electrophile Synthesis I – Tosyl Homoserine

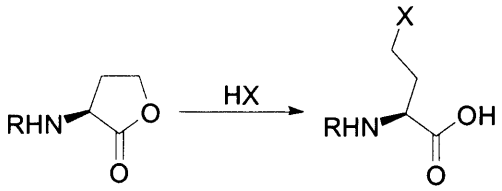
Published syntheses of amino acid molecules containing a leaving group at the γ position have often used radical decarboxylative halogenation[18,19,20,21].



Scheme 32: Synthesis of iodide 154 by decarboxylative halogenation

Protected glutamic acid **151** is coupled to hydroxylamine **152** using DCC (Scheme 32). The intermediate Barton ester **153** is then subjected to either reflux temperatures, light or a chemical radical initiator such as AIBN to encourage homolytic bond cleavage. This is followed by decarboxylation to give a primary carbon based radical which is quenched with CHI_3 to give the primary iodide **154**. To synthesise starting

glutamic acid **151** requires acid differentiation and a multi step synthesis. The yields reported within the literature for the final step are varied and generally below 50%[20]. We decided early on to eliminate this route due to these low yields. Also there were concerns that the harshness of conditions might affect the integrity of the chiral centre and the claim that the product was very difficult to purify[22].

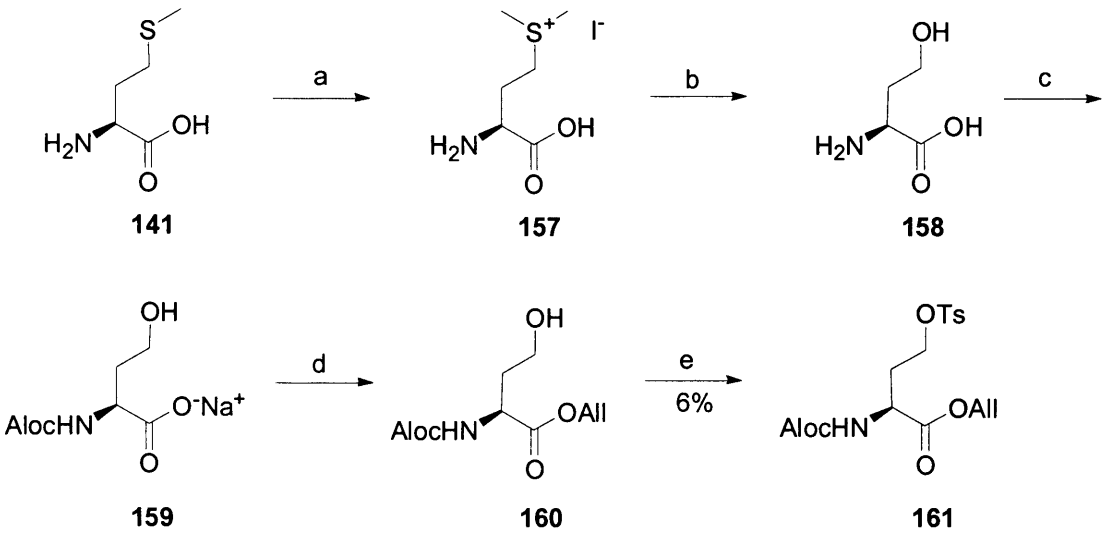


155 **156**

Scheme 33: Synthesis of iodide 156 by homoserine lactone ring opening

Another reaction used for synthesising derivatives such as **138** involves regioselective ring opening of homoserine lactone **155** with either HCl[23], HBr[24] or HI[25] (Scheme 33). The reaction using HI has been shown to racemize the chiral centre during the idodination but HCl and HBr are reported to give enantiopure products. The reaction was not attempted because of reasons such as the difficulty in making homoserine lactone[26], possible racemization during the ring opening reaction due to the harsh conditions and the requirement of amine protecting groups that are resilient to <pH 1.

A synthesis that appeared to suit requirements was found which over 5 steps produced tosylated homoserine derivative **161** (Scheme 34)[27]. The only change to the published route was the protecting groups used. The paper had used a Boc and benzyl ester for the amine and acid protection respectively whereas we chose to use aloc and an allyl ester.



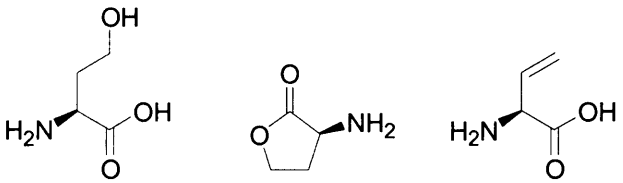
a) MeI, H_2O/MeOH (10:1), rt, 24 h b) K_2CO_3, H_2O, reflux, 4 h c) allyl chloroformate, Na_2CO_3, dioxane:H_2O (1:1), rt, 24 h d) allyl bromide, DMF, rt, 48 h e) p-TsCl, Et_3N, CH_2Cl_2, rt, 3 h

Scheme 34: Synthesis of protected tosyl homoserine 161

L-Methionine was reacted with methyl iodide to form sulfonium salt **157**. Subsequent nucleophilic displacement of the sulfine by a hydroxide ion, by refluxing **157** in water with potassium carbonate, yielded unprotected homoserine **158**, liberating dimethyl sulfide. By TLC this step produced three separate products all approximately equal in intensity. Due to **158** being extremely polar no chromagraphic separation was attempted. Protection of the amine with allyl chloroformate and reaction of the subsequent sodium salt of the acid with allyl bromide gave **160**. Alcohol **160** was then protected and activated with tosyl chloride to give **161** before purification was attempted. After work up the isolated brown oil consisted of over 20 spots by TLC.

After two very slow gradient elutions of flash column chromatography **161** was obtained pure as a red brown oil in only 6% yield over 5 steps.

The synthesis was repeated but there was no improvement of the yield. It was hypothesised that the low yield may be due to the early step where three spots of equal intensity are observed by TLC. Unprotected homoserine is known to lactonise[28,29] and common side reactions of S_N2 displacements are eliminations so it is possible that a combination of **158**, **162** and **163** were produced, though no spectral evidence was obtained.



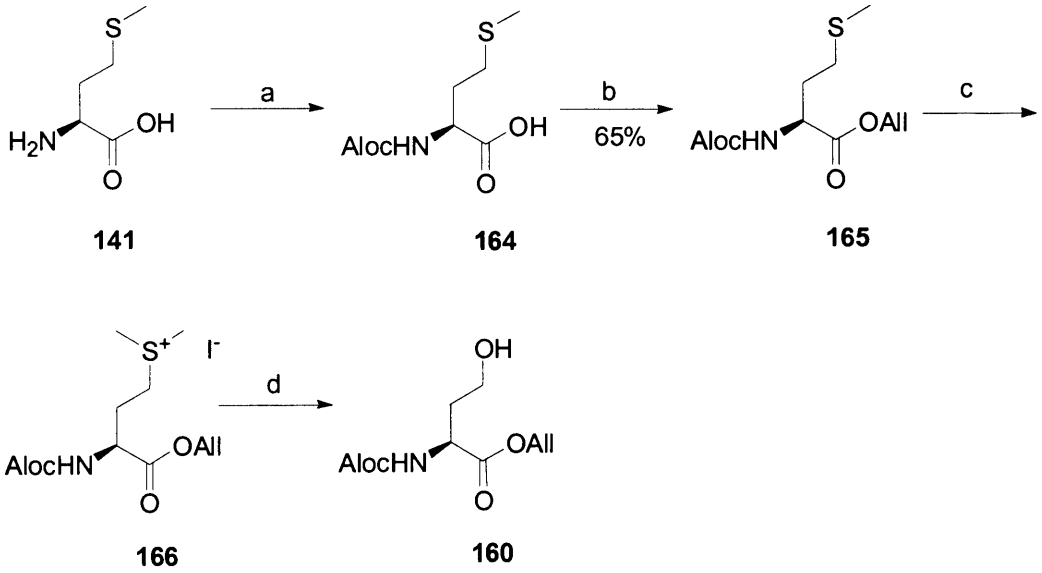
158 **162** **163**

Figure 21: Possible products from sulfonium salt displacement reaction, homoserine 158, homoserine lactone 162 and vinyl glycine 163

With three separate products created during the conversion of **157** to homoserine a low yield was experienced from an early stage in the route. The molecule was also able to lactonise throughout the synthesis and this too would contribute to the low yields observed. Another drawback of this synthesis is the lack of purification after each step. Due to the polar nature and the constant lactonisation it was felt that purification would only reduce the yields further. This though produces a very

difficult and slow chromatographic isolation of **161**. The low yields and the problems associated with the synthesis drove us to search for a new route to electrophile **138**.

To circumvent the alcohol group being free for long amounts of time a pathway was attempted repeating the synthesis but changing the order of the steps. By first protecting methionine we envisaged that we could then perform the displacement as the last step and minimise the amount of lactonisation (Scheme 35).



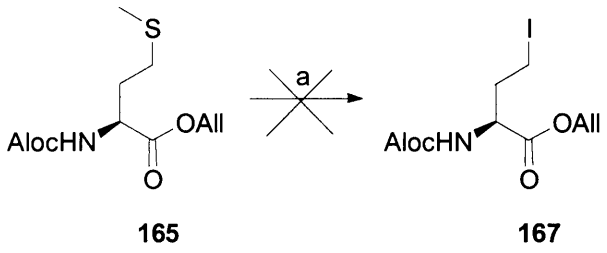
a) Allyl chloroformate, Na_2CO_3, THF:H_2O (1:1), rt, 24 h b) i) K_2CO_3, MeOH ii) allyl bromide, DMF, rt, 48 h c) MeI, H_2O:MeOH (10:1) d) K_2CO_3, H_2O, reflux, 4 h

Scheme 35: Synthesis towards protected homoserine 160

Protection of methionine as its allyl carbamate and allyl ester was performed as before to give **165**. After sulfonium salt formation it was subjected to the same

conditions as in Scheme 6. The TLC after two hours showed the products to be very polar. Increasing the polarity of the TLC solvent system with AcOH showed a complex mixture of over six spots. It was reasoned that among other reactions saponification of the allyl ester had occurred. The reaction was then performed at a lower temperature and over extended times but there appeared to be very little reaction. It seems likely that the reaction requires reflux temperatures to proceed in the allyl ester and sulfine displacement. We therefore attempted new pathways.

2.3.2 Electrophile Synthesis II - Halide Displacement of Sulfonium Salts



165 **167**

a) MeI, NaI, $(CH_3)_2CO$

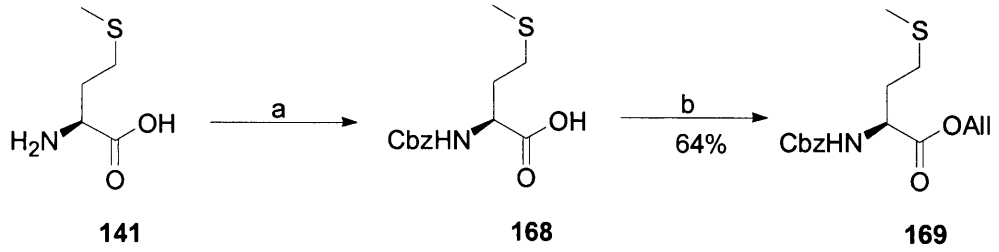
Scheme 36: Synthesis towards protected iodide 167

As discussed, the formation of sulfonium salts from sulfides creates good leaving groups for nucleophilic attack. A literature search revealed that these groups could also be displaced by halide ions[30,31]. This reaction was appealing as it would give a direct route to **167** from **165** and there would be no risk of saponification (Scheme 36). Sulfonium salt was prepared *in situ* and reacted with sodium iodide in acetone. The reaction showed only starting material after 24 hours at room temperature. It was

then refluxed for a further two hours and after this time TLC, using three different solvent systems to ensure they were the same spot, showed only starting material **165**.

The reaction was performed over a range of temperatures and concentrations but results showed no indication of the iodide **167**. A one pot sealed tube reaction, where the nucleophile was added with the alkyl iodide and protected methionine, also yielded no desired products.

Most of the literature reactions being followed had used phenyl or benzyl sulfides as the starting material[32,33,34]. This was then reacted to form the sulfonium salt with methyl iodide or methyl bromide. From the lack of positive results presented here it can be concluded that the presence of the aryl group creates a more susceptible centre for halogen attack. Attempts were then made to get around this problem by increasing the temperature of the reactions. Using solvents with high boiling points it was hoped that the activation point of the reaction could be reached using just alkyl substituents. The solvents chosen were toluene and diglyme which boil at 111°C and 160°C respectively. It appeared at the high temperatures that the allyl carbamate protecting group was unstable.

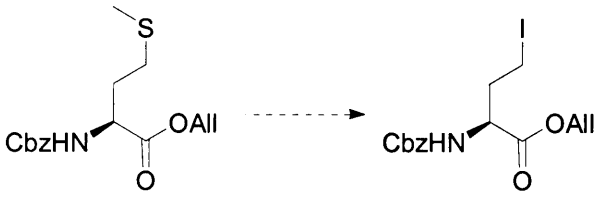


141 168 169

a) Benzyl chloroformate, THF:H_2O (1:1), Na_2CO_3, 0°C to rt, 24 h b) i) K_2CO_3, MeOH ii) allyl bromide, DMF, rt, 24 h

Scheme 37: Synthesis of protected methionine 169

169 was therefore synthesised containing a benzyl carbamate (Scheme 37). No problems were found with this molecule when it was heated to reflux in diglyme. Unfortunately no positive results were obtained using sodium iodide or lithium bromide with preformed sulfonium salt or with *in situ* addition of methyl iodide.



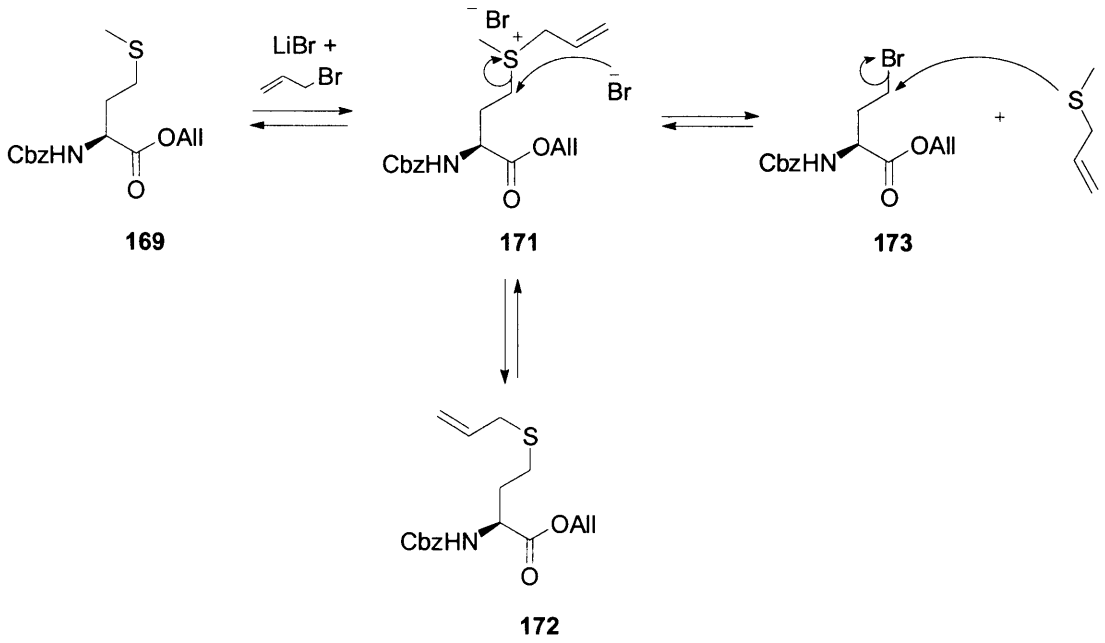
169 **170**

Scheme 38: Synthesis of towards protected iodide 170

The decision was then made to change the type of alkyl halide added. By changing the source from methyl iodide to the more electron rich allyl bromide and benzyl bromide it was anticipated that there would be an increase in the reactivity of the sulfine as a leaving group. Conditions were varied but there appeared to be no effect using toluene. However bromide **173** was observed by electrospray mass

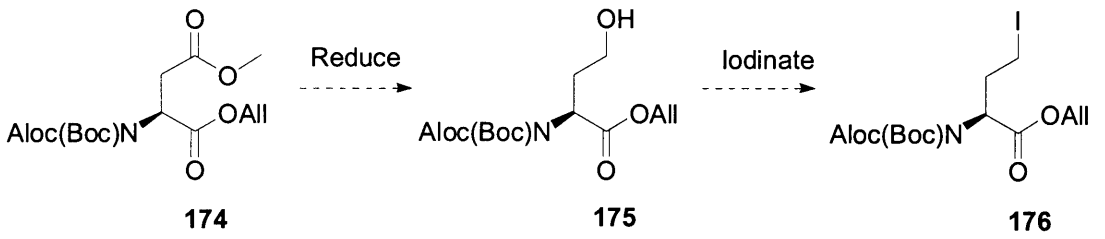
spectrometry when diglyme was used with allyl bromide (Scheme 39). Unfortunately after removal of diglyme *in vacuo* only starting sulfide **169** and **172** were observed.

Scheme 39 shows the proposed mechanism for the synthesis of **172**. Formation of bromide **173** occurs with concurrent formation of high boiling allyl methyl sulfide (b.p. 91-93°C). The high temperatures required to remove diglyme *in vacuo* would also encourage nucleophilic attack of the bromide by the unsymmetrical sulfide. Once sulfonium salt **171** has been reformed either the methyl or the allyl group can be lost to reform the sulfide with loss of the respective alkyl bromide. The higher boiling temperatures of the electron rich alkyl halides which initially aids the formation of bromide **173** finally inhibits its isolation. Other techniques of removing diglyme such as ice-ether extractions were not attempted as the high boiling sulfide would remain in the same layer as the iodo alanine and the same result would be reached. Research of this route ceased at this point as a more promising synthesis of the target bridged ether amino acid was found.



Scheme 39: Synthesis of bromide 173 and mechanism of formation of 172

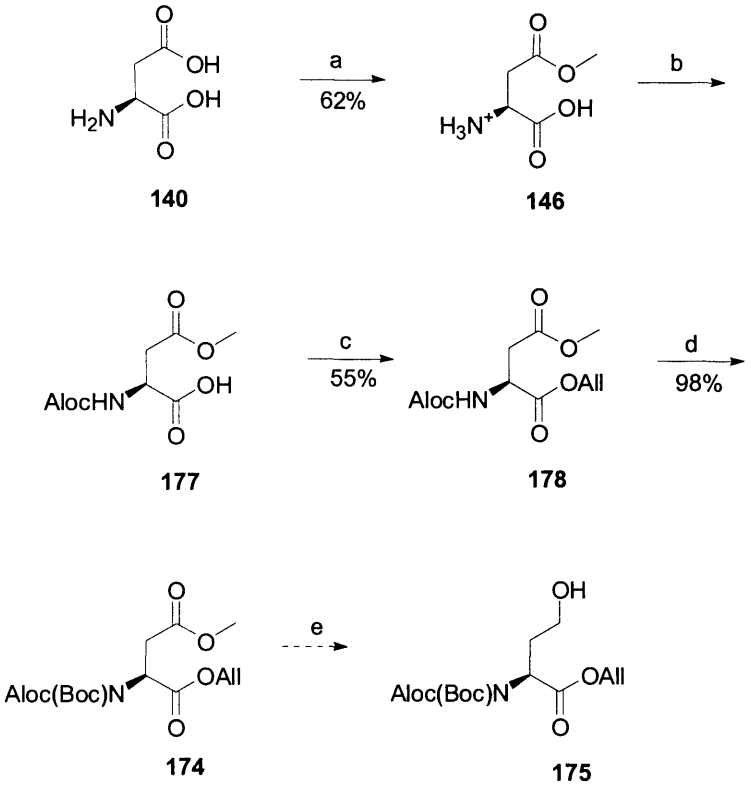
2.3.3 Electrophile Synthesis III - Reduction of Aspartic Acid



Scheme 40: Planned synthesis of iodide 176

The methodology used to create nucleophile **150** had worked well. Revision of this synthesis was planned but this time using orthogonal groups (Scheme 40), replacing

the Boc and ^{t}butyl groups with Aloc and Allyl to give **174**. The molecule would still require a second carbamate to stop the secondary amine interfering with the reduction. The Boc group was chosen again as there was methodology to put it on and literature evidence suggested it could be removed without removal of the Aloc group[17]. It was anticipated that the presence of two reasonably bulky carbamates and an allyl ester would create a crowded α center therefore hindering reduction. The use of the bulky reducing agent would also encourage site specific reduction of the sterically small β methyl ester. To encourage selectivity in the reduction the temperature was lowered to further emphasise the steric difference. The free alcohol produced from this route could then be turned into an electrophile for ether synthesis by mesylation and subsequent iodination.



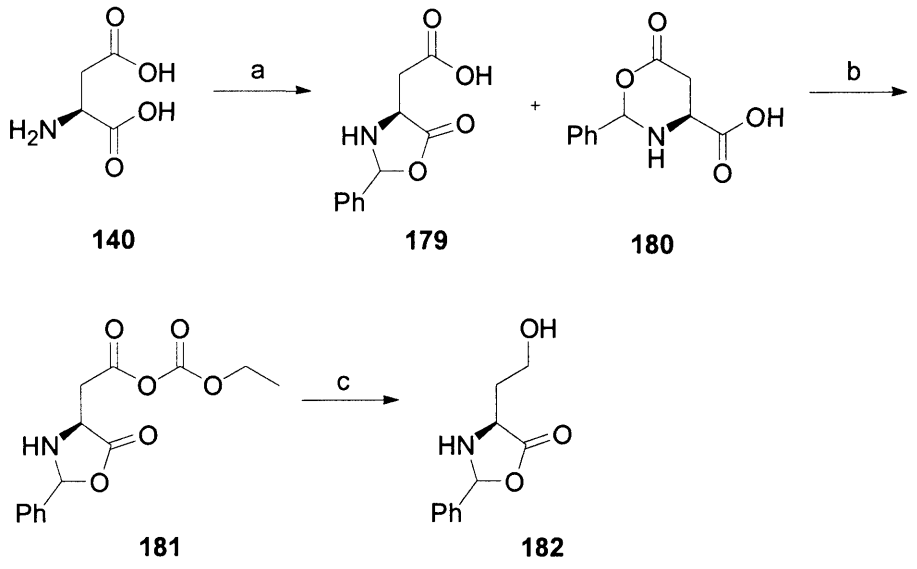
a) $SOCl_2$, MeOH, -10°C to rt b) allyl chloroformate, Na_2CO_3, THF:H_2O (1:1), rt, 24 h c) i) $NaHCO_3$, MeOH ii) allyl bromide, DMF, rt, 24 h d) $(Boc)_2O$, DMAP, MeCN, rt, 24 h e) DIBAL, CH_2Cl_2, -78°C, 1 h

Scheme 41: Synthesis of protected homoserine 175

Aspartic acid was again reacted in methanol with thionyl chloride to yield **146** (Scheme 41). Protection of the amine was achieved with allyl chloroformate and Na_2CO_3 in THF and H_2O. The sodium carboxylate salt was then protected with allyl bromide in DMF to give **178**. The Boc group was then added as the second carbamate using the same conditions as before. Reaction of **174** with DIBAL yielded a large mixture of products by TLC when first attempted in CH_2Cl_2 at –78°C. After some trial reactions optimum dilutions and addition rates were achieved. The resultant TLC showed five products. Isolation of the major three spots by flash chromatography

followed by TLC showed that one spot rapidly lost purity and became two spots after the column. This occurred too quickly to obtain a good NMR. The other spots isolated were a mixture of deprotected and direduction products. The main spot was hypothesised to be alcohol **175** but was rapidly lactonising. The yield after chromatography was very poor and therefore trapping of the alcohol as the tosylate or mesylate would not have produced an appreciable amount of material.

Work performed in parallel to this also indicated that the presence of the free γ alcohol resulted in lactonisation.

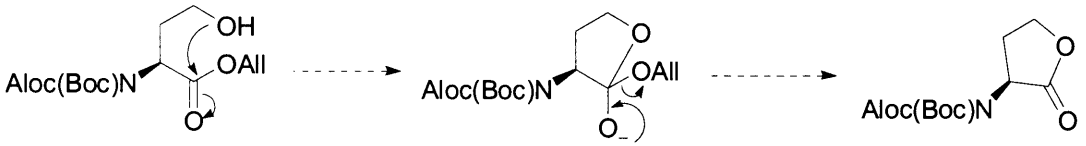


a) Benzaldehyde dimethyl acetal, pTsOH, 1,1,1-trichloroethane, reflux, 18 h b) ethyl chloroformate, Et_3N, THF, -5°C, 1 h c) $NaBH_4$, EtOH, rt, 4 h

Scheme 42: Synthesis of oxazolidine protected homoserine 182

Reaction of aspartic acid with benzaldehyde under Dean-Stark conditions (Scheme 42) gave a mixture of two products believed to be **179** and β acid regioisomer **180**[35].

This protecting group was chosen as it can be deprotected with base and is therefore orthogonal to those used on the potential coupling partner **150**. The two molecules were isolated by acidic work up. Their similar R_f values and high polarity led to the delaying of purification until after the reduction to the less polar **182**. The mixture was therefore subjected to mixed anhydride formation with ethyl chloroformate followed by sodium borohydride reduction[36]. Alcohol **182** was never isolated as the molecules produced were not stable and decomposed on work up, on standing or during attempts at flash column chromatography.



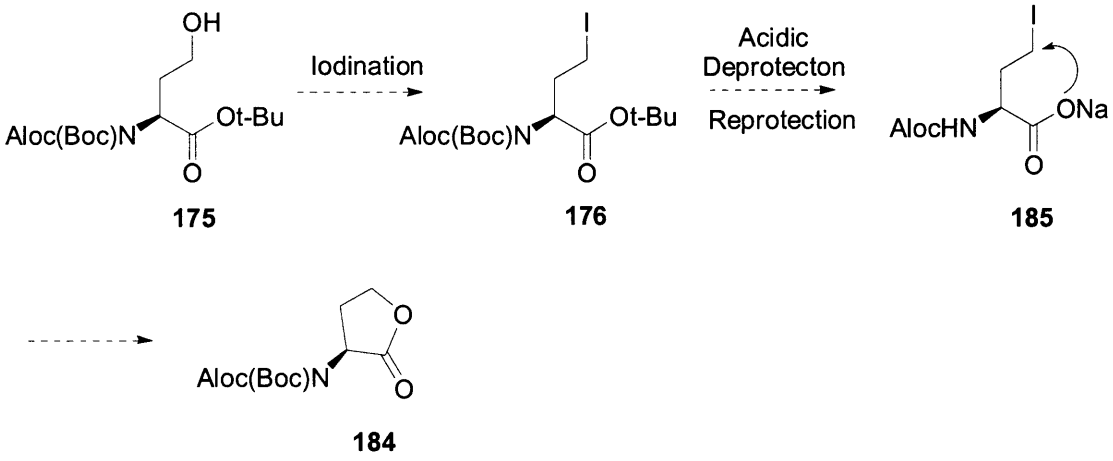
175 **183** **184**

Scheme 43: Synthesis of homoserine lactone 184 from homoserine 175

From these experiments and literature precedent there was no doubt that if the γ alcohol was free and unless the α carboxylate group was protected as a ^{t}butyl ester lactonisation would occur (Scheme 43)[37]. These groups are well known to be resilient to attack by nucleophiles.

The planned synthesis requires that there must be orthogonal protecting groups present in **138** therefore this protecting group could not be used again on both the nucleophile and electrophile. If the ^{t}butyl ester were used and then the γ position converted to an electrophile such as an iodide, subsequent attempted protecting group

exchange would surely result in failure as the molecule would lactonise with the acid acting as nucleophile (Scheme 44).

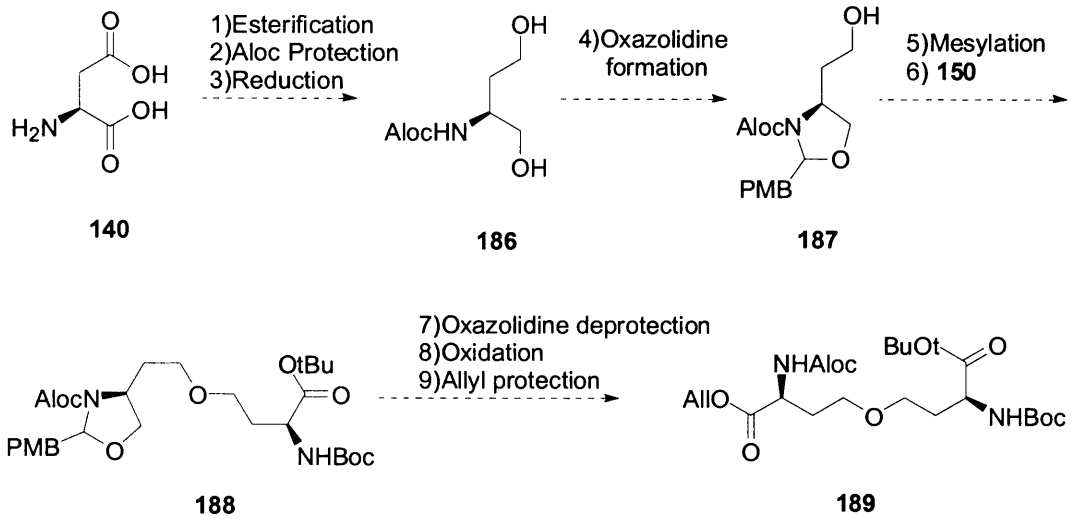


175 **176** **185** **184**

Scheme 44: Synthesis of homoserine lactone 184 by carboxylic acid attack of terminal iodide 185

The planned synthesis of the electrophile then had to be changed to ensure that there was no way in which lactonisation could occur.

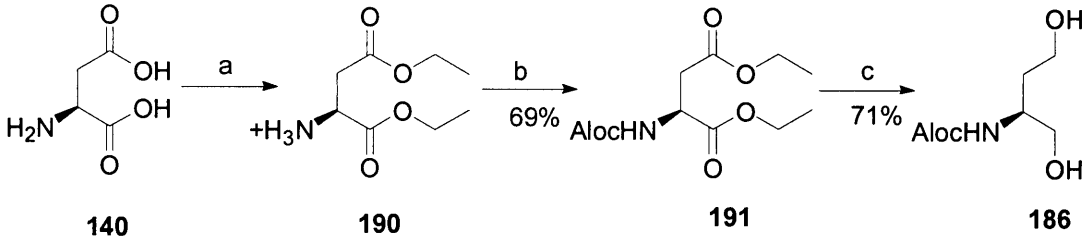
2.3.4 Electrophile Synthesis IV - Reduction and Oxidation of Aspartic Acid



Scheme 45: Planned synthesis of protected ether bridged bis-amino acid 189

The reactions outlined in Scheme 45 show one way of eliminating the problem of lactonisation. Reducing both the acid groups of a protected aspartic acid species gives diol **186** where there is no electrophilic carbonyl onto which the γ alcohol can lactonise. The alcohols can be differentiated by oxazolidine formation via reaction utilising Dean-Stark apparatus with an aldehyde. Using anisaldehyde would give a wide variety of options for steps later in the synthesis as there are many deprotection procedures for this group[17]. Electrophilic derivativization of the free alcohol as the mesylate would produce an adequate partner for Williamson ether coupling with **150**. Oxazolidine deprotection would then lead to possibly the most problematic step of the scheme, oxidation of the alcohol back up to the acid. This would have to be performed in the presence of many sensitive protective groups but it was anticipated

that using methods such as H_5IO_6/CrO_3[38], TPAP/NMO[39] or $Ca(OCl)_2/HOAc$[40] we could effect this transformation.



140 190 191 186

a) $SOCl_2$, EtOH, 0°C to rt, 3 h b) allyl chloroformate, Na_2CO_3, MeCN:H_2O (1:2), rt, 24 h c) $NaBH_4$, LiBr, diglyme, rt, 4 h

Scheme 46: Synthesis of Aloc protected diol 186

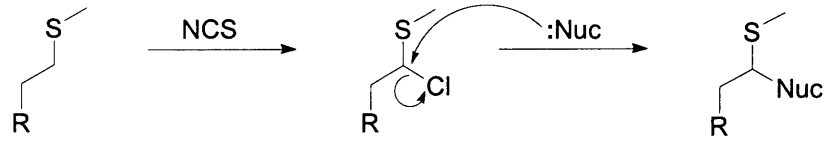
Diesterification of aspartic acid **140** (Scheme 46) with thionyl chloride in ethanol gives **190**. The reaction in methanol to give the dimethyl ester of aspartic acid was also performed. However this was a very low yielding reaction as there was a strong preference for the mono ester. Pushing the reaction towards completion required excessive amounts of thionyl chloride and long reaction times. It was therefore decided that the synthesis should be performed using the ethyl ester. Protection of the amine as the allyl carbamate was achieved to give **191** in good yield over two steps. Literature methods for the reduction of this molecule used sodium borohydride in ethanol[41,42]. Following this precedent attempts were made to reduce the ester. After many reactions to optimise the very poor yields experienced the conditions were changed. Using the powerful reductant $LiBH_4$ formed *in situ* by premixing $NaBH_4$ and LiBr in diglyme[43], the diol **186** was produced in 3.5 hours giving a good yield after flash column chromatography. Unfortunately all attempts at oxazolidine formation of **186** with anisaldehyde failed, as it was highly insoluble in non protic

solvents and therefore all Dean-Stark reactions performed using toluene, benzene and 1,1,1-trichloroethane failed. Changing the dilution and adding excess reagent made no difference to the reaction.

At this point continuation of this scheme was stopped. Even with further attempts at performing the Dean-Stark reaction it was unlikely that a high yielding reaction would occur due to the poor solubility of **186**. Encountering such problems early on in the synthesis with more uncertain steps to follow, such as the oxidation, a new route to the electrophile was found.

2.3.5 Electrophile Synthesis V - Hemithioacetal Formation of Methionine

Literature precedent showed that thioethers could be reacted with NCS to form α-chloro sulfides[44] **193** (Scheme 47).

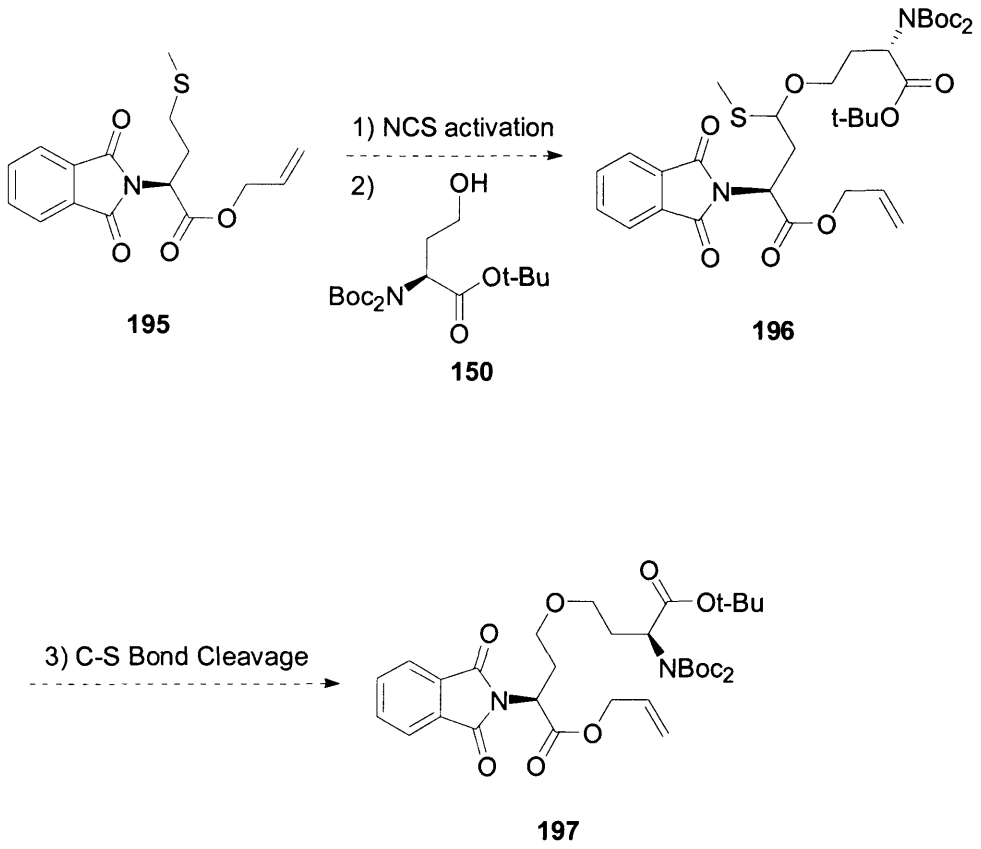


192 **193** **194**

Scheme 47: α-Chlorination of a sulfide and displacement by a nucleophile

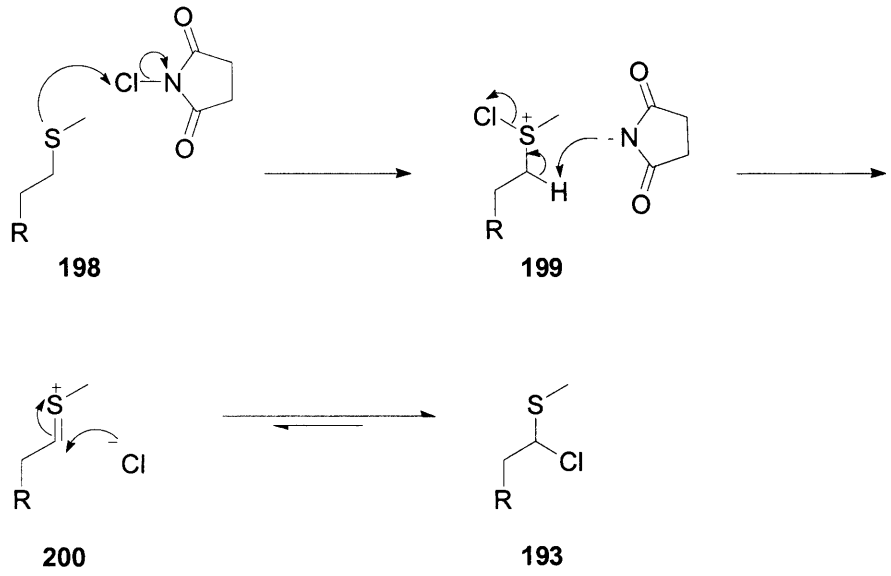
These highly reactive species provided good coupling partners for alcohol attack and hemithioacetal formation[45]. Using this chemistry it was envisaged that a protected

methionine species could be made to react with alcohol **150** and form hemithioacetal **196** (Scheme 48). Cleavage of the carbon sulfur bond would yield target ether **197**.



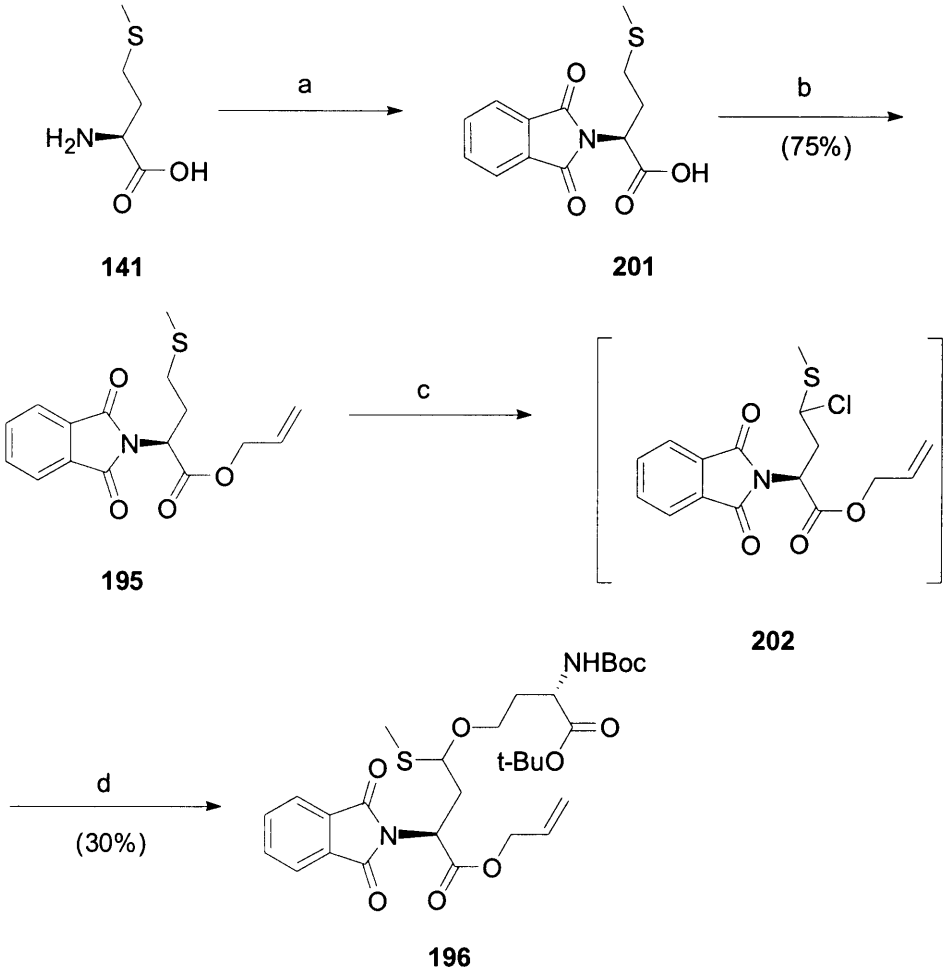
Scheme 48: Planned synthesis of protected ether bridged bis-amino acid 197

The reaction of sulfides with NCS[46] to form α-chlorosulfides is richly precedented in the literature[44,47,48]. The reaction has been shown to work in natural product synthesis and also in amino acid chemistry. It can be carried out with a range of reagents such as sulfuryl chloride[49], thionyl chloride[49], trichloroisocyanuric acid (Chloreal)[50], iodobenzene[51] dichloride and benzenesulfenyl chloride[52], but NCS is by far the mildest.



Scheme 49: Mechanism of α-chlorination reaction with sulfides

Scheme 49 shows the mechanism of the reaction. Chlorination of the sulfur to form a sulfonium salt (**199**) occurs by nucleophilic attack of the chlorine atom by the sulfur lone pair. The succinimide counter ion then deprotonates α to the sulfide which results in loss of a chloride ion. The positively charged sulfide is then neutralized by attack of the chloride ion α to the sulfide (**200**). The product **193** is generally considered to be unstable and often used straight away without purification. By using NCS and carbon tetrachloride as solvent crude purification can be achieved as succinimide is not soluble and can be filtered off. α-Chlorosulfides are potent electrophiles and react with a range of nucleophilic reagents such as sulfides, alcohols, Grignard reagents and silyl enol ethers[44].



141 201 195 202 196

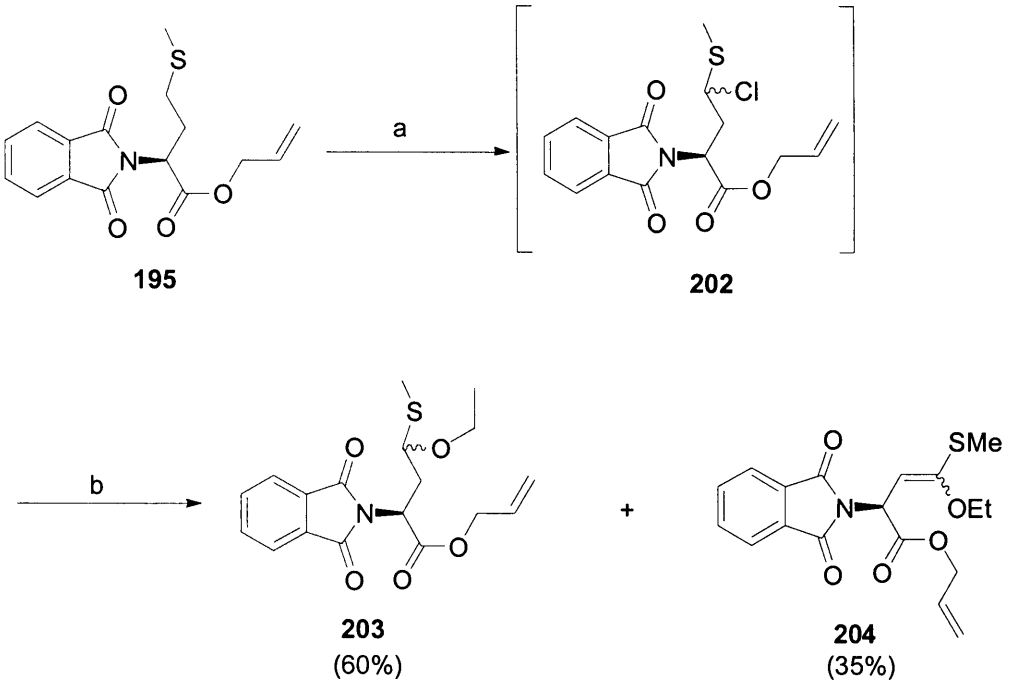
a) Phthalic Anhydride, DMF, microwave, 4 mins b) i) $NaHCO_3$, MeOH ii) allyl bromide, DMF, 48 h c) NCS, CCl_4, rt, 2 h d) **150**, AgOTf, DMAP, CH_2Cl_2, -40°C to -25°C

Scheme 50: Synthesis of protected hemithio acetal bridged bis-amino acid 196

Scheme 50 shows the synthesis of hemithioacetal **196**. Protection of methionine as the phthalimide using microwave irradiation in DMF followed by allyl ester formation yielded **195**. α-Chlorosulfide formation was then performed by the action of NCS in carbon tetrachloride. After 30 minutes the solution was filtered and the solvent removed. The residual oil was taken up in CH_2Cl_2 and dripped slowly into a solution of alcohol **150**, silver triflate, DMAP and CH_2Cl_2 at –45°C[53,54]. Silver triflate

was used as it is well known to increase the activity of alkyl halides in nucleophilic reactions[55]. The literature protocol being followed had used the virtually non nucleophilic bulky base DTBP. In this initial trial reaction DMAP was used as it was the only dry amine base available at the time. The poor final 30% yield of **196** was hypothesised to be due to the use of DMAP and highlighted the advantages of using more bulky and less nucleophilic bases, as the α-chlorosulfide formed is a potent electrophile and therefore quite capable of reacting with DMAP. The yield although poor was acceptable for an unoptimized reaction at this stage. During the course of the reaction one of the two Boc protecting groups was removed. It was hypothesised that this was caused by the Lewis acidic reagent AgOTf. This occurrence made little difference to the planned scheme as the presence of two Boc groups was no longer required.

To study the potentially challenging step of carbon sulfur bond cleavage a model hemithioacetal was synthesised, as alcohol **150** (and therefore **196**) was an expensive resource.



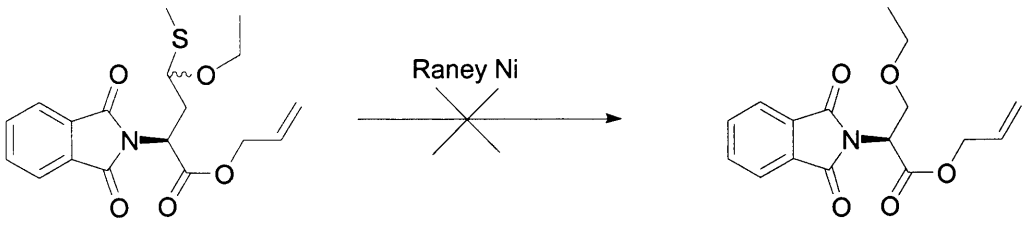
a) NCS, CH_2Cl_2, rt, 1 h b) EtOH, 2,6-lutidine, rt, 24 h

Scheme 51: Synthesis of model hemithioacetal 203

Reaction of previously protected methionine **195** with NCS in CH_2Cl_2 at room temperature for 30 min produced the α-chlorosulfide **202** (Scheme 51) which was not isolated. Addition of 2,6-lutidine and a vast excess of ethanol gave a final yield of 60% for **203**. Though low yielding the reaction used cheap materials that could be scaled up to 15 g and synthesised in 2 days. Reactions performed with DMAP and K_2CO_3 gave yields of 30% and 45% respectively highlighting the importance of the base used in the reaction. The final hemithioacetal and foul smelling by-product, tentatively assigned to be **204** by NMR and mass spectrometry, had very close R_f values and therefore the separation of the two compounds required a minimum of three slowly eluted columns. Similar work conducted on methionine has been

attempted before using different protecting groups and methanol instead of ethanol as the nucleophile[56].

There are many documented methods and reagents for the cleavage of carbon-sulfur bonds[57], Raney nickel[58], radical desulfurization[59,60], oxidation[61], nickel boride[62], zinc/ammonium chloride[63], samarium iodide[64] and novel nickel complexes[65,66]. There are also papers regarding cleavage of carbon-sulfur bonds in hemithioacetals[67]. Unfortunately many of the literature methods were performed on simple alkyl sulfides where very harsh conditions could tolerated. Molecule **196** contained 5 protecting groups and 2 chiral centres and was susceptible to side reactions with acids, bases, nucleophiles and transition metals amongst other reagents. It was therefore important to cast aside the more brutal methods and choose those that appeared mildest.



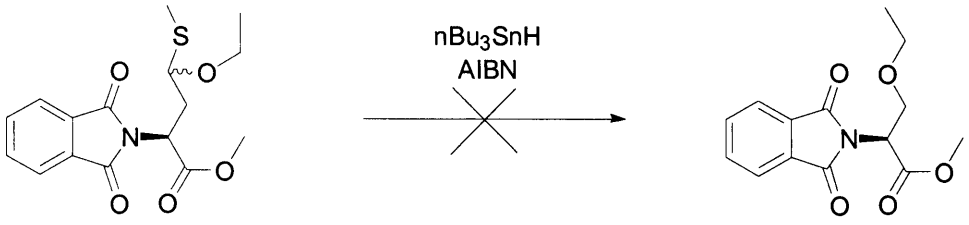
203 **205**

Scheme 52: Attempted cleavage of the C-S bond of 203 using Raney Ni

The first method attempted was cleavage using Raney Ni (Scheme 52). This represented a cheap and well precedented method for sulfur extrusion though it appeared to not have been attempted on hemithioacetals before. Problems were

encountered initially when weighing the material. When developing methodology it is fundamental to know how much of each reagent is used. This is difficult using Raney Ni as it must be kept wet due to its pyrophoric nature at all times and is therefore weighed out as a suspension. The density of Raney Ni within the suspension is not known and therefore the equivalents cannot be accurately measured. It was therefore attempted to perform the same steps each time it was weighed to minimize discrepancies in equivalents. The small volume being used made it very difficult to work out the amount of Raney Ni contained within a certain volume by weight minus the weight of the water due to the accuracy of the scales at low masses.

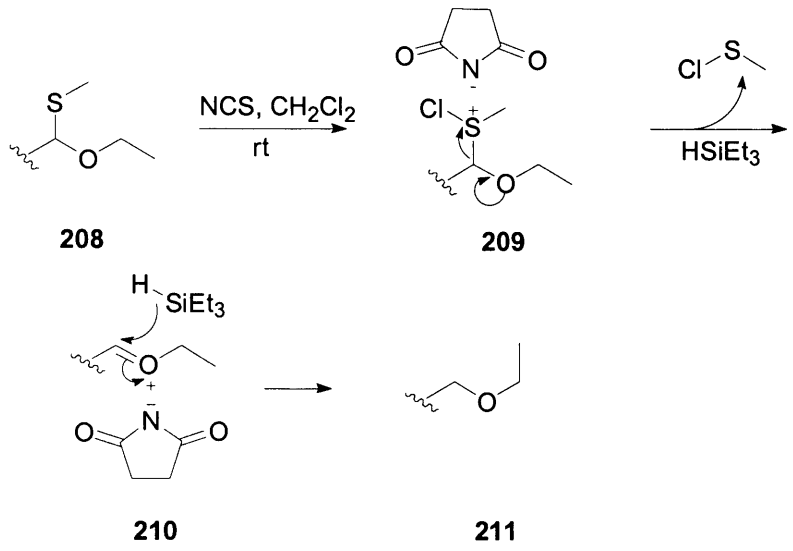
Reports in the literature for desulfurisation using the same grade of Raney nickel vary wildly with respect to time, temperature, solvents and equivalents[58]. Table 2 (page 100) shows the range of conditions that were attempted using this reagent. Reactions were carried out and then passed through a plug of silica to remove any nickel residues. Solvent evaporation followed by NMR showed that none of the experiments carried out yielded anything resembling the target model ether. Reactions generally returned starting material but in the longer reactions left for a number of days at reflux complex decomposition had taken place. The results were surprising considering the generality of the reaction in the literature but as it did not work in this system a second method was chosen.



206 207

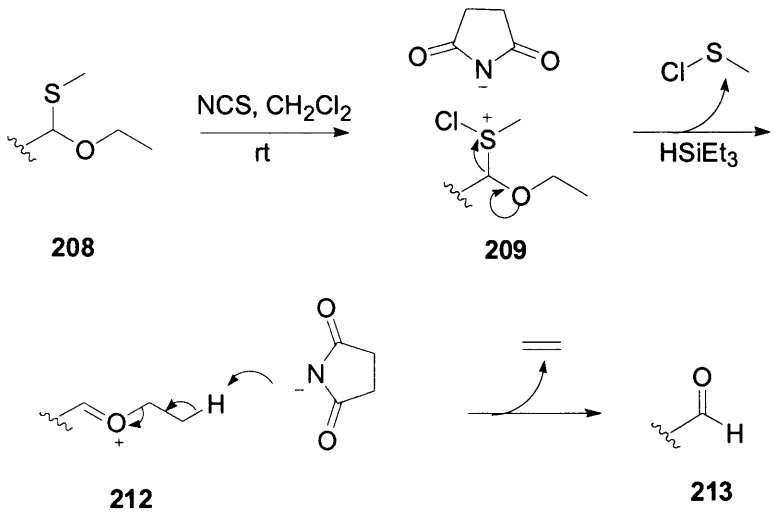
Scheme 53: Attempted cleavage of the C-S bond of 206 using radical desulfurisation

Radical desulfurizations are typically performed with tributyl tin hydride in the presence of a radical initiator such as AIBN[68]. There is a degree of argument over this reaction in the literature, some claim it does not work for the less reactive methyl sulfides[69] and others claim it does[70,71]. Under the reductive radical conditions it was believed that the unsaturated allyl ester may react. Methyl ester **206** was therefore synthesised. The methyl group could be saponified upon completion of the reaction and replaced with an allyl ester, it is also orthogonal to the protecting groups of **150**. Following literature procedures the reaction was attempted over a range of different conditions. The number of equivalents of both hydride and initiator were varied. The solvent, toluene, was degassed using the freeze-thaw method[72] with both argon and nitrogen. The temperature and time of reaction was also varied. After an extensive list of parameters were tried (Table 2) there only ever remained starting hemithioacetal **206**. It was concluded that radical desulfurisation was ineffective for **206**.



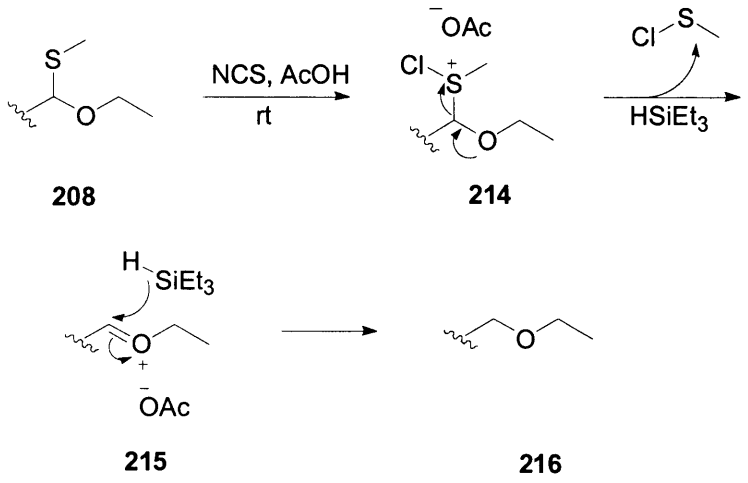
Scheme 54: Mechanism of attempted cleavage of the C-S bond of 206 using NCS and Et_3SiH

Scheme 54 shows the proposed mechanism for the next method attempted. Using an electrophilic halide source it was proposed that upon formation of the sulfonium salt anchimeric assistance from the oxygen would form the oxonium ion **210**. This would then be reduced by a hydride source such as Et_3SiH or $NaBH_4$ to yield the ether **211**. The reaction performed was based on the mechanism of DMTSF desulfurisations[73,74]. Reactions were performed using NBS and NCS in parallel. Half an equivalent of either reagent was used in each reaction as the initially formed methanesulfenyl chloride goes on to chlorinate a second equivalent of hemithioacetal. Reactions were carried out in CH_2Cl_2 and although temperature, concentration, timing of addition of reagents and equivalents was varied no desired ether was obtained. The major products from these reactions were starting hemithioacetal **208** and aldehyde **213** (Scheme 64). The reactions were carried out in dry solvents and under an argon atmosphere so aldehyde **213** is generated in the absence of water.



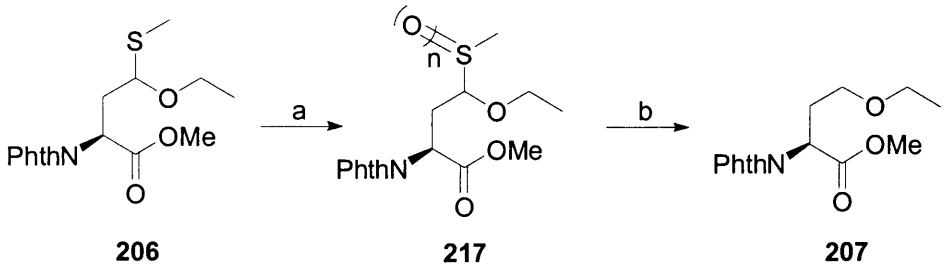
Scheme 55: Postulated mechanism explaining formation of aldehyde 213

Scheme 55 shows our postulated mechanism to explain this. Formation of the chloro sulfonium salt **209**, followed by deprotonation of the ethyl ether by the succinimide counter ion leads to a cascade which results in formation of succinimide, ethene and aldehyde **213**. The presence of starting material can be attributed to incomplete reaction after NCS addition. To minimize the amount of aldehyde produced and increase the likelihood of ether formation a source of acidic protons was introduced. Reactions were carried out in CH_2Cl_2/^{t}butanol mixtures but starting material and aldehyde were still present. The acidity of the solution was then increased by the use of acetic acid as the lone solvent (Scheme 56).



Scheme 56: Mechanism of attempted cleavage of the C-S bond of 206 using NCS, Et_3SiH and AcOH

After addition of NCS for thirty minutes at room temperature, triethylsilane was added and the solution refluxed for 2 hours. After work up and purification ether **207** was isolated in good yield. Upon repeating this reaction using identical conditions only starting sulfide **206** and aldehyde **213** were isolated. Varying all the conditions and using newer reagents also did not help and the ether was never again isolated using these conditions (Table 2).



a) MCPBA, CH_2Cl_2, 0°C, 2 h b) $BF_3.OMe_2$, Et_3SiH, CH_2Cl_2, -45°C, 1 h

Scheme 57: Synthesis of ether 207 using MCPBA and BF_3 dimethyl etherate

Scheme 57 shows the final attempt to cleave the carbon-sulfur bond. The reaction had little literature precedent[61] but what had been performed was in delicate natural product molecules and we therefore considered the conditions mild enough for **196**. The reaction involved oxidation of the sulfur and subsequent addition of a Lewis acid. The presence of the Lewis acid cleaves the sulfur carbon bond. Following the literature MCPBA was used as oxidant. This step proceeded well producing two spots presumed to be the sulfoxide and sulfone which was confirmed by mass spectrometry. Certain literature sources had claimed the oxidation of hemithioacetals led to decomposition products but this was not observed[67]. Boron trifluoride dimethyl ether was then added to the reaction as the Lewis acid. Upon purification a small yield of the ether **207** was isolated. Crude isolation of the two products (**217**) as an oil, and removal of any traces of the MCPBA or water that might hamper the effect of the Lewis acid was then performed, subsequent reaction with boron trifluoride gave ether **207**, isolated in 35% yield. Attempts to optimise the reaction were performed, however no yield greater than the first attempt was achieved.

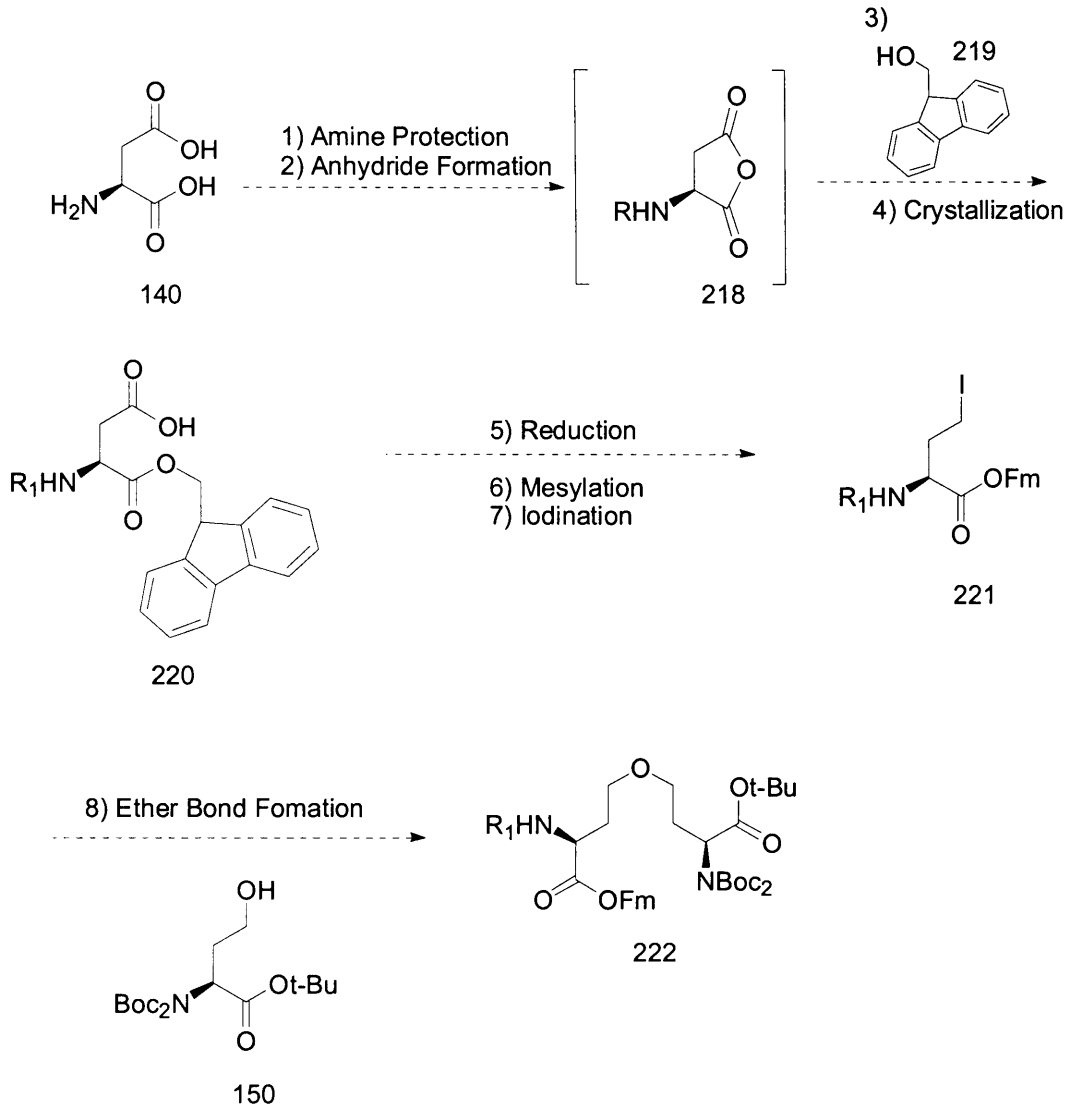
Table 2: List of conditions attempted in cleaving the C-S bond from hemithio acetals 203 and 206

	Reagents	Solvents	Temperature	Time	Gas	Yield
1	Raney Ni	Acetone / EtOH	rt to reflux	0.5h - 7days	Ar / H_2 / N_2	0
2	nBu_3SnH, AIBN	Toluene	Reflux	0.25h - 1days	Ar / H_2 / N_2	0
3	1) NCS, 2) $NaBH_4$	DCM / glyme	rt to Reflux	0.25h - 2days	Ar	0
4	1) NCS, 2) Et_3SiH	DCM	rt to Reflux	0.25h - 3days	Ar	0
5	1) NBS, 2) $NaBH_4$	DCM / glyme	rt to Reflux	0.25h - 2 days	Ar	0
6	1) NCS, 2) Et_3SiH	DCM & ^tBuOH	rt to Reflux	0.25h - 2days	Ar	0
7	1) NCS, 2) Et_3SiH	DCM & ^tBuOH	Reflux	0.25h - 2days	Ar	0
8	1) NCS, 2) Et_3SiH	AcOH	rt to Reflux	0.25h - 2days	Ar	0
9	MCPBA, $BF_3.OMe_2$	DCM	0°C	2h	Ar	<10%
10	1) MCPBA, 2) $BF_3.OMe_2$	DCM	0°C	2h	Ar	36%

Considering this result, the suitability of the pathway was called into question. The low yields observed in the coupling and sulfur cleavage steps were clearly increasing the cost of the synthesis as a large amount of starting material was required to make **207**. Also upon repetition the yields of the reactions were highly variable. No standard could be reached and the success of the reaction appeared to be due to minute untraceable factors. This made the possibility of a scale up using this chemistry extremely risky. Other attempts towards electrophile **138** were therefore tried.

2.3.6 Electrophile Synthesis VI – Successful Iodide Synthesis

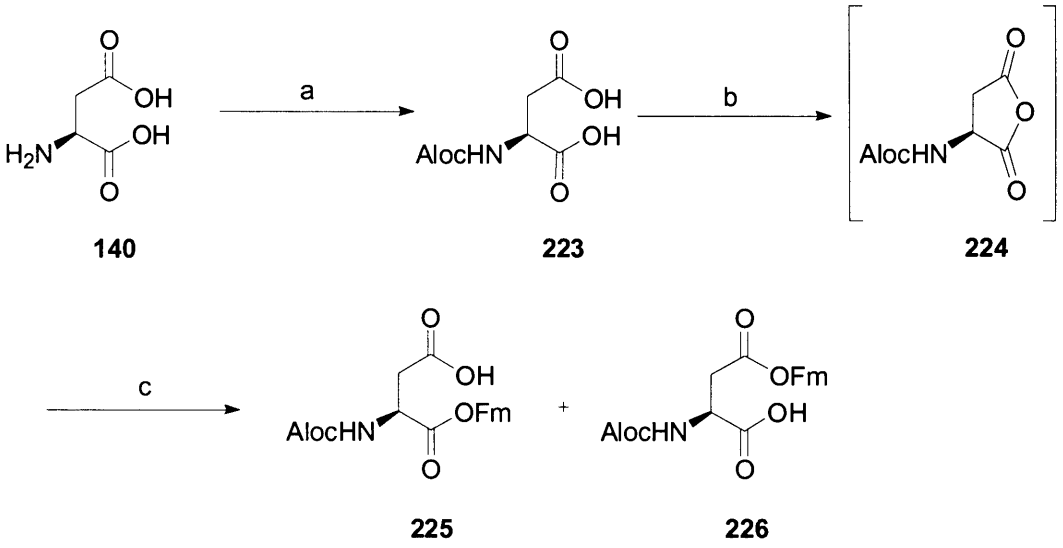
A paper came to our attention which claimed that the problem of lactonisation inherent to homoserine could be prevented using a protecting group orthogonal to ^{t}butyl[75]. Using the base labile Fm group Grieco and co-workers were able to synthesise stable protected derivatives of homoserine. Fm groups are often thought of as the ester variety of the Fmoc group as they are both removed with piperidine in approximately the same time. The literature pathway went on to derivatise the alcohol as an iodide and this too was stable. Scheme 58 shows the planned route to iodide **221** and ether **222**.



Scheme 58: Planned synthesis of protected ether bridged bis-amino acid 222

The literature source initially protected aspartic acid as the Boc derivative. As an orthogonal strategy was needed, we envisaged that this could be changed to an allyl carbamate. The diacid would then be dehydrated to anhydride **218** and opened with Fm alcohol **219** to give a mixture of α and β protected esters. Recrystallisation to yield **220** and subsequent active ester reduction would provide protected homoserine. The primary iodide could then be made by mesylation followed by iodide

displacement. It was envisaged that coupling of alcohol **150** and iodide **221** would form ether **222**.

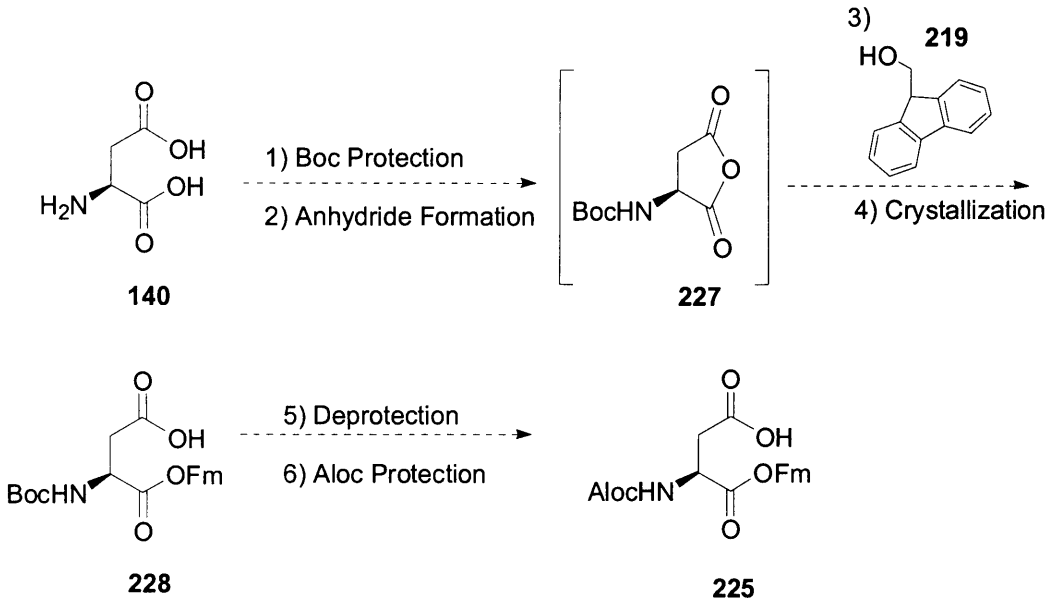


a) Allyl chloroformate, Na_2CO_3, H_2O, rt, 24 h b) Ac_2O, THF, reflux, 4 h c) **219**, $^iPr_2EtN$, THF, rt, 24h

Scheme 59: Synthesis of protected aspartic acid regioisomers 225 and 226

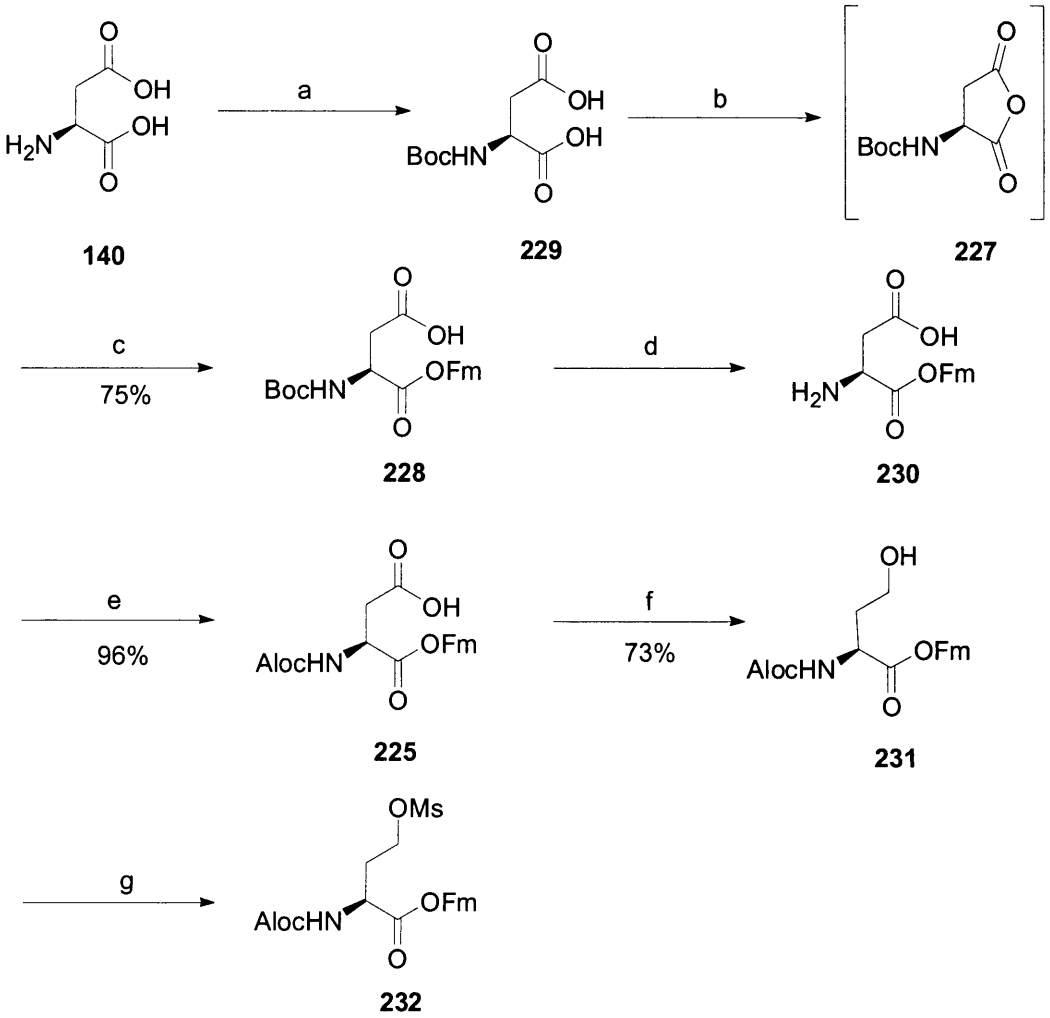
Aspartic acid was protected as its allyl carbamate **223** (Scheme 59) and then dehydrated by heating the acids in THF with Ac_2O[76] to give **224**. After addition of **219**, **225** and **226** were isolated as a waxy solid, attempted recrystalisation of the two regioisomers failed. No attempt was made at isolating a DCHA salt of **225** and **227** as the Fm would be deprotected under the conditions required to form the molecule. Failure also occurred when trying to purify the mixture by flash column chromatography as the two compounds coeluted. Although it was hypothesised that the two regioisomers contained acids that would have differing properties due to their electronic and steric environments no separation was possible. The strategy therefore

had to be changed as the allyl carbamate did not allow for isolation and purification of the α Fm ester **225**.



Scheme 60: Planned synthesis of protected aspartic acid 225

Scheme 60 shows the revised route to **225**. Deprotection of the Boc and reprotection as the Aloc carbmate was to be performed once the desired α Fm ester was isolated. This scheme also requires a change of reagent when performing the cyclisation as the presence of AcOH found within Ac_2O would very likely deprotect the Boc group, so DCC[77] was planned to be used instead.



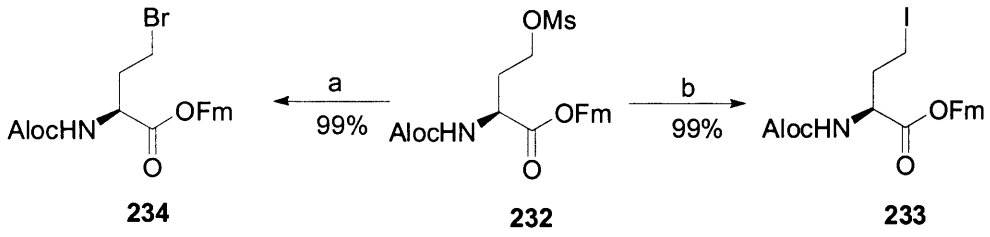
a) $(Boc)_2O$, Na_2CO_3, H_2O, 0°C to rt, 24h b) DIC, $^iPr_2EtN$, THF, rt, 4h c) **219**, $^iPr_2EtN$, THF, rt, 24 h d) TFA:CH_2Cl_2 (1:1), Et_3SiH, 0°C, 2.5h e) allyl chloroformate, $NaHCO_3$, THF:H_2O (1:1), 0C, 2h f) ^{i}butyl chloroformate, $^iPr_2EtN$, CH_2Cl_2, rt, 1hr then MeOH, $NaBH_4$, -78°C 2.5 h g) MsCl, Et_3N, CH_2Cl_2, rt, 2.5 h

Scheme 61: Synthesis of aspartic acid derived mesylate 232

Upon protection of aspartic acid with $(Boc)_2O$ **229** was cyclised with DCC and opened with Fm alcohol (Scheme 61). The reaction produced a very fine brown solid which was resistant to dissolving in EtOAc. After extensive heating a clear brown solution was obtained. Upon addition of a small quantity of hexane, a fine brown suspension started to appear. The solid was isolated and found to be a mixture of

DCU and unwanted side reaction material. Repeating the crystallisation gave a small amount of pure **228** and after 5 more cycles of recrystallisation a reasonable yield of 32% was achieved. Due to the very lengthy and problematic purification procedure using DCC it was decided to attempt the dehydration using a different carbodiimide. Using DIC it was possible to isolate the mixture of regioisomers as a pink crystalline material that was far easier to recrystallise. After a further four recrystallisations, **228** was isolated in excellent 75% yield. This reaction was found to be highly robust when scaled up to 15 g and repeated. The Boc group of **228** was then deprotected with TFA in CH_2Cl_2 and amino acid **230** was rapidly crystallised with cold Et_2O and hexane. NMR spectroscopy of this crude material showed that there had been a small amount of transesterification within the molecule producing the β Fm ester. After optimisation it was possible to stop this happening by increasing the TFA concentration and therefore shortening the length of the reaction. Upon reprotection of the amine with allyl chloroformate and purification it became clear that a small degree of transesterification had again occurred. Optimisation of the conditions by reducing reaction times and lowering the temperature gave pure **225**. This was then reacted with *iso*-butyl chloroformate and NMM to yield a mixed anhydride. The protonated NMM present as a solid in the dichloromethane was initially filtered off but it was found that this step could be omitted and there was no loss of yield. Lowering the temperature to –78°C $NaBH_4$ was then added followed by dropwise addition of MeOH. It was important to maintain the low temperature as reactions performed above this suffered from many side reactions and low yields. After work up homoserine **231** was rapidly purified by flash column chromatography. A small

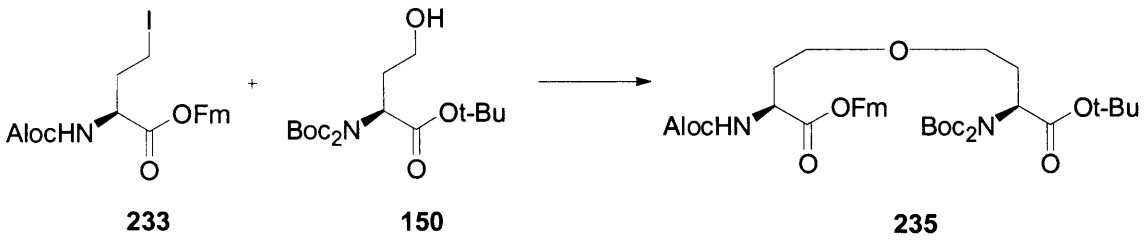
amount of lactonisation could be observed by TLC after **231** had been in a standing solution at room temperature for 2 hours. As an oil it was stable in the freezer indefinitely. Mesylation of **231** was then performed which occurred in good yield and gave **232**. Purification was not attempted on this molecule as only one spot by TLC was observed.



a) LiBr, THF, reflux, 2 h b) NaI, THF, reflux, 2 h

Scheme 62: Synthesis of protected iodide 233 and bromide 234 from mesylate 232

Mesylate **232** was reacted with NaI in refluxing THF to give iodide **233** (Scheme 62) This reaction again was very clean and produced a single spot by TLC. The iodide was stable at room temperature in solution or as a yellow powder. It was decided that bromide **234** should also be made so mesylate **232** was refluxed in THF with LiBr. The reaction was again very clean yielding only one spot by TLC. The reason the bromide was synthesised too was because these are sometimes found to give cleaner etherification reactions over iodides as they are less prone to elimination reactions. Producing the two primary halides it was felt that any attempted ether coupling methodology could be more rigorously probed.

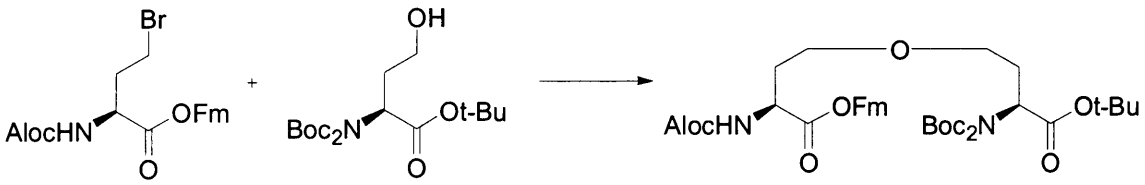


Scheme 63: Attempted synthesis of orthogonally protected ether bridged bis-amino acid 235 using iodide 233 and alcohol 150

With the previously planned molecules **233** and **150** synthesised the stage of attempting to couple the two together had been reached (Scheme 63). After reviewing the literature for etherification reactions a standard Williamson ether coupling via a sodium alkoxide was not chosen to be performed first. This was because although alcohol **150** was stable to lactonisation, if it was deprotonated it was likely to ring close. It was therefore decided to attempt a silver salt mediated etherification[78]. These reactions are often used in natural product syntheses when harsher conditions must be avoided[79,80]. It is most commonly performed with AgOTf[81] and Ag_2O[82,83,84]. Reactions with Ag_2O generally require high temperatures and/or long reactions times[85], whereas those with AgOTf are often performed at low temperatures and are complete in under an hour[86]. Typically a bulky amine base such as DTBP is used in the reactions. This is a very expensive reagent so the cheaper 2,6-lutidine was used which is a hindered base but a slightly more nuclophilic one[43].

The two molecules **233** and **150** were dissolved in CH_2Cl_2 and the temperature lowered to –78°C. 2,6-Lutidine was then added followed by AgOTf. After 10 minutes the TLC showed many products (without a main intense spot), and starting alcohol

150. When reaction times were shortened or dilutions increased it was clear that the many side reactions were occurring faster than any etherification. Attention was therefore turned to performing the reaction with bromide **234** (Scheme 64).



Scheme 64: Attempted synthesis of orthogonally protected ether bridged bis-amino acid 235 using bromide 234 and alcohol 150

The reaction was stirred for one hour at –78°C but no reaction was observed to be occurring by TLC so it was very slowly warmed to room temperature. After being at this temperature for 10 minutes the TLC showed again a multitude of products and starting alcohol **150**. Attempts to optimise the reaction by changing the temperature, dilution, solvent and length of reaction time it became apparent that no etherification was occurring. From the results experienced it appeared that the alcohol **150** was stable to the reaction conditions but the primary halides were reacting with the reagent or with themselves. Hypothesising it may be a problem with the reactive AgOTf the salt was changed to Ag_2O. Reactions using this reagent also resulted in a lack of formation of an ether bond. Even when heating the reaction to 40°C in the presence Ag_2O very little happened. Further reactions were attempted using Ag_2CO_3[87] and $AgNO_3$[88] but no indication that an ether had been formed could be found. Table 3 shows the reactions performed.

Table 3: List of conditions attempted form ether from alcohol 150, iodide 233 and bromide 234

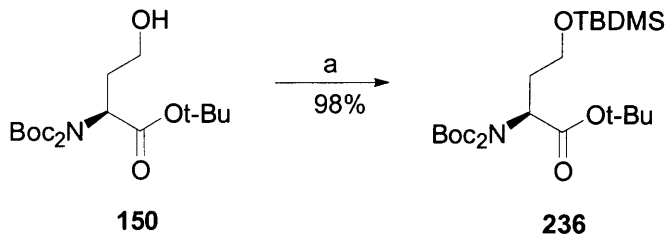
Electrophile	Base	Solvent Used	Silver Salt	Time/hrs	Temperature	Yield of **106**
Iodide	2,6-Lutidine	CH_2Cl_2 / $MeNO_2$ / THF	AgOTf	0.1	-78°C	0
Bromide	2,6-Lutidine	CH_2Cl_2 / $MeNO_2$ / THF	AgOTf	0.1	-78°C to 0°C	0
Iodide	2,6-Lutidine	CH_2Cl_2 / $MeNO_2$ / THF	Ag_2O	0.1 to 120	rt & 50°C	0
Bromide	2,6-Lutidine	CH_2Cl_2 / $MeNO_2$ / THF	Ag_2O	6 to 120	rt & 50°C	0
Iodide	2,6-Lutidine	CH_2Cl_2 / $MeNO_2$ / THF	$AgNO_3$	0.1 to 120	rt & 50°C	0
Bromide	2,6-Lutidine	CH_2Cl_2 / $MeNO_2$ / THF	$AgNO_3$	6 to 120	rt & 50°C	0
Iodide	N/A	CH_2Cl_2 / $MeNO_2$ / THF	Ag_2CO_3	1 to 120	rt & 50°C	0
Bromide	N/A	CH_2Cl_2 / $MeNO_2$ / THF	Ag_2CO_3	1 to 120	rt & 50°C	0

Next a classical Williamson ether coupling with NaH was performed. Although there are competing reactions such as lactonisation and Fm deprotection it was felt that by adding the NaH at low temperature to a solution of **150** and **233** the etherification would be favoured. The reaction was performed at –78°C in CH_2Cl_2 after 20 minutes it was quenched with AcOH and warmed to room temperature. The resultant TLC consisted of many spots and a small amount of starting iodide, there was no sign of the alcohol **150**. Other variations attempted such as using the bromide and changing the solvent had no effect. A reaction run with just alcohol **150** and NaH in THF showed only decomposition products after 10 minutes at –78°C, highlighting the unstable nature of the alkoxide formed. A similar reaction to this was tried which used KF fused onto Celite as the etherification reagent[89]. The reaction involved microwave irradiation and the iodide **233** and alcohol **150** in a very concentrated solution of DMF. It was hypothesised that as the reaction involved no extremes of pH the starting molecules may withstand the conditions. After testing various conditions such as dilution, length of time in the microwave and equivalents of KF-Celite the

reaction did not work The products isolated included **150** minus a Boc group and starting iodide. No positive result was achieved with bromide **234** either.

A relatively new method then came to our attention, reductive etherification of silyl protected alcohols and aldehydes[90,91,92,93]. This reaction has seen considerable interest in recent years as there are limited methods for mild etherifications. The reaction has been performed by a variety of reagents such as $FeCl_3$[94,95], CuOTf[96], $B(C_6H_5)_3$[97], and $BiBr_3$[98,99]. It is believed that the reaction proceeds by formation of an oxonium ion which is then quenched by a hydride source. There has been much debate over the catalytic cycle and reactive species of the etherification[98,90,100].

Before the etherification reaction could be performed the starting materials had to be synthesised. Silylation of alcohol **150** with TBDMSCl[101] was favoured as test reactions with **231** showed that the presence of imidazole deprotected the Ofm group over the course of the reaction.

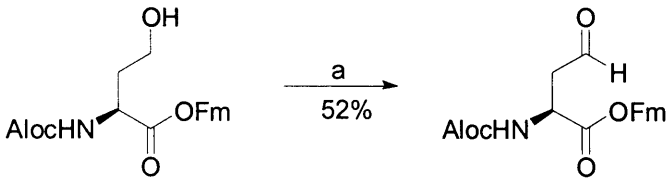


150 **236**

a) TBDMSCl, DMF, imidazole, rt, 18 h

Scheme 65: Synthesis of TBDMS protected homoserine silyl ether 236

Silylation occurred in a concentrated solution of **150** (Scheme 65) in DMF at room temperature overnight. Purification by flash column chromatography gave an excellent yield of 98% for TBDMS ether **236**.

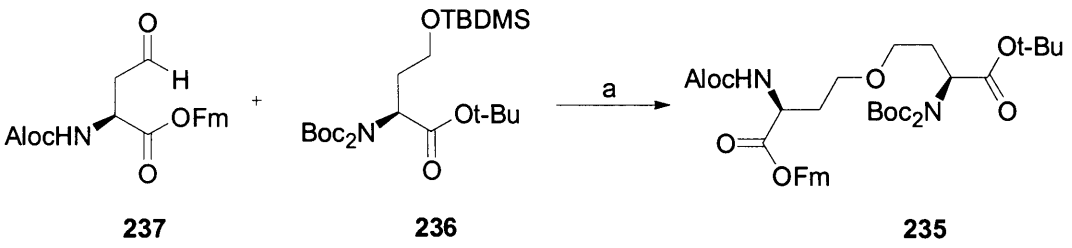


231 **237**

a) Oxalyl Chloride, DMSO, CH_2Cl_2, -78°C, 0.5 h then **231**, Et_3N, to 0°C, 2 h

Scheme 66: Synthesis of aspartate semi-aldehyde 237

To synthesise the desired aldehyde **237**, a Swern reaction was used[102,103]. Oxalyl chloride was cooled to –78°C in CH_2Cl_2 and DMSO was added dropwise (Scheme 66). After 30 minutes alcohol **231** dissolved in CH_2Cl_2 was added dropwise. After another 30 minutes Et_3N was added and the reaction was warmed to 0°C. Post work up, it became clear that there were two major products from the reaction. The side product produced in large quantity is produced if the reaction becomes too warm. Unfortunately no way of stopping this unwanted side reaction was found however slow the addition, or low the temperature was. This gave a poor yield of 53%.



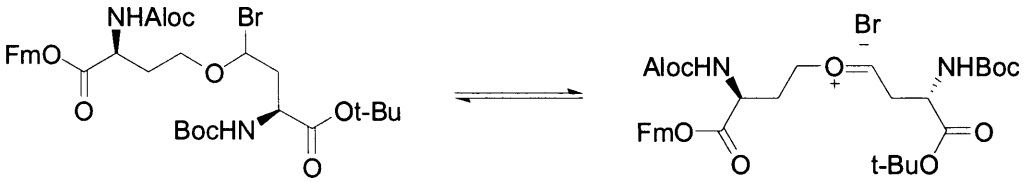
237 **236** **235**

a) $BiBr_3$, Et_3SiH, MeCN, rt, 48h

Scheme 67: Attempted synthesis of orthogonally protected ether bridged bis-amino acid 235 using silyl ether 236 and aldehyde 237

Silyl ether **236** was dissolved in MeCN and stirred at room temperature (Scheme 67)[100]. Et_3SiH was then added followed by $BiBr_3$. Upon addition of the catalyst the reaction went a dark black grey colour. Aldehyde **237** was then dripped into the reaction and the mixture was stirred for 48 hours. The final TLC showed at least 7 products had been made but there was one spot which was both UV active and stained with ninhydrin, indicative of the Fm group and Boc amine being present.

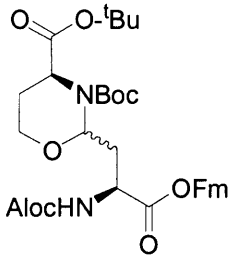
Isolation of this spot gave a product with an NMR which showed a missing Boc group and a proton missing adjacent to the ether linkage. At first it was believed that the compound was **238** (Scheme 68). By mass spectrometry it appeared that the oxonium salt **239** was seen, with elimination of Br^- in the spectrometer. However attempts to quench the bromide with Et_3SiH, or $NaBH_4$ with a Lewis acid, were unsuccessful.



238 **239**

Scheme 68: Postulated products from silyl ether 236 and aldehyde 237

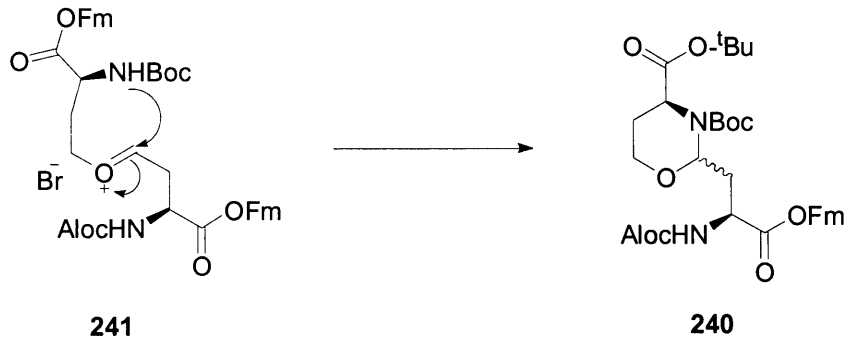
Previous reports on α-halo ethers indicated that they are extremely reactive and would rapidly react with water to form hemi acetals[104]. After reassessing the spectra obtained it was eventually worked out that the correct final structure was not **238,** but **240** (Figure 22).



240

Figure 22: Product of reductive etherification reaction, hemiaminal 240

It was hypothesised that the formation of **240** would occur during the coupling step. The small amount of HBr produced within the reaction would deprotect the Boc group and this would leave a highly nucleophilic nitrogen anion which could attack the oxonium salt reportedly formed during the coupling (Scheme 69).



241 **240**

Scheme 69: Postulated mechanism of silyl ether 236 and aldehyde 237

Reactions performed containing K_2CO_3 to try and stop excessive HBr production returned starting materials **237** and **236**. This is understandable as the acid is believed to be the catalytic species and by removing this we see no reaction.

2.4 Summary

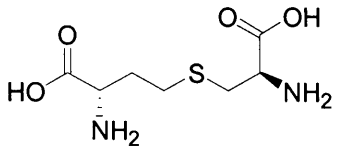
After successfully making target synthons **150** and **233** no way was found of coupling them together. Many reactions and attempts to optimise all of the silver, bismuth and classical Williamson ether coupling reactions had been performed but an ether was never successfully synthesised. The lactonisation that occurs when an alkoxide forms from alcohol **150** is understandable. This molecule is ideally set up to perform this reaction but is stopped by the presence of the bulky ^{t}butyl group. The increase in reactivity that occurs when the alkoxide forms must be greater than the effect of the steric repulsion and lactonisation occurs, even at low temperatures.

Reductive etherification gave good yields, unfortunately of the wrong material. After working out what the final product formed was we attempted to stop its formation and encourage ether formation by addition of base. From the postulated mechanism though it can be seen why this would leave just starting materials.

The failure of any of the silver salt chemistry to work is however surprising. The reactions carried out have much literature precedent. Although the less hindered base 2,6-lutidine was used instead of DTBP reactions were expected to occur but in less than excellent yields. One explanation could be that the iodide and bromide reacts with itself before any coupling occurs when AgOTf is used. This though does not explain the lack of success achieved using Ag_2O. With the failure of any positive

results experienced attempting to couple alcohol and halide we changed our approach and ended the work on etherifications.

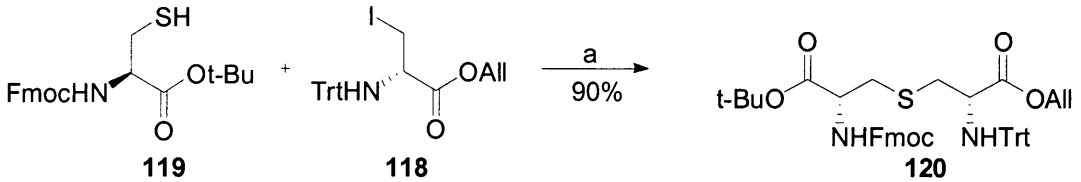
3.0 Synthesis of Protected Homolanthionine



242

Figure 23: Product of reductive etherification reaction, hemiaminal 240

Homolanthionine **242** is a one sulfur mimic of a disulfide bridge. Instead of containing a two sulphur bridge it contains a thioether and is therefore not readily cleaved under physiological conditions. These properties have made it an attractive candidate for stabilisation of small bioactive peptides. To date two groups have performed syntheses of a peptide containing a homolanthionine bridge[105,75]. The two syntheses both installed the bridge as a post synthetic modification of the peptide and potentially therefore limit the amino acids residues that can be used in the sequence. We planned to synthesise an orthogonally protected homolanthionine and incorporate it into the peptide in the same way as Scheme 43.

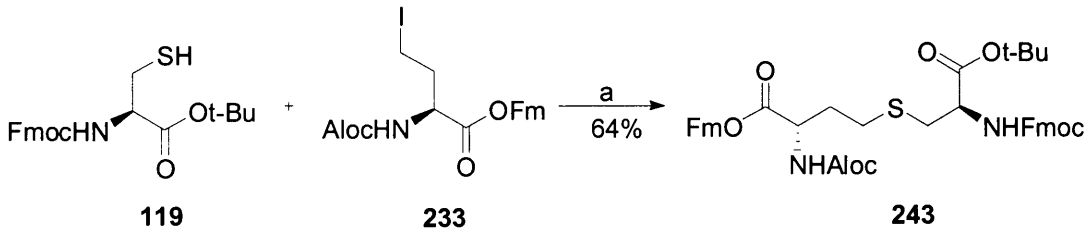


119 **118** **120**

a) Cs_2CO_3, DMF, rt, 2.5 h

Scheme 70: Synthesis of protected lanthionine 120

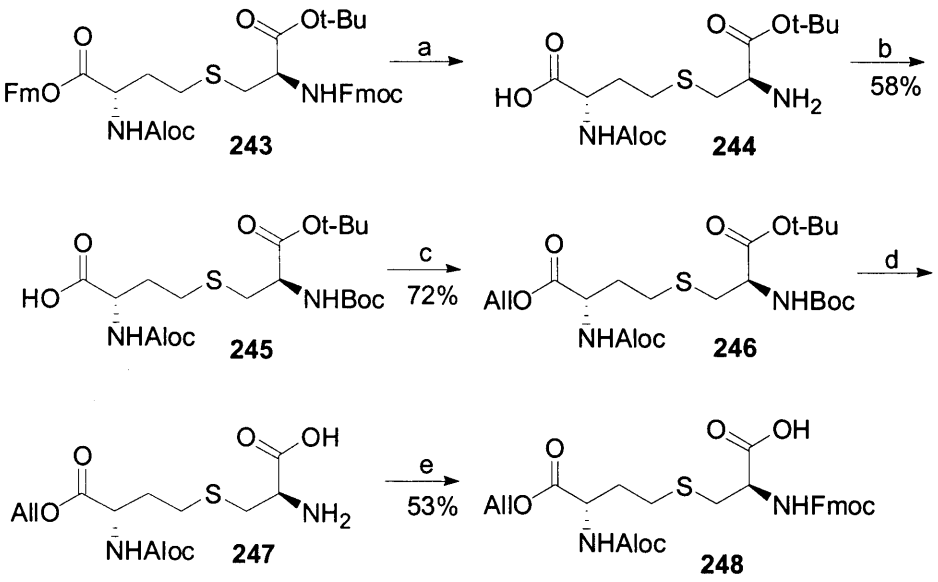
Work within the group to make lanthionine **120** by coupling cysteine **119** with iodoalanine **118** has been shown to work in very good 90% yield (Scheme 70)[4]. Using the same reaction conditions iodide **233** was reacted with cysteine **119** in DMF with Cs_2CO_3 (Scheme 71). After optimisation of time and dilution a reliable 64% yield of **122** could be achieved upon increasing the reaction to a 5 g scale. The success in performing this reaction highlights the increased nucleophilicity of sulphur over oxygen. Whereas none of the etherifications worked this reaction was completed with an ionic base at room temperature.



a) Cs_2CO_3, DMF, rt, 2.5 h

Scheme 71: Synthesis of protected homolanthionine 243

The synthesised sulfide contained protecting groups that had to be changed to the ideal set of **127**, a free acid, Fmoc carbamate, allyl carbamate and an allyl ester (Scheme 72). To achieve this a series of protecting group exchanges were performed .

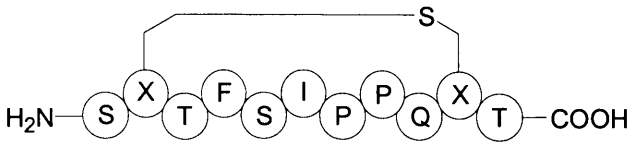


a) Piperidine, DMF, rt, 2.5 h b) $NaHCO_3$, THF:H_2O (1:1), $(Boc)_2O$, rt. 24 h c) i) Cs_2CO_3, MeOH i) allyl bromide, DMF, rt, 24 h d) TFA, Et_3SiH, CH_2Cl_2, 0°C to rt, 3.5 h e) Fmoc chloroformate, $NaHCO_3$, THF:H_2O (1:1), 0°C to rt, 2.5 h

Scheme 72: Synthesis of homolanthionine 248 for peptide synthesis

Firstly both the Fm and Fmoc groups were removed by treatment of **243** with piperidine at room temperature. The primary amine was then Boc protected to ensure that it did not react with the ester made in the next step. The free acid was esterified with allyl bromide to furnish fully protected homolanthionine **246**. The Boc and ^{t}butyl groups were then removed with TFA and the primary amine protected as an Fmoc carbamate **248** in a disappointing 53% yield. The entire pathway of protecting group exchange involving 5 steps was performed in a good overall yield of 22%. Each step of this route was painstakingly optimised as 250 mg of **248** was the minimum mass required for peptide synthesis. After scaling the reaction up three times enough material was obtained to make a peptide.

The peptide that was chosen to be synthesised was an analogue of a Bowman Birk inhibitor[106,107,108,109]. These are naturally-occurring serine protease inhibitors containing a nine-residue β turn constrained by a disulfide loop which is responsible for their inhibitory activity. To increase the stability of the native peptide mimics have been created containing saturated and non saturated carba side chains of varying lengths, although no one has produced a homolanthionine derivative (**249**, Figure 6)[110].

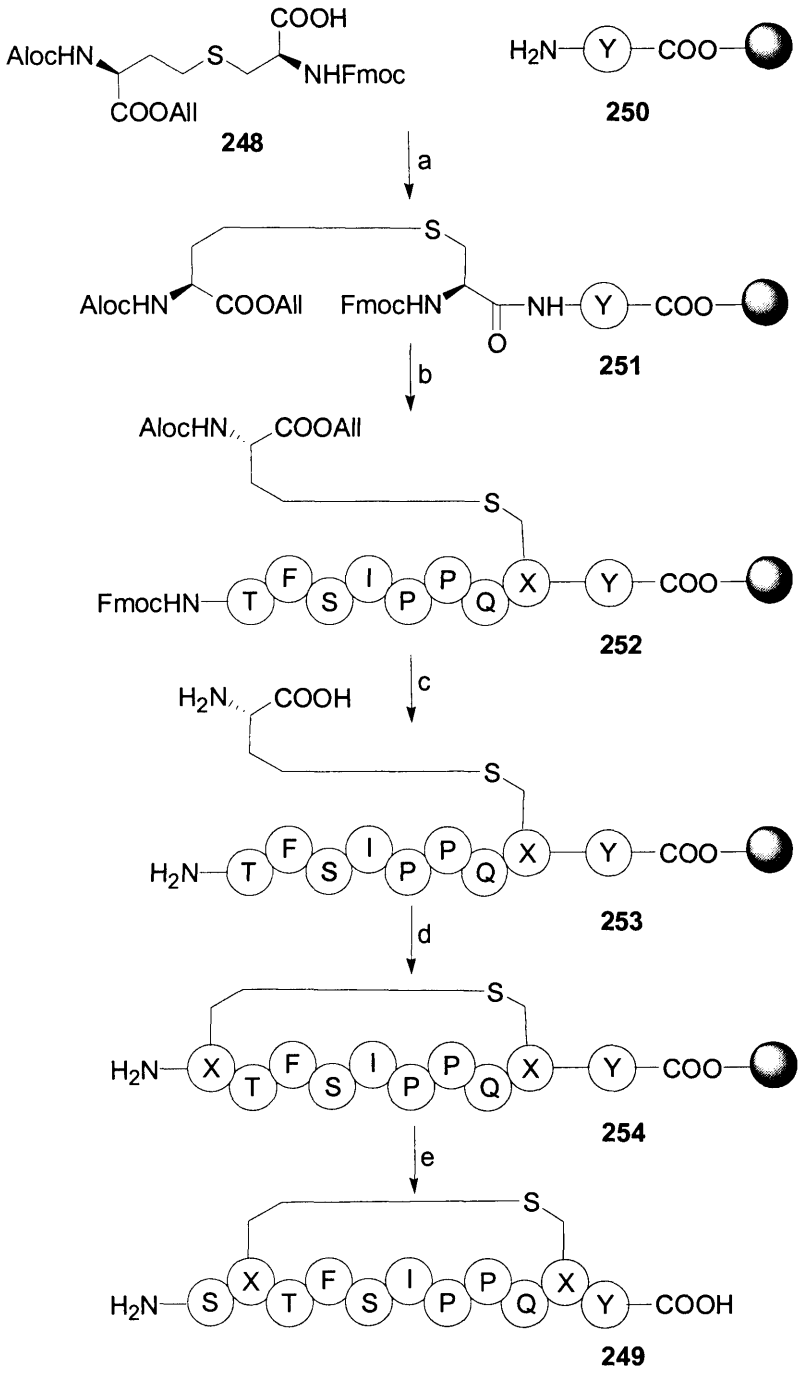


249

Figure 24: Target peptide 249 containing an ether bridged side-chain

Peptide synthesis was then begun towards molecule **249**. A preloaded tyrosine chlorotrityl Sieber resin was used to install the first residue into the peptide sequence (Scheme 73). All peptide synthesis was carried out using a SYRO peptide synthesizer. The first step involved deprotection of the Fmoc group from the resin bound tyrosine. A Kaiser test was then performed which confirmed that an amine was present. The free acid of protected homolanthionine **248** was then activated with PyAOP, HOAt and DIEA and coupled with the amine of the tyrosine residue. The mixture was reacted for 1 hour with a fast vortex of the solution occurring every 2 minutes for 20 seconds. The resin was then washed with several portions of DMF and CH_2Cl_2 before a Kaiser test was again performed. This showed a negative result for the presence of an amine so the synthesis was continued by deprotection of the Fmoc

protecting group. After a positive result from the Kaiser test the amine was then coupled to the HBTU/DIEA activated acid of a glutamine residue. The synthesis was then automated using standard Fmoc SPPS protocols until **252** was arrived at. With the Fmoc group still present on the amine of the tyrosine both allylic protecting species were deprotected with a cocktail of $Pd(PPh_3)_4$, DMF, $CHCl_3$, NMM, AcOH in the absence of light and under nitrogen. After the solution had been reacting for 2 hours with a 1 minute vortex every 3 minutes the resin was washed with a solution of DIEA in DMF and then DMF containing diethyl dithiocarbamate to ensure all allylic species were quenched and removed from the reaction vessel. The Kaiser test at this stage showed only a partial positive result, being a light red as apposed to the dark purple we had encountered for previous positive results. Regardless of this we continued with the synthesis and cleaved the Fmoc group from the resin bound tyrosine at the end of the linear chain. The Kaiser test at this point was firmly positive. The peptide was then cyclised using PyOAP, HOAt and DIEA to give **254**. A similarly unconvincing Kaiser test was then observed but the synthesis was resumed and the final amino acid serine coupled to the free amine and the Fmoc removed. The resin was then treated with a cocktail of TFA, ethanedithiol, Et_3SiH and water to cleave the peptide from the solid phase and remove all side chain protecting groups to give **249**.



a) PyAOP, HOAt, DIEA, DMF b) i) piperidine, DMF ii) Fmoc SPPS c) i) $Pd(PPh_3)_4$, DMF, $CHCl_3$, NMM, AcOH ii) piperidine, DMF d) PyAOP, HOAt, DIEA, DMF e) i) Fmoc-Ser(O^tBu)-COOH, HBTU, DIEA, DMF ii) piperidine, DMF iii) TFA, ethanedithiol, Et_3SiH, H_2O

Scheme 73: Attempted synthesis of peptide 249 by SPPS

The cleavage cocktail was then diluted with ether and cooled to -23°C in the freezer. This caused the peptide to precipitate out as a white solid. The solid was isolated by centrifugation and freeze dried from water to give 15 mg (10 % yield) of crude material .

The solid was dissolved in water and subjected to preparative reverse phase HPLC. The graph created from this contained a series of at least 5 very broad peaks and a rising base line. This indicated that the material was not very soluble in the solvent system of water MeCN being used. Mass spectrometry of samples from the major peaks indicated that there was peptide sized material present but nothing matching the mass of **249**. These same samples were then subjected to NMR and the lack of solubility was confirmed. None of the material readily dissolved in D_2O giving very poor broad peaks. None of the NMR spectrographs showed presence of peptide **249**. This was confirmed by comparison of the spectrographs obtained with those of the natural peptide SCTFSIPPQST synthesised in parallel to **249**. The only sample that looked like it contained most of the amino acids expected to be in **249** gave two peaks by mass spectrometry 12 and 13 mass units higher than the expected mass. The NMR spectrograph of this material showed the presence of allylic material. Postulating that the $Pd(PPh_3)_4$ used to cleave the allylic groups may not have been fully active, as it was from a old batch we decided to repeat the synthesis.

Upon repetition of the synthesis the same results were encountered. The main peak by HPLC gave the same 12 to 13 mass unit increase by mass spectrometry and the NMR

contained allylic signals. Although a newly ordered batch of $Pd(PPh_3)_4$ had been used this time the result was the same. The quality of the $Pd(PPh_3)_4$ was later found to be very poor as it did not deprotect allylic protecting groups on a different peptide after many hours of reacting. This was unfortunate as there was no more protected homolanthionine **248** to try another peptide synthesis.

The low yields of both peptide syntheses produced very little material for spectroscopy. The lack of solubility in D_2O and poor NMR spectrum meant that working out what the mystery structure was not possible. The small increase in mass of 12 and 13 units meant that it was not a simple case of an allylic group still being present either as a protecting group or attached to a nearby nucleophilic amino acid side chain. After calculating many masses accounting for deletion sequences and the presence of a variety of protecting groups attached and removed nothing similar to the observed mass could be found.

3.1 Summary

Synthesis of protected homolanthionine was achieved coupling iodide **233** with a cysteine derivative. The protecting groups were then manipulated to make the molecule ready for SPPS. A peptide was synthesised as a mimic of the Bowman-Birk inhibitor. After cleavage of the peptide from the solid phase and purification by HPLC nothing resembling target peptide **249** was observed by NMR or mass spectrometry. The closest material resembling **249** contained allylic spcecies by NMR and had a mass of 12 and 13 units higher than expected. After much deliberation no conclusion could be reached over what this moiety was.

4.0 Conclusion

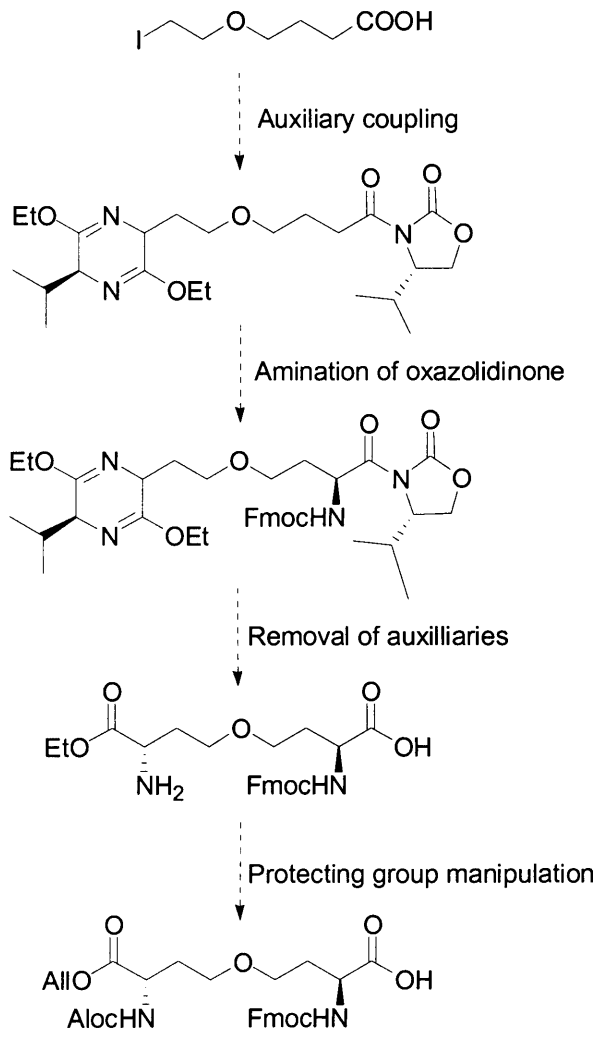
After many failed routes to an ether it can be concluded that making this molecule from simple chiral pool substrates is not easy. The lack of many ether bridged amino acids reported in the literature highlights the difficulty in creating these species. Although target molecules alcohol and iodide were obtained no ether could be synthesised. This therefore also highlights the scarcity of mild etherification methods in the literature. Beyond the Williamson ether coupling and the emerging reductive etherification there does not appear to be any robust preparations for making alkyl ethers.

The two orthogonal pathways to stable protected homoserine molecules are however very useful as shown in the homolanthionine work. Iodides of either molecule can be made easily and reacted with cysteine to give homolanthionine. Further derivatisation of these molecules would be interesting to investigate a whole series of sulfur, nitrogen and carbon bridged bis amino acids.

Although peptide synthesis was undertaken no peptide was obtained. The reasons for this could be either the poor state of the $Pd(PPh_3)_4$ or the large size of the ring closure reaction. This nine amino acid ring closure is the largest that has been attempted in the group; most have consisted of a maximum of five residues between the bridged bis amino acids. Without full characterisation of the final peptide-like species it is very hard to know which of the two were to blame for the failure.

5.0 Future Work

The lack of simple reactions to yield ether means that more challenging synthetic pathways should be attempted. Using asymmetric synthesis it should be possible to install the two stereocentres of a bis amino acid in much the same way as **25** (Scheme 3) but starting from a symmetrical ether rather than an alkyl chain[3] (Scheme 83).



Scheme 74: Planned synthesis of orthogonally protected ether bridged bis-amino acid using asymmetric chemistry

Further work towards homolanthionine **248** containing peptides and the identification of why the peptide synthesis did not work should be performed (Figure 25). Having two stable homoserine molecules means that orthogonally protected bis amino acids such as **255**, **256**, and **257** should be easy to prepare. Changing the heteroatom from sulfur to nitrogen could also give interesting results.

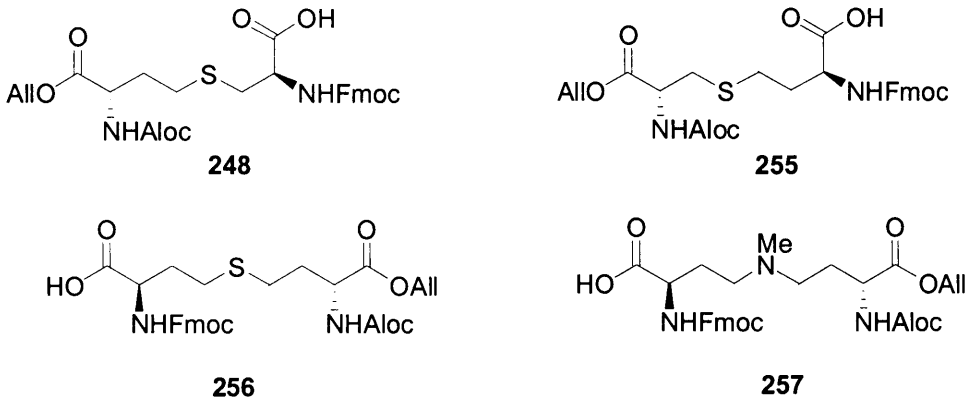


Figure 25: Future target molecules to attempt synthesis of, protected homolanthionines 248 and 255 and bridged sulfide 256 and amine 257

References

[1] Sessions, R. *Personal Communication*, 2001.

[2] McNamara, L. M. A.; Andrews, M. J. I.; Mitzel, F.; Siligardi, G.; Tabor, A. B. *Tetrahedron Lett.* **2001**, *42*, 1591-1593.

[3] McNamara, L. M. A.; Andrews, M. J. I.; Mitzel, F.; Siligardi, G.; Tabor, A. B. *J. Org. Chem.* **2001**, *66*, 4585-4594.

[4] Mustapa, M. F. M.; Harris, R.; Bulic-Subanovic, N.; Elliott, S. L.; Bregant, S.; Groussier, M. F. A.; Mould, J.; Schultz, D.; Chubb, N. A. L.; Gaffney, P. R. J.; Driscoll, P. C.; Tabor, A. B. *J. Org. Chem.* **2003**, *68*, 8185-8192.

[5] Chan, W.C.; White, P.D. *In Fmoc Solid Phase Peptide Synthesis: A Practical Approach*, Oxford University Press, 2000.

[6] Guibe, F. *Tetrahedron* **1998**, *53*, 2967-3042.

[7] Corey, E.J.; Cheng, X-M. In *The Logic of Chemical Synthesis*, Wiley, 1995.

[8] Nefkens, G. H. L.; Zwanenburg, B. *Tetrahedron* **1983**, *39*, 2995-2998.

[9] Gong, B.; Lynn, D. G. *J. Org. Chem.* **1990**, *55*, 4763-4765.

[10] Ledger, R.; Stewart, F.H.C. *Aust. J. Chem.* **1965**, *18*, 1477.

[11] Prestidge, R. L.; Harding, D. R. K.; Battersby, J. E.; Hancock, W. S. *J. Org. Chem.***1975**, *40*, 3287-3288.

[12] Van Heeswijk, W.A.R.; Eenink, M.J.D.; Feijen, J. *Synthesis* **1982**, 744-747.

[13] Schwarz, H.; Bumpus, F. M.; Page, I. H. *J. Am. Chem. Soc.* **1957**, *79*, 5697-5703.

[14] Cox, R. J.; Gibson, J. S.; Martin, M. B. M. *ChemBioChem* **2002**, *3*, 874-886.

[15] Grehn, L.; Gunnarson, K.; Ragnarsson, U. *Acta. Chem. Scandanavia,* **1986**, *B40*, 745.

[16] Gosselin, F.; Lubell, W. D. *J. Org. Chem.* **1998**, *63*, 7463-7471.

[17] Green, T.W.; Wuts, P.G.M. In *Protective Groups in Organic Synthesis*, Wiley-Interscience, New York, 1999.

[18] Barton, D. H. R.; Herve, Y.; Potier, P.; Thierry, J. *J. Chem. Soc., Chem. Commun.* **1984**, 1298-1299.

[19] Barton, D. H. R.; Herve, Y.; Potier, P.; Thierry, J. *Tetrahedron* **1988**, *44*, 5479-5486.

[20] Malachowski, W. P.; Coward, J. K. *J. Org. Chem.* **1994**, *59*, 7625-7634.

[21] Jackson, R. F. W.; Fraser, J. L.; Wishart, N.; Porter, B.; Wythes, M. J. *J. Chem. Soc., Perkin Trans. 1* **1998**, 1903-1912.

[22] Jackson, R. F. W.; Moore, R. J.; Dexter, C. S.; Elliot, J.; Mowbray, C. E. *J. Org. Chem.* **1998**, *63*, 7875-7884.

[23] Hoffmann, M. G.; Zeiss, H. J. *Tetrahedron Lett.* **1992**, *33*, 2669-2672.

[24] Bajgrowicz, J. A.; Elhallaoui, A.; Jacquier, R.; Pigiere, C.; Viallefont, P. *Tetrahedron Lett.***1984**, *25*, 2231-2234.

[25] Frankel, M.; Knobler, Y. *J. Am. Chem. Soc.* **1958**, *80*, 3147-3149.

[26] Uzar, H. C. *Synthesis* **1991**, 526-528.

[27] Baldwin, J. E.; North, M.; Flinn, A. *Tetrahedron* **1988**, *44*, 637-642.

[28] Rudolph, J.; Hannig, F.; Theis, H.; Wischnat, R. *Org. Lett.* **2001**, *3*, 3153-3155.

[29] Meffre, P. *Amino Acids* **1999**, *16*, 251-272.

[30] Knipe, A.C. In *The Chemistry of the Sulfonium Group* Stirling, J.M.; Patai, S., Ed.; Wiley, 1981; Vol 2, p 313-385.

[31] Breuilles, P.; Uguen, D. *Tetrahedron Lett.* **1987**, *28*, 6053-6056.

[32] Corey, E.J.; Jautelat, M. *Tetrahedron Lett.* **1968**, 9, 5787.

[33] Siegel, S.; Graefe, A. F. *J. Am. Chem. Soc.* **1953**, *75*, 4521-4525.

[34] Crosby, G. A.; Kato, M. *J. Am. Chem. Soc.* **1977**, *99*, 278-280.

[35] Seebach, D.; Fadel, A.; *Helv. Chim. Acta* **1985**, *68*, 1243.

[36] Yokoshima, S.; Tokuyama, H.; Fukuyama, T.S. *Angew. Chem. Int. Ed. Engl.* **2000**, *39*, 4073.

[37] Valerio, R.M.; Alewood, P.F.; Johns, R.B. *Synthesis*, **1988**, 786.

[38] Reginato, G.; Mordini, A.; Valacchi, M.; Grandini, E. *J. Org. Chem.* **1999**, *64*, 9211-9216.

[39] Z. Xu, C. W. Johannes, A. F. Houri, D. S. La, D. A. Cogan, G. E. Hofilena, A. H. Hoveyda, *J. Am. Chem. Soc.*, **1997**, *119*, 10302-10316.

[40] Mcdonald, C. E.; Nice, L. E.; Shaw, A. W.; Nestor, N. B. *Tetrahedron Lett.* **1993**, *34*, 2741-2744.

[41] Ksander, G. M.; deJesus, R.; Yuan, A.; Ghai, R. D.; Trapani, A.; McMartin, C.; Bohacek, R. *J. Med. Chem.* **1997**, *40*, 495-505.

[42] Hou, D. R.; Reibenspies, J. H.; Burgess, K. *J. Org. Chem.* **2001**, *66*, 206-215.

[43] Brown, H.C. In *Boranes in Organic Chemistry*; Cornell University Press, 1972; p 216-218

[44] Dilworth, B. M.; Mckervey, M. A. *Tetrahedron* **1986**, *42*, 3731-3752.

[45] Mukaiyama, T.; Sugaya, T.; Marui, S.; Nakatsuka, T. *Chem. Lett.* **1982**, 1555-1558.

[46] Tuleen, D. L.; Stephens, T. B. *Chem. Ind. London* **1966**, 1555.

[47] Paquette, L. A.; Wingard, R. E.; Philips, J. C.; Thompson, G. L.; Read, L. K.; Clardy, J. *J. Amer. Chem. Soc.* **1971**, *93*, 4508.

[48] Tuleen, D. L.; Stephens, T. B. *J. Org. Chem.* **1969**, *34*, 31.

[49] Truce, W. E.; Birum, G. H.; Mcbee, E. T. *J. Amer. Chem. Soc.* **1952**, *74*, 3594-3599.

[50] Mura, A. J.; Bennett, D. A.; Cohen, T. *Tetrahedron Lett.* **1975**, 4433-4436.

[51] Schreiber, K.; Fernande, V. P. *J. Org. Chem.* **1961**, *26*, 2478.

[52] Oki, M.; Kobayashi, K. *Bull. Chem. Soc. Japan* **1970**, *43*, 1223.

[53] Hirama, M.; Oishi, T.; Uehara, H.; Inoue, M.; Maruyama, M.; Guri, H.; Satake, M. *Science* **2001**, *294*, 1904-1907.

[54] Inoue, M.; Xing, G.; Wang, X.; Wang, J.; Hirama, M. *Org. Lett.* **2002**, *4*, 3439-3442.

[55] Kevill, D.N. In *The Chemistry of Functional Groups, Suppl. D.*, S. Patai and Z.

[56] Meffre, P.; Durand, P.; Legoffic, F. *Synthesis* **1995**, 1111.

[57] Caubere, P.; Coutrot, P. In *Comprehensive Organic Synthesis* Trost, B.M., Ed; Pergamon Press, Oxford, 1991, vol. 8, pp. 835-870.

[58] Hauptmann, H.; Walter, W.F. *Chem Rev.* **1962**, *62*, 347.

[59] Neumann, W.P. *Synthesis*, **1987**, 665.

[60] Buynak, J.D.; Rao, M.N.; Ramalakshmi, H.P.; Chandrasekaran, R.Y.; Finn, K. *J. Org. Chem.* **1985**, *50*, 4245.

[61] Nicolaou, K. C.; Duggan, M. E.; Hwang, C. K. *J. Am. Chem. Soc.* **1986**, *108*, 2468-2469.

[62] Ganem, B.; Osby, J.O. *Chem. Rev.* **1986**, *86*, 763.

[63] Russell, G.A.; Mikol, G.J. *J. Am. Chem. Soc.* **1966**, *88* 5498.

[64] Molander, G.A.;Hahn, G. *J. Org. Chem.* **1986**, *51*, 1135.

[65] Becker, S.; Fort, Y.; Vanderesse, R.; Caubere, P. *J. Org. Chem.* **1989**, *54*, 4848.

[66] Caubere, P. *Angew. Chem., Int. Ed. Engl.* **1983**, *22*, 599.

[67] Mandai, T.; Hara, K.; Nakajima, T.; Kawada, M.; Otera, J. *Tetrahedron Lett.* **1983**, *24*, 4993-4996.

[68] Gutierrez, C. G.; Stringham, R. A.; Nitasaka, T.; Glasscock, K. G. *J. Org. Chem.* **1980**, *45*, 3393-3395.

[69] Gutierrez, C. G.; Summerhays, L. R. *J. Org. Chem.* **1984**, *49*, 5206-5213.

[70] Nicolaou, K. C.; Mcgarry, D. G.; Somers, P. K.; Kim, B. H.; Ogilvie, W. W.; Yiannikouros, G.; Prasad, C. V. C.; Veale, C. A.; Hark, R. R. *J. Am. Chem. Soc.* **1990**, *112*, 6263-6276.

[71] Burnett, D. A.; Choi, J. K.; Hart, D. J.; Tsai, Y. M. *J. Am. Chem. Soc.* **1984**, *106*, 8201-8209.

[72] Nolle, A.; Mahendroo, P. *J. Chem. Phys.* **1960**, *33*, 863.

[73] Trost, B. M.; Sato, T. *J. Am. Chem. Soc.* **1985**, *107*, 719-721.

[74] Trost, B. M.; Vaultier, M.; Santiago, M. L. *J. Am. Chem. Soc.* **1980**, *102* , 7929-7932.

[75] Campigalia, P.; Gomez-Monterrey, I.; Longobardo, L.; Lama, T.; Novellino, E.; Grieco, P. *Terahedron Lett.* **2004**, *45*, 1453.

[76] Shibata, N.; Baldwin, J. E.; Jacobs, A.; Wood, M. E. *Tetrahedron* **1996**, *52*, 12839-12852.

[77] Shibata, N.; Baldwin, J.E.; Jacobs, J.; Wood, M.E. *Tetrahedron*, **1996**, *52*, 12839.

[78] Kevill, D.N. In *The Chemistry of Functional Groups, Suppl. D.*, S. Patai and Z. Rappoport, Ed. Wiley, New York, 1983, p. 933

[79] Greene, A. E.; Ledrian, C.; Crabbe, P. *J. Am. Chem. Soc.* **1980**, *102*, 7583-7584.

[80] Baxendale, I. R.; Ley, S. V.; Nessi, M.; Piutti, C. *Tetrahedron* **2002**, *58*, 6285-6304.

[81] Loh, T. P.; Feng, L. C. *Tetrahedron Lett.* **2001**, *42*, 6001-6005.

[82] Martin, S. F.; Rueger, H.; Williamson, S. A.; Grzejszczak, S. *J. Am. Chem. Soc.* **1987**, *109*, 6124-6134.

[83] Mislow, K. *J. Am. Chem. Soc.* **1951**, *73*, 4043-4044.

[84] Finch, N.; Fitt, J. J.; Hsu, I. H. S. *J. Org. Chem.* **1975**, *40*, 206-215.

[85] Chai, Y. H.; Vicic, D. A.; McIntosh, M. C. *Org. Lett.* **2003**, *5*, 1039-1042.

[86] Ham, S.; Birney, D.M. *Tetrahedron Lett.* **1994**, *35*, 8111-8116.

[87] Foland, L. D.; Decker, O. H. W.; Moore, H. W. *J. Am. Chem. Soc.* **1989**, *111*, 989-995.

[88] Pocker, Y.; Kevill, D.N. *J. Am. Chem. Soc.* **1965**, *87*, 4771.

[89] Ando, T; Yamawaki, J. *Chem. Lett.* **1979**, *1*, 45-47

[90] Bajwa, J. S.; Jiang, X. L.; Slade, J.; Prasad, K.; Repic, O.; Blacklock, T. J. *Tetrahedron Lett.* **2002**, *43*, 6709-6713.

[91] Slade, J. S.; Vivelo, J. A.; Parker, D. J.; Bajwa, J.; Liu, H.; Girgis, M.; Parker, D. T.; Repic, J.; Blacklock, T. *Org. Proc. Res. Dev.* **2005**, *9*, 608-620.

[92] Evans, P. A.; Cui, J.; Gharpure, S. J.; Polosukhin, A.; Zhang, H. R. *J. Am. Chem. Soc.* **2003**, *125*, 14702-14703.

[93] Aikins, J. A.; Haurez, M.; Rizzo, J. R.; Van Hoeck, J. P.; Brione, W.; Kestemont, J. P.; Stevens, C.; Lemair, X.; Stephenson, G. A.; Marlot, E.; Forst, M.; Houpis, I. N. *J. Org. Chem.* **2005**, *70*, 4695-4705.

[94] Iwanami, K.; Seo, H.; Tobita, Y.; Oriyama, T. *Synthesis* **2005**, 183-186.

[95] Iwanami, K.; Yano, K.; Oriyama, T. *Synthesis* **2005**, 2669-2672.

[96] Yang, W. C.; Lu, X. A.; Kulkarni, S. S.; Hung, S. C. *Tetrahedron Lett.* **2003**, *44*, 7837-7840.

[97] Chandrasekhar, S.; Chandrashekar, G.; Babu, B. N.; Vijeender, K.; Reddy, K. V. *Tetrahedron Lett.* **2004**, *45*, 5497-5499.

[98] Evans, P. A.; Cui, J.; Gharpure, S. J.; Hinkle, R. J. *J. Am. Chem. Soc.* **2003**, *125*, 11456-11457.

[99] Evans, P. A.; Andrews, W. J. *Tetrahedron Lett.* **2005**, *46*, 5625-5627.

[100] Jiang, X. L.; Bajwa, J. S.; Slade, J.; Prasad, K.; Repic, O.; Blacklock, T. J. *Tetrahedron Lett.* **2002**, *43*, 9225-9227.

[101] Corey, E. J.; Venkates, A. *J. Am. Chem. Soc.* **1972**, *94*, 6190.

[102] Omura, K.; Swern, D. *Tetrahedron* **1978**, *34*, 1651.

[103] Tidwell, T. T. *Synthesis* **1990**, 857-870.

[104] Benneche, T. *Synthesis* **1995**, 1-27.

[105] Yu, L.;Lai,Y.;Wade, J. V.;Coutts, S. M. *Tetrahedron Lett.***1998** ,*39* ,6633-6636.

[106] Jaulent, A. M.; Leatherbarrow, R. J. *Protein Eng. Des. Sel.* **2004**, *17*, 681-687.

[107] Scarpi, D.; McBride, J. D.; Leatherbarrow, R. J. *Bioorg. Med. Chem.* **2004**, *12*, 6045-6052.

[108] McBride, J. D.; Watson, E. M.; Brauer, A. B. E.; Jaulent, A. M.; Leatherbarrow, R. J. *Biopolymers* **2002**, *66*, 79-92.

[109] Brauer, A. B. E.; Kelly, G.; McBride, J. D.; Cooke, R. M.; Matthews, S. J.; Leatherbarrow, R. J. *J. Mol. Biol.* **2001**, *308*, 565-566.

[110] Miles, S. M.; Leatherbarrow, R. J.; Marsden, S. P.; Coates, W. J. *Org. Biomol. Chem.* **2004**, *2*, 281-283.

6.0 Experimental

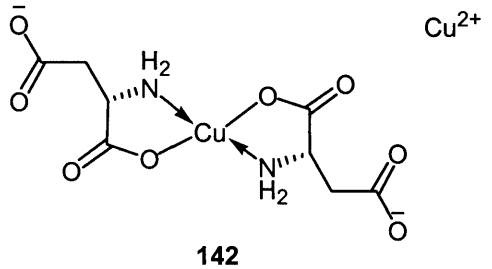
All ^{1}H NMR spectra were recorded on a Bruker 300 MHz NMR machine. ^{13}C NMR were recorded on a Bruker 75 MHz NMR machine. Low resolution mass spectrographs were recorded on a Quattro LC electrospray mass spectrometer. HRMS were recorded on a VG ZAB 2SE. IR spectrographs were recorded on a Shimadzu 8700. Optical rotations were recorded using a Optical Activity AA-100 digital polarimeter using the sodium-D line. Melting points were recorded using a Reichert hot stage apparatus and are given uncorrected.

Unless otherwise noted, solvents used were reagent grade and used without purification. The solvents THF, CH_2Cl_2, toluene and MeCN were dried using solvent drying columns. 2,6-Lutidine, diglyme, piperidine, TMG, $^{i}Pr_2EtN$, Et_3N and NMM were dried by distillation from CaH_2 under argon and stored over molecular sieves. EtOH and MeOH were distilled from Zn/I_2 under an argon atmosphere and used immediately. Acetone was distilled from activated molecular sieves onto activated molecular sieves and used immediately. DMF was purchased dry from Aldrich and stored over molecular sieves. CCl_4 and methanesulfonyl chloride were distilled from P_2O_5.

Flash column chromatography was performed using silica 60 obtained from BDH. TLC was performed using 0.25 mm thick layer Merck 60 F_{254}. Visualisation of the

spots on TLC plates was achieved using UV, phosphomolybdic acid, anisaldehyde, potassium permanganate, vanillin, ninhydrin, bromocresol green or iodine stains.

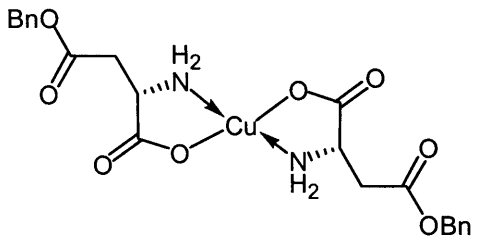
L-Aspartic acid copper (II) complex copper II salt octahydrate (142)[1]



142

A suspension of $CuCO_3$ (2.0 g, 16.2 mmol, 1.1 eq) in H_2O (230 ml) was heated to 70°C and stirred until all the solid $CuCO_3$ had dissolved. To this a solution of L-aspartic acid (2.0 g, 15.0 mmol) in H_2O (85 ml) was added dropwise over 1 hour. The resulting mixture was allowed to cool to room temperature and was stirred for a further 48 hours. The mixture was then suction filtered to isolate the desired solid. This was washed with H_2O (3 × 20 ml), EtOH (3 × 20 ml) and EtOAc (3 × 20 ml). The solid **142** was then dried in a vacuum oven 80°C / 14 mmHg for 48 hours and used crude in following reactions.

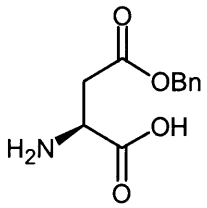
Aspartic acid 4-benzyl ester copper (II) complex (144)[1]



144

To a solution of finely powdered **143** (0.77 g, 1.99 mol) prepared above and aspartic acid (0.52 g, 3.98 mmol) in DMF (5.0ml) and H_2O (0.6 ml) stirred at room temperature was added TMG (1.0 ml, 7.96 mmol) over 30 minutes. After 2 hours further DMF (5.0ml) was added. To this mixture was added benzyl bromide (0.95 ml, 7.96 mol) and the solution stirred for 24 hours at room temperature before dilution with acetone (30 ml). The resultant mixture was stirred for 3 hours and then suction filtered. The isolated solid was washed with acetone (3 × 20 ml) and dried under vacuum in the presence of P_2O_5 for 72 hours and used crude in following reactions.

L-Aspartic acid β -benzyl ester (145)[1]



145

To a solution of $NaHCO_3$ (900 mg) in H_2O (13.0 ml) was added EDTA (2.0 g) and the mixture stirred at room temperature for 1 hour. To this was added **144** (2.0 g) in

one portion and the resulting suspension was shaken vigorously for 20 minutes, before being left to stir for 3 hours at room temperature. After dilution with acetone (10 ml) and cooling to 10°C the suspension was suction filtered. The collection flask was changed and the resulting solid was washed with H_2O (20 ml). Removal of the solvent *in vacuo* yielded white solid **145** (445 mg, 46.9%). Identical by ^1H NMR and ^{13}C NMR to lit.[2]

R_f = 0.62 (silica gel, 50% ^nBuOH, 25% AcOH, 25% H_2O)

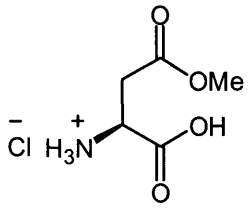
m.pt. = 216-218°C [lit m.pt. = 218°-219° C][2]

$[\alpha]_D^{21}$ = +5.15° (0.012M in H_2O) [lit $[\alpha]_D^{20}$ = +6.9° (c = 0.89 in H_2O)][2]

^1H NMR (300 MHz, DMSO-d_6) δ 7.35 (5H, m, Ar), 5.07 (2H, s, CH_2Ph), 3.48 (1H, m, CH α), 2.88 (1H, dd, J = 7.6Hz, CHHβ), 2.58 (1H, dd, J = 5.2, 16.8 Hz, CHH β), 2.47 (2H, s, NH_2)

^{13}C NMR (75 MHz, DMSO-d_6) δ 175.2 (*C*O Acid), 172.5 (*C*O Ester), 142.1 (*C*q Ar), 128.9 (*C*-H Ar), 127.4 (*C*-H Ar), 127.1 (*C*-H Ar), 73.1 (*C*H_2 COOC$H_2C_6H_5$), 55.3 (*C*H α), 36.9 (*C*H_2 β)

L-Aspartic acid β-methyl ester (146)[3]



146

A solution of L-aspartic acid (2.0 g, 15.0 mmol) in dry MeOH (10.0 ml) at –10°C was treated with dropwise addition of $SOCl_2$ (1.2ml, 16.8 mmol, 1.1eq) over 10 minutes. After 20 minutes at –10°C the mixture was warmed to room temperature and stirred for a further 25 minutes. Upon addition of Et_2O (50 ml) white crystals appeared. After cooling to 0°C and vigorous shaking the solid was removed by suction filtration. The resulting residue was recrystalised twice from Et_2O/MeOH with addition of a few drops of 1N HCl to yield pure white solid **146** (1.65 g, 60%). Identical by ^1H NMR and ^{13}C NMR to lit.[3]

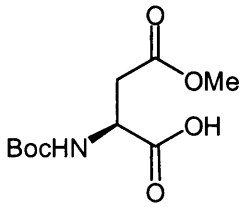
R_f = 0.51 (silica gel, 50% ^{n}BuOH, 25% AcOH, 25% H_2O)

m.pt. = 189-191°C dec [lit. m.pt. = 193°C][3]

^1H NMR (300 MHz, D_2O) δ 4.17 (1H, t , J = 5.7 Hz, C*H* α), 3.64 (3H, s, COOC*H*$_3$), 3.11 (2H, d, J = 5.6 Hz, C*H*$_2$ *β*)

^{13}C NMR (75 MHz, D_2O) δ 172.1 (*C*O Acid), 169.8 (*C*O Ester), 54.5 (*C*H α), 49.7 (O*C*H_3), 34.2 (*C*H_2 *β)*

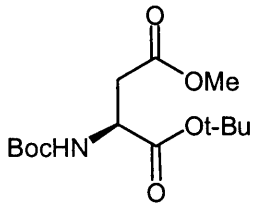
<u>*N*-α-Boc-L-aspartic acid β-methyl ester (147)</u>



147

A solution of **146** (24.0 g, 0.131 mol) in THF (200 ml) and H_2O (100 ml) at 0°C was treated with Na_2CO_3 (13.8 g, 0.131 mol, 1 eq). After effervescence of CO_2, further Na_2CO_3 (13.8 g, 0.131 mol, 1 eq) was added, followed by careful addition of $(Boc)_2O$ (31.5 g, 0.144 mol, 1.1 eq). The mixture was warmed to room temperature and stirred for 48 hours, before removal of THF *in vacuo*. The resulting mixture was poured onto ice/water and extracted with Et_2O (100 ml) to remove non-acidic impurities. The aqueous layer was then acidified to pH 2.0 with 0.1M HCl and extracted with diethyl ether (3 × 150 ml). The combined ether extracts were washed with saturated brine and dried ($NaSO_4$). The solvent was removed *in vacuo* to yield **147** as an oil which was used crude in the following step.

<u>*N*-α-Boc-L-aspartic acid β-methyl ester ^{t}butyl ester (148)</u>[4]



148

To a solution of crude **147** (0.131 mol) in dry CH_2Cl_2 (250 ml) at 0°C was added DMAP (1.20 g, 9.83 mmol, 0.07 eq) followed by ^{t}BuOH (13.0 ml, 0.144 mol, 1.1 eq) and DCC (32.3 g, 0.157 mol, 1.2 eq). The mixture was warmed to room temperature and stirred for 48 hours, before being filtered and washed with CH_2Cl_2 (80 ml) to remove DCU. The resulting solution was washed with saturated aqueous $NaHCO_3$ (3 × 150 ml), saturated brine (2 × 150 ml), dried (Na_2SO_4) and concentrated *in vacuo*. The residual oil was purified by flash column chromatography (silica gel, 5% EtOAc in CH_2Cl_2) to provide **148** as a solid (28.1 g, 71%).

R_f = 0.55 (silica gel, 5% EtOAc in CH_2Cl_2)

m.pt. = 65°-69°C

$[\alpha]_D^{22}$: -39.1° (c = 0.012M in CH_2Cl_2)

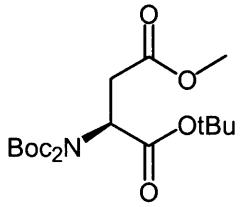
IR ν_{max}; 3435 (N-H), 2923 (C-H), 1751, 1728 (C=O)

^{1}H NMR (300 MHz, $CDCl_3$) δ 5.40 (1H, br m, N*H*), 4.41 (1H, br m, C*H* α), 3.59 (3H, s, OCH_3), 2.88 (1H, dd, *J* = 16.5, 4.5 Hz, C*H*H β), 2.71 (1H, dd, *J* = 16.5, 5.0 Hz, CH*H* β), 1.43 (9H, s, NCOOC(CH_3)$_3$), 1.41 (9H, s, NCOOC(CH_3)$_3$)

^{13}C NMR (75 MHz, $CDCl_3$) δ 171.6 (*C*O ^{t}Butyl), 170.3 (*C*O Methyl), 158.3 (*C*O Boc), 82.7 (OCO*C*(CH_3)$_3$), 80.2 (NCOO*C*(CH_3)$_3$), 52.1 (*C*H α), 51.0 (O*C*H_3), 37.4 (*C*H_2 β), 28.7 (*C*H_3 ^{t} OCOC(*C*H_3)$_3$), 28.2 (NCOOC(*C*H_3)$_3$)

HRMS $C_{14}H_{26}NO_6$ calcd. for $[M + H]^+$ 304.17600 found 304.17712.

<u>*N*-α-DiBoc-L-aspartic acid β-methyl ester ^{t}butyl ester (149)</u>[5]



149

To a solution of **148** (200 mg, 0.660 mmol) in dry MeCN (5.0 ml) was added DMAP (16 mg, 0.132 mmol, 0.2 eq) and $(Boc)_2O$ (576 mg, 2.64 mmol, 4 eq). After stirring for 24 hours at room temperature the solvent was removed *in vacuo*. The residual oil was taken up in EtOAc (35 ml) and washed with 0.01M $KHSO_4$ (2 × 35 ml),

saturated aqueous $NaHCO_3$ (2 × 35 ml), saturated brine (3 × 35 ml), dried (Na_2SO_4) and concentrated *in vacuo*. The residual oil was purified by flash column chromatography (silica gel, 20% Et_2O in hexane) to provide yellow solid **149** (160 mg, 60%). Identical by ^1H NMR, ^{13}C NMR and IR to lit.[4]

R_f = 0.45 (silica gel, 20% Et_2O in hexane)

m.pt. = 63 - 66° C [lit. m.pt. = 64°- 66°C][4]

$[\alpha]_D^{19}$: -72.1° (c = 0.012M in CH_2Cl_2) [lit. $[\alpha]_D^{25}$: -13.0° (c = 2.01 in CH_2Cl_2)][4]

IR ν_{max} (Nujol, cm^{-1}) = 2952, 2922, (C-H), 1741 (C=O)

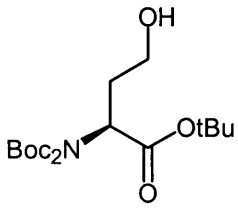
^1H NMR (300 MHz, $CDCl_3$) δ 5.33 (1H, dd, *J* = 7.2, 6.4 Hz, C*H* α), 3.69 (3H, s, COOCH_3), 3.21 (1H, dd, *J* = 16.5, 7.2 Hz C*H*H β), 2.67 (1H, dd, *J* = 16.5, 6.3 Hz CH*H* β), 1.50 (18H, s, 2 × NCOOC(CH_3)$_3$), 1.43 (9H, s, OCOC(CH_3)$_3$)

^{13}C NMR (75 MHz, $CDCl_3$) δ 171.4 (*C*O ^{t}Bu), 168.7 (*C*O Me), 152.0 (CO Boc), 83.2 (OCO*C*(CH$_3$)$_3$), 82.0 (NCOO*C*(CH$_3$)$_3$), 55.6 (*C*H α), 51.9 (COO*C*H$_3$), 35.5 (*C*H$_2$ *β*), 28.0 (OCOC(*C*H$_3$)$_3$), 27.9 (NCOOC(*C*H$_3$)$_3$)

ES+ $C_{18}H_{33}NO_7Na$ *m/z* $[M + Na]^+$ 426, [M – Boc + Na] 326.

HRMS $C_{19}H_{33}NO_8Na$ calcd. for $[M + Na]^+$ 426.21038 found 426.20902.

<u>*N*-α-Di-t.Boc-L-Homoserine t.-butyl ester (150)</u>[4]



150

To a solution of **149** (100 mg, 0.248 mmol) in dry CH_2Cl_2 (8.0 ml) at –78°C was added DIBAL (1.0 M solution in toluene, 520 µl, 0.521 mmol, 2.1 eq) slowly over 8 minutes. The reaction mixture was stirred for 1 hour before being quenched with acetone (5.0 ml) and then H_2O (1.0 ml), allowed to warm to room temperature, dried (Na_2SO_4) and filtered through Celite. The solvent was removed *in vacuo* and the residual oil purified by flash column chromatography (silica gel, 20% EtOAc in hexane) to yield a thick colorless oil **150** (91 mg, 98%). Identical by ^1H NMR, ^{13}C NMR and IR to lit.[4]

R_f = 0.30 (silica gel, 20% EtOAc in hexane)

$[\alpha]_D^{22}$: -23.3° (c = 0.010M in CH_2Cl_2) [lit. $[\alpha]_D^{25}$: -13.0° (c = 2.01 in CH_2Cl_2)][4]

IR ν_{max} (neat, cm^{-1}) = 3543 (O-H), 2979, 2935, (C-H), 1789, 1747 (C=O)

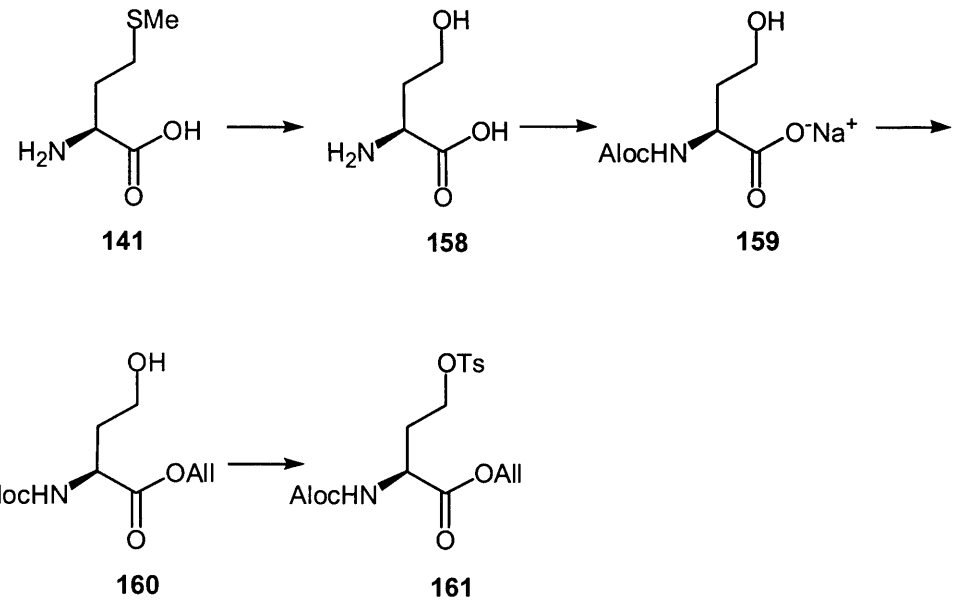
^1H NMR (300 MHz, $CDCl_3$) δ 4.90 (1H, dd, *J* = 9.5, 4.9 Hz, C*H* α), 3.68 (2H, m, CH_2 γ), 2.30 (1H, m, CH_2 β), 1.95 (1H, m, CH_2 β), 1.46 (18H, s, 2 × NCOOC(CH_3)$_3$), 1.39 (9H, s, OCOC(CH_3)$_3$)

^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.9 (*C*O ^{t}Bu), 153.0 (CO Boc), 83.6 (*C*q ^{t}Bu), 81.9 (*C*q Boc), 59.8 (*C*H$_2$ γ), 56.9 (*C*H α), 32.9 (*C*H$_2$ *β*), 28.4 (OCOC(*C*H$_3$)$_3$), 28.3 (NCOOC(*C*H$_3$)$_3$)

ES+ $C_{18}H_{33}NO_7Na$ *m/z* $[M + Na]^+$ 398.

HRMS $C_{18}H_{33}NO_7Na$ calcd. for $[M+Na]^+$ 398.21546 found 398.21600.

<u>*N*-α-Aloc-L-Homoserine allyl ester tosylate (161)</u>[6]



A solution of L-methionine (2.0 g, 13.4 mmol) in H_2O (131 ml) and MeOH (26.0 ml) was stirred at –10°C and was then treated with MeI (2.5 ml, 40.2 mmol, 3 eq). The reaction mixture was warmed to room temperature and stirred for 24 hours. The solvent was then concentrated *in vacuo*, until approximately 50 ml remained. To this was added $NaHCO_3$ (1.13 g, 13.4 mmol, 1 eq), the resulting solution was heated at reflux for 4 hours. The solution was cooled to room temperature and **158** was used unpurified in the next reaction.

A solution of crude **158** (13.4 mmol) in H_2O (50.0 ml) was cooled to 0°C and $NaHCO_3$ (4.50 g, 53.6 mmol, 4 eq) was added. This was followed by dropwise addition of a solution of allyl chloroformate (2.8 ml, 26.8 mmol, 2 eq) in dioxane (50.0 ml) over a period of 10 minutes. The resulting mixture was stirred at 5°C for 48

hours. The solvents were then removed *in vacuo* and the crude solid **159** was used in the next reaction without purification.

To a solution of crude **159** (13.4 mmol) in DMF (40.0 ml) was added allyl bromide (0.57 ml, 13.5 mmol, 1.01 eq). The resulting mixture was stirred for 60 hours at room temperature before the solvent was removed in vacuo. The residual oil was dissolved in CH_2Cl_2 (50 ml), washed with saturated aqueous $NaHCO_3$ (3 × 50 ml), dried ($MgSO_4$) and concentrated *in vacuo*. The crude oil **160** was taken into the next step without purification.

Crude **160** (13.4 mmol), was dissolved in dry CH_2Cl_2 (35.0 ml) at 0°C and tosyl chloride (3.07 g, 16.0 mmol, 2 eq) was added. Dry Et_3N (6.0 ml) was then added and the mixture was stirred for 3 hours at room temperature. The solution was then washed with 0.05M aqueous citric acid (3 × 50 ml), aqueous saturated $NaHCO_3$ (3 × 100 ml), dried (Na_2SO_4) and evaporated to give a colorless oil. This was purified by flash column chromatography (silica gel, 80% CH_2Cl_2 in hexane) to isolate a collection of spots. Further purification by flash column chromatography (silica gel, 40% EtOAc in hexane) provided pure brown oil **161** (585 mg, 6%).

$R_f = 0.5$ (silica gel, 40% EtOAc in hexane)

$[\alpha]^{20}_D = -15.1^\circ$ (c = 0.015M in CH_2Cl_2)

IR ν_{max} (neat, cm^{-1}) = 3327 (N-H), 2972, 2947 (C-H), 1647 (C=C), 1596 (C=C, Ar)

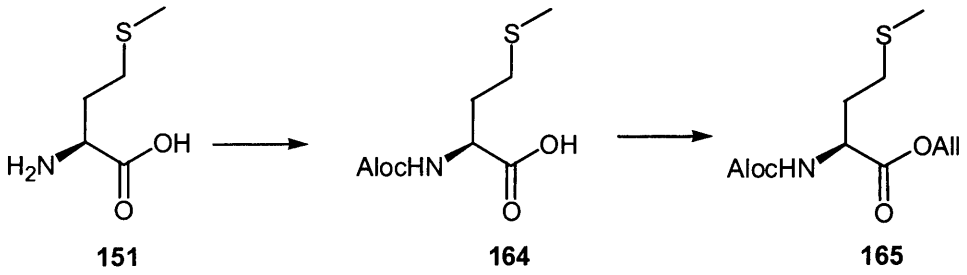
^{1}H NMR (300 MHz,$CDCl_3$) δ 7.63 (2H, d, *J* = 8.3 Hz, C*H* Ar), 7.20 (2H, d, *J* = 8.1 Hz, C*H* Ar), 5.7-5.8 (2H, m, 2 × CH_2CH=CH_2), 5.1-5.2 (4H, m, 2 × CH_2CH=CH_2), 4.5 (4H, m, 4H, 2 × CH_2CH=CH_2), 4.49 (1H, m, C*H* α), 3.97 (2H, t, *J* = 5.8 Hz, CH_2 γ), 2.30 (3H, s, CH_3Ar), 2.01-2.15 (2H, m, CH_2 β).

^{13}C NMR (75 MHz , $CDCl_3$) δ 171.3 (*C*O Allyl) 156.8 (*C*O Alloc), 145.3 (CH_3*C*Ar), 133.1 (SO_2*C*Ar), 132.8 (CH_2*C*H=CH_2), 131.7 (CH_2*C*H=CH_2), 130.3 (*C*H Ar), 128.4 (*C*H Ar), 119.6 (CH_2CH=*C*H_2), 118.3 (CH_2CH=*C*H_2), 66.8 (*C*H_2CH=CH_2), 66.4 (*C*H_2CH=CH_2), 53.8 (*C*H_2 γ), 51.4 (*C*H α), 31.8 (*C*H_2 β), 22.0 (*C*H_3Ar)

ES+ $C_{18}H_{23}NO_7S$ *m/z* $[M + Na]^+$ 420.

HRMS $C_{18}H_{23}NO_7SNa$ calcd. for $[M+Na]^+$ 420.10929 found 420.10797.

<u>*N*-α-Aloc-L-Methionine allyl ester (165)</u>



151 **164** **165**

To a stirred solution of L-methionine (5.0 g, 33.5 mmol) and Na_2CO_3 (3.55 g, 33.5 mmol, 1 eq) in THF:H_2O (1:1, 30.0 ml) at 0°C was added allyl chloroformate (3.6ml, 33.5 mmol, 1 eq) dropwise over 20 minutes. After slow warming to room temperature the mixture was stirred for 24 hours. The solution was then acidified to pH 2 with 1M HCl at 0°C and extracted with EtOAc (5 × 50 ml), dried (Na_2SO_4) and the solvent removed *in vacuo*. The remaining white solid **164** was used without further purification

To a solution of **164** (7.90 g, 33.5 mmol) in dry MeOH (20.0 ml) was added K_2CO_3 (4.62 g, 33.5 mmol, 1 eq). After 5 minutes of agitation the solution was concentrated *in vacuo* to yield a white solid which was subsequently taken up in dry DMF (8 ml). To this was added allyl bromide (2.83 ml, 33.5 mmol, 1 eq) and the mixture was stirred overnight at room temperature under an Argon atmosphere. The solvent was removed *in vacuo* and the residual solid taken up in CH_2Cl_2, washed with saturated aqueous $NaHCO_3$ (3 × 100 ml), saturated brine (3 × 100 ml) and dried (Na_2SO_4). Upon solvent evaporation the residual oil was purified by flash column chromatography (silica gel, 30% EtOAc in Hexane) to provide clear yellow oil **165** (3.24 g, 65%) over two steps.

R_f = 0.50 (silica gel, 20% EtOAc in Hexane)

$[\alpha]^{20}_D$ = +98.0° (c = 0.015 M in CH_2Cl_2)

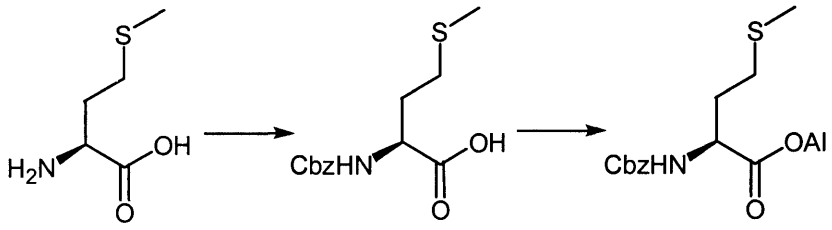
IR ν_{max} (neat, cm^{-1}) = 3357 (N-H), 2950, 2900 (C-H), 1751 (C=O), 1647 (C=C)

^{1}H NMR (300 MHz, $CDCl_3$) δ 5.87 (2H, m, CH_2CH=CH_2), 5.53 (1H, br s, N*H*), 5.2 (4H, m, CH_2CH=CH_2), 4.59 (2H, d, *J* = 5.7 Hz, CH_2CH=CH_2), 4.52 (2H, d, *J* = 5.7 Hz, CH_2CH=CH_2), 4.44 (1H, br. m, C*H* α), 2.50 (2H, t, *J* = 7.5 Hz, CH_2 γ), 2.13 (1H, m, CH_3 β), 2.05 (3H, s, SCH_3), 1.97 (1H, m, CH_3 β)

^{13}C NMR (75 MHz, $CDCl_3$) δ 171.7 (*C*O Allyl), 155.8 (*C*O Alloc), 132.6 (CH_2CH=CH_2), 131.5 (CH_2CH=CH_2), 119.0 (CH_2CH=CH_2), 117.8 (CH_2CH=CH_2), 66.1 ($OCOCH_2CH$=CH_2) 65.8 (CH_2CH=CH_2), 53.2 (*C*H α), 31.9 (CH_2 β), 29.9 (CH_2 γ), 15.4 (SCH_3)

HRMS $C_{12}H_{20}NO_4S$ calcd. for $[M+H]^+$ 274.1113 found 274.1153.

<u>*N*-α-Cbz-L-Methionine allyl ester (169)</u>



151 **168** **169**

To a stirred solution of L-methionine (10.0 g, 67.0 mmol) and Na_2CO_3 (10.6 g, 0.10 mol, 1.5 eq) in THF:H_2O (1:1, 30.0 ml) at 0°C was added benzyl chloroformate (11.5 ml, 80.4 mmol, 1.2 eq) dropwise over 20 minutes. After slow warming to room

temperature the mixture was stirred for 24 hours. The solution was then acidified to pH 2.5 with 0.01 M HCl at 0°C and extracted with EtOAc (5 × 50 ml), dried (Na_2SO_4) and the solvent removed *in vacuo*. The remaining solid **168** was used without further purification

To a solution of **168** (19.0 g, 67.2 mmol) in dry MeOH (30.0 ml) was added K_2CO_3 (9.28 g, 67.2 mmol, 1 eq). After 5 minutes of agitation the solution was concentrated in vacuo to yield a white solid which was subsequently taken up in dry DMF (15 ml). To this was added allyl bromide (5.69 ml, 67.2 mmol, 1 eq) and the mixture was stirred overnight at room temperature under an argon atmosphere. The solvent was removed *in vacuo* and the residual solid taken up in CH_2Cl_2, washed with saturated aqueous $NaHCO_3$ (3 × 100 ml), saturated brine (3 × 100 ml) and dried (Na_2SO_4). Upon solvent evaporation the residual oil was purified by flash column chromatography (silica gel, 30% EtOAc in hexane) to provide **169** as a clear yellow oil (13.9 g, 64%) over two steps.

R_f = 0.50 (silica gel, 20% EtOAc in Hexane)

$[\alpha]^{20}_D$ = +79.7° (c = 0.013 M in CH_2Cl_2)

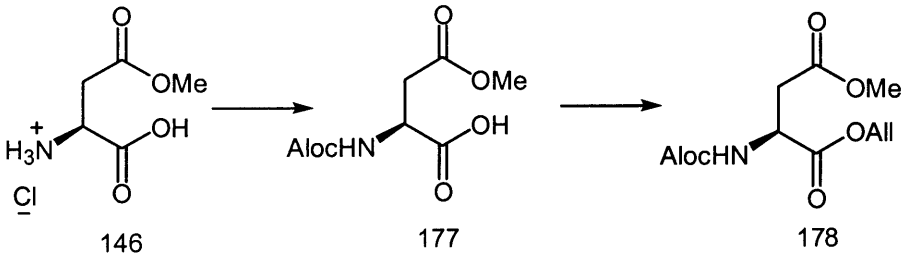
IR ν_{max} (neat, cm^{-1}) = 3342 (N-H), 2947, 2918 (C-H), 1728 (C=O), 1662 (C=C), 1525 (C=C, Ar)

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.29 (5H, m, C*H* Ar) 5.90 (1H, m, CH_2CH=CH_2), 5.33 (3H, m, N*H*, CH_2CH=CH_2), 5.23 (2H, s, NCOOCH_2Ar), 4.63 (2H, m, CH_2CH=CH_2), 4.48 (1H, br m, C*H* α), 2.52 (2H, t, *J* = 6.3 Hz, CH_2 γ), 2.15 (1H, m, C*H*H β), 2.06 (3H, s, SCH_3), 2.02 (1H, m, CH*H* β)

^{13}C NMR (75 MHz , $CDCl_3$) δ 171.4 (*C*O Allyl), 155.8 (*C*O Cbz), 136.5 (*C*q Ar Cbz), 131.6 (CH_2CH=CH_2), 128.4 (*C*H Ar), 128.0 (*C*H Ar), 127.9 (*C*H Ar), 118.7 (CH_2CH=CH_2), 67.0 (*C*H_2CH=CH_2) 65.9 (COO*C*H_2Ar), 53.5 (*C*H α), 32.2 (*C*H_2 β), 30.0 (*C*H_2 γ), 15.3 (S*C*H_3)

HRMS $C_{16}H_{22}NO_4S$ calcd. for $[M+H]^+$ 324.26950 found 324.27119.

<u>*N*-α-Aloc-L-Aspartic acid β-methyl ester allyl ester (178)</u>



146 177 178

To a stirred solution of acid **146** (800 mg, 4.37 mmol) and $NaHCO_3$ (1.83 g, 21.9 mmol, 5 eq) in THF:H_2O (1:1, 10.0 ml) at 0°C was added allyl chloroformate (510 μl,

4.81 mmol, 1.1 eq) dropwise over 5 minutes. The solution was slowly warmed to room temperature and after 24 hours was complete by TLC. The THF was removed *in vacuo* and the resulting aqueous solution was acidified to pH 2 with $KHSO_4$ and washed with EtOAc (5 × 50 ml). The organic layer was then washed with brine (3 × 50 ml), dried (Na_2SO_4) and evaporated to give thick colourless oil **177** which was used without any further purification.

R_f = 0.9 (silica gel, n-butanol:acetic acid:water, 2:1:1)

Acid **177** (1.01 g, 4.37 mmol) was dissolved in dry MeOH (10.0 ml) and $NaHCO_3$ (403 mg, 4.81 mmol, 1.1 eq) was added. After 5 minutes of agitation the solution was concentrated in vacuo to yield a white solid which was subsequently taken up in dry DMF (3.0 ml). To this was added allyl bromide (407 µl, 4.81 mmol, 1.1 eq) and the mixture was stirred overnight at room temperature under an argon atmosphere. The solution was then diluted with CH_2Cl_2 (100 ml) and washed with saturated aqueous $NaHCO_3$ (3 × 100 ml). The aqueous layer was then extracted with CH_2Cl_2 (3 × 50 ml) before the organic layer was washed with brine (3 × 50 ml), dried (Na_2SO_4) and concentrated *in vacuo* to yield a clear colourless oil. This was purified by flash column chromatography (silica gel, 10% EtOAc, 10% CH_2Cl_2, 80% hexane) to yield clear yellow oil **178** (890 mg, 55% over two steps).

R_f = 0.64 (silica gel, 40% EtOAc in Hexane)

$[\alpha]^{19}_D = +72.4°$ (0.016M in CH_2Cl_2)

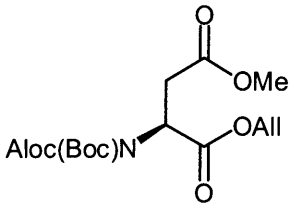
IR ν_{max} (neat, cm^{-1}) = 3356 (N-H), 2952 (C-H), 1732, 1739 (C=O), 1647 (C=C)

^1H NMR (300 MHz, $CDCl_3$) δ 5.96 - 5.84 (2H, m, CH_2CH=CH_2), 5.83 (1H, br s, N*H*), 5.34 - 5.18 (4H, m, 2 × CH_2CH=CH_2), 4.64 - 4.63 (m, 5H, 2 × CH_2CH=CH_2, C*H* α), 3.67 (3H, s, OCH_3), 3.0 (1H, dd, *J* = 16.9, 4.7 Hz, C*H*H β), 2.8 (1H, dd, *J* = 16.9, 4.7 Hz, CH*H* β)

^{13}C NMR (75 MHz , $CDCl_3$) δ 171.1 (*C*O Allyl) 170.3 (*C*O Methyl), 155.8 (*C*O Alloc), 132.5 (CH_2CH=CH_2), 131.5 (CH_2CH=CH_2), 118.8 (CH_2CH=CH_2), 117.8 (CH_2CH=CH_2), 66.3 (CH_2CH=CH_2), 65.9 (CH_2CH=CH_2), 52.0 ($COOCH_3$), 50.5 (*C*H α), 36.5 (CH_2 β)

HRMS $C_{12}H_{18}NO_6$ calcd. for $[M+H]^+$ 272.11341 found 272.11262.

<u>*N*-α-Boc-Aloc-L-Aspartic acid β-methyl ester allyl ester (174)</u>[5]



174

To a solution of **178** (100 mg, 0.369 mmol) in dry MeCN (5.0 ml) was added DMAP (45mg, 0.0738 mmol, 0.02 eq) and $(Boc)_2O$ (323 mg, 1.48 mmol, 4 eq). After stirring for 24 hours at room temperature the solvent was removed *in vacuo*. The residual oil was taken up in CH_2Cl_2 (30 ml) and washed with 1M $KHSO_4$(3 × 50 ml), saturated aqueous $NaHCO_3$ (3 × 50ml), saturated brine (3 × 50 ml), dried (Na_2SO_4) and concentrated *in vacuo*. The residual oil was purified by flash column chromatography (silica gel, 20% EtOAc, 10% CH_2Cl_2, 70% hexane) to provide **174** clear yellow oil (134 mg, 98%).

R_f = 0.45 (silica gel, 40% EtOAc in Hexane)

$[\alpha]^{19}_D$ = -62.4° (c = 0.014M in CH_2Cl_2)

IR ν_{max} (neat, cm^{-1}) = 2981, 2952 (C-H), 1747 (C=O), 1649 (C=C)

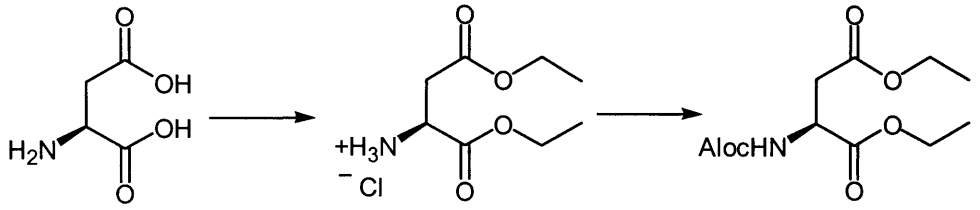
^1H NMR (300 MHz, $CDCl_3$) δ 5.85 (2H, m, 2 × CH_2CH=CH_2), 5.49 (1H, t, *J* = 7.0, C*H* α), 5.29 – 5.18 (4H, m, 2 × CH_2CH=CH_2), 4.69 – 4.67 (4H, m, 2 × CH_2CH=CH_2), 3.66 (3H, s, OCH_3), 3.24 (1H, dd, *J* = 16.5, 7.0 Hz, C*H*H β), 2.75 (1H, dd, *J* = 16.5, 7.0 Hz, CH*H* β)

^{13}C NMR (75 MHz, $CDCl_3$) δ 170.9 (*C*O Allyl), 169.1 (*C*O Methyl), 153.3 (N*C*(O)O), 150.9 (*C*O N*C*(O)O), 131.4 (CH_2CH=CH_2), 131.3 (CH_2CH=CH_2), 118.9

($CH_2CH{=}CH_2$), 118.6 ($CH_2CH{=}CH_2$), 84.1 ($C(CH_3)_3$), 67.9 ($CH_2CH{=}CH_2$), 66.2 ($CH_2CH{=}CH_2$), 55.2 (CH α), 50.9 ($COOCH_3$), 35.4 (CH_2 β), 27.9 ($C(CH_3)_3$)

HRMS $C_{17}H_{26}NO_8$ calcd. for $[M+H]^+$ 372.16583 found 372.16448.

<u>*N*-α-Aloc-L-Aspartic acid di-ethyl ester (191)</u>



140 190 191

To a solution of L-aspartic acid (5.0 g, 37.6 mmol) in dry EtOH (20.0 ml) was added $SOCl_2$ (10.9 ml, 15.2 mmol, 0.4 eq) dropwise over a period of 30 minutes at 0°C. The mixture was warmed to room temperature and stirred for 2 hours, before being basified to pH 5.0 by slow addition of saturated aqueous $NaHCO_3$ and H_2O. Removal of the solvent *in vacuo* yielded crude solid **190** which was used unpurified in the next reaction.[7]

ES+ $C_8H_{15}NO_4Na$ *m/z* $[M + Na]^+$ 212.

HRMS $C_8H_{16}NO_4$ calcd. for $[M+H]^+$ 190.10793 found 190.10812.

To a solution of crude **190** (37.6 mmol) in MeCN (35.0 ml) and H_2O (70.0 ml) was added Na_2CO_3 (3.99 g, 37.6 mmol, 1 eq) and allyl chloroformate (3.99 ml, 37.6 mmol, 1 eq). The mixture was stirred at room temperature for 24 hours before the solvents were removed *in vacuo*. The residual oil was dissolved in CH_2Cl_2 (50 ml). The organic layer was then washed with saturated aqueous citric acid (2 × 50 ml), saturated aqueous $NaHCO_3$ (2 × 50 ml), saturated brine (2 × 50 ml), dried (Na_2SO_4) and concentrated *in vacuo*. The residual oil was purified by flash column chromatography (silica gel, 6% EtOAc in CH_2Cl_2) to yield clear colourless oil **191** (7.1 g, 69.3%) over two steps.

R_f = 0.45 (silica gel, 5% EtOAc in CH_2Cl_2);

$[\alpha]^{20}_D$ = +65.7° (c = 0.025 M in CH_2Cl_2)

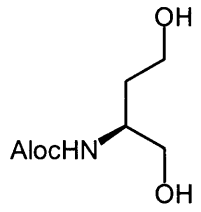
IR ν_{max} (neat, cm^{-1}) = 3358 (N-H), 2984, 2941 (C-H), 1732 (C=O), 1647 (C=C)

^{1}H NMR (300 MHz, $CDCl_3$) δ 5.91 (1H, m, CH_2CH=CH_2), 5.83 (2H, d, *J* = 5.5 Hz, N*H*), 5.25 (2H, m, CH_2CH=CH_2), 4.57 (3H, m, CH_2CH=CH_2, C*H* α), 4.14 (4H, m, 2 × CH_2CH_3), 2.95 (1H, dd, *J* = 17.0, 4.6Hz, C*H*H β), 2.78 (1H, dd, *J* = 17.0, 4.7 Hz, CH*H* β), 1.22 (6H, m, CH_2CH_3)

^{13}C NMR (75 MHz, $CDCl_3$) δ 170.7 (2 × *C*O Ethyl), 155.8 (CO Aloc), 132.6 ($NCOCH_2CH{=}CH_2$), 117.8 ($NCOCH_2CH{=}CH_2$), 65.9 ($NCOCH_2CH{=}CH_2$), 61.8(CH_2CH_3), 61.0 (CH_2CH_3), 50.4 (*C*H α), 36.7 (CH_2 β), 14.0 (2 × CH_2CH_3)

HRMS $C_{12}H_{20}NO_6$ calcd. for $[M+H]^+$ 274.12906 found 274.12841.

2-(S)-[[(allyloxycarbonyl]amino]-1,4-butanediol (186)



186

To a solution of $NaBH_4$ (280 mg, 7.40 mmol, 2 eq) in dry diglyme (10.0 ml) was added LiBr (643 mg, 7.40 mmol, 2 eq) and the milky solution stirred for 0.5 hours. Diester **191** (1.0 g, 3.66 mmol) dissolved in dry diglyme (10.0 ml) was then added dropwise over 10 minutes. The resulting mixture was stirred at room temperature for 3.5 hours. The reaction was quenched with acetone (5.0 ml) followed by H_2O (5.0 ml) and the solvent removed *in vacuo* to give a oil which was purified by flash column chromatography (silica gel, 10% MeOH in $CHCl_3$) to give clear colourless oil **186** (994 mg, 71%).[8]

R_f = 0.15 (silica gel, 4% MeOH in CH_2Cl_2)

$[\alpha]^{20}_{D}$ = -72.3° (0.041 M in CH_2Cl_2)

IR ν_{max} (neat, cm^{-1}) = 3606 (O-H), 3346 (N-H), 3009, 2977, (C-H), 1760 (C=O), 1651 (C=C)

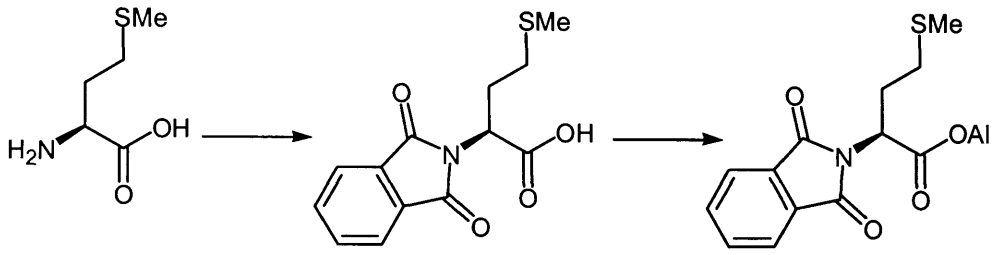
^{1}H NMR (300 MHz, $CDCl_3$) δ 5.89 (1H, m, CH_2CH=CH_2), 5.78 (1H, br. d, *J* = 8.3 Hz, N*H*) 5.26 (2H, m, CH_2CH=CH_2), 4.55 (2H, d, *J* = 5.6 Hz, CH_2CH=CH_2), 3.85 (1H, br m, C*H* α), 3.68 (4H, m, CH_2OH), 1.81 (1H, m, CH_2CH_2OH),1.67 (1H, m, CH_2CH_2OH)

^{13}C NMR (75 MHz, $CDCl_3$) δ 158.7 (*C*O Alloc), 133.2 (CH_2CH=CH_2), 117.6 (CH_2CH=CH_2), 66.1 (CH_2CH=CH_2), 64.3 (NCHCH_2OH), 58.3 (CH_2CH_2OH), 50.3 (N*C*H), 33.4 (CH_2CH_2OH)

ES+ $C_8H_{15}NO_6Li$ *m/z* $[M + Li]^+$ 196, $[M + Na]^+$ 212.

HRMS $C_8H_{16}NO_4$ calcd. for $[M+H]^+$ 190.10793 found 190.10778.

<u>*N*-α-Phthalyl-L-methionine allyl ester (195)</u>



141 201 195

To a solution of L-methionine (1.0 g, 6.70 mmol) in dry DMF (20.0ml) was added phthalic anhydride (0.99 g, 6.70 mmol, 1 eq). The flask was then heated in a 600 W commercial kitchen microwave on full power for 20 seconds with subsequent stirring. This was repeated until there was no more solid phthalic anhydride remaining. The solvent was removed *in vacuo* and crude yellow oil **201** was used without further purification.

Acid **201** (1.86 g, 6.67 mmol) was dissolved in dry MeOH (5.0 ml) and $NaHCO_3$ (617 mg, 7.33 mmol, 1.1 eq) was added. After 5 minutes of agitation the solution was concentrated *in vacuo* to yield a white solid which was subsequently taken up in dry DMF (3.0 ml). To this was added allyl bromide (564 µl, 7.33 mmol, 1.1 eq) and the mixture was stirred overnight at room temperature under an argon atmosphere. The solution was then diluted with CH_2Cl_2 (100 ml) and washed with saturated aqueous $NaHCO_3$ (3 × 100 ml). The aqueous layer was then extracted with CH_2Cl_2 (3 × 50 ml) before the organic layer was washed with brine (3 × 50 ml), dried (Na_2SO_4) and concentrated *in vacuo* to yield a clear colourless oil. This was purified by flash column chromatography (silica gel, 30% EtOAc in hexane) to yield clear yellow oil **195** (1.32 g, 62% over two steps).

R_f = 0.45 (silica gel, 30% EtOAc in hexane)

$[\alpha]^{20}_D$ = 26.1° (c = 0.011M in CH_2Cl_2)

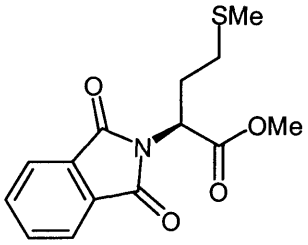
IR ν_{max} (neat, cm^{-1}) = 2918 (C-H), 1716 (C=O), 1647 (C=C), 1525 (C=C, Ar)

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.84 (2H, m C*H* Ar), 7.75 (2H, m, C*H* Ar), 5.88 – 5.79 (1H, m, CH_2CH=CH_2), 5.28 – 5.09 (3H, m, CH_2CH=CH_2, C*H* α), 4.62 (2H, m, CH_2CH=CH_2), 2.60 – 2.20 (4H, br m, CH_2 β, CH_2 γ), 2.06 (3H, s, SCH_3)

^{13}C NMR (75 MHz, $CDCl_3$) δ 168.8 (*C*O Allyl), 167.6 (*C*O Phthalyl), 134.3 (*C*q Ar), 131.8 (*C*H Ar), 131.4 (CH_2CH=CH_2), 123.6 (*C*H Ar), 118.7 (CH_2CH=CH_2), 66.4 (CH_2CH=CH_2), 51.0 (*C*H α), 30.9 (CH_2 β), 28.0 (CH_2 γ), 15.4 (SCH_3)

HRMS $C_{16}H_{18}NO_4S$ calcd. for $[M+H]^+$ 320.09565 found 320.09518.

<u>*N*-α-Phthalyl-L-methionine methyl ester (258)</u>[9]



258

To a solution of **201** (17.0 mmol) in dry MeOH (25.0 ml) was added K_2CO_3 (2.35 g, 17.0 mmol, 1 eq). After 5 minutes of agitation the solution was concentrated *in vacuo* to yield a yellow solid which was subsequently taken up in dry DMF (4.0 ml). To this was added methyl iodide (1.1 ml, 17.0 mmol, 1 eq) and the mixture was stirred overnight at room temperature under an argon atmosphere. The solvent was removed *in vacuo* and the residual solid taken up in CH_2Cl_2, washed with saturated aqueous $NaHCO_3$ (3 × 100 ml), saturated brine (3 × 100 ml) and dried (Na_2SO_4). Upon solvent evaporation the residual oil was purified by flash column chromatography (silica gel, 30% EtOAc in hexane) to provide **258** as a thick clear yellow oil (3.24 g, 65%) over two steps.

R_f = 0.45 (silica gel, 30% EtOAc in hexane)

$[\alpha]^{20}_D$ = -60.1° (c = 0.028 M in CH_2Cl_2)

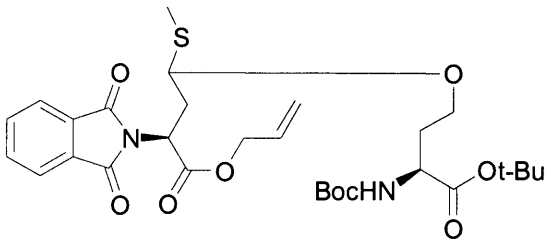
IR ν_{max} (neat, cm^{-1}) = 2916 (C-H), 1776, 1716 (C=O), 1612 (C=C, Ar)

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.84 (2H, m, C*H* Ar), 7.72 (2H, m, C*H* Ar), 5.07 (1H, C*H* α), 3.71 (3H, s, OC*H*$_3$), 2.57 – 2.28 (4H, br m, C*H*$_2$ β, C*H*$_2$ γ), 2.04 (3H, s, SC*H*$_3$)

^{13}C NMR (75 MHz, $CDCl_3$) δ 169.6 (*C*O Methyl), 167.6 (*C*O Phthalyl), 134.3 (*C*q. Ar), 131.8 (*C*H Ar), 123.6 (*C*H Ar), 52.9 (O*C*H_3), 50.8 (*C*H α), 30.8 (*C*H_2 β), 28.0 (*C*H_2 γ), 15.3 (S*C*H_3)

HRMS $C_{14}H_{15}NO_4S$ calcd. for $[M]^+$ 293.07218 found 293.07240.

4-(*S*)-(3-tert-Butoxycarbonyl-3-tert-butoxycarbonylamino-propoxy)-2-(S)-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-4-methylsulfanyl-butyric acid allyl ester (196)[10]



196

To a stirred solution of **195** (175 mg, 0.597 mmol) in CH_2Cl_2 (0.6 ml) at room temperature was added NCS (80 mg, 0.597 mmol, 1 eq). After 20 minutes the reaction was cooled to –78°C and added to a premixed solution of **150** (336 mg, 0.896 mmol, 1.5 eq), AgOTf (257 mg, 0.716 mmol, 1.2 eq) and 2,6-lutidine (43 µl, 0.597 mmol, 1 eq) in CH_2Cl_2 (0.6 ml) at –78°C. The mixture was stirred at this temperature for 2 hours before being slowly warmed to room temperature and diluted with CH_2Cl_2 (50 ml). This was subsequently filtered through silica gel and concentrated *in vacuo*. The residual oil yielded was purified by flash column chromatography (silica gel, 30% EtOAc in hexane) to provide clear yellow oil **196** (153 mg, 37%).

R_f = 0.20 (silica gel, 30% EtOAc in hexane)

IR ν_{max} (neat, cm^{-1}) = 2979, 2910 (C-H), 1764 (C=O), 1649 (C=C), 1630 (C=C, Ar)

Diastereoisomer I

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.86 (2H, m, C*H* Ar), 7.74 (2H, m, C*H* Ar), 5.85 (1H, m, CH_2CH=CH_2), 5.28 – 5.18 (4H, m, CH_2CH=CH_2, PthNC*H* α, BocN*H*), 4.64 (2H, m, CH_2CH=CH_2), 4.45 (2H, m, C*H*(SMe)OCH_2, BocNHC*H* α), 3.80 (1H, m, OCH_2CH_2), 3.26 (1H, m, OCH_2CH_2), 2.80 – 2.50 (2H, br. m, CH_2CH(SMe)), 2.02 (4H, br. s, SCH_3, OCH_2CH_2), 1.67 (1H, m, OCH_2CH_2), 1.47 (9H, s, CH_3 Boc), 1.44 (9H, s, CH_3 ^{t}Bu)

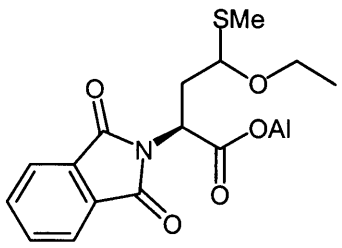
Diastereoisomer II

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.86 (2H, m, C*H* Ar), 7.74 (2H, m, C*H* Ar), 5.85 (1H, m, CH_2CH=CH_2), 5.28 – 5.18 (4H, m, CH_2CH=CH_2, PthNC*H* α, BocN*H*), 4.64 (2H, m, CH_2CH=CH_2), 4.45 (1H, m, BocNHC*H* α), 4.22 (1H, m, C*H*(SMe)OCH_2), 3.80 (1H, m, OCH_2CH_2), 3.26 (1H, m, OCH_2CH_2), 2.80 – 2.50 (2H, br. m, CH_2CH(SMe)), 2.02 (4H, br. s, SCH_3, OCH_2CH_2), 1.67 (1H, m, OCH_2CH_2), 1.47 (9H, s, CH_3 Boc), 1.44 (9H, s, CH_3 ^{t}Bu)

ESMS *m/z* $C_{29}H_{40}N_2O_9SNa$ *m/z* $[M - Boc + Na]^+$ 615.

(Not enough material for further spectroscopy)

2-(*S*)-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-4-ethoxy-4-methylsulfanyl-butyric acid allyl ester (203)



203

To a solution of **195** (100 mg, 0.313 mmol) in CH_2Cl_2 (5.0 ml) was added NCS (46 mg, 0.344 mmol, 1.1 eq) and the mixture was stirred for 1 hour in the dark under an argon atmosphere. To this was added EtOH (10.0 ml) and the solution was stirred overnight. The reaction was then washed with saturated brine (3 × 50 ml), dried (Na_2SO_4) and concentrated *in vacuo*. The resulting colourless oil was purified by a series of gradient eluted flash column chromatography runs (silica gel, 1% to 5% EtOAc, 29% to 25% CH_2Cl_2 in hexane) to provide **203** as a thick clear yellow oil and a mixture of inseparable diastereoisomers (68 mg, 60%).

R_f = 0.40 (silica gel, 20% EtOAc in hexane).

$[\alpha]^{20}_D$ = -46.0° (c = 0.018 M in CH_2Cl_2)

IR ν_{max} (neat, cm^{-1}) = 2976, 2925 (C-H), 1778, 1716 (C=O), 1663 (C=C), 1601 (C=C, Ar)

Diastereoisomer I

^1H NMR (300 MHz, $CDCl_3$) δ 7.78 (2H, m, C*H* Ar), 7.71 (2H, m, C*H* Ar), 5.80 (1H, m, $CH_2CH{=}CH_2$), 5.24 – 5.14 (3H, m, $CH_2CH{=}CH_2$, C*H* α), 4.61 (2H, d, *J* = 5.4 Hz, $CH_2CH{=}CH_2$), 4.25 (1H, dd, *J* = 9.8, 3.2 Hz, C*H*(SMe)OEt), 3.71 (1H, m, OCH_2CH_3), 3.23 (1H, m, OCH_2CH_3), 2.68 (2H, m, CH_2 β), 1.96 (3H, s, SCH_3), 0.87 (3H, t, *J* = 7.0 Hz, OCH_2CH_3)

^{13}C NMR (75 MHz, $CDCl_3$) δ 168.8 (*C*O Allyl), 167.5 (*C*O Phthalyl), 134.0 (*C*q Ar), 131.4 (*C*H Ar), 131.3 ($CH_2CH{=}CH_2$), 123.6 (*C*H Ar), 118.8 ($CH_2CH{=}CH_2$), 80.9 (*C*H(SMe)OEt), 66.7 ($CH_2CH{=}CH_2$), 63.5 (OCH_2CH_3) 49.6 (*C*H α), 34.8 (CH_2 β), 14.6 (OCH_2CH_3), 9.3 (SCH_3)

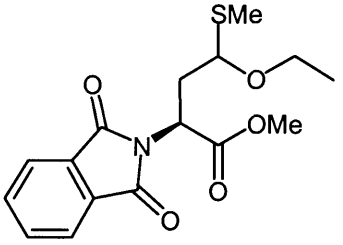
Diastereoisomer II

^1H NMR (300 MHz, $CDCl_3$) δ 7.78 (2H, m, C*H* Ar), 7.71 (2H, m, C*H* Ar), 5.80 (1H, m, $CH_2CH{=}CH_2$), 5.24 – 5.14 (3H, m, $CH_2CH{=}CH_2$, C*H* α), 4.61 (2H, d, *J* = 5.4 Hz, $CH_2CH{=}CH_2$), 4.59 (1H, t, *J* = 6.9Hz, C*H*(SMe)OEt), 3.71 (1H, m, OCH_2CH_3), 3.23 (1H, m, OCH_2CH_3), 2.68 (2H, m, CH_2 β), 1.98 (3H, s, SCH_3), 1.13 (3H, t, *J* = 7.0 Hz, OCH_2CH_3)

^{13}C NMR (75 MHz, $CDCl_3$) δ 168.8 (*C*O Allyl), 167.5 (*C*O Phthalyl), 134.0 (*C*q Ar), 131.4 (*C*H Ar), 131.3 ($CH_2CH{=}CH_2$), 123.6 (*C*H Ar), 118.8 ($CH_2CH{=}CH_2$), 82.9 (*C*H(SMe)OEt), 66.7 ($CH_2CH{=}CH_2$), 63.9 (OCH_2CH_3) 50.2 (*C*H α), 35.1 (CH_2 β), 14.8 (OCH_2CH_3), 9.4 (SCH_3)

HRMS $C_{18}H_{21}NO_4SNa$ calcd. for $[M + H]^+$ 386.10377 found 386.10304.

2-(*S*)-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-4-ethoxy-4-methylsulfanyl-butyric acid methyl ester (206)



206

To a solution of **258** (100 mg, 0.341 mmol) in CH_2Cl_2 (5 ml) was added NCS (50 mg, 0.380 mmol, 1.1 eq) and the mixture was stirred for 1 hour in the dark under an argon atmosphere. To this was added EtOH (10 ml) and the solution was stirred overnight. The reaction was then washed with saturated brine (3 × 50 ml), dried (Na_2SO_4) and concentrated *in vacuo*. The resulting colourless oil was purified by a series of gradient eluted flash column chromatography runs (silica gel, 1% to 5% EtOAc, 29% to 25% CH_2Cl_2 in hexane) to provide **206** as an oil and a mixture of inseparable diastereoisomers (62%, 71mg).

R_f = 0.40 (silica gel, 20% EtOAc in hexane)

$[\alpha]^{20}_D$ = -20.5° (c = 0.018 M in CH_2Cl_2)

IR ν_{max} (neat, cm^{-1}) = 2976, 2925 (C-H), 1778, 1716 (C=O), 1612 (C=C, Ar)

Diastereoisomer I

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.87 (2H, m, C*H* Ar), 7.74 (2H, m, C*H* Ar), 5.12 (1H, C*H* α), 4.50 (1H, t, *J* = 6.7 Hz, C*H*(SMe)OEt), 3.74 (4H, m, OC*H*$_3$, OC*H*$_2$CH$_3$), 3.25 (1H, m, OC*H*$_2$CH$_3$), 2.70 – 2.40 (2H, m, C*H*$_2$ β), 2.03 (3H, s, SC*H*$_3$), 1.18 (3H, t, *J* = 7.0 Hz, OCH$_2$C*H*$_3$)

^{13}C NMR (75 MHz, $CDCl_3$) δ 169.6 (*C*O Methyl), 167.5 (*C*O Phthalyl), 134.3 (*C*q Ar), 131.8 (*C*H Ar), 123.6 (*C*H Ar), 82.9 (*C*H(SMe)OEt), 63.8 (O*C*H$_2$CH$_3$), 52.9 (O*C*H$_3$), 50.0 (*C*H α), 35.1 (*C*H$_2$ β), 14.7 (OCH$_2$*C*H$_3$), 9.4 (S*C*H$_3$)

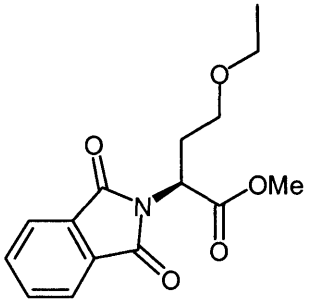
Diastereoisomer II

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.87 (2H, m, C*H* Ar), 7.74 (2H, m, C*H* Ar), 5.12 (1H, C*H* α), 4.27 (1H, dd, *J* = 9.9, 3.7 Hz, C*H*(SMe)OEt), 3.74 (4H, m, OC*H*$_3$, OC*H*$_2$CH$_3$), 3.25 (1H, m, OC*H*$_2$CH$_3$), 2.70 – 2.40 (2H, m, C*H*$_2$ β), 2.05 (3H, s, SC*H*$_3$), 0.91 (3H, t, *J* = 7.0 Hz, OCH$_2$C*H*$_3$)

^{13}C NMR (75 MHz, $CDCl_3$) δ 169.6 (*C*O Methyl), 167.5 (*C*O Phthalyl), 134.3 (*C*q Ar), 131.8 (*C*H Ar), 123.6 (*C*H Ar), 80.9 (*C*H(SMe)OEt), 63.5 (OCH_2CH_3), 52.9 (OCH_3), 49.4 (*C*H α), 34.9 (CH_2 β), 14.6 (OCH_2CH_3), 9.3 (SCH_3)

HRMS $C_{16}H_{20}NO_5S$ calcd. for $[M + H]^+$ 338.10621 found 338.10675.

2-(*S*)-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-4-ethoxy-butyric acid methyl ester (207)



207

To a stirred solution of **206** (64 mg, 0.189 mmol) in CH_2Cl_2 (3 ml) at 0°C was added MCPBA (72mg, 0.416 mmol 2.2 eq). After 1 hour Et_3SiH (150 μl, 0.945 mmol) and $BF_3.OMe_2$ (17 μl, 0.189 mmol, 1 eq) were added dropwise. After a further 1 hour the reaction was diluted with CH_2Cl_2 (50 ml) and warmed to room temperature before being filtered through celite. The filtrate was washed with 0.01 M aqueous $KHSO_4$ (3 × 50 ml), saturated brine (3 × 50 ml), dried (Na_2SO_4) and concentrated in vacuo. The residual oil was purified by flash column chromatography (silica gel, 20% EtOAc in hexane) to yield clear colourless oil **207** (17 mg, 30%).[11]

R_f = 0.35 (silica gel, 20% EtOAc in hexane)

$[\alpha]^{20}_D$ = 52.5° (c = 0.027 M in CH_2Cl_2)

IR (Too small an amount of material for reliable spectrograph)

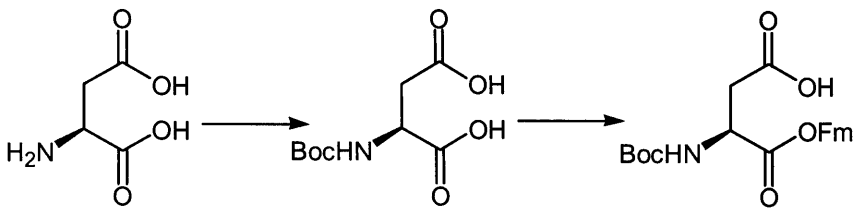
^{1}H NMR (300 MHz,$CDCl_3$) δ 7.87 (2H, m, C*H* Ar), 7.75 (2H, m, C*H* Ar), 5.10 (1H, dd, *J* = 10.6, 4.7 Hz, C*H* α), 3.73 (3H, s, O*CH*$_3$), 3.50 (1H, m, C*H*$_2$ γ), 3.35 (3H, m, O*CH*$_2$CH$_3$, C*H*$_2$ γ), 2.46 (2H, m, C*H*$_2$ β), 0.976 (3H, t, *J* = 6.9 Hz, OCH$_2$*CH*$_3$)

^{13}C NMR (75 MHz, $CDCl_3$) δ 169.2 (*C*O Methyl), 165.2 (*C*O Phthalyl), 134.7 (*C*q Ar), 134.1 (*C*H Ar), 123.5 (*C*H Ar), 67.1 (O*C*H$_2$CH$_3$), 66.3 (O*C*H$_2$CH$_2$), 52.8 (*C*H α), 49.8 (O*C*H$_3$), 28.9 (*C*H$_2$ β), 14.3 (S*C*H$_3$)

ES+ $C_{15}H_{17}NO_5$ Na *m/z* $[M + Na]^+$ 314.

HRMS $C_{15}H_{18}NO_5$ calcd for $[M + H]^+$ 292.11078, found 292.11042.

<u>*N*-α-t.-Boc-L-Aspartic acid fluorenylmethyl ester (228)</u>[12]



140 229 228

To a stirred solution of L-aspartic acid (20.0 g, 0.150 mol) and Na_2CO_3 (47.7 g, 0.450 mol, 3 eq) in H_2O (1.0 l) at 0°C was added $(Boc)_2O$ (32.8 g, 0.150 mol, 1 eq) over 20 minutes. After slow warming to room temperature the reaction was stirred for 24 hours. The mixture was then cooled to 0°C and acidified to pH 2.0 with 0.05 M $KHSO_4$ before being extracted with EtOAc (5 × 150 ml). The organic layer was then washed with brine (3 × 200 ml), dried (Na_2CO_3) and concentrated in vacuo to give a clear colourless oil **229** which was used in the next step without further purification.

To a stirred solution of **229** (10.0 g, 0.0429 mol) in dry THF (175 ml) under an argon atmosphere was added DIC (7.32 ml, 0.0473 mol, 1.1 eq) dropwise over 20 minutes. After 4 hours the reaction mixture was filtered through a dry sinter funnel and concentrated to approximately 100ml *in vacuo*. To the solution was added 9-fluorenylmethanol (9.28 g, 0.0473 mol, 1.1 eq) in one portion and then $^{i}Pr_2EtN$ (4.1 ml, 0.0452 mol, 1.05 eq) dropwise over 20 minutes. The reaction was then stirred for 24 hours under argon before being diluted with toluene (50 ml) and quenched with AcOH (5 ml). The solution was concentrated *in vacuo* until approximately 10 ml remained and was then diluted with EtOAc (100 ml), washed with 0.05M $KHSO_4$ (3 × 100ml), saturated brine (3 × 100 ml), dried (Na_2SO_4) and the solvent evaporated *in*

vacuo. After 24 hours drying on a high vacuum line a pink solid remained which was subsequently dissolved in a minimum amount of hot EtOAc and recrystallized by addition of hexane. The filtered solid was then washed twice with cold ethyl acetate to give **228**. The filtrate was concentrated *in vacuo* and recrystallised another four times to give solid **228** (13.2 g, 75%). Identical by ^{1}H NMR to lit.[12]

R_f = 0.1 to 0.3 (silica gel, 50% EtOAc in hexane)

m.pt. = 138° to 142°C [lit. m.pt. = 156°C][13]

$[\alpha]^{20}_{D}$ = +19.1° (0.011 M in MeOH)

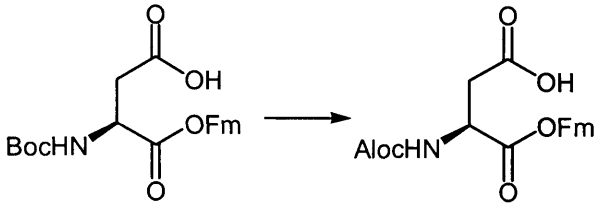
IR ν_{max} (Nujol, cm^{-1}) = 3290 (N-H), 2952, 2922 (C-H), 1737 (C=O), 1504 (C=C, Ar)

^{1}H NMR (300 MHz, $CDCl_3$/DMSO) δ 7.45 (2H, m, C*H* Ar), 7.26 (2H, m, C*H* Ar), 7.10 (4H, m, C*H* Ar), 5.74 (1H, br. d, *J* = 9.0 Hz, N*H*), 4.30 (1H, m, C*H* α), 4.02 (2H, d, *J* = 6.4 Hz, COOC*H*$_2$CHAr), 3.98 (1H, d, *J* = 7.2 Hz,COOCH$_2$C*H*Ar), 2.66 (1H, dd, *J* = 17.2, 5.2 Hz, C*H*H β) 2.43 (1H, m, CH*H* β), 1.11 (9H, s, NCOOC(C*H*$_3$)$_3$)

^{13}C NMR (75 MHz, $CDCl_3$/DMSO) δ 171.8 (*C*O OFm), 171.3 (*C*O Acid), 155.3 (*C*O Boc), 143.4 (*C*q. Ar), 140.9 (*C*q. Ar), 127.7 (*C*H Ar), 127.1 (*C*H Ar), 125.0 (*C*H Ar), 119.8 (*C*H Ar), 82.5 (NCOO*C*(CH$_3$)$_3$), 67.3 (COO*C*H$_2$CHAr), 49.9 (*C*H α), 46.4 (COOCH$_2$*C*HAr), 42.7 (*C*H$_2$ β), 28.2 (NCOOC(*C*H$_3$)$_3$)

HRMS $C_{23}H_{25}NO_6Na$ calcd. For $[M+Na]^+$ 434.15795 found 434.15697.

_N_-α-Aloc -L-Aspartic acid fluorenylmethyl ester (225)



228 225

To a solution of CH_2Cl_2:TFA (1:1, 5.0 ml) at 0°C was added **228** (200 mg, 0.486 mmol). The mixture was stirred at this temperature for 2.5 hours before being poured into ether (300 ml) and cooled to -23°C in the freezer overnight. After this time a solid precipitate was formed which was isolated by filtration. The filtrate was diluted with hexane (100 ml) and again cooled to -23°C in the freezer overnight to produce more solid. The solid was isolated by filtration and this process was performed once more to yield amino acid **230** which was used crude in the following reaction.

To a solution of amine **230** (270 mg, 0.657 mmol) and $NaHCO_3$ (330 mg, 3.94 mmol, 6 eq) in H_2O:THF (1:1, 16 ml) being stirred at 0°C was added allyl chlorofomate (67 μl, 0.723 mmol, 1.1 eq). After 2 hours the reaction was quenched by careful addition of 0.05M $KHSO_4$ (20ml) and diluted with EtOAc (50 ml). The organic layer was washed with 0.05M aqueous $KHSO_4$ (3 × 40 ml), saturated brine (3 × 40 ml), dried

(Na_2SO_4) and the solvent removed *in vacuo* to give a thick oil. Purification with flash column chromatography (silica gel, 1% AcOH, 50% EtOAc in hexane) provided a cloudy pink crystalline glass **225** (249 mg, 96%).

R_f= 0.1 to 0.3 (silica gel, 50% EtOAc in hexane)

m.pt. = 68°C

$[\alpha]^{20}_D$ = -16.7° (0.015 M in MeOH)

IR ν_{max} (Nujol, cm^{-1}) = 3305 (N-H), 2952, 2922 (C-H), 1747 (C=O), 1645 (C=C),1506 (C=C, Ar)

^{1}H NMR (300 MHz,$CDCl_3$) δ 7.73 (2H, d, *J* = 7.5 Hz, C*H* Ar), 7.53 (2H, t, *J* = 6.6 Hz, C*H* Ar), 7.38 (2H, m, C*H* Ar), 7.28 (2H, m, C*H* Ar), 5.89 (2H, m, N*H*, CH_2CH=CH_2), 5.35 – 5.2 (2H, m, CH_2CH=CH_2), 4.68 (1H, m, C*H* α), 4.66 (2H, m, CH_2CH=CH_2), 4.54 (2H, m, COOCH_2CHAr), 4.22 (1H, t, *J* = 6.3 Hz $COOCH_2CH$Ar), 2.89 (1H, dd, J = 17.7, 4.5 Hz, C*H*H β), 2.71 (1H, dd, J = 17.7, 4.5 Hz, CH*H* β)

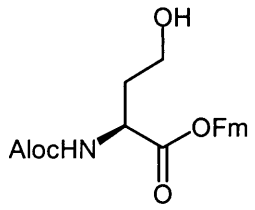
^{13}C NMR (75M MHz, $CDCl_3$/DMSO) δ 175.7 (*C*O OFm), 170.7 (*C*O Acid), 156.0 (*C*O Aloc), 143.4 (*C*q. Ar), 141.3 (*C*q. Ar), 132.4 (CH_2CH=CH_2), 127.9 (*C*H Ar), 127.2 (*C*H Ar), 124.9 (*C*H Ar), 120.0 (*C*H Ar), 118.2 (CH_2CH=CH_2), 67.7

(COOCH_2CHAr), 66.2 (CH_2CH=CH_2), 50.2 (*C*H α), 46.7 (COOCH_2*C*HAr), 36.3 (CH_2 β)

ES+ $C_{22}H_{21}NO_6Na$ *m/z* $[M + Na]^+$ 418

HRMS $C_{22}H_{22}NO_6$ calcd. for $[M+H]^+$ 396.14471 found 396.14642.

<u>*N*-α-Aloc -L-Homoserine fluorenylmethyl ester (231)</u>



231

To a stirred solution of **225** (1.7 g, 4.30 mmol) in dry CH_2Cl_2 (10.0 ml) was added isobutyl chloroformate (614 µl, 4.73 mmol, 1.1 eq) dropwise over 5 minutes and then N-methylmorpholine (373 µl, 4.30 mmol, 1 eq). The reaction was stirred under argon at room temperature for 30 minutes and then cooled to –78°C before $NaBH_4$ (325 mg, 8.6 mmol, 2 eq) was added in one portion followed by dropwise addition of MeOH (10.0 ml) over 10 minutes. After 1.5 hours the reaction was quenched by addition of AcOH (3.0 ml) and stirred for 30 minutes at –78°C before warming to room temperature and dilution with toluene (40 ml). The solvent was removed *in vacuo*

with the water bath not exceeding 30°C to give a thick oil. This was taken up by EtOAc (50 ml) and washed with 0.1 M HCl (3 × 50 ml), saturated brine (3 × 50 ml), dried (Na_2SO_4) and the solvent evaporated *in vacuo* to yield a viscous oil. This was purified by flash column chromatography (silica gel, 40% EtOAc in hexane) to give clear colourless oil **231** (1.3 g, 73%).

R_f = 0.25 (silica gel, 50% EtOAc in hexane)

$[\alpha]^{20}_D$ = -84.7° (0.012 M in CH_2Cl_2)

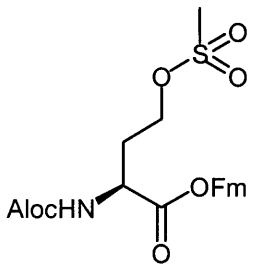
IR ν_{max} (neat, cm^{-1}) = 3419 (N-H), 2899 (C-H), 1758 (C=O), 1683 (C=C), 1576 (C=C, Ar)

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.73 (2H, d, *J* = 7.5, C*H* Ar), 7.57 (2H, m, C*H* Ar), 7.36 (2H, m, C*H* Ar), 7.30 (2H, m, C*H* Ar), 5.89 (2H, m, N*H*, CH_2CH=CH_2), 5.37 – 5.18 (2H, m, CH_2CH=CH_2), 4.57 – 4.43 (5H, m, CH_2CH=CH_2, COOCH_2CHAr, C*H* α), 4.21 – 4.09 (1H br m, $COOCH_2CH$Ar), 3.63 (2H, m, CH_2OH) 2.03 (1H, m, CH_2 β), 1.69 (2H, m, CH_2 β)

^{13}C NMR (75 MHz , $CDCl_3$) δ 172.5 (*C*O OFm), 156.6 (*C*O Aloc), 143.6 (*C*q. Ar), 141.3 (*C*q. Ar), 132.5 (CH_2CH=CH_2), 127.9 (*C*H Ar), 127.2 (*C*H Ar), 125.0 (*C*H Ar), 120.1 (*C*H Ar), 118.0 (CH_2CH=CH_2), 67.1 ($COOCH_2CH$Ar), 66.0 (CH_2CH=CH_2), 58.5 (CH_2, γ), 51.7 (*C*H α), 46.8 ($COOCH_2CH$Ar), 34.9 (CH_2 β)

HRMS $C_{22}H_{23}NO_5Na$ calcd. For $[M+Na]^+$ 404.14738 found 404.14640.

N-α-Aloc -L-Aspartic acid fluorenylmethyl ester mesylate (232)

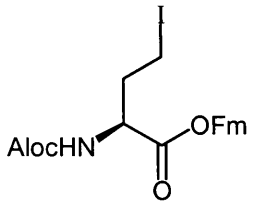


232

To a stirred solution of **231** (2.70 g, 7.08 mmol) in dry CH_2Cl_2 (54.0 ml) under argon was added methanesulfonyl chloride (1.4 ml, 17.7 mmol, 2.5 eq) and then *N*-methylmorpholine (1.7 ml, 17.2 mmol, 2.4 eq). After 2.5 hours at room temperature the reaction was quenched with 0.1 M aqueous $KHSO_4$ (20 ml). The organic layer was washed with 0.1M aqueous $KHSO_4$ (3 × 50 ml), saturated aqueous $NaHCO_3$ (3 × 50 ml), brine (3 × 50 ml), dried (Na_2SO_4) and the solvent concentrated *in vacuo*. The resultant oil **232** was used without any further purification.

R_f = 0.25 (silica gel, 40% EtOAc in hexane)

4-Iodo-2-(*S*)-allyloxycarbonylamino-butyric acid fluoren-9-ylmethyl ester (233)



233

To a stirred solution of **232** (500 mg, 0.956 mmol) in dry THF (50.0 ml) under argon was added NaI (780 mg, 3.82 mmol, 4 eq). The reaction was heated to 70°C and after 2 hours the solvent was removed *in vacuo*. The remaining solid was taken up in CH_2Cl_2:EtOAc (50:3, 53.0 ml) and washed with 0.1M aqueous $Na_2S_2O_3$ (3 × 50 ml), saturated brine:H_2O (1:6, v:v, 3 × 50 ml), dried (Na_2SO_4) and the solvent removed *in vacuo*. The resultant yellow solid **233** (465 mg, 99%) was used without further purification.

R_f = 0.55 (silica gel, 30% EtOAc in hexane)

m.pt. = 112°C

$[\alpha]^{18}_D$ = -39.6° (c = 0.0092 M in CH_2Cl_2)

IR ν_{max} (Nujol, cm^{-1}) = 3312 (N-H), 2922, 2952 (C-H), 1737 (C=O), 1693 (C=C), 1537 (C=C, Ar)

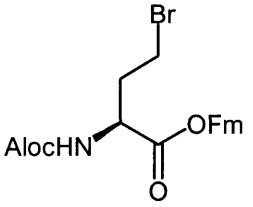
^{1}H NMR (300 MHz,$CDCl_3$) δ 7.77 (2H, d, *J* = 7.4, C*H* Ar), 7.57 (2H, m, C*H* Ar), 7.39 (2H, m, C*H* Ar), 7.34 (2H, m, C*H* Ar), 5.89 (1H, m, CH_2CH=CH_2), 5.3 – 5.2 (2H, m, N*H*, CH_2CH=CH_2), 4.57 (4H, m, CH_2CH=CH_2, COOCH_2CHAr), 4.39 (1H, m, C*H* α), 4.21 (1H, m, $COOCH_2CH$Ar), 3.00 (2H, m, CH_2I), 2.24 (1H, br m, CH_2 β), 2.04 (1H, br m, CH_2 β)

^{13}C NMR (75 MHz, $CDCl_3$) δ 171.2 (*C*O OFm), 155.8 (*C*O Alloc), 143.4 (*C*q. Ar), 141.4 (*C*q. Ar), 132.4 (CH_2CH=CH_2), 128.7 (*C*H Ar), 128.0 (*C*H Ar), 124.9 (*C*H Ar), 120.2 (*C*H Ar), 118.1 (CH_2CH=CH_2), 67.1 ($COOCH_2CHAr$), 66.1 (CH_2CH=CH_2) 54.7 (*C*H α), 46.8 ($COOCH_2CHAr$), 36.8 (*C*H_2 β), -0.8 (*C*H_2I)

HRMS $C_{22}H_{22}NO_4INa$ calcd. for $[M+Na]^+$ 514.04912 found 514.04656.

Anal. Calcd. for $C_{22}H_{22}NO_4I$ (491.319): C, 53.78; H, 4.51; N, 2.85; I, 25.83. Found C, 54.17; H, 4.64; N, 2.83; I, 25.65.

4-Bromo-2-(*S*)-allyloxycarbonylamino-butyric acid fluoren-9-ylmethyl ester (234)



234

To a stirred solution of mesylate **232** (500 mg, 0.956 mmol) in dry THF (50.0 ml) under argon was added NaBr (393 mg, 3.82 mmol, 4 eq). The reaction was heated to 70°C and after 2 hours the solvent was removed *in vacuo*. The remaining solid was taken up in CH_2Cl_2:EtOAc (50:3, 53 ml) and washed with 0.1M aqueous $Na_2S_2O_3$ (3 × 50 ml) and saturated brine:H_2O (1:6, v:v, 3 × 50 ml), dried (Na_2SO_4) and the solvent removed *in vacuo*. The resultant white solid **234** (419 mg, 99%) was used without further purification.

R_f = 0.55 (silica gel, 30% EtOAc in hexane)

m.pt. = 108°C

$[\alpha]^{20}_D$ = -94.9° (0.0071 M in CH_2Cl_2)

IR ν_{max} (Nujol, cm^{-1}) = 3311 (N-H), 2952, 2922 (C-H), 1737 (C=O), 1693 (C=O)

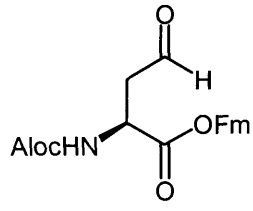
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.77 (2H, d, *J* = 7.5 Hz, C*H* Ar), 7.55 (2H, m, C*H* Ar), 7.41 (2H, m, C*H* Ar), 7.34 (2H, m, C*H* Ar), 5.89 (1H, m, CH_2CH=CH_2), 5.38 – 5.20 (3H, br m, N*H*, CH_2CH=CH_2), 4.60 (5H, m, CH_2CH=CH_2, COOCH_2CHAr, C*H* α), 4.22 (1H, m, $COOCH_2CH$Ar), 3.33 (2H, m, CH_2Br), 2.09 (1H, br m, CH_2 β), 2.04 (1H, br m, CH_2 β)

^{13}C NMR (75 MHz, $CDCl_3$) δ 171.4 (*C*O OFm), 155.8 (*C*O Alloc), 143.0 (*C*q. Ar), 141.4 (*C*q. Ar), 132.2 ($CH_2CH{=}CH_2$), 128.7 (*C*H Ar), 128.0 (*C*H Ar), 124.9 (*C*H Ar), 120.2 (*C*H Ar), 118.4 ($CH_2CH{=}CH_2$), 115.7 ($NCOCH_2CH{=}CH_2$), 67.2 (CH_2 $COOCH_2CHAr$), 66.1 ($CH_2CH{=}CH_2$) 52.7 (*C*H α), 46.8 ($COOCH_2CHAr$), 35.5 (CH_2 β), 28.1 (CH_2Br)

HRMS $C_{22}H_{22}NO_5BrNa$ calcd. for $[M+Na]^+$ 466.06298 found 466.06204.

Anal. Calcd. for $C_{22}H_{22}NO_4Br$ (443.073): C, 59.47; H, 4.99; N, 3.15; Br, 17.98. Found C, 59.73; H, 5.20; N, 3.08; Br, 18.02.

2-(*S*)-allyloxycarbonylamino-4-oxo-butyric acid fluoren-9-ylmethyl ester (237)



237

To a solution of oxalyl chloride (233 µl, 2.76 mmol, 1.5 eq) in dry DCM (0.50 ml) at –78°C was added dropwise DMSO (145 µl, 2.76 mmol, 1.5 eq). The resulting solution was stirred for 30 minutes at this temperature before **231** (700 mg, 1.84

mmol) disolved in DCM (2.0 ml) was added dropwise over 10 minutes. After 30 minutes Et_3N (1.28 ml, 9.2 mmol, 5 eq) was added and the reaction was warmed to 0°C. After 2 hours the solution was quenched with 0.1M $KHSO_4$ (20 ml) and allowed to warm to room temperature. The resulting solution was diluted with DCM (100 ml) and the organic layer washed with 0.1M $KHSO_4$ (3 × 75 ml) , dried ($NaSO_4$) and concentrated *in vacuo*. The residual oil (650mg) was purified by flash column chromatography (silica gel, 30% EtOAc in hexane) to yield dusty brown solid **237** pure (545 mg, 52%).

R_f = 0.35 (silica gel, 30% EtOAc in hexane)

m.pt. = 110°C

$[\alpha]^{20}_D$ = +63.2° (c = 0.0092 M in CH_2Cl_2)

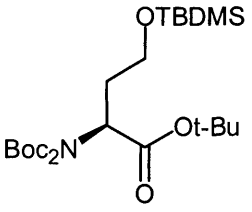
IR ν_{max} (Nujol, cm^{-1}) = 3305 (N-H), 2952, 2922, (C-H), 1737 (C=O), 1645 (C=C), 1531 (C=C, Ar)

^{1}H NMR (300 MHz, $CDCl_3$) δ 9.50 (1H, s, C(=O)*H*), 7.76 (2H, d, *J* = 7.5, C*H* Ar), 7.54 (2H, m, C*H* Ar), 7.41 – 7.25 (4H, m, C*H* Ar), 5.89 (1H, m, CH_2CH=CH_2), 5.61 (1H, d, *J* = 8.6, N*H*), 5.30 – 5.10 (2H, CH_2CH=CH_2), 4.62 – 4.53 (5H, m, CH_2CH=CH_2, $COOCH_2CHAr$, C*H* α), 4.23 (1H, t, *J* = 6.1 Hz, $COOCH_2CHAr$), 2.9 (1H, m, CH_2 β)

^{13}C NMR (75 MHz, $CDCl_3$) δ 199.0 (*C*O Aldehyde) 170.6 (*C*O OFm), 155.8 (*C*O Alloc), 143.4 (*C*q. Ar), 141.3 (*C*q. Ar), 132.4 ($NCOCH_2CH=CH_2$), 127.9 (*C*H Ar), 127.2 (*C*H Ar), 124.9 (*C*H Ar), 120.1 (*C*H Ar), 118.1 ($NCOCH_2CH=CH_2$), 67.3 ($COOCH_2CHAr$), 66.1 ($NCOCH_2CH=CH_2$) 49.0 (*C*H α), 46.7 ($COOCH_2CHAr$), 45.7 (CH_2 β)

HRMS $C_{22}H_{21}NO_5Na$ calcd. for $[M+Na]^+$ 402.13174 found 402.13277.

<u>*N*-α-Di-t.Boc-L-Homoserine t.-butyl ester TBDMS ether (236)</u>



236

To a solution of **150** (500 mg, 1.33 mmol) in dry DMF (0.5 ml) was added imidazole (26 mg, 3.33 mmol, 2.5 eq) and $^tBuMe_2SiCl$ (260 mg, 1.8 mmol, 1.35 eq). The resulting solution was stirred for 18 hours before being diluted with saturated brine (20 ml) and ethyl acetate (20 ml). The organic layer was then washed with saturated brine (2 × 50 ml), dried ($NaSO_4$) and concentrated *in vacuo.* The residual oil (0.98 g)

was purified by flash column chromatography (silica gel, 5% EtOAc, 0.001% Et_3N in hexane) to yield **236** as a clear colourless oil (640 mg, 98.4%).[14]

R_f = 0.25 (silica gel, 5% EtOAc in hexane);

$[\alpha]^{20}_D$ = + 27.7° (c = 0.022 M in CH_2Cl_2)

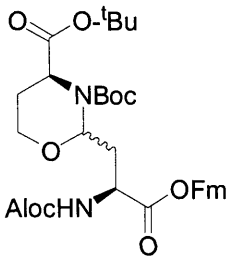
IR ν_{max} (neat, cm^{-1}) = 2979, 2956 (C-H), 1737 (C=O)

^1H NMR (300 MHz, $CDCl_3$) δ 4.90 (1H, dd, *J* = 8.6, 5.3 Hz, C*H* α), 3.67 (2H, m, CH_2 β), 2.34 (1H, m, CH_2 γ), 2.00 (1H, m, CH_2 γ), 1.49 (18H, s, NCOC(CH_3)$_3$), 1.43 (9H, s, OCOC(CH_3)$_3$), 0.87 (9H, s, Si(CH_3)$_2$C(CH_3)$_3$), 0.03 (6H, s, Si(CH_3)$_2$C(CH_3)$_3$)

^{13}C (75 MHz, $CDCl_3$) δ 170.1 (*C*O ^{t}Butyl), 152.4 (CO Boc), 82.6 (*C*q. ^{t}Buyl), 81.1 (*C*q. Boc), 60.2 (*C*H_2 γ), 55.9 (CH α), 33.1 (CH_2 β), 28.0 (CH_3 ^{t}Buyl), 27.9 (*C*H_3 Boc), 26.0 (*C*H_3 Si(CH_3)$_2$C(*C*H_3)$_3$), 18.3 (Si(CH_3)$_2$*C*(CH_3)$_3$)
(Peaks for Si(*C*H_3)$_2$C(CH_3)$_3$ not present on spectrum, expected to be *ca.* – 6 ppm)

HRMS $C_{24}H_{47}NO_7SiNa$ calcd. for $[M+Na]^+$ 512.30193 found 512.30246.

2-[2-tert-Butoxycarbonylamino-2-(9H-fluoren-9-ylmethoxycarbonyl)-ethyl]-[1,3]oxazinane-3,4-dicarboxylic acid di-tert-butyl ester (240)



240

To a stirred solution of **236** (56 mg, 0.114 mmol) in MeCN (0.5 ml) at room temperature was added Et_3SiH (27 µl, 0.171 mmol, 1.5 eq.). After 5 minutes $BiBr_3$ (34.0 mg, 0.076 mmol, 0.67 eq.) was added. **237** (65 mg, 0.171 mmol, 1.5 eq.) dissolved in MeCN (0.5ml) was then added and the solution stirred for 24 hours. The reaction was diluted with EtOAc 50 ml which was then washed with Na_2CO_3 (2 × 50 ml), dried ($NaSO_4$) and concentrated *in vacuo.* The residual oil was purified by flash column chromatography (silica gel, 20% EtOAc in hexane) to yield clear colourless oil **240** pure (50.2 mg, 69.1%).[15]

Rf = 0.17 (silica gel, 20% EtOAc in hexane)

$[\alpha]^{20}_D$ = + 11.2° (c = 0.010 M in CH_2Cl_2)

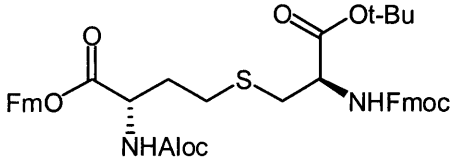
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.85 (2H, m, C*H* Ar), 7.60 (2H, d, *J* = 7.1, C*H* Ar), 7.37 (2H, m, C*H* Ar), 7.26 (2H, m, C*H* Ar), 5.92 (1H, m, CH_2CH=CH_2) 5.87 (1H, m, N*H*), 5.44 (1H, m, AlocNHCHCH$_2$C*H*(O)NBoc) 5.28 – 5.18 (2H, m, CH_2CH=CH_2), 4.71 (1H, br. s, ^{t}BuOOCC*H*NBoc), 4.58 – 4.48 (5H, m, CH_2CH=CH_2,

COOC*H*$_2$CHAr, AlocNHC*H*CH$_2$CH(O)NBoc), 4.29 (1H, br m, COOCH$_2$C*H*Ar), 3.75 (2H, ^{t}BuOOCCHCH$_2$C*H*$_2$O), 2.25 - 2.20 (2H, m, AlocNHCHC*H*$_2$CH(O)NBoc, ^{t}BuOOCCHC*H*$_2$CH$_2$O), 2.10 – 2.02 (2H, m, AlocNHCHC*H*$_2$CH(O)NBoc, ^{t}BuOOCCHC*H*$_2$CH$_2$O), 1.41 (18H, s, NCOC(C*H*$_3$)$_3$, OCOC(C*H*$_3$)$_3$)

^{13}C NMR (75 MHz , $CDCl_3$) δ 172.1 (*C*O OFm), 171.4 (*C*O Al) 156.0 (*C*O Aloc), 153.4 (*C*O Boc), 143.6 (*C*q. Ar), 141.3 (*C*q. Ar), 132.7 (CH$_2$*C*H=CH$_2$), 127.8 (*C*H Ar), 127.2 (*C*H Ar), 125.1 (*C*H Ar), 120.0 (*C*H Ar), 117.8 (CH$_2$CH=*C*H$_2$), 82.0 (*C*q ^{t}Bu), 81.0 (*C*q Boc), 67.6 (COO*C*H$_2$CHAr), 65.8 (*C*H$_2$CH=CH$_2$), 64.3 (AlocNHCHCH$_2$*C*H(O)NBoc), 58.1 (^{t}BuOOCCHCH$_2$*C*H$_2$O), 51.7 (2 × *C*H α), 46.9 (COOCH$_2$*C*HAr), 34.4 (AlocNHCH*C*H$_2$CH(O)NBoc), 34.2 (^{t}BuOOCCH*C*H$_2$CH$_2$O), 28.3 (OCOC(*C*H$_3$)$_3$), 27.9 (NCOC(*C*H$_3$)$_3$)

HRMS $C_{35}H_{44}N_2O_9Na$ calcd. for $[M+Na]^+$ 659.29444 found 659.29494.

3-[(*S*)-2-*tert*-Butoxycarbonyl -2-(fluoren-9-ylmethoxycarbonylamino)ethylsulfanyl]-(*S*)-(allyloxycarbonylamino)butanoic acid fluoren-9-ylmethyl ester (243)[16]



243

To a stirred solution of thiol **119** (2.20 g, 5.51 mmol) and **233** (2.71 g, 5.51 mmol) in dry DMF (10.0 ml) was added Cs_2CO_3 (1.62 g, 4.96 mmol, 1.1 eq). After 2.5 hours at room temperature the reaction was diluted with EtOAc (100 ml) and the organic layer washed with 0.025 M $KHSO_4$ (50 ml), H_2O (6 × 50 ml), dried and the solvent evaporated *in vacuo*. The resultant oil was purified by flash column chromatography (silica gel, 5% Et_2O, in CH_2Cl_2) to give **243** pure (2.7 g, 64%).

R_f = 0.20 (silica gel, 30% EtOAc in hexane)

$[\alpha]^{20}_D$ = +22.0° (0.020 M in CH_2Cl_2)

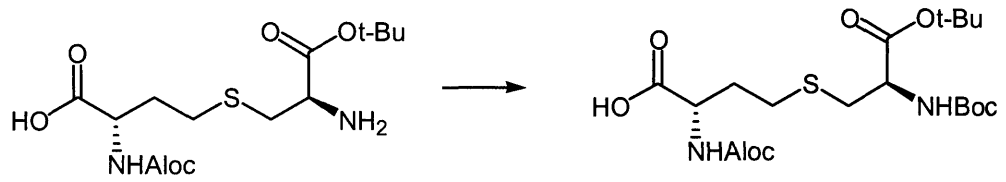
IR ν_{max} (neat, cm^{-1}) = 3415 (N-H), 2901 (C-H), 1733 (C=O), 1680 (C=C), 1522 (C=C, Ar)

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.04 (4H, d, *J* = 7.7 Hz, C*H* Ar), 7.77 (4H, m, C*H* Ar), 7.59 (4H, m, C*H* Ar), 7.43 (4H, m, C*H* Ar), 5.93 (2H, m, N*H*, CH_2CH=CH_2), 5.84 (1H, d, *J* = 9.7 Hz, N*H*), 5.32 – 5.18 (2H, m, CH_2CH=CH_2), 4.56 (6H, m, CH_2CH=CH_2, 2 × C*H* α, CH_2COOCH_2CHAr), 4.96 (2H, m, NCOOCH_2CHAr), 4.22 (2H, m, $NCOOCH_2CHAr$ & CH_2COOCH_2CHAr), 3.00 (2H, br m, CHCH_2S), 2.53 (2H, br m, CH_2 γ), 2.05 (1H, m, CH_2CH_2S), 2.02 (1H, m, CH_2CH_2S), 1.51 (9H, s, $OCOC(CH_3)_3$)

^{13}C NMR (75MHz, $CDCl_3$) δ 171.9 (*C*O OFm), 169.7 (*C*O O^{t}Bu), 159.9 (*C*O Fmoc), 155.8 (*C*O Aloc), 143.9 (*C*q Ar Fm), 143.8 (*C*q Ar Fmoc), 143.5 (*C*q Ar Fm), 143.3 (*C*q Ar Fmoc),132.6 (CH_2CH=CH_2) 128.0 (*C*H Ar Fm), 127.8 (*C*H Ar Fmoc), 127.3 (*C*H Ar Fm), 127.2 (*C*H Ar Fmoc), 125.0 (*C*H Ar Fm), 125.2 (*C*H Ar Fmoc), 120.1 (*C*H Ar Fm), 120.0 (*C*H Ar Fmoc), 117.9 (CH_2CH=CH_2), 83.0 (OCO*C*(CH_3)$_3$), 67.1 (CH_2COOCH_2CHAr & $NCOOCH_2CHAr$), 66.0 (CH_2CH=CH_2), 54.4 (FmocNH*C*H α), 53.0 (AllocNH*C*H α), 47.2 (CH_2COOCH_2CHAr), 46.8 ($NCOOCH_2CHAr$), 34.7 ($CHCH_2S$), 30.4 (CH_2CH_2S), 28.6 (CH_2CH_2S), 28.1 (OCOC(CH_3)$_3$)

HRMS $C_{44}H_{46}N_2O_8SNa$ calcd. for $[M + H]^+$ 785.28724 found 785.28561.

3-[(*S*)-2-*tert*-Butoxycarbonyl -2-(*tert*-butoxycarbonyll amino)ethylsulfanyl]-(*S*)-(allyloxycarbonylamino)butanoic acid (245)



To a stirred solution of **243** (1.20 g, 1.57 mmol) in DMF (10.0 ml) was added piperidine (3.0 ml) dropwise over 5 minutes. The reaction was stirred at room temperature for 2.5 hours before being quenched with AcOH (5.0 ml) and diluted with toluene (100 ml). The solvent was then removed *in vacuo* and after 24 hours at

low pressure (0.5 mmHg) the resultant oil **244** was used crude in the following reaction.

To a stirred solution of **244** (1.57 mmol) and $NaHCO_3$ (317 mg, 3.77 mmol, 2.4 eq) in THF:H_2O (1:1, 10.0 ml) at 0°C was added Boc_2O (480 mg, 2.20 mmol, 1.4 eq). After warming slowly to room temperature the mixture was stirred for 24 hours. The reaction was then diluted with EtOAc (100 ml) and quenched by addition of $KHSO_4$ (50 ml). The organic layer was then washed with $KHSO_4$ (3 × 50 ml), the collected washings were then back extracted with fresh EtOAc (5 × 50 ml) and the combined organic extract then washed with saturated brine (2 × 50 ml). After the solvent was dried (Na_2SO_4) and removed *in vacuo* the remaining solid was purified by flash column chromatography (silica gel, 1 to 10% AcOH, in EtOAc) to give **245** pure (420 mg, 57.9%) over two steps.

R_f = 0.35 (silica gel, 2% AcOH in EtOAc)

$[\alpha]^{20}_D$ = +5.5° (0.003 M in CH_2Cl_2)

IR ν_{max} (neat, cm^{-1}) = 3440 (N-H), 2919 (C-H), 2890 (COO-H), 1758 (C=O), 1657 (C=C)

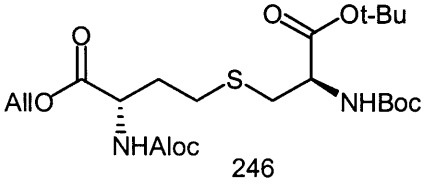
^{1}H NMR (300 MHz, $CDCl_3$) δ 6.28 (1H, br s N*H*), 6.20 (1H, br s, N*H*), 5.93 (1H, m, CH_2CH=CH_2), 5.32 – 5.18 (2H, m, CH_2CH=CH_2), 4.55 (2H, d, *J* = 4.7Hz,

$CH_2CH{=}CH_2$), 4.54 (2H, m, 2 × C*H* α), 3.24 (2H, br m, $CHCH_2S$), 2.94 (2H, br m, CH_2 γ), 2.34 – 2.26 (2H, m, CH_2CH_2S), 1.46 (9H, s, $OCOC(CH_3)_3$), 1.43 (9H, s, $NCOC(CH_3)_3$)

^{13}C NMR (75 MHz, $CDCl_3$) δ 169.4 (*C*O O^{t}Bu), 168.9 (*C*O Acid), 156.2 (*C*O Boc), 155.5 (*C*O Alloc), 132.7 ($CH_2CH{=}CH_2$), 117.9 ($CH_2CH{=}CH_2$), 83.2 ($OCOC(CH_3)_3$), 82.8 ($NCOC(CH_3)_3$), 65.9 ($CH_2CH{=}CH_2$), 53.2 (BocNH*C*H α), 50.1 (AllocNH*C*H α), 34.3 ($CHCH_2S$), 30.2 (CH_2CH_2S), 28.3 ($OCOC(CH_3)_3$), 27.9 ($NCOOC(CH_3)_3$), 25.0 (CH_2CH_2S)

(Unidentifiable by mass spectrometry)

3-[(*S*)-2-*tert*-Butoxycarbonyl -2-(*tert*-butoxycarbonyll amino)ethylsulfanyl]-(*S*)-(allyloxycarbonylamino)butanoic acid allyl ester (246)



246

To a solution of **245** (420 mg, 0.909 mmol) in dry MeOH (10.0 ml) was added Cs_2CO_3 (148 mg, 0.455 mmol, 0.5 eq). After 5 minutes of agitation the solution was concentrated *in vacuo* to yield a white solid which was subsequently taken up in dry DMF (5.0 ml). To this mixture was added allyl bromide (100 µl, 1.18 mmol, 1.3 eq)

and the reaction stirred at room temperature under an atmosphere of argon for 24 hours. The solution was then diluted with EtOAc (100 ml) and quenched by addition of 0.1M $KHSO_4$ (10 ml). The organic layer was washed with 0.1M $KHSO_4$ (3 × 50 ml) the collected washings were then back extracted with fresh EtOAc (5 × 50 ml) and the combined organic extract then washed with saturated brine (2 × 50 ml). After the solvent was dried (Na_2SO_4) and removed *in vacuo* the remaining thick oil was purified by flash column chromatography (silica gel, 50% EtOAc, in hexane) to yield **246** (330 mg, 72.2%).

R_f = 0.20 (silica gel, 50% EtOAc in hexane)

$[\alpha]^{20}_D$ = +17.9° (0.011 M in CH_2Cl_2)

IR ν_{max} (neat, cm^{-1}) = 3478 (N-H), 3002 (C-H), 1755 (C=O), 1654 (C=C)

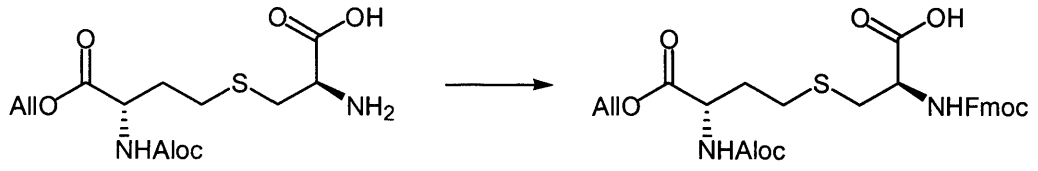
^{1}H NMR (300 MHz,$CDCl_3$) δ 5.95 (1H, br s, N*H*), 5.85 (3H, m, 2 × CH_2CH=CH_2, N*H*), 5.30 (4H, m, 2 × CH_2CH=CH_2), 4.61 – 4.41 (6H, m, 2 × CH_2CH=CH_2, 2 × C*H* α), 3.11 (2H, br m, $CHCH_2S$), 2.81 (2H, br m, CH_2 γ), 2.33 (1H, m, CH_2CH_2S), 2.15 (1H, m, CH_2CH_2S), 1.42 (9H, s, $OCOC(CH_3)_3$), 1.37 (9H, s, $NCOOC(CH_3)_3$)

^{13}C NMR (75 MHz, $CDCl_3$) δ 171.0 (*C*O Allyl), 169.3 (*C*O O^{t}Bu), 156.0 (*C*O Boc), 153.1 (*C*O Alloc), 132.5 (CH_2CH=CH_2), 131.3 (CH_2CH=CH_2), 119.2 (CH_2CH=CH_2), 118.8 (CH_2CH=CH_2), 83.2 ($OCOC(CH_3)_3$), 82.9 ($NCOOC(CH_3)_3$), 66.3

($CH_2CH=CH_2$), 65.9 ($CH_2CH=CH_2$), 53.5 (BocNHCH α), 50.4 (AllocNHCH α), 34.1 (CH_2CH_2S), 30.3 ($OCOC(CH_3)_3$), 28.2 ($NCOOC(CH_3)_3$), 25.6 (CH_2CH_2S)

(Unidentifiable by mass spectrometry)

3-[(*S*)-2-Carboxy-2-(fluoren-9-ylmethoxycarbonyll amino)ethylsulfanyl]-(*S*)-(allyloxycarbonylamino)butanoic acid allyl ester (248)



To a stirred solution of **246** (330 mg, 0.657 mmol) and Et_3SiH (2.0 ml) in CH_2Cl_2 (5.0 ml) at 0°C was added TFA (5.0 ml) dropwise over 15 minutes. The reaction was then slowly warmed to room temperature and stirred for 3.5 hours. The solution was then diluted with toluene (100 ml) and the solvent removed *in vacuo* to give a grey solid **247** used in the next reaction without purification.

To a stirred solution of **247** (0.661 mmol) and $NaHCO_3$ (275 mg, 3.31 mmol, 0.5 eq) in THF:H_2O (1:1, 16.0 ml) at 0°C was added Fmoc chloroformate (203 mg, 0.793 mmol, 1.2 eq). After 2.5 hours stirring at room temperature the reaction was quenched by addition of 0.1M $KHSO_4$ (50 ml). The aqueous solution was then

extracted with EtOAc (6 × 50 ml) before the organic layer was washed with saturated brine (3 × 50 ml), dried (Na_2SO_4) and concentrated *in vacuo* to give a white solid **248** which was purified by flash column chromatography (silica gel, 1 to 10% Ac_2O, in EtOAc) to yield (199 mg, 53%).

R_f = 0.45 (silica gel, 2% AcOH in EtOAc)

$[\alpha]^{20}_D$ = +10.1° (0.010 M in CH_2Cl_2)

IR ν_{max} (neat, cm^{-1}) = 3440 (N-H), 2933 (C-H), 2906 (COO-H), 1741 (C=O), 1689 (C=C), 1520 (C=C, Ar)

^{1}H NMR (300 MHz, CD_3OD) δ 7.77 (2H, d, *J* = 7.4 Hz, C*H* Ar), 7.65 (2H, d, J = 7.0 Hz, C*H* Ar), 7.52 (2H, m, C*H* Ar), 7.26 (2H, C*H* Ar), 5.89 (2H, m, 2 × CH_2CH=CH_2), 5.35 – 5.22 (4H, m, 2 × CH_2CH=CH_2), 4.63 (2H, d, *J* = 5.6 Hz, NCOOCH_2CHAr), 4.51 (2H, m, 2 × CH_2CH=CH_2), 4.50 – 4.35 (4H, br. m, C*H* α, CH_2CH=CH_2, NCOOCH$_2$C*H*Ar), 4.23 (1H, m, C*H* α), 3.31 (1H, br. m, CHCH_2S), 3.19 (1H, br m, CHCH_2S), 2.90 (2H, br m, CH_2 γ), 2.31 (1H, m, CH_2CH$_2$S), 2.18 (1H, m, CH_2CH$_2$S)

^{13}C NMR (75 MHz, $CDCl_3$) δ 172.0 (*C*OOH), 169.1 (*C*O Allyl), 157.2 (*C*O Fmoc), 157.2 (*C*O Aloc), 143.8 (*C*q Ar), 141.2 (*C*q Ar), 134.0 (CH_2CH=CH_2), 132.9 (CH_2CH=CH_2), 128.9 (*C*H Ar), 128.3 (*C*H Ar), 126.4 (*CH* Ar), 121.1 (*C*H Ar), 119.1 (CH_2CH=CH_2), 118.3 (CH_2CH=CH_2), 68.3 (NCOOCH_2CHAr), 67.3 (CH_2CH=CH_2),

67.0 ($CH_2CH=CH_2$), 54.4 (FmocNH*C*H α), 49.6 (AllocNH*C*H α), 48.4 (NCOOCH_2*C*HAr), 35.0 (CH*C*H_2S), 30.9 (*C*H_2CH_2S), 25.5 (CH_2*C*H_2S)

ES+ $C_{29}H_{32}NO_8SK$ *m/z* $[M + K]^+$ 607.

(Unidentifiable by HRMS)

Peptide Synthesis

Peptide **249** was synthesised on a MultiSynTech Syro Peptide Synthesiser (Model MP-60) using Fmoc-Tyr(^{t}Bu)-NovaSyn TGT resin with a 0.2 mmol/g loading. DMF was used as the primary solvent throughout the peptide synthesis. Determination of free amines was achieved through a Kaiser test.

Kaiser Test

The resin was washed with CH_2Cl_2 to remove all traces of DMF. A small amount of resin was then removed and placed in an Eppendorf vial. Four drops from each Kaiser test solution (shown below) were delivered to the vial and the clear yellow mixture then heated for 5 minutes at 110°C. A positive test gave a dark purple solution which indicated the presence of primary amines, a negative result produced no colour change.

Solution 1: KCN (2ml, 0.001 M) in pyridine (98ml)

Solution 2: Ninhydrin (5% w/v) in ^{t}butanol

Solution 3: Phenol (80% v/v) in ^{t}butanol

Syro Protocols

All masses and volumes stated in the pre-programmed MultiSynTech Syro protocols are used throughout unless otherwise stated. The peptide synthesiser contains an agitation block that holds the 2 ml reaction syringes. These are vessels containing frits, the base of which is connected to a vacuum pump so solutions can be removed while the resin remains in the syringe. DMF/piperidine mixes were generated from stock solutions of DMF and piperidine by the peptide synthesizer. Amino acid and reagent concentrations were calculated with respect to the amount of starting homolanthionine **243** available.

Fmoc Deprotection

Automated Fmoc deprotection was carried out first with piperidine (40% v/v) in DMF for 3 minutes (500 μL per syringe), followed by piperidine (20% v/v) in DMF for 10 minutes (500 μL per syringe) before a series of washing steps with DMF (6 x 550 μL per syringe). During each deprotection step a fast vortex of the reaction for 20 seconds every 1 minute was performed to ensure thorough mixing.

Automated Amino acid coupling

Amino acid solution in DMF (250 μL per syringe) was transferred from the amino acid block on the synthesiser to the reaction syringes, followed by addition of HBTU in DMF (250 μL per syringe, 0.4 M). A solution of DIPEA in NMP (100 μL per syringe, 1.6 M) was then added to take the total volume to 600 μL and the mixture reacted for 40 minutes (fast vortex for 20 seconds every 4 minutes). After this time the solution was drained from the syringe and the resin washed with DMF (4 x 550 μL) before Fmoc deprotection of the amino acid and washing with DMF (6 x 550 μL).

Amino Acid	MolWeight [g/mol]	Material [mg(ml)]	Dissolved Volume [ml]	Concentration [mol/l]
Fmoc-Gln-(Trt)	610.7	553	2.26	0.40
Fmoc-Pro	337.4	306	2.26	0.40
Fmoc-Pro	337.4	306	2.26	0.40
Fmoc-Ile	353.4	320	2.26	0.40
Fmoc-Ser(^{t}Bu)	383.4	347	2.26	0.40
Fmoc-Phe	387.4	351	2.26	0.40
Fmoc-Thr(^{t}Bu)	397.5	360	2.26	0.40
Fmoc-Ser(^{t}Bu)	383.4	347	2.26	0.40
HBTU/DMF	379.3	1700	11.3	0.40
^{i}Pr$_2$EtN/NMP	129.30	(0.789)	2.83	1.60

Homolanthionine Coupling

3-[(*S*)-2-Carboxy-2-(fluoren-9-ylmethoxycarbonylamino)ethylsulfanyl]-(*S*)-(allyloxycarbonylamino)butanoic acid allyl ester **248** (257 mg, 0.454 mmol, 4 eq) was dissolved in DMF (0.60 ml) and added to a solution of PyAOP (0.118 g, 0.227 mmol, 2 eq) and HOAt (0.31 mg, 0.227 mmol, 2 eq) in DMF (1.2 ml). This solution was added equally to each syringe (0.36 ml) and the reaction left for 1 hour (vortex 20 seconds every 2 minutes). The syringes were then drained and any unreacted bis amino acid **248** collected and purified.

Alloc / Allyl deprotection

Deprotection of both allyl ester and carbamate groups was performed using $Pd(PPh_3)_4$ (0.1725 g, 0.150 mmol, 2 eq., 0.05 M) dissolved in a solution of DMF/$CHCl_3$/AcOH/NMM (18.5:18.5:2:1 volume, 1.38/1.38/0.15/0.75 ml, total volume, 3 ml). This mixture was added to each reaction syringe (500 µL) and the resultant suspension reacted for 2 hours in the dark under nitrogen (vortex 20 seconds every 2 minutes).

Upon evacuation of the reaction syringe the resin was washed with DMF (2 x 500 µL per syringe), $^{i}Pr_2EtN$ in DMF (0.5% v/v, 10 ml, 4 x 500 µL per syringe), DMF (4 x 500 µL per syringe), sodium diethyldithiocarbamate trihydrate in DMF (0.5% w/v, 10 ml, 4 x 500 µL per syringe) and DMF (4 x 500 µL). All washes were vortexed for 20 seconds every 1 minute.

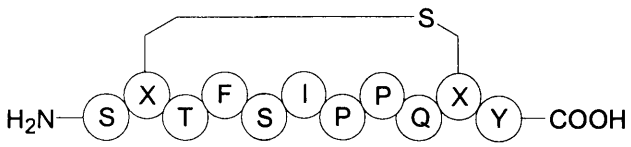
Cyclisation

PyAOP (295 mg, 0.567 mmol, 5 eq) and HOAt (77 mg, 0.567 mmol, 5 eq) were dissolved in DMF (1.8 ml) and the resultant solution (0.36 ml) added to each syringe. $^{i}Pr_2EtN$ (0.018ml, 5 x 10^{-4} mol, 10 eq.) was then added to each syringe and the reaction was carried out for 1 hour with a fast vortex for 20 seconds every 2 minutes.

Control Peptide – H_2N-SCTFSIPPQST-COOH

Linear control peptide was synthesised containing two cysteine molecules, instead of the homolanthionine bridge. This peptide was synthesised by automated methods and no Kaiser tests were performed. The control peptide was oxidatively cyclised on resin using I_2 dissolved in H_2O (0.02 M). Cleavage conditions and work up were identical for that of peptide **249**.

Synthesis of Peptide 249



Three syringes (2 ml) were loaded with resin (3 x 189 mg) before DMF (1 ml) was added and the resin allowed to swell for 30 minutes. After several washings with DMF (4 x 500 μL) the Fmoc group of the resin bound tyrosine was removed followed by a Kaiser test. Homolanthionine **248** was then coupled to the free amine and a Kaiser test was performed. Subsequent Fmoc deprotection and Kaiser test was performed before the peptide synthesis was automated. Fmoc-glutamine(Trt) (0.553 g, 0.11 mmol, 8 eq) was first to be coupled followed by Fmoc-proline (0.306 g, 0.11 mmol, 8 eq), Fmoc-proline (0.306 g, 0.11 mmol, 8 eq), Fmoc-isoleucine (0.320 g, 0.11 mmol, 8 eq), Fmoc-serine-(^{t}Bu) (0.347 g, 0.11 mmol, 8 eq), Fmoc-phenylalanine (0.351 g, 0.11 mmol, 8 eq) and Fmoc-threonine-(^{t}Bu) (0.361 g, 0.11 mmol, 8 eq) The Fmoc group of the threonine residue added was not removed at this time and the allyl

ester and carbamate deprotected using the previously mentioned $Pd(PPh_3)_4$ reagent cocktail.

After a Kaiser test the Fmoc group of the terminal serine was deprotected. A further Kaiser test was performed before the peptide was cyclised on resin. Another Kaiser test confirmed the presence of the primary amine before the final amino acid, Fmoc-serine (0.347 g, 0.11 mmol, 8 eq) was coupled and the Fmoc group removed.

The resin was then washed with CH_2Cl_2 (2 x 1 ml per syringe) and treated with a cocktail solution (94% TFA, 2.5% H_2O, 2.5% ethanedithiol, 1% triisopropylsilane) for 3 hours to cleave the peptide from the resin and remove side-chain protecting groups. The solutions were collected and each syringe was washed with TFA (2 x 500 μL per syringe). These were combined and concentrated *in vacuo* until a small amount of solvent remained in the flask. Cold ether (-23°C) was then added and precipitation of the peptide was observed. The mixture was cooled in the freezer for 1 hour before centrifugation (20 minutes at 3500 rpm at 0°C). The supernatant ethereal layer was then decanted and the pellet produced by centrifugation resuspended in cold ether. The centrifugation procedure was repeated producing a second pellet which was dissolved in the minimum volume of distilled water (5 ml) and freeze-dried over night.

References

[1] Vanheeswijk, W. A. R.; Eenink, M. J. D.; Feijen, J. *Synthesis* **1982**, 744-747.

[2] Tennant-Eyles, R. J.; Fairbanks, A. J. *Tetrahedron: Asymmetry* **1999**, *10*, 391-401.

[3] Schwarz, H.; Bumpus, F. M.; Page, I. H. *J. Am. Chem. Soc.* **1957**, *79*, 5697-5703.

[4] Cox, R. J.; Gibson, J. S.; Martin, M. B. M. *ChemBioChem* **2002**, *3*, 874-886.

[5] Grehn, L.; Gunnarson, K.; Ragnarsson, U. *Act. Chem. Scandanavia,* **1986**, *B40*, 745.

[6] Baldwin, J. E.; North, M.; Flinn, A. *Tetrahedron* **1988**, *44*, 637-642.

[7] Williams, R. M.; Sinclair, P. J.; Zhai, D.; Chen, D. *J. Am. Chem. Soc.* **1988**, *110*, 1547-1557.

[8] Brown, H.C. In *Boranes in Organic Chemistry*; Cornell University Press, 1972; p 216-218

[9] Clarke, S.; Hider, R. C.; John, D. I. *J. Chem. Soc., Perkin Trans.1* **1973**, 230-234.

[10] Inoue, M.; Xing, G.; Wang, X.; Wang, J.; Hirama, M. *Org. Lett.* **2002**, *4*, 3439-3442.

[11] Nicolaou, K. C.; Duggan, M. E.; Hwang, C. K. *J. Am. Chem. Soc.* **1986**, *108*, 2468-2469.

[12] Campigalia, P.; Gomez-Monterrey, I.; Longobardo, L.; Lama, T.; Novellino, E.; Grieco, P. *Tetrahedron Lett.* **2004**, *45*, 1453.

[13] Spatola, A. F.; Crozet, Y.; deWit, D.; Yanagisawa, M. *J. Med. Chem.* **1996**, *39*, 3842-3846.

[14] Corey, E. J.; Venkates, A. *J. Am. Chem. Soc.* **1972**, *94*, 6190.

[15] Evans, P. A.; Cui, J.; Gharpure, S. J.; Hinkle, R. J. *J. Am. Chem. Soc.* **2003**, *125*, 11456-11457.

[16] Mustapa, M. F. M.; Harris, R.; Bulic-Subanovic, N.; Elliott, S. L.; Bregant, S.; Groussier, M. F. A.; Mould, J.; Schultz, D.; Chubb, N. A. L.; Gaffney, P. R. J.; Driscoll, P. C.; Tabor, A. B. *J. Org. Chem.* **2003**, *68*, 8185-8192.