University of Reading

Synthetic Studies on Thiomorpholinones for the Application in Peptide Coupling

Doctor of Philosophy

The School of Chemistry, Food and Pharmacy

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October 2018

Declaration

I confirm that this is my own work and the use of material from other sources has been properly and fully acknowledged.

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Abstract

This study addresses synthetic approaches to access a glycine-derived thiomorpholinone template and the application of this template for the production of peptides via *C*-terminus extension.

The first chapter surveys strategies in peptide synthesis, including stepwise elongation and convergent approaches. Several important amino and carboxy protecting groups are described. The development of methodologies in peptide synthesis, beginning with solid phase peptide synthesis (SPPS) towards chemical ligation, native chemical ligation (NCL) and other ligation techniques such as Staudinger ligation and click chemistry, are considered. The racemization problem in peptide synthesis via direct enolization and oxazolone formation are highlighted. Several widely used coupling reagents such as acyl chloride, carbodiimide and phosphonium reagents are discussed. The development of a morpholinone to thiomorpholinone-based peptide synthesis strategy is summarized.

Chapter 2 focuses on an alternative synthetic route to obtain glycine-derived thiomorpholinone templates. A seven step synthetic route was successfully developed to access C-3 unsubstituted thiomorpholinones for the first time. The synthesis starts with the nucleophilic ring opening of Boc-protected morpholinone to give an ester. The sulfur introduction was achieved via Mitsunobu reaction, followed by hydrolysis, ring closure with DCC and deprotection to give the desired glycine-derived thiomorpholinone template. Attempts to improve the initially developed method successfully reduced the synthetic sequence to six steps by preparing an ester in a one

pot strategy. An attempt at incorporation of a 5-(4-methoxyphenyl) substituent into the thiomorpholinone system was able to give an ester by adapting an improved six step procedure.

Chapter 3 concentrates on several other routes investigated with the aim to access the glycine-derived thiomorpholinone template. In a reductive amination approach, benzyl *N*-Fmoc 2-(4-methoxyphenyl)thioglycine was generated based on a six step procedure requiring two more steps including benzyl removal before ring closing to obtain the more synthetically attractive 5-(4-methoxyphenyl) C-3 unsubstituted thiomorpholinone template. The use of thioacetate as an alternative starting material in this approach successfully led to the thioacid in three steps. A thionation approach was also considered for the conversion of the morpholinone precursor to the thiomorpholinone. Several thionating agents were studied for this purpose, including Lawesson's reagent, a P_4S_{10}–HMDO combination and the P_4S_{10}-pyridine complex using Fmoc-protected morpholinone as a starting material.

Chapter 4 describes an application of the C-3 unsubstituted thiomorpholinone template in peptide synthesis. This is exemplified by the preparation of a tripeptide derivative ala-gly-ala. The *N*-terminus peptide extension on the template was carried out using an acid chloride to give a dipeptide adduct ala-gly. The nucleophilic ring opening of *N*-acylated thiomorpholinone was achieved using a carboxyl protected amino acid under mild conditions to give the ala-gly-ala tripeptide precursor. This study demonstrates that a glycine-derived thiomorpholinone can be used as a template to carry out *C*-terminus peptide coupling under mild conditions without epimerization as oxazolone formation is prevented.

Chapter 5 summarises the main findings in this study including the development and improvement of the synthetic method to access the desired glycine-derived thiomorpholinone template and also the use of this template towards peptide generation.

Chapter 6 outlines the experimental procedures and spectroscopic data of the compounds synthesized during the course of these studies.

Acknowledgements

First and foremost, I would like to express my sincere gratitude to my supervisor Professor Laurence M. Harwood for giving me the opportunity to learn and having a valuable experience in organic chemistry. I truly appreciate the continuous support, advice, motivation, guidance and discussion we had during my study.

My sincere thanks also to Dr John McKendrick for the lecture in peptide chemistry as well as Dr Chris Smith and Dr Philippa Cranwell for the discussion during weekly meeting. I would also like to thank Martin Reeves and Nicholas Michael for mass spectra, Dr Radoslaw M. Kowalczyk for NMR spectra at variable temperature and all members in the department for their support.

I would like to express my appreciation to members in the Harwood group including Dr Rui Gu, Dr Ashfaq Asfar, Dr Andy Smith, James Westwood and Dr Joe Cowell for their help and kindness. Also, I would like to thank Ministry of Education of Malaysia for financial support of my study.

Last but not least, I would like to thank my family, parents and friends for their continuous support and encouragement throughout this study.

Abbreviation

AcOH	Acetic acid
Ala	Alanine
AOP	(7-aza-benzotriazolyloxy)-tris-(dimethylamino)phosphonium hexafluorophosphate
Bn	Benzyl
Boc	*tert*-butyloxycarbonyl
BOP	(benzotriazolyloxy)-tris-(dimethylamino)phosphonium hexafluorophosphate
DCC	Dicyclohexylcarbodiimide
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCM	Dichloromethane
DIAD	Di*iso*propyl azodicarboxylate
DIPEA	*N*,*N*-Di*iso*propylethylamine
DMAP	4-dimethylaminopyridine
DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide
Et	Ethyl
FDA	Food and Drug Administration
Fmoc	9-Fluorenylmethyloxycarbonyl
Gly	Glycine
HIV	Human immunodeficiency virus
HMDO	Hexamethyldisiloxane
HMPA	Hexamethylphosphoramide
HOBt	1-hydroxybenzotriazole
i-Pr	*iso*propyl
KEH	Potassium 2-ethyl hexanoate
Me	Methyl
MOM	Methoxymethyl
NCL	Native chemical ligation

Pd	Palladium
ppm	parts per million
PyBop	(benzotriazolyloxy)-tris-(pyrrolidino)phosphonium hexafluorophosphate
PyBrop	Bromotripyrrolidinophosphonium hexafluorophosphate
Rf	Retention factor
RNase A	Ribonuclease A
S_N2	Nucleophilic substitution, second order
SPPS	Solid phase peptide synthesis
STAB	Sodium triacetoxyborohydride
TMS	Trimethylsilyl
t-Bu	*tert*-butyl
TFA	Trifluoroacetic acid
TFAA	Trifluoroacetic anhydride
THF	Tetrahydrofuran
TLC	Thin layer chromatography
Z	Benzyloxycarbonyl

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Chapter 1

Introduction

1.1 Introduction to the strategy for chemical synthesis of peptides

Proteins play an important role in biological processes including enzymatic reactions, transport/storage (haemoglobin), immune protection (antibodies) and mechanical support (collagen). With significant roles in various biochemical reactions by demonstrating high specificity towards their molecular target,[1] proteins and peptides have attracted a lot of attention from synthetic chemists and pharmacists in exploring their potential as drug candidates. Based on information from the THPdb database in 2017, since the 1980s, 239 therapeutic peptides and proteins have been approved by the United States Food and Drug Administration (FDA) for clinical use.[1] More than 60 peptide-based drugs are currently available on the market and several other peptides are under clinical development.[1,2] There are various applications of peptide drugs in the medical and pharmaceutical field, delivering treatments for cancer, diabetes, obesity, osteoporosis, allergies, immunological disorders and cardiovascular disease.[1,2]

As a result, the chemical synthesis of proteins has become an important area of research, resulting in the rapid development of new techniques and methodologies over recent years, with the aim of creating novel peptides with desirable properties based on a range of methods such as *de novo* design, template-based modelling or by altering natural frameworks. The chemical synthesis of proteins allows the facile incorporation of unnatural functionality into these structures. Many peptide-based products that demonstrate interesting biological activities contain amino acid building blocks that may not be easily introduced by traditional peptide coupling reagents and reactions.[3] Consequently, the development of new peptide coupling reagents and novel

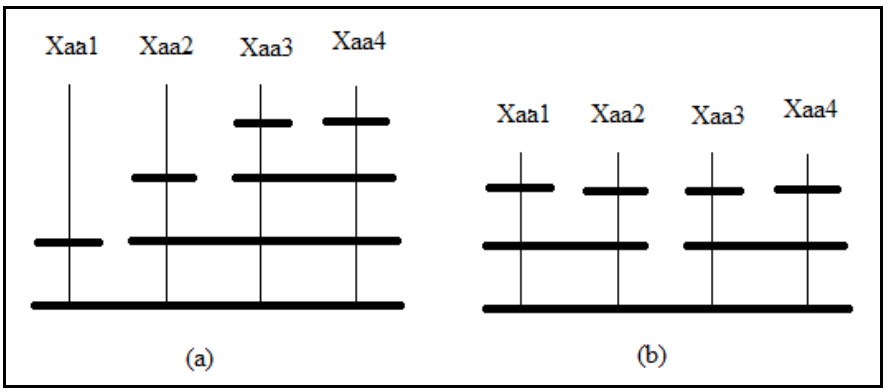
methodologies continues to be required so that difficult peptide coupling reactions are rendered more efficient. Above all, in any process, the peptide bond should be formed rapidly and quantitatively under mild conditions, avoiding side reactions without interfering with the integrity of adjacent stereogenic centres and producing only easily removed by-products.

The advances in peptide synthetic methodology have brought about the growth and expansion of protein synthesis. Many peptides have been successfully synthesized and reported. For example, the octapeptide hormone oxytocin was successfully synthesized through solution phase synthesis and an enzyme ribonuclease (RNase A) was synthesized through solid phase methodology.[4] Nowadays, peptide synthesis through solid phase methodology is the most common strategy and the efficiency of this method continues to improve. Unfortunately, solid phase peptide synthesis is generally limited to peptides that contain fewer than 40 residues; the synthesis of peptides and proteins with greater length being far more difficult to accomplish. Most proteins are simply too long to synthesize by the simple repetitive stepwise coupling of amino acid monomers.[4] It has been reported that the convergent coupling of protected or partially protected peptides in solution and on solid phase is a better approach to access proteins with more than 40 residues.[4] This strategy is claimed to minimize side reactions, increase the efficiency of amino acid coupling and more importantly can reduce the risk of epimerization.

1.2 Strategy in peptide synthesis

In principle, peptides can be synthesized based on either a stepwise elongation strategy or a convergent fragment condensation strategy. For example, a tetrapeptide could be synthesised by any four stepwise tactics as described as (1+[2+(3+4)]), [(1+2)+3]+4,

[1+(2+3)]+4 and 1+[(2+3)+4] in which one residue is added for coupling at a time (Figure 1.1).[5] Alternatively, in a convergent approach, dipeptide fragments could be prepared before condensing them together into a tetrapeptide (Figure 1.1).[5] From a simple arithmetic point of view, the convergent method will give a better yield of approximately 64 % if an 80 % yield is obtained at each stage, compared to a stepwise elongation strategy that will only produce around 52 % overall yield. Peptides produced through a convergent strategy are generally easier to purify than those prepared by the stepwise method as stepwise synthesis can lead to deletion products where an amino acid residue has only incompletely coupled. Furthermore, the synthesis of peptide fragments can be carried out simultaneously in parallel, in contrast with the stepwise method that must be performed sequentially. Thus, a set of precursor peptides with variable sequences can be synthesized before assembling them into a larger peptide. However, the selection of fragment position is crucial in a fragment condensation approach, introducing constraints because the coupling reaction may only work efficiently in terms of yield or stereoisomeric purity at certain positions.



(a) Stepwise elongation (1+[2+(3+4)]) (b) Convergent, fragment condensation

Figure 1.1 Strategy in peptide synthesis

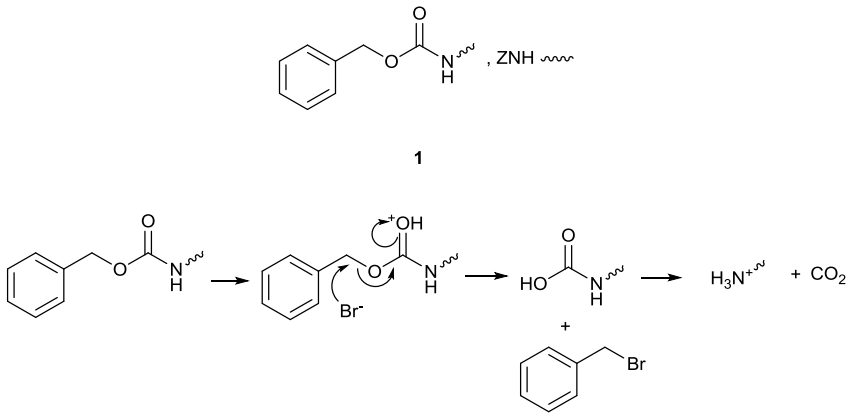
1.3 Protection in peptide synthesis

1.3.1 α-Amino protection

Amino group protection can be achieved by suppressing its nucleophilic reactivity with an appropriate substituent that diminishes electron density on the nitrogen. The protecting group should be able to remain in position as long as it is required and then readily removed without affecting the integrity of the structure.

1.3.1.1 Benzyloxycarbonyl (Z) protection

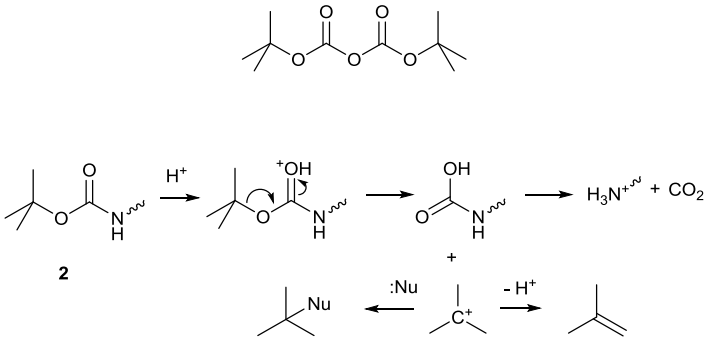
The benzyloxycarbonyl (Z) group **1** was the first amino protecting group developed in 1932 by Bergmann and Zervas.[5,6] The Z group was initially introduced onto the amino group as the chloroformate reagent, but this protocol leads to unwanted side reactions such as dipeptide formation and so the use of less reactive acylating agents is now preferred. This protecting group is stable under mildly basic and acidic condition and to nucleophilic reagents such as amines, hydrazine and dilute alkali. The deprotection reaction can be carried out with HBr/AcOH by a S_N2 mechanism (Scheme 1.1).[5] Most protected peptides are readily soluble in acetic acid and the deprotected products can be precipitated as their hydrobromide salts using ether.



Scheme 1.1 Mechanism of removal of the Z group with HBr/AcOH

1.3.1.2 *tert*-Butyloxycarbonyl (Boc) protection

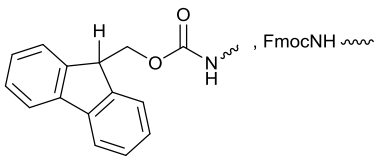
In 1957, the *tert*-butyloxycarbonyl (Boc) **2** protecting group was introduced by McKay and Albertson.[7] The precursor anhydride is considered safer to use than the corresponding chloroformate and is stable on storage for long periods if kept in a refrigerator. The Boc group removal can be reliably carried out under mild conditions by dissolving the substrate either in neat TFA or TFA in dichloromethane at room temperature for half an hour (Scheme 1.2).[5] This protecting group is stable towards catalytic hydrogenolysis, reducing agents, basic and nucleophilic reagents even on prolonged exposure.



Scheme 1.2 Mechanism of removal of the Boc group with TFA

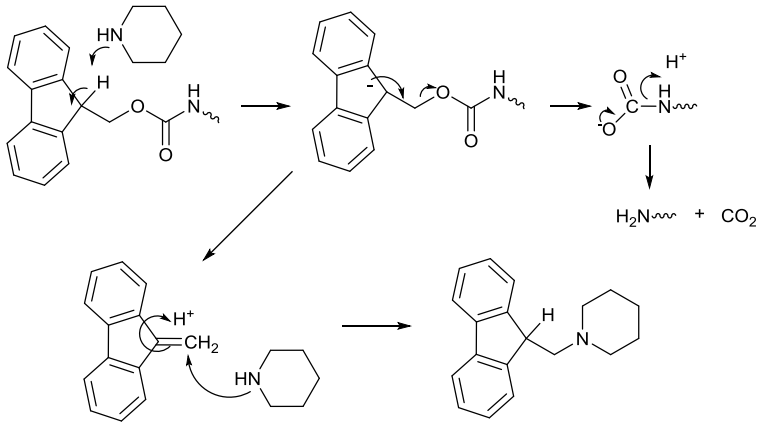
1.3.1.3 9-Fluorenylmethoxycarbonyl (Fmoc) protection

The 9-Fluorenylmethoxycarbonyl (Fmoc) **3** was developed as an amino protecting group in 1970 by Carpino and Han.[8] The Fmoc group is usually introduced onto the amino group using the chloroformate precursor but this has a tendency to cause side reactions, such as dipeptide and tripeptide production, similarly to benzyl chloroformate. However, the development of less reactive derivatives such as the succinimido ester derivative is now preferred as it generally gives better yields.



3

The Fmoc group is very stable towards acids, while deprotection is effectively carried out under basic conditions using piperidine (20 % in DMF), usually taking only a few seconds to complete at room temperature (Scheme 1.3).[5]

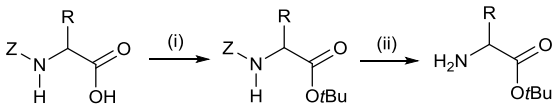


Scheme 1.3 Mechanism of removal of the Fmoc group with 20 % piperidine/DMF

1.3.2 Carboxylic protection

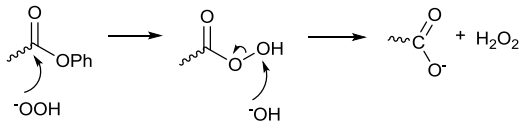
Carboxylic protection is required when the carboxylic component of the other amino acid or peptide is activated in the presence of the amino component and all such protection is based on esterification. Regeneration of the parent carboxylic acid from the ester is accomplished by acyl-oxygen or alkyl-oxygen cleavage depending on the ester used. Carboxylic protection as a methyl ester can be performed with hot methanolic hydrogen chloride to afford the methyl ester hydrochloride, generally as a crystalline product.[5] Treatment with thionyl chloride in methanol is a convenient means of generating the reaction medium. Methyl group protection provides good stability, not only during the formation of the peptide bond, but also during the *N*-deprotection reaction as methyl esters demonstrate high stability towards HBr/AcOH, TFA, catalytic hydrogenolysis, thiols and amines.[5] However, problems such as racemization may be encountered during methyl group removal as it has to be carried out under relatively harsh condition.

Protection as a *t*-butyl ester can be performed directly from the amino acid by acid catalysed treatment with a solution of isobutene as shown in Scheme 1.4.[5] The amino acid *t*-butyl ester is stable towards nucleophilic and basic attack, while treatment with TFA removes the *t*-butyl ester.



Scheme 1.4 (i) DCM sat. with $Me_2C=CH_2$, H_2SO_4 (cat.), 20 ^oC, 3 days (ii) H_2/Pd(C)/EtOH.

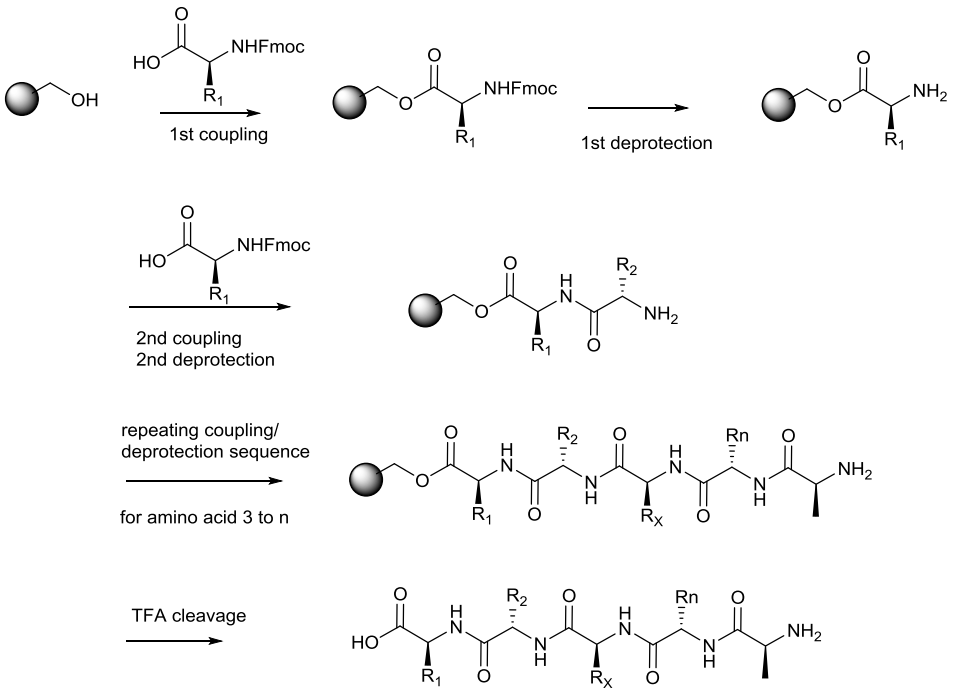
Phenyl esters are readily prepared and the resultant ester is commonly obtained as a crystalline material. These esters are stable towards acids and catalytic hydrogenation and can be removed with alkali or alkaline peroxide as they are susceptible to nucleophilic attack (Scheme 1.5).[5]



Scheme 1.5 pH 20.5, 20 ^{o}C, aq. DMF

1.4 Solid phase peptide synthesis

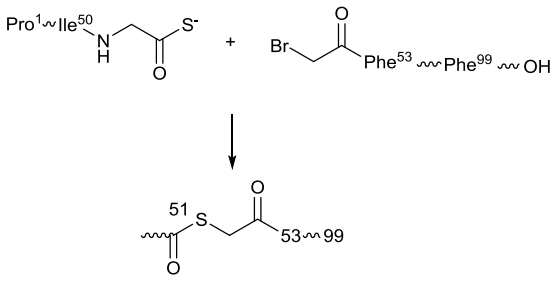
In 1963, Merrifield established a novel approach to synthesize peptides based on solid phase peptide synthesis (SPPS).[9] In this approach, the *N*-protected amino acid is bound onto a resin followed by removal of the *N*-protecting group. The unprotected amino acid is then coupled with the second *N*-protected amino acid and the process is repeated until the desired sequence of amino acids is obtained, with the final steps involving side chain deprotection and cleavage of the desired peptide from the polymer support. The principle of amide bond formation on solid support is illustrated in Scheme 1.6.[10] The repeated coupling and deprotection procedures and the nature of the growing peptide chain entail an appropriate selection of resin and protecting groups to avoid premature cleavage or alteration of the growing peptide. This methodology is now carried out on automated synthesizers and can be applied routinely to the coupling of up to 40 - 50 amino acids.



Scheme 1.6 Solid phase peptide synthesis

1.5 Chemical ligation

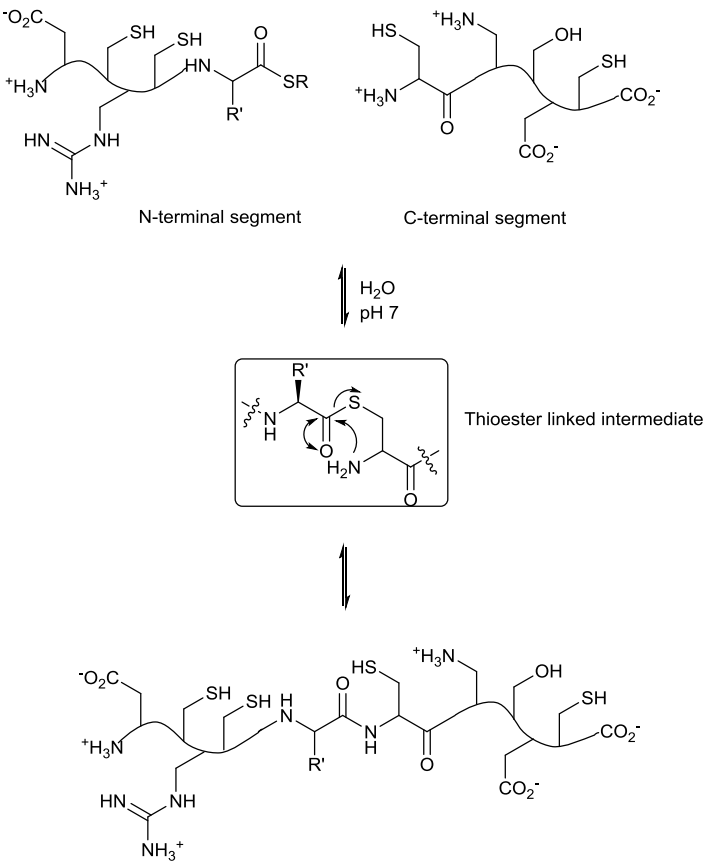
The absolute limitation of protein length to about 50 amino acids in advanced SPPS methodologies has led to the development of a new protocol in protein synthesis called "chemical ligation".[11] This method allows the coupling of two unprotected peptide fragments chemoselectively in aqueous solution to form a much longer peptide chain.[11] A chaotropic agent such as 6 M guanidine-HCl is used to increase the solubility of the reacting segments and thereby accelerate the reaction.[12] The principle of this chemical ligation technique is exemplified in the synthesis of an HIV-protease enzyme (Scheme 1.7).[12] In this approach, thiocarboxylate and bromoacetyl peptides containing approximately 50 residues each were reacted through a thioester-forming ligation to yield the thioester HIV-protease enzyme containing 99 residues.



Scheme 1.7 Chemical ligation in peptide synthesis

1.6 Native chemical ligation and other ligation techniques

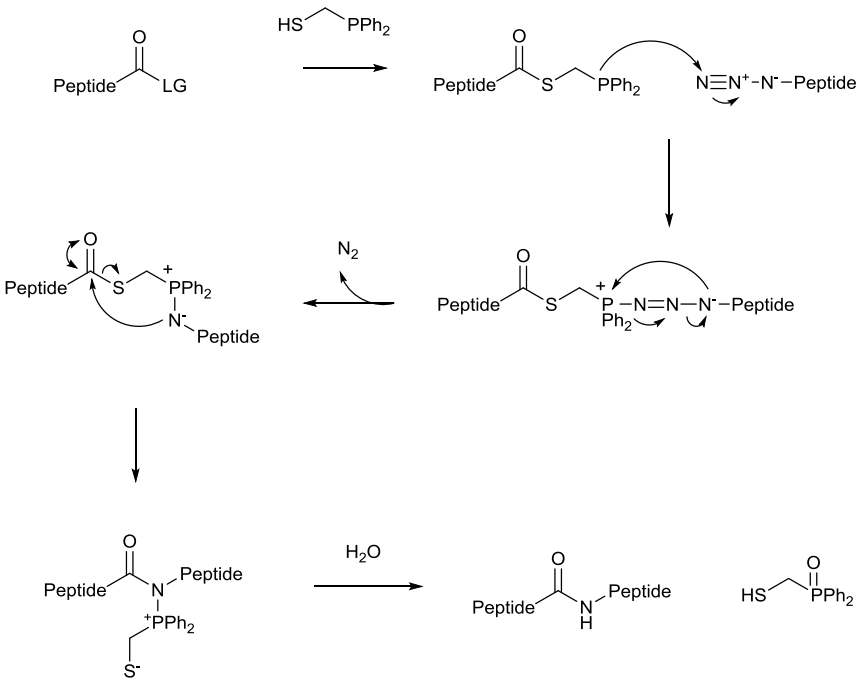
In 1994, Dawson et al. developed a native chemical ligation (NCL) technique that could assemble two unprotected peptide segments chemoselectively at neutral pH in aqueous solution to form a large polypeptide in which the newly formed linkage is an amide.[13] In this method, an *N*-terminal cysteine peptide and peptide-α-thioester are reacted, initially to give the thioester-linked intermediate that subsequently undergoes intramolecular nucleophilic rearrangement, resulting in the formation of the amide bond at the ligation side (Scheme 1.8).[12]



Scheme 1.8 Native chemical ligation in peptide synthesis

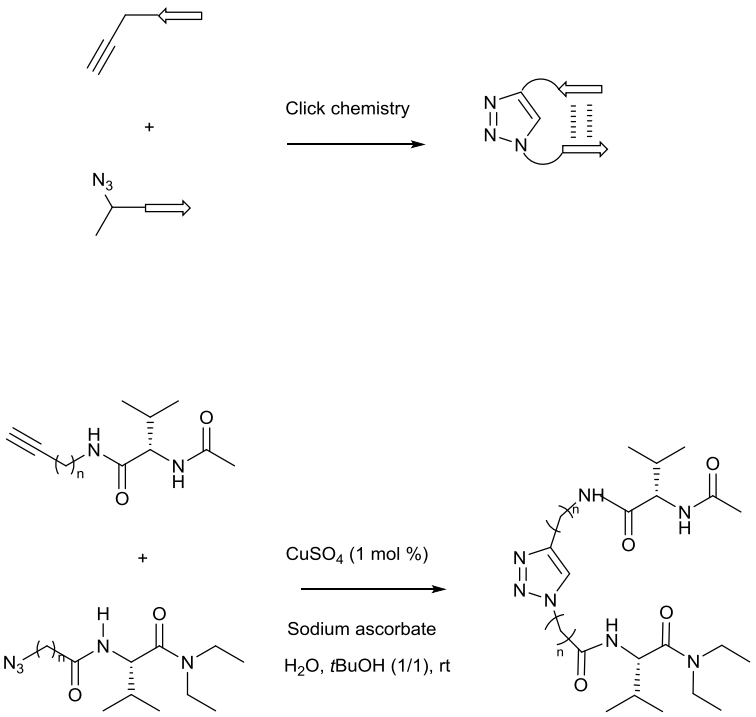
This ligation technique has been widely applied in peptide synthesis. However, it is limited by its reliance on the presence of a cysteine residue at the *N*-terminus of one of the fragments to be coupled, but cysteine is the second least common residue in peptide sequences.[14]

In 2000, Staudinger ligation was introduced by Raines and Bertozzi.[15] This method is based on the reaction between an azide and a phosphine to produce an iminophosphorane that then forms an amidophosphonium salt via an acyl shift reaction. This salt subsequently undergoes hydrolysis to form the amide product and phosphine oxide (Scheme 1.9). [10, 16]



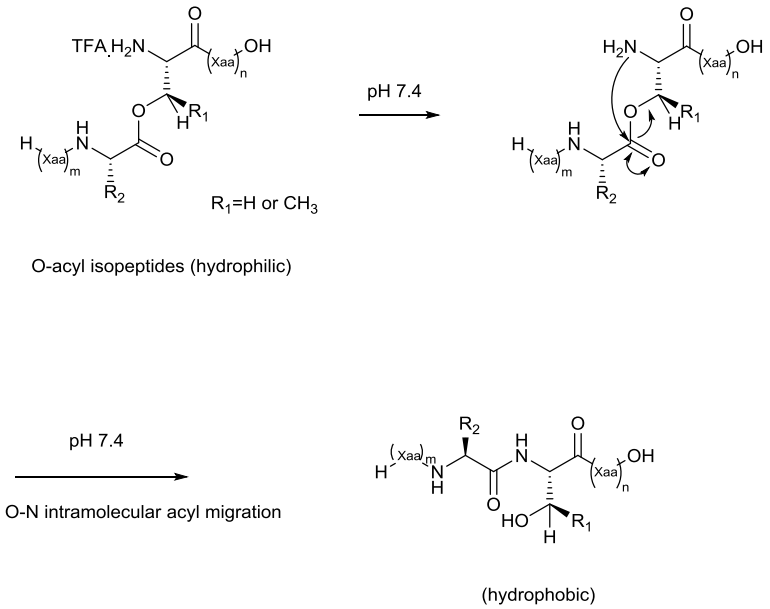
Scheme 1.9 Staudinger ligation in peptide synthesis

The concept of click chemistry was developed by Sharpless and co-workers in 2001 as another ligation tool, involving the copper (I)-catalysed addition of acetylenes to azides to form 1,2,3-triazoles.[17] Triazole ligation allowed the construction of cyclic tetrapeptides in good yield and it was reported that formation of the triazole ring could facilitate the cyclization (Scheme 1.10).[18]



Scheme 1.10 Synthesis of tetrapeptides using a copper(I)-catalysed azide-alkyne cycloaddition (click reaction)

In 2003, the generation of an *O*-acyl ester linkage within the peptide backbone instead of the *N*-acyl amide linkage was reported to increase the solubility of a readily aggregated long sequence peptide by changing the secondary structure of the native peptide. The target peptide was then generated through *O*-*N* intramolecular acyl migration reaction (Scheme 1.11). [19]



Scheme 1.11 *O*-Acyl isopeptide method in peptide synthesis

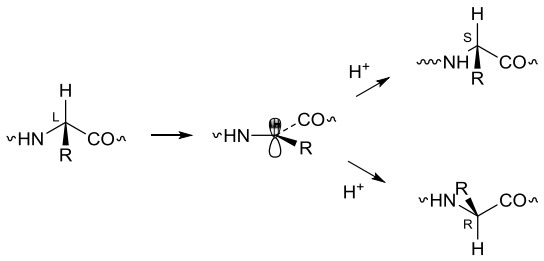
1.7 The racemization problem in peptide synthesis

Racemization is one of the primary concerns in peptide chemistry. The loss of chiral integrity at one or more stereogenic centres will form a mixture of epimers that will lead to difficult purification problems. Due to its highly repetitive nature, efficient coupling is vital in peptide synthesis and so it is imperative to optimize the appropriate conditions at each coupling step to avoid epimerization and minimize side reactions in order to facilitate purification. Loss of chiral integrity can occur by two different mechanisms, direct enolization and the oxazolone formation.

1.7.1 Direct enolization

Deprotonation of the α-carbon of an α-amino acid will generate the carbanion intermediate that could reprotonate to lead to either the *S*- or *R*- configuration of the amino acid (Scheme 1.12).[5] Amino acid racemization occurs most rapidly with good

leaving groups and the use of unhindered strong bases in dipolar aprotic solvents such as DMSO and DMF.[5] Generally however, this mechanism only contributes slightly, if at all, to racemization during peptide synthesis.

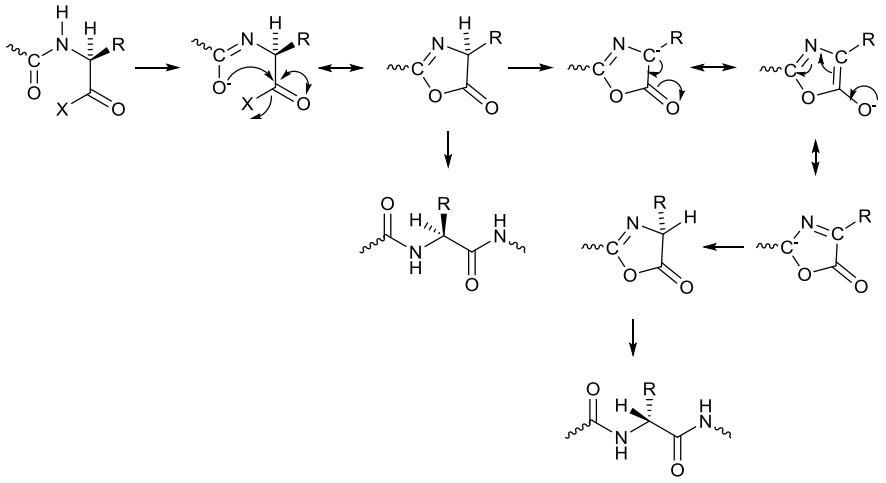


Scheme 1.12 Racemization in a peptide based on direct enolization

1.7.2 Epimerization by oxazolone mechanism

Oxazolone formation is encountered in peptide synthesis when a *C*-terminus activated acylamino acid cyclizes under mildly basic conditions (Scheme 1.13).[5] The oxazolone acts as it were a *C*-terminus activating group and can therefore react with the amino group of another amino acid to produce a peptide bond. However, racemization of the oxazolone through its aromatic enol tautomer can occur to varying degrees alongside peptide bond formation. As a result, the peptide produced is, to a greater or lesser degree, epimerized at what had been the activated *C*-terminus. This reaction occurs rapidly if the amino nitrogen is acylated with a simple acyl group such as acetyl or benzoyl group, but also occurs if the acyl substituent is a peptide chain and can even result in complete epimerization with the use of good leaving groups that facilitate the cyclization to the oxazolone. Interestingly, if the acyl subsituent is replaced with an alkoxycarbonyl protecting group, oxazolone formation is largely blocked. Racemization

does not occur during the activation and coupling stages when using Z, Boc and Fmoc protected amino acids under normal conditions.[5]

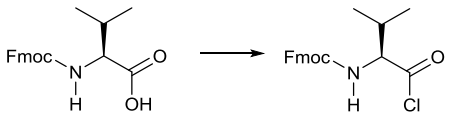


Scheme 1.13 Racemization in a peptide chain based on oxazolone mechanism

1.8 Carboxyl activation strategies

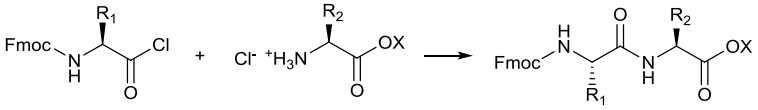
1.8.1 Acyl chlorides

The most simple and convenient approach to peptide synthesis is the activation of *N*-protected amino acid by conversion to an acyl chloride followed by treatment with the *C*-teminus protected amino ester, either under Schotten-Baumann conditions or in an organic solvent. Thionyl chloride and phosphorus pentachloride are commonly used in acyl chloride formation but simple acyl amino acid chlorides tend to cyclize to oxazolones giving racemic material.[5] This limitation to acyl chloride application in peptide chemistry was overcome through the development by Carpino of Fmoc acid chlorides, which are more stable and easier to prepare under mild conditions (Scheme 1.14).[20]



Scheme 1.14 $SOCl_2$, CH_2Cl_2, reflux, 15 minutes

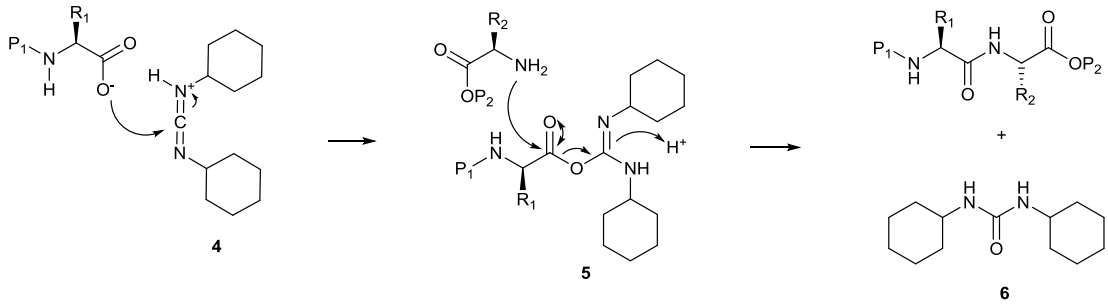
Various peptides have been successfully synthesized in good yields by employing Fmoc acid chloride methodology in the presence of zinc dust (Scheme 1.15).[21] The amino acid ester hydrochloride is neutralized *in situ* using zinc dust instead of base prior to coupling, resulting in a peptide free from epimerization and with minimum side reactions. Coupling is usually fast and is normally complete within 15 minutes.



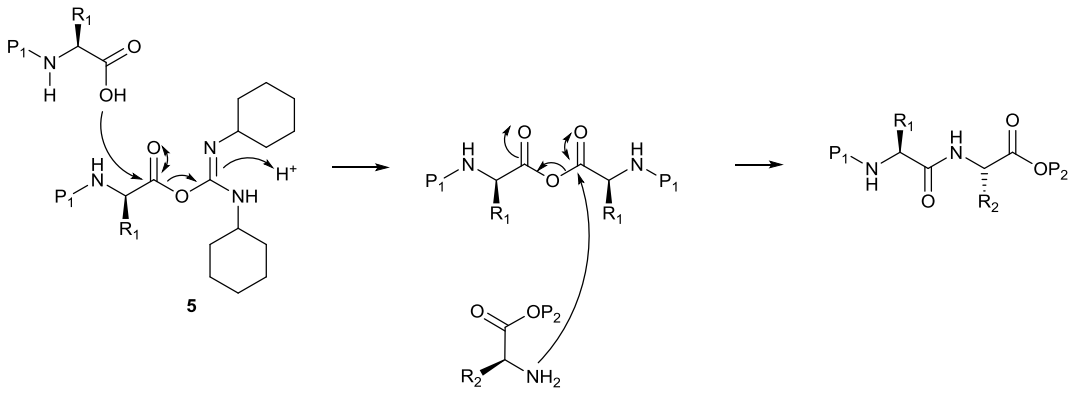
Scheme 1.15 Zn, THF, room temperature, 15 minutes

1.8.2 Carbodiimide coupling

Dicyclohexyl carbodiimide (DCC) **4** was introduced by Sheehan and Hess in 1955[22] as a carboxy- group activating agent. The discovery of this reagent led to a simple peptide coupling method involving addition of the *N*-protected amino acid to a solution of DCC in an organic solvent to give the *O*-acylisourea **5**, a potent acylating agent (Scheme 1.16).[5] The *O*-acylisourea subsequently forms the peptide bond, either through direct aminolysis, or via the symmetrical anhydride with formation of dicyclohexylurea **6** (Scheme 1.17).[5] The separation of this by-product is generally not an issue as it has a very low solubility in most organic solvents and can be removed by filtration.[22]

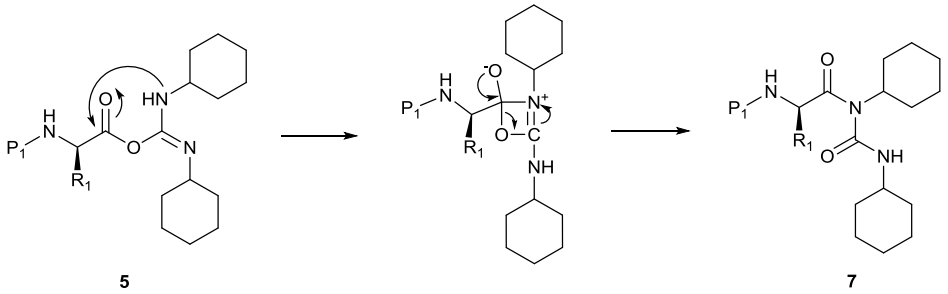


Scheme 1.16



Scheme 1.17

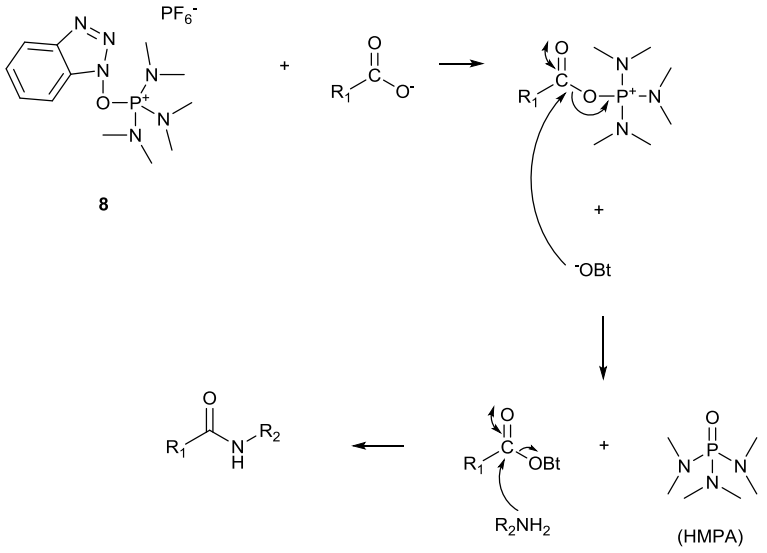
However, an intramolecular acyl transfer of the *O*-acylisourea may occur to a significant extent to form unreactive *N*-acylurea **7** and this reduces the yield and leads to purification problems (Scheme 1.18).[5,10] The use of 1-hydroxybenzotriazole (HOBt) as an additive as α-nucleophiles to react preferentially with the *O*-acylisourea to generate an active ester reduces the risk of racemization and other side reactions.[10]



Scheme 1.18

1.8.3 Phosphonium reagents

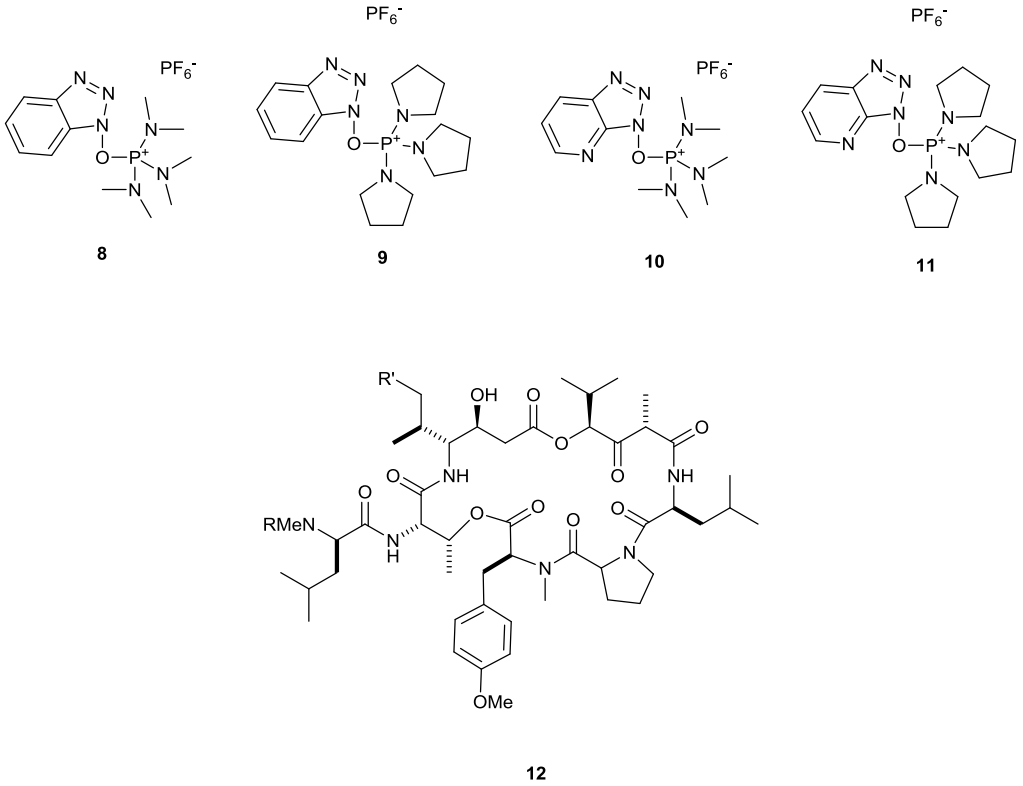
The phosphonium salt, (benzotriazolyloxy)-tris-(dimethylamino)phosphonium hexafluorophosphate) (BOP, **8**) reagent was developed by Castro in 1975[23,24] as a stable coupling reagent and started gaining popularity over DCC as it greatly lowered the risk of racemization, due to the rapid rate of coupling of this system, giving excellent yields and producing easily removed co-products (Scheme 1.19).[5,25] The *N*-protected amino acid is deprotonated to generate the carboxylate anion and this subsequently attacks the positively charged phosphonium salt resulting in a nucleophilic BtO^- leaving group that reacts with the carboxyl group of the first intermediate in the presence of a tertiary amine such as triethylamine or di*iso*propylethylamine (DIPEA). The *C*-protected amino acid then attacks the BtO-ester to form the desired peptide.



Scheme 1.19

However, use of the BOP reagent generates the known carcinogen hexamethylphosphoramide (HMPA), thus limiting its application in peptide chemistry.[25] As an alternative, the (benzotriazolyloxy)-tris-(pyrrolidino)phosphonium hexafluorophosphate) (PyBop, **9**) reagent was developed and is reported to demonstrate all the advantages of the BOP reagent and, more importantly, to produce a less harmful by-product.[25]

Two other phosphonium salts that are claimed to be even more efficient as coupling reagents are (7-aza-benzotriazolyloxy)-tris-(dimethylamino)phosphonium hexafluorophosphate (AOP, **10**) and the slightly more reactive (7-aza-benzotriazolyloxy)-tris-(pyrrolidino)phosphonium hexafluorophosphate (PyAOP, **11**), neither of which generates HMPA.[25] The pyrrolidino- derivative PyAOP was successfully used in a cyclization to produce the anticancer agent, didemnin **12** in an excellent 70 % yield through solution phase methodology.[25]

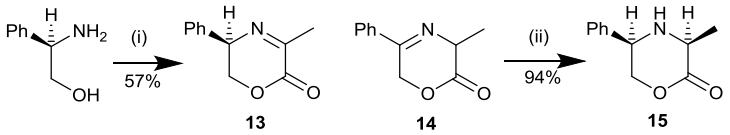


1.9 Development of a morpholinone-based peptide synthesis strategy

1.9.1 The morpholinone template

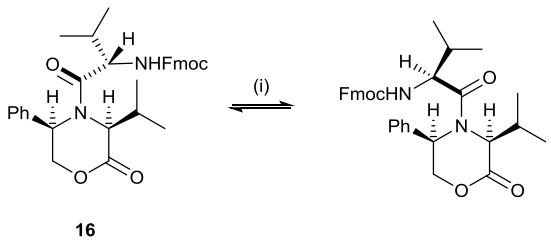
Due to the epimerization problems associated with *C*-terminus-activated, *N*-acylated amino acids, linear peptide synthesis is limited to *C*- to *N*-terminus direction in a linear extension protocol. The Harwood group has discovered the possibility for peptide coupling in a manner similar to that Nature carries it out, namely in the *N*- to *C*-terminus direction, based on the morpholinone template. The (3*S*,5*R*)-3-methyl-5-phenylmorpholinone template was constructed based on the method developed by Harwood and Vines (1996) (Scheme 1.20).[26] This template was synthesized by reacting (*R*)-2-phenylglycinol with ethyl pyruvate for 24 hours in 2,2,2-trifluoroethanol to produce mainly dehydromorpholinone **13**. The crude material was then purified by chromatography to remove the racemic isomer **14** that was formed as a by-product.

Hydrogenation of **13** under an atmosphere of hydrogen in dichloromethane using PtO_2 catalyst for 5 hours furnished the desired template **15**.



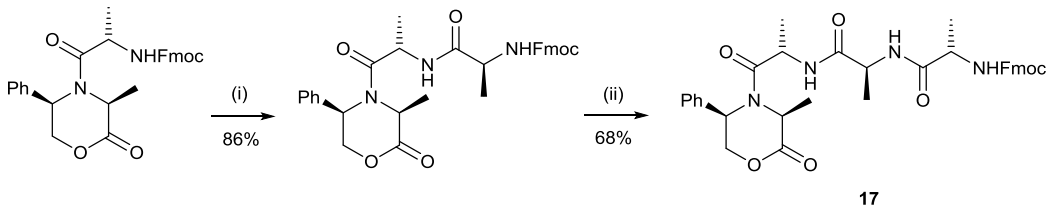
Scheme 1.20 (i) Ethyl pyruvate, 4 Å molecular sieves, TFE, Δ, 24 hours (ii) H_2, 1 atmosphere, PtO_2, CH_2Cl_2, 5 hours

(3*S*,5*R*)-Morpholinones such as **15** can act as *C*-terminus activated amino acids, but an *N*-acyl substituent on the nitrogen is not able to reach the lactone carbonyl and therefore cannot form an oxazolone. This was demonstrated by heating *N*-acetyl morpholinone **16** in DMSO-d_6 at 120 ^oC for 3 weeks with no evidence of formation of the epimer at C-3 (Scheme 1.21).[27] A mixture of conformers was observed at temperature in a range of 20 – 80 ^oC, due to slow interconversion between the two conformations of the amide as a result of restricted rotation around the amide bond, perhaps further induced by the steric interactions of the amide substituent with the substituent at C-3.[28] However, when the temperature was increased to 120 ^oC, the free rotation around the amide bond showed the presence of only a single diastereoisomer indicating that the morpholinone based model for *C*-terminus based peptide synthesis should indeed be free from epimerization.

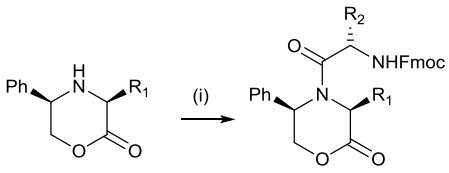


Scheme 1.21 (i) DMSO-d_6, 120 ^oC, 3 weeks

Furthermore, the *N*-terminus of the morpholinone template was successfully extended using classical solution phase methodology to form tetrapeptide derivative **17**. In the first step of the chain extension, DBU in THF was used to remove the Fmoc protecting group from the morpholinone and then coupling with Fmoc-L-alanine was carried out using bromotripyrrolidinophosphonium hexafluorophosphate and *N*,*N*-diisopropylethylamine (Scheme 1.22).[27] Moreover, it was discovered that the *N*-terminus extension of the morpholinone template was not limited to morpholinones with less bulky groups at C-3 and it was demonstrated that *N*-extended products were successfully furnished with bulky C–3 side chains such as propyl, benzyl or even *tert*-butyl (Scheme 1.23).[27] These findings indicated that *N*-terminus extension of the morpholinone template was not significantly affected by the sterically hindered environment.



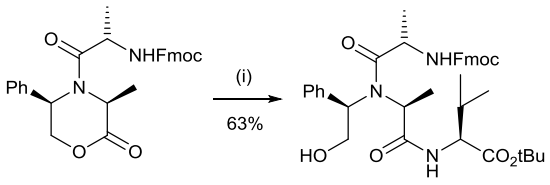
Scheme 1.22 (i) 10 mol % DBU, THF, 5 hours; DIPEA, PyBrop, Fmoc-L-alanine, THF, 18 hours; (ii) 50 mol % DBU, THF, 5 hours; DIPEA, PyBrop, Fmoc-L-alanine, THF, 18 hours



			Yield
18	R_1 = *i*Pr	**19** R_1 = *i*Pr, R_2 = CH_3	82%
		20 R_1 = *i*Pr, R_2 = *i*Pr	52%
21	R_1 = *t*Bu	**22** R_1 = *t*Bu, R_2 = CH_3	51%
		23 R_1 = *t*Bu, R_2 = *i*Pr	18%
24	R_1 = Bn	**25** R_1 = Bn, R_2 = CH_3	36%

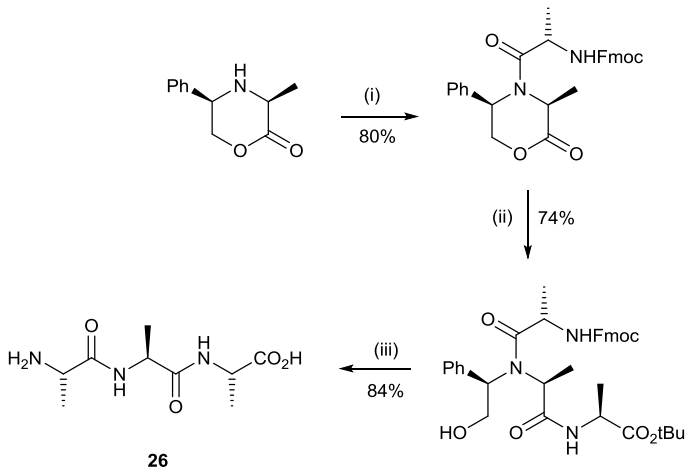
Scheme 1.23 (i) *N*-Fmoc-L-alanine acid chloride, Na_2CO_3, CH_2Cl_2,1 - 48 hours

It was known that the *N*-acylated morpholinone ring can react with nucleophilic species, leading to opening of the lactone ring (Scheme 1.24).[27] Furthermore, it was suggested that the degree of substitution on the free amine might not have a significant steric effect on this opening[27] and that the *N*-acylated morpholinone could thus play the role of a *C*-terminus activated dipeptide that would prevent the formation of oxazolone and therefore epimerization of the activated amino acid**.** However, by increasing the steric hindrance at C-3 with an *iso*propyl or benzyl morpholinone template, it was found that the aminolysis could not occur under the same conditions.

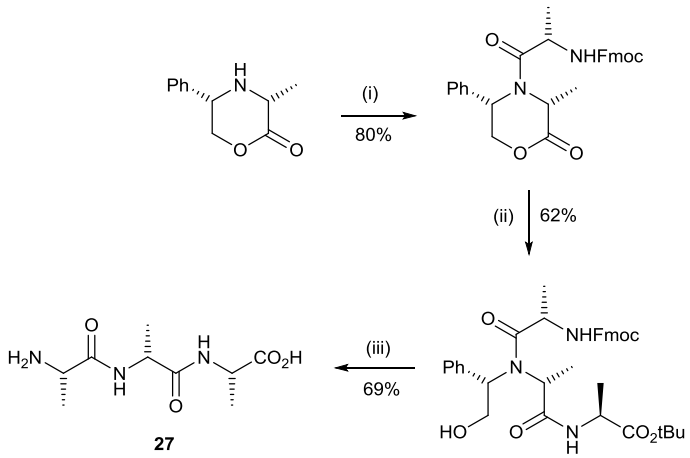


Scheme 1.24 (i) *t*-butyl-L-valinate, $Al(CH_3)_3$, CH_2Cl_2, 24 hours

Nevertheless, two simple tripeptides, L-ala-L-ala-L-ala **26** and L-ala-D-ala-L-ala **27** were successfully synthesized by adopting this approach. The synthetic pathways to these peptides are illustrated in Schemes 1.25 and 1.26.[27,29]



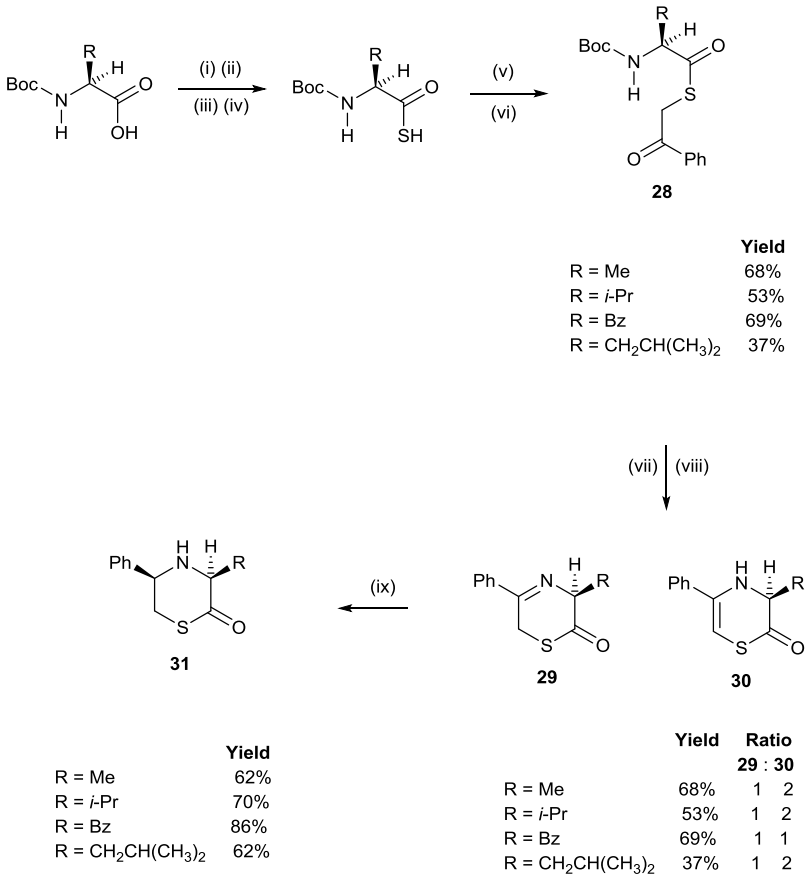
Scheme 1.25 (i) *N*-Fmoc-L-alanine acid chloride, Na_2CO_3, CH_2Cl_2, 1 hour; (ii) *t*-butyl-L-alanate, $Al(CH_3)_3$, CH_2Cl_2 , 24 hours (iii) Li, liquid NH_3, *t*-BuOH, THF, -78 ^oC, 15 minutes



Scheme 1.26 (i) *N*-Fmoc-L-alanine acid chloride, Na_2CO_3, CH_2Cl_2, 1 hour; (ii) *t*-butyl-L-alanate, $Al(CH_3)_3$, CH_2Cl_2 , 24 hours (iii) Li, liquid NH_3, *t*-BuOH, THF, -78 ^oC, 15 minutes

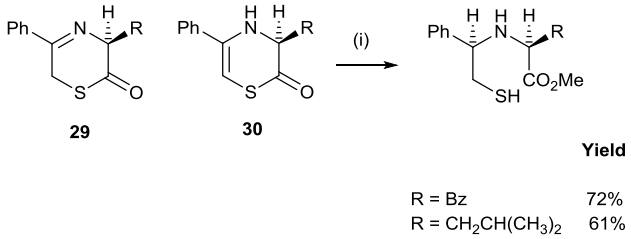
1.9.2 The thiomorpholinone template

Thiolactones are known to be more prone to nucleophilic attack than the lactone ring in the morpholinone template. Therefore, a synthetic route to novel thiomorpholinone analogues was developed in the Harwood group to investigate the reactivity of this system towards amide bond formation by thiolactone ring opening with the aim of finding milder and more efficient conditions for *C*-terminus extension. The original synthetic route for preparing the novel chiral thiomorpholinones is described in Scheme 1.27.[30,31] The Boc-protected L-amino acid was reacted with ethyl chloroformate at 0 ^{o}C for 30 minutes to activate the *C*-terminus. The resulting solution was then treated with sodium hydrosulfide hydrate to form the thioacid to which potassium 2-ethyl hexanoate was added. The potassium salt was subsequently reacted with 2-bromoacetophenone to form the thioester **28**. The Boc-protecting group was then removed by adding 20 % (v/v) trifluoroacetic acid in dichloromethane followed by cyclization on treating the deprotected amine with anhydrous potassium carbonate or cesium carbonate to generate the corresponding isomeric dehydrothiomorpholinones **29** and **30**. These were then diastereoselectively reduced with sodium cyanoborohydride in THF to furnish the desired *syn*-3(*S*)-substituted-5(*R*)-phenylthiomorpholinones **31**.



Scheme 1.27 (i) Et_3N, DMF, 0 ^oC, 30 minutes, (ii) $ClCO_2Et$, 0 ^oC, 30 minutes, (iii) NaHS, 0 ^oC, 30 minutes, (iv) 1 M HCl, 0 ^oC (v) KEH, Et_2O, 30 minutes (vi) 2-bromo-acetophenone, DMF, 18 hours (vii) TFA, CH_2Cl_2, room temperature, 2 hours (viii) Cs_2CO_3 or K_2CO_3, CH_2Cl_2, room temperature, 24 hours (ix) (i) $NaBH_3CN$, AcOH, THF, room temperature, 48 hours

This study revealed that the thiolactone was readily attacked by nucleophilic solvents such as methanol, a process that did not occur in the morpholinone system (Scheme 1.28)[30] and the greater tendency of this template towards nucleophilic attack lent support to the proposal that the thiomorpholinone template could facilitate amide bond formation and improve the *C*-terminus extension peptide coupling reaction.



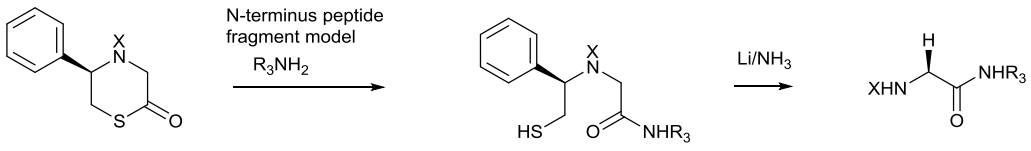
Scheme 1.28 (i) $NaBH_3CN$, AcOH, MeOH, 24 hours

1.10 Aims of this project

Several derivatives of 3,5-disubstituted thiomorpholinones have been successfully constructed in the Harwood group incorporating benzyl, *iso*propyl, butyl and methyl groups at C-3.[30] However, it was found that the parent thiomorpholinone, with C-3 unsubstituted, could not be accessed through that procedure. Attempts at developing alternative synthetic routes to obtain this C-3 unsubstituted thiomorpholinone with a 5-(4-methoxyphenyl) auxillary had been carried out by previous researchers in the Harwood group and it was reported to be more challenging to synthesize than other thiomorpholinone derivatives. Therefore, it was proposed that attempts to synthesize C-3 unsubstituted thiomorpholinones **32** would be continued by considering other pathways and the reactivity of these templates, both as the free amine and as the *N*–acylated form, would be studied with regard to ring opening amide bond formation (Scheme 1.29). In particular the stereochemical integrity of position 3 would be investigated in the case of the *N*–acyl derivatives.

Thus, the development of thiomorpholinones as novel reagents for amide bond formation would be explored, to assess their capability to be used as templates for peptide coupling reagents. Using this model, the peptide chain could be extended, not only from the *N*-terminus side of the template, but also from the thiolactone function

acting as the activated *C*-terminus without epimerization by analogy with the *N*-acylated morpholinones, where intramolecular interaction to form an oxazolone is prevented. It was therefore expected that the 5-phenylthiomorpholinone motif could generate an amide bond by *C*-terminus extension without *C*-terminus epimerization and then be *N*-debenzylated under conditions more amenable to peptide synthesis.



Scheme 1.29 Proposed pathways for peptide synthesis using the glycine-derived thiomorpholinone template

References

1 Usmani, S. S., Bedi, G., Samuel, J. S., Singh, S., Kalra, S., Kumar, P., Ahuja, A. A., Sharma, M., Gautam, A. and Raghava, G. P. S. *PLoS One*, 2017, **12**, 1–12.

2 Chandrudu, S., Simerska, P. and Toth, I. *Molecules*, 2013, **18**, 4373–88.

3 Han, S. Y. and Kim, Y. A. *Tetrahedron*, 2004, **60**, 2447–2467.

4 Bradley, L. N., Matthew, B. S. and Ronald, T. R. *Annu. Rev. Biophys. Biomol. Struct.*, 2005, **34**, 91–118.

5 Jones, J. *Amino Acid and Peptide Synthesis*, Oxford University Press Inc., New York, 2002.

6 Bergmann, M. and Zervas, L. *Berichte*, 1932, **65**, 1192–1201.

7 McKay, F. C. and Albertson, N. F. *J. Am. Chem. Soc.*, 1957, **79**, 4686–4690.

8 Carpino, L. A. and Han, G. Y. *J. Am. Chem. Soc.*, 1970, **92**, 5748–5749.

9 Merrifield, R. B. *Biochemistry*, 1964, **3**, 1385–1390.

10 Montalbetti, C. A. G. N. and Falque, V. *Tetrahedron*, 2005, **61**, 10827–10852.

11 Schnölzer, M. and Kent, S. B. *Science*, 1992, **256**, 221–225.

12 Dawson, P. E. and Kent, S. B. *Annu. Rev. Biochem.*, 2000, **69**, 923–60.

13 Dawson, P. E., Muir, T. W., Clark-Lewis, I. and Kent, S. B. *Science*, 1994, **266**, 776–779.

14 Mühlberg, M., Jaradat, D. M. M., Kleineweischede, R., Papp, I., Dechtrirat, D., Muth, S., Broncel, M. and Hackenberger, C. P. R. *Bioorg. Med. Chem.*, 2010, **18**, 3679–86.

15 Saxon, E., Armstrong, J. I. and Bertozzi, C. R. *Org. Lett.*, 2000, **2**, 2141–2143.

16 Soellner, M. B., Nilsson, B. L. and Raines, R. T. *J. Org. Chem.*, 2002, **67**, 4993–4996.

17 Kolb, H. C., Finn, M. G. and Sharpless, K. B. *Angew. Chemie, Int. Ed.*, 2001, **40**, 2004–2021.

18 Lutz, J. F. and Zarafshani, Z. *Adv. Drug Deliv. Rev.*, 2008, **60**, 958–970.

19 Yoshiya, T., Taniguchi, A., Sohma, Y., Fukao, F., Nakamura, S., Abe, N., Ito, N., Skwarczynski, M., Kimura, T., Hayashi, Y. and Kiso, Y. *Org. Biomol. Chem.*, 2007, **5**, 1720–30.

20 Carpino, L. A., Cohen, B. J., Stephens, K. E., Sadat-Aalaee, S. Y., Tien, J. H. and Langridge, D. C. *J. Org. Chem.*, 1986, **51**, 3732–3734.

21 Gopi, H. N. and Suresh Babu, V. V. *Tetrahedron Lett.*, 1998, **39**, 9769–9772.

22 Sheehan, J. C. and Hess, G. P. *J. Am. Chem. Soc.*, 1955, **77**, 1067–1068.

23 Castro, B., Dormoy, J. R., Evin, G. and Selve, C. *Tetrahedron Lett.*, 1975, **16**, 1219–1222.

24 Fournier, A., Wang, C. and Felix, A. M. *Int. J. Pept. Protein Res.*, 1988, **31**, 86–97.

25 Al-Warhi, T. I., Al-Hazimi, H. M. A. and El-Faham, A. *J. Saudi Chem. Soc.*, 2012, **16**, 97–116.

26 Harwood, L. M., Vines, K. J. and Drew, M. G. B. *Synlett*, 1996, **11**, 1051–1053.

27 Harwood, L. M., Mountford, S. J. and Yan, R. *J. Pept. Sci.*, 2009, **15**, 1–4.

28 Yan, R., Phd Thesis. University of Reading, 2006.

29 Harwood, L. M. and Yan, R., 2008, *U.S. Patent No. WO 2008/0281075 Al*. Washington, DC: U.S. Patent and Trademark Office.

30 Drew, M. G. B., Harwood, L. M. and Yan, R. *Synlett*, 2006, **2006** (19), 3259–3262.

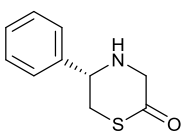
31 Harwood, L. M., Wellings, D. A. and Moody, J. D., 2012, *U.S. Patent No. WO 2012/020231 Al*. Washington, DC: U.S. Patent and Trademark Office.

Chapter 2

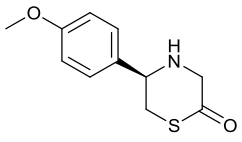
Synthesis of glycine derived thiomorpholinones

Several 3(*S*)-*R*-5(*R*)-phenylthiomorpholinone derivatives possessing bulky substituents such as benzyl, *iso*propyl, butyl and the less bulky methyl group have been successfully synthesized in previous work within the Harwood group in good overall yields that range from 62 – 86 %.[1] However, the synthesis of the glycine-derived, C-3 unsubstituted thiomorpholinone **33** could not be achieved through the procedure developed to prepare 3,5-disubstituted thiomorpholinones. The formation of a mixture of imine and enamine isomers was reported to be successful but the final reduction step to the unsubstituted thiomorpholinone was claimed problematic, despite many attempts being carried out, as all attempts ended with the thiomorpholinone dimer.[2] At this stage, it was therefore decided to focus on developing an alternative synthetic route to obtain glycine thiomorpholinones **32** and **33** although it seemed likely that the lack of steric hindrance at C-3 would present a great challenge to access the parent thiomorpholinone templates.

In this study, several alternative synthetic routes were explored to access the C-3 unsubstituted thiomorpholinone system including lactone ring opening, Mitsunobu reaction, hydrolysis, thiolactonization, amination and thionation.



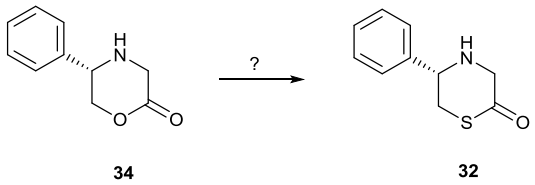
32



33

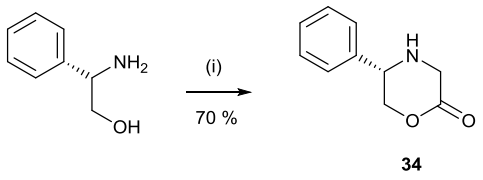
2.1 Ring opening of the morpholinone template and Mitsunobu reaction

It was planned to use monosubstituted 5(*S*)-phenylmorpholinone **34** as a model substrate for the conversion to 5(*S*)-phenylthiomorpholinone **32**.



34 **32**

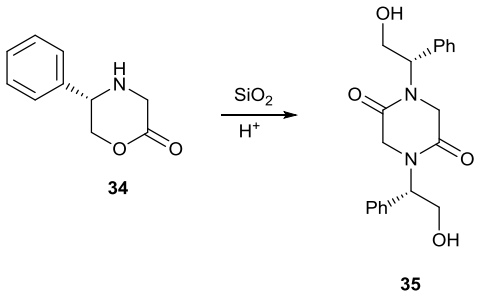
Starting material **34** was prepared by the standard procedure,[3] dissolving (*S*)-2-phenylglycinol and *N*,*N*-di*iso*propylethylamine in anhydrous acetonitrile under an atmosphere of nitrogen (Scheme 2.1). To this was then added a solution of phenyl bromoacetate in anhydrous acetonitrile drop-wise over 10 minutes and the mixture was subsequently stirred for 24 hours at room temperature and concentrated under reduced pressure to give the crude product **34** as a colourless gum.



34

Scheme 2.1 (i) Phenyl bromoacetate, DIPEA, CH_3CN, room temperature, 24 hours

The crude product **34** was then subjected to purification. Several attempts were performed to purify the crude product by column chromatography on silica, eluting with petroleum ether - diethyl ether (2 : 1) and also crystallization from ethyl acetate. However, the desired compound was found to have decomposed after both attempted purification procedures. It is known that the target compound is sensitive to acidic conditions and also that heating crude material to more than 40 ^{o}C can promote dimerization to the diketopiperazine **35** (Scheme 2.2).[3]



Scheme 2.2 Dimerization of **34** to diketopiperazine **35**

Subsequently, when the column chromatography was repeated, approximately 2 cm of anhydrous potassium carbonate was added to the top of a column of Florisil® to attempt to neutralize any acid in the crude material before elution. The crude material **34** was immediately loaded onto the column to avoid dimerization of **34** to **35**[3] and the column eluted with petroleum ether – diethyl ether (1 : 2) to remove impurities followed by acetone to elute **34** in 70 % yield as a pale yellow oil.

Evidence that 5(*S*)-phenylmorpholinone **34** had been successfully synthesized was based on observation of the characteristic pair of double doublets at δ 4.42 ppm (J = 11.0 Hz, J' = 4.5 Hz) and δ 4.20 ppm (J = 11.0 Hz, J' = 4.0 Hz), and a apparent triplet at δ 4.31 ppm (J = 11.0 Hz) corresponding to the ABX system at C-5 and C-6 of the desired product (Figure 2.1). The AB protons appeared at δ 3.98 ppm (J = 18.0 Hz) and δ 3.86 ppm (J = 18.0 Hz) as two doublets. A mass ion peak of [MH^+] at 178.0863 gave further support that the desired product had been obtained.

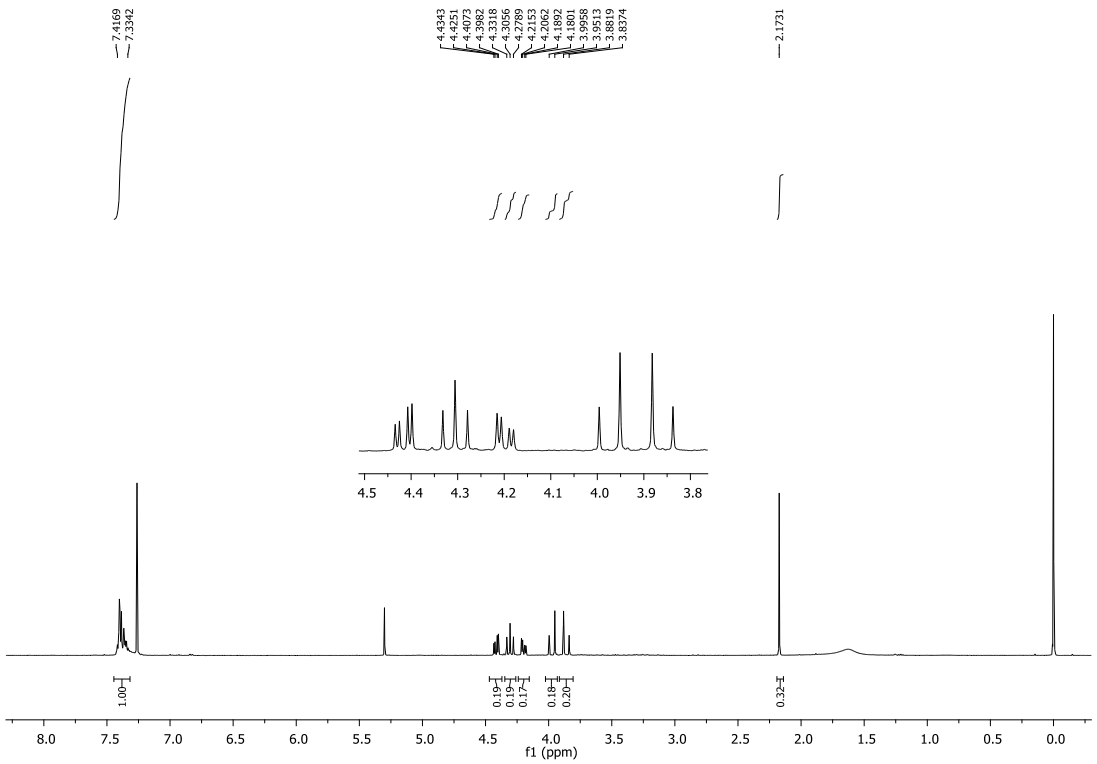
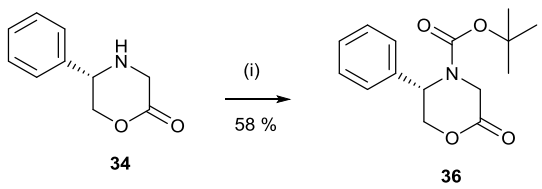


Figure 2.1 ^{1}H NMR spectrum of 5(*S*)-phenylmorpholinone **34** in $CDCl_3$

The *N*-protection of **34** was performed immediately with di-*tert*-butyl dicarbonate **2** based on the method reported by Dellaria and Santarsiero[3] to avoid any loss due to dimerization (Scheme 2.3). The 5(*S*)-phenylmorpholinone **34**, di-*tert*-butyl dicarbonate **2** and triethylamine were dissolved in diethyl ether at room temperature and the mixture left for 6 hours. The reaction mixture was then worked up and purified over silica, eluting with diethyl ether, to afford *N*-Boc-5(*S*)-phenylmorpholinone **36** as a white solid in 58 % yield.



Scheme 2.3 (i) Di-*tert*-butyl dicarbonate, Et_3N, EtOAc, room temperature, 6 hours

Boc-protected morpholinone **36** gave broadened signals on recording the 400 MHz NMR spectrum, considered to be due to the restricted rotation of the acyl group attached to the nitrogen. An attempt at further characterization at 500 MHz NMR at elevated temperatures from 25 ^{o}C to 85 ^{o}C was able to improve the appearance of the spectrum, leading to the broad signals becoming more, but not totally, resolved (Figure 2.2).

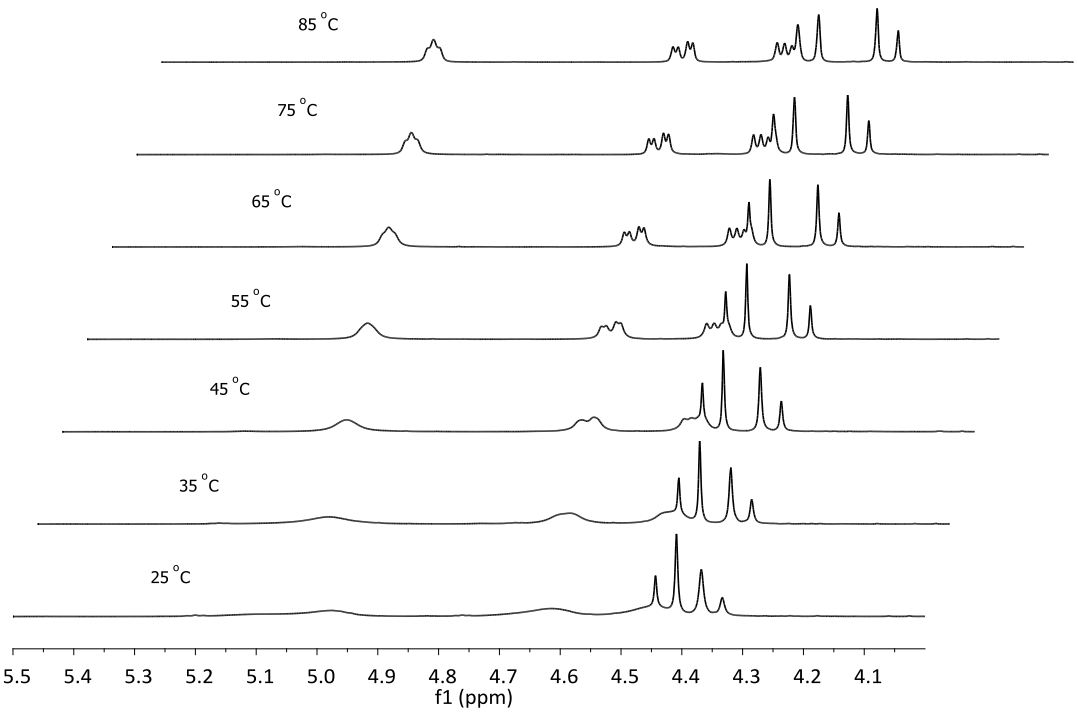


Figure 2.2 ^{1}H NMR spectra of *N*-Boc-5(*S*)-phenylmorpholinone **36** in DMSOd_6 at variable temperature on 500 MHz (Region from 4.0 to 5.5 ppm expanded; the AB and ABX components are partially resolved at the highest temperature)

Variable temperature facilities were not available on the two 400 MHz spectrometers, but at the higher field strength and at 85 ^{o}C, the broad signals that appeared centred at δ 4.64 ppm and δ 4.48 ppm were simplified into two double doublets at δ 4.64 ppm (J = 8.0 Hz, J' = 4.0 Hz) and δ 4.47 ppm (J = 12.0 Hz, J' = 4.0 Hz) and the broad resonance centred at δ 5.05 ppm became a broad triplet resonating at δ 5.05 ppm (J = 4.0 Hz) corresponding to the ABX system for the protons at C-5 and C-6 (Figure 2.3). Two doublets were also observed centred on δ 4.43 ppm (J = 12.0 Hz) and δ 4.31 ppm (J = 12.0 Hz), indicating the presence of the AB system at C-3. All ABX and AB protons of **36** were shifted downfield from the parent compound **34** due to the deshielding effect of the Boc group. So, despite having to use a greater field strength, analysing at higher temperature improved the ^{1}H NMR spectrum of **36,** causing the broad signals to become more defined and separated. A new carbonyl carbon resonance appeared at δ 154.0 ppm in addition to that at δ 168.8 ppm in the ^{13}C NMR spectrum signifying the presence of the Boc-carbonyl group. Two strong absorptions at 1768 cm^{-1} and 1700 cm^{-1} in the IR spectrum provided further support for the presence of two carbonyl groups. A mass ion at 276.1241 [MH^{-}] gave additional evidence that the Boc protection of 5(*S*)-phenylmorpholinone had been obtained.

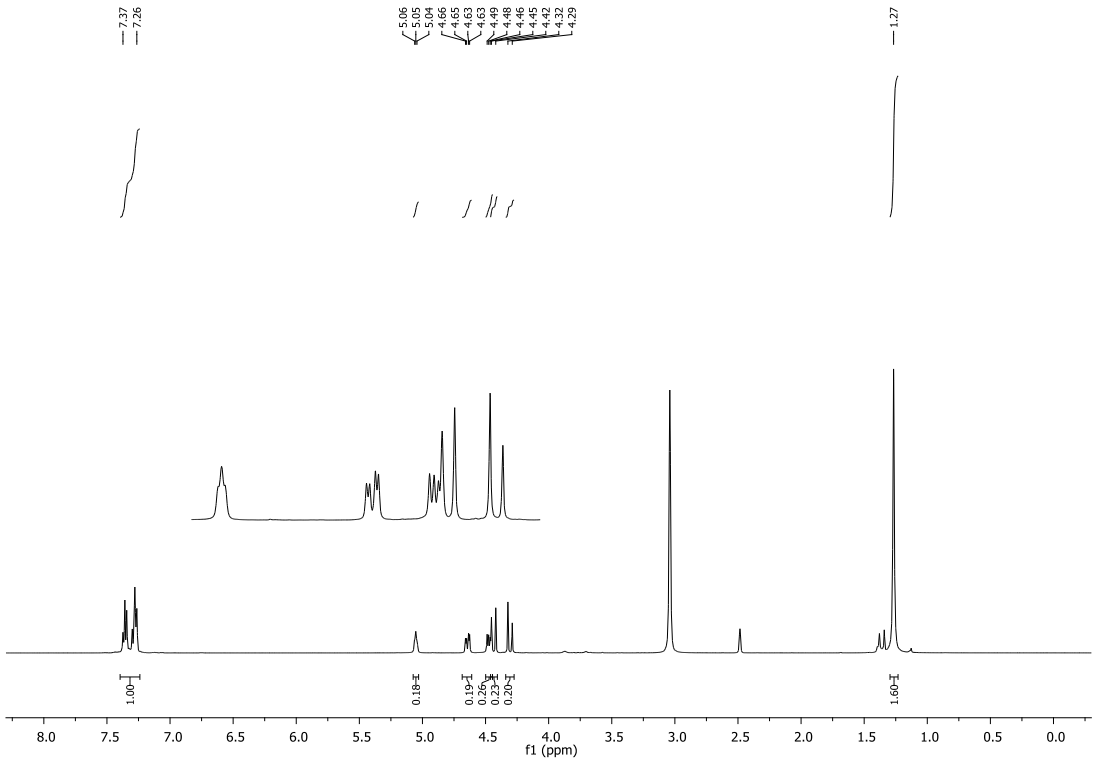
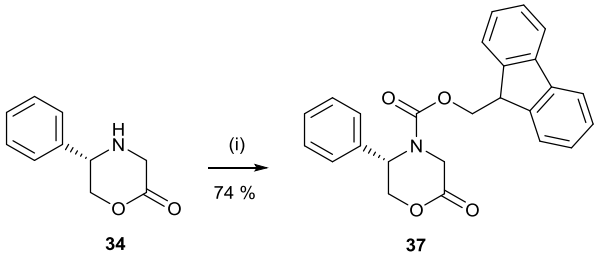


Figure 2.3 ^{1}H NMR spectrum of *N*-Boc-5(*S*)-phenylmorpholinone **36** in DMSOd_6 at 85 ^{o}C at 500 MHz

N-Protection with the Fmoc protecting group was carried out as well as *N*-Boc protection, to give more options in our synthetic approach going forward, as Boc and Fmoc protecting groups demonstrate orthogonal reactivity under acidic and basic conditions respectively. The *N*-Fmoc protection was carried out by stirring a mixture of morpholinone **34** with Fmoc-Cl and a suspension of K_2CO_3 in dichloromethane to give *N*-Fmoc-5(*S*)-phenylmorpholinone **37** in 74 % yield (Scheme 2.4).



Scheme 2.4 (i) Fmoc-Cl, K_2CO_3, CH_2Cl_2, overnight, room temperature

Once again, undefined broadened resonances were observed in the ^{1}H NMR spectrum analysed at room temperature at 400 MHz. Analysis at variable temperature at 500 MHz was therefore carried out in order to attempt to characterize the compound spectroscopically.

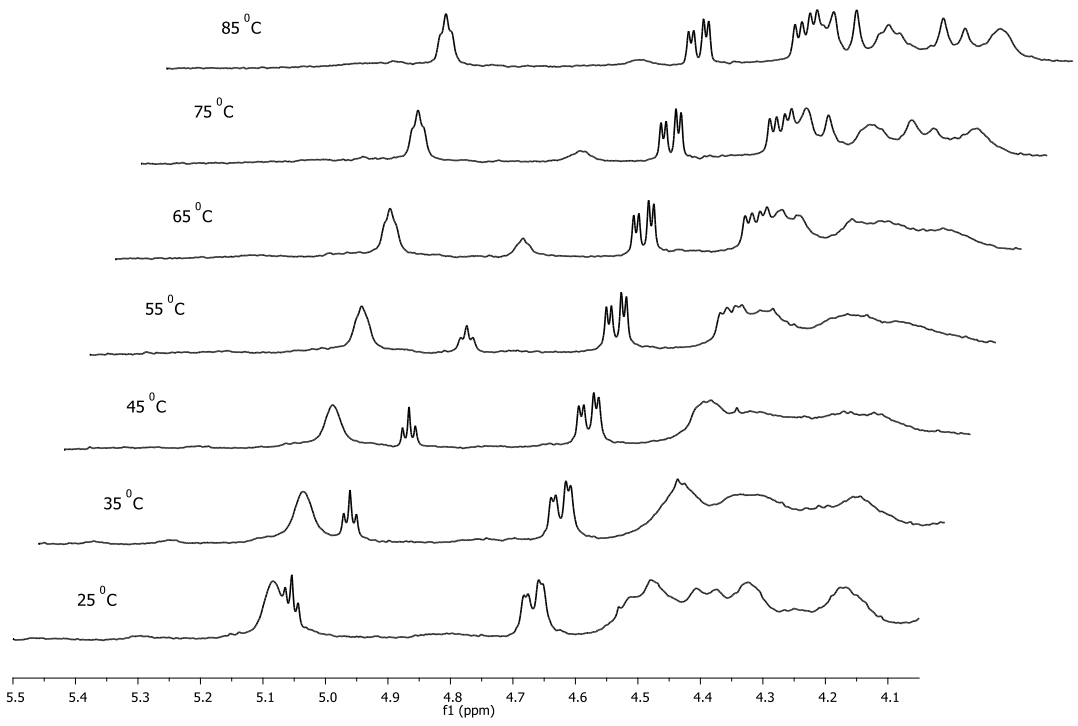


Figure 2.4 ^{1}H NMR spectrum of *N*-Fmoc-5(*S*)-phenylmorpholinone **37** in DMSOd_6 at variable temperature at 500 MHz (Region from 4.0 to 5.5 ppm expanded showing partial resolution of the ABX and AB systems)

At 85 ^{o}C, the ABX protons for C-5 and C-6 could be partially resolved into a broad triplet at δ 5.05 ppm (J = 4.0 Hz) and two double doublets at δ 4.65 ppm (J = 12.0 Hz, J' = 4.0 Hz) and δ 4.47 ppm (J = 12.0 Hz, J' = 4.0 Hz) (Figure 2.4; Figure 2.5). All the ABX resonances were shifted downfield compared to the parent compound **34**, as with the previous *N*-Boc protection, indicating the electron withdrawing properties of the

Fmoc group. The two AB protons could be detected as partially resolved mutually coupled doublets centred on δ 4.42 ppm (J = 16.0 Hz) and δ 4.24 ppm (J = 16.0 Hz). Mass spectrometry further supported the formation of *N*-Fmoc-5(*S*)-phenylmorpholinone **37** with a molecular ion at 422.1362 [MNa^+] being observed.

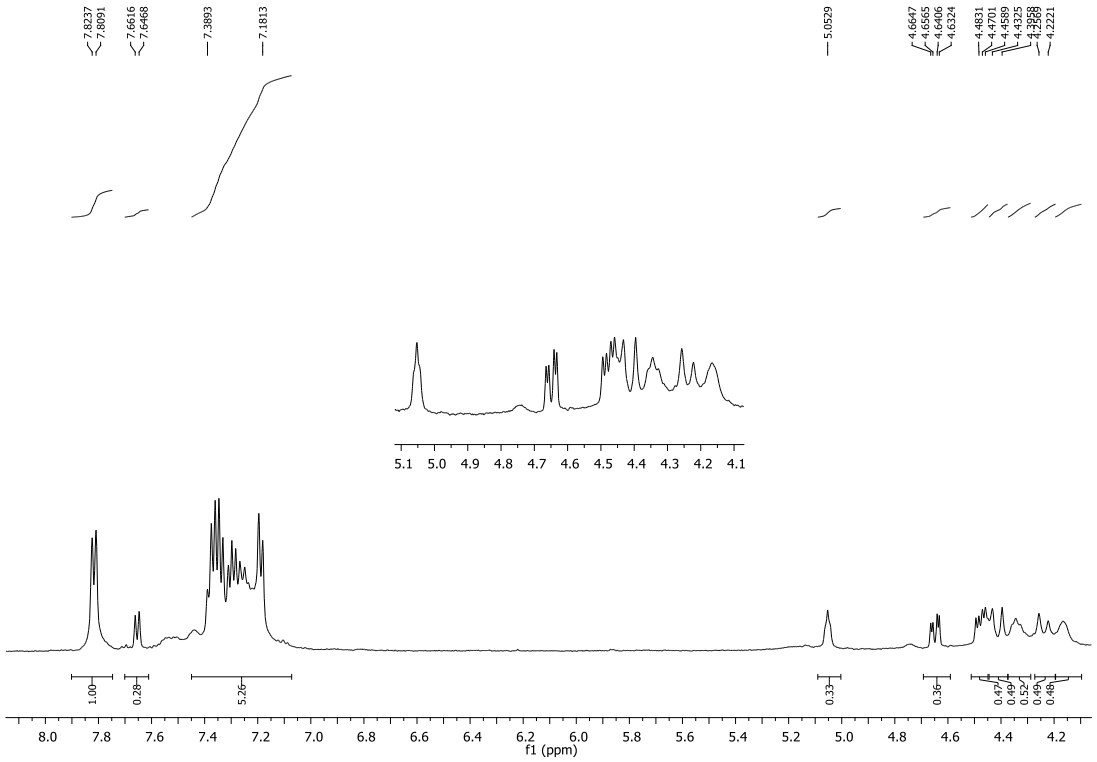
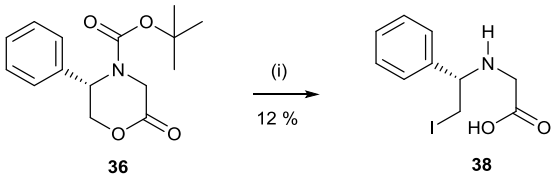


Figure 2.5 ^{1}H NMR spectrum of *N*-Fmoc-5(*S*)-phenylmorpholinone **37** in DMSOd_6 at 85 ^{o}C at 500 MHz

The key step for the next stage was lactone ring opening. This step posed a great challenge as the chosen reagent should be able to cleave the ester without stripping off the protecting group. Our initial attempts at opening the morpholinone **36** template with benzyl mercaptan and installing sulfur in one go by adapting Lumma's methodology failed.[4] Our attention then turned to the use of iodotrimethylsilane (TMSI) based on the methodology detailed by Deslongchamps and Guay (Scheme 2.5).[5]

After several unsuccessful attempts, the ring cleavage of **36** was finally induced with TMSI in $CHCl_3$ when the reaction mixture was heated to reflux at 48 ^oC for 24 hours to afford iodo acid **38**, but in a disappointing 12 % yield. However, the Boc-protecting group was also removed under these conditions. Under less forcing conditions, the ring opening failed; reaction at room temperature, even after five days, leaving the starting material untouched.



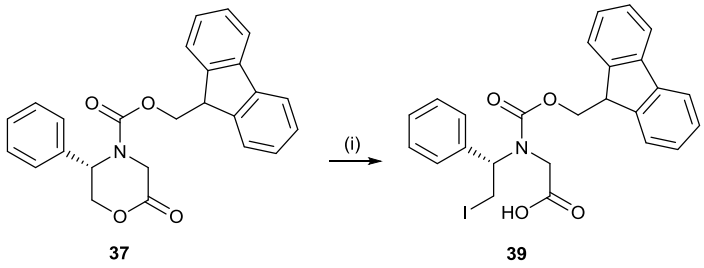
36 **38**

Scheme 2.5 (i) TMSI, $CHCl_3$, 48 ^oC, 24 hours

Three apparent triplets at δ 5.05 ppm (J = 8.5 Hz), δ 4.72 ppm (J = 8.5 Hz) and δ 4.16 ppm (J = 8.5 Hz) signified the presence of the freely rotating –CH–CH_2– system and two doublets at 4.32 ppm (J = 18.5 Hz) and 3.42 ppm (J = 18.5 Hz) indicated the AB system of iodoacid **38**. The mass ion at 305.9985 [MH^+] gave further support that iodoacid **38** had been isolated. Unfortunately, we found that the ring opening only occurred at 48 ^oC and the Boc-protecting group was easily removed at temperatures as low as 38 ^oC. Therefore, we decided to discontinue this approach to **38** due to the poor yield at an early stage and the need for reprotection of the nitrogen.

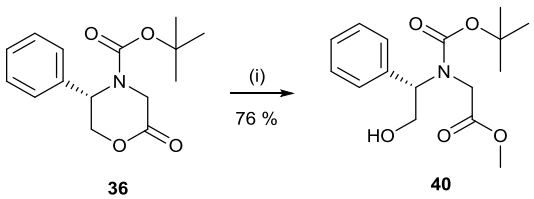
A similar ring opening procedure was performed on **37** by refluxing with TMSI but all attempts ended up with recovered starting material, despite several attempts having been carried out. Heating **37** with TMSI even at 98 ^oC overnight still gave mostly recovered starting material and Fmoc-protected iodoacid **39** could only be detected as a

trace from mass spectrometry with the molecular ion peak at 528.0687 [MH^+] being present when the crude product mixture was analysed. However, from this experiment, it was found that *N*-Fmoc protection could withstand high temperatures, giving us more options for further attempts at lactone ring opening.



Scheme 2.5 (i) TMSI, $CHCl_3$, 98 ^oC, 24 hours

Therefore, we decided to change our strategy by converting the protected morpholinone to opened δ-hydroxy methyl ester under basic conditions following an adaption of Corey's methodology.[6] With a large excess of methanol, the nucleophilic ring opening was carried out at room temperature for 24 hours in the presence of 6.0 equivalents of triethylamine to give crude δ-hydroxy methyl ester as a colourless oil. Attempts at purification of the crude material over silica, eluting with petroleum ether – diethyl ether (1 : 2) gave slightly impure **40** in ~76 % yield along with some starting material being recovered (Scheme 2.6).



Scheme 2.6 (i) MeOH, Et_3N, room temperature, 24 hours

The methanolysis of **36** was then extended from 24 hours to 48 hours and 72 hours, with the reaction being monitored by ^{1}H NMR spectroscopy, based on the decrease of the proton resonance in **36** at δ 4.97 ppm (J = 4.0 Hz) (Figure 2.6). However, this showed that there was no significant change in the ratio of **36** and **40** over that period of time suggesting that both **36** and **40** exist in equilibrium under these conditions. This conclusion was supported by Choji and Kazuo, who reported that several morpholin-2-one derivatives exist in equilibrium with their open chain methyl esters during methanolysis, with the lactone being favoured by increased ring substitution, most likely due to steric factors related to the Thorpe-Ingold effect.[7,8]

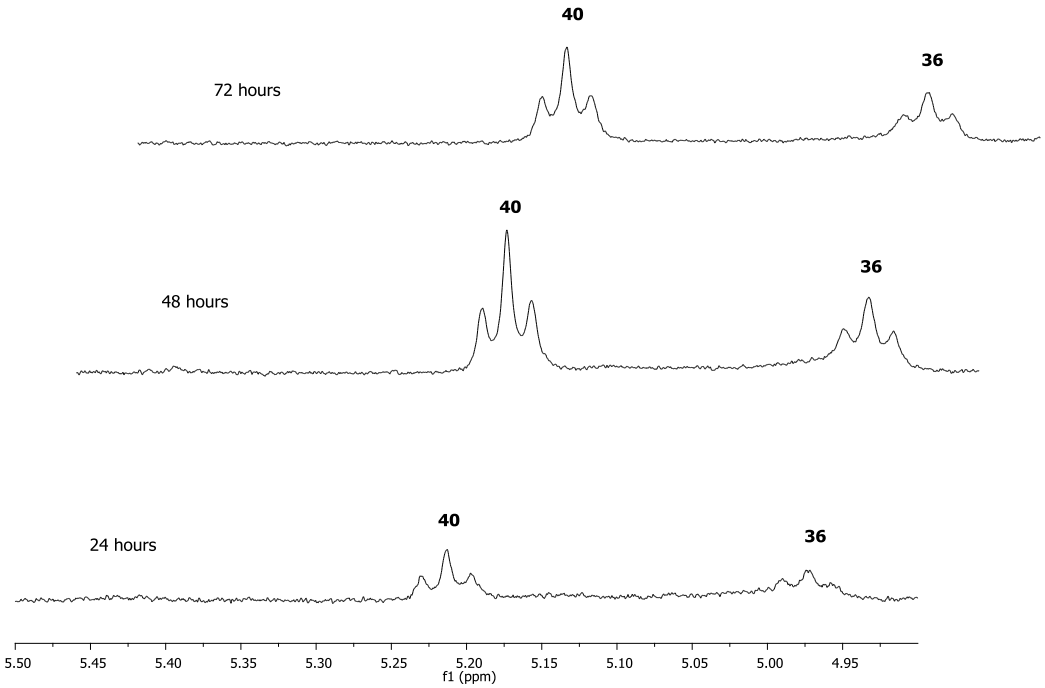


Figure 2.6 ^{1}H NMR spectra of a mixture of morpholinone **36** and δ-hydroxy methyl ester **40** in DMSOd_6, range δ 5.50 ppm to δ 4.85 ppm, after methanolysis for 24, 48 and 72 hours

The partial success of ring opening resulting in conversion to δ-hydroxy methyl ester **40** was indicated by a singlet appearing at δ 3.58 ppm (Figure 2.7) revealing the presence of the methyl ester and this was supported by a mass ion observed at 332.1468 [MNa^+]. A broad absorption centred on 3486 cm^{-1} in the IR spectrum, corresponding to the free alcohol gave further support for the formation of **40**.

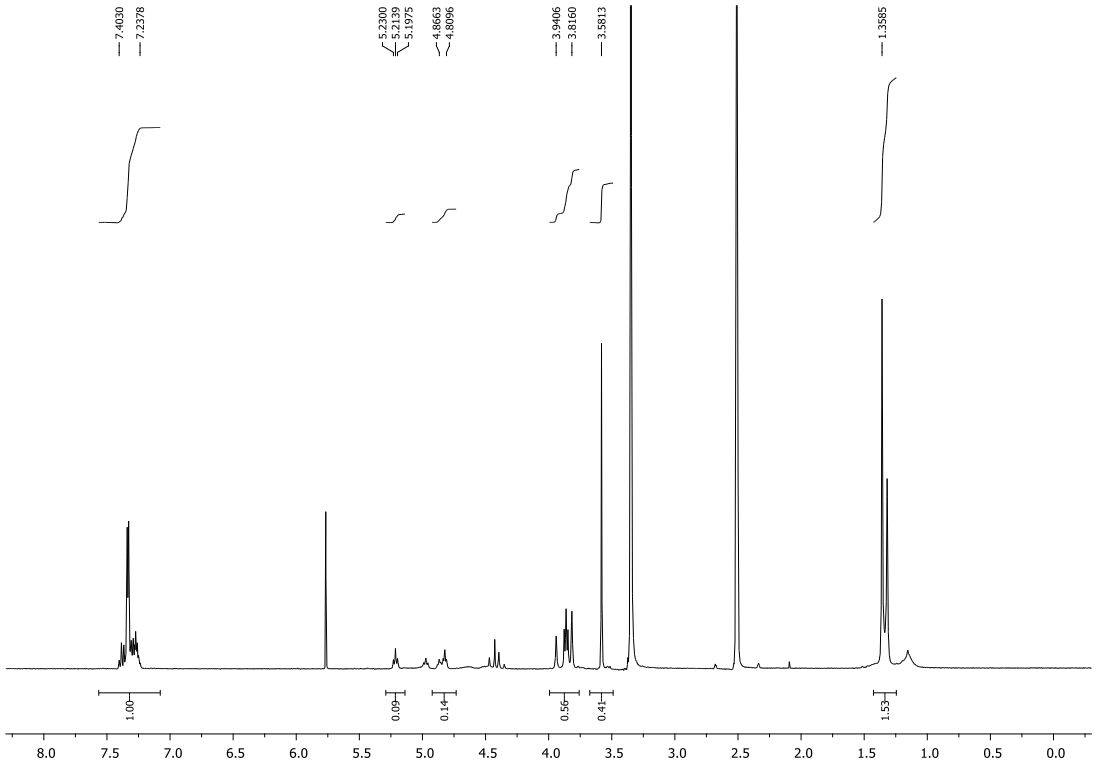
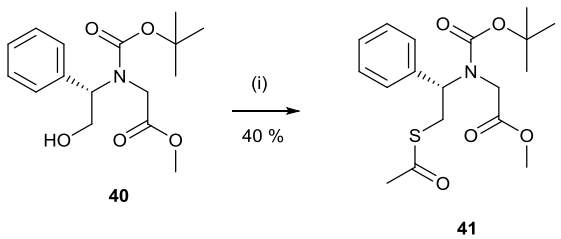


Figure 2.7 ^{1}H NMR spectrum of a mixture of δ-hydroxy methyl ester **40** and *N*-Boc-5-phenylmorpholinone **36** in DMSOd_6

For the sulfur introduction in the next step, we were considering an approach that would not require any heating as our previous work had indicated that the Boc group could not withstand high temperatures in this system. Thus, with δ-hydroxy methyl ester **40** in hand, we decided to apply a Mitsunobu reaction procedure to attempt to prepare the δ-thioacetate methyl ester at temperatures from 0 ^{o}C to room temperature (Scheme 2.7).[9]



Scheme 2.7 (i) DIAD, triphenylphosphine, dry THF, 0 ^{o}C, 30 minutes, N_2 atmosphere (ii) thioacetic acid, dry THF, 0 ^{o}C, 1 hour, room temperature, 24 hours, N_2 atmosphere

Treatment of δ-hydroxyl methyl ester **40** with thioacetic acid, in the presence of triphenylphosphine and diisopropylazodicarboxylate (DIAD) afforded thioacetate methyl ester **41** as a yellow oil in 40 % yield after 24 hours. The reaction required anhydrous THF and an inert atmosphere for the DIAD to be reduced and this could be observed when a milky mixture was formed after about 15 minutes. Separation of thioacetate methyl ester **41** from the triphenylphosphine oxide and hydrazine side products was best achieved by column chromatography and slow elution with hexane – ethyl acetate (7 : 1). In the beginning, it proved difficult to separate **41** from triphenylphosphine oxide as both compounds had similar Rf values of 0.3 and 0.4 respectively in hexane – ethyl acetate (4:1). Trituration with dichloromethane and hexane to precipitate triphenylphosphine oxide followed by purification through column chromatography using hexane – ethyl acetate (4 : 1) were also tried, but **41** could only been obtained in very poor yield and purity. Fortunately, chromatography on silica using the less polar solvent combination of hexane – ethyl acetate (7 : 1) successfully afforded pure **41** as a yellow oil in a yield of 40 %. However, prior to carrying out the column chromatography, the crude product required around 20 – 25 minutes for complete dissolution in dichloromethane before loading onto the column.

Heating to speed up the dissolution rate did not help as a white precipitate would then form on top of the column during elution, rendering the column ineffective.

A singlet observed at δ 2.33 ppm in the ^{1}H NMR spectrum signified the presence of an acylthio group in **41** (Figure 2.8). An ABX system was indicated by the presence of a broadened triplet at δ 5.54 ppm (J = 8.0 Hz) and a broad resonance integrating to two protons at δ 3.44 – 3.38 ppm. Two signals resonating at δ 3.79 ppm (J = 16.0 Hz) as a doublet and at δ 3.56 ppm (J = 16.0 Hz) as a broad doublet could be assigned to the AB system of **41**. From the ^{1}H - ^{1}H COSY NMR spectrum, there were correlation peaks among the ABX protons as well as between the AB protons indicating these couplings more clearly. The presence of a carbonyl carbon resonance at δ 195.4 ppm on the ^{13}C NMR spectrum in addition to the two existing carbonyl carbons at δ 170.7 ppm and δ 154.9 ppm provided further support for the formation of **41**. Finally, mass spectrometry confirmed the structure with mass ions observed at 368.1524 [MH^{+}] and 390.1346 [MNa^{+}], matching with the desired thioacetate methyl ester **41**. In addition, mass ions at 279.0933 [MH^{+}] and 205.1180 [MH^{+}] for triphenylphosphine oxide and the hydrazine from the other fractions respectively suggested those side products were obtained during purification of **41**.

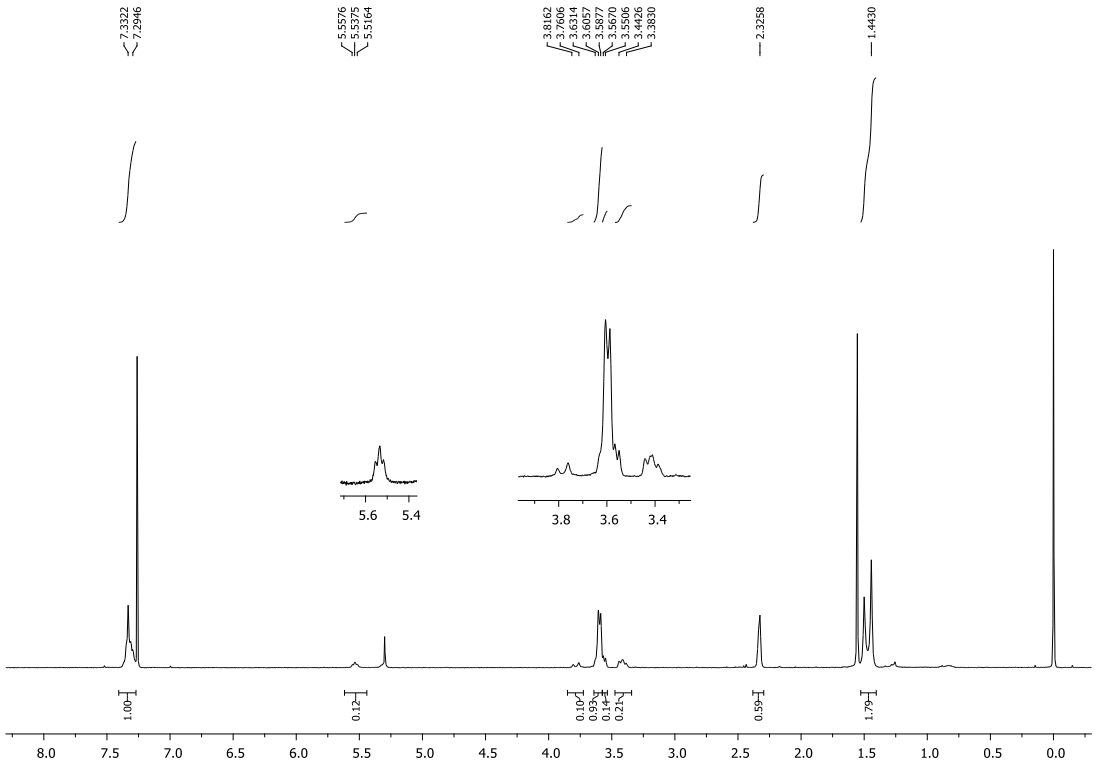
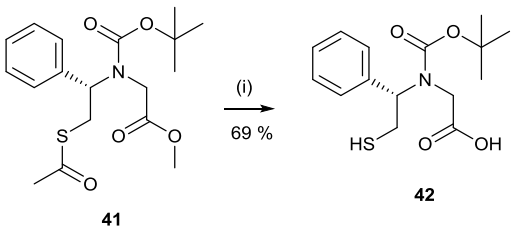


Figure 2.8 ^{1}H NMR spectrum of *N*-Boc-thioacetate methyl ester **41** in $CDCl_3$

The thioacetate methyl ester **41** was then hydrolysed with lithium hydroxide under an atmosphere of nitrogen overnight to give crude thiol acid **42** (Scheme 2.8). The purification of crude thiol acid was carried out using hexane – ethyl acetate (4 : 1) on silica with the addition of 1 % formic acid to the solvent system to facilitate elution of the relatively polar thioacid from the column (TLC Rf value of 0.2 in this solvent). With this procedure, thiol acid **42** was successfully obtained as a yellow oil in 69 % yield.



41 42

Scheme 2.8 (i) LiOH, water, *i*-PrOH, room temperature, overnight

The absence of singlets at δ 2.34 ppm and δ 3.61 ppm in the ^{1}H NMR spectrum showed that the acyl and methoxy groups respectively had been completely removed (Figure 2.9). The ABX signals were detected at δ 5.28 ppm (J = 8.0 Hz) as a triplet and centred on δ 3.09 ppm as a broad signal. Furthermore, two doublets at δ 3.63 ppm (J = 17.5 Hz) and δ 3.55 ppm (J = 17.5 Hz) could be assigned to the AB system of **42**. Further evidence for mutual coupling within these ABX and AB multiplets was based on the correlations observed in the ^{1}H – ^{1}H COSY NMR spectrum. The absence of a carbonyl carbon resonance at δ 195.4 ppm and observation of a methoxyl carbon resonance at δ 70.6 ppm in the ^{13}C NMR spectrum provided further evidence for the removal of the acyl and methoxy groups. All of this was consistent with the observation of a mass ion peak at 312.1264 [MH^{+}] and 334.1084 [MNa^{+}] corresponding to the desired product.

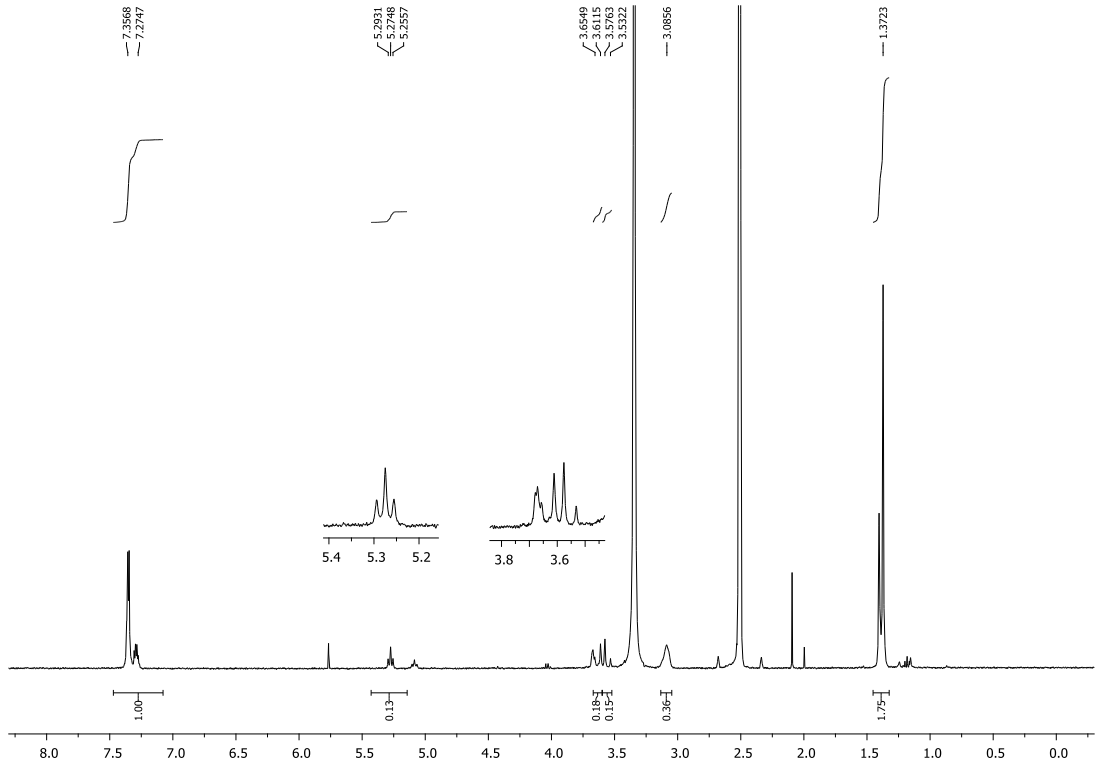
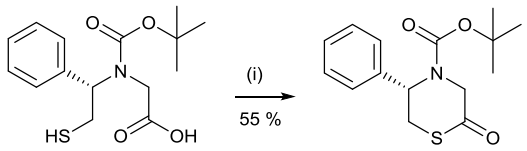


Figure 2.9 ^{1}H NMR spectrum of *N*-Boc- thioacid **42** in DMSOd_6

For the initial attempt to complete the final step of thiolactonization, we considered dicyclohexylcarbodiimide as the condensing agent. Other approaches for thiolacid ring closure using 10-camphorsulfonic acid or tosylic acid required heating to reflux at 110 ^{o}C, which could not be applied to our *N*-Boc protected compound.[10,11] Sixty years ago, Woodward *et al*. demonstrated that treatment of hydroxyacids with DCC in the presence of pyridine resulted in very effective conditions for lactonization.[12,13] Furthermore, in 1969, Steglich and Höfle demonstrated that 4-dimethylaminopyridine (DMAP) was superior to pyridine.[14] Therefore, the cyclization of thiol acid **42** was performed by reacting it with DCC in dichloromethane at 0 ^{o}C for 15 minutes before adding DMAP and leaving the mixture at room temperature overnight (Scheme 2.9). The formation of a white precipitate, presumed to be dicyclohexyl urea **6**, was observed and the resulting mixture was filtered with suction through Celite®, the pad washed

three times with dichloromethane and the filtrate concentrated to give the crude cyclized product as a yellow oil. Purification of the crude product over silica, eluting with hexane – ethyl acetate (4 : 1), afforded **43** as a yellow oil in 55 % yield.



42 **43**

Scheme 2.9 (i) DCC, CH_2Cl_2, 0 ^oC, 15 minutes (ii) DMAP, room temperature overnight

As expected, Boc-protected thiomorpholinone **43** gave broadened signals on ^1H NMR analysis at 400 MHz, once again requiring further analysis at variable temperature. A sample, dissolved in DMSOd_6, was analysed from 25 ^oC to 85 ^oC at 500 MHz (Figure 2.10), with analysis at 85 ^oC giving a spectrum in which the resonances had become better defined.

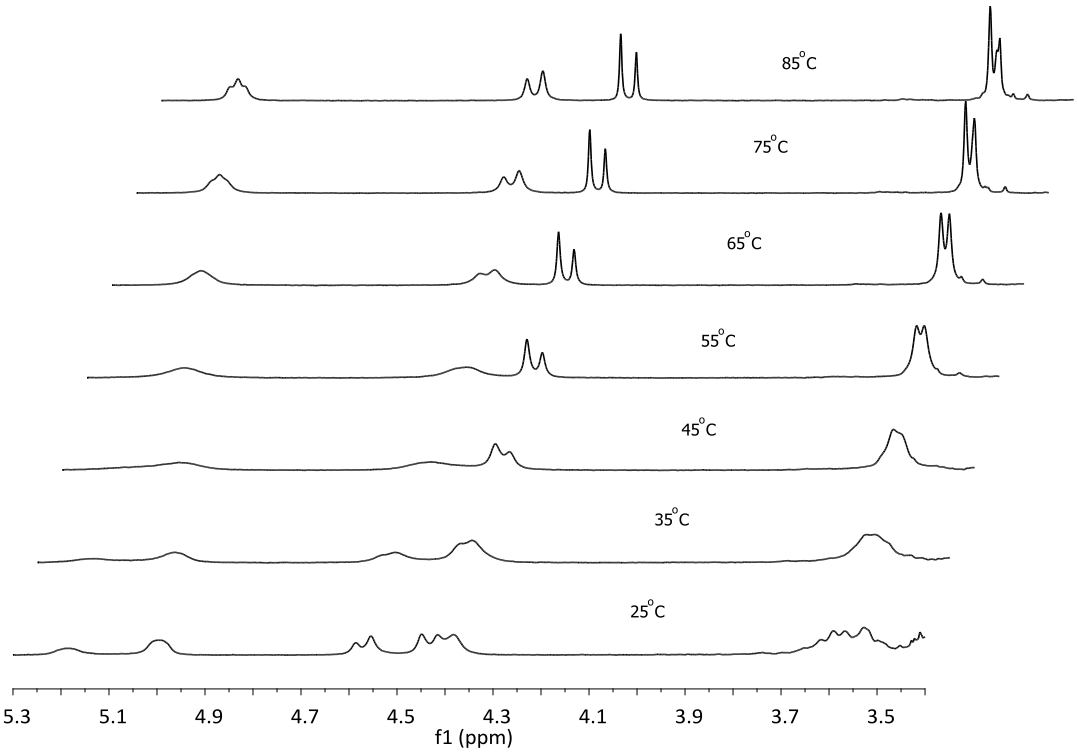


Figure 2.10 ^{1}H NMR spectra of *N*-Boc-protected thiomorpholinone **43** in DMSOd_6 at variable temperature at 500 MHz (region from 3.4 to 5.3 ppm expanded)

Two broad ABX signals resonating at δ 5.02 ppm and δ 3.65 – 3.56 ppm in the 400 MHz NMR spectrum were simplified into a broadened triplet at δ 5.14 ppm (J = 7.0 Hz) and a broad doublet at δ 3.57 (J = 7.0 Hz), integrating to one proton and two protons respectively (Figure 2.11). Two doublets at δ 4.52 ppm (J = 16.0 Hz) and δ 4.33 ppm (J = 16.0 Hz) were also observed, corresponding to the AB system. The appearance of a resonance at δ 200.2 ppm in the ^{13}C NMR spectrum that could be assigned to a thiolactone further suggested that the ring closure had occurred. This was further supported by an absorption at 1156 cm^{-1} in the infra-red spectrum that revealed the presence of the C-S-C bond of the lactone ring, while strong overlapping absorptions around 1697 cm^{-1} signified the existence of the thiolactone and Boc carbonyl groups. Further supporting evidence was obtained from the the mass spectrum

that showed a molecular ion at 316.0978 [MNa^+], also indicating that *N*-Boc-protected thiomorpholinone **43** had been successfully synthesized.

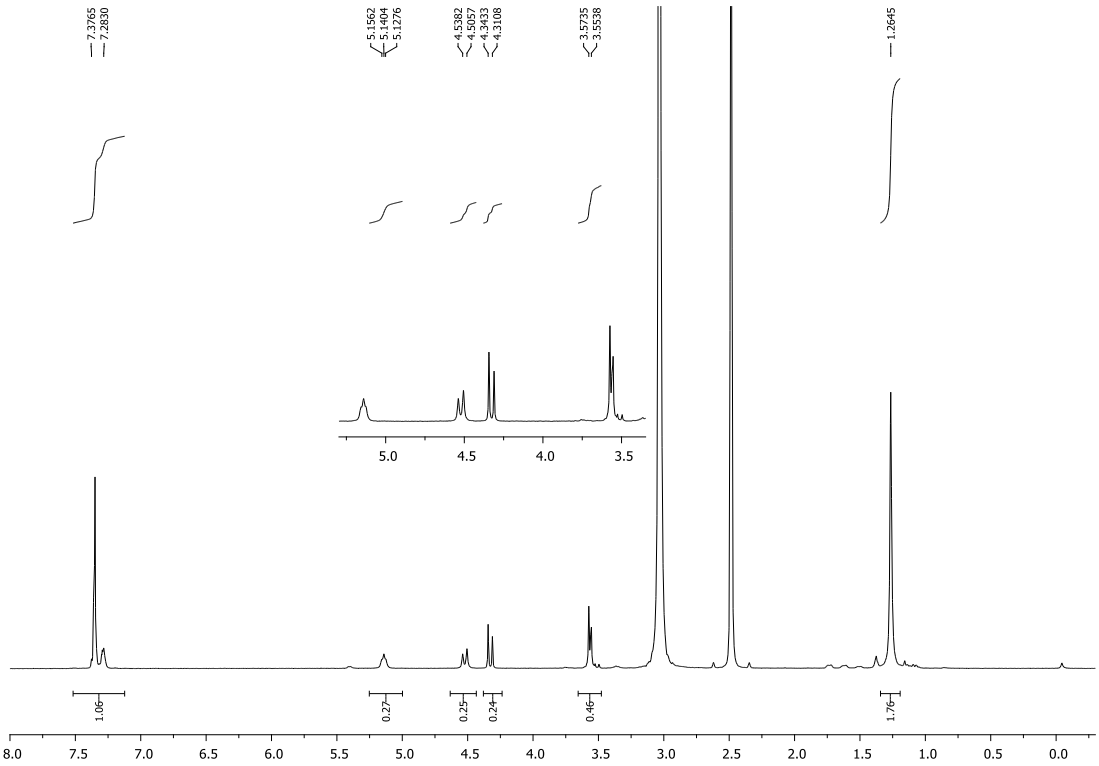
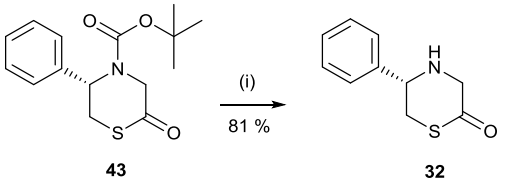


Figure 2.11 ^{1}H NMR spectrum of *N*-Boc-5(S)-phenylthiomorpholinone **43** in DMSOd_6 at 85 ^{o}C at 500 MHz

Removal of the Boc protecting group from Boc-thiomorpholinone **43** was conveniently achieved using 50 % (v/v) trifluoroacetic acid in dichloromethane (Scheme 2.10). The desired 5-phenylthiomorpholinone **32** was obtained as a yellow oil in 81 % yield. The deprotection was also examined using 4 M HCl in dioxane as these conditions were reported to improve the yields and lead to shorter reaction times.[15] However, this resulted in a lower yield than trifluoroacetic acid – dichloromethane (1 : 1) mixture (42 %), even though reaction was complete in 30 minutes to give the thiomorpholinone HCl salt.



43 32

Scheme 2.10 (i) TFA, CH_2Cl_2, 0 ^oC, 30 minutes, room temperature, 1 hour 30 minutes

The disappearance of the resonance at δ 1.26 ppm in the ^1H NMR spectrum showed that the Boc group had been completely removed (Figure 2.12). The formation of **32** was verified by the presence of the thiomorpholinone characteristic resonances in the ^1H NMR spectrum, with two double doublets at δ 4.24 ppm (J = 11.0 Hz, J' = 3.5 Hz) and δ 3.19 ppm (J = 12.0 Hz, J' = 3.5 Hz) and a triplet at δ 3.48 ppm (J = 12.0 Hz) corresponding to the ABX system. All three ABX protons were shifted upfield in comparison to the *N*-Boc precursor **43**. In addition, the two AB coupled protons had also shifted upfield to δ 3.86 ppm (J = 17.5 Hz) and δ 3.81 ppm (J = 17.5 Hz) due to the removal of the deshielding effect of the *N*–Boc group. The absence of resonances due to the Boc carbonyl carbon and *tert*-butyl carbonyl carbon in the ^{13}C NMR spectrum of **43** at δ 154.7 ppm and δ 28.2 ppm respectively provided more support for the removal of the Boc protecting group. Furthermore, the IR spectrum showed a broad N-H stretching centred around 3320 cm^{-1}, indicating the presence of a free secondary amine, and a single carbonyl stretching absorption at 1664 cm^{-1}. Final evidence came from the mass spectrum, with a molecular ion observed at 194.0634 [MH^+] and so we were able to conclude that the desired glycine derived thiomorpholinone **32** had at last been obtained.

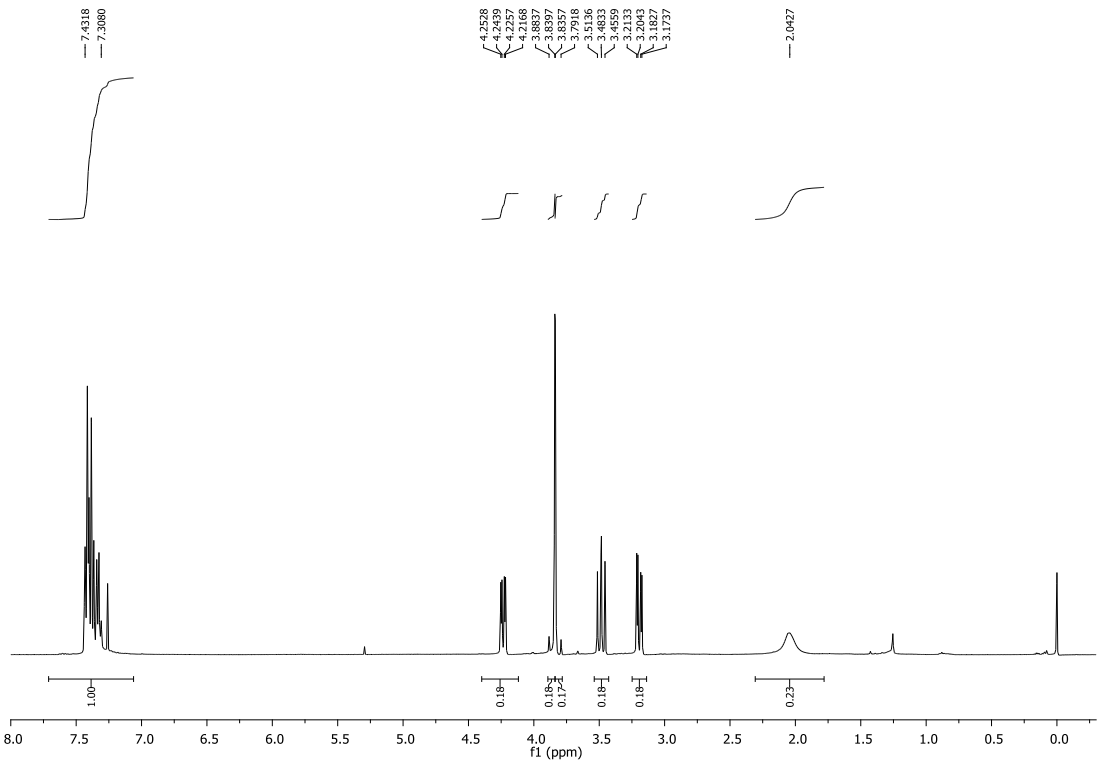
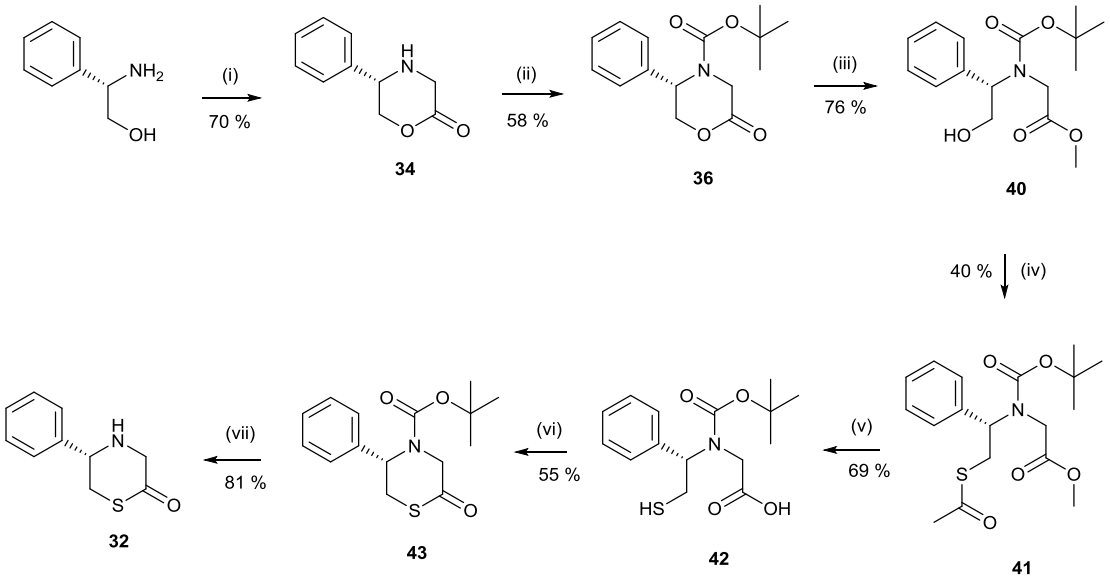


Figure 2.12 ^{1}H NMR spectrum of glycine derived thiomorpholinone **32** in $CDCl_3$

It is important to note that the *N*-protection was essential throughout the synthetic route to ensure the success of template preparation. This approach provides the first synthetic route to access the C-3 unsubstituted 5-phenylthiomorpholinone template that could be used as a key structural motif for convergent synthesis of peptides containing a glycine amino acid residue at the coupling site at either *C*- or *N*- terminus.

2.2 Attempts at improvement of the developed method for C-3 unsubstituted thiomorpholinone template synthesis

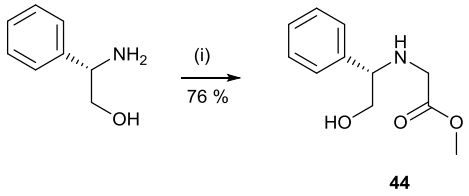
Overall, a 7 step procedure to access the template of glycine derived thiomorpholinone **32** had been developed for the first time. The procedure starts with the preparation of 5-phenylmorpholinone followed by *N*-Boc protection and then proceeds via nucleophilic ring opening of the lactone to give a methyl ester. The sulfur incorporation step is performed via Mitsunobu reaction to obtain a thioacetate methyl ester which is then hydrolysed to generate the corresponding thiol acid. Ring closure of the thiol acid is achieved using DCC and the synthesis is completed with acidic Boc-deprotection to give the desired template **32** (Scheme 2.11).



Scheme 2.11 (i) Phenyl bromoacetate, DIPEA, CH_3CN, room temperature, 24 hours (ii) Di-*tert*-butyl dicarbonate, Et_3N, EtOAc, room temperature, 6 hours (iii) MeOH, Et_3N, room temperature, 24 hours (iv) DIAD, triphenylphosphine, dry THF, 0 ^oC, 30 minutes, N_2 atmosphere, thioacetic acid, dry THF, 0 ^oC, 1 hour, room temperature, 24 hours, N_2

atmosphere (v) LiOH, water, *i*-PrOH, room temperature, overnight (vi) DCC, CH_2Cl_2, 0 ^oC, 15 minutes, DMAP, room temperature, overnight (vii) TFA, CH_2Cl_2, 0 ^oC, 30 minutes, room temperature, 1 hour 30 minutes

Wishing to shorten and improve this pathway, we turned our intention to explore a two step procedure to prepare *N*-Boc-hydroxy methyl ester **40**. In this approach, (*S*)-phenylglycinol and methyl bromoacetate were reacted according to the methodology developed by Panek and Masse.[16] This *N*-alkylation procedure successfully generated δ-hydroxy methyl ester **44** as a yellow oil in 76 % yield (Scheme 2.12). This more direct preparation of **44** thus allows a more direct access to the glycine-derived thiomorpholinone **32** without needing to prepare morpholinone **34**.



44

Scheme 2.12 (i) Methyl bromoacetate, Et_3N, dry THF, 0 ^oC to room temperature, 24 hours

The success of the *N*-alkylation step was indicated by a singlet appearing at δ 3.70 ppm in the ^1H NMR spectrum of the product, corresponding to the ester methoxy group (Figure 2.14). Three ABX resonances were detected at δ 3.80 ppm (J = 8.5 Hz, J' = 4.5 Hz), δ 3.74 ppm (J = 11.0 Hz, J' = 4.5 Hz) and δ 3.61 ppm (J = 11.0 Hz, J' = 8.5 Hz) as double doublets. Two doublets resonating at δ 3.40 ppm (J = 17.5 Hz) and δ 3.30 ppm (J = 17.5 Hz) could be assigned to the AB system. The carbonyl carbon resonance was observed at δ 172.7 ppm in the ^{13}C NMR spectrum and a molecular ion observed at 210.1125 [MH^+] completed the structural assignment.

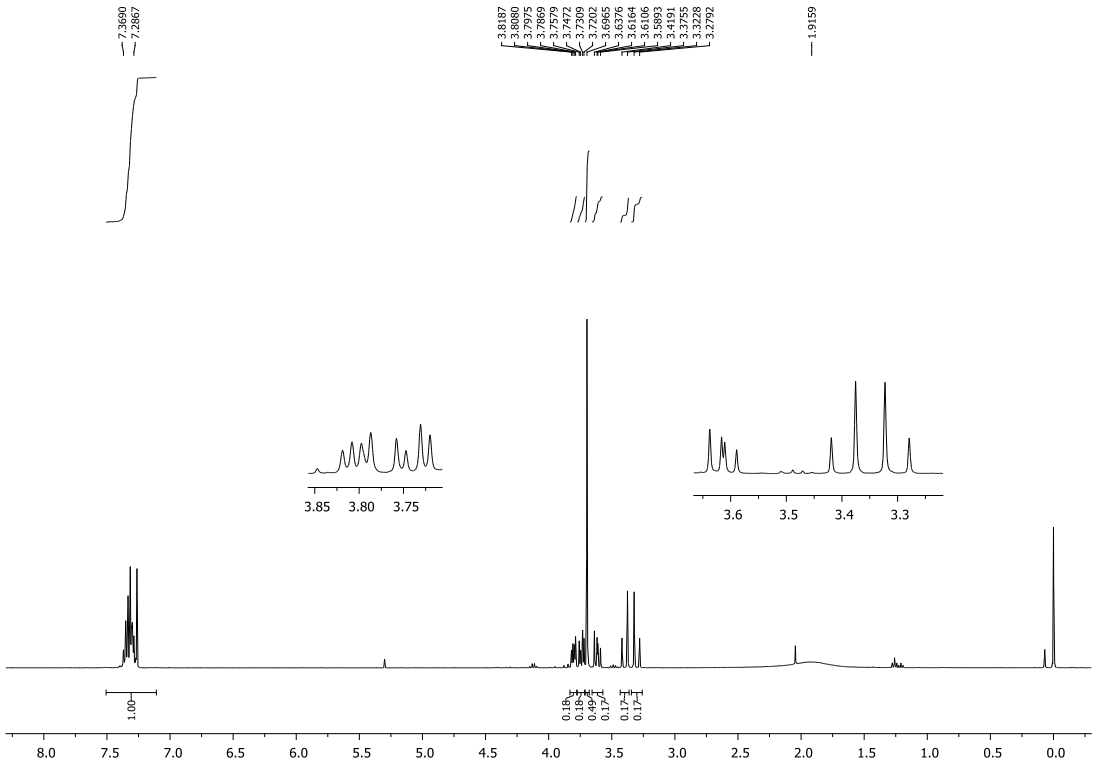
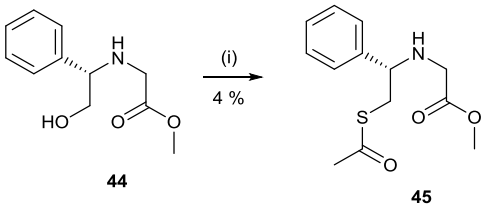


Figure 2.14 ^{1}H NMR spectrum of *N*- hydroxy methyl ester **44** in $CDCl_3$

The Boc protection of **44** could then be carried out to prepare *N*-Boc-δ-hydroxy methyl ester **40** and feed in to the following steps to generate the target template. Nevertheless, the successful *N*-alkylation of **44** led us attempt to undertake sulfur installation by adapting the Mitsunobu reaction as described in the previous protocol without protecting the nitrogen (Scheme 2.13).[9] After 24 hours of reaction, *N*-thioacetate methyl ester **45** was obtained but, frustratingly, could only be isolated in a disappointing 4 % yield. We propose that the low yield can most likely be attributed to the strong nucleophilic properties of nitrogen causing most of the starting material **44** to dimerize.



Scheme 2.13 (i) DIAD, triphenylphosphine, dry THF, 0 ^{o}C, 30 minutes, N_2 atmosphere, thioacetic acid, dry THF, 0 ^{o}C, 1 hour, room temperature, 24 hours, N_2 atmosphere

Examination of the this material found a new singlet appearing at δ 2.48 ppm in the ^{1}H NMR spectrum, showing that the sulfur substitution had been successful (Figure 2.15). The ABX coupling system resonated at δ 5.18 ppm (J = 7.0) and δ 3.10 ppm as a triplet and multiplet respectively. The two protons from the methylene group were observed as doublets at δ 3.91 ppm (J = 17.0 Hz) and δ 3.37 ppm (J = 17.0 Hz). The formation of **45** was further supported by mass spectrometry, which showed a molecular ion peak at 268.1002 [MH^{+}] and 290.0821 [MNa^{+}].

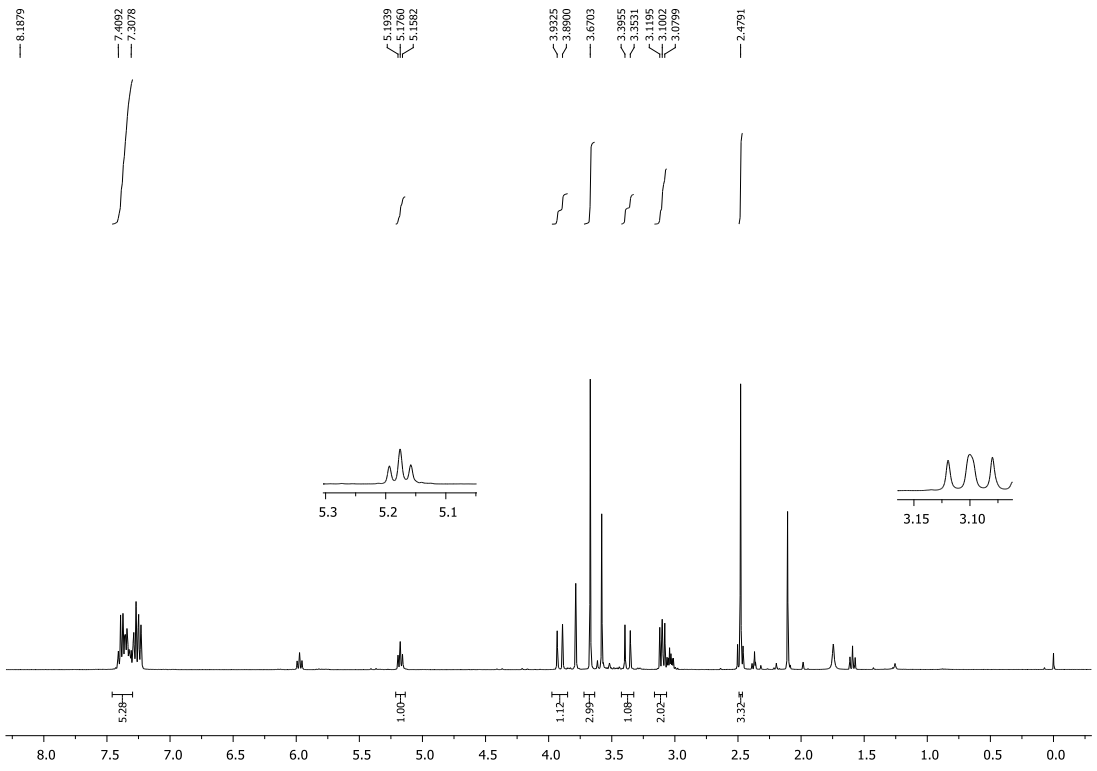
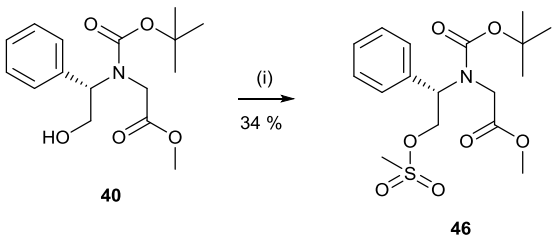


Figure 2.15 ^{1}H NMR spectrum of *N*-thioacetate methyl ester **45** in $CDCl_3$

We then aimed to improve the yield of the sulfur introduction step with *N*-Boc hydroxy methyl ester **40** by converting the hydroxyl group to a mesylate and then treating with potassium thioacetate to give thioacetate methyl ester **41**. The mesylate substituent is well known in organic chemistry as one of better leaving groups.[17] Thus, methanesulfonyl chloride was added dropwise to a solution of **40** and DIPEA in dichloromethane at 0 ^{o}C (Scheme 2.14). The reaction mixture was stirred for an hour at 0 ^{o}C and stirring was then continued for another hour at room temperature. Unfortunately, after work up, this reaction provided **46** as a yellow oil but only in 34 % yield.



Scheme 2.14 (i) Methanesulfonyl chloride, DIPEA, DCM, 0 ^{o}C, 1 hour, room temperature, 1 hour

The conversion of the hydroxyl group to its corresponding mesylate was indicated by the appearance of an additional singlet resonance in the ^{1}H NMR spectrum at δ 3.68 ppm (Figure 2.16). The presence of the ABX system was verified by three quasi triplets resonating at δ 5.06 ppm (J = 8.5 Hz), δ 4.72 ppm (J = 8.5 Hz) and δ 4.15 ppm (J = 8.0 Hz). Two doublets appeared at δ 4.30 ppm (J = 18.0 Hz) and δ 3.39 (J = 18.0 Hz) signifying the presence of the AB system. The molecular ion peak observed at 410.1244 [MNa^{+}] in the mass spectrum gave further evidence to confirm that mesylate **46** had been synthesized, albeit in disappointing yield.

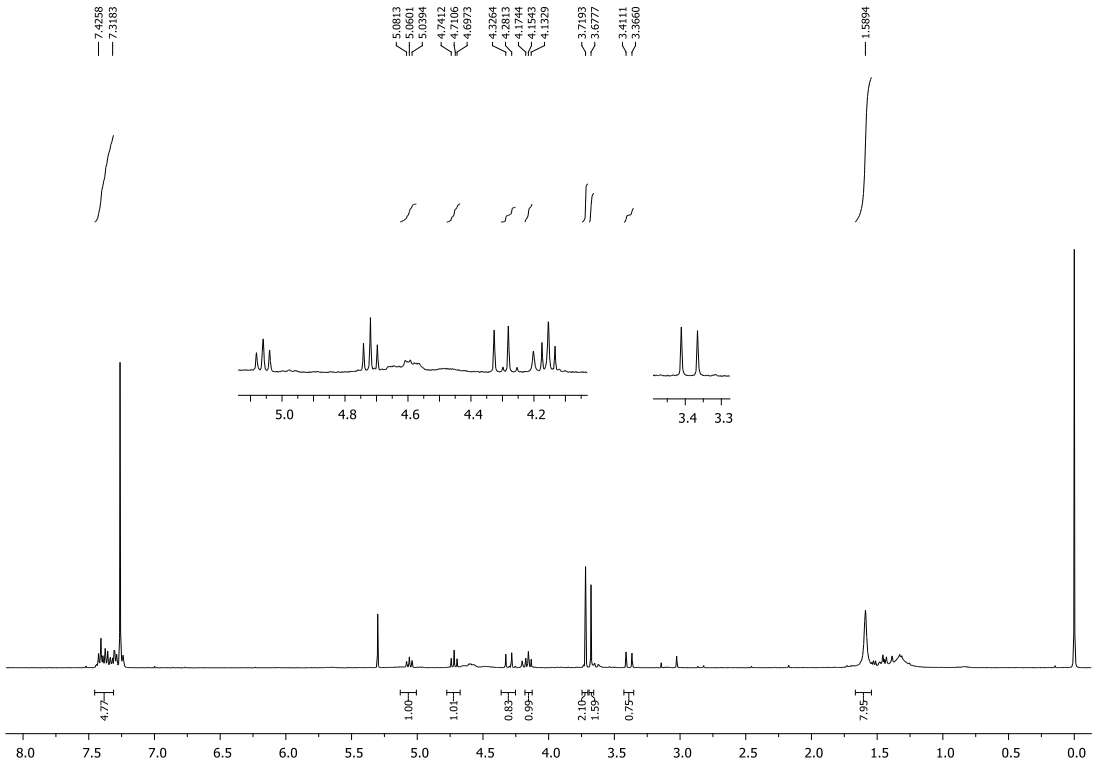
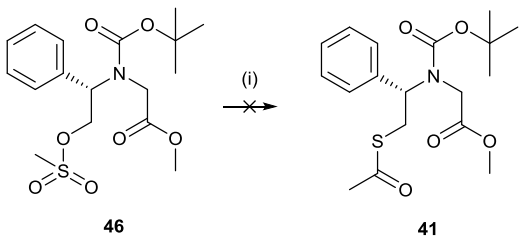


Figure 2.16 ^{1}H NMR spectrum of *N*-Boc-mesylate methyl ester **46** in $CDCl_3$

Due to worries about stability, mesylate **46** was used directly for the next step without purification. In this step, mesylate **46** was dissolved in DMF and the solution subsequently heated with potassium thioacetate at 40 ^{o}C for 16 hours (Scheme 2.15).[18] After work up and purification over silica, eluting with hexane – ethyl acetate (4 : 1), none of the desired product could be isolated. We therefore decided to discontinue this approach due to the poor yield during the formation of the mesylate. For future work, the use of other leaving groups to replace the mesylate should be continued to investigate whether this could improve the yield.

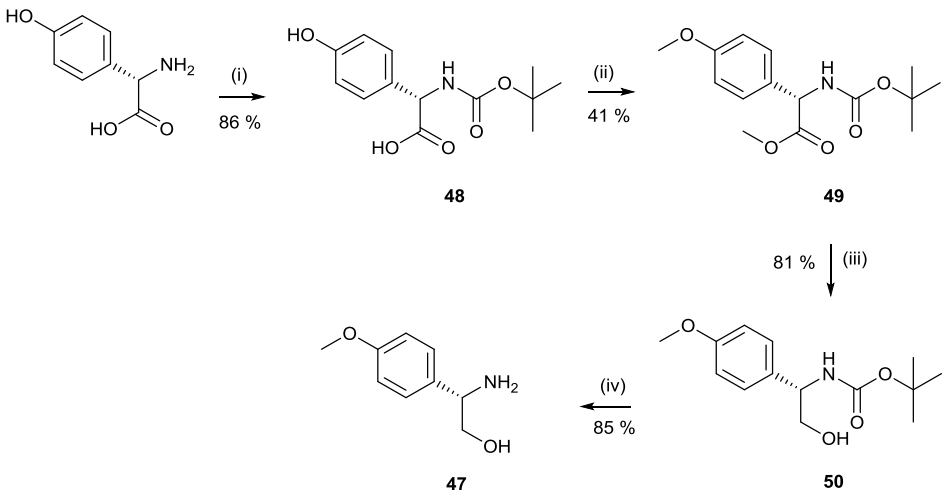


Scheme 2.15 (i) Potassium thioacetate, DMF, 40 ^{o}C, 16 hours

2.3 Approaches towards the 5-(4-methoxyphenyl) thiomorpholinone template

After the successful glycine derived thiomorpholinone **32** generation, the next plan was to incorporate a methoxy group onto the 5-aryl substituent. In a previous study, the *t*-butyl, Fmoc protecting group and phenyl substituent on the morpholinone-based peptide precursor were all removed in one pot under harsh conditions using lithium in liquid ammonia after formation of the peptide linkage (Scheme 1.25 and 1.26).[19] This approach was demonstrated by the Harwood group when preparing the L-ala-L-ala-L-ala and L-ala-D-ala-L-ala tripeptides.[20] The incorporation of a 5-(4-methoxyphenyl) group into the thiomorpholinone system would potentially allow for much more mild debenzylation conditions after amide bond formation.

To achieve this target, we decided to use 4-methoxyphenylglycinol **47** as a starting material, which could be prepared through a four step procedure as summarized in Scheme 2.16. The preparation of starting material began with amino group protection of commercially available 4-hydroxyphenylglycine with di-*tert*-butyl dicarbonate **2** in the presence of sodium hydroxide to afford **48** as a white solid in 86 % yield.



Scheme 2.16 (i) Di-*tert*-butyl dicarbonate, NaOH, H_2O – dioxane (2 : 1), room temperature, 4 hours (ii) MeI, K_2CO_3, acetone, 50 ^oC, 16 hours (iii) $NaBH_4$, LiCl, ethanol, 0 ^oC, 10 minutes, THF, room temperature, 3 hours (iv) TFA, CH_2Cl_2, 0 ^oC, 30 minutes, room temperature, 90 minutes

The *N*-Boc protection of **48** was determined based on the presence of a singlet resonance at δ 1.38 ppm (Figure 2.17) in the ^1H NMR spectrum corresponding to the *tert*-butyl resonance. The successful *N*-protection was also indicated by the observation of a molecular ion peak at 290.0999 [MNa^+] in the mass spectrum.

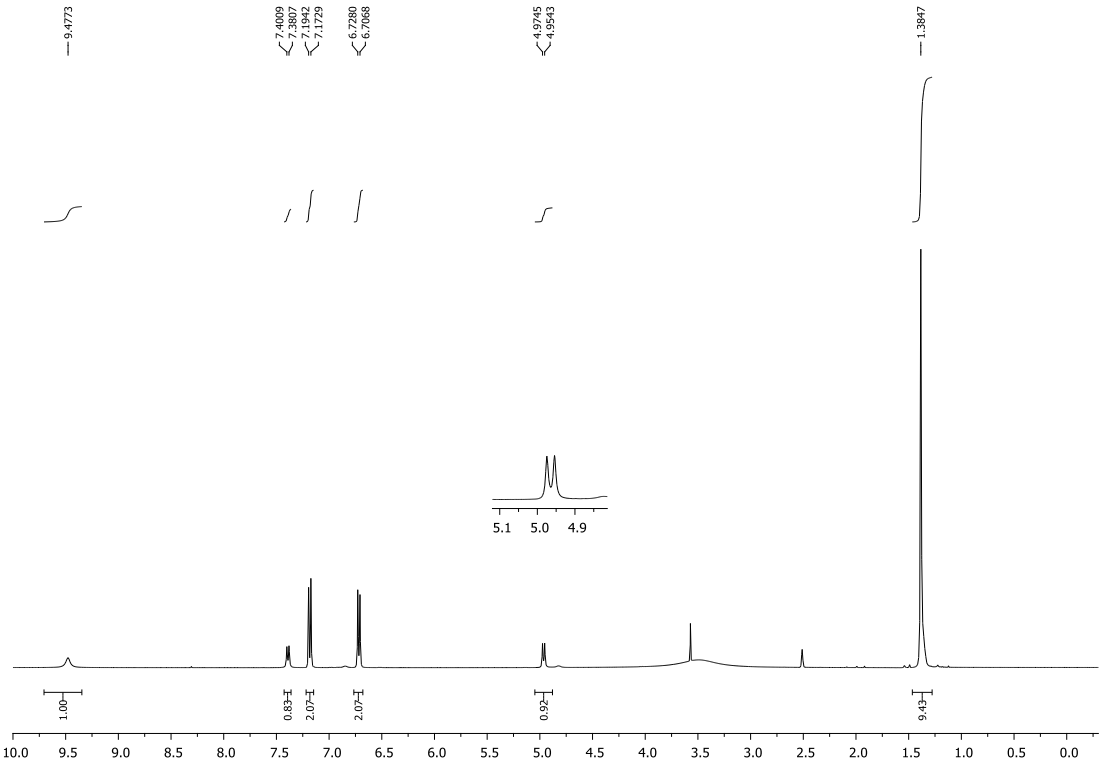


Figure 2.17 ^1H NMR spectrum of *N*-Boc-4-hydroxyphenylglycine **48** in DMSOd_6

Our next task was to convert the phenol to a methoxy group via *O*-methylation with iodomethane according to the method developed by Leermann *et al*.[21] Treatment of **48** with iodomethane in the presence of K_2CO_3 at 50 ^oC for 16 hours successfully led to methoxy installation on the phenyl ring of **49** as well as esterification of the carboxylic acid. Purification of the crude material over silica, eluting with petroleum ether – diethyl ether (2 : 1), furnished **49** as a white solid in 41 % yield. The presence of two singlets at δ 3.79 ppm and δ 3.71 ppm in the ^1H NMR spectrum indicated the existence of the two methoxy groups in **49** (Figure 2.18). Further evidence was obtained from mass spectrometry, with a molecular ion at 318.1312 [MNa^+] being observed.

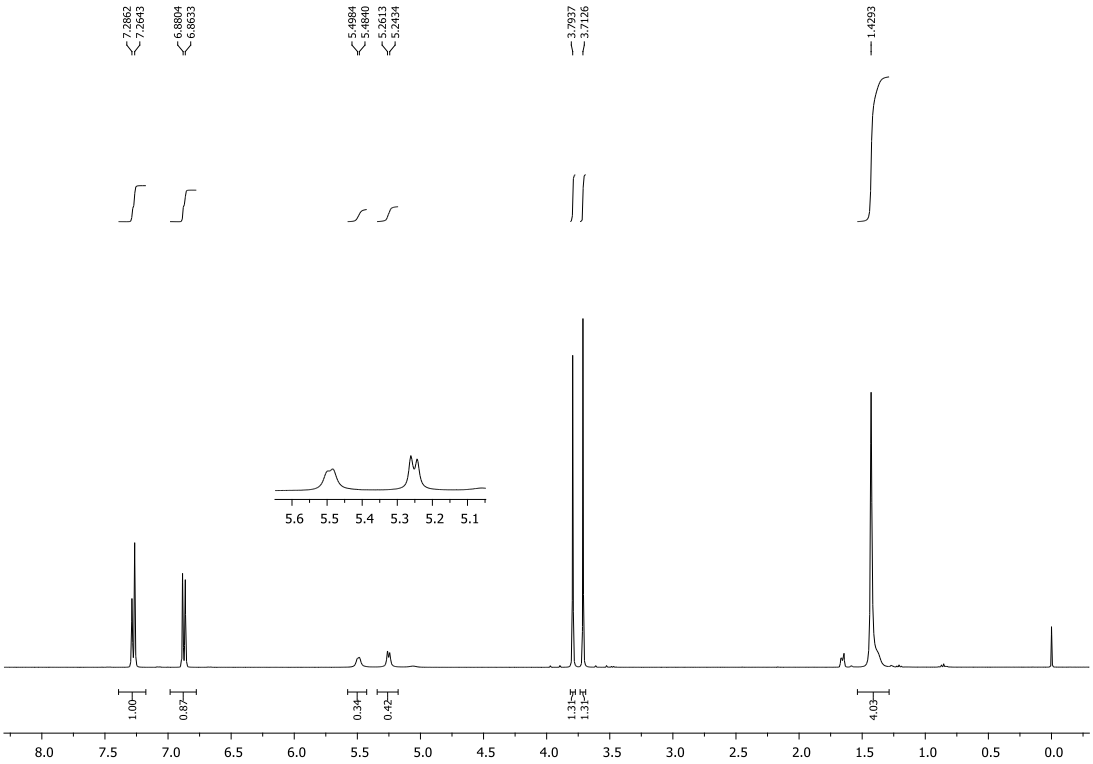


Figure 2.18 ^1H NMR spectrum of *N*-Boc-4-methoxyphenylglycine methyl ester **49** in $CDCl_3$

The resulting methyl ester **49** was then reduced to its corresponding alcohol with lithium aluminium hydride to give *N*-Boc-4-methoxyphenylglycinol **50** as a white solid in 65 % yield. However, previous work had shown that the reduction of a similar ester using $NaBH_4$ as reducing agent could afford the corresponding alcohol in higher yield.[22] By adapting this approach, a solution of **49** in THF was added to a suspension of $NaBH_4$ in ethanol at room temperature for 3 hours, in the presence of LiCl to enhance the reduction efficiency of $NaBH_4$ through coordination of Li to the carbonyl oxygen; thus increasing the rate of hydride attack.[23] After work up and purification over silica, eluting with hexane – ethyl acetate (4 : 1), the desired reduced *N*-Boc-4-methoxyphenylglycinol **50** was obtained as a white solid in an improved yield of 80 %. The structure was confirmed based on the absence of the methoxy resonance at δ 3.71 ppm in the ^{1}H NMR spectrum reflecting the transformation of the ester to the glycinol (Figure 2.19). A broad absorption in the IR spectrum centred at 3402 cm^{-1} indicated the presence of the hydroxy group in **50**. Finally, analysis by mass spectrometry provided more support, with a molecular ion peak detected at 268.1542 [MH^+] and 290.1363 [MNa^+].

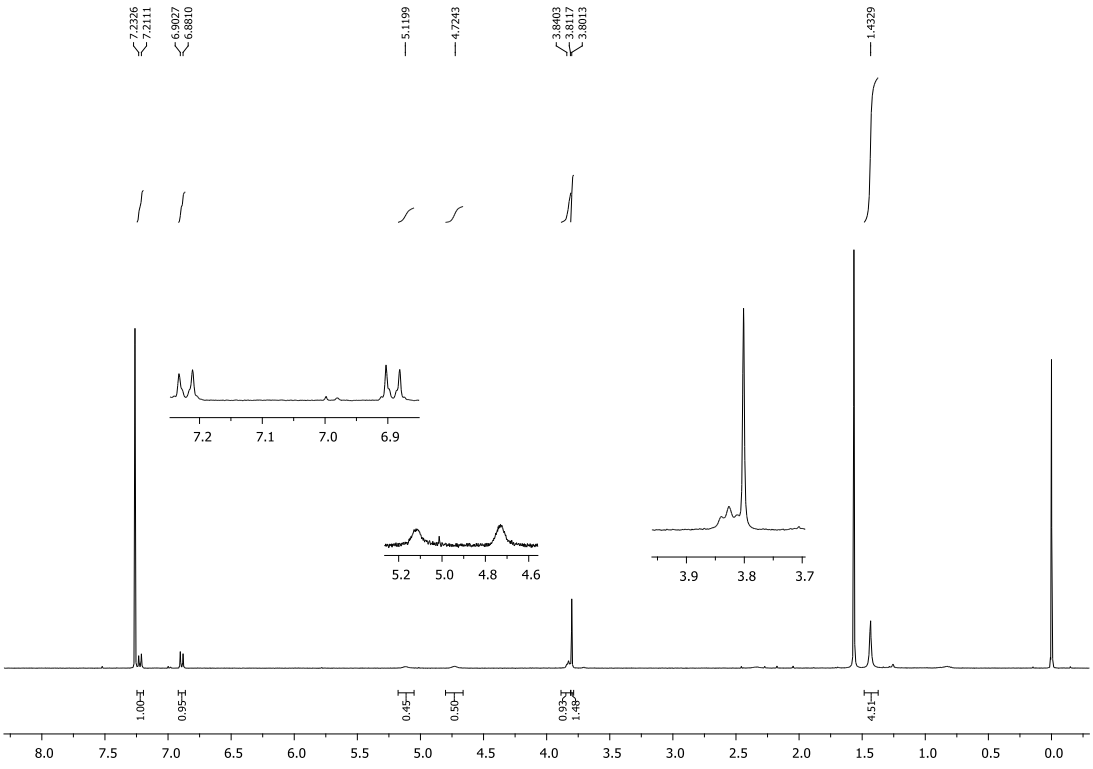


Figure 2.19 ^{1}H NMR spectrum of *N*-Boc-4-methoxyphenylglycinol **50** in $CDCl_3$

Subsequently, Boc deprotection of **50** was carried out with 50 % (v/v) trifluoroacetic acid in dichloromethane to give the desired starting material 4-methoxyphenylglycinol **47** as a yellow oil in 85 % yield. The disappearance of the broad singlet at δ 1.43 ppm from the ^{1}H NMR spectrum indicated the removal of the Boc group (Figure 2.20). Another broad signal appeared at δ 1.85 ppm could be assigned to the free amino and hydroxy group protons and free water in the solvent. A characteristic of the ABX system showed broad resonances at δ 4.06 ppm and δ 3.74 ppm and at δ 3.60 ppm (J = 9.5 Hz) as an apparent triplet. Mass spectrometry confirmed the formation of **47** with a molecular ion peak being observed at 168.1019 [MH^{+}].

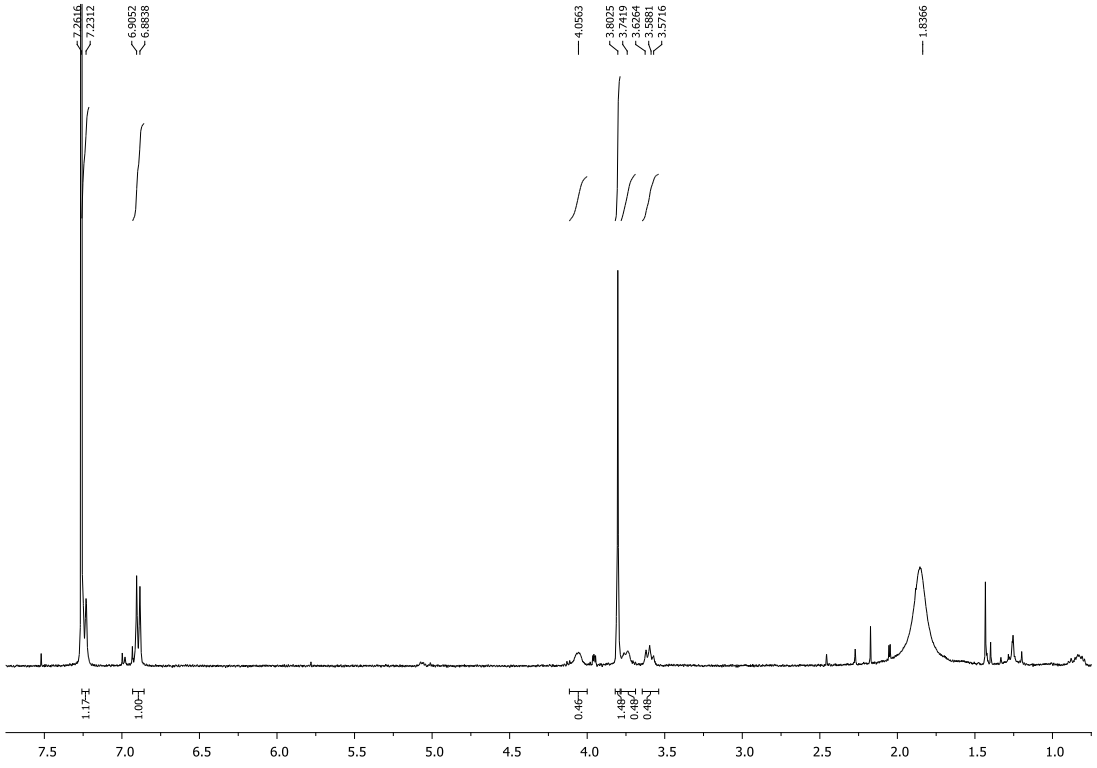
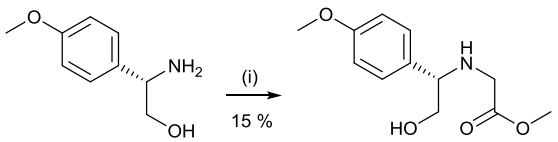


Figure 2.20 ^{1}H NMR spectrum of 4-methoxyphenylglycinol **47** in $CDCl_3$

With **47** in hand, we were keen to consider a shorter pathway to access the glycine thiomorpholinone template without preparing the intermediate morpholinone.

Following the earlier approach with the phenyl analogue, **47** was treated with methyl bromoacetate in dry THF at 0 ^{o}C and the mixture was allowed to warm to room temperature over 24 hours (Scheme 2.17). The crude material was purified over silica, eluting with hexane – ethyl acetate (1 : 1), until **51** began to elute; then ethyl acetate was used for complete elution of the relatively polar **51**. This *N*-alkylation procedure successfully generated hydroxy methyl ester **51**, but in a disappointing 15 % yield. The procedure was repeated by increasing the amount of methyl bromoacetate to 2.0 and 2.5 equivalents but this reaction ended with an equally poor yield of the target compound. For future attempts at improving the yield, it would be worthwhile examining more basic conditions for the *N*-alkylation reaction by increasing the amount of triethylamine to facilitate the nitrogen attack on the methyl bromoacetate.



47 **51**

Scheme 2.17 (i) Methyl bromoacetate, Et_3N, dry THF, 0 ^{o}C to room temperature, 24 hours

Evidence for the successful *N*-alkylation was based on the appearance of an AB resonance as two doublets at δ 3.31 ppm (J = 17.5 Hz) and δ 3.21 ppm (J = 17.5 Hz) and an additional methoxy singlet resonating at δ 3.62 ppm corresponding to the methyl ester (Figure 2.21). The carbonyl carbon resonance observed at δ 173.1 ppm in the ^{13}C NMR spectrum gave further evidence for the presence of an ester. Mass spectrometric analysis of **51** was consistent with the proposed structure, having molecular ions at 240.1230 [MH^+] and 262.1050 [MNa^+].

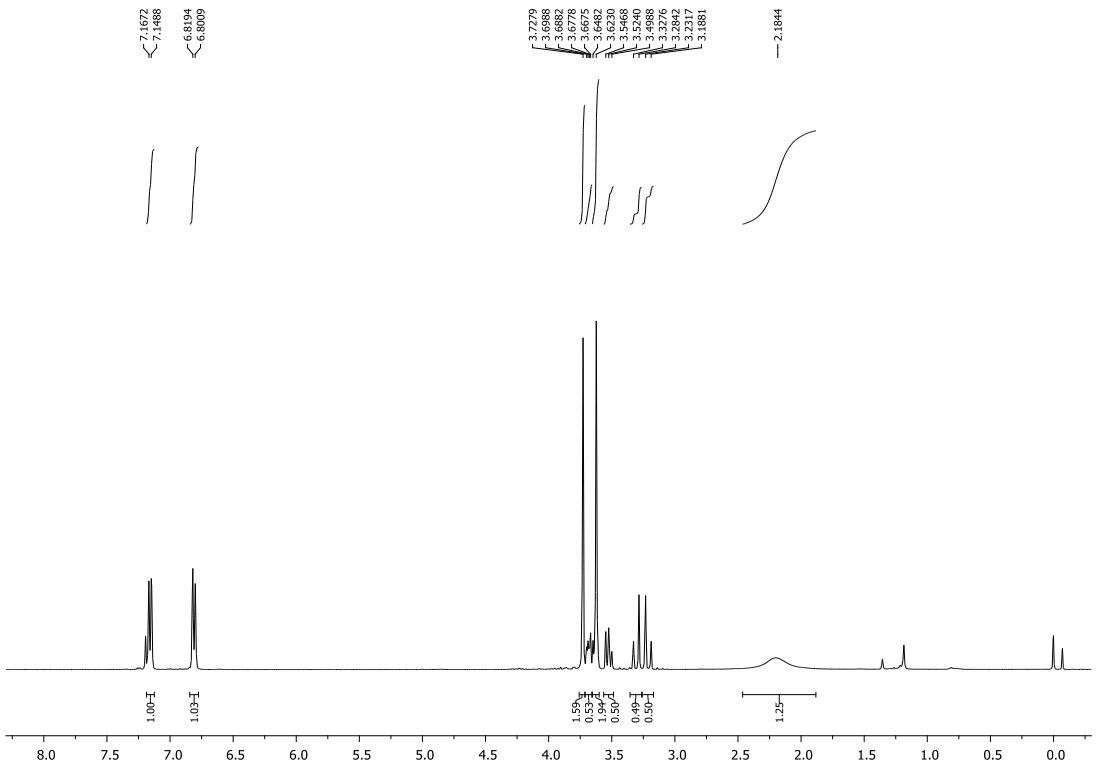
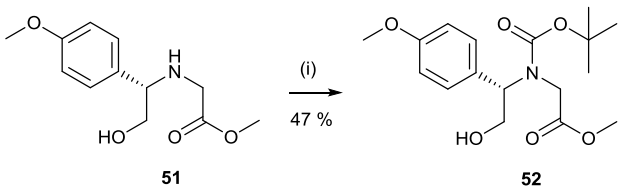


Figure 2.21 ^{1}H NMR spectrum of 4-methoxyphenyl ester **51** in $CDCl_3$

The *N*-Boc protection of **51** then proceeded to give **52** following the standard procedure (Scheme 2.17).[3] The presence of a broad singlet at δ 1.35 ppm in the ^{1}H NMR spectrum showed that *N*-Boc protection of **52** had been achieved and this was also supported by mass spectrometric analysis with a molecular ion being observed at 340.1755 [MH^+] and 362.1574 [MNa^+].

Unfortunately, this synthetic route remains incomplete due to lack of time. For future work in developing this synthetic route, if **52** could tolerate the synthetic route developed to access glycine thiomorpholinone (Scheme 2.11), it most probably requires four more steps along similar lines as already established, and would permit access to the more attractive version of the thiomorpholinone reagent.



Scheme 2.17 (i) Di-*tert*-butyl dicarbonate, Et_3N, EtOAc, room temperature, 6 hours

References

1 Drew, M. G. B., Harwood, L. M. and Yan, R. *Synlett*, 2006, **19**, 3259–3262.

2 Ru, G., Phd Thesis. University of Reading, 2016.

3 Dellaria, J. F. and Santarsiero, B. D. *J. Org. Chem*. 1989, **85**, 3916–3926.

4 Lumma, W. C., Dutra, G. A. and Voeker, C. A., *J. Org. Chem.*, 1970, **35**, 3442–3444.

5 Deslongchamps, P. and Guay, D. *Can. J. Chem.*, 1985, **63**, 2757–2762.

6 Corey, E. J., Albright, J. O., Barton A. E. and Hashimoto, S. I. *J. Am. Chem. Soc.*, 1980, **102**, 1435–1436.

7 Kashima, C. and Harada, K. *J. Org. Chem.*, 1989, **54**, 789–792.

8 Beesley, R. M., Ingold, C. K. and Thorpe, J. F. *J. Chem. Soc.,Trans*., 1915, **107**,1080-1106

9 Kerdesky, F. A. J., Schmidt, S. P., Holms, J. H., Dyer, R. D., Carter, G. W. and Brooks, D. W. *J. Med. Chem.*, 1987, **30**, 1177–1186.

10 Ferraris, D. V., Majer, P., Ni, C., Slusher, C. E., Rais, R., Wu, Y., Wozniak, K. M., Alt, J., Rojas, C., Slusher, B. S. and Tsukamoto, T. *J. Med. Chem.*, 2014, **57**, 243–247.

11 Stoermer, D., Vitharana, D., Hin, N., Delahanty, G., Duvall, B., Ferraris, D. V.,

Grella, B. S., Hoover, R., Rojas, C., Shanholtz, M. K., Smith, K. P., Stathis, M., Wu, Y., Wozniak, K. M., Slusher, B. S. and Tsukamoto, T. *J. Med. Chem.*, 2012, **55**, 5922–5932.

12 Woodward, R. B., Bader, F. E., Bickel, H., Frey, A. J. and Kierstead, R. W. *Tetrahedron*, 1958, **2**, 1–57.

13 Fieser, L. F. and Fieser, M. *Reagents for Organic Synthesis*, John Wiley and Sons, Inc., New York, 1967.

14 Steglich, W. and Höfle, G. *Angew. Chemie Int. Ed. Engl.*, 1969, **8**, 981–981.

15 Han, G., Tamaki, M. and Hruby, V. J. *J. Pept. Res.*, 2001, **58**, 338–341.

16 Panek, J. S. and Masse, C. E. *J. Org. Chem.*, 1998, **63**, 2382–2384.

17 Gordon, I. M. and Maskill, H. *Chem. Soc. Rev.*, 1989, **18**, 123-151.

18 Jason, C. and Satpal, V. 2012. *U.S. Patent No. WO 2012175924 A2*. Washington, DC: U.S. Patent and Trademark Office.

19 Harwood, L. M., Mountford, S. J. and Yan, R. *J. Pept. Sci.*, 2009, **15**, 1–4.

20 Harwood, L. M., Wellings, D. A. and Moody, J. D., 2012, *U.S. Patent No. WO 2012/020231 Al*. Washington, DC: U.S. Patent and Trademark Office.

21 Leermann, T., Broutin, P.E., Leroux, F. R. and Colobert, F. *Org. Biomol. Chem.*, 2012, **10**, 4095-4102.

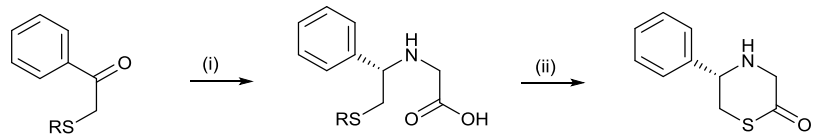
22 Chunyang, J., Ann M, D. and Tiffany, L. L., *Bioorganic & Med. Chem.*, 2017, **25**, 805–812.

23 Zhu, H. J. and Pittman, C. U. *Synth. Commun.*, 2003, **33**, 1733–1750.

Chapter 3

Other attempts to access C3-unsubstituted thiomorpholinone

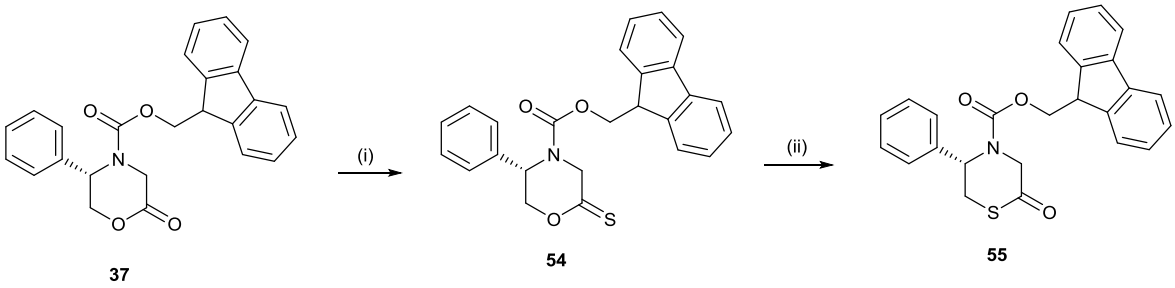
In an attempt to access the C-3 unsubstituted thiomorpholinone, several routes were explored. In one approach, we aimed to construct amino thiol protected acid precursor **53** via reductive amination before ring-closing to access the desired thiomorpholinone template (Scheme 3.1). The details of this pathway are discussed in Section 3.1.



53

Scheme 3.1 Proposed access to the 3-unsubstituted thiomorpholinone template via reductive amination (i) glycine, $NaCNBH_3$, reflux, 24 hours[1] (ii) trifluoroacetic anhydride, reflux, 2 hours[2]

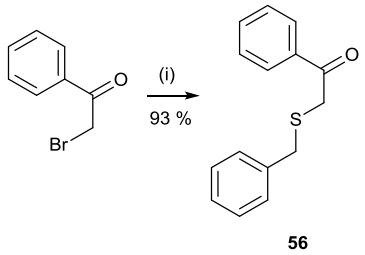
Another potential pathway planned to access the desired thiomorpholinone template was by thionation of the corresponding morpholinone. In principle, this approach required only two more steps from **37** to give thionated product **54** followed by isomerisation to give thiomorpholinone **55** (Scheme 3.2). Several thionating agents were used in the reaction and are discussed further in Section 3.2.



Scheme 3.2 Proposed access to thiomorpholinone **55** via thionation (i) Lawesson's Reagent or P_4S_{10}[3] (ii) $BF_3.Et_2O$, dry toluene, 110 ^oC, N_2 atmosphere[4]

3.1 Thiomorpholinone synthesis via reductive amination

For this synthetic pathway, the thiol group was planned to be introduced earlier in the synthetic sequence. To this end, a benzyl mercaptan was chosen as a source of the thiol group as this prevented the thiol interfering during the reductive amination. To achieve this, we considered the reductive amination reaction between thiobenzyl acetophenone **56** with glycine to generate thiobenzyl acid skeleton **53**. The starting material **56** was prepared by adding benzylthiol dropwise to a solution of 2-bromoacetophenone and triethylamine in acetone cooled in an ice – salt bath (Scheme 3.3).[5] The crude product was purified over silica eluting with hexane – ethyl acetate (4 : 1) to give **56** as a white solid in an excellent 93 % yield.



Scheme 3.3 (i) benzylthiol, Et_3N, acetone solvent, ice – salt bath, 1 hour

The incorporation of the thiobenzyl group in **56** was indicated by an additional singlet appearing at δ 3.68 ppm in the ^{1}H NMR spectrum corresponding to the presence of the -SCH_2- group (Figure 3.1). The preparation of **56** was also supported from the mass spectrometric analysis with a molecular ion observed at 243.0838 [MH^+].

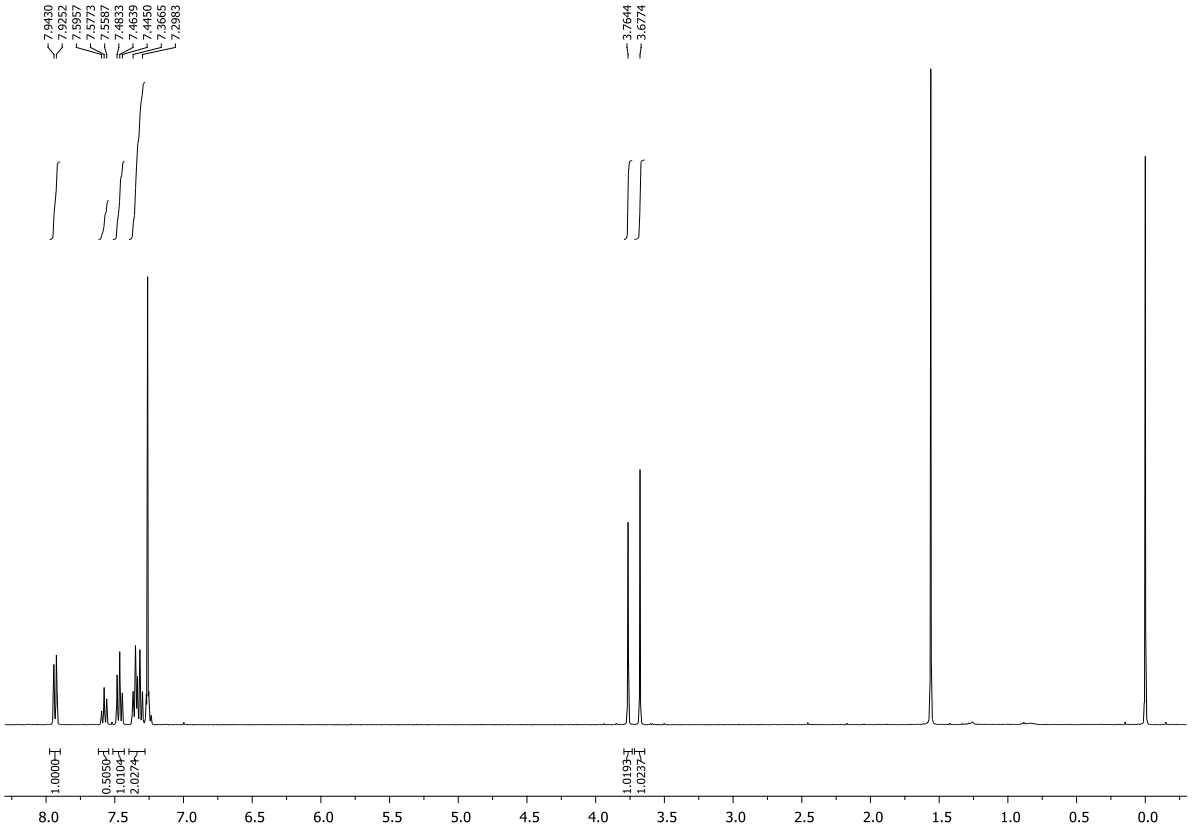
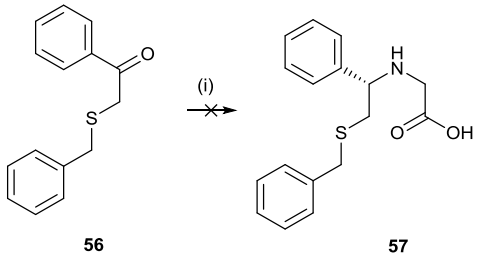


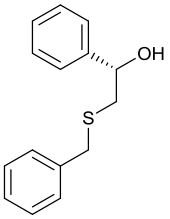
Figure 3.1 ^{1}H NMR spectrum of thiobenzyl **56** in $CDCl_3$

With starting material **56** in hand, the reductive amination was carried out with glycine using sodium cyanoborohydride as the reducing agent (Scheme 3.4).[1] The mixture was heated to reflux for 24 hours, but it was found that the starting material **56** had simply been reduced to alcohol **58**.



56 57

Scheme 3.4 (i) glycine, $NaCNBH_3$, reflux, 24 hours



58

The reduction to alcohol **58** was revealed by the presence of an ABX system with double doublets observed at δ 4.66 ppm (J = 9.0 Hz, J' = 3.5 Hz), δ 2.78 ppm (J = 14.0 Hz, J' = 3.5 Hz) and δ 2.65 ppm (J = 14.0 Hz, J' = 9.0 Hz). A molecular ion at 227.0890 [$(M-H_2O)\ H^+$] in the mass spectrum further supported the fact that alcohol **58** had been obtained.

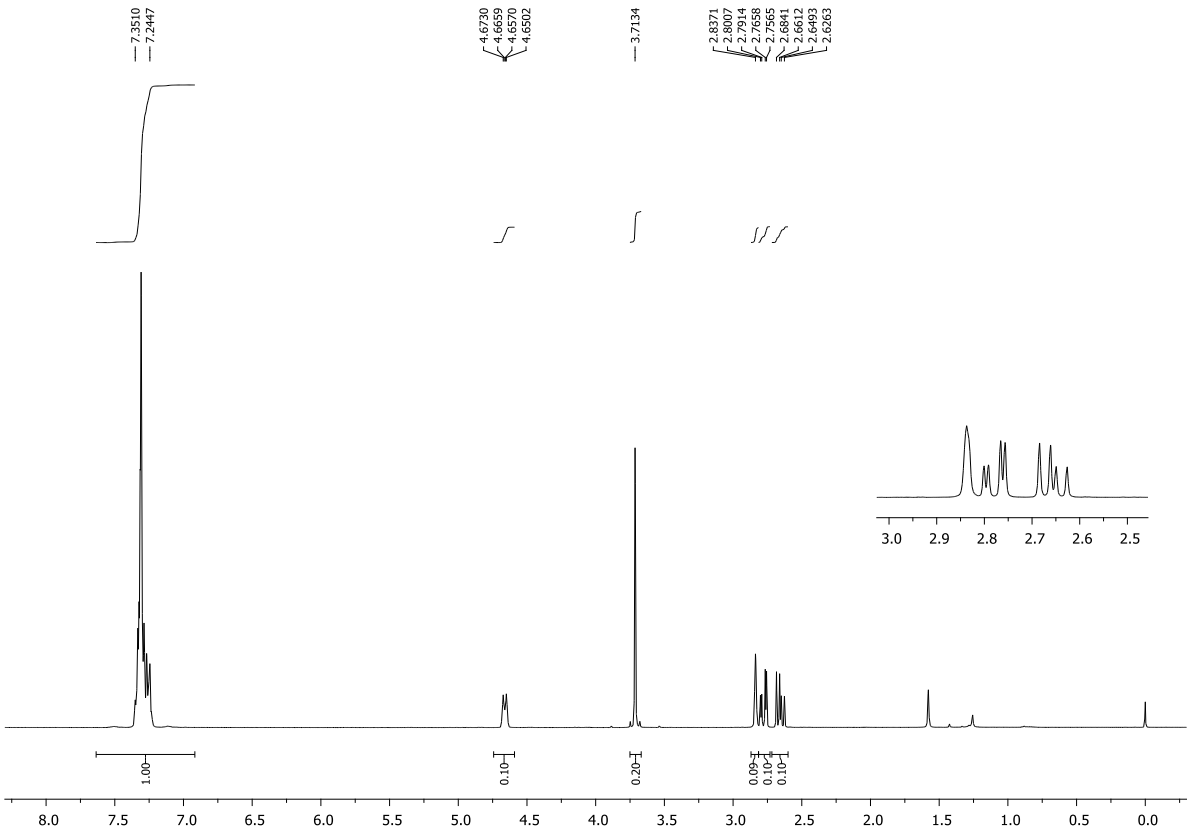
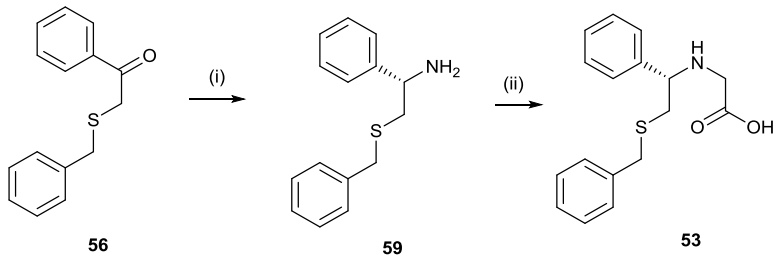


Figure 3.2 ^{1}H NMR spectrum of reduced product **58** in $CDCl_3$

Another option that could be carried out would be the amination of thiobenzyl acetophenone **56** to prepare aminothioether **59** that could undergo an alternative reductive amination reaction with glyoxylic acid to generate the thiobenzyl acid skeleton as summarised in Scheme 3.5.[1,6]



Scheme 3.5 (i) ammonium acetate, $NaCNBH_3$, methanol, reflux, 24 hours (ii) glyoxylic acid, $NaCNBH_3$, 5 M methanolic HCl, methanol, room temperature, 24 hours

Thus, treatment of **56** with ammonium acetate and sodium cyanoborohydride successfully produced aminothioether **59** as a yellow oil in 73 % yield. The success of this amination was signified by an ABX system appearing at δ 2.86 – 2.76 ppm as a two-proton multiplet and a one-proton double doublet at δ 4.08 ppm (J = 8.0 Hz, J' = 6.0 Hz) occurring upfield compared to alcohol **58** (Figure 3.3). The formation of aminothioether **59** was further supported by mass spectrometric analysis with a molecular ion being observed at 244.1154 [MH^+].

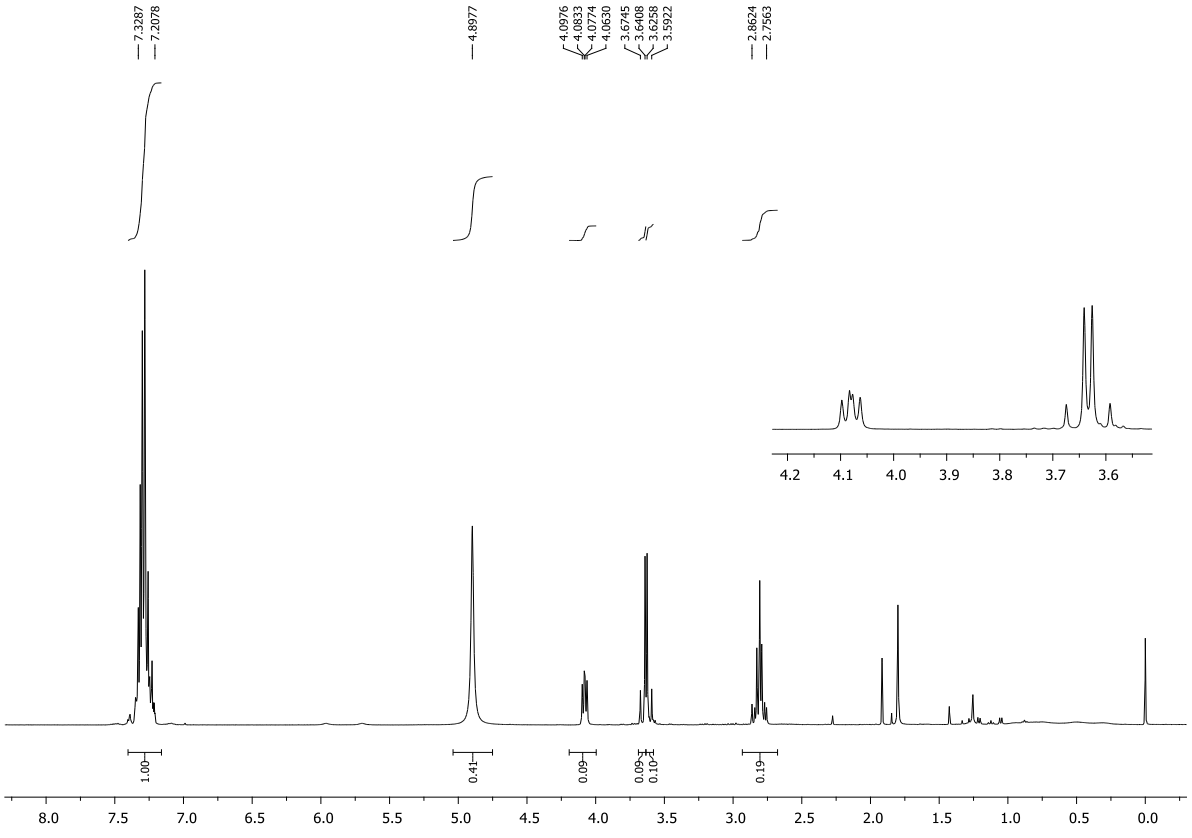
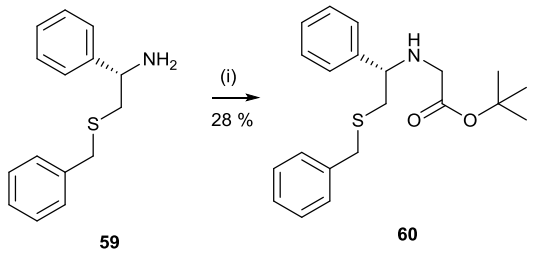


Figure 3.3 ^{1}H NMR spectrum of aminothioether **59** in $CDCl_3$

The next step was to perform the second reductive amination reaction between aminothioether **59** and glyoxylic acid using the same reducing system.[6] However, the reaction failed, despite many attempts. We then decided to perform the *N*-alkylation reaction between aminothioether **59** with *tert*-butyl bromoacetate to construct an

alternative precursor **60** with the acid protected as a *t*-butyl ester (Scheme 3.6). To this end, *tert*-butyl bromoacetate was added dropwise to a solution of aminothioether **59** and triethylamine in dry THF at 0 ^{o}C.[7] The mixture was allowed to warm at room temperature and stirred overnight. After workup and purification over silica, eluting with hexane - ethyl acetate (4 : 1), the desired *N*-alkylated product **60** was obtained as a yellow oil in 28 % yield.



59 **60**

Scheme 3.6 (i) *tert*-butyl bromoacetate, Et_3N, dry THF, 0 ^{o}C to room temperature, overnight

The presence of an AB coupling system in the ^{1}H NMR spectrum of the purified product at δ 3.19 ppm (*J* = 17.5 Hz) and δ 2.99 ppm (*J* = 17.5 Hz) indicated that the desired *N*-alkylated precursor had been obtained (Figure 3.4). Furthermore, signals resonating at δ 3.72 ppm as a multiplet and double doublets at δ 2.69 ppm (*J* = 13.5 Hz, *J'* = 4.5 Hz) and δ 2.59 ppm (*J* = 13.5 Hz, *J'* = 9.5 Hz) could be assigned to the ABX system. Supporting evidence was obtained from the mass spectrum, which showed molecular ions at 358.1835 [MH^+] and 380.1655 [MNa^+] corresponding to the structure **60**.

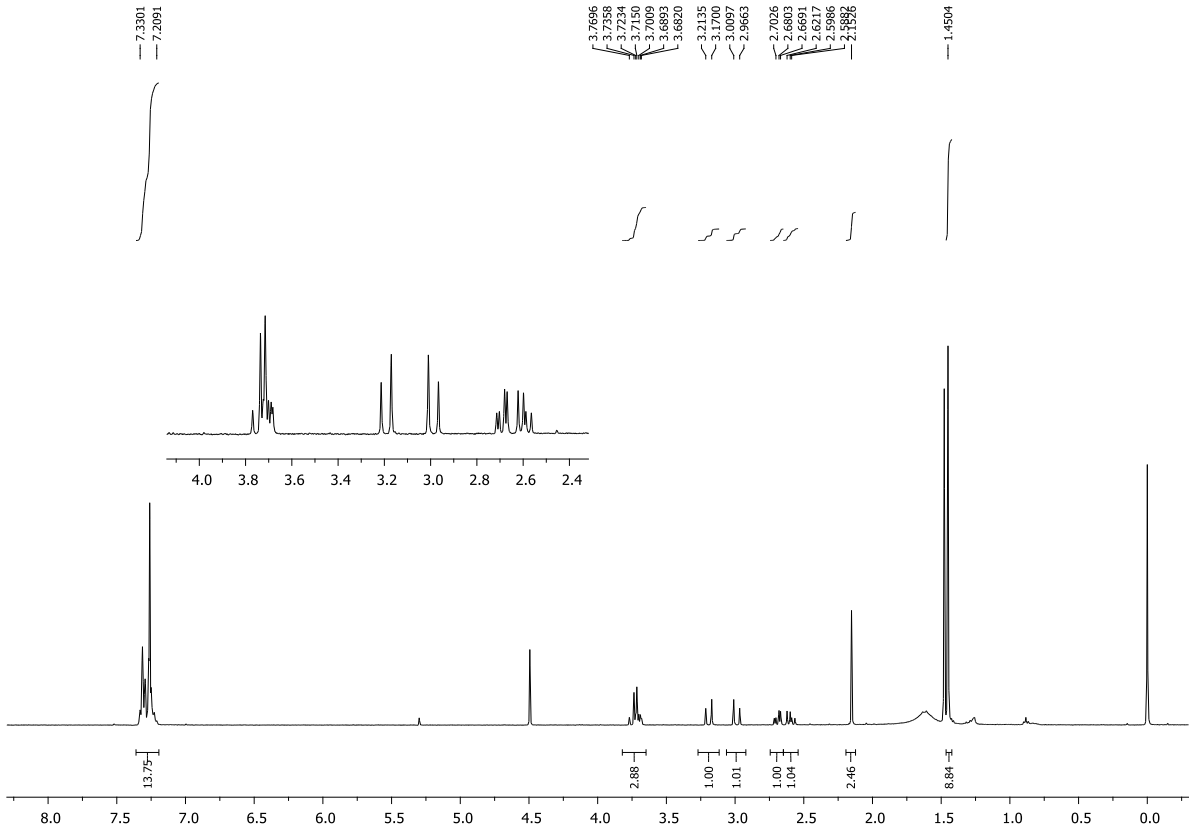
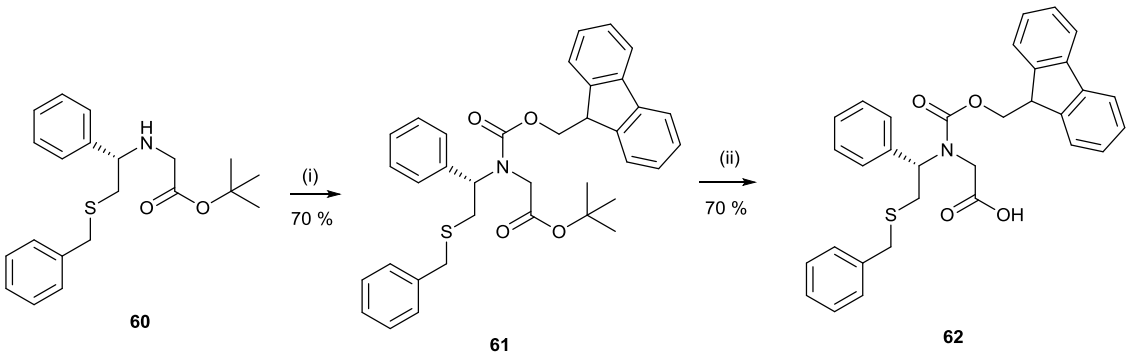


Figure 3.4 ^{1}H NMR spectrum of *N*-alkylated product **60** in $CDCl_3$

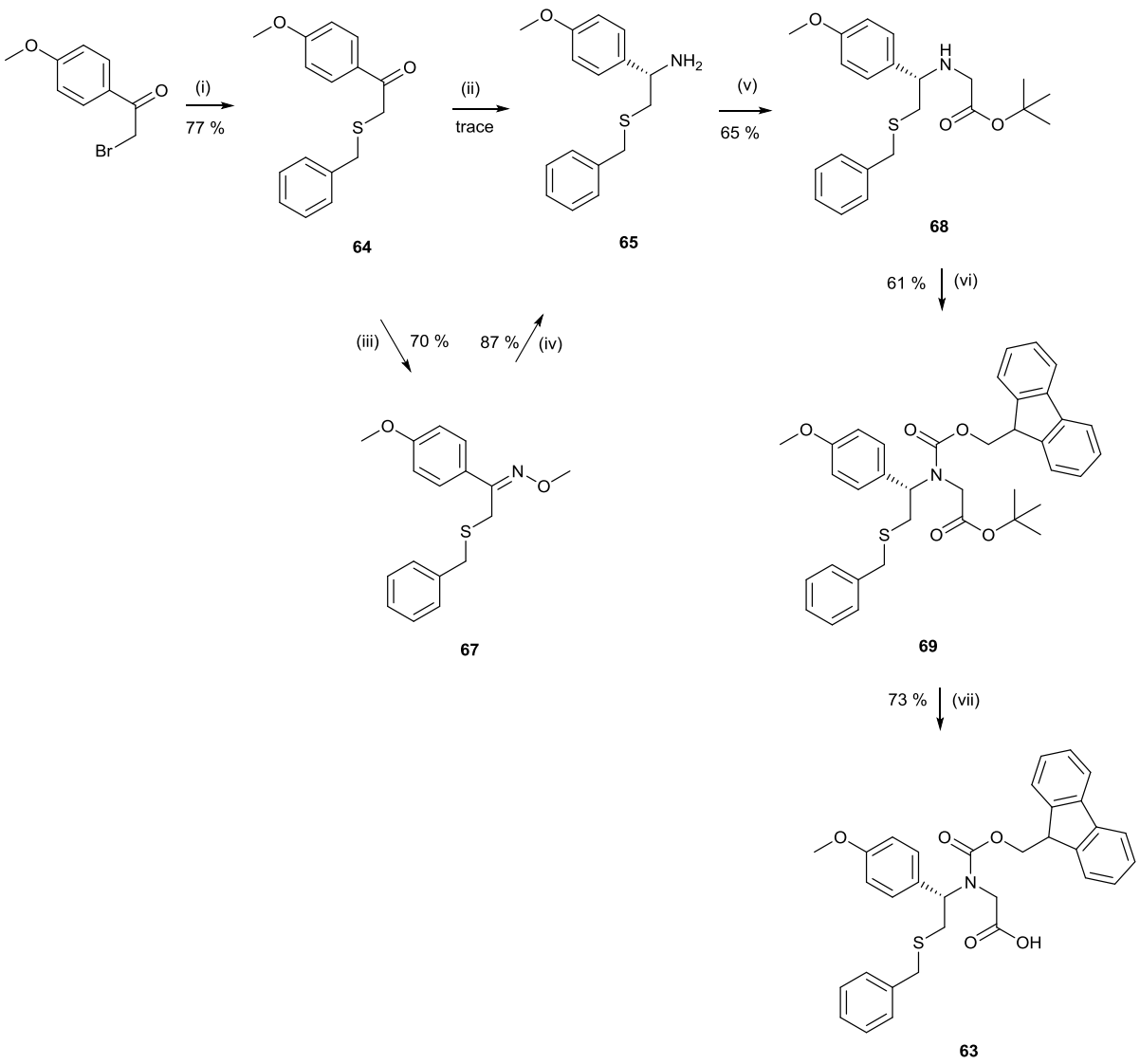
Our next plan was to perform ring closure using TFAA by adapting Ferraris' methodology.[2] The use of an acidic reagent and heating at 60 ^{o}C in the procedure led us to decide upon *N*-Fmoc protection for **60** as the Fmoc-protecting group demonstrated stability at higher temperatures based on our experience with Fmoc-morpholinone **37**, as described in Chapter 2. *N*-Fmoc protection was carried out on **60** with Fmoc-Cl with a suspension of K_2CO_3 in dichloromethane at room temperature overnight (Scheme 3.7). The crude product was purified over silica, eluting with hexane – ethyl acetate (7 : 1), to give *N*-Fmoc *t*Bu glycinate **61** as a yellow oil in 70 % yield. The molecular ion observed at 580.2516 [MH^+] and 602.2336 [MNa^+] confirmed the success of the *N*-Fmoc protection. The removal of the *t*Bu protecting group was then performed using 50 % (v/v) trifluoroacetic acid in dichloromethane to give *N*-Fmoc thiobenzyl acid **62** as a

yellow oil in 70 % yield (Scheme 3.7). The absence of a singlet at δ 1.26 ppm in the ^{1}H NMR spectrum showed that the *t*-butyl protecting group had been removed. Further supporting evidence was obtained from mass spectrometric analysis, with a molecular ion being observed at 546.1710 [MNa^{+}], which was consistent with the assigned structure **62**.



Scheme 3.7 (i) Fmoc-Cl, K_2CO_3, CH_2Cl_2, overnight, room temperature (ii) TFA, CH_2Cl_2, 0 ^{o}C, 30 minutes, room temperature, 1 hour

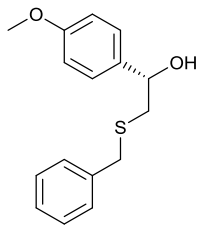
At this point in the work, we had already successfully developed a synthetic route to access glycine-derived thiomorpholinone starting from 5-phenylmorpholinone as described in Chapter 2. We therefore decided to repeat the same procedure to prepare *N*-Fmoc thiobenzyl acid **62** using 4-methoxybromoacetophenone as the starting material, with a slight modification in order to introduce the methoxyphenyl group into the thiomorpholinone system. The synthetic pathway used to access the 4-(methoxyphenyl) *N*-Fmoc thiobenzyl acid **63** is outlined in Scheme 3.8.



Scheme 3.8 (i) benzyl mercaptan, Et_3N, acetone, ice – salt bath, 1 hour (ii) ammonium acetate, $NaCNBH_3$, methanol, reflux, 24 hours (iii) *O*-methyl hydroxylamine hydrochloride, pyridine, ethanol, reflux, 2 hours (iv) BH_3-THF, THF, 0 ^oC; reflux, 3.5 hours; reflux → room temperature (v) *tert*-butyl bromoacetate, Et_3N, dry THF, 0 ^oC to room temperature, overnight (vi) Fmoc-Cl, K_2CO_3, CH_2Cl_2, overnight, room temperature (vii) TFA, CH_2Cl_2, 0 ^oC, 30 minutes, room temperature, 1 hour

The thiobenzyl substitution reaction was carried out following the previous protocol using benzylthiol to give **64** as a white solid in 77 % yield.[5] The appearance of a new

singlet at δ 3.63 ppm in the ^{1}H NMR spectrum indicated the success of the thiobenzyl installation and this was supported by mass spectrometric analysis, with quasi molecular ions detected at 273.0944 [MH^{+}] and 295.0763 [MNa^{+}]. In the second step, however, the reductive amination on **64** with ammonium acetate only gave the desired amino thiobenzyl **65** as a trace product, based on analysis by mass spectrometry with a molecular ion peak being observed at 274.1262 [MH^{+}]. Instead, it was found that most of the starting material had been reduced to alcohol **66**.



66

The formation of alcohol **66** was shown by the appearance of an ABX coupling system with double doublets resonating at δ 3.96 ppm (*J* = 9.0 Hz, *J'* = 4.5 Hz), δ 2.71 ppm (*J* = 13.5 Hz, *J'* = 4.5 Hz) and δ 2.58 ppm (*J* = 13.5 Hz, *J'* = 9.0 Hz) and was further supported by mass spectrometric analysis with a molecular ion peak at 257.0996 [(M-H_2O) H^{+}].

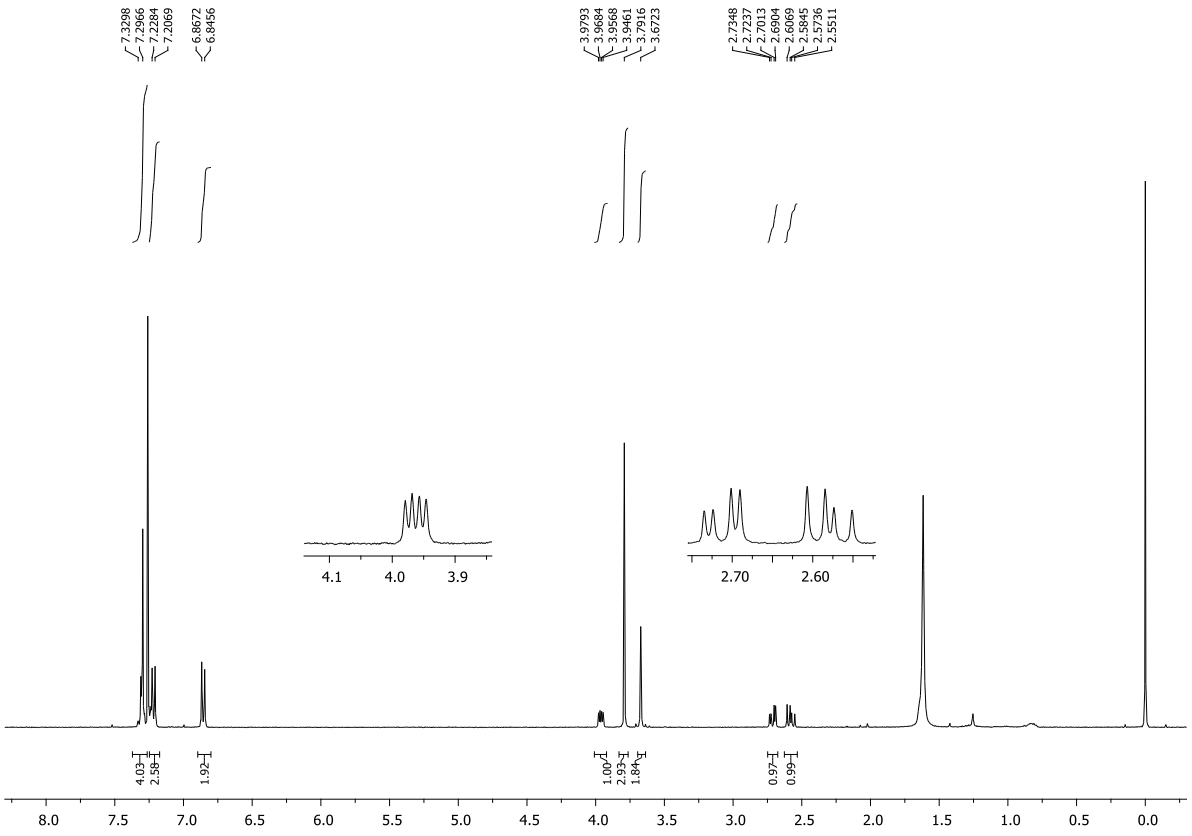


Figure 3.5 ^{1}H NMR spectrum of reduced product **66** in $CDCl_3$

In an alternative approach, we decided to prepare 4-methoxyphenylaminoethyl thiobenzyl ether **65** following the procedure developed by Tchertchian *et al.*[6] In this methodology, **64** was converted to oxime **67** by heating to reflux in ethanol with *O*-methyl hydroxylamine hydrochloride and pyridine before reducing to the aminothiobenzyl derivative **65** (Scheme 3.8).[6] In this way, oxime **67** was obtained as a colourless oil in 70 % yield, the presence of a new methoxy singlet at δ 3.97 ppm in addition to that at δ 3.82 ppm signifying the success of conversion (Figure 3.6). A molecular ion peak detected at 302.1209 [MH^+] by mass spectrometric analysis gave additional evidence to confirm the formation of oxime **67**.

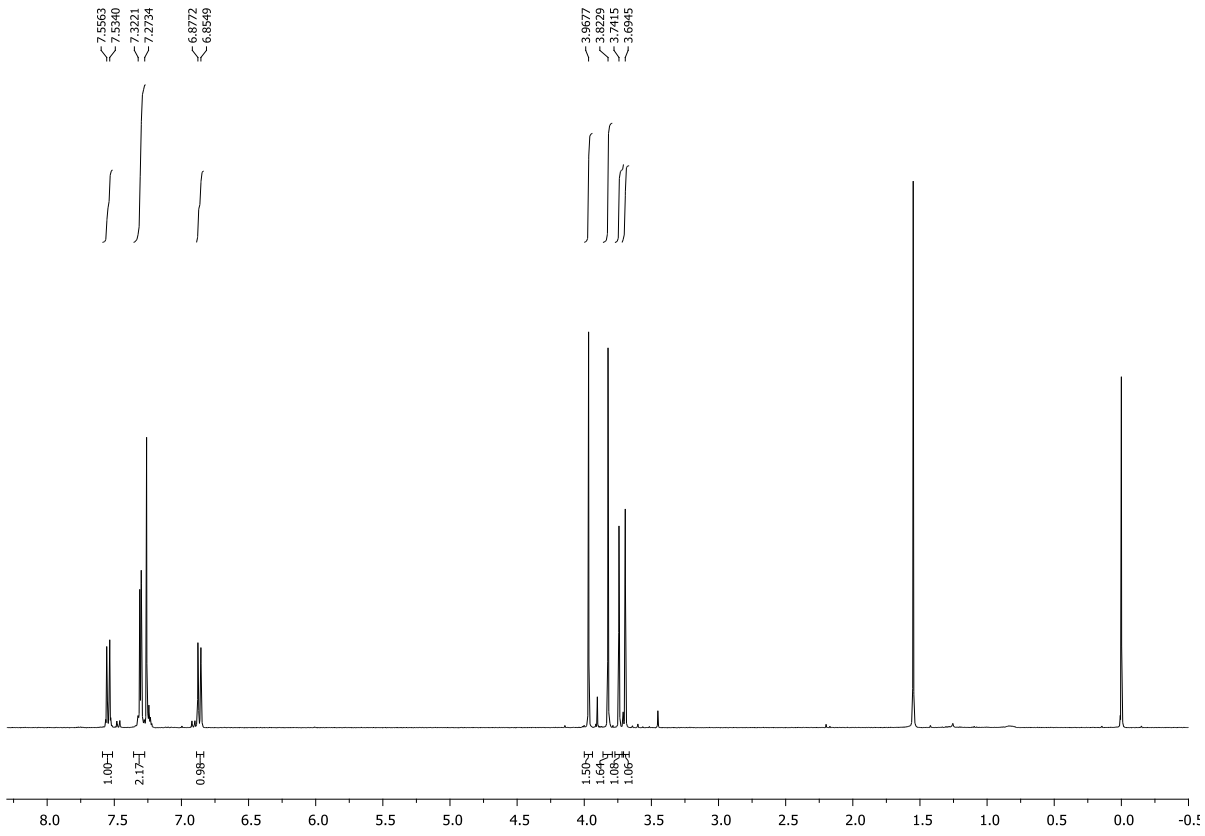


Figure 3.6 ^{1}H NMR spectrum of oxime **67** in $CDCl_3$

Our next task was to reduce the oxime functionality to 4-methoxyphenylamino ethyl thiobenzyl ether derivative **65**. The reduction was carried out using a solution of BH_3-THF and the mixture was heated to reflux for 3.5 hours (Scheme 3.8).[6] After work up and purification on silica, eluting with ethyl acetate - methanol (4 : 1), the desired product **65** was obtained in 87 % yield as a yellow oil.

The disappearence of the methoxy singlet at δ 3.97 ppm in the ^{1}H NMR spectrum indicated that the desired reduction had occurred. A characteristic ABX coupling system appeared at δ 4.10 ppm (J = 7.0 Hz) as an apparent triplet and at δ 2.94 ppm (J = 14.0 Hz, J' = 7.0 Hz) and δ 2.82 ppm (J = 14.0 Hz, J' = 7.0 Hz) as double doublets (Figure 3.7). Further evidence was obtained from the mass spectrum with a molecular

ion peak observed at 274.1260 [MH^+], which was consistent with the proposed structure **65**.

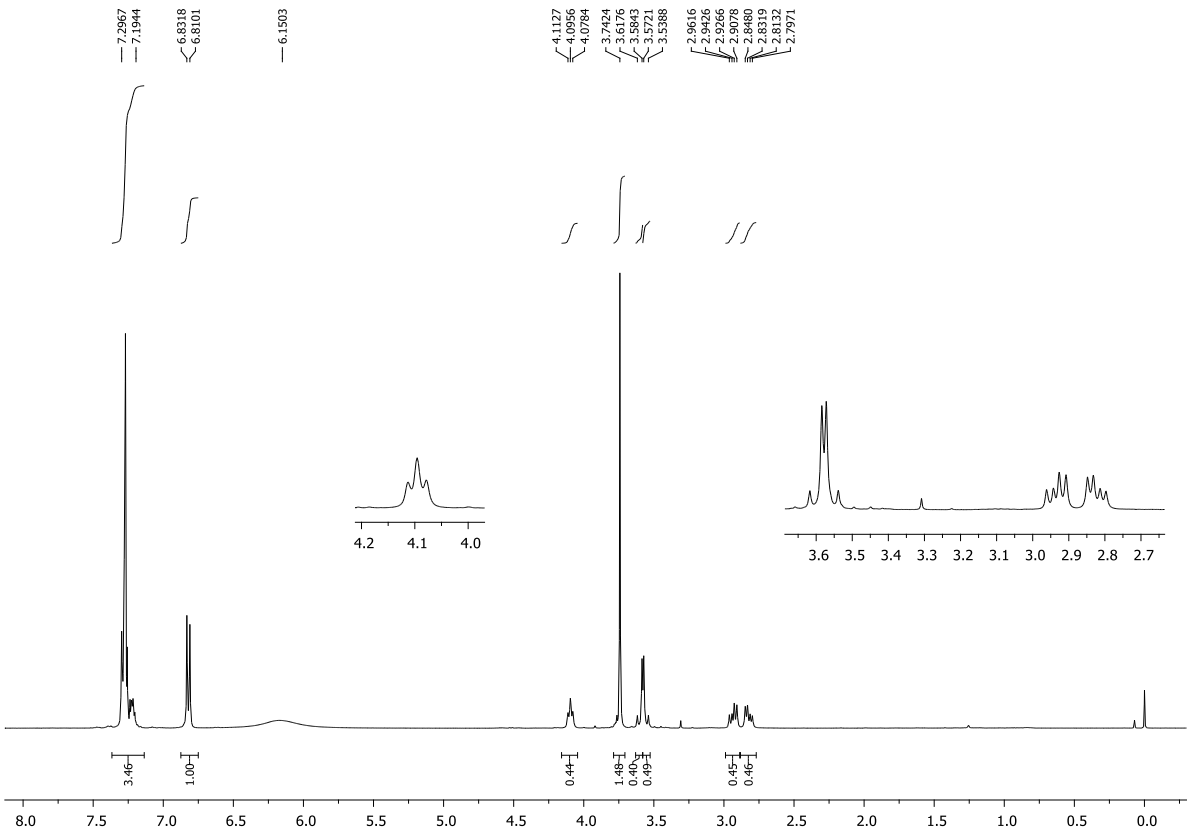


Figure 3.7 ^{1}H NMR spectrum of 4-(methoxyphenyl)aminothiobenzyl derivative **65** in $CDCl_3$

The *N*-alkylation of **65** was performed following the same procedure as before, using *tert*-butyl bromoacetate to give **68** as a yellow oil in 65 % yield.[7] The structure was confirmed based on the two doublets detected at δ 3.17 ppm (J = 17.5 Hz) and δ 2.98 ppm (J = 17.5 Hz) corresponding to the AB resonance system (Figure 3.8). The molecular ion peaks observed at 388.1941 [MH^+] and 410.1760 [MNa^+] confirmed that the desired *N*-alkylated product had been obtained.

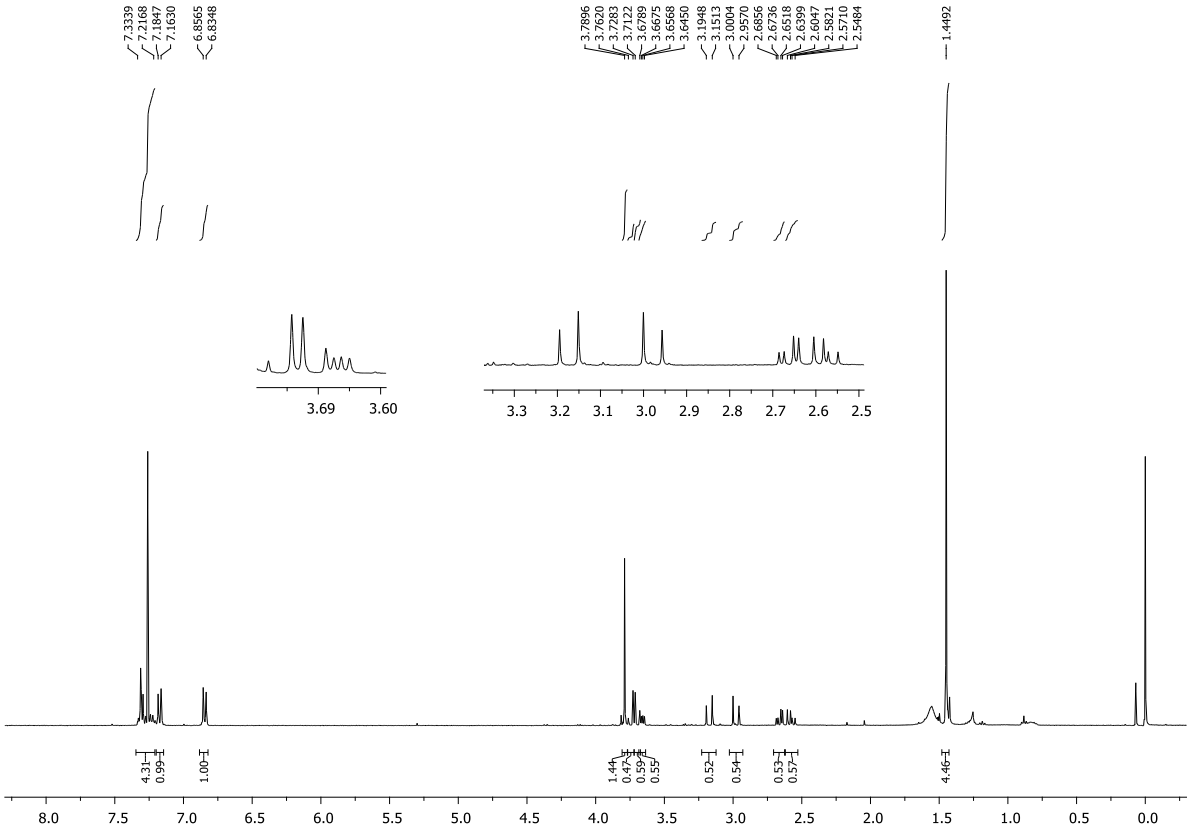
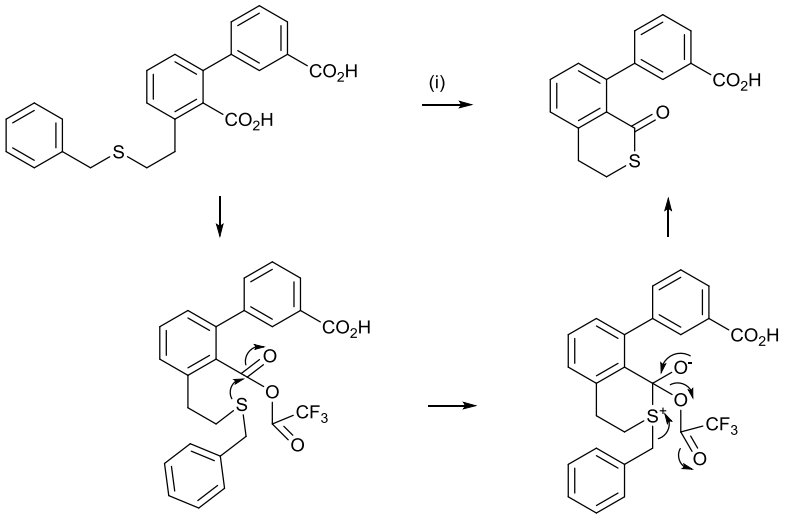


Figure 3.8 ^{1}H NMR spectrum of 4-methoxy *N*-alkylated product **68** in $CDCl_3$

The *N*-Fmoc protection on **68** was subsequently carried out to give **69** in 61 % yield. The appearance of a triplet at δ 5.56 ppm (J = 8.0 Hz) and a doublet at δ 2.97 ppm (J = 8.0 Hz) indicated the success of the *N*-Fmoc attachment. The *tert*-butyl removal on **69** was performed using a similar procedure to previously with 50 % (v/v) trifluoroacetic acid in dichloromethane to give **63** in 73 % yield. The absence of a singlet at δ 1.30 ppm showed that the *tert*-butyl group had been completely removed. The success of the concommitant *N*-Fmoc protection and *tert*-butyl removal was supported by mass spectrometric analysis with a molecular ion peak at 632.2441 [MH^+] and 576.1815 [MNa^+] being observed for **69** and **63** respectively.

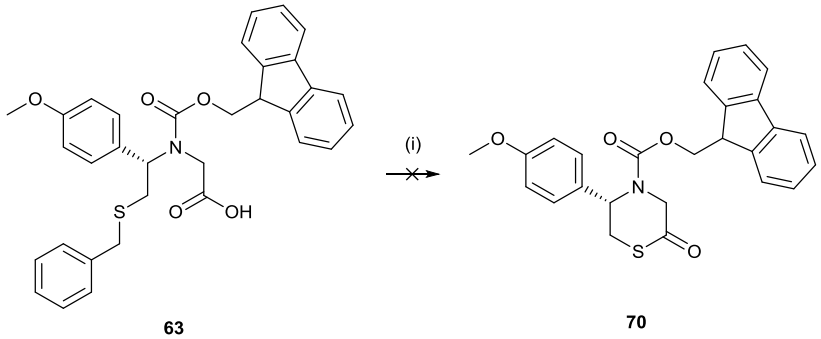
The cyclisation of **63** was then attempted by adapting the Ferraris procedure,[2] involving heating to reflux in trifluoroacetic anhydride at 60 ^{o}C.[2] According to Ferraris *et al.*, with

their system, the ring closure proceeds by initial generation of the corresponding mixed anhydride.[2] This anhydride is then converted to a sulfonium intermediate that undergoes subsequent double elimination to produce the thiolactone (Scheme 3.9).[2]



Scheme 3.9 (i) TFAA, reflux

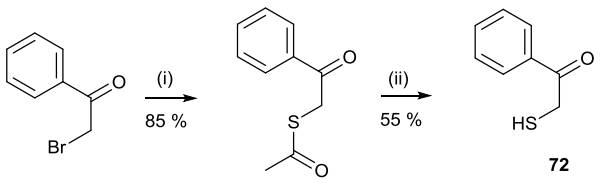
Following this protocol, **63** was heated to reflux in trifluoroacetic anhydride for 2 hours (Scheme 3.10). The resulting mixture was evaporated *in vacuo*, quenched with saturated $NaHCO_3$ at 0 ^oC, acidified with 1 M HCl and extracted with ethyl acetate. Attempted purification of the crude product was carried out over silica with a gradient elution starting with hexane - ethyl acetate (4 : 1) and increasing to 100 % ethyl acetate; however none of the fractions contained the desired cyclised product. Treatment of **63** with trifluoroacetic anhydride was extended to 4 and 6 hours but then simply led to decomposition.



Scheme 3.10 (i) trifluoroacetic anhydride, reflux, 2 hours

At this stage, we decided to abandon this cyclisation approach. In future attempts, alternative procedures for benzyl removal would be worth exploring with **69**, where the carboxyl group protected as its *tert*-butyl ester.

At the same time as attempting to develop approaches proceeding through a reductive amination strategy, we also considered thioacetate **71** as an alternative starting material. This was prepared by treating 2-bromoacetophenone with potassium thioacetate in THF (Scheme 3.11). The mixture was heated at 40 ^{o}C for 24 hours and after work up, the crude product was concentrated *in vacuo* to give **71** as a yellow oil in 85 % yield. The appearance of a new singlet at δ 2.41 ppm in the ^{1}H NMR spectrum indicated successful of thioacylation (Figure 3.9). The formation of **71** was further confirmed from by mass spectrometric analysis with quasi molecular ion peaks being observed at 195.0474 [MH^{+}] and 217.0294 [MNa^{+}].



71 72

Scheme 3.11 (i) potassium thioacetate, THF, reflux, 24 hours (ii) 1 M NaOH in MeOH, room temperature, 24 hours

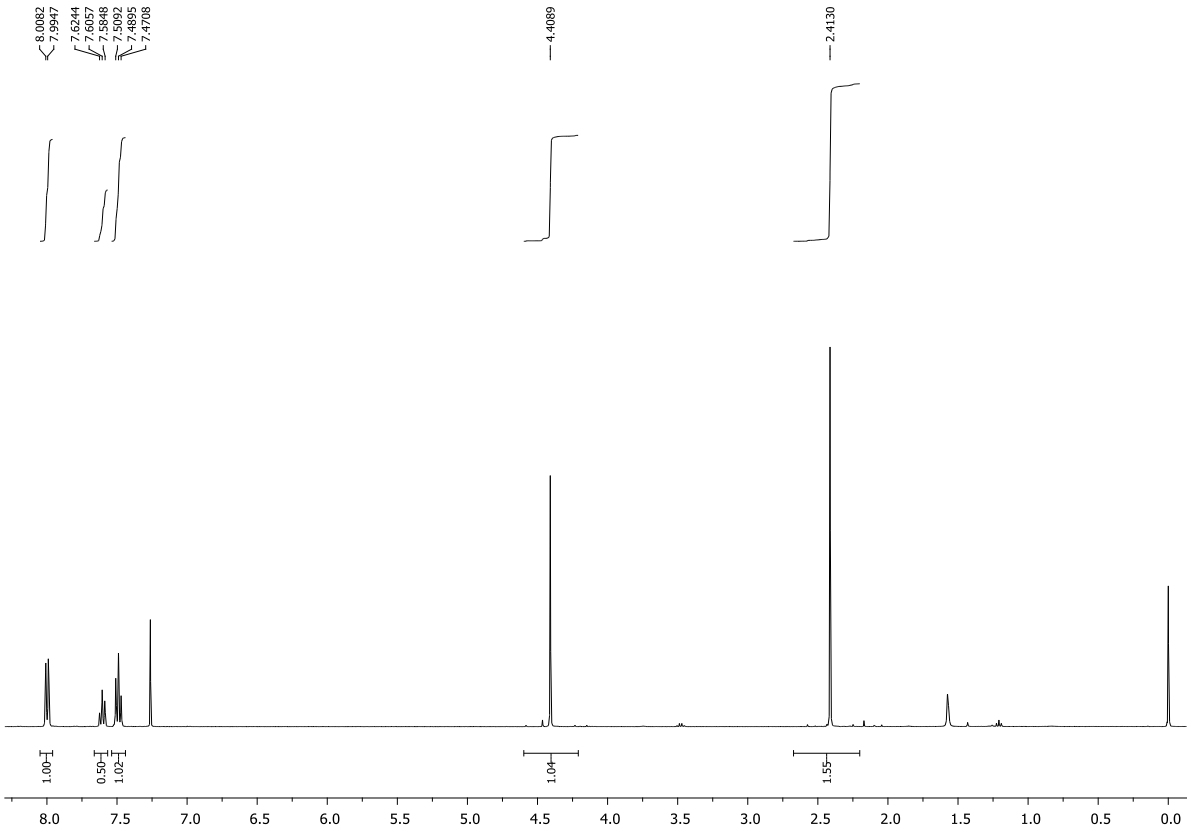


Figure 3.9 ^{1}H NMR spectrum of thioacetate **71** in $CDCl_3$

The thioacetate **71** was then hydrolysed with 1 M NaOH in methanol at room temperature for 24 hours to give thioacid **72** as a yellow oil in 55 % yield. The conversion to **72** was verified by the disappearance of the acyl singlet at δ 2.41 ppm in

the ^{1}H NMR spectrum (Figure 3.10) and a molecular ion peak detected at 153.0367 [MH^{+}] confirmed that the thioacid **72** had been obtained.

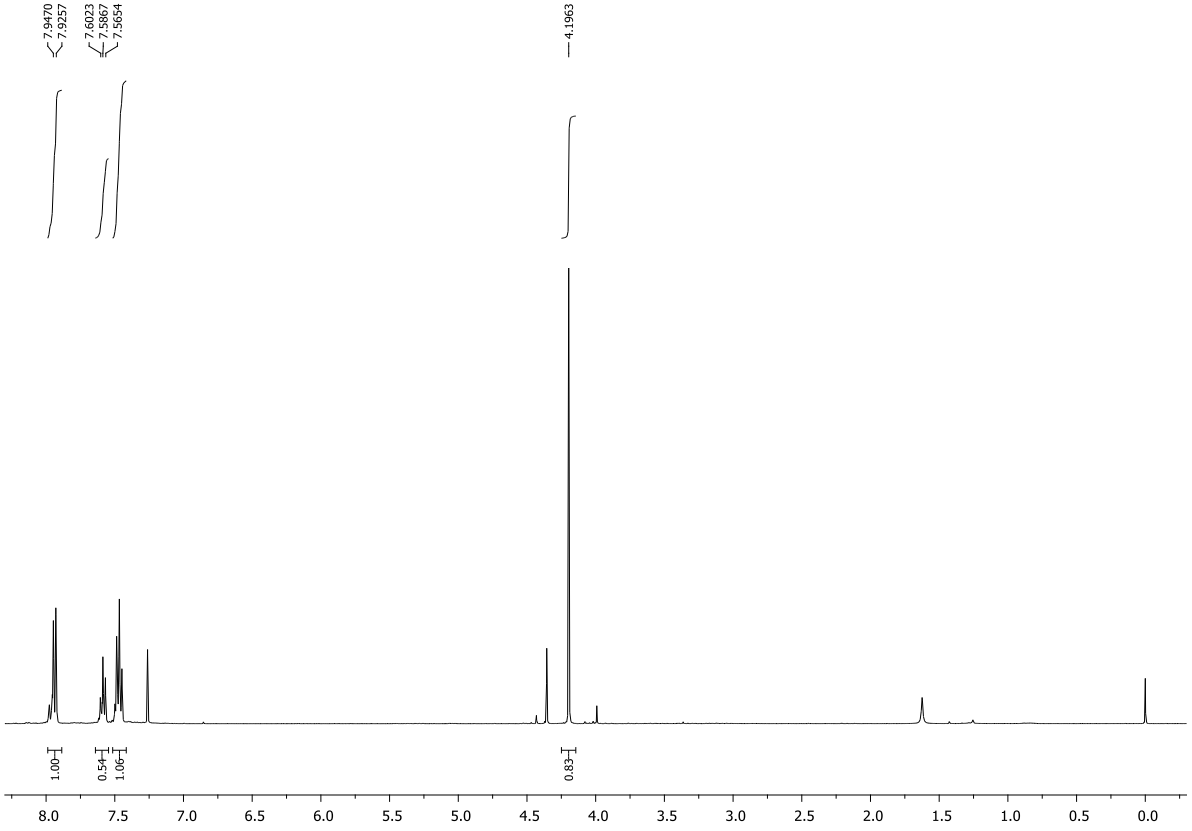
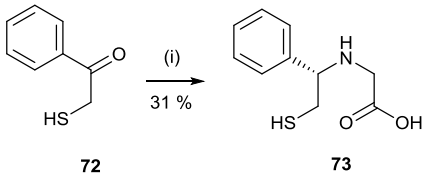


Figure 3.10 ^{1}H NMR spectrum of thioacid **72** in $CDCl_3$

Direct reductive amination of **72** was then performed with glycine using sodium triacetoxyborohydride (STAB) as the reducing agent. Abdel-Magid *et al*. reported that the use of sodium triacetoxyborohydride in reductive amination of certain ketones could give a better yield and fewer side products in comparison to $NaBH_3CN$/MeOH, borane-pyridine or catalytic hydrogenation.[8] Therefore, treatment of **72** with glycine using STAB as the reducing agent, in the presence of acetic acid as catalyst, was carried out at room temperature for 24 hours (Scheme 3.12). The reaction mixture was quenched with saturated $NaHCO_3$, extracted into diethyl ether, washed with brine, dried and purified over silica eluting with hexane – ethyl acetate (1 : 4).



72 73

Scheme 3.12 Glycine, STAB, acetic acid, room temperature, 24 hours

The presence of AB and ABX coupling systems in the ^{1}H NMR spectrum as two doublets (J = 14.0 Hz) at δ 4.17 ppm and δ 4.10 ppm accompanied by two double doublets at δ 3.11 ppm (J = 14.0 Hz, J' = 3.0 Hz) and δ 2.88 ppm (J = 14.0 Hz, J' = 9.5 Hz) and a broadened double triplet at δ 4.91 ppm (J = 9.5 Hz, J' = 3.0 Hz) led us to assume that reductive amination had been successfully achieved (Figure 3.11). Further supporting evidence obtained from the ^{1}H – ^{1}H COSY NMR spectrum indicating the cross coupling between the AB and ABX protons.

However, no molecular ion peak of desired product **73** or any possible dimerised or reduced product of **73** could be observed in the mass spectrum. We presume that **73** exists as zwitterionic compound. According to Jones, the zwitterionic character of similar compounds to **73** causes them to be very involatile requiring high temperature for vapourization.[9] Furthermore, several amino acids and peptide derivatives have been reported to lose their carboxyl group during mass spectrometric analysis.[10] From the mass spectrum of proposed product **73**, a molecular ion peak of 167.0126 [(M-CO_2H)H^+] was observed supporting the fact that the **73** might have lost its carboxyl group during fragmentation.

At this point, based on the evidence from ^{1}H NMR and COSY spectrum, we proposed that the product obtained from the reductive amination of **72** with glycine was the desired product **73**.

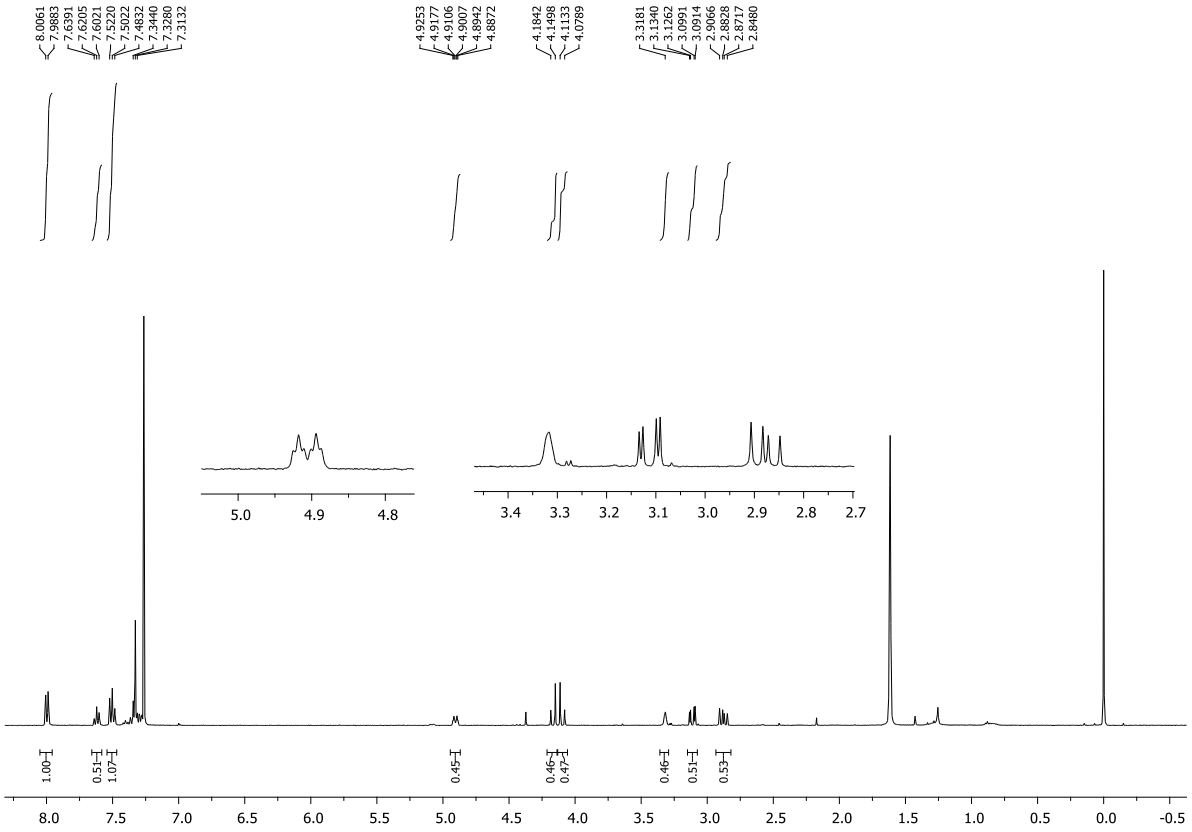


Figure 3.11 ^{1}H NMR spectrum of proposed product **73** in $CDCl_3$ from attempted STAB reductive amination of **72** with glycine

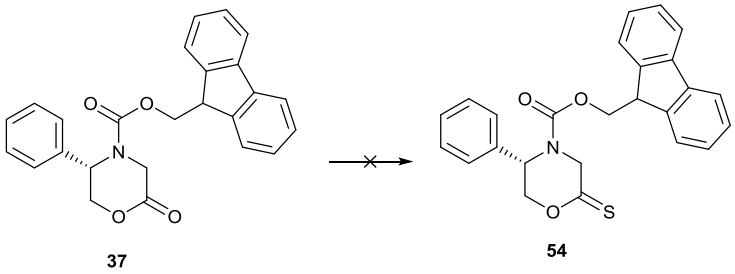
For future work along this synthetic route, it is recommended the protection of the SH group is carried out on **72** before proceeding to the reductive amination and the starting material containing the 4-methoxyphenyl group should be used. Since the thiol group is more sensitive than a hydroxyl group, not just under acidic conditions. but also demonstrates instability to basic conditions and oxidizing agents, this protection seems likely to be important to ensure the success of the reductive amination.[11] The thiol protection could be performed by preparing the methoxymethyl (MOM) thioether based on the procedure suggested by Jae *et al*. and removed with $ZnBr_2$ and a mercaptan according to the procedure developed by Hyun *et al*.[11, 12] After thiol protection, the

approach should need only five steps to access the desired thiomorpholinone via precursor **73** following the established synthetic route outlined in Scheme 2.11.

3.2 Thiomorpholinone synthesis via thionation of morpholinone

Phosphorus pentasulfide (P_4S_{10}) and Lawesson's reagent are commonly used as thionating agents that can convert lactones to thionolactones.[3,13] Furthermore, in 2006, Filippi *et al*. discovered that boron trifluoride etherate could efficiently catalyse the isomerisation of thionolactones to thiolactones.[4] By adopting both procedures, we were interested to explore the thionation reaction to prepare thionomorpholinone **54** and then proceed to isomerization to thiomorpholinone **55** as exemplified in Scheme 3.2. Since both processes require the use of heating to higher than 80 ^{o}C in the standard procedures, we decided to use *N*-Fmoc-5(*S*)-phenylmorpholinone **37** as the starting material.

Following the standard procedure,[3] **37** and Lawesson's reagent were heated at reflux in acetonitrile for 4 hours under an atmosphere of nitrogen (Scheme 3.13). The mixture was concentrated *in vacuo* and purified over silica eluting with hexane - ethyl acetate (4 : 1), but only starting material was recovered from the reaction. A change of solvent to toluene and extending the duration of reflux to 24 hours still failed to give the thionated product.



Scheme 3.13 (i) Lawesson's Reagent, MeCN, 80 ^{o}C, 4 hours, N_2 atmosphere (ii) Lawesson's Reagent, CH_3Ph, 110 ^{o}C, 24 hours, N_2 atmosphere (iii) P_2S_5, HMDO, MeCN, 82 ^{o}C, 24 hours, N_2 atmosphere (iv) P_2S_5, HMDO, CH_3Ph, 108 ^{o}C, 24 hours, N_2 atmosphere (v) P_4S_{10}-pyridine, MeCN, 82 ^{o}C, 24 hours (vi) P_4S_{10}-pyridine, dimethylsulfone, 170-175 ^{o}C, 15 minutes

Curphey has reported that the use of hexamethyldisiloxane (HMDO) in thionations could increase the selectivity of phosphorus pentasulfide (P_4S_{10}) to produce thionolactones.[14] Adopting this approach, **37** was heated to reflux with a P_4S_{10}–HMDO combination in acetonitrile for 3 hours under a nitrogen atmosphere (Scheme 3.13). Unfortunately, once again, the starting material remained untouched. The procedure was repeated by changing the solvent to toluene but no reaction was observed. In 2011, a P_4S_{10}-pyridine complex was reported to achieve even more selective thionations at high temperatures (165 – 175 ^{o}C) at which the Lawesson's reagent would decompose.[15] Following this protocol, **37** was heated to reflux with the P_4S_{10}-pyridine complex in acetonitrile for 24 hours but again gave only recovered starting material. The thionation procedure was repeated, but this time at a temperature between 170 – 175 ^{o}C in dimethyl sulfone for 15 minutes.[15] However, yet again, only starting material was recovered at the end of the heating period.

On the positive side, even though these thionation procedures failed to give the desired thionated product, we have found that *N*-Fmoc-5(*S*)-phenylmorpholinone **37** can withstand temperatures up to 175 ^{o}C. As a result of this degree of thermal stability, for work in the future, it would be worth attempting a thionation procedure developed by Filippi *et al*. that applies microwave irradiation in the reaction.[16] The exposure of direct microwave irradiation to the reaction mixture under solventless conditions might help with the formation of the desired thionomorpholinone.

References

1 Macmillan, D. and Anderson, D. W. *Org. Lett.*, 2004, **6**, 4659 – 4662.

2 Ferraris, D. V, Majer, P., Ni, C., Slusher, C. E., Rais, R., Wu, Y., Wozniak, K. M., Alt, J., Rojas, C. and Slusher, B. S. *J. Med. Chem.*, 2014, **57**, 243 – 247.

3 Filippi, J. J., Fernandez, X., Lizzani-Cuvelier, L. and Loiseau, A. M. *Flavour Fragr. J.*, 2006, **21**, 175–184.

4 Filippi, J.J., Fernandez, X. and Dunach, E. *Tetrahedron Lett.*, 2006, **47**, 6067 – 6070.

5 Zhdanko, A. G., Gulevich, A. V and Nenajdenko, V. G. *Tetrahedron*, 2009, **65**, 4692–4702.

6 Tchertchian, S., Hartley, O. and Botti, P. *J. Org. Chem.*, 2004, **69**, 9208–9214.

7 Arnott, G., Clayden, J. and Hamilton, S. D. *Org. Lett.*, 2006, **8**, 5325 – 5328.

8 Abdel-Magid, A. F., Carson, K. G., Harris, B. D., Maryanoff, C. A. and Shah, R. D. *J. Org. Chem.*, 1996, **61**, 3849–3862.

9 Jones, J. H. *Q. Rev. Chem. Soc.*, 1968, **22**, 302 – 316.

10 Aplin, R. T. and Jones, J. H. *Chem. Commun.*, 1967, 261–263.

11 Hyung, J. J., Eun-Young, Y., Min, K. K., Ji-Hyun, L., Yong-Jin, Y. and Sang-Gyeong, L., *Bull. Korean Chem. Soc.*, 2003, **24**, 1689–1691.

12 Jae, H. H., Young, E. K., Jeong-Hun, S. and Do, H. R., *Tetrahedron*, 2010, **66**, 1673–1677.

13 Ozturk, T., Ertas, E. and Mert, O. *Chem. Rev.*, 2007, **107**, 5210–5278.

14 Curphey, T. J. *J. Org. Chem.*, 2002, **67**, 6461–6473.

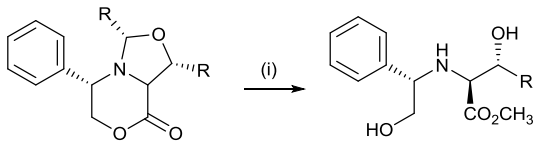
15 Bergman, J., Pettersson, B., Hasimbegovic, V. and Svensson, P. H. *J. Org. Chem.*, 2011, **76**, 1546–1553.

16 Filippi, J. J., Fernandez, X., Lizzani-Cuvelier, L. and Loiseau, A. M. *Tetrahedron Lett.*, 2003, **44**, 6647–6650.

Chapter 4

Application of glycine derived thiomorpholinone templates to amide bond formation

After generation of C-3 unsubstituted thiomorpholinone template **32**, we decided to explore its reactivity towards peptide development. In a previous study, it was shown that the *N*-acylated morpholinone is prevented from intramolecular attack on the carbonyl moiety on the lactone ring to form an oxazolone (Scheme 1.21).[1] Furthermore, the study showed that the non-acylated morpholinone was sensitive to attack by nucleophilic species that will lead to ring opening of the lactone (Scheme 4.1).[2]



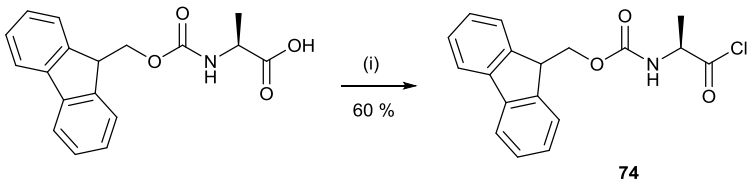
Scheme 4.1 (i) 1 M HCl, Methanol, reflux, 2 hours, N_2 atmosphere

With the thiolactone function in the thiomorpholinone system, that is known to show greater propensity towards nucleophilic attack,[3] we therefore planned our strategy to begin with *N*-acylation at the nitrogen moiety on the thiomorpholinone template **32**, circumventing the problem associated with oxazolone formation and then to proceed to the *C*-terminus extension, thus generating the peptide adduct.

4.1 *N*-terminus extension

After deprotection, the glycine derived thiomorpholinone template **32** was ready for amide bond generation at the *N*-terminus. Thus, template **32** was immediately used for *N*-acylation to avoid any loss to dimerization. There are several methods developed for *N*-acylation in peptide synthesis using activating agents such as carbodiimide,[4]

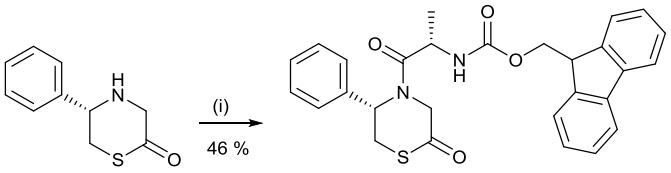
phosphonium reagents[5] and acyl chlorides.[6] In a previous study, the *N*-acylation of morpholinone via carbodiimide and phosphonium salt PyBrop was reported to fail.[1] However, the *N*-acylation of morpholinone was demonstrated using *N*-Fmoc amino acid chlorides.[1] The development of Fmoc-amino acid chloride as coupling agents by Carpino allowed the rapid synthesis of smaller peptides under mild conditions without loss of chiral integrity.[6] Following Carpino's approach, Fmoc-alanine acid chloride **74** was prepared by reacting *N*-Fmoc-alanine with thionyl chloride in dry dichloromethane for 2 hours under an atmosphere of nitrogen (Scheme 4.2).[6] The crude product was purified over silica eluting with hexane – ethyl acetate (6 : 1) to give **74** as a white solid in 60 % yield.



74

Scheme 4.2 (i) $SOCl_2$, dry dichloromethane, reflux, 2 hours, N_2 atmosphere

The *N*-terminus peptide extension on glycine derived thiomorpholinone template **32** was then carried out by adapting Carpino's approach and that developed by the Harwood group.[1] In this methodology, a solution of Fmoc-alanine acid chloride **74** was added drop-wise to a suspension of thiomorpholinone template **32** and Na_2CO_3 in dry dichloromethane and the mixture was left at room temperature overnight (Scheme 4.3). The resulting mixture was suction filtered through a short pad of Celite® and the filtrate was concentrated and purified over silica, eluting with hexane – ethyl acetate (1 : 1) to afford coupled product **75** as a yellow oil in 46 % yield.



32 75

Scheme 4.3 (i) Fmoc-alanine acid chloride **74**, Na_2CO_3, dry dichloromethane, room temperature, overnight, N_2 atmosphere

The success of the peptide coupling was verified by observation of a doublet at δ 1.19 ppm (J = 7.0 Hz) and a broad signal at δ 4.27 – 4.20 ppm on ^{1}H NMR analysis, corresponding to the methyl and CH groups of the alanine constituent (Figure 4.1). Although not resolved in the ^{1}H NMR spectrum, correlation between these two signals was observed in the ^{1}H – ^{1}H COSY NMR spectrum. The characteristic ABX proton coupling system was again shifted downfield from the parent compound **32** to δ 5.38 ppm (J = 12.5 Hz, J' = 7.0 Hz) and δ 3.67 – 3.59 ppm, appearing as a double doublet and a broad multiplet respectively, indicating that *N*-acylation had indeed occurred. A similar effect was displayed by the AB coupling protons that resonated downfield compared to the starting material at δ 4.63 ppm (J = 17.0 Hz) and δ 4.51 ppm (J = 17.0 Hz). Although, once again poorly resolved in the ^{1}H NMR spectrum, the ABX and AB coupled protons were correlated based on the observation of cross peaks in the ^{1}H – ^{1}H COSY NMR spectrum. Further evidence was obtained from the ^{13}C NMR spectrum with two new carbonyl carbon resonances appearing at δ 171.9 ppm and δ 164.6 ppm, reflecting Fmoc-alanine attachment on the *N*-terminus of thiomorpholinone template **32**. A molecular ion observed at 487.1686 [MH^+] and 509.1505 [MNa^+] confirmed that peptide adduct **75** had been successfully obtained.

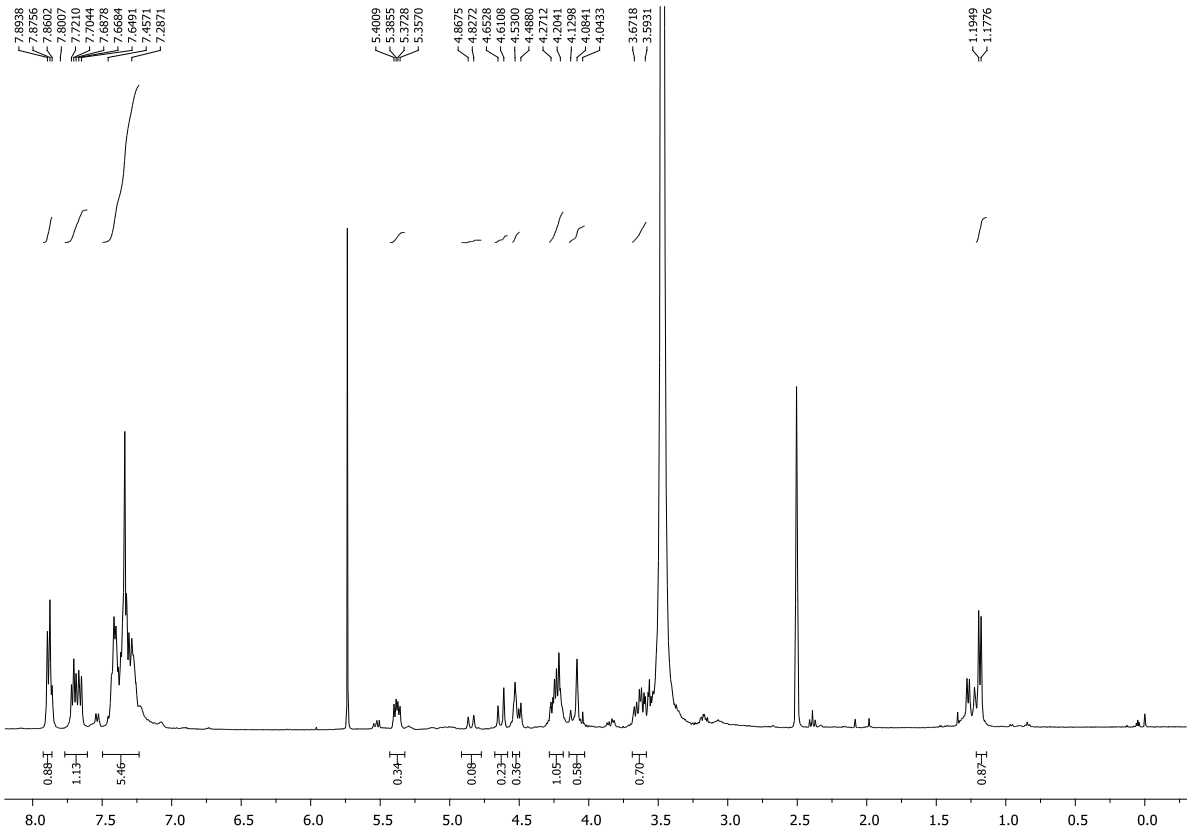


Figure 4.1 ^{1}H NMR spectrum of dipeptide adduct **75** in DMSOd_6

The ^{1}H NMR analysis was repeated at a spectrometer frequency of 500 MHz using variable temperature in an attempt to resolve the broad peaks in the region δ 4.24 – 4.15 ppm (Figure 4.2). However, as the temperature was raised from 25 ^{o}C to 85 ^{o}C, most of the signals broadened and the AB coupled resonances coalesced, most likely due to restricted rotation of Fmoc-alanine on the *N*-terminus of dipeptide adduct **75,** meaning that the system was reaching but not passing through its coalescence temperature.

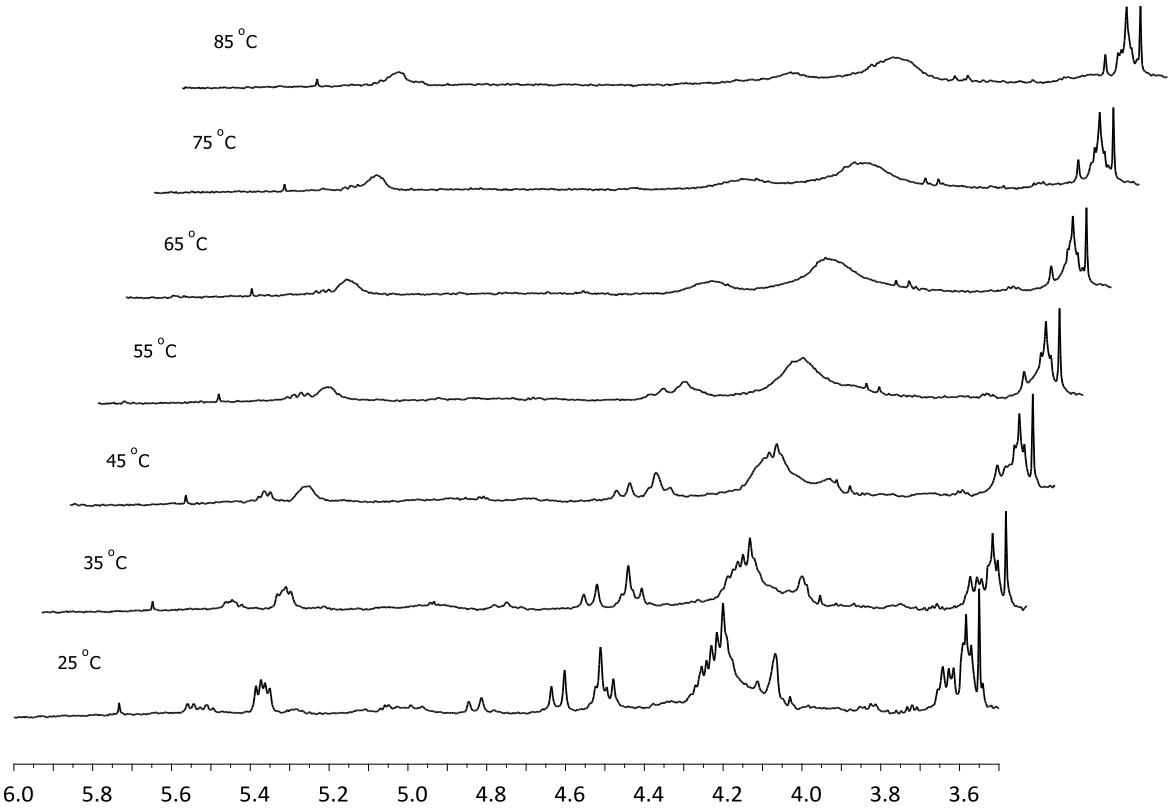


Figure 4.2 ^{1}H NMR spectrum of adduct **75** in DMSOd_6 at variable temperature at 500 MHz (region δ 3.5 to 6.0 ppm expanded)

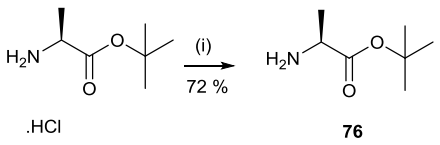
4.2 *C*-terminus extension

In a previous study, the ring opening of *N*-acylated morpholinone templates was achieved using Weinreb's modified dimethylaluminium amide method, relatively harsh condition found necessary due to the relative unreactivity of the lactone to nucleophilic ring opening.[1] The drawbacks of this protocol are that the intermediate dialkylaluminium amide is hydrolytically unstable and the reagent used trimethylaluminium is very sensitive to air and moisture, requiring rigorously anhydrous conditions.

Initial attempts at ring opening of the *N*-acylated morpholinone template using simple carboxyl-protected amino acids was unsuccessful.[1] However, by applying ultra-high pressure (19 kbar) to the reaction over 48 hours, the nucleophilic ring opening was found to occur, although it should be noted that such equipment is not generally available.[7] The morpholinone side chain appeared to be a source of steric hindrance blocking the coupling at *C*-terminus to occur. This was demonstrated by failure of the aminolysis reaction if the bulky *iso*propyl or benzyl morpholinone were used as a template.[8]

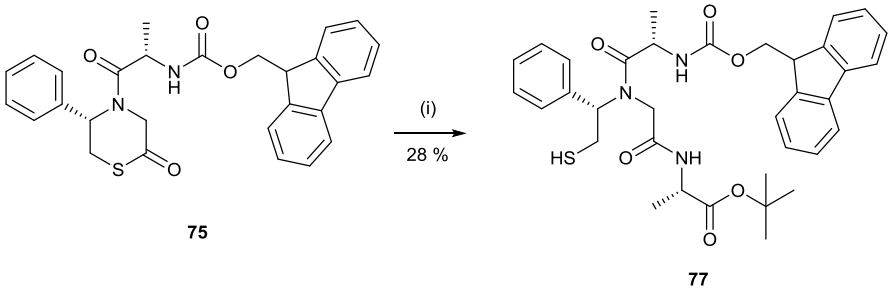
It was proposed that the use of the more electrophilic thiomorpholinone template[3] would allow the aminolysis of the template with a similar procedure using carboxyl-protected amino acids without subjecting the system to ultra-high pressure.[9] Initial results in this area had been disclosed by the Harwood group in 2012, when they reporting the ring opening of *N*-acylated of 3,5-disubstituted thiomorpholinones with carboxyl-protected amino acids.[9] With less steric hindrance at the C-3 position of **75**, only possessing hydrogen at a side chain, we presumed the *C*-terminus extension on the thiolactone function would work by adapting the similar approach.

Thus, the carboxyl protected amino acid was prepared by adding alanine *t*Bu ester hydrochloride to a suspension of sodium carbonate in a water – diethyl ether (1 : 1) (Scheme 4.4). After 1 hour, the resulting solution was extracted into diethyl ether, dried, filtered and concentrated to give alanine *t*Bu ester **76** as a colourless oil in 72 % yield. The preparation of **76** was supported from mass spectrometric analysis showing a molecular ion peak at 146.1176 [MH^+].



Scheme 4.4 (i) $NaCO_3$, deionized water – diethyl ether (1 : 1), room temperature, 1 hour

The nucleophilic ring opening of *N*-acylated thiomorpholinone **75** was then attempted with alanine *t*Bu ester **76** (Scheme 4.5). The reaction was stirred vigorously in dry dichloromethane at room temperature overnight and the crude product was purified over silica, eluting with diethyl ether – dichloromethane (6 : 1), to give tripeptide precursor **77** as a yellow oil in 28 % yield.



Scheme 4.5 (i) alanine *t*Bu ester **76**, dry dichloromethane, room temperature, overnight, N_2 atmosphere

The success of *C*-terminus peptide extension via thiolactone ring opening was verified by the observation of two doublets resonating at δ 1.44 ppm (J = 6.5 Hz) and δ 1.39 ppm (J = 6.0 Hz), indicating the presence of two alanine methyl side chains (Figure 4.3). Furthermore, two broad multiplet signals appearing at δ 4.39 – 4.29 ppm and δ 4.27 – 4.17 ppm could be assigned to the CH protons of the two alanine residues. Although unresolved in the ^{1}H NMR spectrum, both broad signals were shown to couple with the two alanine methyl groups respectively based on ^{1}H – ^{1}H COSY

analysis. In the ^{1}H NMR spectrum, an ABX system was observed at δ 5.79 ppm (J = 10.0 Hz, J' = 6.5 Hz) and δ 3.12 – 3.00 ppm as a double doublet and multiplet integrating to one proton and two protons respectively. A pair of AB coupled resonances was also detected at δ 3.93 ppm (J = 16.5 Hz) and δ 3.61 ppm (J = 16.5 Hz). Further analysis by mass spectrometry confirmed that the desired *N*-Fmoc ala-gly-ala *C*-*t*-butyl tripeptide **77** had been obtained with a molecular ion being observed at 632.2789 [MH^{+}] and 654.2608 [MNa^{+}].

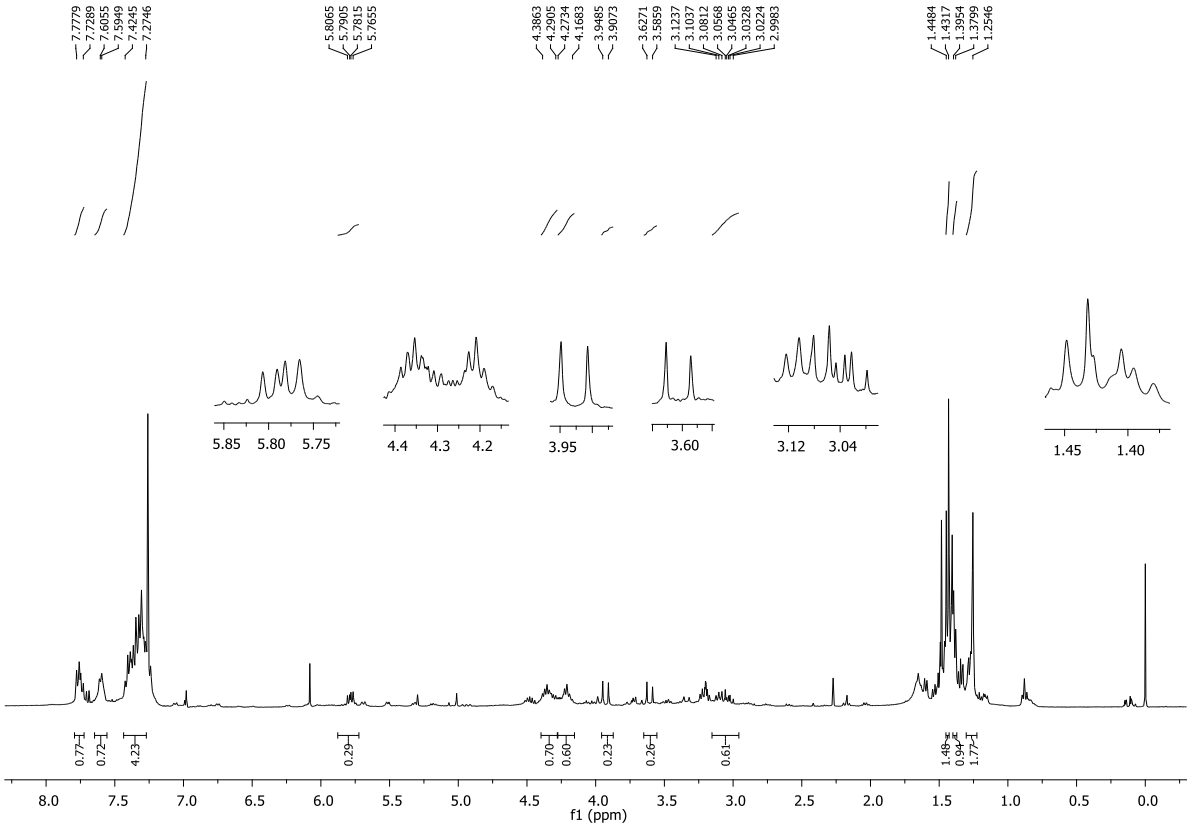


Figure 4.3 ^{1}H NMR spectrum of a tripeptide precursor **77** in $CDCl_3$

Unfortunately, the tripeptide derivative ala-gly-ala **77** with a simple benzylamine protection, rather than a 4-methoxybenzylamine auxiliary means that mild removal of the benzyl substituent is not possible, although global removal of the Fmoc and *tert*-butyl protecting group and benzyl substituent could probably be achieved under harsh

conditions using lithium in liquid ammonia as exemplified in the synthesis of the L-ala-L(D)-ala-L-ala tripeptides based on the morpholinone template protocol.[1] The presence of the substituent methoxy group on the phenyl ring would allow deprotection of **77** under milder conditions using TFA and piperidine.[9] Attempts at incorporating the methoxy group into the glycine thiomorpholinone system were carried out concurrently to this work are described in Chapter 2. Despite this, the short study carried out here demonstrates that the thiomorpholinone template is indeed a viable means of carrying out *C*-terminus peptide extension under mild conditions without causing epimerization at the *C*-terminus residue due to oxazolone formation. This finding also suggests that the synthesis of a longer peptide could be applied in a convergent strategy using the thiomorpholinone template methodology rather than stepwise linear synthesis, where the overall yield reduces with each reaction step. This approach would allow two peptide fragments to be combined using *N*- and *C*- terminus of thiomorpholinone templates to generate a larger peptide derivative.

References

1 Harwood, L. M., Mountford, S. J. and Yan, R. *J. Pept. Sci.*, 2009, **15**, 1–4.

2 Alker, D., Hamblett, G., Harwood, L. M., Robertson, S. M., Watkin, D. J. and Williams, C. E. *Tetrahedron*, 1998, **54**, 6089-6098.

3 Drew, M. G. B., Harwood, L. M. and Yan, R. *Synlett*, 2006, **19**, 3259–3262.

4 Sheehan, J. C. and Hess, G. P. *J. Am. Chem. Soc.*, 1955, **77**, 1067–1068.

5 Castro, B., Dormoy, J. R., Evin, G. and Selve, C. *Tetrahedron Lett.*, 1975, **16**, 1219–1222.

6 Carpino, L. A., Cohen, B. J., Stephens, K. E., Sadat-Aalaee, S. Y., Tien, J. H. and

Langridge, D. C. *J. Org. Chem.*, 1986, **51**, 3732–3734.

7 Harwood, L. M. and Yan, R., 2008, *U.S. Patent No. WO 2008/0281075 Al*. Washington, DC: U.S. Patent and Trademark Office.

8 Yan, R., Phd Thesis. University of Reading, 2016.

9 Harwood, L. M., Wellings, D. A. and Moody, J. D., 2012, *U.S. Patent No. WO 2012/020231 Al*. Washington, DC: U.S. Patent and Trademark Office.

Chapter 5

Conclusions and future work

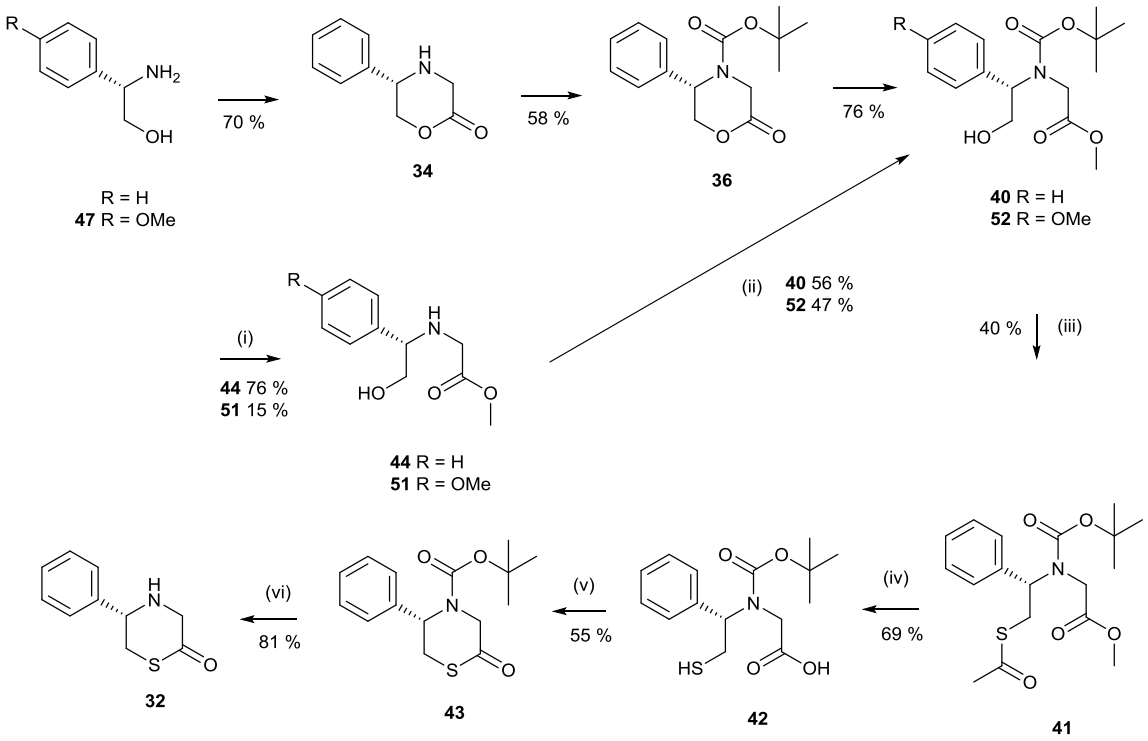
In conclusion, a synthetic route to access C-3 unsubstituted thiomorpholinone template **32** has been developed for the first time, comprising seven steps beginning with commercially available 2-amino-2-phenylethanol. The key step involves the ring opening of Boc-phenylmorpholinone **36** to give methyl ester **40**. The sulfur introduction was accomplished through a Mitsunobu reaction to yield thioacetate methyl ester **41,** which was then hydrolysed to provide thioacid **42**. Cyclisation of the thioacid **42** was achieved under mild conditions using DCC in the presence of DMAP, to give **43,** and subsequent deprotection afforded the desired C-3 unsubstituted thiomorpholinone **32**.

Attempts at improvement of this developed pathway have been carried out. The improved synthetic route was reduced to six steps by preparing methyl ester **44** in a one pot strategy via *N*-alkylation of 2-amino-2-phenylethanol with methyl bromoacetate (Scheme 5.1). This shorthened pathway allows more straightforward access to the desired thiomorpholinone template **32** without having to prepare morpholinone **34**.

A plan for the incorporation of a 4-methoxy group on the thiomorpholinone system was initiated with the successful preparation of Boc-hydroxy methyl ester **52** by adapting the shorter procedure for synthesis of **32**. For the continuation of this work in the future, it is recommended to adopt this approach as it most probably would require four more steps to access 5-(4-methoxyphenyl)thiomorpholinone.

With the C-3 unsubstituted thiomorpholinone template available, the preparation of a tripeptide containing glycine was exemplified by preparing a tripeptide derivative ala-gly-ala **77** using both *N*- and *C*-terminus extensions under mild conditions without

epimerization due to oxazolone formation during the *C*-terminus extension. This study demonstrates that thiomorpholinones can act as *N*-acylated *C*-terminus activated amino acids. It is recommended to carry out the convergent synthesis of a longer peptide in the future to demonstrate its utility as an alternative to the standard linear repetitive *N*-terminus extension strategy most commonly used.



Scheme 5.1 Improved synthetic route to access glycine-derived thiomorpholinone (i) Methyl bromoacetate, Et_3N, dry THF, 0 ^oC to room temperature, 24 hours (ii) Di-*tert*-butyl dicarbonate, Et_3N, EtOAc, room temperature, 6 hours (iii) DIAD, triphenylphosphine, dry THF, 0 ^oC, 30 minutes, N_2 atmosphere, thioacetic acid, dry THF, 0 ^oC, 1 hour, room temperature, 24 hours, N_2 atmosphere (iv) LiOH, water, *i*-PrOH, room temperature, overnight (v) DCC, CH_2Cl_2, 0 ^oC, 15 minutes, DMAP, room temperature, overnight (vi) TFA, CH_2Cl_2, 0 ^oC, 30 minutes, room temperature, 1 hour 30 minutes

Chapter 6

Experimental

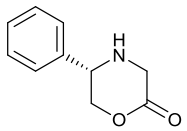
General techniques

All chemicals and reagents were purchased from Sigma-Aldrich, Alfa Aesar and Tokyo Chemical Industries. Acetonitrile was dried over 4 Å molecular sieves and tetrahydrofuran was dried by distillation from sodium benzophenone ketyl.

All ^{1}H NMR spectra were recorded either at Bruker Avance DPX400 spectrometer or at 500 MHz on a Bruker AVIII500 spectrometer. ^{13}C NMR spectra were recorded at 100 MHz on the first spectrometers. NMR solvents used were chloroform-*d*, dimethyl sulfoxide-d_6 and methanol-d_4. Spectra are reported on the δ scale referenced to tetramethylsilane (TMS). The abbreviations s, d, t, m, dd, br, app are used to denote singlet, doublet, triplet, multiplet, double doublet, broad and apparent respectively.

Infrared spectra were recorded on a Perkin-Elmer spectrometer in the frequency range 4000 - 400 cm^{-1}. Mass spectrometry (m/z) analysis and accurate mass measurements (HRMS) were determined on a Thermo Scientific LTQ-Orbitrap-XL mass spectrometer using electrospray ionisation (ESI) technique. Specific rotations were determined on a Perkin-Elmer polarimeter at the sodium D line (589 nm). Melting points were determined with a Buchi melting point apparatus. Thin layer chromatography (TLC) analyses were performed using 0.25 mm thick silica gel 60 F_{254} plates in the appropriate solvent system. Compound visualization was carried out using a UV source at 254 nm and staining with potassium permanganate solution. Column chromatography was conducted either using silica gel 60 (230-400 mesh) or Florisil®.

Synthesis of (*S*)-5-phenylmorpholin-2-one (34)[1]

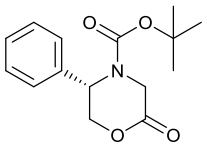


34

(*S*)-2-phenylglycinol (4.0 g, 29.2 mmol, 1.0 equiv) and *N*,*N*-diisopropylethylamine (12.5 mL, 73.0 mmol, 2.5 equiv) were dissolved in dry acetonitrile (100 mL) under an atmosphere of nitrogen at room temperature. Phenyl bromoacetate (6.9 g, 32.1 mmol, 1.1 equiv) in dry acetonitrile (25 mL) was added dropwise over 10 minutes and the mixture was stirred for 24 h at room temperature, then concentrated under reduced pressure to give the crude product. Purification by chromatography on Florisil®, with a 2 cm layer of K_2CO_3 on top of the column, eluting with petroleum ether – diethyl ether (1 : 2) followed by acetone afforded morpholin-2-one **34** (3.6 g, 70 % yield) as a pale yellow oil.

IR (ATR): ν_{max} 3298, 3043, 2953, 1735, 1217 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 7.42 - 7.34 (5H, m, Ph), 4.42 (1H, dd, *J* = 11.0 Hz, *J'* = 4.5 Hz, C$\underline{H}_2$O), 4.31 (1H, app t, *J* = 11.0 Hz, PhC$\underline{H}$), 4.20 (1H, dd, *J* = 11.0 Hz, *J'* = 4.0 Hz, C$\underline{H}_2$O), 3.98 (1H, d, *J* = 18.0 Hz, C$\underline{H}_2$NH), 3.86 (1H, d, *J* = 18.0 Hz, C$\underline{H}_2$NH), 2.18 (1H, s, NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 155.8, 129.6, 128.8, 128.0, 127.4, 66.8, 64.2, 52.0. HRMS calcd for $C_{10}H_{12}NO_2$: 178.0868, found 178.0863 (MH^+).

Synthesis of *tert*-butyl (*S*)-2-oxo-5-phenylmorpholine-4-carboxylate (36)[1]

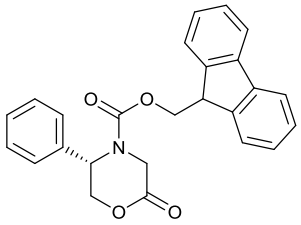


36

(*S*)-5-phenylmorpholin-2-one **34** (3.53 g, 19.9 mmol, 1.0 equiv), di-*tert*-butyl dicarbonate (4.78 g, 21.9 mmol, 1.1 equiv) and triethylamine (2.78 mL, 19.9 mmol, 1.0 equiv) were dissolved in diethyl ether (50 mL) at room temperature and the resulting mixture was stirred for 6 hours. The reaction mixture was then concentrated under reduced pressure, diluted with diethyl ether (25 mL), washed with 5 % aqueous HCl (15 mL) and saturated aqueous $NaHCO_3$ (15 mL), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo* to give the crude product as a yellow oil. Purification by chromatography over silica, eluting with diethyl ether, yielded **36** (3.2 g, 58 %) as a white solid. m.p. 87 – 88 ^{o}C (Lit[1] 87 – 88 ^{o}C).

IR (ATR): ν_{max} 3011, 2985, 2940, 1768, 1700, 1403, 1370, 1162, 1118 cm^{-1}. ^{1}H NMR (500 MHz, DMSO d_6, 85 ^{o}C) δ 7.37 - 7.26 (5H, m, Ph), 5.05 (1H, app t, *J* = 4.0 Hz, PhC<u>H</u>), 4.64 (1H, dd, *J* = 8.0 Hz, *J'* = 4.0 Hz, C<u>H</u>$_2$O), 4.47 (1H, app. dd, *J* = 12.0 Hz, *J'* = 4.0 Hz, C<u>H</u>$_2$O), 4.43 (1H, d, *J* = 12.0 Hz, C<u>H</u>$_2$N), 4.31 (1H, d, *J* = 12.0 Hz, C<u>H</u>$_2$N), 1.27 (9H, s, *t*-butyl). ^{13}C NMR (500 MHz, DMSO d_6): 168.8, 154.0, 139.8, 129.1, 128.1, 126.7, 81.0, 70.1, 54.7, 45.3, 28.6. HRMS calcd for $C_{15}H_{18}NO_4$: 276.1236, found 276.1241 (M-H$^-$). $[\alpha]_D^{20}$ -31.9 (c 0.5, $CHCl_3$).

Synthesis of (9*H*-fluoren-9-yl)methyl (*S*)-2-oxo-5-phenylmorpholine-4-carboxylate (37)

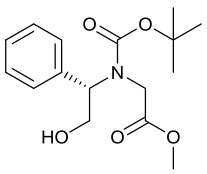


37

To a solution of **34** (2.8 g, 15.8 mmol, 1.0 equiv) in dichloromethane (50 mL) was added K_2CO_3 (10.9 g, 79.0 mmol, 5 equiv) and the solution was stirred for 10 minutes. Fmoc-Cl (4.9 g, 18.9 mmol, 1.2 equiv) was then added and the mixture left overnight. The resulting mixture was quenched with water (20 mL), extracted with dichloromethane (50 mL x 3), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo* to give the crude product that was purified over silica, eluting with approximately 0.5 L of chloroform then acetone to afford **37** as pale yellow oil (4.7 g, 74 %).

IR (ATR): ν_{max} 3058, 2949, 1752, 1721, 1513, 1250, 1188 cm^{-1}. ^1H NMR (500 MHz, DMSO d_6, 85 ^oC) δ 7.82 (2H, d, *J* = 4.0 Hz, Ph), 7.65 (2H, d, *J* = 4.0 Hz, Ph), 7.39 – 7.18 (9H, bm, Ph), 5.05 (1H, app bt, *J* = 4.0 Hz, PhCH), 4.65 (1H, dd, *J* = 12.0 Hz, *J'* = 4.0 Hz, PhCHC$\underline{H}_2$), 4.47 (1H, dd, *J* = 12.0 Hz, *J*' = 4.0 Hz, PhCHC$\underline{H}_2$), 4.42 (1H, d, *J* = 16.0 Hz, C$\underline{H}_2$CO), 4.34 (1H, app t, *J* = 4.0 Hz, C$\underline{H}$CH$_2$O), 4.24 (1H, d, *J* = 16.0 Hz, C$\underline{H}_2$CO), 4.17 (2H, bs, CHC$\underline{H}_2$O). ^{13}C NMR (100 MHz, DMSO d_6): 168.7, 154.3, 145.9, 144.0, 141.2, 129.2, 128.1, 127.6, 126.4, 125.7, 125.4, 120.6, 79.7, 70.3, 64.2, 55.0, 45.1. HRMS calcd for $C_{25}H_{21}NO_4Na$: 422.1368, found 422.1362 (MNa^+).

Synthesis of methyl (*S*)-*N*-(*tert*-butoxycarbonyl)-*N*-(2-hydroxy-1-phenylethyl)glycinate (40)

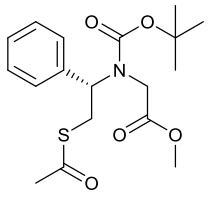


40

To a solution of Et_3N (6.99 g, 69.1 mmol, 6.0 equiv) in MeOH (50 mL) was added a solution of **36** (3.19 g, 11.5 mmol, 1.0 equiv) in MeOH (5 mL). The reaction mixture was stirred for 24 h and then concentrated *in vacuo* to give a crude product that was purified over silica eluting with petroleum ether – diethyl ether (1 : 2) to afford ester **40** (2.72 g, 76 %) as a colourless oil.

IR (ATR): ν_{max} 3486, 3032, 2978, 1762, 1698 cm^{-1}. ^1H NMR (400 MHz, DMSO d_6) δ 7.40 - 7.24 (5H, m, Ph), 5.22 (1H, app t, J = 8.0 Hz, PhC<u>H</u>), 4.87 – 4.81 (2H, m, <u>CH_2</u>OH), 3.91 – 3.84 (2H, m, CH_2N), 3.58 (3H, s, OCH_3), 1.35 (9H, bs, *t*-butyl). ^{13}C NMR (100 MHz, DMSO d_6): δ 170.9, 154.3, 138.6, 128.1, 79.4, 60.6, 51.6, 45.5, 27.9. HRMS calcd for $C_{16}H_{24}NO_5$: 310.1654 and $C_{16}H_{23}NO_5Na$: 332.1474, found 310.1649 (MH^+) and 332.1468 (MNa^+).

Synthesis of methyl (*S*)-*N*-(2-(acetylthio)-1-phenylethyl)-*N*-(*tert*-butoxycarbonyl)glycinate (41).

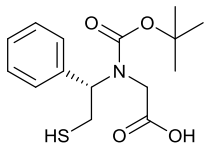


41

DIAD (3.49 g, 17.3 mmol, 2.0 equiv) was added dropwise to a solution of triphenylphosphine (4.53 g, 17.3 mmol, 2.0 equiv) in dry tetrahydrofuran (100 mL) under an atmosphere of nitrogen at 0 ^{o}C for 30 minutes. A milky white mixture resulted. A solution of **40** (2.67 g, 8.64 mmol, 1.0 equiv) and thiolacetic acid (1.32 g, 17.3 mmol, 2.0 equiv) in dry tetrahydrofuran (25 mL) was then added dropwise and the stirring was continued for 1 h at 0 ^{o}C and then left overnight at room temperature resulting in a clear yellow solution. Ether (25 mL) was added and the solution was washed with water (50 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by column chromatography, eluting with hexane – ethyl acetate (7 : 1) to give **41** (1.28 g, 40 %) as a yellow oil.

IR (ATR): ν_{max} 3029, 2977, 1756, 1690 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 - 7.30 (5H, m, Ph), 5.54 (1H, t, *J* = 8.0 Hz, PhCH), 3.79 (1H, d, *J* = 16.0 Hz, CH_2N), 3.61 (3H, s, OCH_3), 3.56 (1H, app d, *J* = 16.0 Hz, CH_2N), 3.44 – 3.38 (2H, bm, CH_2S), 2.33 (3H, s, CH_3COS), 1.44 (9H, bs, *t*-butyl). ^{13}C NMR (500 MHz, $CDCl_3$): 195.4, 170.7, 154.9, 152.9, 129.0, 128.1, 126.2, 81.7, 72.4, 70.6, 69.8, 28.4, 25.3, 21.9. HRMS calcd for $C_{18}H_{26}NO_5S$: 368.1532 and $C_{18}H_{25}NO_5SNa$: 390.1351, found 368.1524 [MH^+] and 390.1346 (MNa^+). $[\alpha]_D^{20}$ -10.4 (*c* 0.3, $CHCl_3$).

Synthesis of (*S*)-*N*-(*tert*-butoxycarbonyl)-*N*-(2-mercapto-1-phenylethyl)glycine (42).

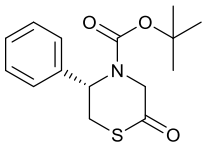


42

To a solution of **41** (1.16 g, 3.16 mmol, 1.0 equiv) in *i*-PrOH (80 mL) was added dropwise a solution of lithium hydroxide (0.53 g, 12.6 mmol, 4.0 equiv) in water (50 mL) at room temperature under an atmosphere of nitrogen. The reaction mixture was then stirred at room temperature overnight. Water (50 mL) was then added and the pH of the mixture was adjusted to pH 5 with 1 M HCl. The aqueous mixture was extracted with ethyl acetate (3 x 100 mL) and the combined organic extracts were dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by column chromatography, eluting with hexane - ethyl acetate - formic acid (4 : 1 : 0.01) afforded **42** (0.68 g, 69 %) as a yellow oil.

IR (ATR): ν_{max} 3494, 3034, 2977, 1732, 1686 cm^{-1}. ^1H NMR (400 MHz, DMSO d_6) δ 7.35 - 7.27 (5H, m, Ph), 5.28 (1H, t, J = 8.0 Hz, PhCH), 3.63 (1H, d, J = 17.5 Hz, CH_2N), 3.55 (1H, d, J = 17.5 Hz, CH_2N), 3.09 (2H, bm, $\underline{CH_2}SH$), 1.37 (9H, bs, *t*-butyl). ^{13}C NMR (500 MHz, $CDCl_3$): 173.4, 154.6, 137.7, 129.0, 128.4, 127.7, 81.3, 61.1, 45.3, 29.8, 28.4. HRMS calcd for $C_{15}H_{22}NO_4S$: 312.1270 and $C_{15}H_{21}NO_4SNa$: 334.1089, found 312.1264 (MH^+) and 334.1084 (MNa^+).

Synthesis of *tert*-butyl (*S*)-2-oxo-5-phenylthiomorpholine-4-carboxylate (43).

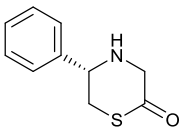


43

DCC (0.45 g, 2.19 mmol, 1.1 equiv) was added to a solution of **42** (0.62 g, 1.99 mmol, 1.0 equiv) in dichloromethane (50 mL) and the reaction mixture was stirred at 0 ^{o}C for 15 minutes. DMAP (0.27 g, 2.19 mmol, 1.1 equiv) was then added and the mixture was left overnight at room temperature. Dicyclohexylurea, formed as a white precipitate, was removed by filtration, the filtrate was concentrated under reduced pressure and the residue purified over silica, eluting with hexane – ethyl acetate (4 : 1) giving **43** (0.32 g, 55 %) as pale yellow oil.

IR (ATR): ν_{max} 3029, 2980, 1697, 1391, 1156 cm^{-1}. ^{1}H NMR (500 MHz, DMSO d_6, 85 ^{o}C) δ 7.35 (5H, m, Ph), 5.14 (1H, app t, J = 7.0 Hz, PhC<u>H</u>), 4.52 (1H, d, J = 16.0 Hz, CH_2N), 4.33 (1H, d, J = 16.0 Hz, CH_2N), 3.57 (2H, br d, J = 7.0 Hz, CH_2S), 1.26 (9H, s, *t*-butyl). ^{13}C NMR (500 MHz, DMSO d_6): 200.2, 154.7, 142.8, 129.0, 128.1, 127.8, 82.7, 58.1, 51.6, 28.2. HRMS calcd for $C_{15}H_{19}NO_3SNa$: 316.0983, found 316.0978 (MNa^+). $[\alpha]_D^{20}$ -38.2 (*c* 0.3, $CHCl_3$).

Synthesis of (*S*)-5-phenylthiomorpholin-2-one (32)



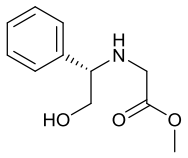
32

To a solution of **43** (0.3 g, 1.02 mmol, 1.0 equiv) in dichloromethane (5 mL) was added TFA (5 mL) at 0 ^{o}C and the mixture stirred at room temperature for 2 hours. The reaction was monitored by TLC until the starting material was consumed. The resulting

mixture was evaporated *in vacuo*, redissolved in dichloromethane (5 mL), washed with $NaHCO_3$ (2 mL) and brine (2 mL), dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to give **32** as a yellow oil (0.16 g, 81 %).

IR (ATR): ν_{max} 3320, 2931, 1664 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 7.43 – 7.20 (5H, m, Ph), 4.24 (1H, dd, *J* = 11.0 Hz, *J*' = 3.5 Hz, PhCH), 3.86 (1H, d, *J* = 17.5 Hz, CH_2CO), 3.81 (1H, d, *J* = 17.5 Hz, CH_2CO), 3.48 (1H, t, *J* = 12.0 Hz, CH_2S), 3.19 (1H, dd, *J* = 12.0 Hz, *J*' = 3.5 Hz, CH_2S), 2.16 (1H, bs, NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 198.2, 141.7, 129.0, 128.3, 126.3, 58.5, 58.3, 36.8. HRMS calculated for $C_{10}H_{12}NOS$: 194.0640, found 194.0634 (MH^+). $[\alpha]_D^{20}$ -119 (*c* 0.5, $CHCl_3$).

Synthesis of methyl (*S*) (2-hydroxy-1-phenylethyl)glycinate (44)[2]

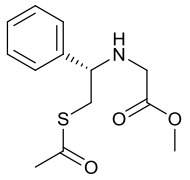


44

To a solution of (*S*)-2-phenylglycinol (5 g, 36.4 mmol, 1.0 equiv) in dry tetrahydrofuran (100 mL) was added triethylamine (6.1 mL, 43.7 mmol, 1.2 equiv) at 0 ^oC. A solution of methyl bromoacetate (6.1 g, 40.0 mmol, 1.1 equiv) in dry tetrahydrofuran (40 mL) was added dropwise and the reaction mixture was left to warm to room temperature over 24 h. The mixture was then diluted with saturated NH_4Cl solution (30 mL), extracted with diethyl ether (3 x 100 mL), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo* to give the crude product. The crude product was purified by column chromatography over silica eluting with hexane – ethyl acetate (1 : 1) to give **44** as a pale yellow oil (5.8 g, 76 %).

IR (ATR): ν_{max} 3384, 2954, 1718 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 – 7.29 (5H, m, Ph), 3.80 (1H, dd, *J* = 8.5 Hz, *J'* = 4.5 Hz, PhCH), 3.74 (1H, dd, *J* = 11.0 Hz, *J'* = 4.5 Hz, CH_2OH), 3.70 (3H, s, OCH_3), 3.61 (1H, dd, *J* = 11.0 Hz, *J'* = 8.5 Hz, CH_2OH), 3.40 (1H, d, *J* = 17.5 Hz, $HNCH_2$), 3.30 (1H, d, *J* = 17.5 Hz, $HNCH_2$), 1.92 (1H, bs, NH). ^{13}C NMR (100 MHz, $CDCl_3$): 172.7, 139.5, 128.8, 128.0, 127.4, 66.7, 64.2, 52.0, 48.2. HRMS calcd for $C_{11}H_{16}NO_3$: 210.1130, found 210.1125 (MH^+).

Synthesis of methyl (*S*)-(2-(acetylthio)-1-phenylethyl)glycinate (45)

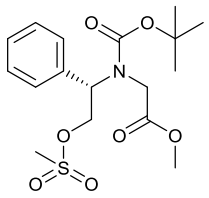


45

To a solution of triphenylphosphine (14.2 g, 54.1 mmol, 2.0 equiv) in dry tetrahydrofuran (250 mL) was added DIAD (10.9 g, 54.1 mmol, 2.0 equiv) under an atmosphere of nitrogen at 0 ^{o}C over 30 minutes. A milky white mixture resulted. A solution of **44** (5.65 g, 27.0 mmol, 1.0 equiv) and thiolacetic acid (4.11 g, 54.1 mmol, 2.0 equiv) in dry tetrahydrofuran (50 mL) was then added dropwise, stirring was continued for 1 hour at 0 ^{o}C and then the mixture was left overnight at room temperature. A clear yellow solution was obtained. Diethyl ether (50 mL) was then added and the mixture was washed with water (100 mL), dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to give the crude product. Purification of the crude product over silica, eluting with hexane – ethyl acetate (7 : 1) afforded **45** (0.3 g, 4 %) as a yellow oil.

IR (ATR): ν_{max} 3474, 3061, 3033, 2970, 1741, 1641, 1431, 1402, 1203 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 - 7.30 (5H, m, Ph), 5.18 (1H, t, *J* = 7.0 Hz, PhCH), 3.91 (1H, d, *J* = 17.0 Hz, $HNCH_2$), 3.67 (3H, s, OCH_3), 3.37 (1H, d, *J* = 17.0 Hz, $HNCH_2$), 3.10 (2H, m, CH_2S), 2.48 (3H, s, CH_3COS). ^{13}C NMR (400 MHz, $CDCl_3$): 194.2, 170.1, 137.5, 129.1, 128.8, 127.2, 64.3, 52.2, 46.2, 44.1, 26.4. HRMS calcd for $C_{13}H_{18}NO_3S$: 268.1007 and $C_{13}H_{17}NO_3SNa$: 290.0827, found 268.1002 (MH^+) and 290.0821 (MNa^+).

Synthesis of methyl (*S*)-*N*-(*tert*-butoxycarbonyl)-*N*-(2-((methylsulfonyl)oxy)-1-phenylethyl)glycinate (46)



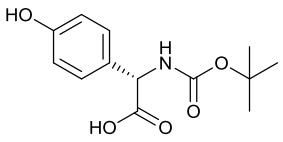
46

To a solution of **40** (1.0 g, 3.24 mmol, 1.0 equiv) and *N*,*N*-diisopropylethylamine (0.84 g, 6.48 mmol, 2.0 equiv) in dichloromethane (15 mL) was added dropwise methanesulfonyl chloride (0.45 g, 3.89 mmol, 1.2 equiv) at 0 ^{o}C. The reaction mixture was stirred for 1 hour at 0 ^{o}C and stirring then continued for 2 hours at room temperature. The resulting mixture was diluted with dichloromethane (15 mL), washed sequentially with saturated NH_4Cl (15 mL) and brine (15 mL), dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to give **46** (0.42 g, 34 %) as a yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 - 7.32 (5H, m, Ph), 5.06 (1H, t, *J* = 8.5 Hz, PhCH), 4.72 (1H, t, *J* = 8.5 Hz, OCH_2), 4.30 (1H, d, *J* = 18.0 Hz, NCH_2), 4.15 (1H, t, *J* = 8.0 Hz, OCH_2), 3.72 (3H, s, OMe), 3.68 (3H, s, CH_3), 3.39 (1H, d, *J* =

18.0 Hz, NCH_2), 1.59 (9H, s, *t*Bu). ^{13}C NMR (400 MHz, $CDCl_3$): 167.6, 154.4, 139.3, 129.5, 128.2, 127.2, 79.5, 70.3, 68.2, 59.9, 50.7, 43.0, 28.1. HRMS calcd for $C_{17}H_{25}NO_7SNa$: 410.1249, found 410.1244 (MNa^+).

Synthesis of (*S*)-*N*-(*tert*-Butyloxycarbonyl)-4-hydroxyphenyl-glycine (48)[3]

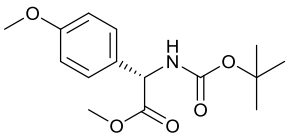


48

A solution of di-*tert*-butyl dicarbonate (12.9 g, 59.2 mmol, 1.1 equiv) in dioxane (54 mL) was added to a solution of *S*-(4-hydroxyphenyl)-glycine (9.0 g, 53.8 mmol, 1.0 equiv) and NaOH (2.15 g, 53.8 mmol, 1.0 equiv) in H_2O - dioxane 2 : 1 (162 mL). The reaction mixture was stirred for 4 hours at room temperature, the dioxane was then evaporated and the aqueous phase was acidified to pH 3 with $KHSO_4$. This was extracted with ethyl acetate (3 x 150 mL), the combined organic phases dried over anhydrous $MgSO_4$, filtered and concentrated to give product **48** as white solid (12.3 g, 86 %). mp 199 - 201 ^oC.

IR (ATR): ν_{max} 3340, 3010, 2981, 1681, 1612 cm^{-1}. ^1H NMR (400 MHz, DMSO d_6) δ 9.48 (1H, bs, OH), 7.39 (1H, *J* = 8.0 Hz, NHBoc), 7.18 (2H, d, *J* = 8.5 Hz, Ph), 6.72 (2H, d, *J* = 8.5 Hz, Ph), 4.96 (1H, d, *J* = 8.0 Hz, PhCH), 1.38 (9H, s, *t*Bu). ^{13}C NMR (100 MHz, DMSO d_6): δ 172.7, 157.0, 155.1, 128.9, 127.5, 115.0, 78.2, 58.4, 28.2. HRMS calcd for $C_{13}H_{17}NO_5Na$: 290.1004, found 290.0999 (MNa^+).

Synthesis of (*S*)-*N*-(*tert*-Butyloxycarbonyl)-4-methoxyphenylglycine methyl ester (49)[3]

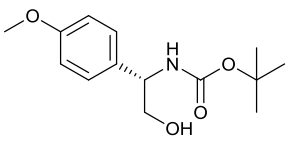


49

K_2CO_3 (24.9 g, 180 mmol, 4.0 equiv) and MeI (19.2 g, 135 mmol, 3.0 equiv) were added to a solution of **48** (12.0 g, 45.0 mmol, 1.0 equiv) in acetone (200 mL). The reaction mixture was heated at reflux for 16 hours, cooled and then filtered through a short pad of Celite®. The solution was washed with brine (100 mL), the organic phase dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure to give the crude product that was purified by column chromatography over silica, eluting with petroleum ether – diethyl ether (2 : 1), to afford **49** as a white solid (5.50 g, 41 %). mp 73 - 75 ^oC.

IR (ATR): ν_{max} 3377, 3004, 2976, 1744, 1707 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 7.27 (2H, d, *J* = 9.0 Hz, Ph), 6.87 (2H, d, *J* = 9.0 Hz, Ph), 5.49 (1H, bd, *J* = 6.0 Hz, NH), 5.25 (1H, d, *J* = 7.0 Hz, PhC<u>H</u>), 3.79 (3H, s, OCH_3), 3.71 (3H, s, OCH_3), 1.43 (9H, s, *t*Bu). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.9, 159.7, 154.8, 129.0, 128.4, 114.3, 80.1, 57.0, 55.3, 52.6, 28.3. HRMS calcd for $C_{15}H_{22}NO_5$: 296.1498 and $C_{15}H_{21}NO_5Na$: 318.1317, found 296.1492 (MH^+) and 318.1312 (MNa^+).

Synthesis of *tert*-butyl (*S*)-(2-hydroxy-1-(4-methoxyphenyl)ethyl)carbamate (50)[4]



50

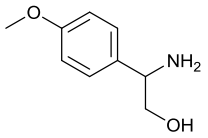
Method 1: To a suspension of lithium aluminium hydride (1.68 g, 44.1 mmol, 4.0 equiv) in tetrahydrofuran (150 mL) was added a solution of **49** (3.25 g, 11.0 mmol, 1.0 equiv) in tetrahydrofuran (50 mL). The mixture was stirred for 30 minutes. Ethyl acetate (60 mL) and 10 % aqueous KOH (w/w) (50 mL) were slowly added to the reaction and the mixture was left for 1 hour. The organic solvent was dried, redissolved in ethyl acetate (100 mL) and separated from aqueous layer. The resulting mixture was then dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure to give **50** as white solid (2.54 g, 65 %).

Method 2: LiCl (2.05 g, 48.5 mmol, 2.6 equiv) was added to a suspension of $NaBH_4$ (1.83 g, 48.5 mmol, 2.6 equiv) in ethanol (69 mL) at 0 ^oC under an atmosphere of nitrogen and the mixture stirred for 10 minutes. A solution of **49** (5.50 g, 18.6 mmol, 1.0 equiv) in tetrahydrofuran (69 mL) was subsequently added and the reaction left for 3 hours at room temperature. The mixture was quenched with saturated NH_4Cl solution (30 mL), water (100 mL) and extracted with ethyl acetate (3 x 150 mL). The organic extracts were combined and washed with brine (100 mL), dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product. Purification by column chromatography on silica gel, eluting with hexane – ethyl acetate (4 : 1), afforded **50** as a white solid (4.01 g, 80.5 %). mp 140 – 142 ^oC.

IR (ATR): ν_{max} 3402, 3377, 3007, 2987, 1681 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 7.22 (2H, d, *J* = 8.5 Hz, Ph), 6.89 (2H, d, *J* = 8.5 Hz, Ph), 5.12 (1H, bs, NH), 4.73 (1H, bs, PhC<u>H</u>), 3.84-3.81 (2H, m, C<u>H</u>$_2$OH), 3.80 (3H, s, OCH_3), 2.34 (1H, bs, OH), 1.43 (9H, s,

*t*Bu). ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.1, 156.2, 131.5, 127.7, 114.2, 80.0, 67.0, 56.4, 55.3, 28.4. HRMS calcd for $C_{14}H_{22}NO_4$: 268.1549 and $C_{14}H_{21}NO_4Na$: 290.1368, found 268.1542 (MH^+) and 290.1363 (MNa^+).

Synthesis of (*S*)-2-amino-2-(4-methoxyphenyl)ethan-1-ol (47)[4]

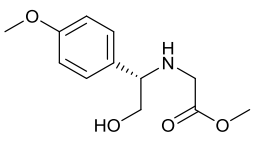


47

To a solution of **50** (0.55 g, 2.08 mmol, 1.0 equiv) in dichloromethane (6 mL) was added TFA (6 mL) at 0 ^oC and the mixture allowed to stir at room temperature until the starting material was consumed, monitoring by TLC. The resulting mixture was evaporated *in vacuo*, redissolved in dichloromethane (15 mL), washed with saturated $NaHCO_3$ (10 mL) and brine (10 mL), dried over anhydrous $MgSO_4$, filtered and concentrated to give **47** as a yellow oil (295 mg, 85 %).

IR (ATR): ν_{max} 3295, 3083, 2938, 1705 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24 (2H, d, *J* = 8.5 Hz, Ph), 6.89 (2H, d, *J* = 8.5 Hz, Ph), 4.06 (1H, bs, PhCH), 3.80 (3H, s, OCH_3), 3.74 (1H, bs, C$\underline{H_2}$OH), 3.60 (1H, t, *J* = 9.5 Hz, CH_2OH), 1.85 (3H, bs, NH_2 and OH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.4, 131.1, 128.8, 114.0, 63.6, 55.8, 55.1. HRMS calcd for $C_9H_{14}NO_2$: 168.1024, found 168.1019 (MH^+).

Synthesis of methyl (*S*)-(2-hydroxy-1-(4-methoxyphenyl)ethyl)glycinate (51)

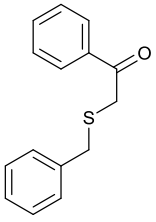


51

Triethylamine (0.28 mL, 1.99 mmol, 1.2 equiv) was added to a solution of **47** (0.28 g, 1.66 mmol, 1.0 equiv) in dry tetrahydrofuran (7 mL) at 0 ^{o}C. A solution of methyl bromoacetate (0.28 g, 1.82 mmol, 1.1 equiv) in tetrahydrofuran (2 mL) was then added dropwise and the reaction mixture was allowed to warm to room temperature over 24 hours. The resulting mixture was diluted with saturated NH_4Cl solution (2 mL), extracted with diethyl ether (3 x 10 mL), dried over anhydrous $MgSO_4$, filtered and concentrated to give the crude product as a yellow oil. This was purified by column chromatography over silica, eluting with hexane – ethyl acetate (1 : 1), followed by ethyl acetate to afford **51** as a yellow oil (60.3 mg, 15 %).

IR (ATR): ν_{max} 3324, 2953, 1739 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.16 (2H, d, *J* = 7.5 Hz, Ph), 6.81 (2H, d, *J* = 7.5 Hz, Ph), 3.73 (3H, s, OMePh), 3.68 (1H, dd, *J* = 8.5 Hz, *J'* = 4.0 Hz, PhCH), 3.65 – 3.62 (1H, bm, CH_2OH), 3.62 (3H, s, OCH_3), 3.52 (1H, t, *J* = 9.0 Hz, CH_2OH), 3.31 (1H, d, *J* = 17.5 Hz, HNCH_2), 3.21 (1H, d, *J* = 17.5 Hz, HNCH_2), 2.20 (1H, bs, NH). ^{13}C NMR (100 MHz, $CDCl_3$): 173.1, 159.3, 131.6, 128.5, 114.1, 67.0, 63.5, 55.3, 51.8, 48.3. HRMS calcd for $C_{12}H_{18}NO_4$: 240.1236 and $C_{12}H_{17}NO_4Na$: 262.1055, found 240.1230 (MH^+) and 262.1050 (MNa^+).

Synthesis of 2-(benzylthio)-1-phenylethan-1-one (56)[5]



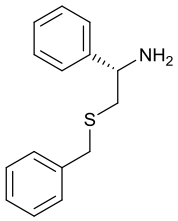
56

To a solution of 2-bromoacetophenone (5.0 g, 25.1 mmol, 1.0 equiv) in acetone (60 mL) cooled in an ice-salt bath was added triethylamine (3.85 mL, 27.6 mmol, 1.1

equiv). Benzyl thiol (2.98 mL, 25.4 mmol, 1.01 equiv) was then added dropwise and a white precipitate was immediately formed. The reaction mixture was left for 1 hour and concentrated *in vacuo* to give the crude product. Purification of the crude product over a short silica column, eluting with hexane – ethyl acetate (4 : 1), afforded **56** as a white solid (5.63 g, 93 %). mp 87 - 89 ^{o}C. (Lit[6] 86 - 88 ^{o}C)

IR (ATR): ν_{max} 3060, 3028, 1670 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 (2H, d, *J* = 7.0 Hz, Ph), 7.58 (1H, t, *J* = 7.5 Hz, Ph), 7.46 (2H, t, *J* = 7.5 Hz, Ph), 7.37 – 7.25 (5H, m, Ph), 3.76 (2H, s, CH_2CO), 3.68 (2H, s, SCH_2Ph). ^{13}C NMR (100 MHz, $CDCl_3$): 192.1, 138.6, 134.4, 132.5, 128.7, 128.5, 128.2, 43.2. 37.6. HRMS calcd for $C_{15}H_{15}OS$: 243.0844, found 243.0838 (MH^+).

Synthesis of (*S*)-2-(benzylthio)-1-phenylethan-1-amine (59)[7]

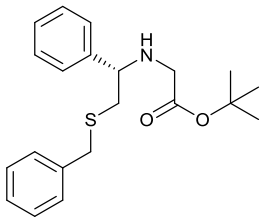


59

To a solution of **56** (1.0 g, 4.13 mmol, 1.0 equiv) in methanol (35 mL) was added ammonium acetate (3.18 g, 41.3 mmol, 10.0 equiv) and sodium cyanoborohydride (0.18 g, 2.89 mmol, 0.7 equiv) and the reaction mixture heated to reflux for 24 hours. The mixture was allowed to cool and washed with 5 % HCl (20 mL), 5 M NaOH (20 mL), extracted with ethyl acetate (3 x 50 mL), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo* to give the crude product. Purification of the crude product over silica, eluting with ethyl acetate – methanol (4 : 1), afforded **59** as yellow oil (0.73 g, 73 %).

IR (ATR): ν_{max} 3314, 3064, 3027, 2922, 1559 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 – 7.21 (10H, m, Ph), 4.90 (2H, bs, NH_2), 4.08 (1H, dd, *J* = 8.0 Hz, *J*' = 6.0 Hz, PhCH), 3.66 (1H, d, *J* = 13.5 Hz, SCH_2Ph), 3.61 (1H, d, *J* = 13.5 Hz, SCH_2Ph), 2.86 – 2.76 (2H, m, CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): 142.7, 138.1, 129.5, 128.9, 128.3, 128.1, 127.7, 127.4, 56.2, 37.2, 36.5. HRMS calcd for $C_{15}H_{18}NS$: 244.1160, found 244.1154 (MH^+).

Synthesis of *tert*-butyl (*S*)-(2-(benzylthio)-1-phenylethyl)glycinate (60)



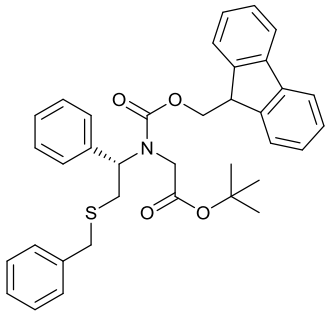
60

To a solution of **59** (1.5 g, 5.49 mmol, 1.0 equiv) in dry tetrahydrofuran (28 mL) was added triethylamine (1.92 mL, 13.7 mmol, 2.5 equiv). The mixture was cooled to 0 ^{o}C and *tert*-butyl bromoacetate (2.68 g, 13.7 mmol, 2.5 equiv) was then added dropwise. The reaction mixture allowed to warm to room temperature and the stirring was continued for 24 hours. The resulting mixture was quenched with brine (15 mL), extracted with ethyl acetate (3 x 50 mL), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo* to give the crude product. Purification of the crude product over silica, eluting with hexane – ethyl acetate (4 : 1), afforded **60** as a pale yellow oil (0. 617 g, 28 %).

IR (ATR): ν_{max} 3314, 3061, 3027, 2979, 2930, 1732, 1456, 1368, 1146 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 – 7.21 (10H, m, Ph), 3.75 (1H, d, *J* = 13.5 Hz, SCH_2Ph), 3.70 (1H, d, *J* = 13.0 Hz, SCH_2Ph), 3.72 (1H, m, PhCH), 3.19 (1H, d, *J* = 17.5 Hz, $HNCH_2$), 2.99 (1H, d, *J* = 17.5 Hz, $HNCH_2$), 2.69 (1H, dd, *J* = 13.5 Hz, *J*'= 4.5 Hz, CH_2CHPh),

2.59 (1H, dd, *J* = 13.5 Hz, *J*' = 9.5 Hz, CH_2CHPh), 2.15 (1H, s, NH), 1.45 (9H, s, *t*Bu). ^{13}C NMR (100 MHz, $CDCl_3$): 171.6, 138.2, 128.9, 128.6, 128.5, 127.7, 127.3, 127.0, 81.1, 61.2, 49.3, 39.4, 36.0, 28.1. HRMS calcd for $C_{21}H_{28}NO_2S$: 358.1841 and $C_{21}H_{27}NO_2SNa$: 380.1660, found 358.1835 (MH^+) and 380.1655 (MNa^+).

Synthesis of *tert*-butyl (*S*)-*N*-(((9H-fluoren-9-yl)methoxy)carbonyl)-N-(2-(benzylthio)-1-phenylethyl)glycinate (61)



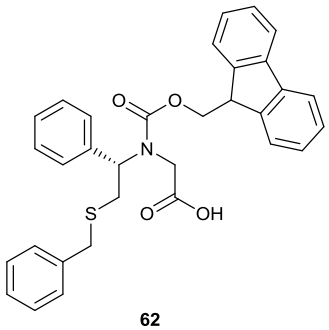
61

To a solution of **60** (0.36 g, 1.01 mmol, 1.0 equiv) in dichloromethane (50 mL) was added K_2CO_3 (0.70 g, 5.06 mmol, 5.0 equiv) and the mixture was stirred for 10 minutes. Fmoc-Cl (0.31 g, 1.21 mmol, 1.2 equiv) was then added and the stirring was continued overnight. The mixture was quenched with water (50 mL), extracted with dichloromethane (3 x 50 mL), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo* to give the crude product as pale yellow oil. Purification of the crude product over silica, eluting with hexane – ethyl acetate (7 : 1), afforded **61** as a pale yellow oil (0.41 g, 70 %).

IR (ATR): ν_{max} 3061, 2976, 1744, 1698, 1449, 1217, 1146 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 7.75 (4H, d, *J* = 8.0 Hz, Ph), 7.60 (4H, d, *J* = 8.0 Hz, Ph), 7.45 – 7.13 (28H, m, Ph), 5.60 (1H, t, *J* = 7.5 Hz, CH), 5.05 (1H, t, *J* = 7.5 Hz, CH), 4.80 (1H, dd, *J* = 11.0 Hz, *J*' = 5.5 Hz, PhCH), 4.60 (1H, dd, *J* = 11.0 Hz, *J* = 5.5 Hz, SCH_2), 4.53 – 4.48

(1H, m, PhCH), 4.45 – 4.39 (1H, m, SCH_2), 4.28 – 4.22 (2H, bm, SCH_2), 3.78 (2H, s, SCH_2Ph), 3.68 – 3.54 (4H, m, NCH_2), 3.38 (1H, s, SCH_2Ph), 2.99 (2H, d, *J* = 7.5 Hz, OCH_2), 2.78 (2H, d, *J* = 7.5 Hz, OCH_2), 1.26 (9H, bs, *t*Bu). ^{13}C NMR (100 MHz, $CDCl_3$): 168.4, 168.3, 156.4, 155.9, 144.1, 144.0, 141.6, 141.4, 138.2, 137.5, 129.1, 129.0, 128.5, 128.2, 128.0, 127.8, 127.3, 127.2, 127.1, 125.3, 124.9, 124.8, 120.2, 120.1, 81.6, 81.4, 68.0, 67.2, 58.1, 47.4, 45.9, 45.7, 36.5, 36.2, 32.5, 32.3, 27.9. HRMS calcd for $C_{36}H_{38}NO_4S$: 580.2522 and $C_{36}H_{37}NO_4SNa$: 602.2341, found 580.2516 (MH^+) and 602.2336 (MNa^+).

Synthesis of (*S*)-*N*-(((9*H*-fluoren-9-yl)methoxy)carbonyl)-*N*-(2-(benzylthio)-1-phenylethyl)glycine (62)



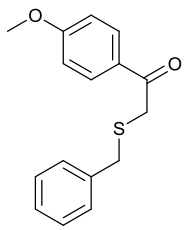
62

A solution of **61** (0.2 g, 0.34 mmol, 1.0 equiv) in dichloromethane (2 mL) was cooled to 0 ^oC, TFA (2 mL) was then added the mixture stirred for 30 minutes at 0 ^oC and then allowed to stir for 1 hour at room temperature. The resulting mixture was evaporated *in vacuo* and redissolved in dichloromethane (5 mL). The solution was then washed with saturated $NaHCO_3$ (2 mL) and brine (2 mL), dried over anhydrous $MgSO_4$, filtered and concentrated to afford **62** as a yellow oil (0.13 g, 70 %).

IR (ATR): ν_{max} 3675, 3078, 2971, 2901, 1785, 1704, 1453, 1222, 1066 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 7.75 (2H, d, *J* = 7.5 Hz, Ph), 7.59 (2H, d, *J* = 7.5 Hz, Ph), 7.41 – 7.08 (14H, m, Ph), 4.44 (2H, d, *J* = 6.5 Hz, OCH_2), 4.34 (1H, dd, *J* = 8.0 Hz, *J*' = 5.0

Hz, PhCH), 4.24 (1H, t, *J* = 6.5 Hz, CH), 3.87 (1H, d, *J* = 15.5 Hz, NCH_2), 3.82 (1H, d, *J* = 15.5 Hz, NCH_2), 3.72 (2H, s, SCH_2Ph), 3.19 (1H, dd, *J* = 13.0 Hz, *J*' = 5.0 Hz, SCH_2), 3.00 (1H, dd, *J* = 13.0 Hz, *J*' = 8.0 Hz, SCH_2). ^{13}C NMR (100 MHz, $CDCl_3$): 170.6, 156.8, 144.6, 143.4, 137.5, 136.9, 130.6, 129.3, 129.1, 128.9, 128.7, 128.4, 127.4, 126.9, 126.6, 120.1, 60.9, 58.1, 45.8, 36.6, 34.4, 30.4. HRMS calcd for $C_{32}H_{29}NO_4SNa$: 546.1715, found 546.1710 (MNa^+).

Synthesis of 2-(benzylthio)-1-(4-methoxyphenyl)ethan-1-one (64)[5]

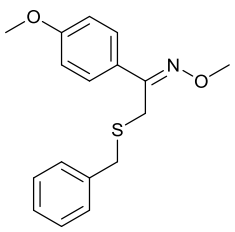


64

4-Methoxybromoacetophenone (8.4 g, 36.7 mmol, 1.0 equiv) was dissolved in acetone (90 mL) and the solution cooled in an ice-salt bath. Triethylamine (5.6 mL, 40.3 mmol, 1.1 equiv) was added, followed by dropwise addition of benzyl mercaptan (4.4 mL, 37.0 mmol, 1.01 equiv). The mixture was stirred for an hour and concentrated *in vacuo* to give the crude product. Purification of the crude product over a short silica column, eluting with hexane – ethyl acetate (4 : 1), afforded **64** as a white solid (7.7 g, 77 %). mp 65 – 67 ^oC.

IR (ATR): ν_{max} 3058, 2930, 2839, 1658 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 7.91 (2H, d, *J* = 9.0 Hz, Ph), 7.36 – 7.23 (5H, m, Ph), 6.93 (2H, d, *J* = 9.0 Hz, Ph), 3.87 (3H, s, OMe), 3.76 (2H, s, CH_2CO), 3.63 (2H, s, SCH_2Ph). ^{13}C NMR (100 MHz, $CDCl_3$): 193.3, 163.7, 137.4, 131.1, 129.3, 128.4, 127.2, 113.8, 55.5, 36.2, 35.7 HRMS calcd for $C_{16}H_{17}O_2S$: 273.0949 and $C_{16}H_{16}O_2SNa$: 295.0769, found 273.0944 (MH^+) and 295.0763 [MNa^+].

Synthesis of (*Z*)-2-(benzylthio)-1-(4-methoxyphenyl)ethan-1-one *O*-methyl oxime (67)[8]

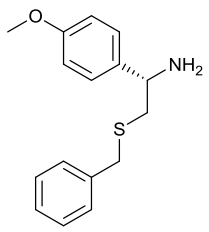


67

To a solution of **64** (6.6 g, 24.4 mmol, 1.0 equiv) in absolute ethanol (200 mL) was added pyridine (29.7 mL, 366.6 mmol, 15 equiv) and *O*-methyl hydroxylamine hydrochloride (6.12 g, 73.3 mmol, 3.0 equiv) and the mixture was heated to reflux for 2 hours. After cooling to room temperature, the resulting mixture was concentrated *in vacuo*, dissolved in dichloromethane (100 mL), washed sequentially with 5 % HCl (70 mL), brine (70 mL) and dried over anhydrous $MgSO_4$. After filtration and removal of solvent *in vacuo*, purification of the crude product over silica, eluting with petrol ether – diethyl ether (2 : 1), afforded **67** as a colourless oil (5.12 g, 70 %) as a mixture of *syn*-/*ant-i* (8/92) isomers.

IR (ATR): ν_{max} 3030, 2936, 2836, 1607, 1513, 1249 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 7.54 (2H, d, *J* = 9.0 Hz, Ph), 7.32 – 7.22 (5H, m, Ph), 6.87 (2H, d, *J* = 9.0 Hz, Ph), 3.97 (3H, s, OMe), 3.82 (3H, s, OMe), 3.74 (2H, s, $CH_2C{=}N$), 3.70 (2H, s, SCH_2Ph). ^{13}C NMR (100 MHz, $CDCl_3$): 160.6, 154.1, 138.1, 130.1, 129.0, 128.4, 127.8, 127.1, 113.9, 62.2, 55.3, 37.0, 24.9. HRMS calcd for $C_{17}H_{20}NO_2S$: 302.1215, found 302.1209 (MH^+).

Synthesis of (*S*)-2-(benzylthio)-1-(4-methoxyphenyl)ethan-1-amine (65)[8]

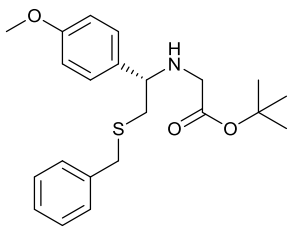


65

To a solution of **67** (3.40 g, 11.3 mmol, 1.0 equiv) in tetrahydrofuran (100 mL) was added 1 M BH_3-THF (56.5 mL, 56.5 mmol, 5.0 equiv) at 0 ^oC. The mixture was then heated to reflux for 3.5 hours. After cooling to room temperature, water (70 mL) and 5 M NaOH (70 mL) were added at 0 ^oC and the mixture was then stirred for 30 minutes at room temperature. The resulting mixture was acidified with 10 M HCl and the aqueous layer was extracted with ether (3 x 150 mL), basified with 28 % NH_4OH (100 mL) and extracted with dichloromethane (3 x 100 mL). The organic extracts were combined, dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo* to give the crude product that was purified over silica, eluting with ethyl acetate – methanol (4 : 1), to afford **65** as a yellow oil (2.68 g, 87 %).

IR (ATR): ν_{max} 3209, 3030, 2962, 2833, 1613, 1513 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 7.29 – 7.19 (7H, m, Ph), 6.82 (2H, d, *J* = 9.0 Hz, Ph), 4.10 (1H, t, *J* = 7.0 Hz, PhCH), 3.74 (3H, s, OMe), 3.60 (1H, d, *J* = 13.5 Hz, SCH_2Ph), 3.56 (1H, d, *J* = 13.0 Hz, SCH_2Ph), 2.94 (1H, dd, *J* = 14.0 Hz, *J'* = 7.0 Hz, CH2S), 2.82 (1H, dd, *J* = 14.0 Hz, *J'* = 7.0 Hz, CH_2S). ^{13}C NMR (100 MHz, $CDCl_3$): 159.7, 137.9, 130.2, 129.1, 128.6, 128.5, 127.2, 114.2, 55.2, 54.8, 37.5, 36.6. HRMS calcd for $C_{16}H_{20}NOS$: 274.1265 and $C_{16}H_{19}NOSNa$: 296.1085, found 274.1260 (MH^+) and 296.1079 (MNa^+).

Synthesis of *tert*-butyl (*S*)-(2-(benzylthio)-1-(4-methoxyphenyl)ethyl)glycinate (68)

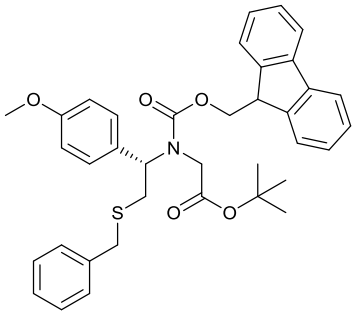


68

Triethylamine (0.96 mL, 6.85 mmol, 2.5 equiv) was added to a solution of **65** (0.75 g, 2.74 mmol, 1.0 equiv) in dry tetrahydrofuran (22 mL). The mixture was cooled to 0 ^{o}C and *tert*-butyl bromoacetate (1.02 mL, 6.85 mmol, 2.5 equiv) was added dropwise. The reaction mixture was then allowed to warm to room temperature and left for overnight. The resulting mixture was subsequently added to brine (50 mL), extracted with ethyl acetate (3 x 80 mL), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo* to give the crude product. Purification of the crude product over silica, eluting with petrol ether – diethyl ether (2 : 1), afforded **68** as a yellow oil (0.69 g, 65 %).

IR (ATR): ν_{max} 3312, 3003, 2979, 1732, 1515, 1244, 1147 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 – 7.22 (5H, m, Ph), 7.17 (2H, d, *J* = 8.5 Hz, Ph), 6.84 (2H, d, *J* = 8.5, Ph), 3.79 (3H, s, OMe), 3.75 (1H, d, *J* = 13.5 Hz, SCH_2Ph), 3.70 (1H, d, *J* = 13.5 Hz, SCH_2Ph), 3.66 (1H, dd, *J* = 9.0 Hz, *J*' = 5.0 Hz, PhCH), 3.17 (1H, d, *J* = 17.5 Hz, CH_2), 2.98 (1H, d, *J* = 17.5 Hz, CH_2), 2.66 (1H, dd, *J* = 13.5 Hz, *J'*= 5.0 Hz, CH_2S), 2.58 (1H, dd, *J* = 14.0 Hz, *J'* = 9.0 Hz, CH_2S), 1.55 (1H, bs, NH), 1.45 (9H, s, *t*Bu). ^{13}C NMR (100 MHz, $CDCl_3$): 171.7, 159.1, 138.3, 134.1, 129.0, 128.5, 127.0, 114.0, 81.0, 60.0, 55.3, 49.3, 39.5, 36.0, 28.1. HRMS calcd for $C_{22}H_{30}NO_3S$: 388.1946 and $C_{22}H_{29}NO_3SNa$: 410.1765, found 388.1941 (MH^+) and 410.1760 (MNa^+).

Synthesis of *tert*-butyl (*S*)-*N*-(((9H-fluoren-9-yl)methoxy)carbonyl)-*N*-(2-(benzylthio)-1-(4-methoxyphenyl)ethyl)glycinate (69)



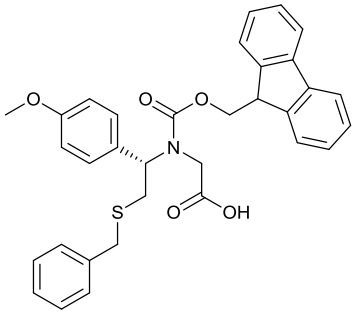
69

To a solution of **68** (0.41 g, 1.06 mmol, 1.0 equiv) in dichloromethane (60 mL) was added K_2CO_3 (0.73 g, 5.31 mmol, 5.0 equiv) and the mixture was allowed to stir for 10 minutes. Fmoc-Cl (0.33 g, 1.27 mmol, 1.2 equiv) was subsequently added and the reaction mixture was left stirring overnight. The resulting mixture was quenched with water (30 mL) and extracted with dichloromethane (60 mL x 3). The combined extracts were dried over anhydrous $MgSO_4$ and concentrated *in vacuo* to give the crude product that was purified by chromatography on silica, eluting with hexane – ethyl acetate (4 : 1), to afford **69** as a yellow oil (0.39 g, 61 %).

IR (ATR): ν_{max} 3001, 2973, 1747, 1698, 1454, 1252, 1155 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 7.80 – 7.72 (4H, m, Ph), 7.64 – 7.59 (4H, m, Ph), 7.46 – 7.12 (18H, m, Ph), 6.84 (4H, d, *J* = 8.50 Hz, Ph), 6.75 (4H, d, *J* = 8.5 Hz, Ph), 5.56 (1H, t, *J* = 8.0 Hz, CH), 5.00 (1H, t, *J* = 8.0 Hz, CH), 4.80 (1H, dd, *J* = 11.0 Hz, *J'* = 5.5 Hz, PhCH), 4.60 (1H, dd, *J* = 11.0 Hz, *J'* = 5.5 Hz, SCH_2), 4.49 (1H, dd, *J* = 10.5 Hz, *J'* = 7.0 Hz, PhCH), 4.40 (1H, dd, *J* = 10.5 Hz, *J'* = 7.0 Hz, SCH_2), 4.28 (1H, t, *J* = 5.5 Hz, SCH_2), 4.23 (1H, t, *J* = 7.0 Hz, SCH_2), 3.79 (3H, s, OCH_3), 3.78 (3H, s, OCH_3), 3.64 (1H, d, *J* = 18.0 Hz, NCH_2), 3.56 (1H, d, *J* = 15.0 Hz, NCH_2), 3.54 (2H, s, SCH_2Ph), 3.37 (2H, s, SCH_2Ph),

2.97 (2H, d, J = 8.0 Hz, OCH_2), 2.75 (2H, d, J = 8.0 Hz, OCH_2), 1.30 (9H, bs, *t*Bu). ^{13}C NMR (100 MHz, $CDCl_3$): 168.5, 159.3, 156.3, 144.0, 141.3, 138.2, 129.8, 129.3, 129.0, 128.5, 127.7, 127.0, 125.2, 120.1, 113.9, 81.5, 67.9, 55.3, 47.4, 45.7, 36.5, 32.6, 27.9. HRMS calcd for $C_{37}H_{39}NO_5SNa$: 632.2447, found 632.2441 (MNa^+).

Synthesis of (*S*)-*N*-(((9*H*-fluoren-9-yl)methoxy)carbonyl)-*N*-(2-(benzylthio)-1-(4-methoxyphenyl)ethyl)glycine (63)



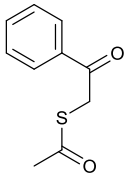
63

To a solution of **69** (0.27 g, 0.44 mmol, 1.0 equiv) in dichloromethane (2 mL) was added TFA (2 mL) at 0 ^oC. The reaction was stirred for 30 minutes at 0 ^oC and the stirring was continued for an hour at room temperature. The mixture was evaporated *in vacuo*, redissolved in dichloromethane (5 mL), washed with saturated $NaHCO_3$ (2 mL) and brine (2 mL), dried over anhydrous $MgSO_4$, filtered and concentrated to give **63** as a yellow oil (0.18 g, 73 %).

IR (ATR): ν_{max} 3420, 3058, 3027, 2954, 2932, 1780, 1723, 1511, 1247, 1171 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 7.73 (2H, d, J = 7.5 Hz, Ph), 7.57 (2H, d, J = 7.0 Hz, Ph), 7.38 – 7.06 (9H, m, Ph), 6.83 (2H, d, J = 8.5 Hz, Ph), 6.68 (2H, dd, J = 11.0 Hz, J'= 8.5 Hz, Ph), 4.38 (2H, d, J = 7.0 Hz, OCH_2), 4.26 (1H, dd, J = 7.5 Hz, J' = 5.0 Hz, PhCH), 4.20 (1H, t, J = 7.0 Hz, CH), 3.80 (2H, s, SCH_2Ph), 3.75 (3H, s, OCH_3), 3.70 (1H, d, J = 17.0 Hz, NCH_2), 3.63 (1H, d, J = 17.0 Hz, NCH_2), 3.14 (1H, dd, J = 13.0 Hz, J' = 5.0 Hz, SCH_2), 2.94 (1H, dd, J = 13.0 Hz, J' = 8.0 Hz, SCH_2). ^{13}C NMR (100 MHz,

$CDCl_3$): 170.0, 160.3, 158.4, 143.9, 141.4, 139.4, 136.7, 130.5, 129.9, 128.7, 127.8, 127.2, 126.2, 125.2, 120.1, 113.9, 67.3, 61.9, 58.4, 55.3, 44.9, 34.5, 30.4. HRMS calcd for $C_{33}H_{31}NO_5SNa$: 576.1821, found 576.1815 (MNa^+).

Synthesis of *S*-(2-oxo-2-phenylethyl)ethanethioate (71)[9]

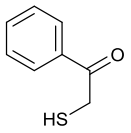


71

To a solution of potassium thioacetate (2.87 g, 25.1 mmol, 1.0 equiv) in tetrahydrofuran (65 mL) was added 2-bromoacetophenone (5 g, 25.1 mmol, 1.0 equiv) and the mixture was heated to 40 ^oC for 24 hours. The resulting mixture was then added to deionized water (50 mL), extracted with ethyl acetate (3 x 65 mL), the combined extracts dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to give **71** as a yellow oil (4.14 g, 85 %).

IR (ATR): ν_{max} 3072, 2989, 1698, 1620, 1542 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 8.00 (2H, d, *J* = 7.0 Hz, Ph), 7.61 (1H, t, *J* = 7.5 Hz, Ph), 7.49 (2H, t, *J* = 7.5 Hz, Ph), 4.41 (2H, s, CH_2S), 2.41 (3H, s, CH_3CO). ^{13}C NMR (100 MHz, $CDCl_3$): 192.4, 190.7, 132.7, 130.4, 127.1, 43.6, 28.5. HRMS calcd for $C_{10}H_{11}O_2S$: 195.0480 and $C_{10}H_{10}O_2SNa$: 217.0299, found 195.0474 (MH^+) and 217.0294 (MNa^+).

Synthesis of 2-mercapto-1-phenylethan-1-one (72)[10]

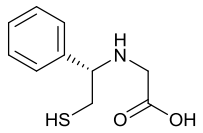


72

A solution of **71** (4.0 g, 20.6 mmol, 1.0 equiv) in 1 M NaOH (20 mL) and MeOH (40 mL) was stirred at room temperature for 24 hours. Deionized water (20 mL) was then added and the mixture was neutralized with 1 M HCl. The neutral mixture was extracted with ethyl acetate (60 mL x 3), the combined extracts dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to give the crude product that was purified over silica, eluting with petroleum ether – ethyl acetate (9 : 1), to afford **72** as yellow oil (1.72 g, 55 %).

IR (ATR): ν_{max} 3034, 2983, 1632, 1553 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 8.00 (2H, d, *J* = 7.0 Hz, Ph), 7.61 (1H, t, *J* = 7.5 Hz, Ph), 7.49 (2H, t, *J* = 7.5 Hz, Ph), 4.41 (2H, s, CH_2S), 2.41 (3H, s, CH_3CO). ^{13}C NMR (100 MHz, $CDCl_3$): 194.3, 135.4, 133.7, 128.8, 45.4. HRMS calcd for C_8H_9OS: 153.0374 and $C_{16}H_{15}O_2S_2$: 303.0513, found 153.0367 (MH^+) and 303.0511 (dimer).

Synthesis of (*S*)-(2-mercapto-1-phenylethyl)glycine (73)

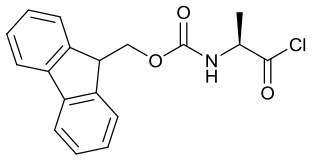


73

To a solution of **72** (0.15 g, 0.96 mmol, 1.0 equiv) in 1,2-dichloroethane (15 mL) was added glycine (72.3 mg, 0.96 mmol, 1.0 equiv). Sodium triacetoxyborohydride (0.29 g, 1.35 mmol, 1.4 equiv) and acetic acid (0.06 g, 0.96 mmol, 1.0 equiv) were then added. The reaction mixture was stirred at room temperature under an atmosphere of nitrogen for 24 hours. The resulting mixture was quenched with $NaHCO_3$ (15 mL), extracted with ether (3 x 30 mL), washed with brine (50 mL), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo* to give the crude product. Purification of the crude product over silica, eluting with hexane – ethyl acetate (1 : 4), afforded **73** as a yellow oil (62.5 mg, 31 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 ppm (2H, d, *J* = 7.0 Hz, Ph), 7.62 ppm (1H, t, *J* = 7.0 Hz, Ph), 7.50 ppm (2H, t, *J* = 7.5 Hz, Ph), 4.91 ppm (1H, bdt, *J* = 9.5 Hz, *J'* = 3.0 Hz, PhCH), 4.17 ppm (1H, d, *J* = 14.0 Hz, CH_2), 4.10 ppm (1H, d, *J* = 14.0 Hz, CH_2), 3.32 ppm (1H, b, NH), 3.11 ppm (1H, dd, *J* = 14.0 Hz, *J'* = 3.0 Hz, CH_2SH), 2.88 ppm (1H, dd, *J* = 14.0 Hz, *J'* = 9.5 Hz, CH_2SH). HRMS calcd for $C_9H_{13}NS$: 167.0769, found 167.0126 [$(M\text{-}CO_2H)H^+$)

Synthesis of (9*H*-fluoren-9-yl)methyl (*S*)-(1-chloro-1-oxopropan-2-yl)carbamate (74)[11]



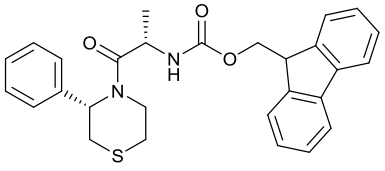
74

To a solution of *N*-Fmoc-*S*-alanine (1.89 g, 6.07 mmol, 1.0 equiv) in dry dichloromethane (30 mL) was added thionyl chloride (7.22 g, 60.7 mmol, 10.0 equiv) and the mixture was heated at reflux for 2 hours under an atmosphere of nitrogen. The resulting mixture was evaporated *in vacuo* to give the crude product which was then purified over silica eluting with hexane – ethyl acetate (6 : 1) to afford **74** as a white solid (1.2 g, 60 %). mp. 88 – 90 ^{o}C.

IR (ATR): ν_{max} 3328, 3040, 1794, 1694 cm^{-1}. ^{1}H NMR (400 MHz, DMSO d_6) δ 7.90 (2H, d, *J* = 7.5 Hz, Ph), 7.67 (2H, d, *J* = 7.5 Hz, Ph), 7.43 (2H, t, *J* = 7.5 Hz, Ph), 7.34 (2H, t, *J* = 7.5 Hz, Ph), 4.29 (2H, d, *J* = 6.5 Hz, OCH_2), 4.23 (1H, app q, *J* = 7.0 Hz, $\underline{CH}CH_3$), 4.01 (1H, t, *J* = 7.0 Hz, $\underline{CH}CH_2O$), 1.28 (3H, d, *J* = 7.0 Hz, $CH\underline{CH_3}$). ^{13}C

NMR (100 MHz, $CDCl_3$): 175.3, 155.6, 144.0, 141.0, 127.8, 127.1, 125.1, 119.7, 66.5, 65.2, 47.4, 18.4. HRMS calcd for $C_{18}H_{17}ClNO_3$: 330.0897, found 330.0903 (MH^+).

Synthesis of (9*H*-fluoren-9-yl)methyl ((*S*)-1-oxo-1-((*S*)-3-phenylthiomorpholino)propan-2-yl) carbamate (75)

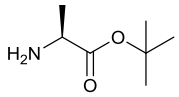


75

To a solution of **32** (92.8 mg, 0.48 mmol, 1.0 equiv) in dry dichloromethane (8 mL) was added Na_2CO_3 (255 mg, 2.40 mmol, 5.0 equiv). A solution of *N*-Fmoc-L-alanine acid chloride **74** (222 mg, 0.67 mmol, 1.4 equiv) in dry dichloromethane (2 mL) was then added dropwise under an atmosphere of nitrogen and the mixture was left overnight. The resulting mixture was filtered through a short pad of Celite® and the filtrate concentrated *in vacuo* to give the crude product that was purified over silica, eluting with hexane – ethyl acetate (1 : 1), to afford **75** as a yellow oil (0.11 g, 46 %).

IR (ATR): ν_{max} 3310, 2987, 1752, 1654, 1450, 1076 cm^{-1}. ^1H NMR (400 MHz, DMSO d_6) δ 7.88 (2H, br d, *J* = 7.0 Hz, Ph), 7.72 – 7.65 (2H, m, Ph), 7.46 – 7.29 (9H, m, Ph), 5.38 (1H, dd, *J* = 12.5 Hz, *J'* = 7.0 Hz, PhCH), 4.85 (2H, d, *J* = 16.0 Hz, OCH_2), 4.63 (1H, d, *J* = 17.0 Hz, NCH_2), 4.51 (1H, d, *J* = 17.0 Hz, NCH_2), 4.27 – 4.20 (1H, br, CH_3CH), 4.09 (1H, t, *J* = 16.5 Hz, CH), 3.67 – 3.59 (2H, br, SCH_2), 1.19 (3H, d, *J* = 7.0 Hz, $CHCH_3$). ^{13}C NMR (100 MHz, $CDCl_3$): 195.5, 171.9, 164.6, 143.8, 141.3, 138.8, 129.6, 129.2, 127.7, 127.1, 125.8, 125.2, 120.0, 67.1, 60.4, 54.7, 53.0, 47.1, 32.7, 19.0. HRMS calcd for $C_{28}H_{27}N_2O_4S$: 487.1692 and $C_{28}H_{26}N_2O_4SNa$: 509.1511, found 487.1686 (MH^+) and 509.1505 (MNa^+). $[\alpha]_D^{20}$ -80.1 (*c* 1.03, $CHCl_3$).

Synthesis of *tert*-butyl *S*-alaninate (76)[12]

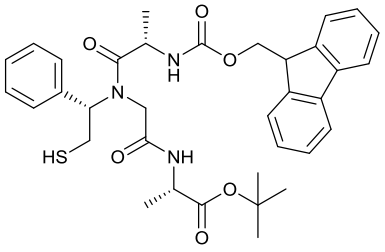


76

To a mixture of Na_2CO_3 (2.9 g, 27.5 mmol, 5.0 equiv) in deionized water – diethyl ether (1:1) (20 mL) was added *S*-alanine *tert*-butyl ester hydrochloride (1.0 g, 5.5 mmol, 1.0 equiv) and the mixture was stirred for an hour under an atmosphere of nitrogen. The resulting mixture was then extracted with diethyl ether (3 x 20 mL), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo* to give **76** as a colourless oil (0.58 g, 72 %).

IR (ATR): ν_{max} 3377, 2978, 1729 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 3.42 (1H, q, *J* = 7.0 Hz, CH), 1.57 (2H, br, NH_2), 1.46 (9H, s, *t*Bu), 1.29 (3H, d, *J* = 7.0 Hz, $\underline{CH_3}CH$). ^{13}C NMR (100 MHz, $CDCl_3$): 176.1, 80.8, 50.7, 28.0, 20.8. HRMS calcd for $C_7H_{16}NO_2$: 146.1181, found 146.1176 (MH^+).

Synthesis of *tert*-butyl-*N*-((((9*H*-fluoren-9-yl)methoxy)carbonyl)alanyl)-*N*-((*S*)-2-mercapto-1-phenylethyl)glycyl-*S*-alaninate (77)



77

To a solution of **75** (0.12 mg, 0.25 mmol, 1.0 equiv) in dry dichloromethane (10 mL) was added a solution of L-alanine *tert*-butyl ester **76** (89.5 mg, 0.62 mmol, 2.5 equiv) in dry dichloromethane (5 mL) under an atmosphere of nitrogen at room temperature and

the reaction was left overnight. The resulting mixture was then filtered through a short pad of Celite® and concentrated *in vacuo* to give the crude product. Purification of the crude product over silica eluting with diethyl ether – dichloromethane (6 : 1) afforded **77** as yellow oil (43.3 mg, 28 %).

IR (ATR): ν_{max} 3312, 3067, 2979, 2930, 1718, 1656, 1448, 1149 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 – 7.76 (2H, m, Ph), 7.61 – 7.60 (2H, br, Ph), 7.42 – 7.28 (9H, m, Ph), 5.79 (1H, dd, *J* = 10.0 Hz, *J'* = 6.5 Hz, PhCH), 4.39 – 4.29 (1H, br m, CH_3CH), 4.27 – 4.17 (1H, br m, CH_3CH), 3.93 (1H, d, *J* = 16.5 Hz, NCH_2), 3.61 (1H, d, *J* = 16.5 Hz, NCH_2), 3.12 – 3.00 (2H, m, CH_2SH), 1.44 (3H, d, *J* = 6.5 Hz, $CHCH_3$), 1.39 (3H, d, *J* = 6.0 Hz, $CHCH_3$), 1.25 (9H, bs, *t*Bu). ^{13}C NMR (100 MHz, $CDCl_3$): 177.6, 172.3, 171.4, 153.2, 143.8, 141.2, 135.9, 129.0, 128.7, 127.8, 127.2, 125.2, 120.0, 82.0, 68.1, 67.2, 49.8, 48.7, 47.2, 45.2, 29.7, 27.9, 26.2, 24.4. HRMS calcd for $C_{35}H_{42}N_3O_6S$: 632.2794 and $C_{35}H_{41}N_3O_6SNa$: 654.2614, found 632.2789 (MH^+) and 654.2608 (MNa^+).

References

1 Dellaria, J. F. and Santarsiero, B. D. *J. Org. Chem*. 1989, **85**, 3916–3926.

2 Panek, J. S. and Masse, C. E. *J. Org. Chem.*, 1998, **63**, 2382–2384.

3 Leermann, T., Broutin, P.E., Leroux, F. R. and Colobert, F. *Org. Biomol. Chem.*, 2012, **10**, 4095-4102.

4 Chunyang, J., Ann M, D. and Tiffany, L. L., *Bioorganic Med. Chem.*, 2017, **25**, 805–812.

5 Zhdanko, A. G., Gulevich, A. V and Nenajdenko, V. G. *Tetrahedron*, 2009, **65**, 4692–4702.

6 Holland, H. L., Brown, F. M., Barrett, F., French, J and Johnson, D. V. *J. Ind.*

Microbiol. Biotechnol., 2003, **30**, 292 – 301.

7 Wu, J., Hou, X. L. and Dai, L. X. *J. Chem. Soc. Perkin Trans. 1*, 2001, **1**, 1314–1317.

8 Tchertchian, S., Hartley, O. and Botti, P. *J. Org. Chem.*, 2004, **69**, 9208–9214.

9 Venkateswararao, E., Jalani, H. B., Manoj, M. and Jung, S. H. *J. Heterocycl. Chem.*, 2016, **53**, 1449–1456.

10 Dehmel, F., Ciossek, T., Maier, T., Weinbrenner, S., Schmidt, B. and Zoche, M. *Bioorg. Med. Chem. Lett.*, 2007, **17**, 4746–4752.

11 Carpino, L. A., Cohen, B. J., Stephens, K. E., Sadat-Aalaee, S. Y., Tien, J. H. and Langridge, D. C. *J. Org. Chem.*, 1986, **51**, 3732–3734.

12 Harwood, L. M., Wellings, D. A. and Moody, J. D., 2012, *U.S. Patent No. WO 2012/020231 Al*. Washington, DC: U.S. Patent and Trademark Office.