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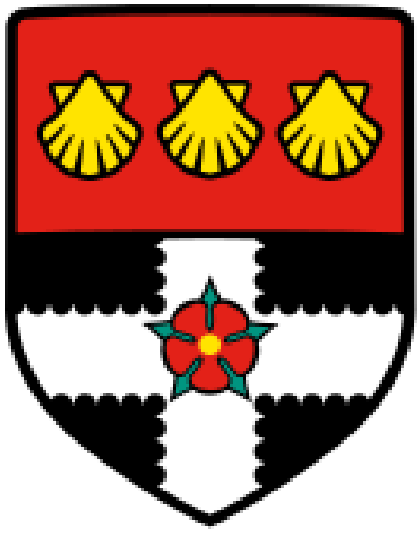

Investigations into the use of the Thiamorpholinone Template for Peptide Synthesis

Doctor of Philosophy

The School of Chemistry, Food and Pharmacy

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September 2016

Declaration

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

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Abstract:

The report herein describes approaches to the synthesis of peptides by using thiamorpholinone templates.

The first chapter considers the importance of peptide bond formation and protein synthesis. Several problems in classical peptide synthesis including epimerization, purification, solubility and yield are discussed. Subsequently, some important protecting and coupling reagents such as the benzyloxycarbonyl protecting group, *t*-butyl chloroformate group, 9-fluorenylmethoxycarbonyl group, benzyl ester, *t*-butyl ester, carbodiimide reagent, phosphonium reagent, uronium reagent and immonium reagent are considered. The development of the methodology of protein synthesis from solid phase peptide synthesis to native chemical ligation is described. Finally, previous syntheses of morpholinone templates and thiamorpholinone templates are considered.

Chapter 2 focuses on the synthesis of *iso*-leucine, alanine and phenylalanine based thiamorpholinones. All of them have been successfully synthesized with decent yields. The stereoselectivity of the formation of the thiamorpholinone template was also studied. L-Selectride as a stereoselective reducing agent was not effective in this case. The glycine-based thiamorpholinone was not successfully synthesized, but formation of the dehydrothiamorpholinone precursor was confirmed.

Chapter 3 concentrates on a range of attempts at the synthesis of the 3-unsubstituted thiamorpholinone. Starting with 4-hydroxyphenylglycine, the corresponding 3-unsubstituted morpholinone was successfully synthesized. However, the direct thionation of this morpholinone was not successful, and the morpholinone decomposed if the temperature was raised over 100 ^{o}C. The next approach was to synthesize the thioester first, then prepare the thiamorpholinone through intramolecular nucleophilic substitution. However, a side reaction related to native chemical ligation happened instead under all conditions tried. Secondly, an episulfide-mediated route was proposed, but the ring-opening

of the episulfide was not successful. Direct amination of a ketone precursor failed but amination via an oxime intermediate was achieved. However, all deprotection attempts to provide the free thiol group failed.

Chapter 4 describes two attempted one-pot strategies to synthesize the desired thiamorpholinone. The first was to use nitromethane, an aziridine and phenyl isothiocyanate and the second was to use nitromethane, carbon disulfide and an aziridine. Neither of these approaches generated the desired six-membered ring product, but gave the five-membered ring product instead.

The fifth chapter outlines the experimental procedures used, and spectroscopic data of the compounds synthesized are presented.

The last chapter lists all the reference quoted in this thesis.

Acknowledgements

Firstly, I would sincerely thank my supervisor Professor Laurence M. Harwood for offering a position in his group. He has provided me continuous help and encouragement in this project.

Secondly, I would also like to thank Dr John McKendrick, Martin Reeves and Nicholas Michael for mass spectra, Pete Heath, Dr Radoslaw M. Kowalczyk and Dr Geoff Brown for all the NMR spectra. Thanks are also due to Dr Elizabeth Page, Mrs Barbara Parr, Dr Philippa Cranwell, Dr Chris Smith and all the members in this department for their help and kindness.

Special thanks to Dr Elizabeth Lander, Dr Ashfaq Afsar, James Westwood, Dr Joe Cowell, Diana Kertini Binti Monir and all the members in Harwood's group for their great help to me and all the happy moments we have had. Thanks to Dr Qiang Zeng, Kai Liu, Chen Long, Yuanyuan Wang for all the badminton we have played together.

Last but not least, I would like to thank my parents and my grandparents for their endless love, encouragement and support. Without them I would not have gone this far.

Abbreviation

Ala	Ala
Bn	Benzyl
Boc	*tert*-butyloxycarbonyl
Cbz/Z	Carboxybenzyl
CDI	1,1'-Carbonyldiimidazole
Cys	Cysteine
DCC	Dicyclohexylcarbodiimide
DCM	Dichloromethane
DIAD	Diisopropyl azodicarboxylate
DIEA	*N*,*N*-Diisopropylethylamine
DMF	Dimethylformamide
DNA	Deoxyribonucleic acid
E1cb	Elimination Unimolecular conjugate Base
Et_2O	Diethyl ether
Et_3N	Triethylamine
EtOH	Ethanol
Fmoc	9-Fluorenylmethyloxycarbonyl
h	Hour
HATU	1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate
HBTU	2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
HMPA	Hexamethylphosphoramide
HOBt	1-Hydroxybenzotriazole
KEH	Potassium 2-ethyl hexanoate
LDA	Lithium diisopropylamide
Leu	Leucine
LG	Leaving group
Me	Methyl
MeCN	Acetonitrile
MeOH	Methanol
min	Minute
n	*normal*
N.O.e	Nuclear Overhauser effect
NBSH	2-Nitrobenzenesulfonylhydrazide
NCL	Native chemical ligation
NMR	Nuclear Magnetic Resonance
p	*para*
PG	Protecting group
ppm	parts per million

SPPS	Solid phase peptide synthesis
t	*tert*
TATU	2-(7-Azabenzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate
TBTU	2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
Thz	1,3-Thiazolidine-4-carboxyl
TLC	Thin layer chromatography
TMU	Tetramethylurea
Tosyl/Ts	4-Toluenesulfonyl

Contents

Chapter 1 Introduction

1.1 Proteins in the biological world

Proteins, as indispensable constituents of living things, play a central role in the biological world. A protein molecule produced naturally can consist of a combination of any of 20 proteinogenic amino acid residues, resulting in hundreds of thousands of functional polypeptide chains depending on the sequence and three-dimensional folded structure. It is relatively easy to obtain a series of protein molecules from the cell by genetic manipulation, but there are still many limitations if chemists want to prepare them by total synthesis. During the last five decades or so, technologies have dramatically developed from synthesizing linear polypeptide chains to synthesizing large protein like molecules possessing the appropriately folded structure. By using NMR and X-ray crystallography techniques, it is possible to discover the three-dimensional structure of proteins and finding positions where alteration of an amino acid residue could modulate activity, and thus, total synthesis presents us with the possibility of being able to modify a single residue from the chain at will.

1.2 Problems in protein synthesis

In the early years of the twentieth century, the total synthesis of enzymes and other proteins was considered as one of the "grand challenges" for organic chemistry. The great German chemists Emil Fischer and Franz Hofmeister worked out a way of total chemical synthesis to produce peptide bonds, and termed linear protein chains of α-amino acids as polypeptides.[1] The first peptide containing 9 amino acid residues was eventually synthesized by Vigneaud.[2] After this, many synthetic polypeptides were produced chemically. However this highly repetitive methodology was soon to show its limitations for even quite short peptide chains.

Figure 1.1 describes how classical organic synthesis works in solution. This linear strategy requires selective and orthogonal protection of the amino group of one coupling partner and the carboxyl group of the other. Coupling then occurs in a controlled manner by activating the unprotected carboxyl terminus and allowing condensation with the amino terminus. Subsequent deprotection of the *N*-terminus of the dipeptide thus formed means the whole process can be repeated with the next *N*-terminus protected *C*-terminus activated amino acid residue. Further protection of potentially reactive side chains (CH_2SH, CH_2OH *etc*.) is necessary in order to stop them interfering with the coupling reaction. In the end, all these protecting groups need to be removed, in one step at best. Thus, not only should there be *N*- and *C*-terminus protection, but also side chains of the polypeptide would need to be protected.

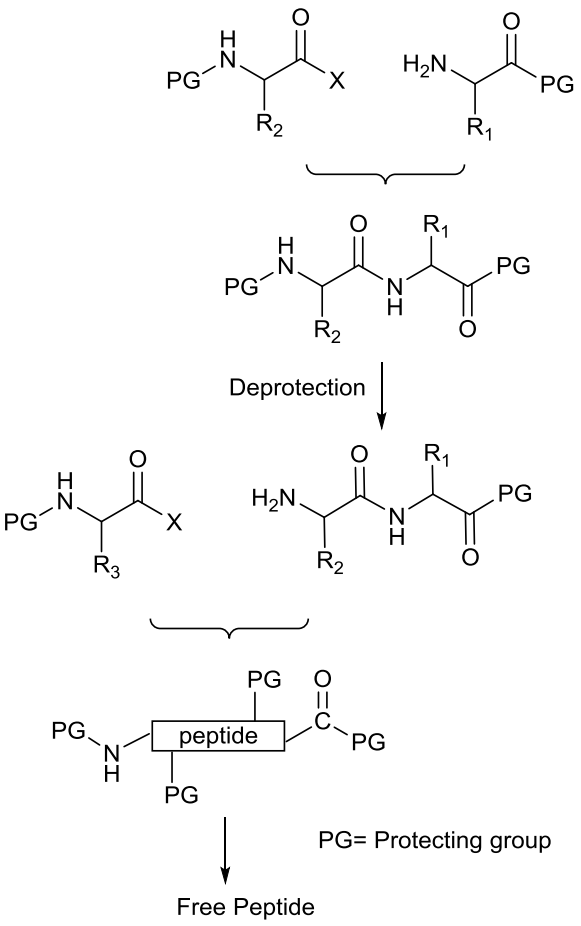


Figure 1.1 Classical solution protein synthesis

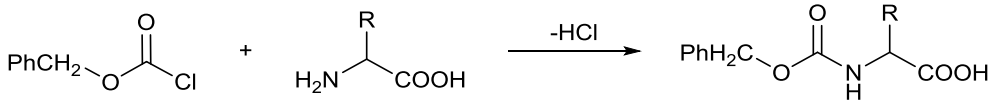
However, there are problems associated with this repetitive approach and these become worse as the peptide chain grows.[3] These include:

(1). Losing chiral integrity during the peptide forming procedure. Epimerization can happen while activating the *C*-terminus of a protected peptide chain.
(2). Difficulty in purifying and characterizing protected peptides. Adding more protecting groups makes it hard to purify and characterize effectively the solution phase products.
(3). Poor solubility in organic solvents. A fully-protected polypeptide may only dissolve partially in most organic solvents, which will result in low reactivity with reactants, leading to low yields and "deletion products".
(4). Need for very high yields at each step. The highly repetitive nature of peptide synthesis requires that each step occur with extremely high efficiency (quantitative conversion if possible) and this is particularly difficult to achieve in solution phase chemistry.

1.2.1 Protecting groups

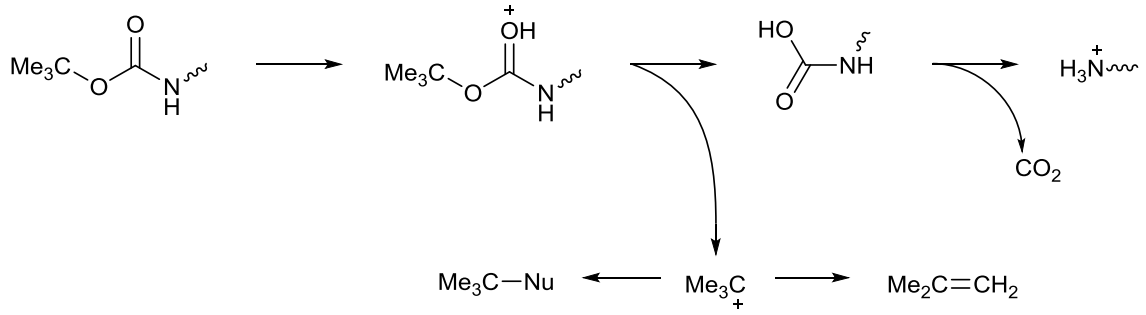
To synthesize a dipeptide of specific sequence, not only must the *C*-terminus of one residue be activated, but also *N*-terminus and the *C*-terminus of the other amino acid must be blocked. Thus, protecting groups are essential to peptide synthesis.

The benzyloxycarbonyl protecting group (Cbz) or (Z) was first introduced by Bergman and Zervas in 1932.[4] As part of a carbamic ester, the nitrogen atom does not have nucleophilic properties and will not take part in peptide bond formation. The amino acid is easily protected by reaction with benzyloxycarbonyl chloride, as shown in **Scheme 1.1**. Equally important, the protecting group is removable under a variety of conditions including palladium catalyzed hydrogenolysis under netural conditions.



Scheme 1.1 *N*-terminus protection by Cbz group

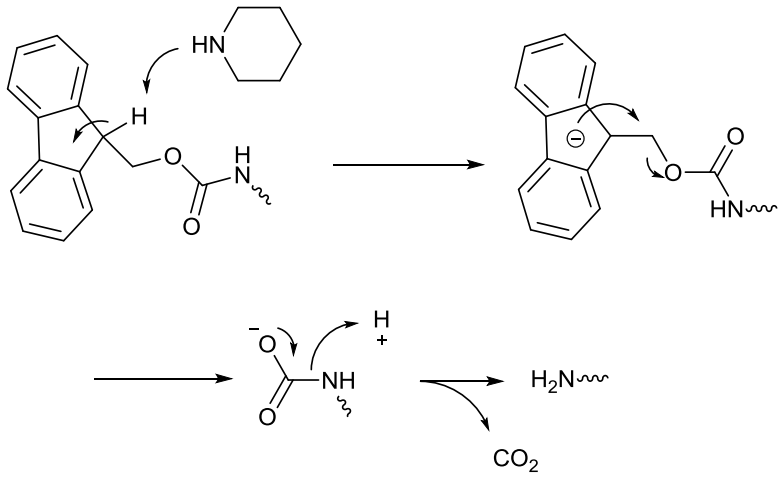
The *t*-butyl carbamate (*t*-Boc) group is also a widely used protecting group in peptide synthesis. This group is completely stable to catalytic hydrogenolysis conditions (and reducing agents generally), but it is more labile to acid than the Z group, to which it is therefore completely orthogonal. Basic and nucleophilic reagents have no effect at all on the Boc group, even on prolonged exposure. In this respect its stability is better than the Z group. Boc groups can be removed by dissolution in trifluoroacetic acid (TFA) (either neat or diluted with dichloromethane) at ambient temperature. These are mild reliable conditions and the mechanism is shown in **Scheme 1.2**.



Scheme 1.2 *N*-terminus deprotection of *t*-Boc group

Another important protecting group is the 9-fluorenylmethoxycarbonyl (Fmoc) group which was developed by Carpino.[5] The Fmoc group is very stable to acidic reagents, but is cleaved swiftly under certain basic condition, normally treatment with 20% piperidine in dimethylformamide. The mechanism of cleavage is E1cb as shown in **Scheme 1.3**.

As for *C*-terminus protection, the carboxyl group is usually converted to its corresponding ester. Two widely used derivatives are benzyl esters (**A**) and *t*-butyl esters (**B**) (**Figure 1.2**). The benzyl group can be removed by HF or hydrogenolysis; whereas the *t*-butyl group can be removed by treatment with TFA. Again it is important to have two protecting groups on the same amino acid residue removable under different conditions because of the need for differential protection of amino acids with carboxylic acid side chains.



Scheme 1.3 *N*-terminus deprotection of Fmoc group

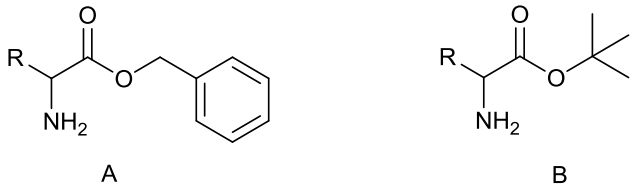


Figure 1.2 Benzyl esters (A) and *t*-butyl esters (B)

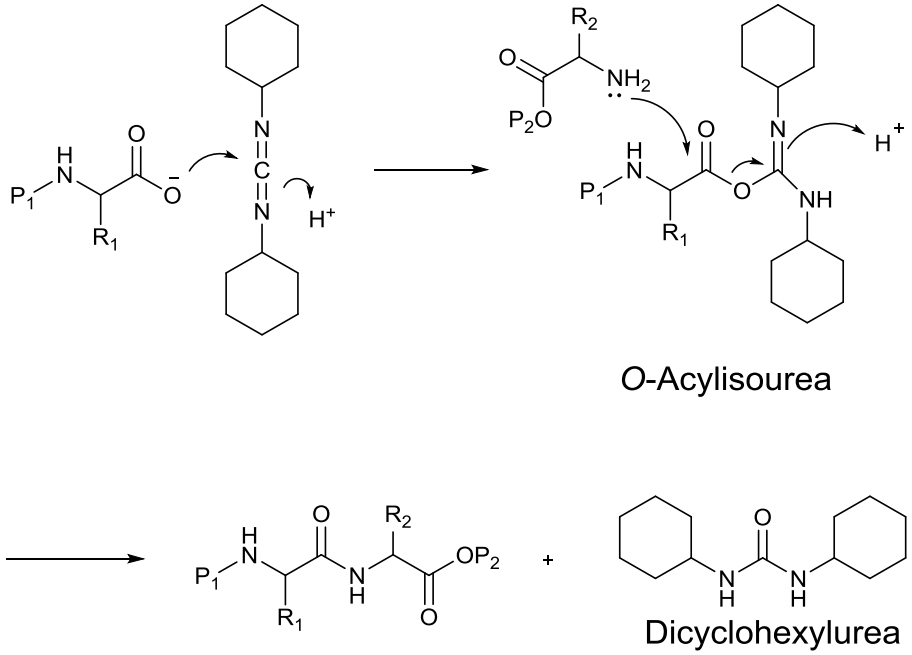
1.2.2 Coupling groups

The basis of peptide bond formation is the conversion of the carboxylic acid function of one amino acid to a reactive acyl derivative that is susceptible to nucleophilic attack by the amino group of the second amino acid. Recently, the accelerating development of new peptide coupling reagents has had a great impact on peptide coupling reactions in organic synthesis. Furthermore the discovery of racemization suppressants has played a key role in the development of peptide synthesis. Here are only some representative types of coupling reagents including carbodiimide, phosphonium, uronium and immonium systems.

1.2.2.1 Carbodiimide reagents

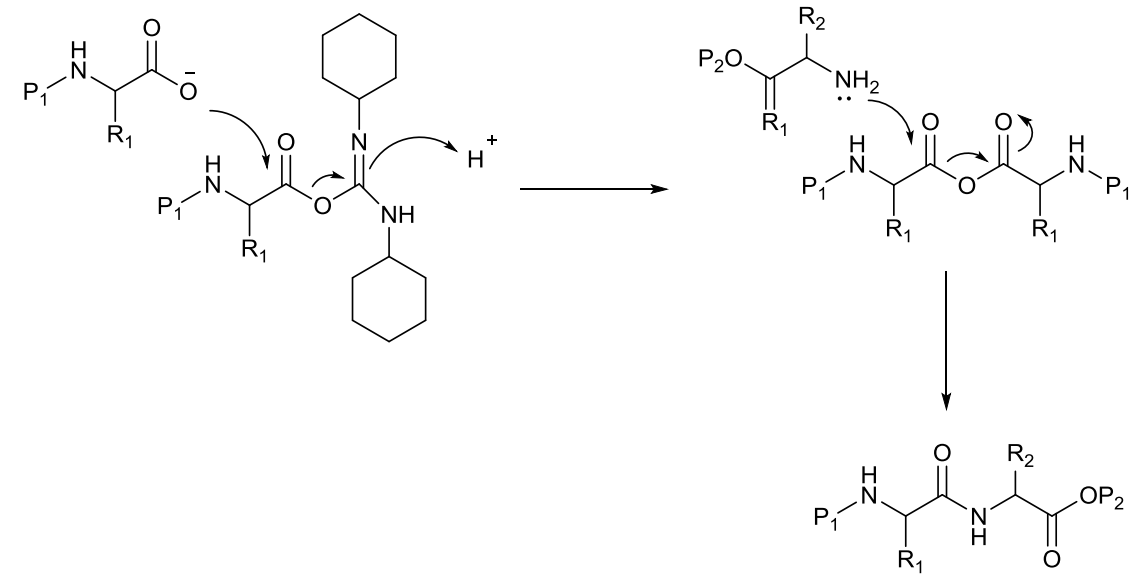
Dicyclohexylcarbodiimide (DCC) was one of the earliest reagents developed specifically to active amino acids in peptide synthesis, and was introduced by Sheehan and Hess in 1955.[6]

In this process (**Scheme 1.3**), the carboxylic acid function of the protected amino acid couples with the DCC to generate an *O*-acylisourea intermediate. Then the *C*-protected amino acid attacks the carbonyl function of the intermediate to form a peptide bond and also give dicyclohexylurea.



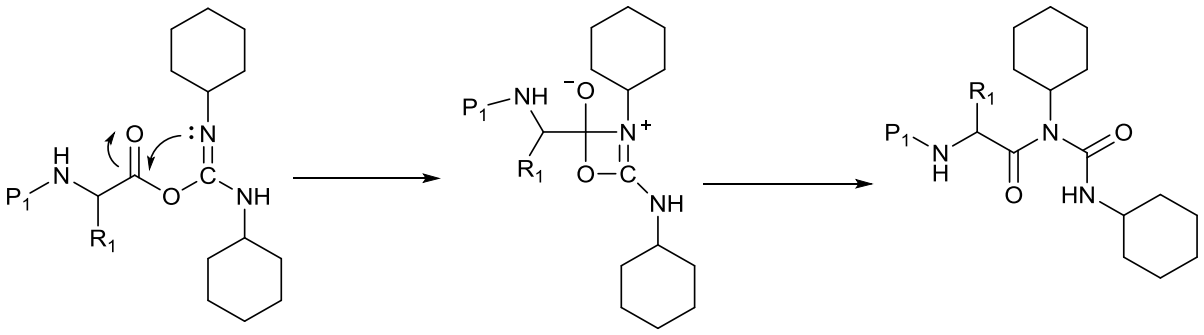
Scheme 1.3 Coupling with DCC

There is another possible pathway to attack shown in **Scheme 1.4**. Once the *O*-acylurea intermediate is formed it can also react with the carboxyl group of the *N*-protected amino acid to give a symmetrical anhydride. However, the anhydride can also react with the *C*-protected amino acid to give the desired product.



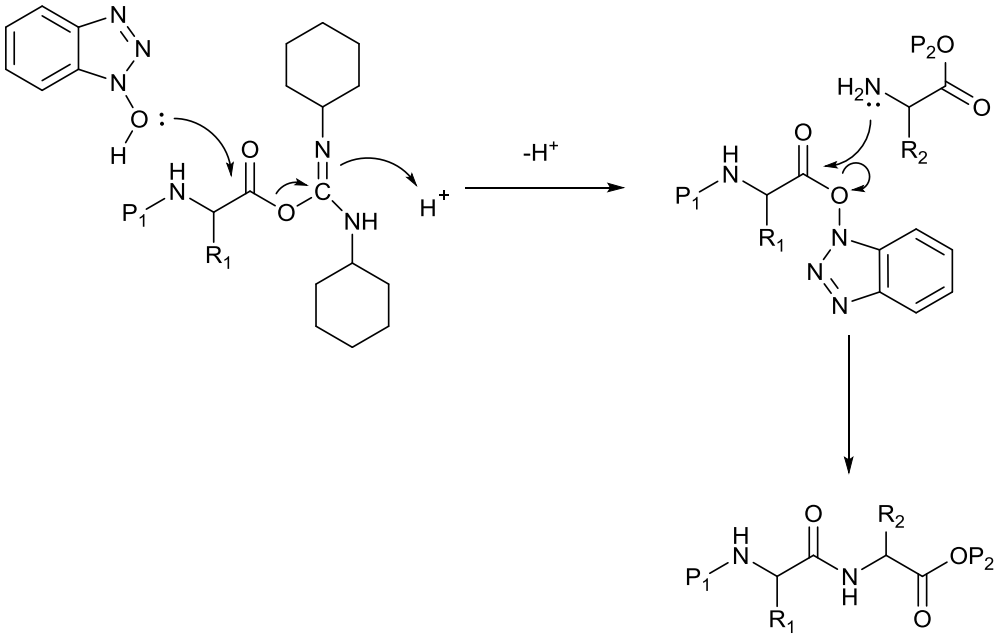
Scheme 1.4 Symmetric anhydride

One problematic side reaction is the intramolecular rearrangement of the *O*-acylisourea intermediate to give an unreactive *N*-acylurea, which can be controlled by appropriate choice of solvent.



Scheme 1.5 The formation of the unreactive *N*-acylurea

A more recent method has been applied in peptide bond formation by introducing 1-hydroxybenzotriazole (HOBt) in the coupling process, which can generate an active benzotriazolyl ester. The pre-formed ester is then reacted with the free amino group of the other *C*-protected amino acid. This method can effectively reduce the chance of side-reactions and epimerization.[6]



Scheme 1.6 Introduction of HOBt

1.2.2.2 Phosphonium reagents

To avoid the racemization and side reactions that can occur with carbodiimide reagents, CloP[7] and BroP[8] as peptide coupling reagents were introduced by Castro in the early 1970s. The new CloP-HOBt combined coupling reagent, known as BOP, is a non-hygroscopic crystalline compound that can easily be prepared in large quantities.[9]

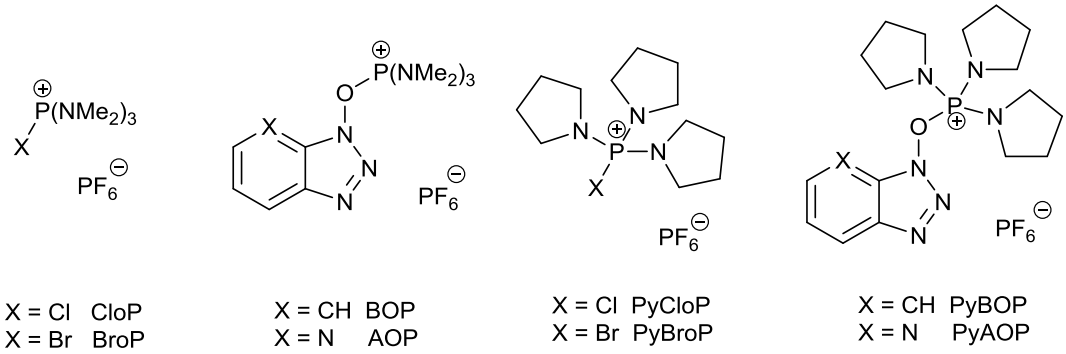
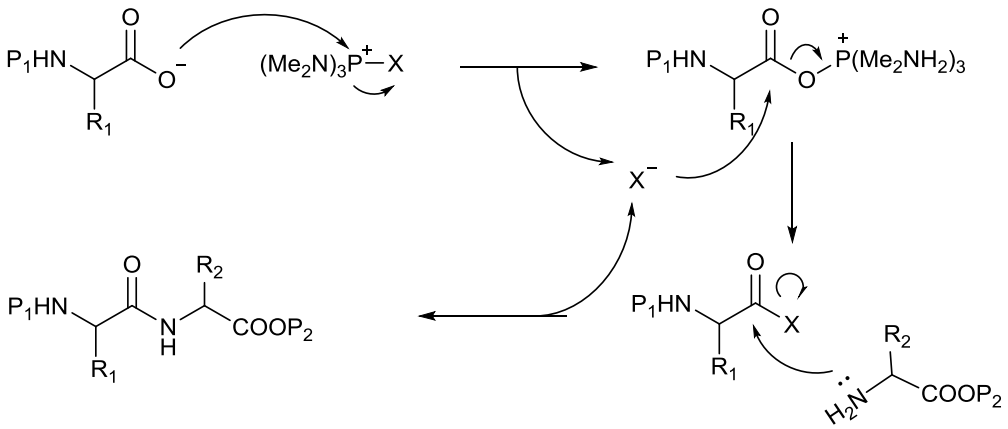


Figure 1.3 Phosphonium reagents

The mechanism is shown in **Scheme 1.7**. Deprotonation of the *N*-protected amino acid by a

tertiary amine liberates a carboxylate anion. This couples with the positively charged phosphonium salt generating the leaving group X which acts as a nucleophile to react with the carboxyl group of the intermediate to give the second reactive intermediate. Then the *N*-terminus of the *C*-protected amino acid attacks this carbonyl group to form the desired peptide bond.

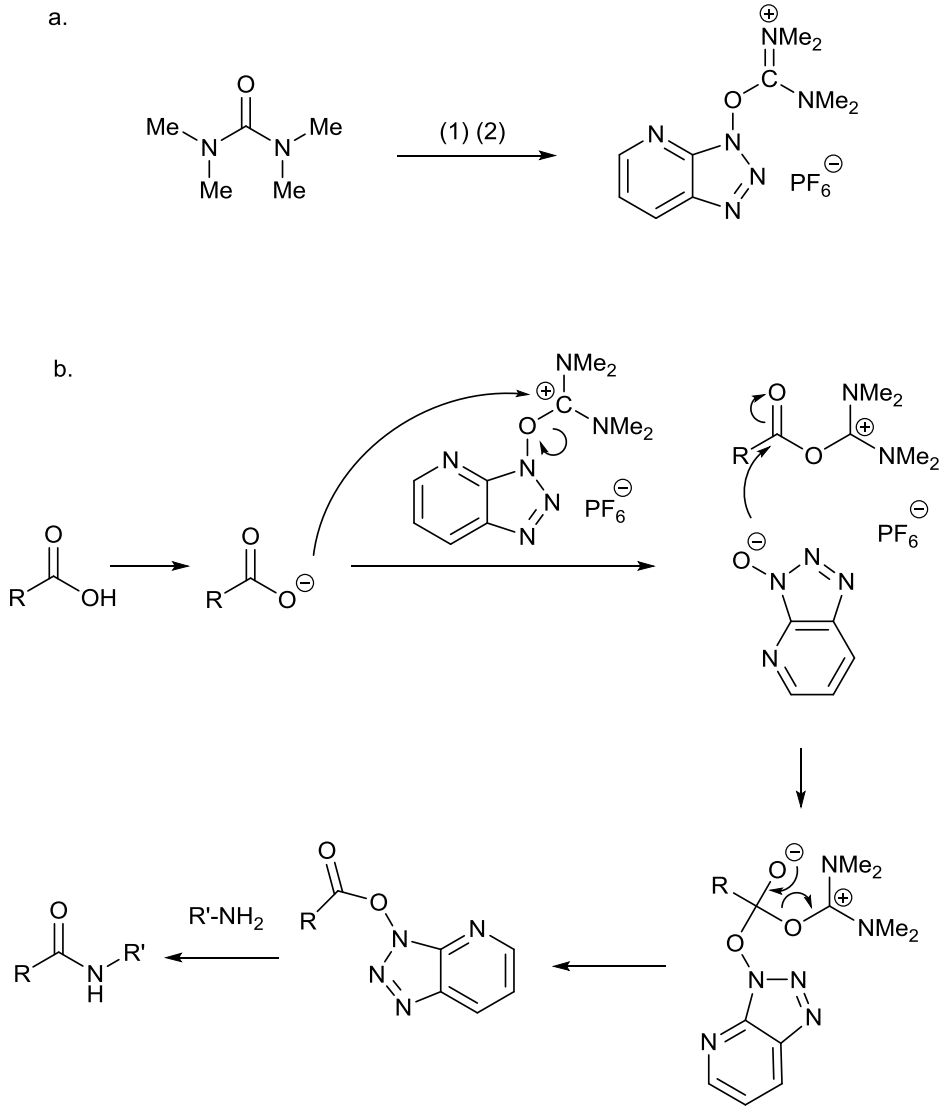


Scheme 1.7 General mechanism of phosphonium reagents

Subsequently PyCloP, PyBroP, and PyBOP were introduced, where the dimethylamine part was replaced by pyrrolidine.[10] Unlike BOP which must be handled carefully and will give carcinogenic hexamethylphosphoramide (HMPA) as a by-product in coupling reactions, these reagents are less hazardous and coupling reactions are fast, being finished within a few minutes.

1.2.2.3 Uronium reagents

Gross synthesized 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) as the progenitor of uronium reagents in 1978 by treating tetramethylurea(TMU) with phosgene at 0 ^{o}C and then HOBt and NH_4PF_6.[11] The activation process using HBTU is shown in **Scheme 1.8**.



Scheme 1.8 a. Preparation of HBTU; b. Activation process using HBTU. (1) $COCl_2$, Toluene, 0 ^oC; (2) HOBt, THF, NH_4PF_6.

Since then, various analogues of HBTU have been prepared and investigated by Knorr.[12] The tetrafluoroborate or hexafluorophosphate anion is generally used as the non-nucleophilic counterion in uronium reagents. A comparison study between HBTU and TBTU showed that the counterion had no significant influence on the coupling rate or racemization. Carpino disclosed the true structure of the active HBTU and its family as the *N*-guanidium rather than the *O*-uronium salt in an elegant study.[13]

The structural modification of HBTU provided several related new peptide coupling reagents with good activity.[14, 15] Firstly, alterations on the HOBt moiety generated HATU,

TATU, and TOTU (**Figure 1.4**). Secondly, alteration on the *O*-uronium moiety gave HBPyU. Thirdly, alteration on both HOBt and *O*-uronium moieties resulted in PyClU, TPyClU, HAPyU, HPyOPfp, HPySPfp, HAPipU, and TAPipU (**Figure 1.4**).

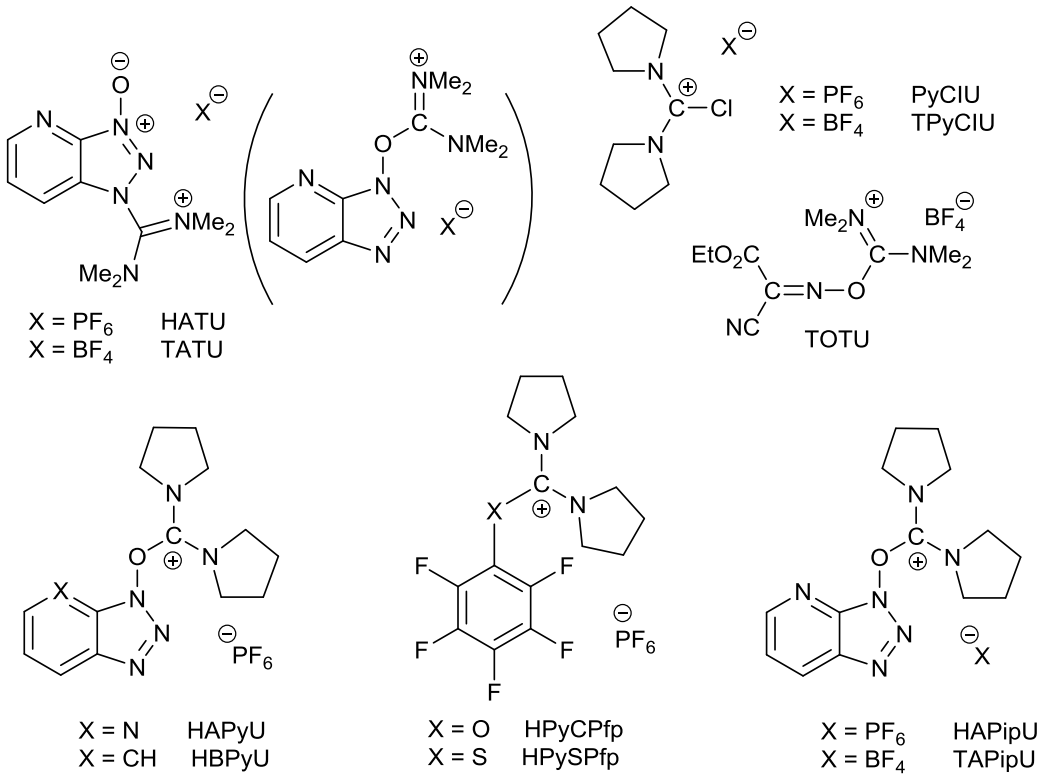


Figure 1.4 Uronium reagents

1.2.2.4 Immonium reagents

Xu designed new immonium reagents by modifying known uronium reagents.[16] The structural distinction of immonium reagents is the replacement of the amino group of the central carbon atom in uronium reagents with a hydrogen, an alkyl, or an aryl group. BOMI was shown to exist as the *N*-amidine derivative instead of the *O*-uronium compound by X-ray single crystal analysis. Some representative immonium reagents are shown in **Figure 1.5**.

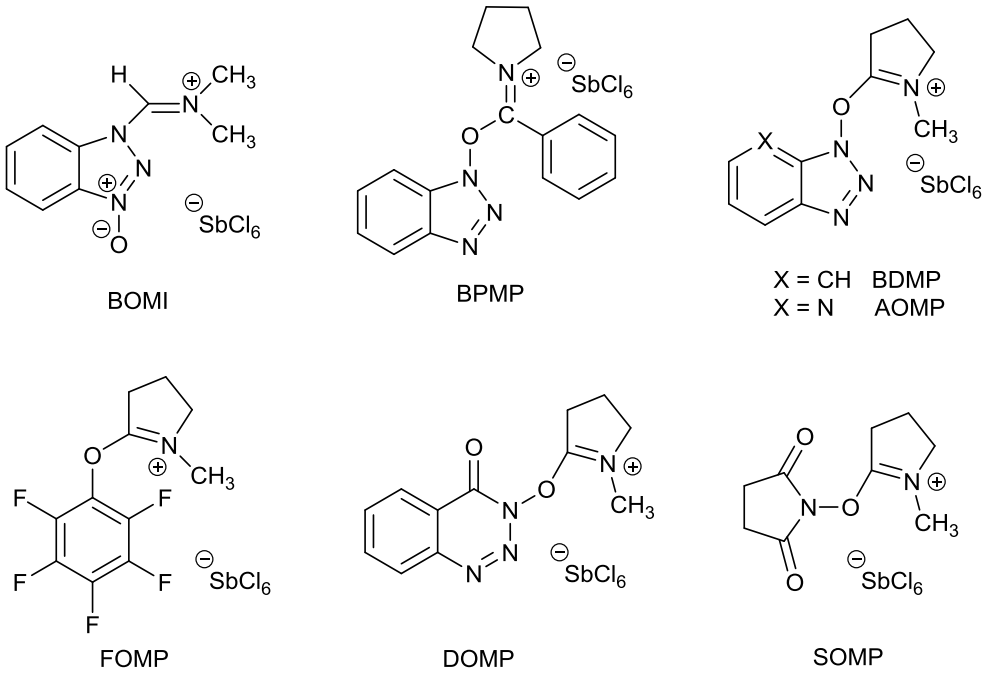


Figure 1.5 Immonium reagents

BOMI and BDMP showed a higher reactivity than other immonium reagents such as AOMP, FOMP, DOMP, BPMP, and SOMP during the synthesis of a tripeptide.[17] Interestingly, immonium reagents gave better results than uronium compounds such as HAPyU and HBPyU, presumably due to the fact that resonance stabilisation of uronium reagents from the amine substituent on the central carbon atom contributed to the retardation of reactivity and such a nitrogen atom was not available in the immonium reagents.

1.2.3 Racemization

It has been well studied that racemization would be observed during the formation of peptide bonds.[18] The mechanisms for racemization in amide bond formation in peptides are shown in **Figure 1.6**, and the most likely racemization path is through the formation of an azlactone.[19] After the amino acid is activated by a coupling reagent, there are two available reaction paths. The activated amino acid[20] could react directly with the amino group of the next amino acid residue, leading to the formation of the desired product **A** without any racemization (**Figure 1.6**). The other path is through an intramolecular

displacement, leading to formation of the azlactone,[10] the carbonyl group of which is prone to enolisation as the oxazole is aromatic. This planarizes the stereogenic centre leading to scrambling of the stereochemistry at this site. However, both epimeric azlactones are still activated towards coupling and each will lead to its corresponding amide.

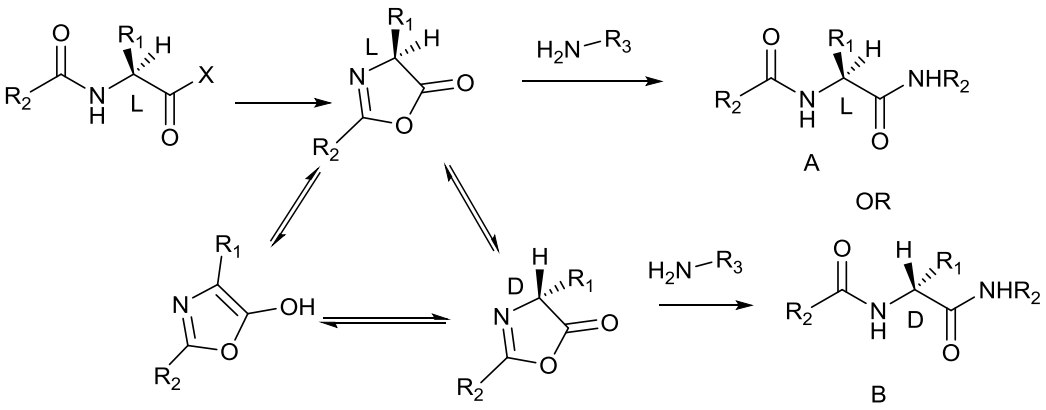


Figure 1.6 Racemization during peptide formation. Amine as nucleophile for amide formation (A) and as base to cause racemization (B)

1.3 Solid phase peptide synthesis

In 1963, a novel method was introduced to greatly improve the peptide synthesis situation by Merrified.[3] This liberated chemists from tedious purification processes by simply washing out the by-products and other impurities, which achieved a higher yield than classic peptide synthesis. The scheme of solid phase peptide synthesis (SPPS) is shown in **Figure 1.7**. Firstly the *N*-protected amino acid is linked to an insoluble polymeric support at the *C*-terminus, then the second amino acid is introduced in excess, in its *N*-protected *C*-terminus activated form after removing the *N*-protecting group on the first residue attached to the solid phase. The solid phase adduct is then purified by filtration and washing. This procedure is repeated until all segments are incorporated and yields can be forced to as near quantitative as possible by using excess of the soluble reactants as these can be easily washed off the immobilised growing chain. The final steps involve removing all protection groups and cleaving the covalent bond between the solid support and polypeptide chain.

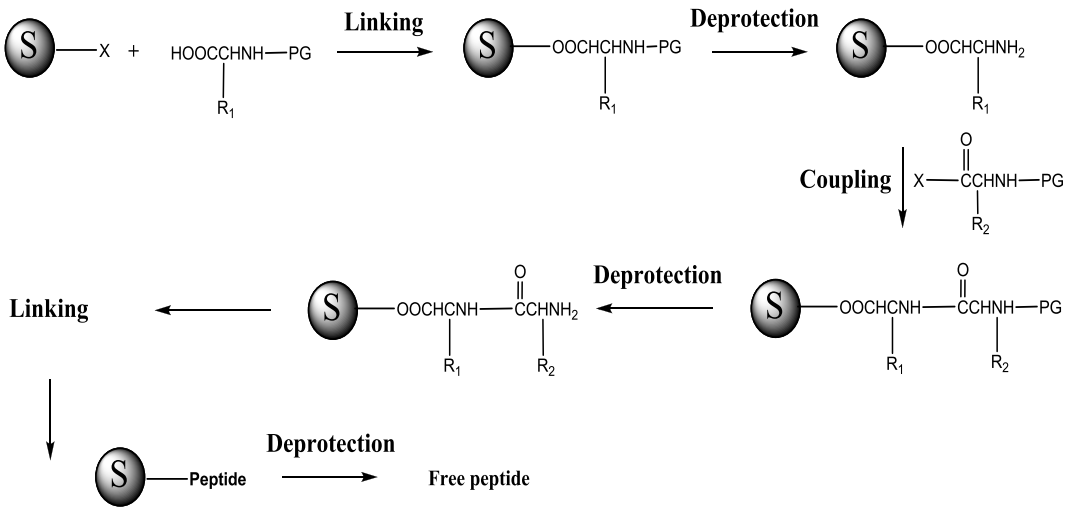


Figure 1.7 Solid-phase peptide synthesis

However, although speeding up and improving purity in peptide synthesis, this method still did not effectively increase the maximum size of polypeptide chains achievable, meaning that about 50 residues is a maximum for total synthesis using SPPS. Apparently, a more effective method was still needed.

1.4 Chemical ligation

In 1992, a novel concept was proposed by Kent to enable the use of unprotected peptide segments for the total synthesis of protein molecules, which represented the next big breakthrough in long peptide synthesis.[21] This chemical ligation approach (**Figure 1.8**) was based on two unique, mutually reactive *C*- and *N*- terminus functional groups on the two peptide segments, which would react at the specific linking site instead of other functional groups on both chains. This totally chemoselective incorporation allows the use of two unprotected peptide segments, which can be more readily purified and characterized. This reaction is carried out in aqueous solution containing a chaotropic reagent such as 6 M guanidine-HCl, to maximize the solubility of the reacting peptide segments.[22]

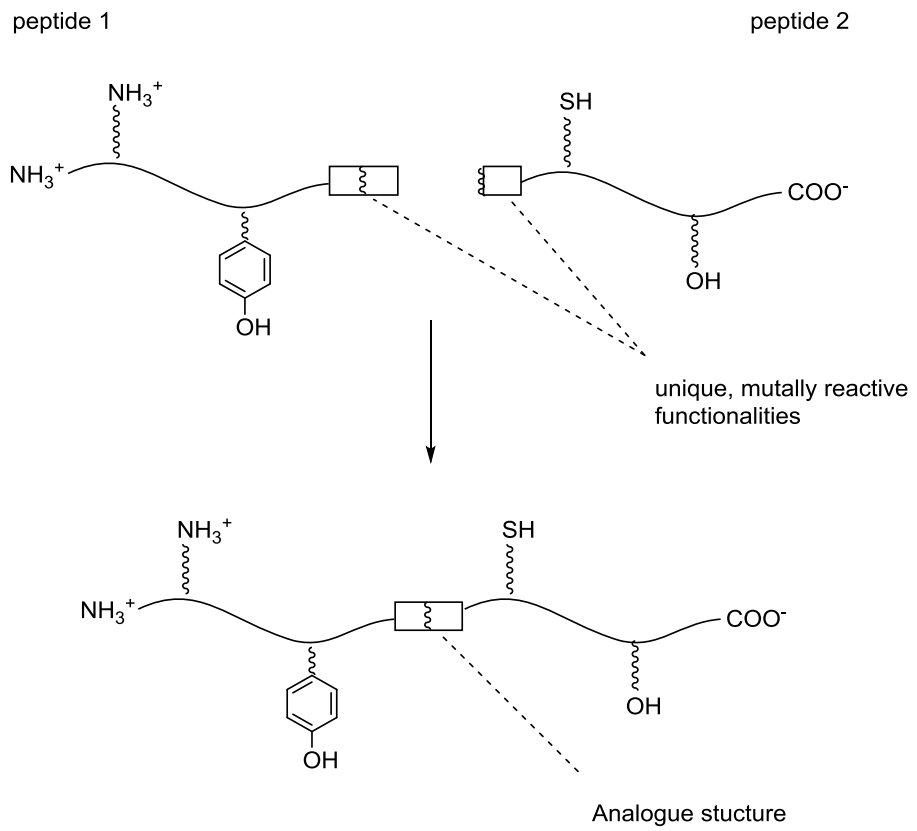


Figure 1.8 Chemical ligation

Various chemistries have been used at the ligation site listed including thioester-forming ligation;[21] oxime-forming ligation;[23] thioether-forming ligation;[24] directed disulfide formation;[25] thiazolidine-forming ligation[26, 27] and peptide bond-forming ligation.[28] The ligation site is therefore often an unnatural linkage, although those protein molecules which have been synthesized by chemical ligation, still demonstrated good protein-like activity after folding the polypeptide structure.

Thus, chemical ligation provides a straightforward way of avoiding poor solubility of longer peptide chains caused by side chain protecting groups, in order to make functionalised small proteins and enzymes.[29]

There was a further extension of the original method by Dawson *et al* in 1994;[28] wherein thioester-forming ligation chemistry was developed to give natural polypeptide products, by means of a thioester-to-amide rearrangement. The principle of native chemical ligation

(NCL) is pictured in **Figure 1.9**. Initially, a reversible *trans*-thioesterification occurs between the *C*-terminus thioester on one peptide fragment and the free thiol group of an *N*-terminal cysteine (Cys) on the other fragment. This thioester-linked material involving an *N*-terminus cysteine residue can then undergo an irreversible S-to-N rearrangement, by intramolecular nucleophilic attack, to form the desired native peptide bond. Native chemical ligation is highly chemoselective, even when there are other cysteine residues present in either or both segments as any *trans*-thioesterification occurring at these sites cannot lead to rearrangement to form an amide bond.[28, 30] Therefore, even without side chain protection, by-product formation is minimized during the reaction. The reaction is carried out in aqueous 6 M guanidine-HCl at neutral pH (pH 6.8-7.0) under effectively physiological conditions and gives good yields.[22] However, an obligate requirement of this native chemical ligation method is that ligation occurs at a unique *N*-terminus Cys residue. However, Cysteine is the least common of all residues in natural protein molecules (*circa* 1%) and is either unlikely to be present at a desirable ligating region of the target or may be absent altogether. Nevertheless, this technique marked an important step forward as cysteine-rich proteins can now be accessible by using native chemical ligation.

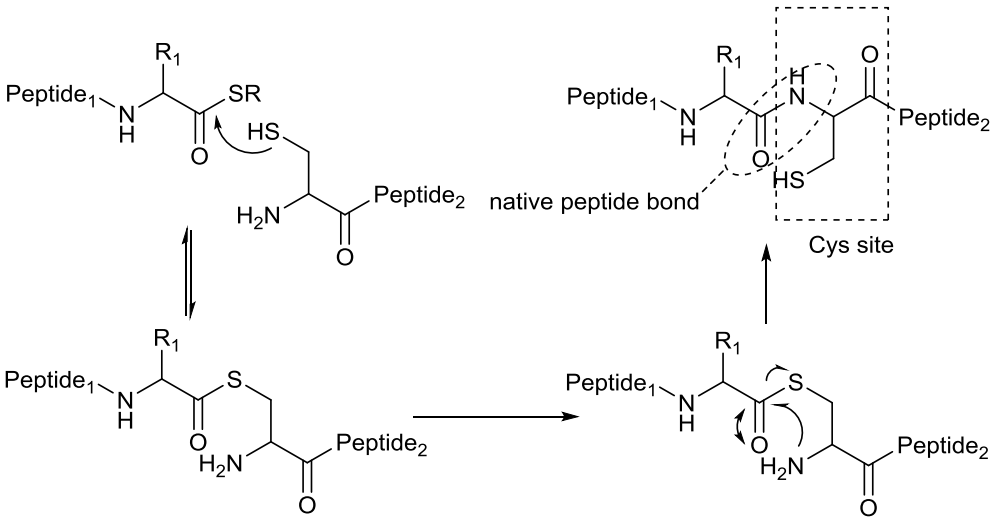


Figure 1.9 Native chemical ligation.

Subsequently, much work has been carried out by Kent and his group, who have focused on modification of both thioester peptide precursors and thiol additives, to improve the

efficiency of native ligation reactions.[31, 32]

Based on the fact that cysteine residues are the least common amino acid found in natural proteins, several attempts had been made to find a cysteine residue free solution to synthesize a native polypeptide chain. One approach to such a ligation is the catalytic desulfurization of the cysteine residue at the ligation site, to produce an alanine residue (**Figure 1.10 A**).[33] Alanine is one of the most common residues in natural proteins and its smaller steric hindrance compared to other substituted amino acids is also advantageous for this process.

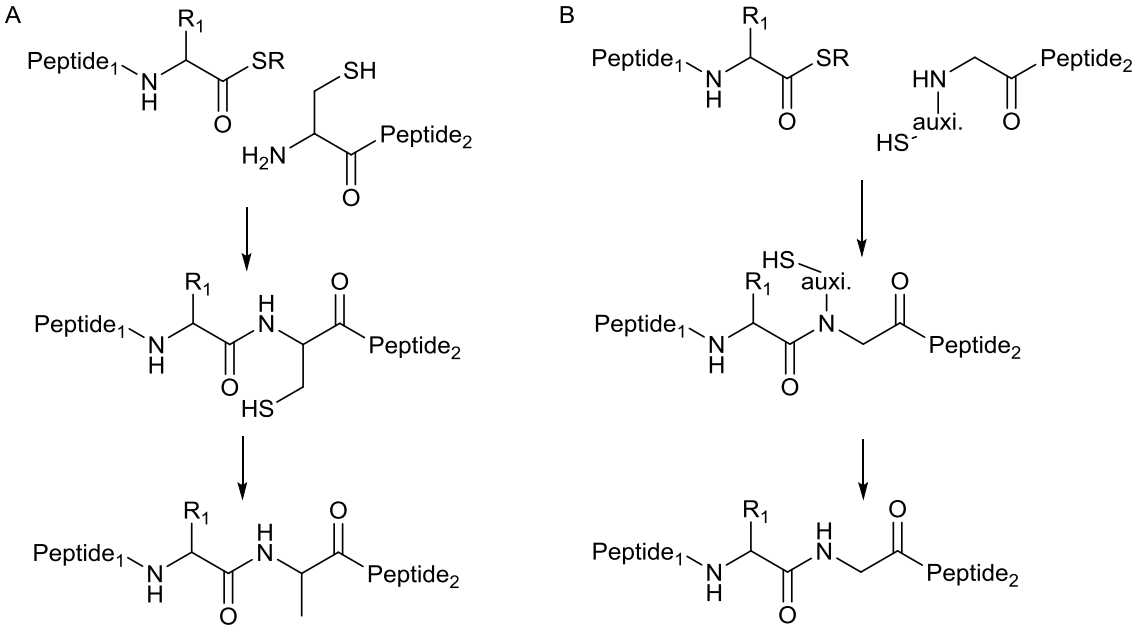


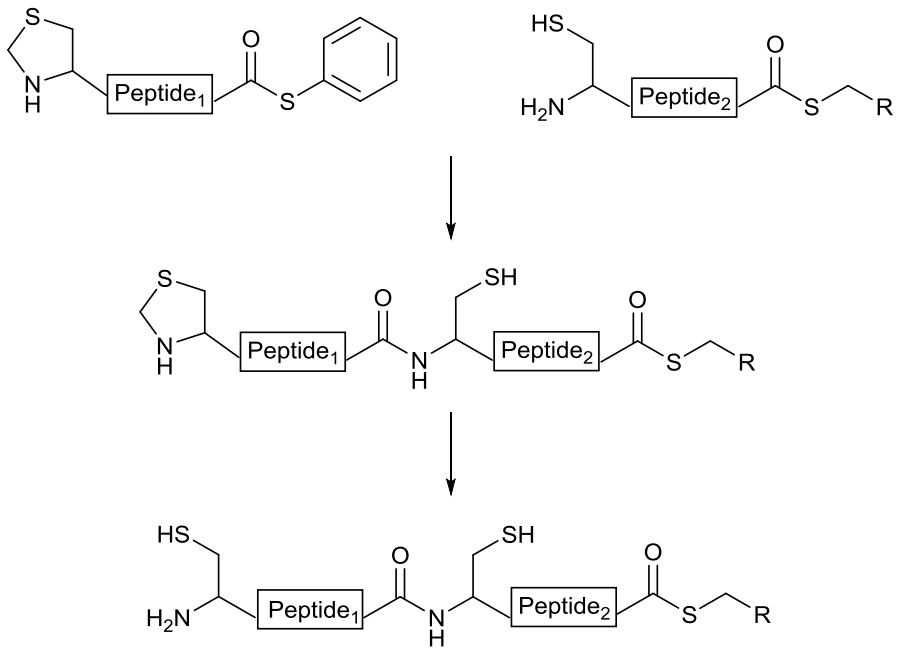
Figure 1.10: A. using desulfurization for NCL at non-Cysteine sites B. using a removable auxiliary for NCL at non-Cysteine sites.

Subsequently, auxiliary-mediated native chemical ligations that enable peptide ligation to be applied to protein sequences lacking cysteine have been investigated (**Figure 1.10 B**). In this approach a removable auxiliary is added to the *N*-terminus of one peptide segment to assist formation of the amide bond. This auxiliary must contain a thiol group to mimic the side chain of the cysteine residue and initiate a thioester-mediated NCL reaction. On the one hand, the auxiliary should be stable under the conditions for removing protecting

groups from the solid-phase synthesis generated peptide; on the other hand, it should be cleavable after the formation of amide bonds.[34, 35]

Native chemical ligation and its extended developments have thus proved a big step forward for the total synthesis of proteins. Currently, NCL has been extended to the use of polypeptide–thioesters produced by recombinant DNA microbial expression, which is termed ‘expressed protein ligation’ and this can be expected to be widely used in chemical-biological research areas.[36, 37]

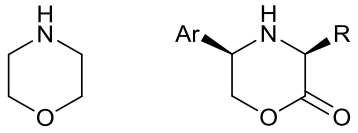
An important recent development in chemical ligation methods for the total synthesis of proteins is the kinetically controlled ligation reaction;[38] the major challenge here being to control the dual reactivity of a bifunctional peptide chain under competitive reaction conditions so that there will be different reactivity at different sites. Herein, a bifunctional Cys-peptide2-($^{\alpha}$thioester), with a *C*-terminus being a peptide-($^{\alpha}$$COSCH_2CH_2CO$)-Leu thioester (i.e. an alkyl thioester), is reacted with a peptide1-($^{\alpha}$thiophenylester) under native chemical ligation reaction conditions to yield a single product (**Scheme 1.9**), then the Thz-peptide-($^{\alpha}$thioalkylester) could be converted into a Cys-peptide-($^{\alpha}$thioalkylester) to perform the next ligation. The polypeptide chain could also be extended from the thioalkylester site via standard native chemical ligation. It was confirmed that a preformed peptide-($^{\alpha}$thiophenylester) would react with a Cys-peptide so much faster than a peptide-($^{\alpha}$thioalkylester) under the same conditions.



Scheme 1.9 Kinetically controlled chemical ligation reaction

1.5 Morpholinone–based amino acid and analogue synthesis

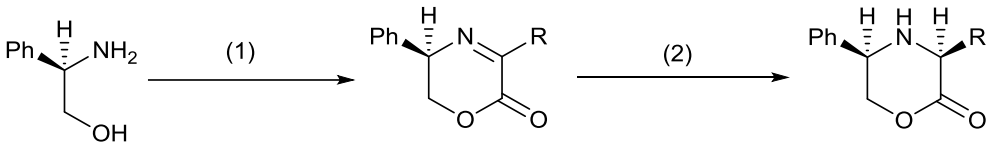
Morpholinones may be considered as derivatives of morpholine **1** (**Figure 1.11**), and may have a carbonyl group attached to either position 2 or 3 of morpholine. Many chiral morpholin-2-one analogues **2** (**Figure 1.11**) have been synthesised within the Harwood group.[39]



(**1**) (**2**)

Figure 1.11: 1. morpholine; 2. morpholinone analogues.

The morpholinone template can be constructed by using phenylglycinol with desired α-ketoester in trifluoroethanol under the presence of 4 Å molecular sieves (**Scheme 1.10**). The resulting dehydromorpholinone can be hydrogenated to give the 3*S*,5*R* template.[40]



Scheme 1.10 Synthesis of morpholinone from phenylglycinol. (1) α-ketoester, 4 Å MS, TFE, reflux, 20 h. (2) H_2, 1 atm, PtO_2, DCM, 5 h

It was recognised by Harwood that, although morphplinones act as activating templates, intramolecular attack of a second amino acid unit onto the morpholinone carbonyl is not possible and so the stereochemistry at C-3 will remain unaltered during coupling. (**Figure 1.12**)

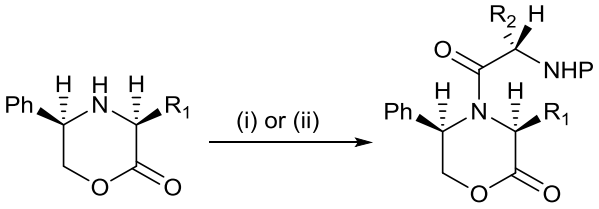
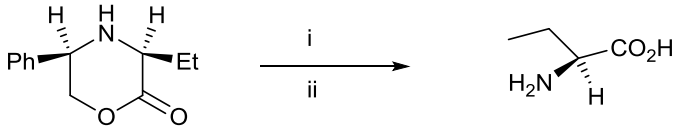


Figure 1.12 The *N*-terminus extension of morpholinone template. (i) N-*t*-Boc-L-amino acid fluorides, *i*Pr_2NEt, CH_2Cl_2; or (ii) *N*-Fmoc-L-amino acid chlorides or azides, $NaHCO_3$, Na_2CO_3, CH_2Cl_2/H2O

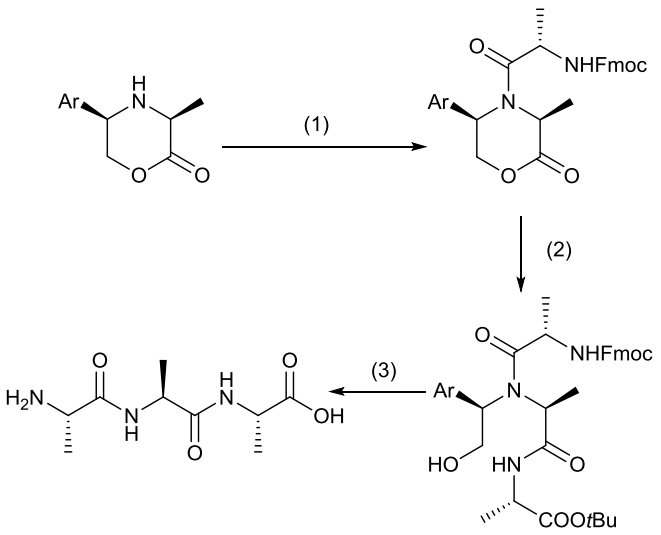
By using Cox's method,[41] morpholinone was cleaved under the condition of Pearlman's catalyst and high pressure. The aqueous solution of the crude amino acid salt was extracted with ethyl acetate followed by freeze-drying of the aqueous layer and ion-exchange chromatography on Dowex-50W acidic resin eluting with 2M ammonia to yield the pure amino acid (**Scheme 1.11**).[40, 41]



Scheme 1.11 Synthesis of amino acid from morpholinone. i. Pd(OH), H (6 atm), THF (1 equiv.), aq. MeOH (1:10); ii. ion exchange

It has been known for a long time that peptide chain elongation must be constructed at the *N*-terminus because of epimerization of activated carboxyl group attacking its stereogenic carbon centre to give oxazolone structure.[42] Within the Harwood group, much research[39] has concluded that morpholinone based systems allow peptide extension from *N*-terminal to *C*-terminal whilst retaining the stereochemistry of the amino acid at ligation site.

Several modifications have been carried out on this morpholinone template (**Scheme 1.12**). It was reported that Boc and Fmoc protected amino acids could be coupled to the morpholinone using DCC[43] and the morpholinone could also be coupled with Fmoc-amino acid chlorides. The acylated morpholinone then underwent a ring opening reaction which was attacked by *t*-butyl-L-alanate, followed by removal of the *N*-benzyl side chain with *t*-butanol in the presence of lithium in liquid ammonia to generate a tri-peptide.[44] In this sequence, the middle amino acid residue was synthesised *ab initio*, being part of the morpholinone template, the *N*-terminus residue was introduced using standard methodology and, most importantly, the *C*-terminus extension occurs without epimerising the adjacent residue as azlactone formation is not possible in this situation.



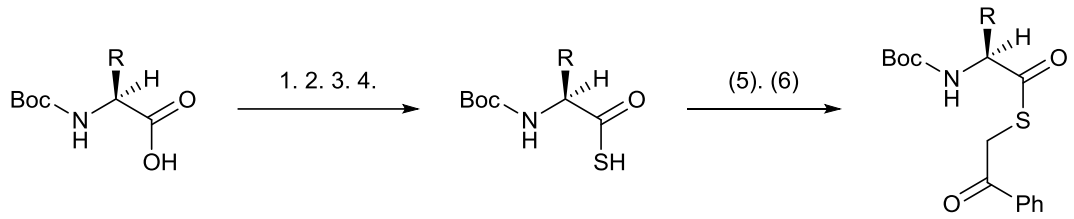
Scheme 1.12 Peptide synthesis by using morpholinone template. (1) *N*-Fmoc-L-alanine chloride, Na_2CO_3, DCM, 1Hr. (2) *t*-Butyl-L-alanate, $Al(CH_3)_3$, DCM, 24 h. (3) Li, liq. NH_3, *t*-BuOH, THF, -78 ^oC, 15 min

Harwood and co-workers further outlined the potential application of morpholinones in the area of solid-phase peptide synthesis whereby morpholinones are loaded onto a polymeric support using Mitsunobu chemistry.[45]

1.6 Thiamorpholinones

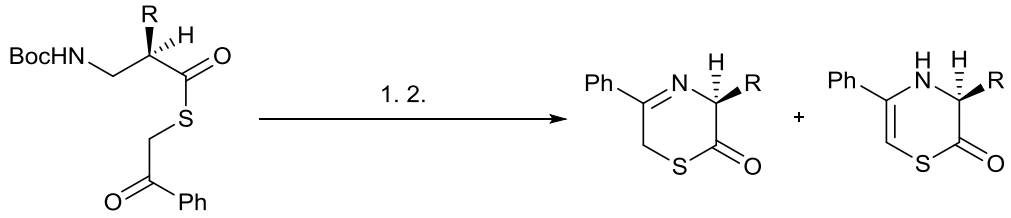
Many successful modifications have been achieved using morpholinone template. However, the morpholinone system was not prone to nucleophilic ring opening under mild conditions, which was not suitable for peptide synthesis as the harsh conditions could damage the side chains of a peptide. Therefore, it was decided to use thiamorpholinone as the template as it was proposed that thiamorpholinones would more readily undergo ring opening reactions.[46] Because the sulfur in the thioester linkage is less able to participate in electron delocalization through the acyl group, and this makes the thioester bond less stable than the ester bond, hence, the thioesters are more reactive than oxygen esters, undergoing more facile nucleophilic displacement reactions at the acyl group.

The synthetic approach towards the previously unknown thiamorpholinone system was developed at Reading by Yan[45] starting with Boc protected L-amino acids. In the first stage, Boc-amino acid were basified by triethylamine and then activated by ethyl chloroformate at 0℃, followed by addition of sodium hydrosulfide and hydrochloric acid subsequently. The resulting thioacids were then basified to their potassium salts with potassium 2-ethyl hexanoate (KEH) in diethyl ether. 2-bromoacetophenone was added to form the desired thioesters (**Scheme 1.13**).



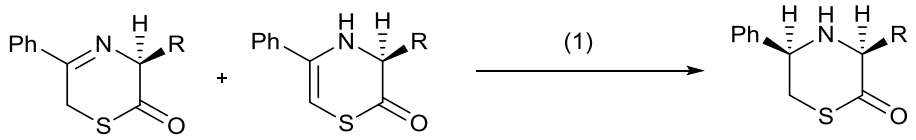
Scheme 1.13 Synthesis of thioester. (1), Et_3N, DMF, 0 ^oC, 30 min; (2), $ClCO_2Et$, 0 ^oC, 30 min; (3), NaSH, 0 ^oC, 30 min; (4), 1 M HCl, 0 ^oC; (5), KEH, Et_2O, 30 min; (6), DMF, 2-bromoacetophenone, 18 h

The next stage was to remove the Boc group of the thioester using 50% TFA in DCM. Then cyclization using anhydrous potassium carbonate generated two isomers, the imine and the enamine in a varying ratio depending on the starting amino acid (**Scheme 1.14**).



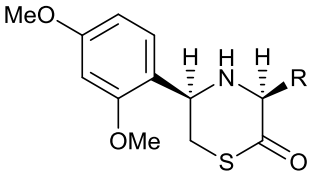
Scheme 1.14 Synthesis of dehydrothiamorpholinone. 1, TFA, DCM, r.t., 2h; 2, K_2CO_3, DCM, r.t., 2h

At last, reduction was needed to achieve the thiamorpholinone template. The first trial was to use palladium to hydrogenate the double bond. However, the sulfur contained in the thiamorpholinone poisoned the catalyst and reduction was not observed. However, when sodium cyanoborohydride in the presence of acetic acid was applied (**Scheme 1.15**) diastereoselective reduction to the 3,5-*syn* disubstituted thiamorpholinone was observed when R = isopropyl. In the first attempt, methanol was used as solvent. But the thiolactone was found to undergo ring opening reaction when the C-3 substituent was methyl due to methanol acting as a nucleophile. Whilst, at first sight a set-back, this at least demonstrated that the thiamorpholinone system was more reactive to ring opening chemistry than the morpholinone system, which was stable towards methanol. Methanol was replaced by THF as the solvent to stop this ring opening occurring.



Scheme 1.15 Reduction of dehydrothiamorpholinone via $NaBH_3CN$. (1) $NaBH_3CN$, AcOH, THF, 24 h

To ease the final debenzylation step the 2,4-methoxyphenyl substituent was initially chosen to replace the phenyl group in the thiamorpholinone. In the previous study, the morpholinone was substituted with a phenyl group at C-5, and the debenzylation of the protected tripeptide required Birch reduction, which would be too harsh for a peptide with any active side chain. The 2,4-methoxyphenyl can be cleaved by TFA, which would be much more compatible with peptide synthesis.[46]

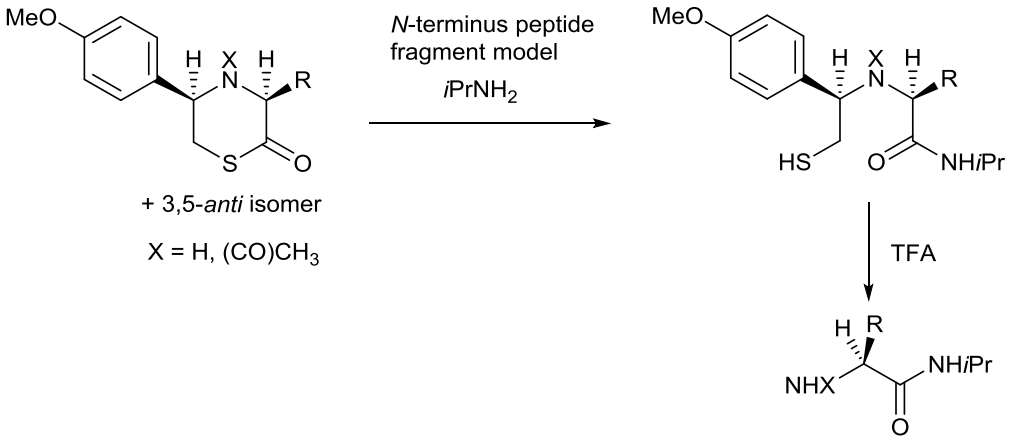


(3)

1.7 Project aims

A range of thiamorpholinones were successfully synthesized by former researchers in the Harwood group and the potential of these templates to synthesize peptides by extending from both *N*- and *C*- terminus has been explored.[45, 46] In this project, the initial aim was to continue the investigation into the methodology of thiamorpholinone synthesis, and modify the 2,4-methyoxyphenyl group to a 4-methoxyphenyl group as it turned out that the presence of the 2,4-dimethoxyphenyl group resulted in lability of the thiamorpholinones. The mono-substituted aromatic ring can also be cleaved under mild conditions compatible with peptide synthesis. A subsequent goal was to synthesize thiamorpholinones based on the glycine amino acid and so unsubstituted at C-3. Attempts to access this C-3

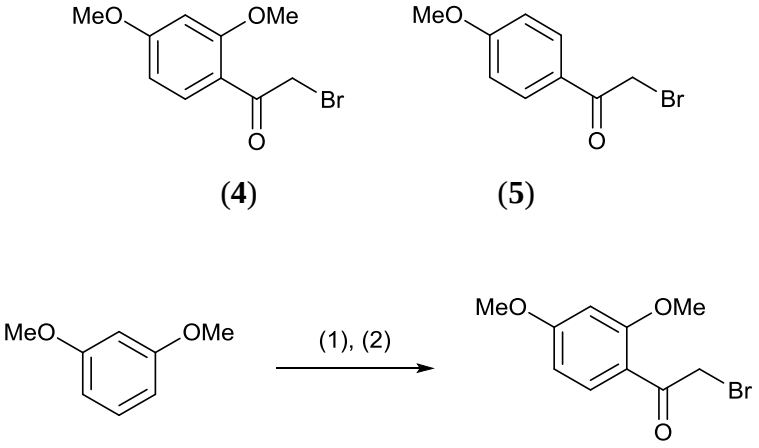
unsubstituted thiamorpholinone had previously been investigated and it had been found to be difficult to synthesize. Furthermore, the reactivity of these templates, both as the free amine and as the *N*–acylated form, would be studied with regard to ring opening amide bond formation.



Scheme 1.16 Thiamorpholinone ring opening amide bond formation

Chapter 2 Synthesis of 5-(4-methoxyphenyl)thiamorpholinones

To prepare the target 5-(2,4-methoxyphenyl)thiamorpholinones, it was first necessary to obtain the corresponding 2'-bromo-2,4-dimethoxyacetophenone (**4**). This chemical is commercially available, but the price in 2014 was £52.30 per gram.[47] However, it can also be prepared in a straightforward procedure by Friedel-Crafts acylation of 1,3-dimethoxybenzene with bromoacetyl bromide (**Scheme 2.1**). After many attempts, the yield of this reaction optimized to 52%.[46]



(**4**) (**5**)

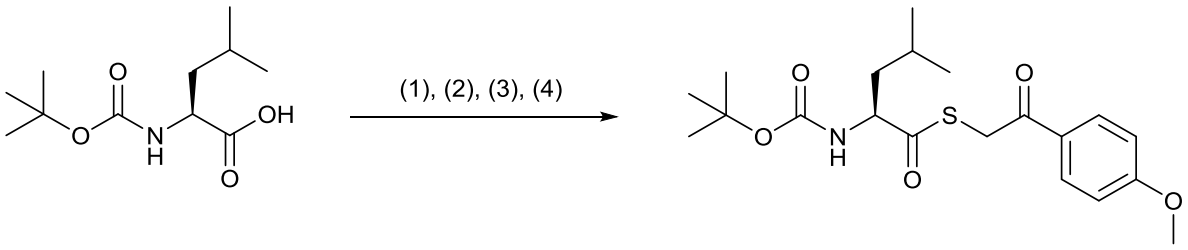
Scheme 2.1 Synthesis of 2'-bromo-2,4-dimethoxyacetonphenone. (1), $AlCl_3$, bromoacetyl bromide, 0 ^oC (2), overnight, r.t.

Compared to 2'-bromo-2,4-dimethoxyacetonphenone, 2'-bromo-4-methoxyacetonphenone (**5**) is more readily commercially available and means that we could circumvent the low yield problems associated with the synthesis of (**4**).

2.1 Synthesis of 3-*iso*butyl-5-(4-methoxyphenyl)thiamorpholin-2-one (7)

The first choice of amino acid was Boc-leucine and the desired 3-*iso*butyl-5-(4-methoxyphenyl)thiamorpholin-2-one (**9**) was synthesized following a modification of the Harwood group's published approach to 5-phenyl thiamorpholinones.[46] In this approach a one pot synthesis generated and reacted the thioacid sodium salt directly

with 2'-bromo-4-methoxyacetophenone instead of isolating the intermediate thioacid and then treating it with base.



(**6**)

Scheme 2.2 Synthesis of thioester (6) from Boc-Leu-OH. (1) Et_3N, 0 ^oC (2) ClCOOEt, 0 ^oC (3) NaSH, 0 ^oC (4) 2-bromo-4'-methoxyacetophenone, r.t.

Thus, Boc-Leu-OH was mixed with triethylamine in dry THF and the mixture stirred for 30 minutes on ice. Ethyl chloroformate was added at 0 ^oC to activate the *C*-terminus, the mixture stirred for a further 10 minutes and then sodium hydrosulfide was added and the mixture left for 2 hours to form a yellow solution of the thioacid as its sodium salt. After this, the mixture was removed from ice and treated with 2'-bromo-4-methoxyacetophenone. The reaction was then stirred overnight and purified by extraction and column chromatography to give the thioester (**6**) in 49% overall yield.

The new compound (**6**) was identified by ^1H NMR spectroscopic analysis wherein two doublets at δ 7.94 and 6.93 ppm corresponding to the aromatic protons, a singlet at δ 3.87 ppm corresponding to the methoxy group and a singlet at δ 1.44 ppm corresponding to the Boc group were observed.

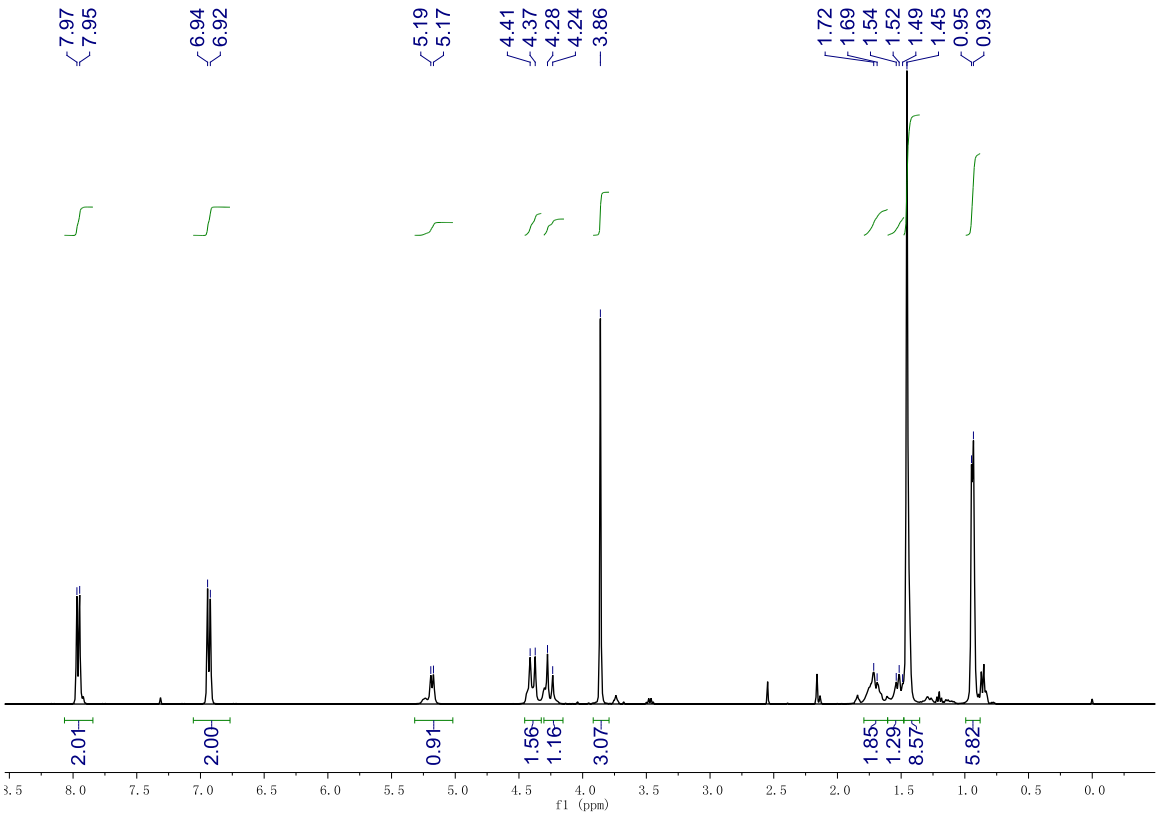
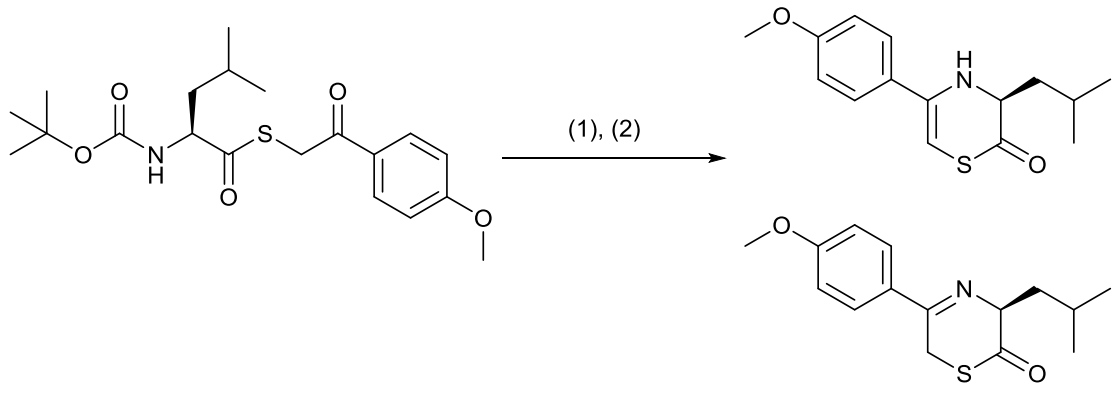


Figure 2.1 ^{1}H NMR spectrum of *iso*-leucine thioester (6)



(**6**) (**7**) and (**8**)

Scheme 2.3 Cyclization of thioester. (1) CF_3COOH:DCM 1:1, (2) K_2CO_3, 4Å molecular sieves, DCM

After removing the Boc group with a 1: 1 mixture of trifluoroacetic acid and dichloromethane, the cyclization was accomplished in the presence of excess potassium carbonate. 4Å Molecular sieves were added to remove the resulting water and move the equilibrium towards cyclization. A new component was observed by TLC analysis and the

reaction was completed within 24 hours, giving rise to a mixture of two isomeric components, (**7**) and (**8**), estimated to be present in a ratio of 1: 1, when examined by ^{1}H NMR spectroscopy.

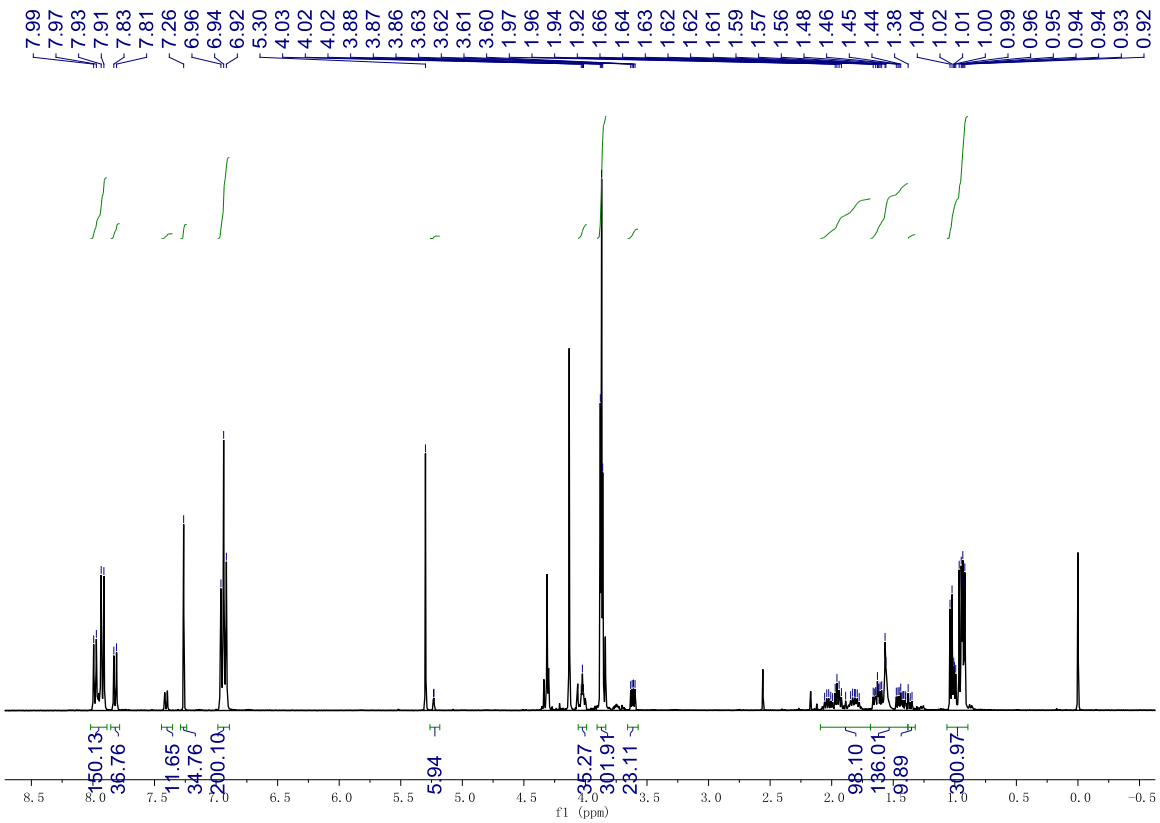
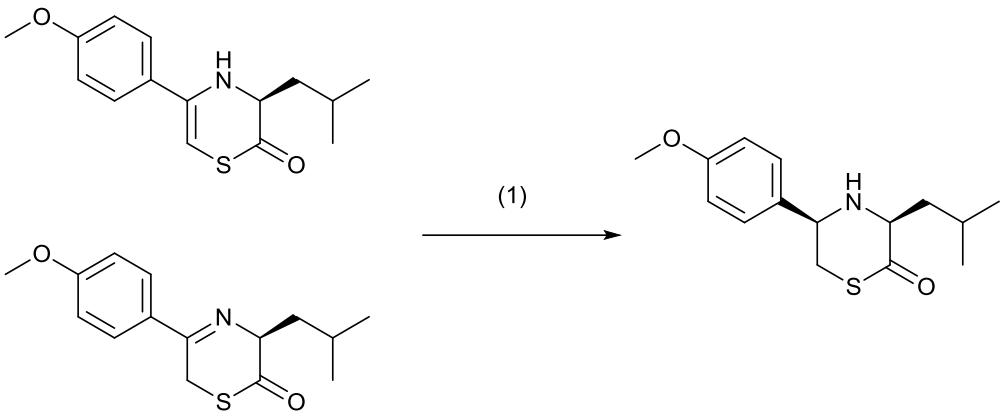


Figure 2.2 ^{1}H NMR spectrum of mixture obtained from cyclizing *iso*-leucine thioester (6)

The two groups of multiplets at δ 7.80 – 7.97 ppm and δ 6.91 – 6.96 ppm were caused by the protons of the aromatic rings of the two products and a narrow doublet centred on δ 5.23 ppm (J = 5.0 Hz) showing a long range coupling with the NH proton corresponded to the C6 vinylic proton of the enamine (**7**). A broadened singlet at δ 3.83 ppm corresponded to the methoxy groups on the aromatic rings of both cyclized materials.



(**7**) and (**8**) (**9**)

Scheme 2.4 Synthesis of leucine thiamorpholinone. (1) $NaBH_3CN$, CH_3COOH, THF

Previous work had shown that the cyclized thioesters with structures related to (**7**) and (**8**) are not stable to silica and decompose on attempted purification by column chromatography, so the mixture was directly used in the next step without any further purification. The next task was to reduce the cyclized product, and sodium cyanoborohydride was chosen as the reducing reagent. After 24 hours, the reduced compound (**9**) was isolated by column chromatography in a low yield of 20% and identified as the desired 3-*iso*butyl-5-(4-methoxyphenyl)thiamorpholinone by NMR analysis. In the ^{1}H NMR spectrum, four peaks appeared at δ 4.22 (dd, J = 11.0, 3.0 Hz, 1H), 3.76 (dd, J = 9.0, 3.5 Hz, 1H), 3.50 (t, J = 11.5 Hz, 1H) and 3.10 (dd, J = 11.5, 3.0 Hz, 1H) ppm corresponding to the four protons on the thiamorpholinone ring. Further evidence was obtained from the mass spectrum in which the molecular ion $[M+H]^+$ could be observed at 280 mass units.

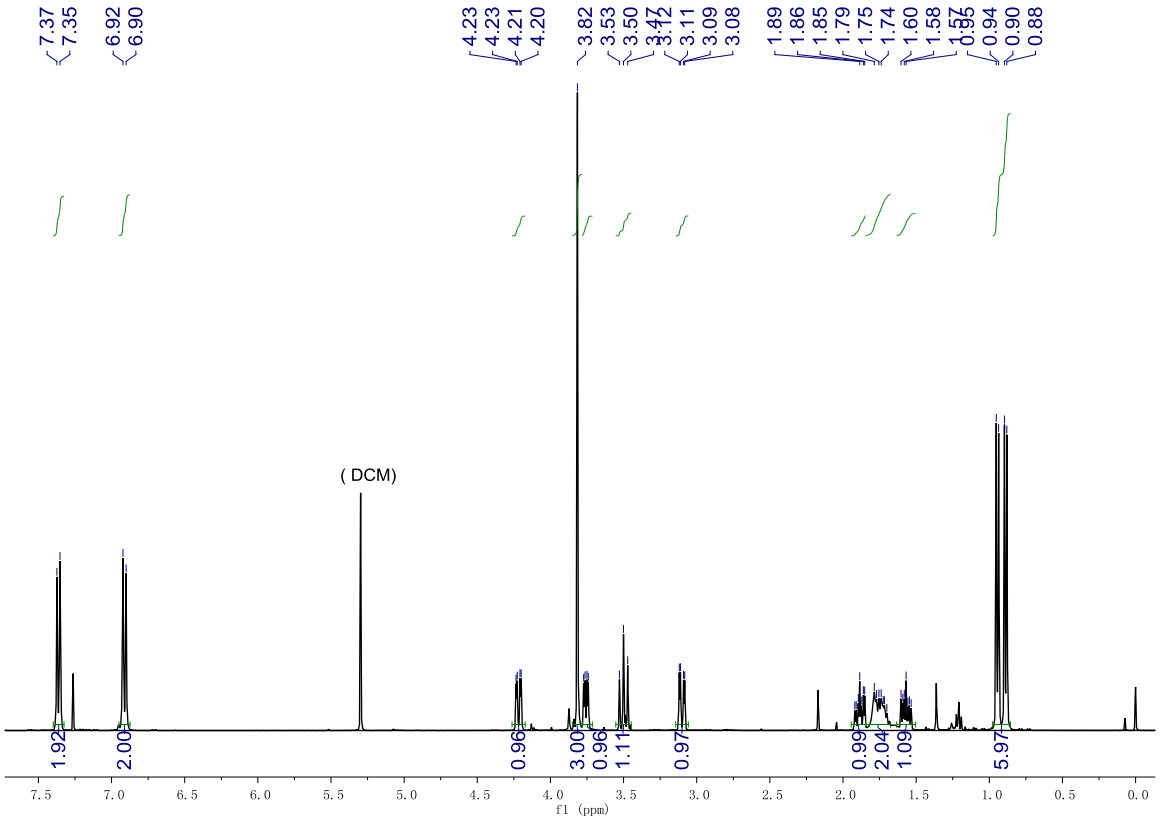


Figure 2.3 ^{1}H NMR spectrum of *iso*-leucine thiamorpholinone (9)

It was also found that this reaction was highly diastereoselective, giving the (3*S*, 5*R*) diastereoisomer (**10**). This was proven by a nuclear Overhauser effect experiment when mutual enhancements of protons at positions 3 and 5 were observed when they were excited

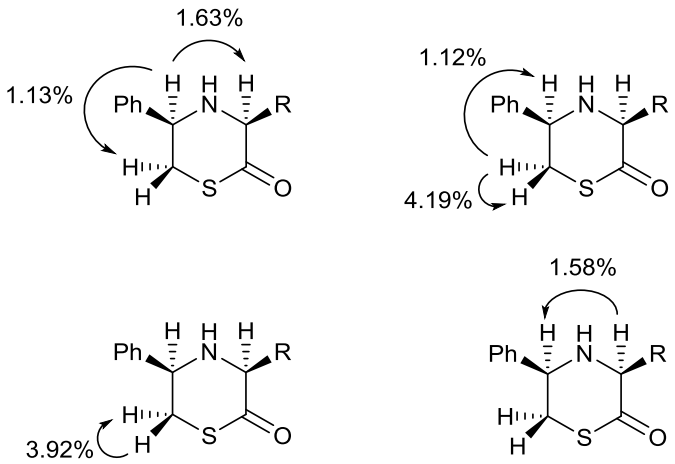
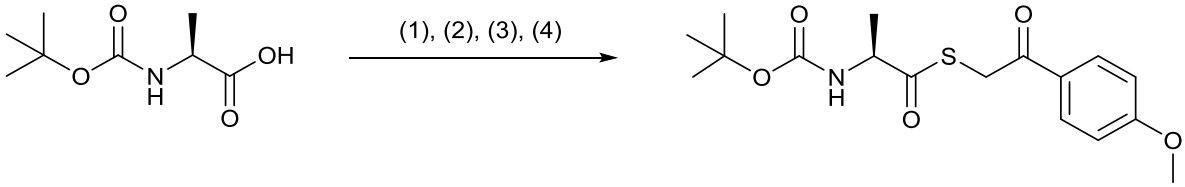


Figure 2.4 Nuclear Overhauser effect experiment on (9)

separately. From this, we could conclude that the cyanoborohydride had attacked the double bond from the opposite face of side chain at C3 giving the 3,5-*syn*-disubstituted thiamorpholinone, in keeping with precedent.

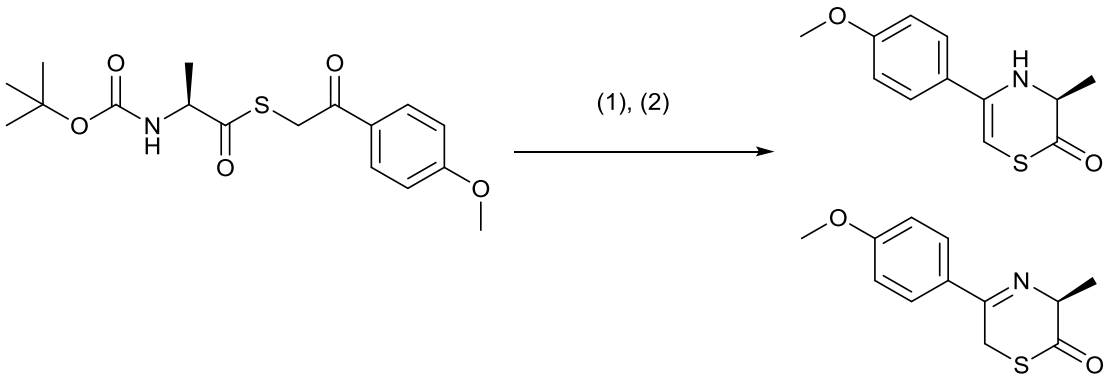
2.2 Synthesis of 5-(4-methoxyphenyl)-3-methylthiamorpholin-2-one (12)



(11)

Scheme 2.5 Synthesis of thioester (11) from Boc-Ala-OH. (1) Et_3N, 0 ^oC; (2) ClCOOEt, 0 ^oC (3) NaSH, 0 ^oC (4) 2-bromo-4'-methoxyacetophenone, r. t.

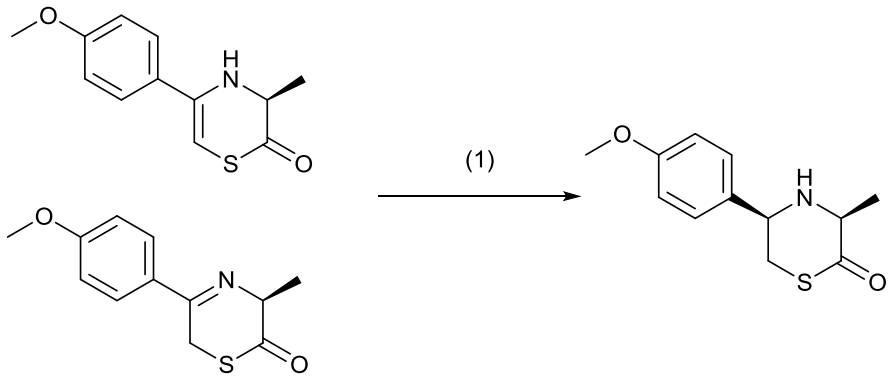
Another investigation into this synthetic route started with Boc-alanine which has a smaller side chain compared to *iso*-leucine as it was proposed that the more steric bulk of the C-3 substituent might be influencing the cyclization equilibrium. The corresponding thioester (**11**) was synthesized under the same conditions as previously. After evaporating the solvent, the crude product was purified by a column chromatography. The resulting colourless solid was characterized by ^1H NMR analysis with the expected singlet due to the methoxy group at δ 3.88 ppm and the other slightly broadened 9H singlet between δ 1.50 - 1.35 ppm due to the Boc group observed. It was found that, after column chromatography, the product crystallized from a mixture of diethyl ether and petrol. After multiple attempts, ethyl acetate and hexane was found to be a better combination for the recrystallization giving a better yield. This recrystallization method was therefore also applied to the *iso*-leucine thioester (**9**), which improved the yield from 49 % to 58 %.



(**11**) (**12**) and (**13**)

Scheme 2.6 Cyclization of thioester. (1) CF_3COOH:DCM 1:1, (2) K_2CO_3, 4Å molecular sieves, DCM

The same procedure as previously was used to the cyclize the alanine thioester, using TFA/DCM to remove the Boc group and potassium carbonate in the presence of 4 Å molecular sieves for the cyclization. The desired cyclic products (**12**) and (**13**) were obtained in a combined yield of 47% and their structures were confirmed by ^{1}H NMR analysis. A doublet at δ 5.22 ppm corresponded to the alkene proton and proved the presence of enamine (**12**). Two doublets at δ 4.32 and 4.11 were due to the proton on methylene group of the imine isomer (**13**), and a multiplet at 4.43 ppm was assigned to the single hydrogen between the NH and methyl group of (**13**).



(12) and (13) **(14)**

Scheme 2.7 Synthesis of alanine-derived thiamorpholinone. (1) $NaBH_3CN$, CH_3COOH, THF

The reduction of these isomeric dehydrothiamorpholinones was again carried out with sodium cyanoborohydride and one equivalent of acetic acid in THF. Analysis by T.L.C. indicated the mixture was converted to a slightly less polar compound that was isolated by column chromatography on silica, eluting with petrol and diethyl ether (2:1), in a yield of 25%. The resulting product was characterized by ^{1}H NMR spectroscopy. Two double doublets at δ 4.25 ppm (J = 11.0, 3.0 Hz) and δ 3.09 ppm (J = 11.0, 3.0 Hz), one quartet at δ 3.86 ppm (J = 6.5 Hz) and one triplet at δ 3.53 ppm (J = 11.5 Hz) were observed, corresponding to the four protons on the thiamorpholinone ring, and a doublet at δ 1.38 ppm (J = 6.5 Hz) was assigned to be the methyl group. Additionally, the mass spectrum contained a mass ion at 238 of $[M+H]^{+}$, further evidence for the formation of the thiamorpholinone (**14**).

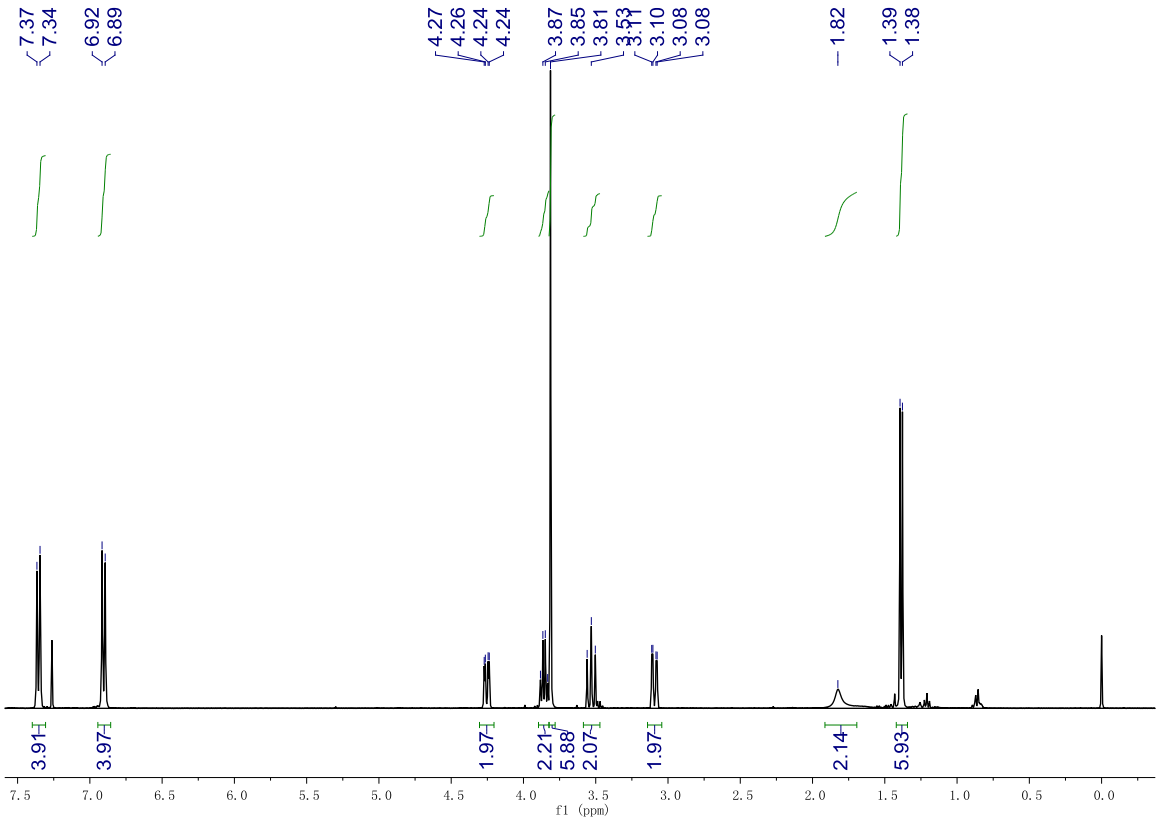
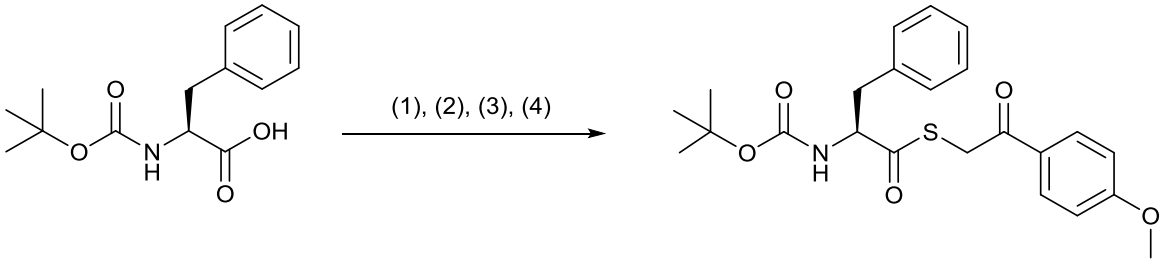


Figure 2.5 ^{1}H NMR spectrum of the 3-(*S*)-methyl-5-(*R*)-5-(4-methoxyphenyl)thiamorpholin-2-one (14)

The material was found to be diastereoisomerically pure and so, in accordance with the previous observation, the cyanoborohydride was presumed to attack the double bond from the opposite face of the C3 methyl group to give the (3*S*,5*R*) diastereoisomer.

2.3 Synthesis of phenylalanine-based thiamorpholinone

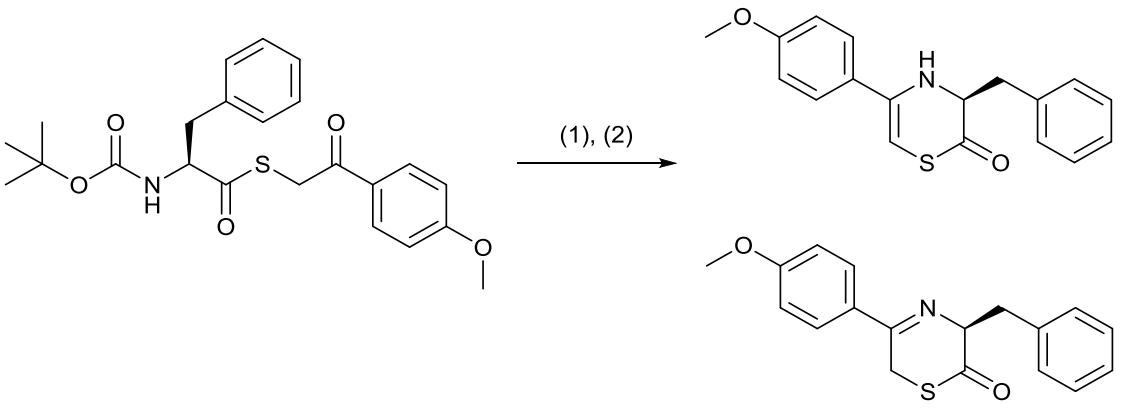
After successful synthesis of the isoleucine and alanine derived 5-(4-methoxyphenyl)thiamorpholinones, attention turned to synthesis of the phenylalanine-derived 5-(4-methoxyphenyl)thiamorpholinone.



(**15**)

Scheme 2.8 Synthesis of thioester (15) from phenylalanine. (1) Et_3N, 0 ^oC; (2) ClCOOEt, 0 ^oC (3) NaSH, 0 ^oC (4) 2'-bromo-4-methoxyacetophenone, r.t.

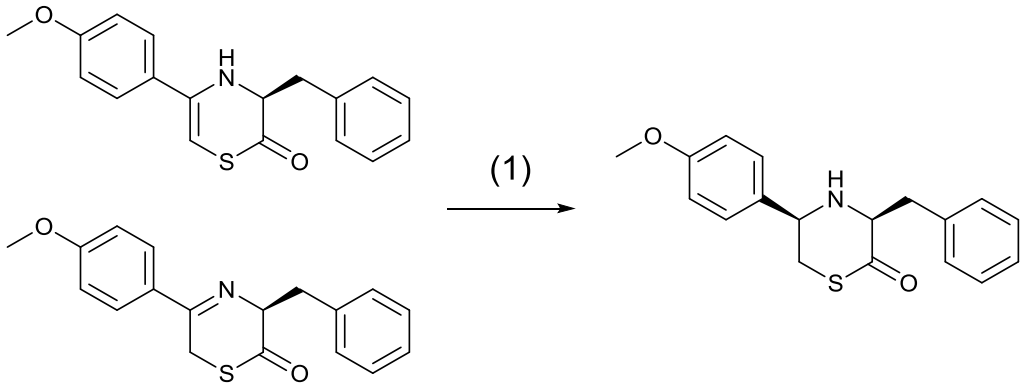
Using same conditions that afforded the *iso*leucine and alaline-derived 5-(4-methoxyphenyl)-thioesters, Boc-phenylalanine gave thioester (**15**) in 69% purified yield after recrystallization in ethyl acetate and hexane (**Scheme 2.8**). The ^1H NMR spectrum showed the methoxy singlet at δ 3.88 ppm, and the protons of Boc group were observed as a broad singlet at δ 1.42 ppm, with multiplets at δ 7.32 -7.12 ppm and doublets at δ 7.98 and 6.95 ppm corresponding to the aromatic protons of the phenyl and 4-methoxyphenyl groups. Four doublets at δ 4.42, 4.27, 3.18 and 3.06 ppm corresponded to two AB coupling systems, CH_2Ph and SCH_2 respectively. CH was found at δ 4.68 ppm as a multiplet.



(**15**) (**16**) and (**17**)

Scheme 2.9 Cyclization of thioester. (1) CF_3COOH:DCM 1:1, (2) K_2CO_3, 4 Å molecular sieves, DCM

Deprotection was again performed using 1:1 TFA/DCM followed by cyclization in DCM in the presence of potassium carbonate and 4 Å molecular sieves. The generated dehydrothiamorpholinones, enamine (**16**) and imine (**17**), were confirmed by mass spectrometry with a peak at 312 of $[M+H]^+$ being observed.



(**16**) and (**17**) (**18**)

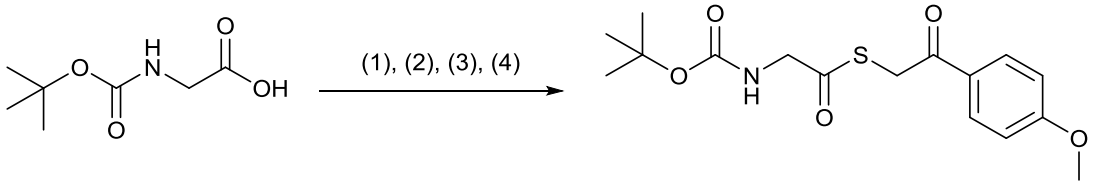
Scheme 2.10 Synthesis of phenylalanine-derived thiamorpholinone (18). (1) $NaBH_3CN$, CH_3COOH, THF

Using the same conditions as previously, the sodium cyanoborohydride and acetic acid, reduction was carried out to generate the 3-benzyl-5-(4-methoxyphenyl)thiamorpholinone (**18**). The product was characterized by ^1H NMR analysis with two double doublets at δ

4.11 ppm (J = 11.0, 3.0 Hz) and 3.01 ppm (J = 11.0, 3.0 Hz) corresponding to CH_2S protons, a triplet at δ 3.44 ppm (J = 11.0 Hz) from CHAr proton, a double doublet at δ 3.94 ppm (J = 10.0, 3.0 Hz) corresponding to NCH proton and two double doublets at δ 3.51 ppm (J = 14.0, 3.0 Hz) and 2.82 ppm (J = 14.0, 10.0 Hz) presenting the protons of CH_2Ph. Unfortunately even through the recovery of material after column chromtography was improved by adding 1 % triethylamine to the eluting solvent, the yield of thiamorpholinone was a disappointing 36%.

2.4 Synthesis of Boc-Glycine-based thiamorpholinone

Glycine is the simplest amino acid and is achiral, with hydrogen forming its side chain. It is also one of the most abundant amino acids. Consequently, it was of great interest to incorporate this amino acid into thiamorpholinone templates, but due to the lack of steric bulk at C-3, it was feared that synthesis of parent thiamorpholinone would prove to be problematic.



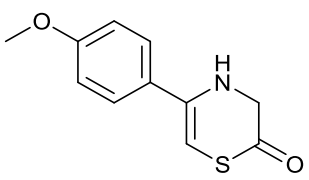
(19)

Scheme 2.11 Synthesis of glycine-derived thioester. (1) Et_3N, 0 ^oC; (2) ClCOOEt, 0 ^oC (3) NaSH, 0 ^oC (4) 2-bromo-4'-methoxyacetophenone, r.t.

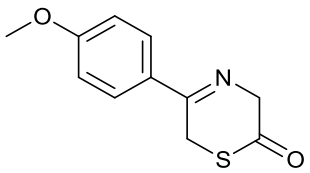
Thus, the Boc-glycine was treated with triethylamine, ethyl chloroformate and sodium hydrosulfide successively to form the corresponding thioacid sodium salt. Then this was reacted *in situ* with 2'-bromo-4-methyacetophenone to give thioester **(19)**, which was purified by recrystallization and analysed by ^1H NMR. Two singlets corresponding to the methoxy group and the Boc protecting group at δ 3.88 and 1.46 ppm respectively were observed and a 2H singlet at δ 4.38 ppm and a 2H doublet at δ 4.13 ppm (J = 6.0 Hz)

corresponding to the two methylene groups were also present. The yield of purified thioester was 56%.

After the standard removal of the Boc group, the thioester was treated with potassium carbonate and 4 Å molecular sieves in DCM in an attempt to generate the cyclized thioesters (**20**) and (**21**). The desired reaction did appear to occur, with two new components being visible on the TLC plate. Based on the evidence of the mass spectrum, which showed molecular ion at 222 of $[M+H]^+$, we concluded that the glycine thioester had cyclized to give (**20**) and (**21**).



(**20**)



(**21**)

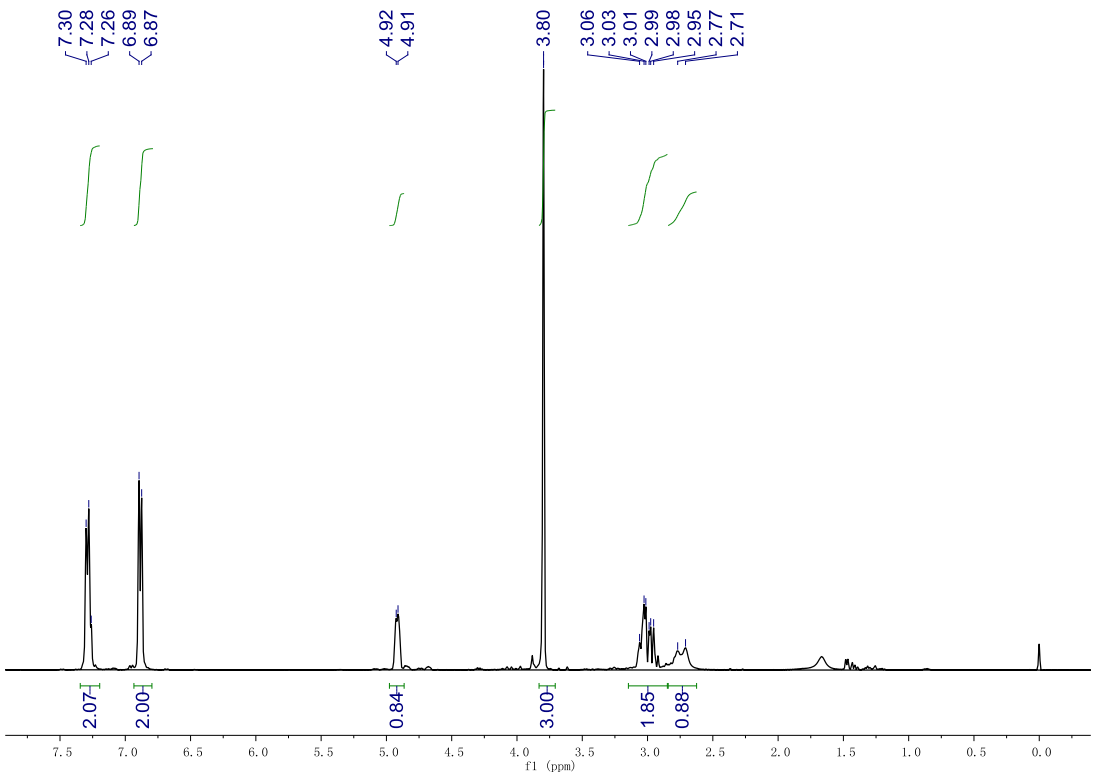


Figure 2.6 ^{1}H NMR spectrum of the first product of reduction of dehydrothiamorpholinones of (20) and (21)

However, following addition of sodium cyanoborohydride and acetic acid, the expected reduction did not appear to have taken place. The characteristic ABX system expected around δ 3.0 to 4.5 ppm and the AB system expected around δ 4.0 ppm were not present in the NMR spectrum of the crude material. From the spectra of two components separated by chromatography, one had only 4 protons in addition to those of the methoxy group and phenyl ring, and the other appeared at first sight to be a mixture of two inseparable compounds. However, the mass spectrum showed a peak at $^{m}/_{z}$ = 385 which could correspond to a fragment peak of a dimer of the thiamorpholinone that has lost a molecule of SO_2. Repeated attempts were made to carry out this reduction, but all failed, so it was decided that new reduction conditions would need to be explored.

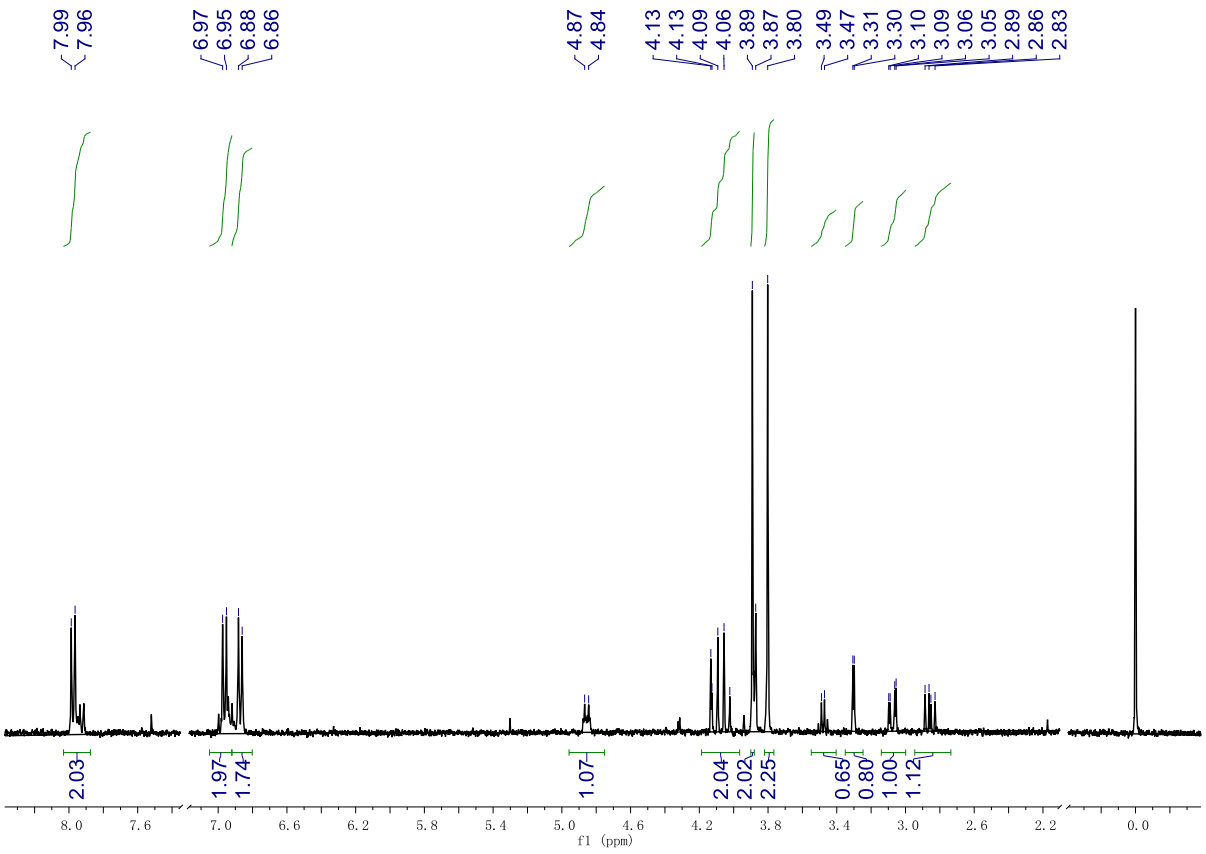
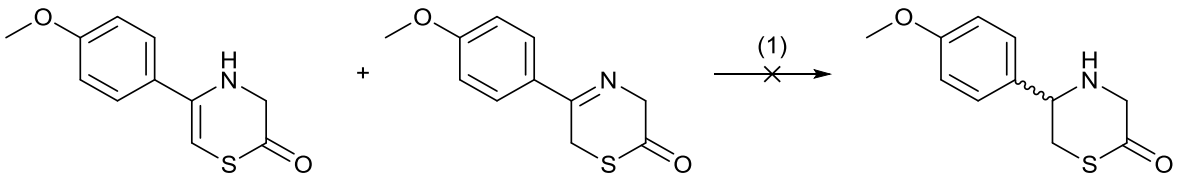


Figure 2.7 ^{1}H NMR spectrum of the second product of reduction of dehydrothiamorpholinones (20) and (21)

2.5 Attempts at preparing the parent thiamorpholinone using other reducing reagents

Palladium is a very well-known hydrogenation catalyst,[48] and our first choice was to use palladium on carbon, even though we were concerned about the possibility of poisoning of the catalyst due to the sulfur contained in the reduction substrates.



(**20**) (**21**) (**22**)

Scheme 2.12 Proposed synthesis of glycine thiamorpholinone (22). (1) Pd/C, H_2

Indeed, after more than 24 hours, starting material still remained, and ^{1}H NMR spectroscopy did not show evidence for the desired ABX coupling around δ 3.0 ppm or the AB system at δ 4.0 ppm. Thus it was concluded that the palladium catalyst had indeed been poisoned by sulfur contained in starting materials (**20**) and (**21**).

Adams's catalyst is less reactive than palladium as a hydrogenation catalyst, but is less liable to be poisoned by sulfur.[49] however, once again the expected hydrogenation did not happen with spectroscopic analysis of the crude material indicating that hydrogenolysis of the benzylamine bond might have occurreded instead.

Wilkinson's catalyst is a homogeneous hydrogenation catalyst[50] with the mechanism involving initial dissociation of triphenylphosphine ligands, followed by oxidative addition of H_2, insertion of alkene, intramolecular hydride transfer, and reductive elimination (**Figure 2.8**).

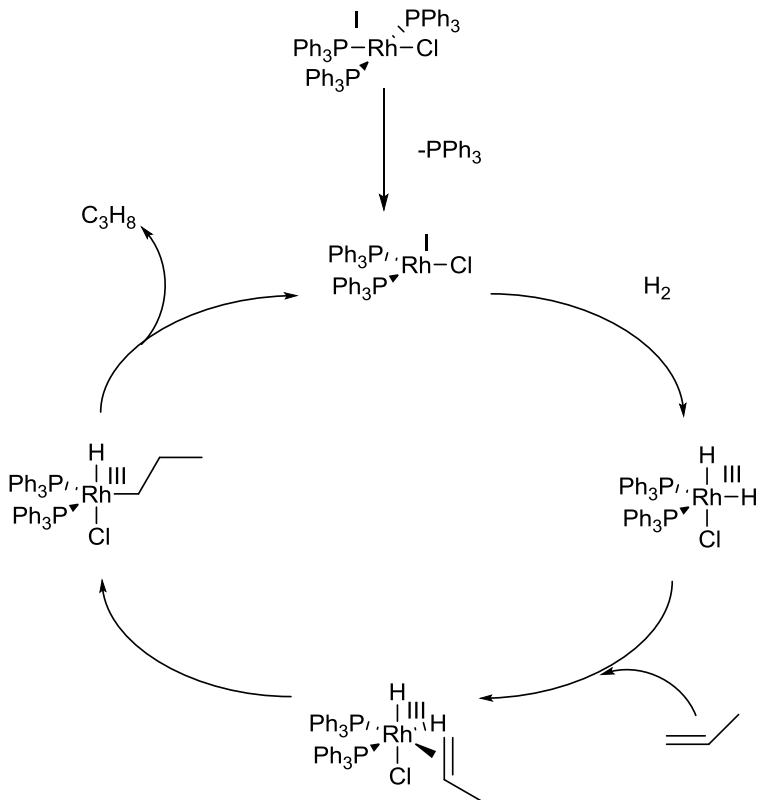


Figure 2.8 Catalytic cycle for Wilkinson's catalyst reduction of an alkene

The reaction was carried out in toluene that had been purged with a stream of hydrogen for 10 min. Then starting materials (**20**) and (**21**) were added and the solution was allowed to stir under a balloon of nitrogen. However, after 24 hours, only the same product was recovered as from the reaction using Adam's catalyst.

Diimide generated by oxidizing hydrazine with a copper (II) salt at 60 ^{o}C has been used to reduce alkenes in the presence of other sensitive, potentially reducible functionalities,[51] but it was feared that high temperature could cause decomposition of any thiamorpholinone formed. So an alternative approach was considered in which the hydrazine is reacted with the 2-nitrobenzenesulfonyl chloride at 0 ^{o}C,[52] to generate the 2-nitrobenzenesulfonylhydrazide (NBSH). This NBSH reagent reduces alkene under much milder conditions than those required to generate diimide from hydrazine itself, which it was hope would prevent the decomposition of any thiamorpholinone. But after 18 hours, no reaction had taken place.

Diborane has the capacity to reduce alkenes, alkynes, cyclopropanes, organic halides and epoxides,[53] and so it was hoped that diborane would have the ability to reduce the enamine (**20**) and imine (**21**). Fresh diborane was prepared by adding a solution of sodium borohydride in diglyme to a solution of boron trifluoride in the same solvent,[54] then the resulting diborane was introduced into methanol to form a diborane-methanol system.[55] It was reported that diborane reacted smoothly with methanol to give dimethoxyborane via methoxyborane and to reduce imines.[56] However, once again, the proton spectrum showed absence of the ABX coupling system and AB coupling system. However, according to mass spectrometry, the crude product was found to contain the same compound as that from cyanoborohydride reduction, a thiamorpholinone dimer.

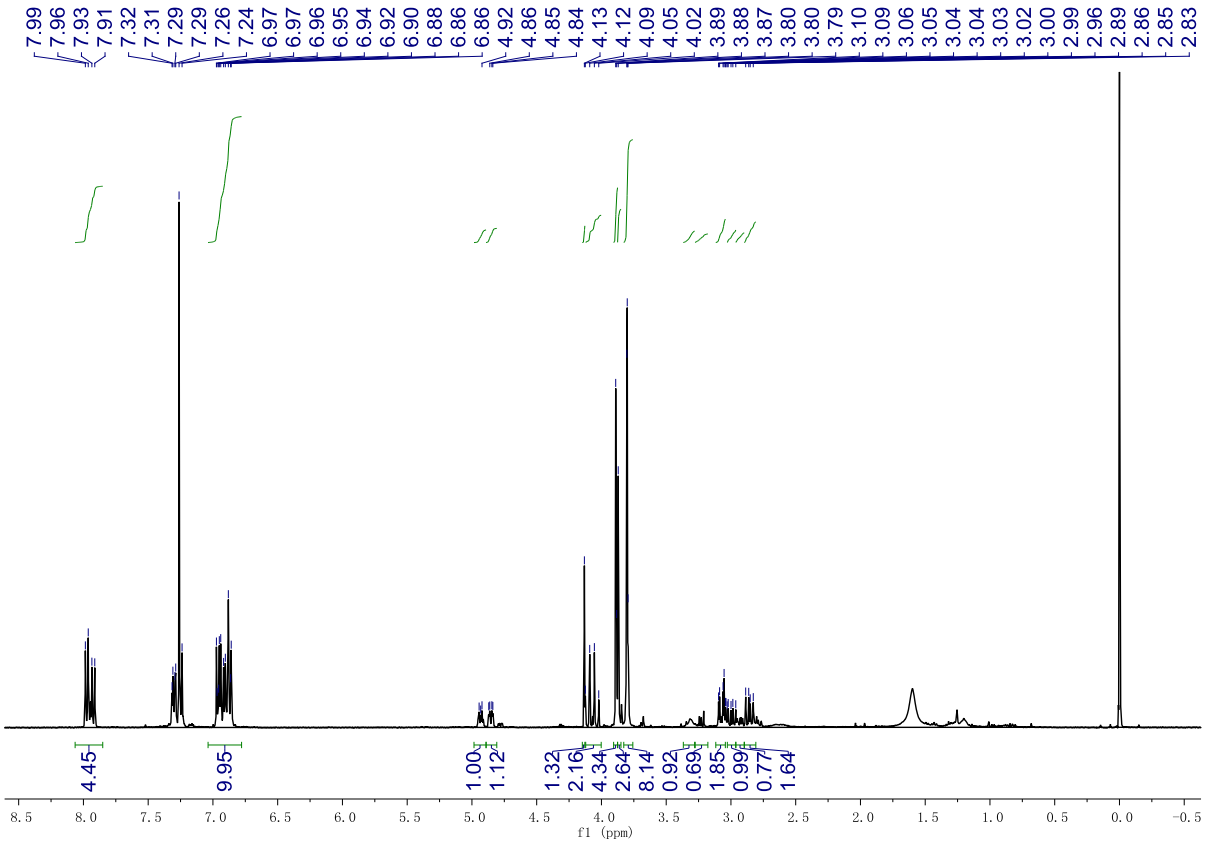


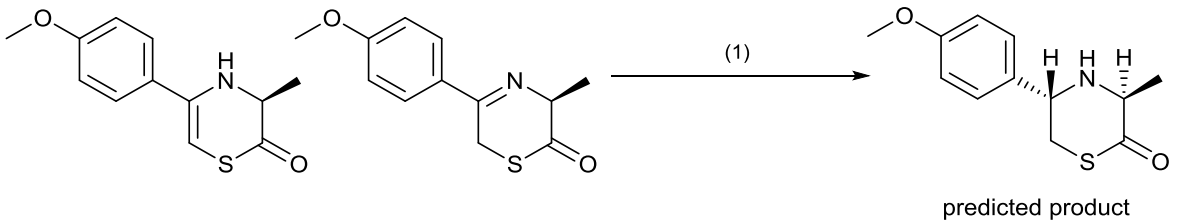
Figure 2.9 ^{1}H NMR spectrum of the diborane reduction product

In conclusion, the direct reduction of the enamine/imine mixture of (**20**) and (**21**) to parent thiamorpholinone was not successful with only dimerized product being isolated. More reduction conditions need to be explored, and another synthetic route to C-3 unsubstituted

thiamorpholinone should be considered as well.

2.6 Attempted alternative stereocontrolled reduction using L-Selectride

By using $NaBH_3CN$ as the reducing agent, the 3,5-disubstituted dehydrothiamorpholinone can be reduced stereoselectively to give the (3*S*, 5*R*)-thiamorpholinones (**9**), (**14**) and (**18**) by axial attack of hydride. However, it is reported that L-Selectride can also reduce cyclic imines by equatorial attack and, with our substrates, this would lead to the alternative (3*R*, 5*R*)-diastereoisomers such as (**24**).[57]



(**12**) (**13**) (**24**)

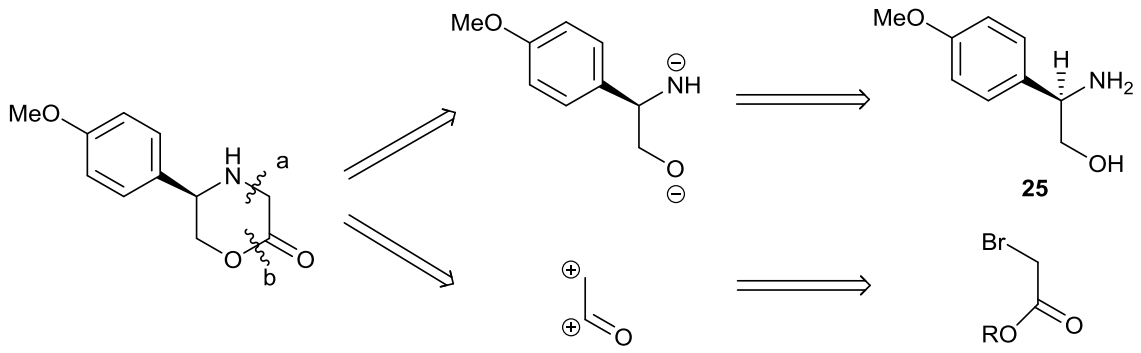
Scheme 2.13 Proposed stereochemical course of reduction of L-Selectride. (1), L-Selectride, THF, -78 ^{o}C, 1.5h

Once again Boc-alanine was used as starting material to form the thioester (**14**), following the previous procedure. After deprotection of the Boc group and cyclization, the crude cyclic enamine-imine mixture (**12**) and (**13**) was dissolved in anhydrous THF and cooled to -78 ℃ under an atmosphere of nitrogen. Then excess L-Selectride was added and the resulting solution left at -78 ℃ for 1.5 hours and then allowed to warm to room temperature. The solution was then quenched by 1 M hydrochloric acid and extracted with DCM. After drying, solvent was removed *in vacuo* to give the crude product. However, ^{1}H NMR analysis of the crude product did not show the expected mutiplets around δ 2 to 4 ppm, which would indicate the presence of reduced product. As a result, the crude product was subjected to column chromatography in an attempt to isolate any components. Combinations of THF or diethyl ether and petrol ether 1:1 were tried, but none of them yielded any identifiable material. Clearly more work is needed to access this more

challenging target.

Chapter 3 Indirect synthesis of C-3 unsubstituted thiamorpholinone

Our investigations have indicated that the C-3 unsubstituted thiamorpholinone system could not be synthesized by cyclizing the corresponding thioester directly. According to an alternative retrosynthetic analysis, one possible solution would be to attempt to prepare the target molecule from the corresponding unsubstituted morpholinone. The morpholinone itself prepared as shown in **Scheme 3.1** with the R group on the bromoacetate being either phenyl or vinyl. Aminoethanol (**25**) could be prepared from the corresponding amino acid (*R*)-4-hydroxyphenylglycine.

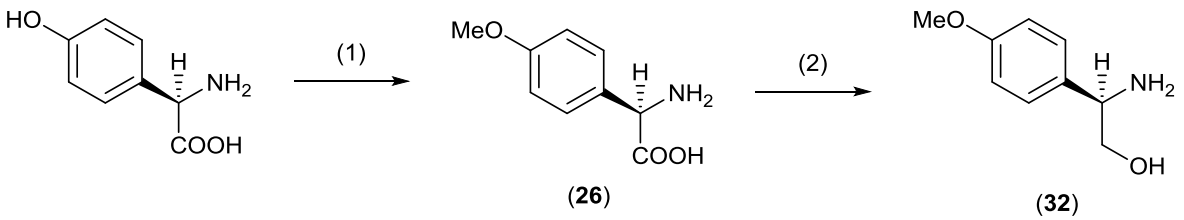


Scheme 3.1 Retrosynthetic analysis of unsubstituted morpholinone

3.1 Synthesis of 5-(4-methoxyphenyl)morpholin-2-one

According to Mountford,[58] the phenolic oxygen of 4-hydroxyphenylglycine can nucleophilically attack benzyl group without any protecting group, but using copper to complex with and protect both carboxylic and amine groups.

In the first step, (*R*)-4-hydroxyphenylglycine was reacted with iodomethane in the presence of copper sulfate and base. The copper selectively coordinates with the carboxylic acid and amine groups, leaving the phenolic hydroxyl group free for methylation.

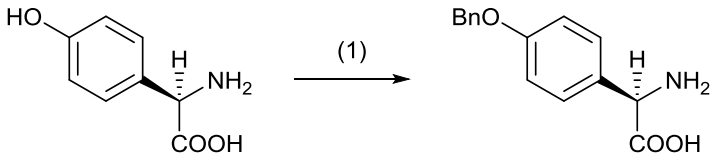


Scheme 3.2 Synthesis of *(R)*-2-amino-2-(4-methoxyphenyl) ethanol (32) via copper coordination. (1), $CuSO_4.5H_2O$, MeI, NaOH(aq), MeOH, reflux; (2) $NaBH_4$, I_2, THF, MeOH, 0 ^oC, then reflux 3 h, r.t., overnight

The first step was to add a solution of $CuSO_4.5H_2O$ in H_2O to a stirred solution of (*R*)-4-hydoxyphenylglycine in 2M NaOH at ambient temperature. The mixture was heated to reflux for 1 h and then cooled to ambient temperature before MeOH and a further 2M NaOH were added to ensure full deprotonation of the phenol. Iodomethane was then added, and the reaction was stirred at ambient temperature overnight. The resulting precipitate was collected and washed with water and methanol, suspended in 1M HCl and stirred for 2 h at ambient temperature to destroy the coordination with copper. After filtration, the crude material was washed with water and aqueous ammonia to remove the copper salts and residual acid. However, all the material was unexpectedly soluble, and no solid remained on the sinter after this treatment.

As this procedure had previously been shown to work for *O*-benzylation, the original procedure was repeated (**Scheme 3.3**). After addition of the solution of $CuSO_4$ to hydroxyphenylglycine in NaOH solution, the mixture was heated to reflux for 1 h and then cooled down to ambient temperature, giving a pale blue solution. Methanol, sodium hydroxide and benzyl bromide were added, and the mixture was stirred overnight. The brown precipitate was washed with water and methanol, suspended in 1 M HCl and stirred for a further 2 hours. The precipitate remaining after washing with water and aqueous ammonia was examined by ^1H NMR spectroscopy. ^1H NMR analysis showed a multiplet at δ 7.36 – 7.43 ppm and two doublets at δ 7.33 ppm and 7.08 ppm corresponding to protons on the mono- and disubstituted benzene rings, and two singlets corresponding to the three

remaining protons, while $–NH_2$ and –COOH protons were invisible in the deuterated methanol. Thus, this method yields the benzylated product, but does not appear to work in the corresponding methylation process.



(**27**)

Scheme 3.3 Synthesis of (*R*)-2-amino-2-(4-(benzyloxy)phenyl)acetic acid. (1), $CuSO_4.5H_2O$, BnBr, NaOH(aq), MeOH, reflux

After searching the literature,[59] it was found that the desired compound (**26**) (**Figure 3.1**) will combine with hydrochloric acid to give a salt, which is soluble in water. The hydrochloric acid can later be eliminated by heating the solution to give desired product (**26**). However, in our system, heating could make the product coordinate with copper again, and such a complex would still be soluble in water. So, the methylation reaction was repeated, the work-up carried out under neutral conditions to retain the amine and carboxylic groups instead of working with an ionic species. Again, no product could be isolated and it would appear that the product is more soluble in water than in ethyl acetate or dichloromethane even at pH 7.

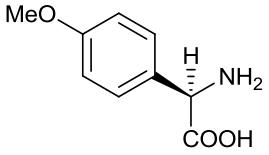
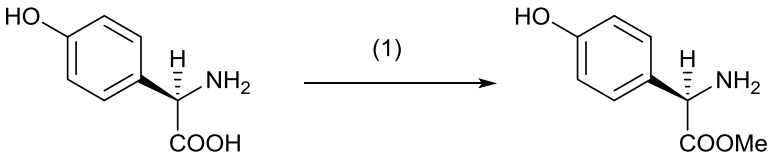


Figure 3.1 Desired compound (26)

Since the method which used copper to coordinate with the amino acid was not applicable with our 4-methoxyphenyl system, the nucleophilic substitution at the phenol group would need to be carried out after protection of both the carboxylic acid and amine groups.

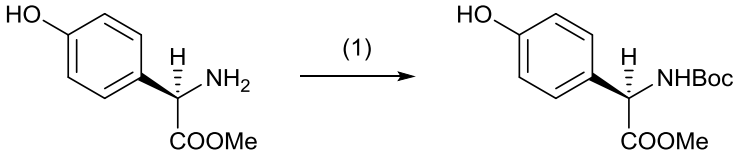


(**28**)

Scheme 3.4 Protection of carboxylic acid group. (1) MeOH, $SOCl_2$, r.t., 12 hr, 100%

The first step was the esterification of (*R*)-(4-hydroxyphenyl)glycine using Seto's method.[60] (*R*)-(4-hydroxyphenyl)glycine was mixed with MeOH under one atmosphere of N_2, and $SOCl_2$ was added. The reaction was stirred overnight at ambient temperature and after removal of the solvent *in vacuo* and washing of the residue with diethyl ether, the desired product was isolated as a pink solid in quantitative yield. ^{1}H NMR spectroscopic analysis indicated a singlet at δ 3.81 ppm corresponding to the three protons of the methyl ester.

Chen's method[60] was then used to protect the amine group using di-*tert*-butyldicarbonate. Sodium hydrogencarbonate was added to a solution of amino acid ester (**28**) in water to deprotonate the hydrogen on the phenol group. Then a solution of di-*tert*-butyldicarbonate in dioxane was added to the mixture, and the reaction stirred in ice for one hour. After removal of the ice bath, the reaction was stirred for 12 hours more when TLC analysis revealed that a single product had been formed with disappearance of the starting material.

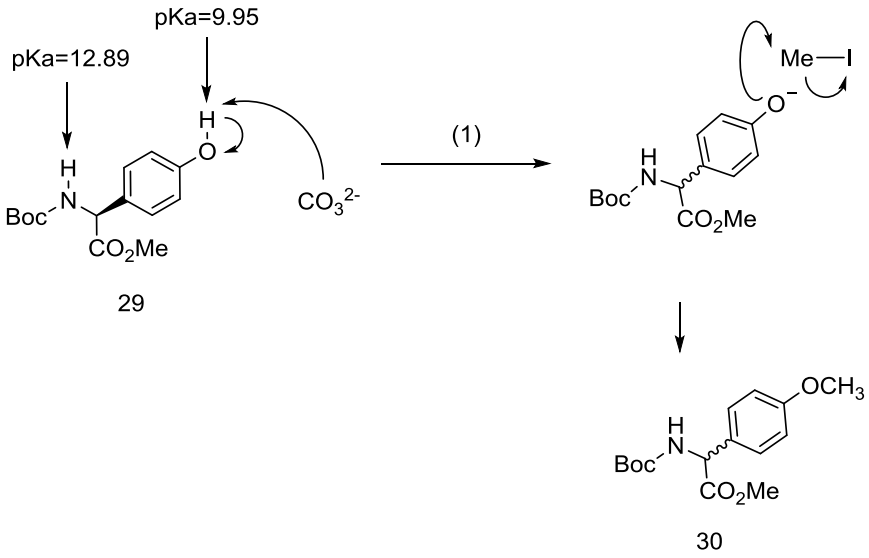


(**28**) (**29**)

Scheme 3.5 Protection of the amine group. (1) $NaHCO_3$ in H_2O, Boc_2O in dioxane, 0 ^oC 1 h, r.t. 12 h, 98%

^{1}H NMR spectroscopy helped to identify the product as evidenced by the appearance of a broad singlet at δ 1.43 ppm integrating for 9 protons.

Caesium carbonate, Cs_2CO_3, a stronger base than K_2CO_3, was our first choice to deprotonate the phenol group prior to methylation.



Scheme 3.6 Methylation of the hydroxyphenyl group. (1) Cs_2CO_3, THF, MeI, r.t., 24 h, 70-80 %

This reaction was found to give a satisfactory yield of 79 % at room temperature over a 24 hour period. The mass ion $[M+H]^+$ at 296 helped to identify product (**30**). In addition the IR spectrum showed two bands at 3365 cm^{-1} and 2989 cm^{-1} corresponding to the N-H and aromatic C-H stretching respectively. Compared with the IR spectrum of compound (**29**), the absence of an O-H group stretch around 3430 cm^{-1} gave further evidence for successful methylation. Finally, the appearance of a second 3-proton singlet at δ 3.72 ppm was conclusive evidence for the aryl methoxy group (**Figure 3.2**).

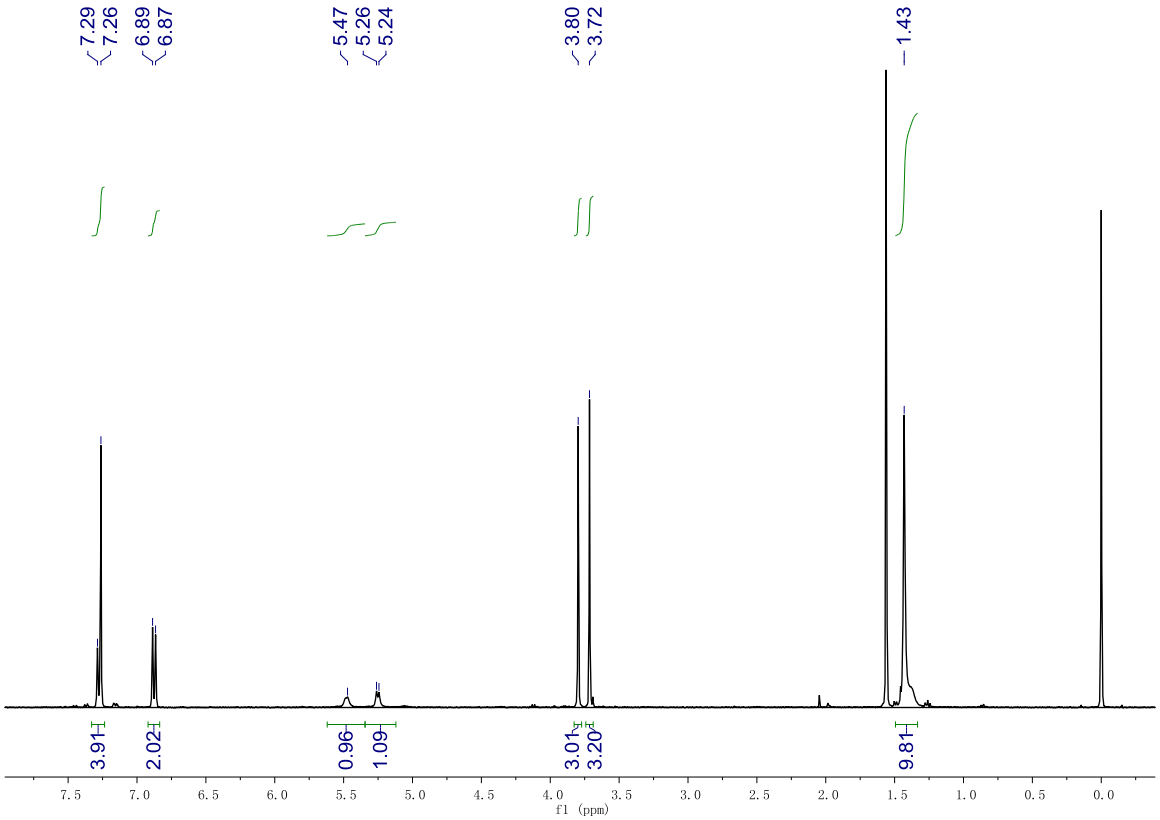
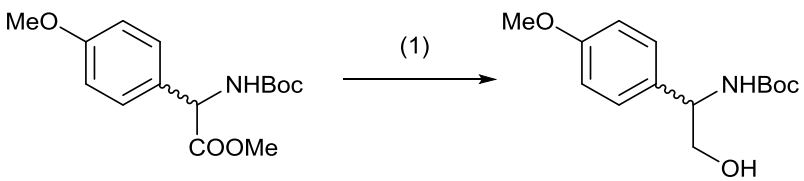


Figure 3.2 The ^{1}H NMR spectrum of (*R*)-methyl 2-(*tert*-butoxycarbonylamino)-2-(4-methoxyphenyl)acetate (30)

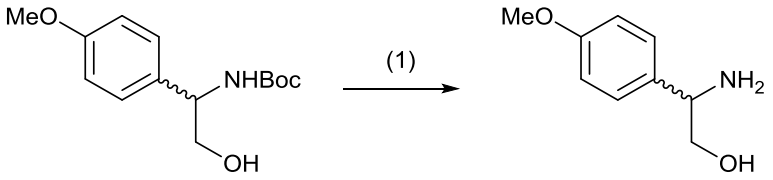
To attempt to improve the yield of this reaction, 5.0 equivalents of iodomethane were added to the reaction. However, no change was observed in the yield after purification. The reaction was also attempted under reflux, but this did not improve the yield either. Instead the product was obtained as a racemate. According to the literature,[61] product (**30**) obtained at room temperature was partially racemized and so in must be concluded that the caesium carbonate can deprotonate (**30**).



(30) **(31)**

Scheme 3.7 Reduction of the ester group. (1) $NaBH_4$, THF, MeOH, 65 ℃ 15 min, 85 ℃ 4 h

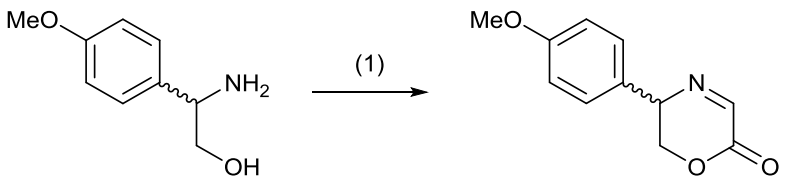
Despite this, the reduction of the ester group using $NaBH_4$ was carried out successfully using a method reported by Saeed *et al*.[62] Sodium borohydride was added to a stirred solution of compound (**30**) in THF, followed by stirring the suspension at 65 ℃ for 15 minutes to speed up dissolution of $NaBH_4$. An equal volume of methanol was added and the mixture was refluxed for 3.5 hours (**Scheme 3.7**). After cooling to room temperature the mixture was quenched with 1 M HCl, basified to pH 10 using potassium hydroxide pellets and extracted with ethyl acetate. The organic phase was dried and concentrated *in vacuo* to obtain a white solid. ^{1}H NMR analysis showed a singlet at δ 3.86 ppm corresponding to the presence of the methylene protons and a broad peak at δ 2.33 ppm corresponding to the OH proton. The IR spectrum showed a broad absorbance centred on 3221 cm^{-1} indicating the presence of an alcohol.



(**31**) (**32**)

Scheme 3.8 Deprotection of the amino group. (1) 50% Trifluoroacetic acid in DCM, r.t., 60 min, quantitative yield

Removal of the Boc group utilised 50% trifluoroacetic acid in DCM at room temperature (**Scheme 3.8**). Monitoring the reaction by TLC showed complete consumption of the starting material after 60 minutes. The solvent was then removed *in vacuo* and the residual oil was dissolved in ethyl acetate and washed with 20% aqueous NaOH. After removal of the solvent, desired product (**32**) was obtained as a white solid in quantitative yield. The ^{1}H NMR spectrum revealed the absence of the characteristic broad singlet at δ 1.43 ppm corresponding to the *t*-butyl protons of the protecting group.



(32) **(33)**

Scheme 3.9 Synthesis of the morpholinone. (1) Ethyl glyoxylate, 4Å molecular sieves, TFE, reflux, 24 h

Following precedent from the Harwood group using pyruvate esters to condense with phenylglycinol,[40] it was proposed to use amino-alcohol (**32**) and ethyl glyoxylate to generate the dehydromorpholinone. Ethyl glyoxylate was added to a solution of amino-alcohol (**32**) in 2,2,2-trifluoroethanol and activated molecular sieves were added to extract the water generated during this reaction. The mixture was refluxed overnight and filtered through Celite®. After concentration *in vacuo*, the crude residue was purified by

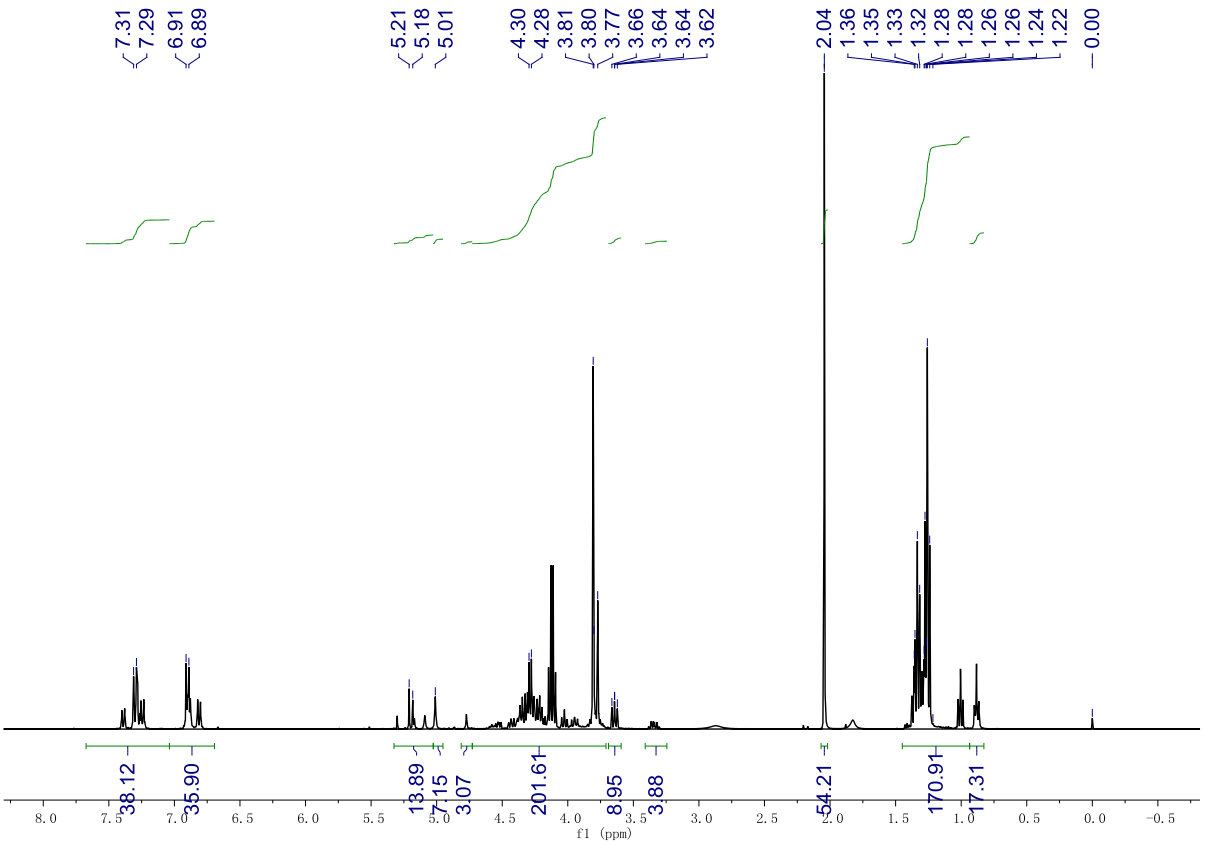
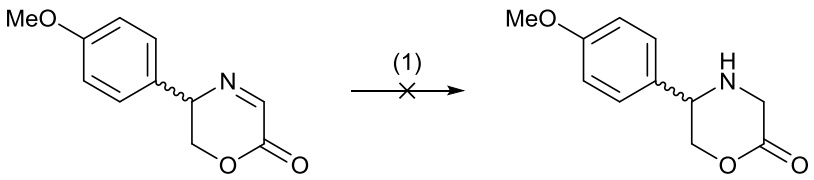


Figure 3.3 The spectrum of the crude material of dehydrogenated morpholinone (33)

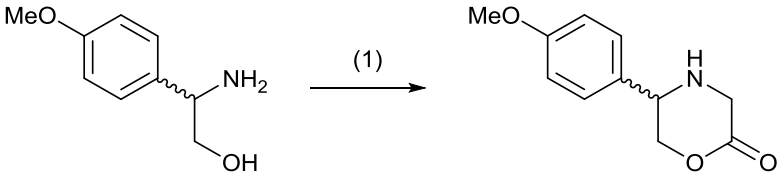
column chromatography eluting with ethyl acetate and petroleum ether (1:3), furnishing two inseparable products. Even after recrystallization, the ^{1}H NMR spectrum was complex but it was considered that the desired product was present as a singlet at δ 5.01 ppm could be assigned to the CH proton of the imine and the doublet at δ 5.20 ppm might result from the proton on the C5 position adjacent to the nitrogen.



(**33**) (**34**)

Scheme 3.10 Hydrogenation of imine double bond. (1) PtO_2-H_2O, H_2, 4 h

As purification proved impossible, the crude mixture was taken through to the catalytic hydrogenation. Following literature precedent, hydrogenation over PtO_2-H_2O was carried out for 4 hours followed by removal of solvent and attempted crystallisation from ethyl acetate-petroleum ether; the resulting material was still unidentifiable and mass spectrometric analysis showed no material with the desired molecular ion.

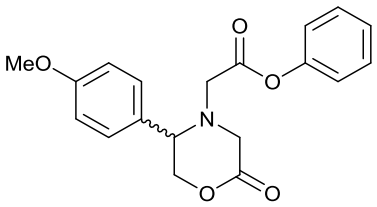


(**32**) (**34**)

Scheme 3.11 Synthesis of (*R*)-5-(4-methoxyphenyl)morpholin-2-one (34). (1) Phenyl bromoacetate, *N*,*N*-diisopropylethylamine, acetonitrile, r.t., 24 h

Since the initial approach had failed, direct synthesis using phenyl bromoacetate was

investigated. After addition of a solution of (*R*)-2-amino-2-(4-methoxyphenyl) ethanol (**32**) and di-isopropylethylamine in anhydrous acetonitrile to a solution of phenyl bromoacetate in anhydrous acetonitrile, the resulting solution was stirred at room temperature for 24 hours under an atmosphere of N_2, during which time a white precipitate, believed to be the ammonium salt, was formed. The solvent was removed *in vacuo* to furnish the crude product that was immediately purified by flash column chromatography on silica, eluting with petrol ether and diethyl ether (1:2) and then acetone. Following recrystallization from diethyl ether and petrol ether, colourless crystals were obtained.



(34)

Figure 3.4 Proposed structure of compound (34)

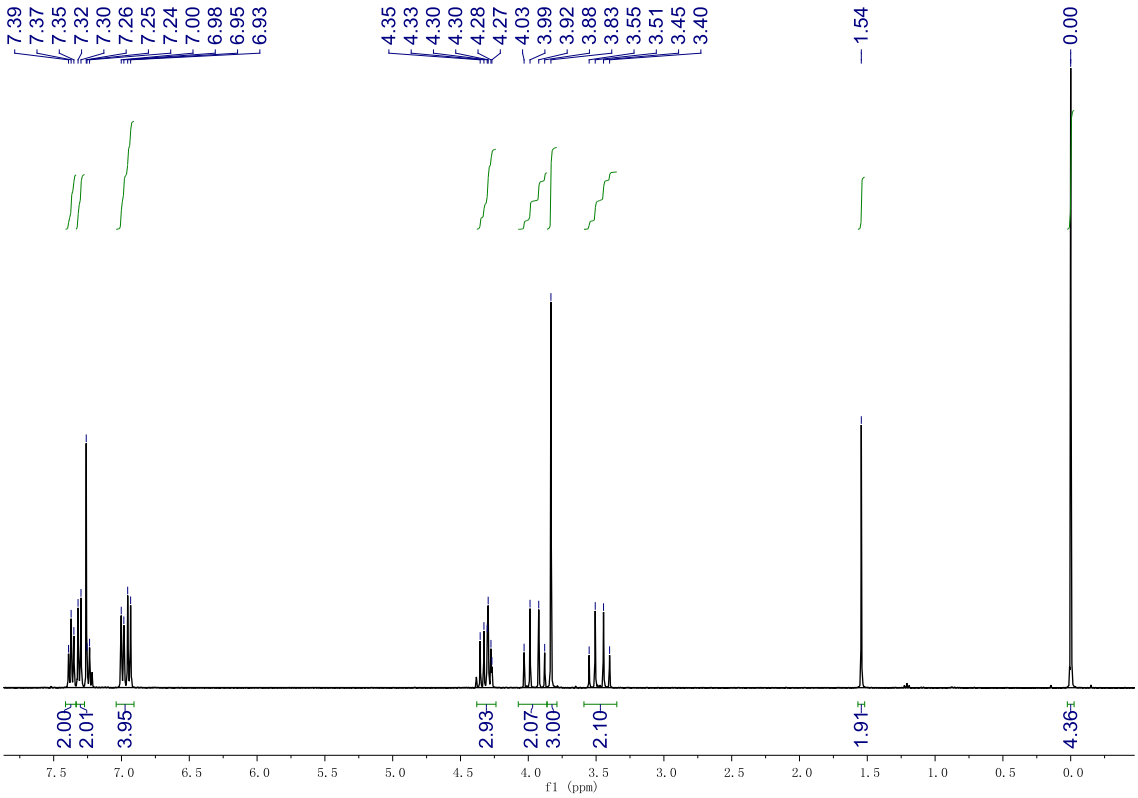
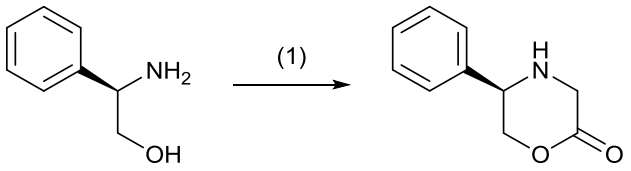


Figure 3.5 The ^{1}H NMR spectrum of solid obtained from the reaction of (32) with phenyl bromoacetate

The ^{1}H NMR spectrum indicated the presence of four doublets and one triplet between δ 6.93 and 7.39 ppm indicating that two phenyl groups were present in this compound (**Figure 3.5**). This led us to propose that we had obtained a over-alkylated morpholin-2-one (**34**) (**Figure 3.4**).



(**36**)

Scheme 3.12 Synthesis of the phenyl morpholinone. (1) Phenyl bromoacetate, di-isopropylethylamine (DIEA), acetonitrile, r.t., 24 h

The same procedure was also investigated using phenylglycinol as originally described to ensure that the procedure would work in our hands. The mixture of phenylglycinol, di-isopropylethylamine and phenyl bromoacetate in acetonitrile was stirred for 24 hours under nitrogen at room temperature. After removal of solvent, the residue was purified by a flash column chromatography with a small pad of Na_2CO_3 on top the silica and purified material recrystallized from petroleum ether / diethyl ether 2:1 to give colourless crystals. ^{1}H NMR spectroscopic analysis showed two double doublets at δ 4.41 and 4.19 ppm corresponding to the two diastereotopic C-6 methylene protons, a triplet at δ 4.30 ppm corresponding to the proton between the NH and phenyl group, and a 2 proton AB quartet was assigned to the methylene group at C-3 (**Figure 3.6**). The IR spectrum showed an amine group NH stretch with a broad peak at 3299 cm^{-1}. Thus the desired morpholinone (**36**) had been synthesized in this case.

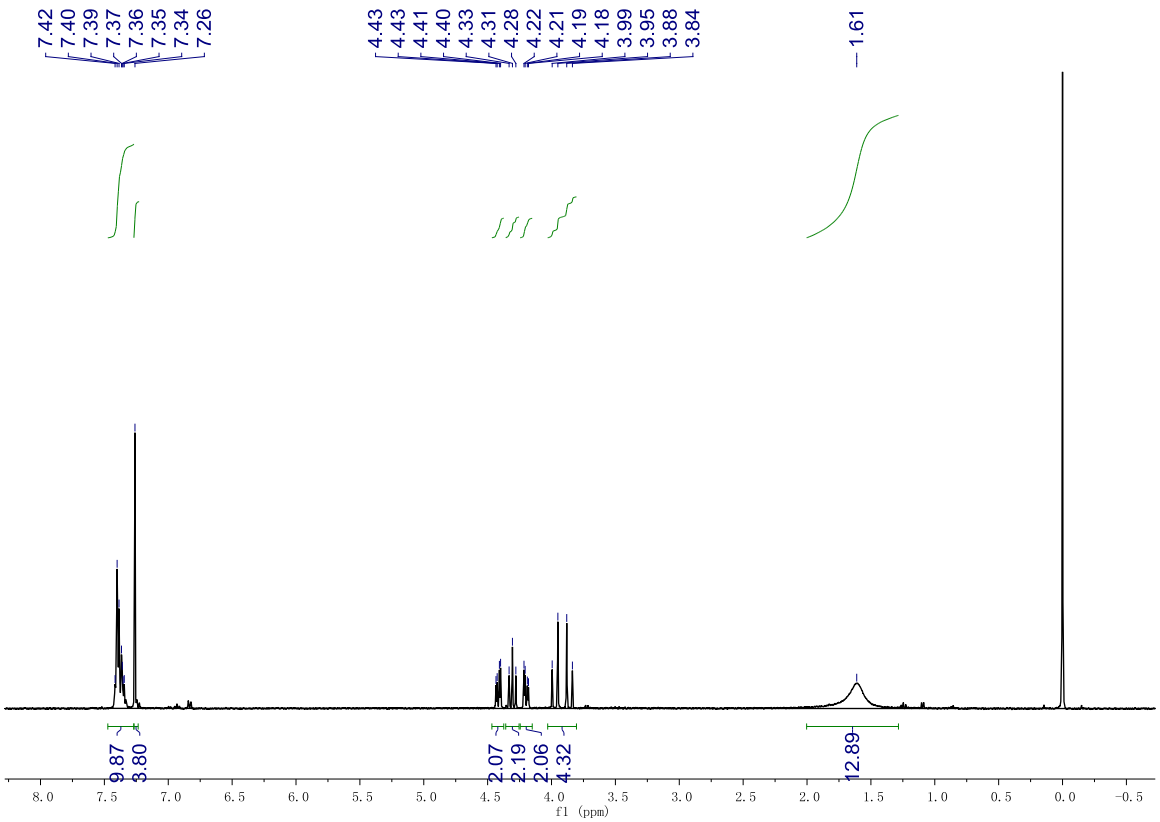


Figure 3.6 ^{1}H NMR spectrum of 5-phenyl morpholin-2-one (36)

Therefore the reaction was repeated with methoxyphenylglycinol (**32**) and one equivalent of phenyl bromoacetate, keeping the temperature at room temperature at all stages. After column chromatography and recrystallization, the ^{1}H NMR spectrum showed two doublets at δ 4.36 and 4.13 ppm corresponding to the two diastereotopic methylene protons of the CH_2O in the morpholinone ring (**Figure 3.7**). An AB quartet centred on δ 3.90 ppm corresponded to the two protons of the NCH_2 group and mass spectrometry showed a peak of $[M+H]^+$ at 208 to give further evidence that we had successfully obtained the desired product (**34)**.

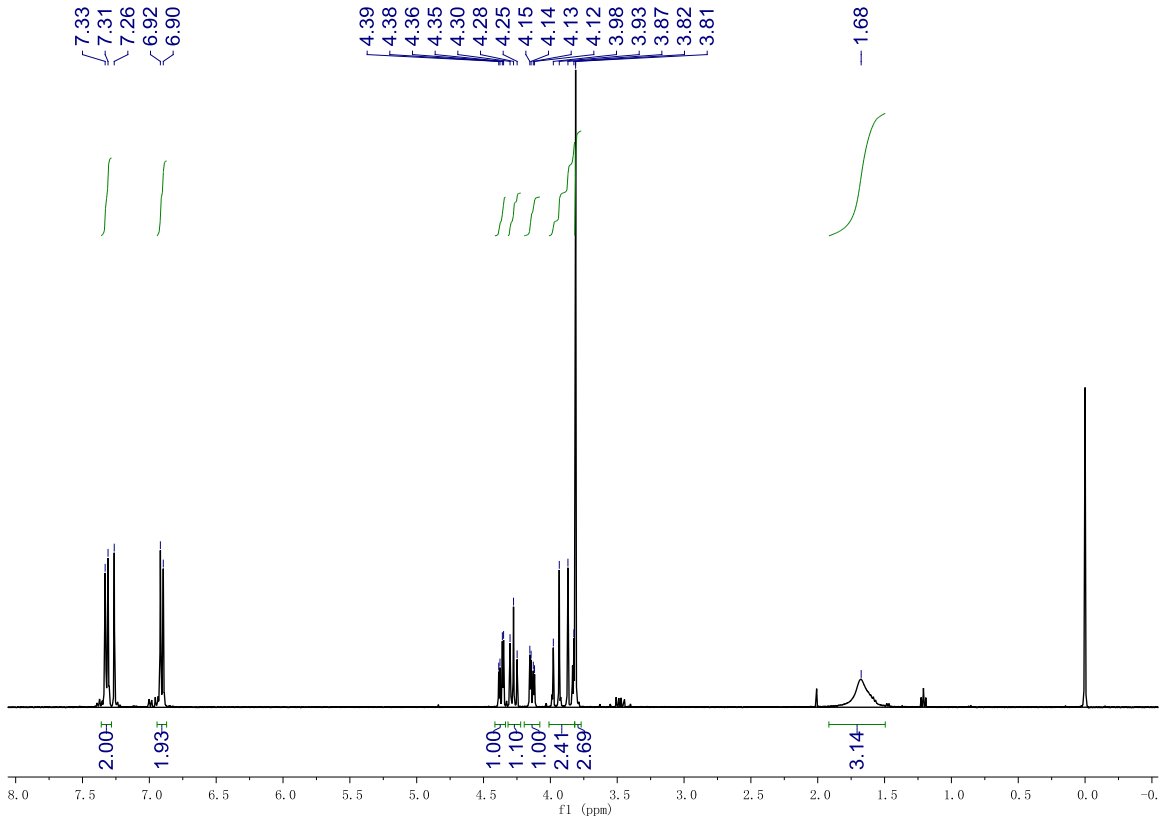
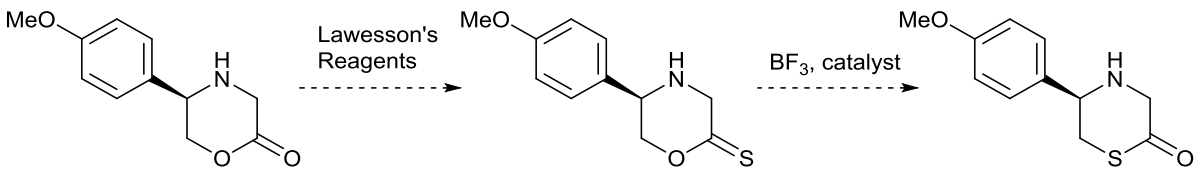


Figure 3.7 ^{1}H NMR spectrum of 5-(4-methoxy)phenylmorpholin-2-one (34)

3.2 Direct thionation of morpholinone

Lawesson's reagent is a well-known thionation reagent in organic chemistry.[63] Lawesson's reagent can convert a carbonyl group to a thiocarbonyl group. Furthermore Filippi *et al.* have reported that boron trifluoride and indium(III) trifluoromethanesulfonate are able to generate thiolactones from thionolactones.[64]

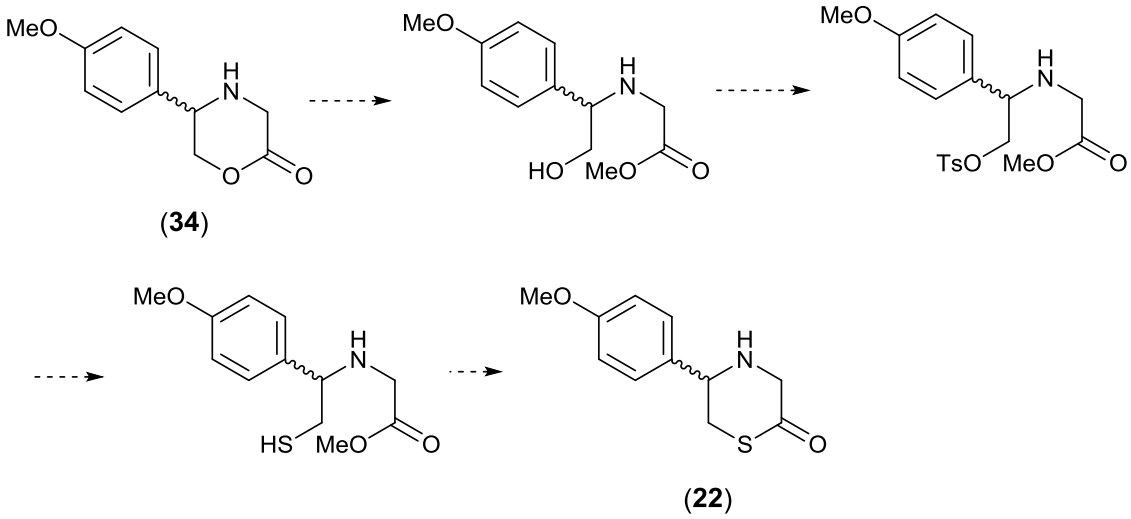


(34) **(37)** **(22)**

Scheme 3.13 Proposed conversion of morpholinone (34) to thiamorpholinone (22) via Lawesson's Reagent and BF_3, indium catalyst.

Based on the above two methods, it was in principle possible to obtain the desired thiamorpholinone from the corresponding morpholinone. Following the literature procedure, morpholinone (**34**) and Lawesson's Reagent were dissolved in toluene under a nitrogen atmosphere. The mixture was heated to reflux for 5 hours. After filtration and removal of the solvent, the ABX system was found to have disappeared from the ^{1}H NMR spectrum of the crude product. This suggested that unsubstituted morpholinone (**34**) was not stable under these conditions.

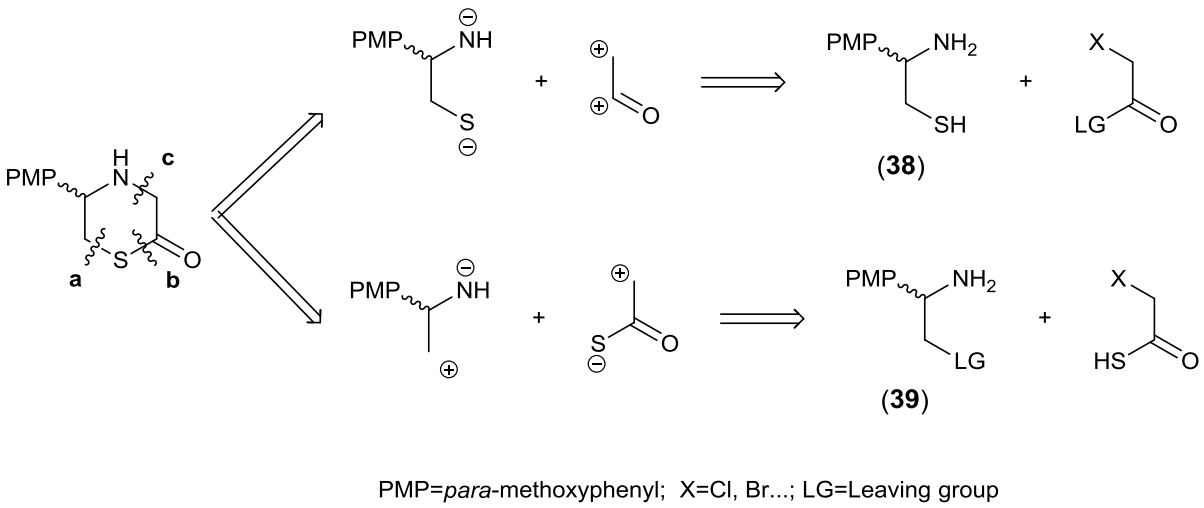
Another solution was to open the morpholinone lactone, and replace the resulting alcohol group with a leaving group and convert the carboxylic acid to a thiocarboxylic group that could then undergo ring-forming reaction to form the desired thiamorpholinone (**Scheme 3.14**).



Scheme 3.14 Proposed conversion of morpholinone (34) to thiamorpholinone (22) via morpholinone ring-opening

3.3 Amino-thiol-mediated thiamorpholinone synthesis

From previous work, it was concluded that thiamorpholinone ccould not easily be synthesized from the morpholinone. According to further retrosynthetic analysis, our proposed plan was to synthesize the thiamorpholinone by a new route (**Scheme 3.15**).

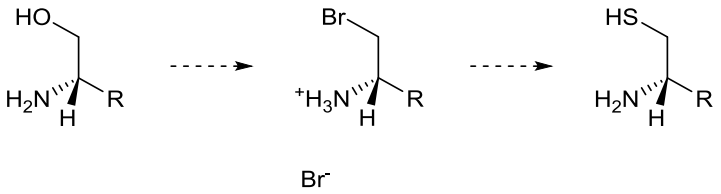


PMP=*para*-methoxyphenyl; X=Cl, Br...; LG=Leaving group

Scheme 3.15 New retrosynthetic analysis of thiamorpholinone

In the first strategy, pre-synthesized methoxyphenylglycinol (**32**) could also be converted to amino-thiol **(38)**, followed by coupling with phenyl bromoacetate to generate the 3-unsubstituted thiamorpholinone. A second strategy would be to cleave bonds **a** and **c**, as it was proposed that corresponding compound (**39**) and an appropriately functionalized thioacetic acid could couple with each other. Since the amino-alcohol (**32**) had been synthesized previously, we only needed to convert the hydroxyl group to a leaving group.

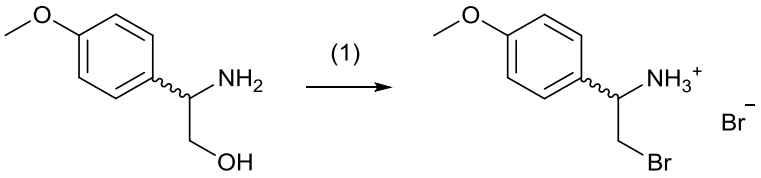
It has been reported by Meinzer *et al*. that amino-thiol (**38**) could be generated from the amino-alcohol (**32**) via a bromoalkanamine hydrobromide intermediate.[65]



Scheme 3.16 Synthesis of amino thiol from amino-alcohol (32)

Following the literature procedure, 4-methoxyphenylglycinol (**32**) was reacted with 48% HBr at 175 ^{o}C for 5 hours. The reaction was diluted with water and decolourized with charcoal. After filtration and concentration, the product was examined by ^{1}H NMR

spectroscopy that showed shifts of the diastereotopic methylene protons to δ 3.50 ppm and the appearance of three protons assigned to ammonium group at δ 8.20 ppm. However, the resonance corresponding to the methoxy group was missing. It is known that 48% HBr can be used to demethylate phenols and it seems that this had happened in this case.[66]

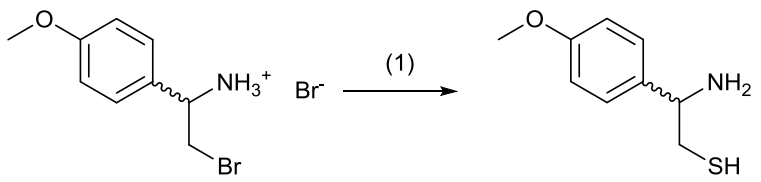


(**32**) (**40**)

Scheme 3.17 Synthesis of bromoalkanamine hydrobromide (40). (1) $SOBr_2$, DCM, 0 ^oC

Therefore, the conditions were modified to use $SOBr_2$ in DCM at 0 ^oC with the reaction being complete within 4 hours (**Scheme 3.17**). The mixture was concentrated and dissolved in EtOH, followed by decolourizing with charcoal followed by recrystallization from EtOH to afford product (**40**).

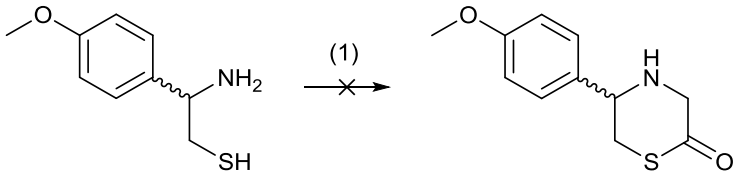
The ^1H NMR spectrum showed that the methoxy group was still present appearing at δ 3.75 ppm as a singlet, the protons of methyline and methylene groups appeared at δ 4.20 and 3.62 ppm respectively, and the ammonium proton was found at δ 8.22 ppm, which suggested preparation of hydrobromide salt (**40**).



(**40**) (**41**)

Scheme 3.18 Synthesis of amino-thiol (41). (1) a.Thiourea, EtOH, reflux, 15 h; b, tetraethylenepentamine, reflux, 1 h.

Then a solution of (**40**) and thiourea in EtOH was heated to reflux for 15 hours, followed by addition of tetraethylenepentamine. The mixture was heated again for another hour before being extracted with ethyl acetate and washed twice with 1M HCl. The organic phase was dried over $MgSO_4$ and concentrated *in vacuo*.



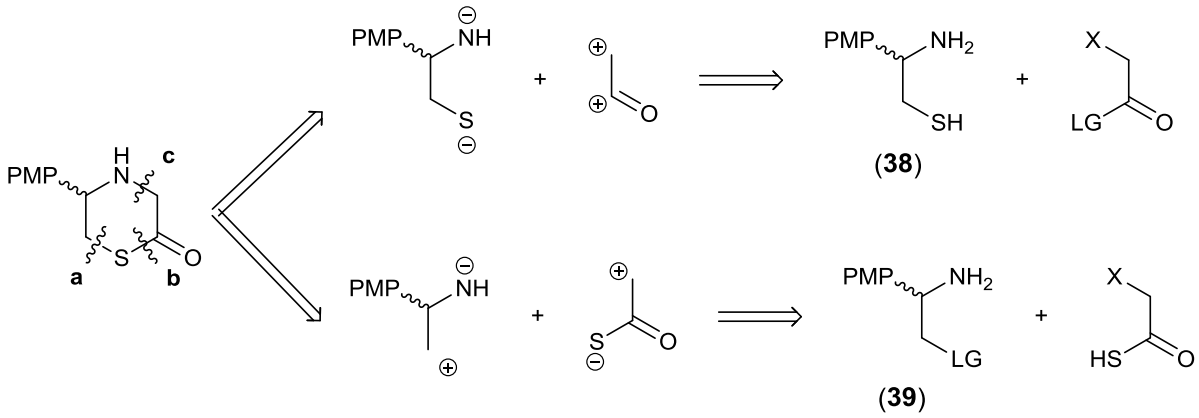
(**41**) (**22**)

Scheme 3.19 Synthesis of thiamorpholinone from amino thiol (41). (1)Phenyl bromoacetate, DIEA, MeCN, r.t.

To avoid oxidative dimerization, the resulting thiol (**41**) was carried through to the next step without further purification. Phenyl bromoacetate and DIEA were added to a solution of intermediate (**41**) in MeCN (**Scheme 3.19**). But after 24 hours at room temperature, only starting material was recovered after column chromatography.

Condensation with ethyl glyxoylate in CF_3CH_2OH was also attempted but this also failed. It is believed that oxidative dimerization had occurred, precluding thiamorpholinone formation and so yet another strategy needed to be considered.

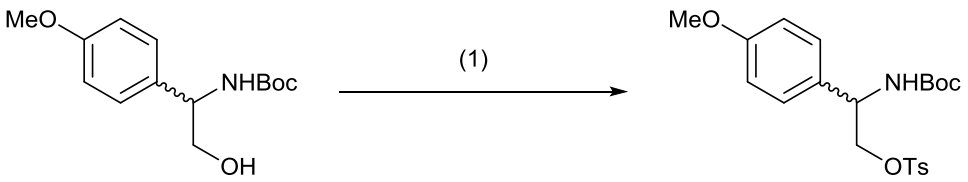
3.4 Thioester mediated thiamorpholinone synthesis



PMP=*para*-methoxyphenyl; X=Cl, Br...; LG=Leaving group

Scheme 3.20 New retrosynthetic analysis of thiamorpholinone.

In order to avoid oxidative dimerization of the thiol it was proposed that a thioester should be formed by the second approach shown in (**Scheme 3.20**).



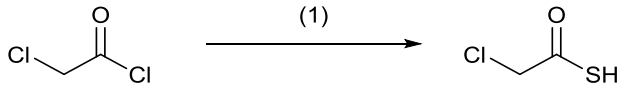
(**31**) (**42**)

Scheme 3.21 Synthesis of 2-((*tert*-butoxycarbonyl)amino)-2-(4-methoxyphenyl)ethyl 4-methylbenzenesulfonate (42). (1), *p*-TsCl, Et_3N, DCM, 0 ^oC

Boc-protected amino-alcohol (**31**) was reacted with *p*-toluenesulfonyl chloride in the presence of triethylamine at 0 ^oC. After filtering off the ammonia salt and removal of solvent, the tosylated product was obtained after column chromatography. In the ^1H NMR spectrum, a singlet at δ 2.44 ppm could be assigned to the methyl group of the tosyl group, and two apparent doublets centred on δ 7.10 and 7.67 ppm equated to the four aryl protons. In the ^{13}C spectrum, resonances around δ 130 ppm (the existence of rotamers caused

resonances more than 8) suggested that two benzene rings were present in the product and a resonance at δ 20.65 indicated the presence of a methyl group. Further evidence from the IR spectrum was a strong absorption at 1366 cm^{-1}, which supported the presence of an S=O double bond. Finally, the mass spectrum showed a molecular ion at 444 corresponding to $[M+Na]^+$.

The synthesis of thioacetic acid (**43**) was proposed using chloroacetyl chloride and sodium hydrosulfide as starting materials.[67] Chloroacetyl chloride was added dropwise to sodium hydrosulfide in the absence of solvent. The reaction was found to be very exothermic and so it was carried out with cooling in ice under a nitrogen atmosphere.

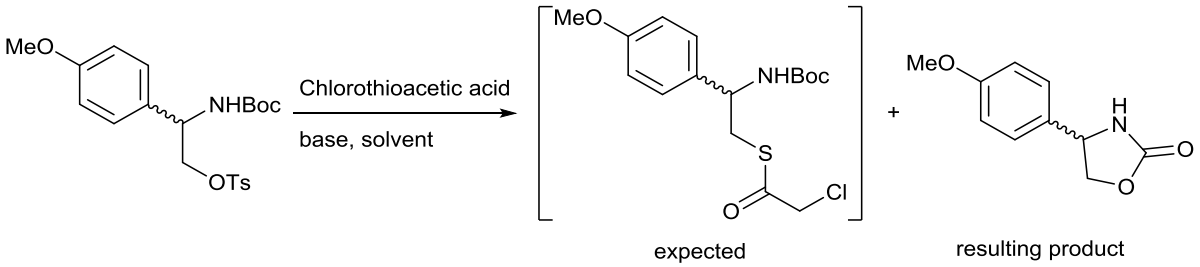


(**43**)

Scheme 3.22 Synthesis of chlorothioacetic acid (43). (1), *p*-TsCl, Et_3N, DCM, 0 ^oC

The product was extracted with THF and dried *in vacuo*, then characterized by ^1H NMR analysis. Singlets at δ 4.11 and 9.68 ppm indicated the two sets of protons on the chlorothioacetic acid. Resonances at δ 40.96 and 170.17 ppm in the ^{13}C spectrum also supported the conclusion that chlorothioacetic acid (**43**) had been successfully prepared.

Then the Boc-protected amino tosylate (**42**) was reacted with chlorothioacetic acid in the presence of base. At first, potassium carbonate and caesium carbonate were both examined in THF, but no reaction was observed. However, when the reaction was carried out in DMF with caesium carbonate as base, the starting material disappeared after 18 hours. After purification by column chromatography, the product was characterized by ^1H NMR spectroscopy that indicated a singlet at δ 3.82 ppm corresponding to the methoxy group, a double doublet at δ 4.16 ppm (J = 8.5, 7.0 Hz) and two triplets at δ 4.91 (J = 8.5 Hz) and 4.70 (J = 8.5 Hz) ppm. On the basis of this data, the only structure that could be proposed was an oxazolidin-2-one (**Scheme 3.23**).

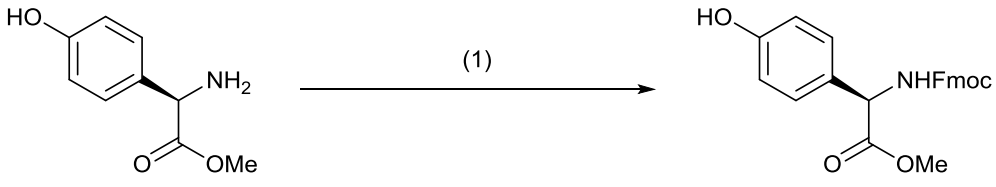


(**42**) (**44**) (**45**)

Scheme 3.23 Attempt to synthesize chlorinated thioester (44).

Somehow the Boc group had been lost during this process. Thus, the procedure was modified by basifying the thioacetic acid first, followed by addition of Boc-protected amino tosylate (**42**). However, the aziridine was still obtained; even though the pH value was verified to be higher than 7 before adding the tosylate (**42**). The reason of the loss of the Boc group under these conditions remains unclear.

At this point we decided to change the amine protecting group to an Fmoc group as this would be stable under acid conditions which could remove Boc protecting group but should also be stable at pH 7.

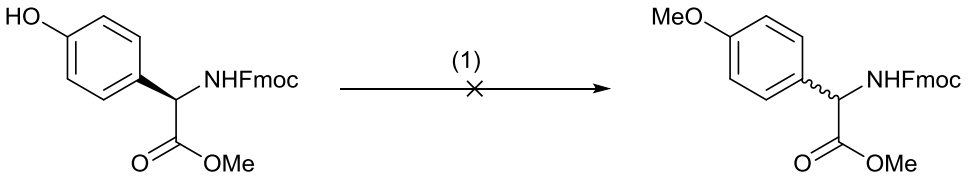


(**28**) (**46**)

Scheme 3.24 Synthesis of methyl 2-((((*9H*-fluoren-9-yl)methoxy)carbonyl)amino)-2-(4-hydroxyphenyl)acetate (46). (1), $NaHCO_3$, H_2O, Fmoc-Cl, dioxane, ice-r.t.

Amino acid ester (**28**) was dissolved in water followed by addition of sodium hydrogen carbonate and a solution of Fmoc-chloride in dioxane and the reaction was stirred on ice for one hour. After removing the ice bath, the reaction was stirred for another 2 hours when analysis by TLC suggested that the reaction was complete.

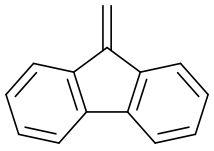
In the ^{1}H NMR spectrum，a series of doublets and triplets at δ 7.72, 7.55, 7.36 and 7.25 ppm showed the presence of fluorene part of the Fmoc protecting group and two multiplets at δ 4.38 and 4.17 ppm corresponded to the aliphatic protons of Fmoc group. The IR spectrum indicated that there were two carbonyl groups present with strong absorptions at 1699 and 1633 cm^{-1}. Finally mass spectrometry confirmed the structure with an molecular ion at 404 of $[M+H]^{+}$.



(**46**) (**47**)

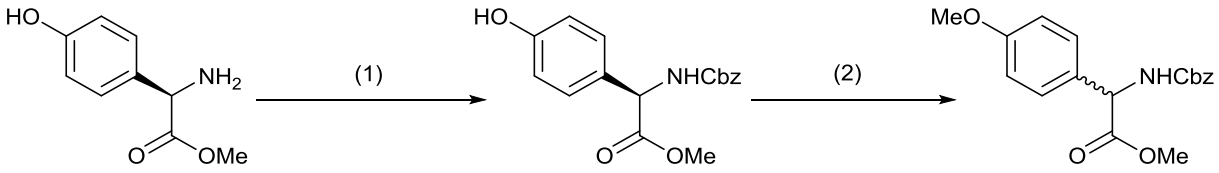
Scheme 3.25 Methylation of methyl 2-((((*9H*-fluoren-9-yl)methoxy)carbonyl)amino)-2-(4-hydroxyphenyl)acetate (46). (1), K_2CO_3, MeI, THF

Fmoc-protected amino ester (**46**) was then reacted with iodomethane in THF in the presence of K_2CO_3 at room temperature for 15 hours.



Compound (48)

After concentrating the organic phase, the resulting crude product was purified by column chromatography but only 9-methylenefluorene (**48**) was obtained. The ^{1}H NMR spectrum showed a singlet at 6.02 ppm corresponding to the two protons of the terminal alkene. It was assumed that the other part of the molecule was soluble in the aqueous phase and not recovered from extraction and that the conditions had been too basic for the Fmoc protection.



(**28**) (**49**)

(**50**)

Scheme 3.26 Cbz protection of (*R*)-(4-hydroxyphenyl)glycine methyl ester with further methylation. (1), $NaHCO_3$, H_2O, CbzCl, dioxane, 0 ^oC-r.t.; (2), K_2CO_3, MeI, THF

Thus, the Cbz protecting group was proposed, which is stable under both acidic and basic conditions. The amino acid ester (**28**) was reacted with benzyl chloride in water and dioxane (1:1) in the presence of $NaHCO_3$ for 18 hours, with monitoring by TLC. The appearance of an AB quartet at δ 5.12 and 5.07 ppm (J_{AB} = 12 Hz) and a multiplet for an aromatic ring at δ 7.34 ppm indicated that the successful preparation of Cbz protected (4-hydroxyphenyl)glycine methyl ester (**49**) which was isolated in a yield of 61%.

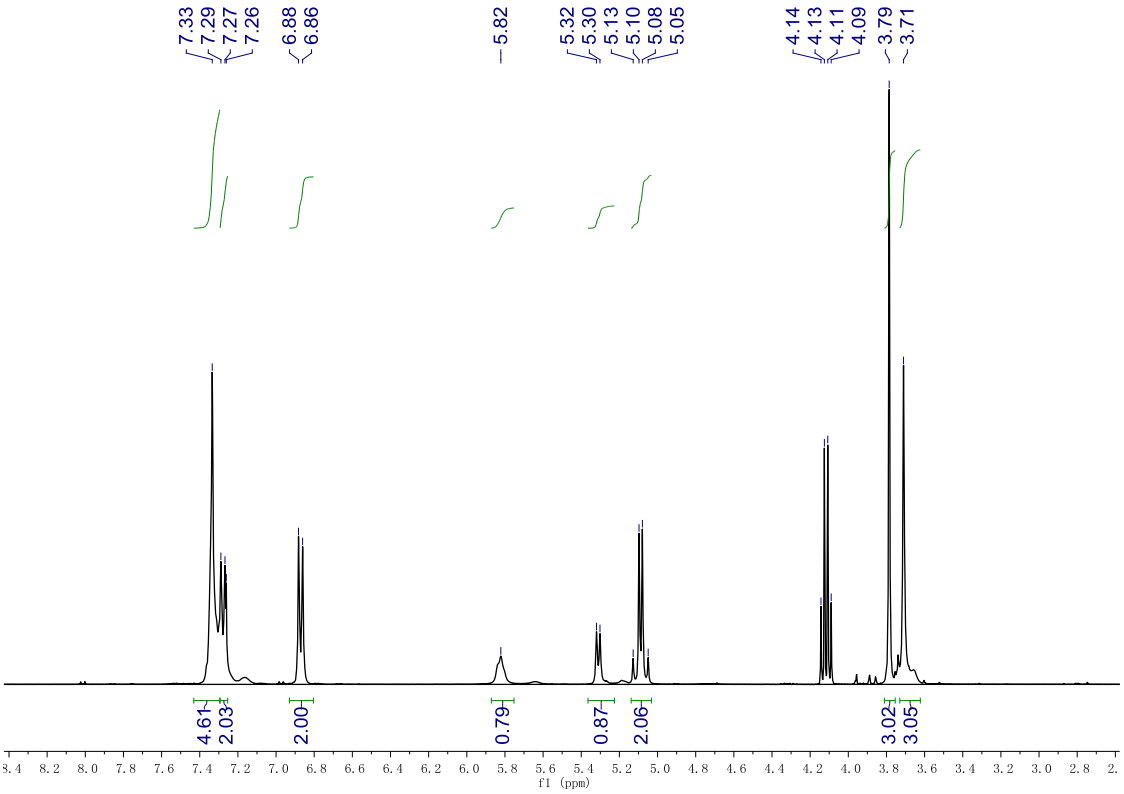
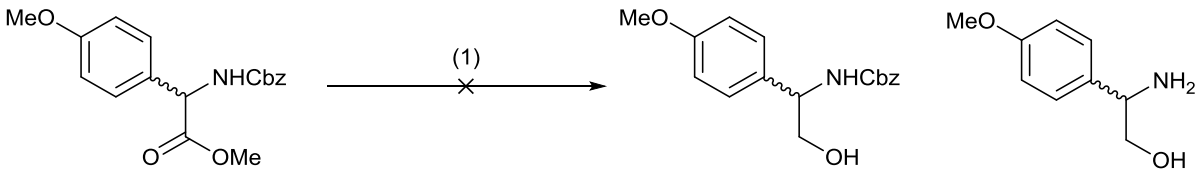


Figure 3.8 ^{1}H NMR spectrum of methyl 2-(((benzyloxy)carbonyl)amino) -2-(4-methoxyphenyl)acetate (50).

The Cbz protected (4-hydroxyphenyl)glycine methyl ester (**49**) finally *O*-methylated with iodomethane in the presence of K_2CO_3. In the ^{1}H NMR of the product (**50**), a 3 proton singlet was observed at δ 3.79 ppm indicating successful methylation and after purification, a colourless solid was obtained in a yield of 76%.



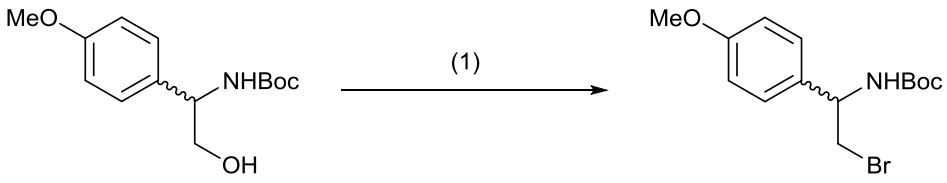
(**50**) (**51**) (**32**)

Scheme 3.27 Reduction of compound (50) by $NaBH_4$. (1), $NaBH_4$, THF, 65 ^oC, 15 min, MeOH, 80 ^oC, 3.5 h

The resulting compound **(50)** was then treated with sodium borohydride in THF to reduce the ester to an alcohol. However, the expected product was not obtained on work-up and extraction; instead, *N*-deprotected amino-alcohol (**32**) was separated out after column chromatography in a yield of 85%, By ^{1}H NMR analysis, it was evident that both the methoxy group of the ester and the benzyl group had disappeared from the spectrum.

In conclusion, three kinds of protecting group were examined, but none of them was able to protect the amine group throughout the whole process. The Boc group was lost during the final substitution step, the Fmoc group was unstable under the basic conditions of the methylation step and the Cbz group was lost during the reduction with sodium boronhydride. Other leaving groups need to be considered if this approach is to be pursued in future.

After failed attempts at the synthesizing the thioester using a tosyl leaving group, it was decided to attempt to prepare the corresponding bromide.

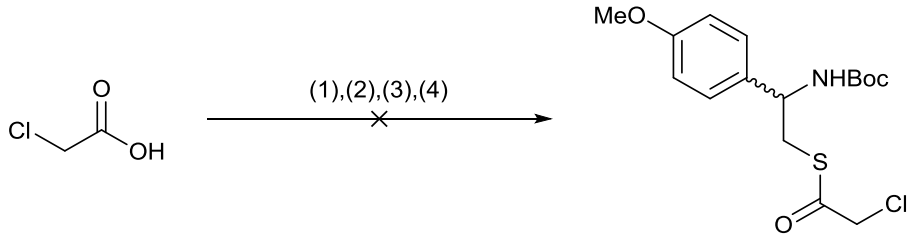


(**31**) (**52**)

Scheme 3.28 Synthesis of *tert*-butyl (2-bromo-1-(4-methoxyphenyl)ethyl)carbamate (52). (1), PPh_3, CBr_4, THF

In the Appel reaction, tetrabromomethane is used to brominate compounds under neutral conditions that are compatible with Boc protection.[68] Thus, Boc protected amino-alcohol (**31**) was treated with tetrabromomethane and triphenylphosphine in dry THF over 18 hours with TLC monitoring. The mixture was diluted with diethyl ether to precipitate the majority of the triphenylphosphine oxide, followed by filtration and concentration *in vacuo*. Pure product (**52**) was achieved by column chromatography.

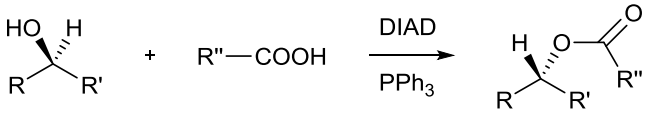
^{1}H NMR analysis showed that the two diastereotopic methylene protons were shifted from δ 3.82 to 3.67 ppm and infra-red spectroscopy showed the absence of alcohol group. Finally presence of bromine was confirmed by mass spectrometry with molecular ions at 659 $[2M(Br_2)+H]^+$, 661 $[2M(Br^{81}Br)+H]^+$ and 663 $[2M(^{81}Br_2)+H]^+$.



(**43**) (**53**)

Scheme 3.29 Synthesis of *S*-(2-((*tert*-butoxycarbonyl)amino)-2-(4-methoxyphenyl)ethyl) 2-chloroethanethioate (53). (1) Et_3N, 0 ^oC; (2) ClCOOEt, 0 ^oC; (3) NaSH, 0 ^oC; (4) compound (52), r.t.

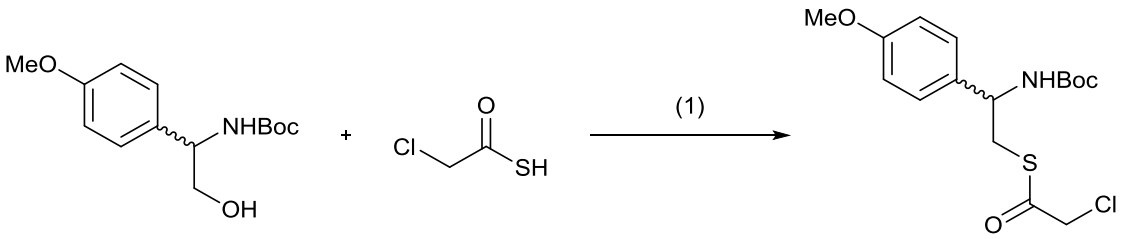
Following the standard thioester forming procedure, chloroacetic acid and trimethylamine were dissolved into THF and stirred for 30 minutes, followed by addition of ethyl chloroformate to form acid anhydride, and a white precipitate was generated immediately. After 10 minutes, sodium hydrosulfide was added at 0 ^{o}C to form sodium chlorothioacetate; then, after 2.5 h, amino-bromide (**52**) was added and the reaction was allowed to warm up to room temperature. After 15 hours, there was still starting material present as determined by TLC and so the reaction was monitored continually by TLC for two days, when no starting material could be observed. However no desired product was isolated on work-up.



Scheme 3.30 Mitsunobu Reaction

The Mitsunobu reaction allows the conversion of primary and secondary alcohols to esters, phenyl ethers, thioethers and various other compounds.[69] In our system, using chlorothioacetic acid (**43**), the desired thioester could be achieved directly, by reacting amino-alcohol (**31**) with chlorothioacetic acid (**43**).

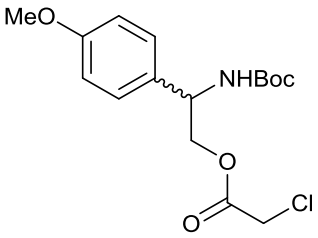
Firstly, triphenylphosphine was dissolved in anhydrous THF at 0 ^{o}C, followed by addition of diisopropyl azodicarboxylate (DIAD) over 15 minutes. A white suspension was observed, indicating formation of the betaine intermediate and the reaction was allowed to stir for another 30 minutes to make sure that DIAD was completely transformed into betaine. At this point, Boc-protected amino-alcohol (**31**) in THF and chlorothioacetic acid (**43**) were added slowly over 30 minutes.



(**31**) (**43**) (**53**)

Scheme 3.31 Synthesis of *S*-(2-((*tert*-butoxycarbonyl)amino)-2-(4-methoxyphenyl)ethyl) 2-chloroethanethioate (53). (1), PPh_3, DIAD, THF

After stirring for 18 hours, TLC analysis showed that the starting material had been consumed. The majority of the triphenylphosphine was precipitated by adding diethyl ether and filtered, and solvent was evaporated *in vacuo*. The residue oil was purified by column chromatography, eluting with diethyl ether and petroleum ether 1:2. The ^{1}H NMR spectrum showed the appearance of a two proton singlet indicating the presence of the chloroacetate group. A two proton multiplet at δ 4.38 ppm corresponding to the CH_2 coupling with a CH, multiplet, at δ 4.99 ppm (**Figure 3.9**). However, the ^{13}C NMR spectrum indicated that carbonyl group chemical shift was δ 167 ppm which did not tally with the presence of a thioester where the resonance would be expected to appear at over δ 190 ppm. Such a chemical shift would be more in keeping with an ester instead of a thioester and mass spectrometry confirmed this with a molecular ion at 366 [$M+Na^+$]. Therefore we concluded that the chlorothioacetic acid preparation had been unsuccessful.



Compound (54)

At this point we considered alternative methods for synthesizing thioacetic acid. It has reported that 1,1'-carbonyldiimidazole (CDI) can be used as a coupling reagent to activate the acetic acid, then react with nucleophilic reagent to give the desired thioacid.[70] In most

cases, H_2S was chosen as the sulfur donor. But we chose to continue using NaSH as it is far more convenient to handle.

Hence, chloroacetic acid was dissolved in DMF, followed by adding 1,1'-carbonyldiimidazole to generate the intermediate acyl imidazolide and then sodium hydrosulfide.

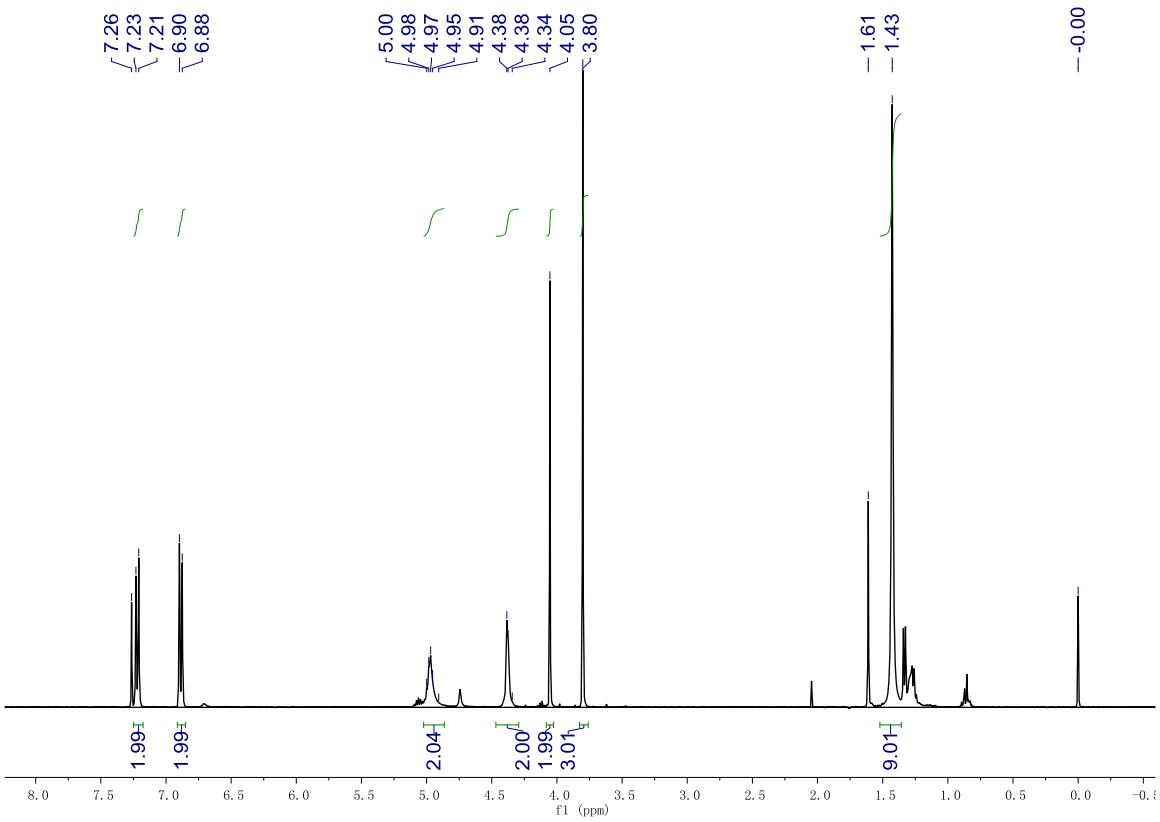
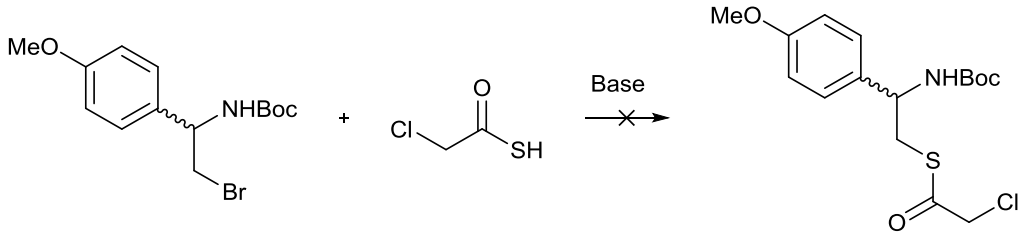


Figure 3.9 ^{1}H NMR spectrum of compound (54)

Upon completion of the reaction, the product was purified by chromatography, eluting with acetonitrile and 1 % formic acid. In the ^{1}H NMR spectrum, the singlet at δ 2.04 ppm was assigned to residual formic acid and the two singlets around δ 3.00 ppm and the singlet at 8.10 corresponded residual DMF. The singlet at δ 4.03 ppm corresponded the chloromethyl protons group and the broad resonance at δ 9.47 ppm was assigned to the SH proton.

Furthermore, ^{13}C NMR analysis also suggested the successful preparation of chlorothioacetic acid, with the carbonyl resonance now appearing at δ 194.2 ppm.

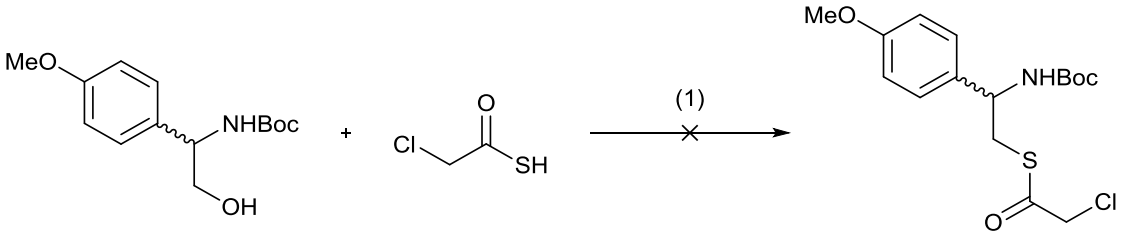
After further clean-up, chlorothioacetic acid (**43**) was used directly in the next reaction (**Scheme 3.32**). Thus, the Boc-protected amino-bromide (**52**) was reacted with chlorothioacetic acid (**43**) in the presence of triethylamine in THF. However, after 18 hours, no new material was detected by TLC analysis and even when "heated to reflux" for another 18 hours, only starting materials were recovered.



(**52**) (**43**) (**53**)

Scheme 3.32 Synthesis of *S*-(2-((*tert*-butoxycarbonyl)amino)-2-(4-methoxyphenyl)ethyl) 2-chloroethanethioate (53).

The same sample of chlorothioacetic acid was also tested in the Mitsunobu reaction (**Scheme 3.33**) but, yet again, only ester (**54**) was recovered from the reaction.

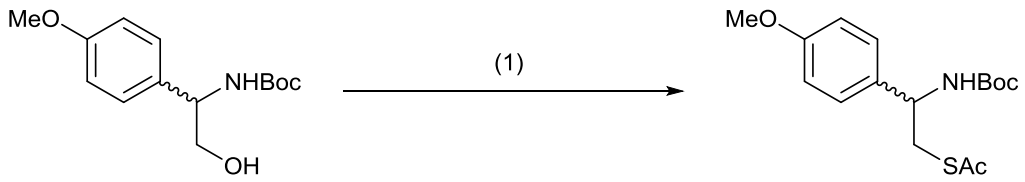


(**31**) (**43**) (**53**)

Scheme 3.33 Attempted synthesis of *S*-(2-((*tert*-butoxycarbonyl)amino)-2-(4-methoxyphenyl)ethyl) 2-chloroethanethioate (53) via Mitsunobu reaction. (1), PPh_3, DIAD, THF

Since it was proving difficult to make the desired thioester from chlorothioacetic acid, an alternative synthetic strategy was proposed. Thus we decided to synthesize the thioacetate and hydrolyse this to be the corresponding amino-thiol. This amino-thiol could then be reacted with chloroacetyl chloride to generate the desired chlorothioester (**53**).

The first step was to use the Mitsunobu reaction to prepare thioacetate (**55**).[71]

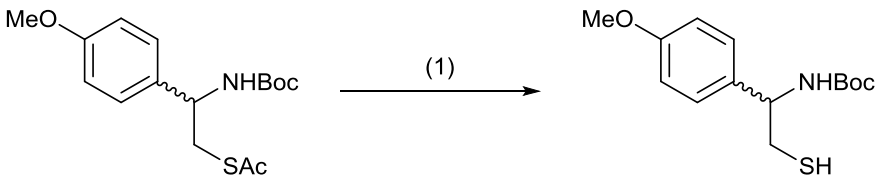


(**31**) (**55**)

Scheme 3.34 Synthesis of *S*-(2-((*tert*-butoxycarbonyl)amino)-2-(4-methoxyphenyl)ethyl) ethanethioate (55). (1), PPh_3, DIAD, thioacetic acid

Triphenylphosphine was dissolved in distilled THF at 0 ^{o}C, followed by dropwise addition of DIAD. A white precipitate formed after a few minutes and, after stirring for 30 minutes on ice, amino-alcohol (**31**) in THF was added to the reaction dropwise, followed by thioacetic acid. The reaction turned clear immediately, and was stirred on ice for 2 hours, then allowed to warm up to room temperature. After leaving overnight, the reaction was shown to be complete by TLC analysis.

The majority of the triphenylphosphine oxide was precipitated out by adding diethyl ether and, after filtration, solvent was removed *in vacuo*. The residue was purified by chromatography, eluting with 10 % ethyl acetate in hexane. The structure of product (**55**) was confirmed by ^{1}H NMR analysis, where the methyl group of the thioester was observed at δ 2.34 ppm. The two diastereotopic methylene protons were shifted to δ 3.24 ppm. In the ^{13}C NMR spectrum a peak at δ 195.9 ppm correlated with the thioester carbonyl carbon.



(55) **(56)**

Scheme 3.35 Synthesis of *tert*-butyl (2-mercapto-1-(4-methoxyphenyl)ethyl)carbamate (56). (1), 1 M NaOH, MeOH

Purified thioacetate (**56**) was then dissolved in methanol, 1 M aqueous sodium hydroxide was added and the mixture was stirred for 1 hour. The reaction was quenched with 1 M hydrochloric acid and stirred for another 1 hour, before the mixture was extracted with DCM and the extract concentrated *in vacuo*.

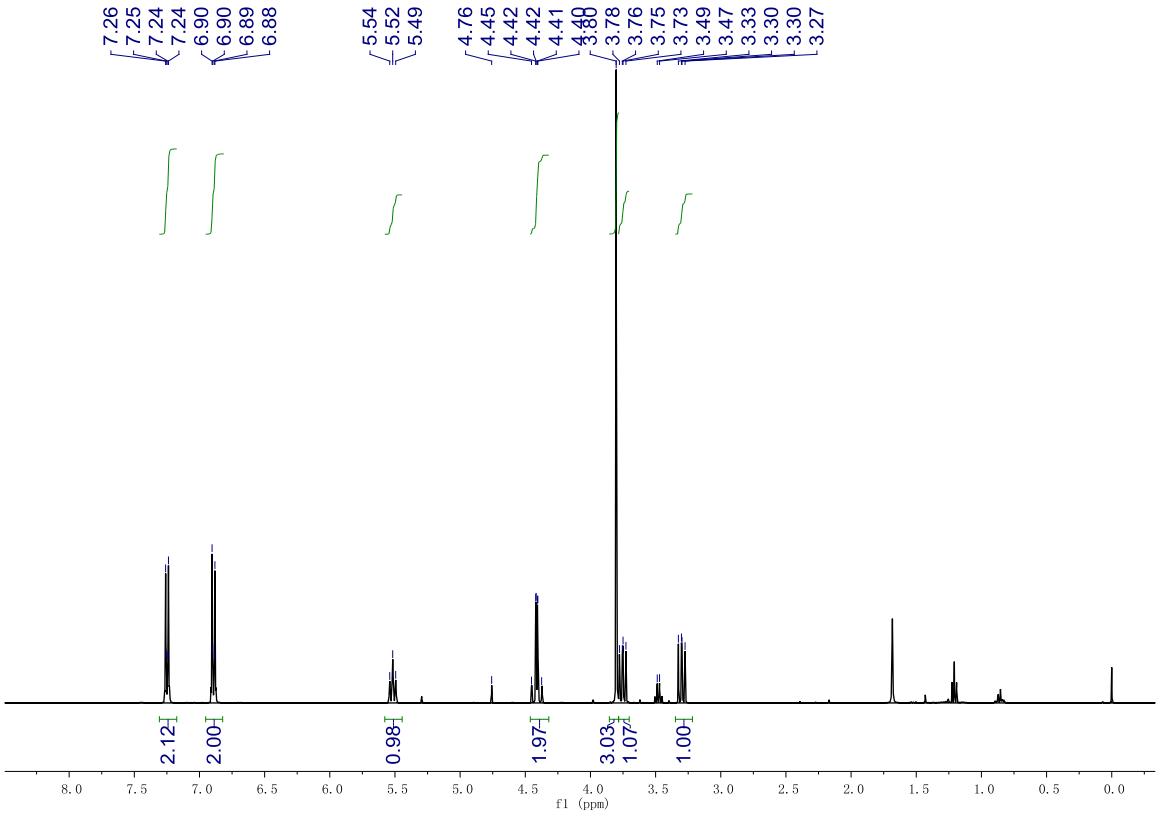
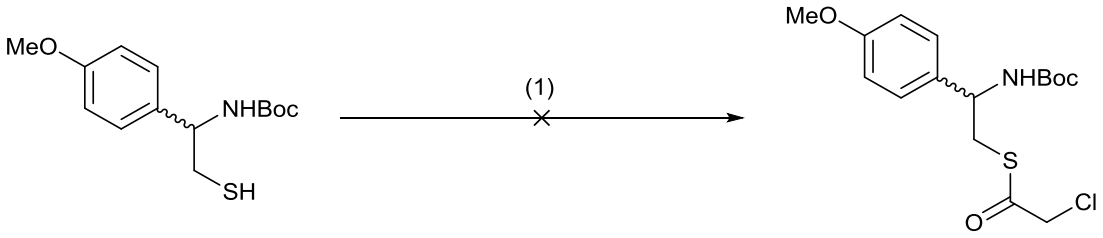


Figure 3.10 ^{1}H NMR spectrum of the hydrolysis product of thioacetate (55).

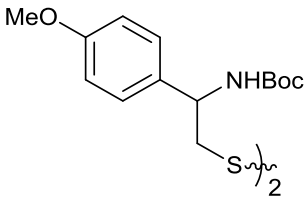
From the ^{1}H NMR spectrum (**Figure 3.10**), it could be seen that the methyl group at δ 2.34 ppm from thioacetate had disappeared, showing that all starting material had been consumed.



(**56**) (**53**)

Scheme 3.36 Synthesis of compound (53) from amino thiol (56). (1), Chloroacetyl chloride, 4 Å MS

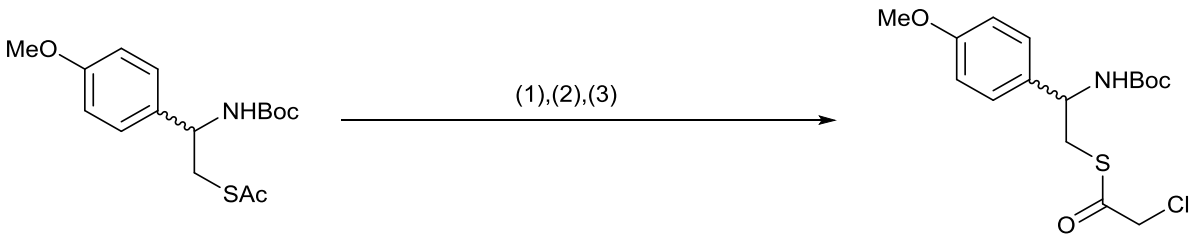
To a solution of the resulting amino thiol (**56**) in chloroform, triethylamine and 4 Å molecular sieves were added and the mixture was cooled to 0 ^{o}C. Chloroacetyl chloride was added dropwise over 10 minutes, resulting in a vigorous reaction. After stirring on ice for 2 hours, the reaction was stirred at room temperature for a further 12 hours. After isolation and purification, no ester could be identified, instead a material that was initially thought to be the starting thiol was isolated.



Disulfide 57

However, although the ^{1}H NMR spectrum was similar to that of the starting material, TLC comparison showed it was not the same compound. Most peaks appeared at the same chemical shift, but the diastererotopic methylene protons had shifted a little to δ 3.06 ppm. It was evident that the thiol had dimerized after having been exposed to air. Subsequently, to avoid oxygen contact, all solutions were de-gassed by purging with nitrogen for at least

20 minutes, and the thiol (**56**) was used immediately after hydrolysis was complete without purification.



(55) **(53)**

Scheme 3.37 Synthesis of (2-((*tert*-butoxycarbonyl)amino)-2-(4-methoxyphenyl)ethyl) 2-chloroethanethioate (53). (1), 1 M NaOH, MeOH; (2), 1 M HCl; (3), Chloroacetyl chloride, Et_3N, 4 Å MS

When the reaction was deemed to be complete by TLC analysis, the reaction was quenched, extracted with ethyl acetate and the organic phase was evaporated *in vacuo*. After column chromatography, desired product (**53**) was obtained in a yield of 68%.

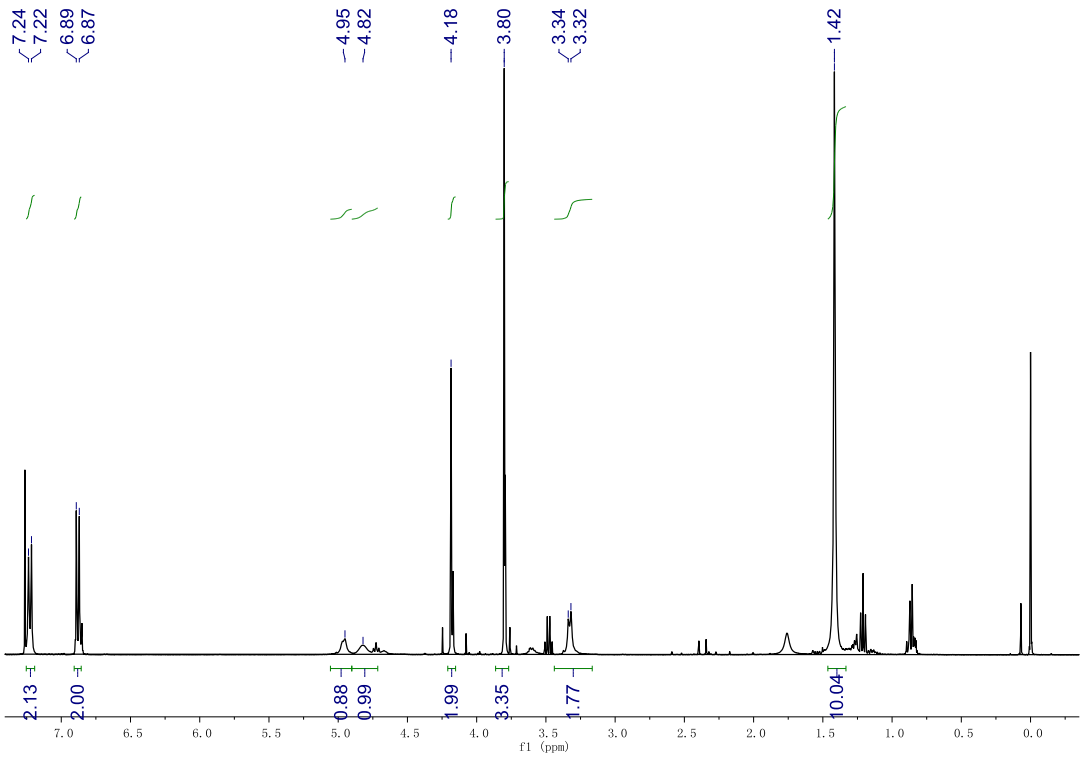
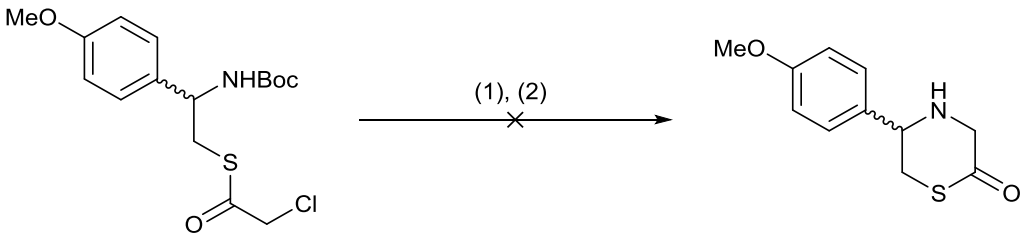


Figure 3.11 ^{1}H NMR spectrum of (2-((*tert*-butoxycarbonyl)amino)-2-(4-methoxyphenyl)ethyl) 2-chloroethanethioate (53)

The ^{1}H NMR spectrum (**Figure 3.11**) possessed a resonance assigned to the CH_2Cl group at δ 4.19 ppm; while the methoxy group and Boc group resonance were present at δ 3.80 and 1.42 ppm respectively. Mass spectrometry also confirmed the structure with a molecular ion at 382 $[M+Na]^+$.

Another fraction that was separated from the column was characterized and shown to be dimerized product (**57**) obtained in 32% yield. It can be concluded that amino thiol (**56**) is very sensitive to oxygen.



(**53**) (**22**)

Scheme 3.38 Attempt to synthesize of glycine-based thiamorpholinone by chlorinated thioester (53). (1), TFA, DCM; (2), K_2CO_3, 4Å MS, DCM

The chlorinated thioester (**53**) was next Boc deprotected using trifluoroacetic acid and DCM. Then after removal of trifluoroacetic acid, the free amine was dissolved in DCM in the presence of potassium carbonate and 4 Å molecular sieves. The mixture was stirred for 48 hours when no starting material remained. After work-up and purification by column chromatography, the major product was analysed by ^{1}H NMR spectroscopy (**Figure 3.12**).

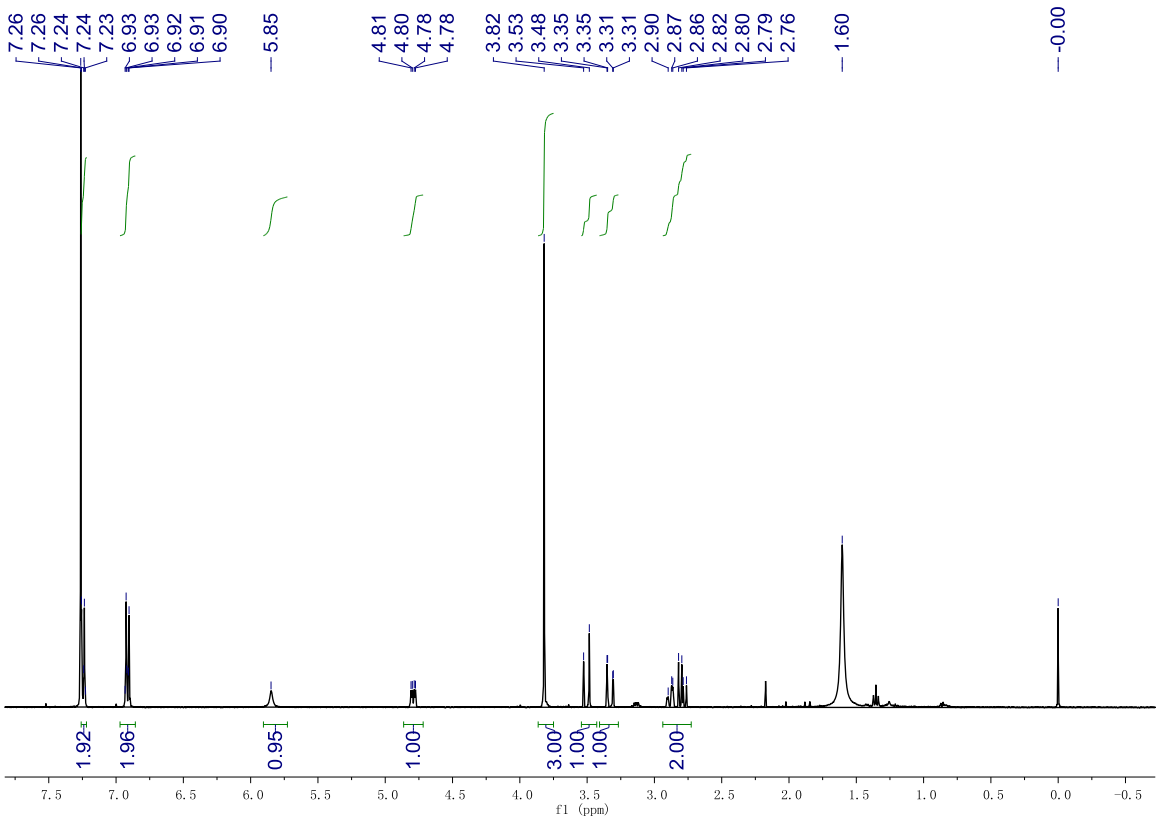
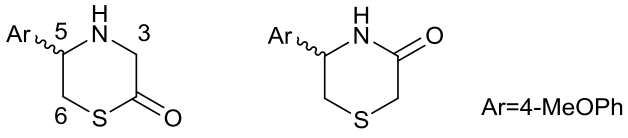


Figure 3.12 ^{1}H NMR spectrum of the product of cyclization

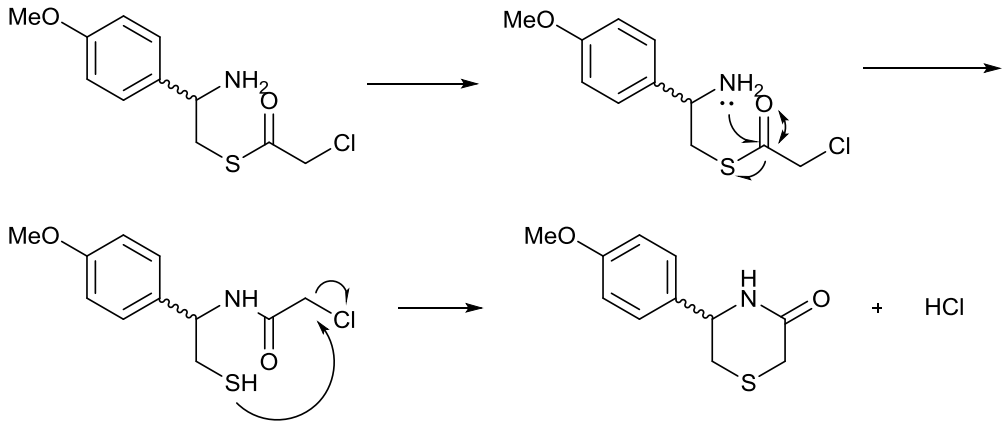
The ^{1}H NMR spectrum presented diastereotopic protons and an ABX system. It appeared that the two doublets at δ 3.33 and 3.50 ppm corresponded to the protons at position 3 of (**22**), the two double doublets at δ 2.79 and 2.88 ppm corresponded to the two diastereotopic protons at position 6 and these two protons coupled with the double doublet at δ 4.79 ppm, which would correspond to the proton on the position 5. The mass spectrum also convinced us we had achieved our goal with a molecular ion at 224 $[M+H]^{+}$.



(**22**) (**58**)

However, in the ^{13}C NMR spectrum, there was no evidence for the presence of a carbonyl carbon of a thioester. Only a resonance at δ 167.2 ppm was found; whereas a thioester carbonyl carbon would be expected to resonate over δ 190 ppm. It was at this point that we

realized that the isomeric amide (**58**) had been obtained instead of desired thiolactione (**22**). A mechanism to explain the course of events that led to the unwanted isomer is shown in **Scheme 3.39**.



Scheme 3.39 Proposed mechanism of cyclization

It would seem that after the amine group was de-protected, the free amine could not only attack the carbon with chlorine leaving group, but also the thioester group.[28] Clearly in this case, the thioester is more prone to be attacked. This rearrangement is directly analogous to that which occurs in native chemical ligation. Then the resulting thiol group can then undergo intramolecular displacement of chloride forming the isomeric lactam.

Before we had realized that an isomer had in fact been prepared, a second attempt was carried out under the same conditions. However, after work-up, the ^{1}H NMR spectrum showed a different ABX system. A triplet was found at δ 5.52 ppm, two double doublets were found at δ 3.75 ppm (J_{AB} = 9.0 Hz) and 3.30 ppm (J_{AB} = 9.5 Hz) respectively, and an AB quartet appeared at δ 4.40 ppm (J_{AB} = 1.5 Hz), with an integration for two protons. Based on the rearrangement-cyclization mechanism we initially proposed and chlorine pattern showed in mass spectrum, this new material was proposed to have structure (**59**) with two molecular ions of 242 $[M(^{35}Cl)+H]^{+}$ and 244 $[M(^{37}Cl)+H]^{+}$ (3:1).

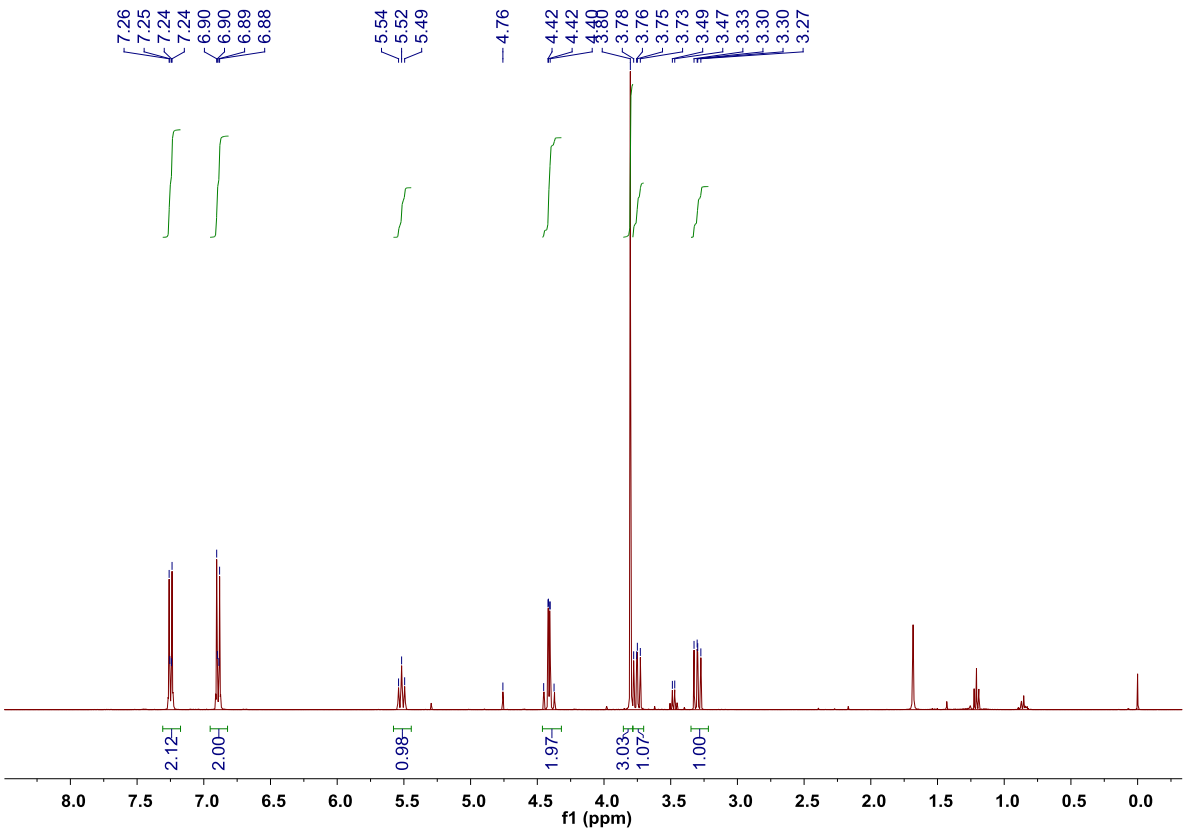
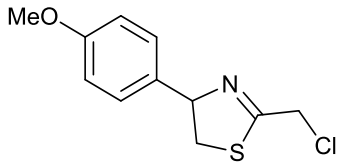


Figure 3.13 ^{1}H NMR spectrum of another product of attempted cyclization

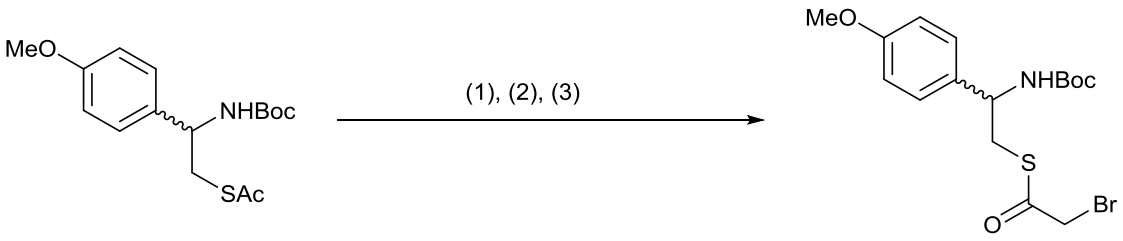


(**59**)

Figure 3.14 Proposed structure (59) of another product of cyclization

Frustratingly, despite multiple attempts, cyclic compound (**58**) was only isolated on a single occasion.

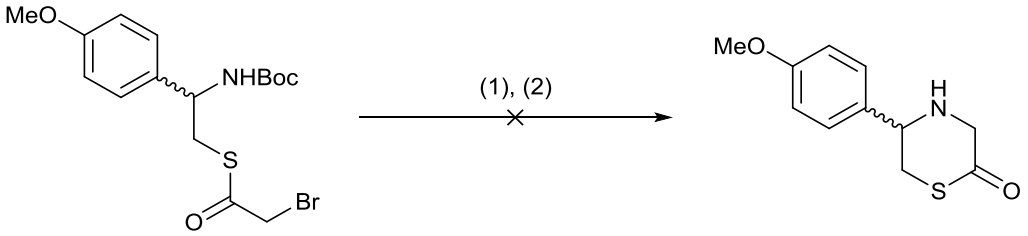
With the understanding that intramolecular attack on the thioester is favoured over nucleophilic substitution of chlorine, we decided to replace the chlorine with bromine in the hope that, with a better leaving group, the desired cyclization might occur.



(**55**) (**60**)

Scheme 3.40 Synthesis of *S*-(2-((*tert*-butoxycarbonyl)amino)-2-(4-methoxyphenyl)ethyl) 2-bromoethanethioate. (1), 1 M NaOH, MeOH; (2), 1 M HCl; (3), Bromoacetyl bromide, 4 Å MS

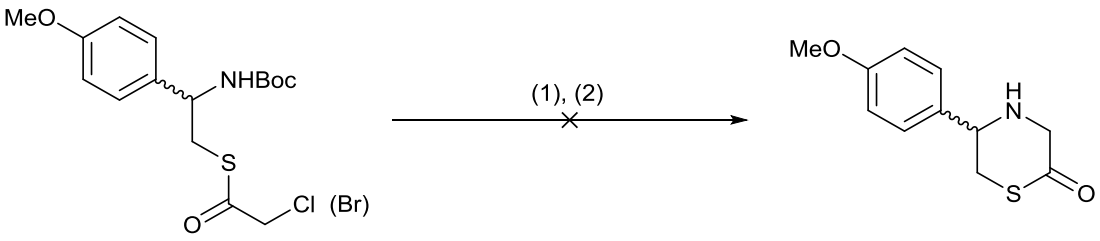
The same protocol for preparing (**60**) was followed as in the preparation of chloroacetate (**53**) (**Scheme 3.40**). After purification by column chromatography, the desired product was separated out and characterized in a yield of 37 %. In the ^{1}H NMR spectrum, a resonance assigned to the CH_2Br was observed at δ 4.03 ppm compared to the corresponding resonance in the chloroacetate (**53**) which appeared at δ 4.19 ppm.



(**60**) (**22**)

Scheme 3.41 Attempt to synthesize of glycine-based thiamorpholinone via the brominated thioester. (1), TFA, DCM; (2), K_2CO_3, 4 Å MS, DCM

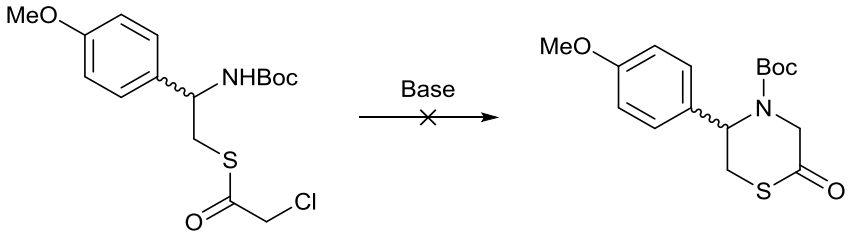
However, when this brominated thioester (**60**) underwent de-protection, the same coupling pattern was observed in the ^{1}H NMR, and ^{13}C NMR spectra as with (**58**) indicating the formation of a lactam.



Scheme 3.42 Attempt of cyclization with $AgNO_3$. (1),TFA, DCM; (2), $AgNO_3$, K_2CO_3, MeCN

In an attempt to favour displacement of the halide, silver nitrate was added to the deprotected ester.[72] However, these attempts also failed, and gave the same lactam product (**58**).

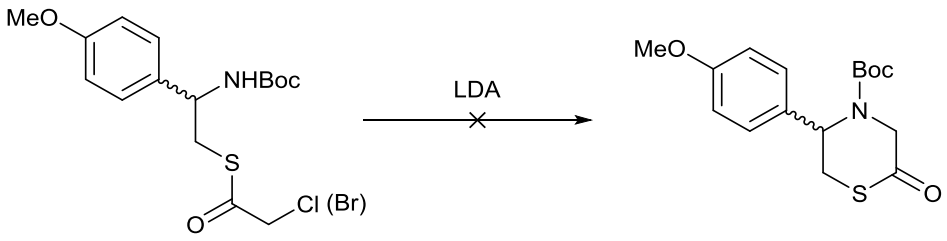
Therefore, to avoid intramolecular attack of the free amine onto the thioester, it was decided that the Boc protecting group had to remain on the amine in order to have any chance of cyclizing to desired thiamorpholinone (**22**).



(**53**) (**61**)

Scheme 3.43 Attempt to synthesize of glycine-based thiamorpholinone (61) without deprotection step.

To this end, chlorothioester (**53**) was treated with potassium carbonate directly in DCM at room temperature for 48 hours, but no reaction was observed. Then the mixture was heated to reflux and stirred for another 48 hours, but still the starting material remained unreacted. Caesium carbonate was also tried, and failed equally.



Scheme 3.44 Attempt to synthesize glycine-based thiamorpholinone by using LDA

Finally it was decided to use the far more basic LDA. Firstly, diisopropylamine was dissolved in anhydrous tetrahydrofuran under nitrogen at -78 ^{o}C, and *n*-butyl lithium was added at -78 ^{o}C. After 30 min, a solution of Boc protected amino thioester in THF was added to the system. The reaction was stirred at -78 ^{o}C for four hours and then warmed up to room temperature for overnight.

The mixture was extracted and purified by chromatography eluting with diethyl ether and petroleum ether (3:2). Unfortunately ^{1}H NMR analysis indicated that the thioester had been hydrolysed during the reaction. The singlet at δ 4.18 ppm representing the CH_2Cl methylene group was gone, and no ABX coupling system was present. Mass spectrometry also showed that disulfide (**57**) had been formed as the result of oxidation of the resulting thiol.

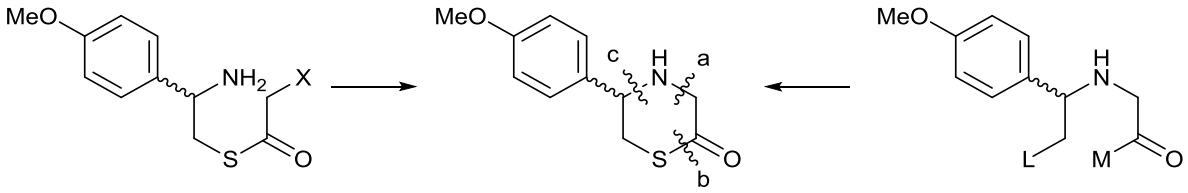
Sodium bis(trimethylsilyl)amide (NaHMDS) is another non-nucleophilic base often used in organic synthesis.[73] Furthermore sodium has less affinity than lithium to bind covalently the carbonyl oxygen, which might give better chance of deprotonating the protected nitrogen.

Starting material (**53**) and NaHMDS were added to anhydrous THF at -78 ^{o}C, the reaction was stirred at -78 ^{o}C for four hours then allowed to warm up to room temperature. However, after isolation and purification, the same hydrolysed dimeric product was recovered.

In conclusion, it has proven unfeasible to synthesize the desired thiamorpholinone in a route via an acyclic thioester as so it was decided to look for an alternative strategy.

3.5 Episulfide mediated thiamorpholinone synthesis

On reviewing the retrosynthetic analysis, there are two possible cleavable bonds **a** and **b**. All previous work had indicated that bond **a** could not be used as the final step in the synthesis of thiamorpholinone (**22**). As the free amine would preferentially undergo intramolecular nucleophilic attack on the thioester, in the stage of this, focus was concentrated on how to form the thioester bond in the final step.



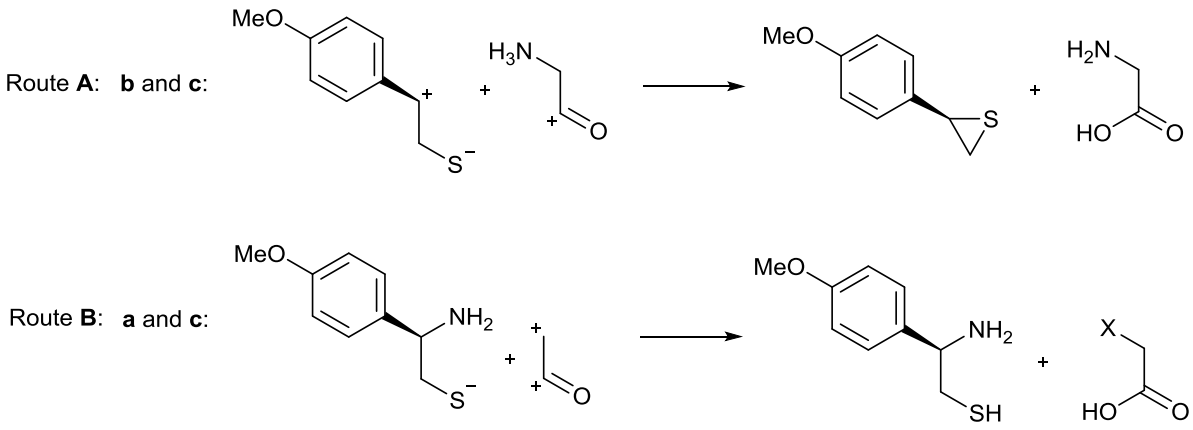
(**22**)

Scheme 3.45 Two pathways to synthesize the thiamorpholinone

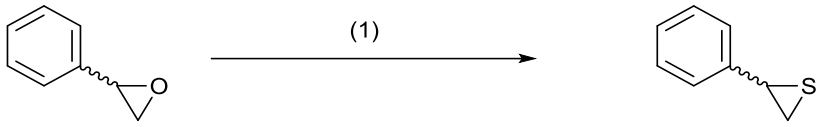
Previously, *p*-methoxyphenyl substituted morpholinone (**34**) had been successfully synthesized. It was therefore considered that this could be subjected to lactone ring opening followed by conversion of the hydroxyl group to a thiol group, and subsequent ring closure to give the desired thiamorpholinone.

An alternative retrosynthesis would be to cleave bond**s a** or **c** (**Scheme 3.46**). If bond **b** and **c** were cleaved (Route **A**), the resulting two parts could be derived from an episulfide and a glycine derivative. If the episulfide was attacked at the benzylic position with the nucleophilic nitrogen, it would generate a thiol, which could couple with an amino acid to give the thiolactone.

If bonds **a** and **c** were cleaved (Route **B**), the resulting two parts could be derived from an amino-thiol and an α-substituted acetic acid derivative in much the same way as the morpholinone derivatives have been synthesized.



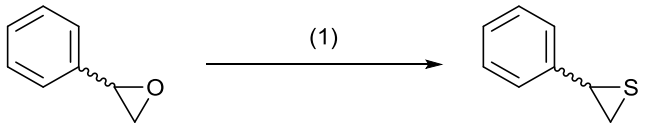
Scheme 3.46 Retrosynthetic analysis of thiamorpholinone



(**64**)

Scheme 3.47 Synthesis of episulfide with thiourea. (1), Thiourea, $CaCO_3$, THF, reflux

It has been reported that aromatic episulfides can be obtained from the corresponding epoxides.[74] According to the literature procedure, styrene oxide was added to a mixture of thiourea and calcium carbonate under the solvent free conditions. The thiourea and calcium carbonate need to be mixed and grounded very well and the reaction was reported to be completed in 5 minutes at 65 ^{o}C. However, in our hands, even after over 4 hours no reaction was observed. On raising the temperature to 90 ^{o}C, the starting material simply converted to styrene. On heating to 65 ^{o}C for 15 hours gave a material of which 54 % of the mixture was styrene, 28 % of the mixture was unreacted styrene oxide, and 18 % was styrene episulfide. Thus, new reaction conditions needed to be explored.

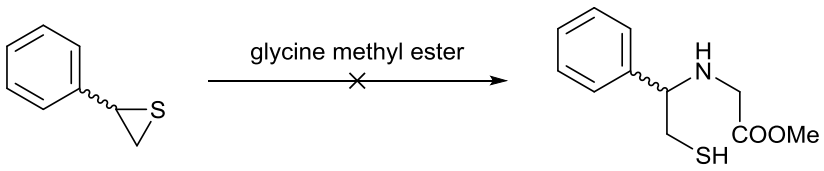


(**64**)

Scheme 3.48 Synthesis of styrene episulfide with NH_4SCN. (1), NH_4SCN, heat

Another research group had reported that ammonium thiocyanate could also be used as the sulfur donor.[75] following this procedure, styrene oxide was mixed with 1.2 equivalents of ammonium thiocyanate, and the neat mixture was heated to 65 ^{o}C for 3 hours. This led to the episulfide being isolated in a 41 % yield. As we believed that low yield might be due to the styrene episulfide decomposing to styrene, the temperature was lowered to 55 ^{o}C, and the reaction was heated for 4 hours to furnish styrene episulfide in an excellent 90 % yield.

Thus, the styrene episulfide was treated with glycine methyl ester (obtained by treating the commercially available hydrochloride salt with triethylamine) in DCM at room temperature.



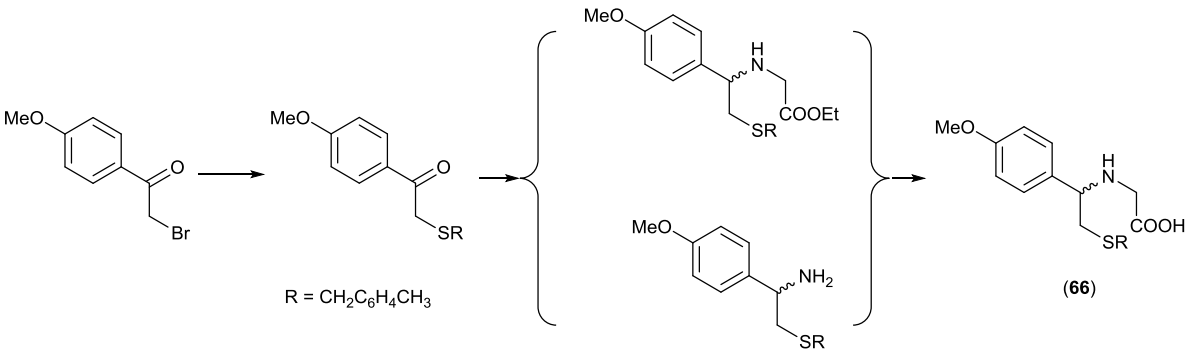
(**64**) (**65**)

Scheme 3.49 Amination of styrene episulfdie

However, after 15 hours, no reaction was observed and so the temperature was increased to 55 ^{o}C, but still no product was observed after 24 hours. When the temperature was increased to 65 ^{o}C, as expected, the styrene episulfide decomposed with the formation of elemental sulfur being observed. Other solvent systems were also tested, such as methanol, water, DMF, and water/dioxane. However, none of them could provide the correct conditions for opening styrene episulfide three-membered ring.

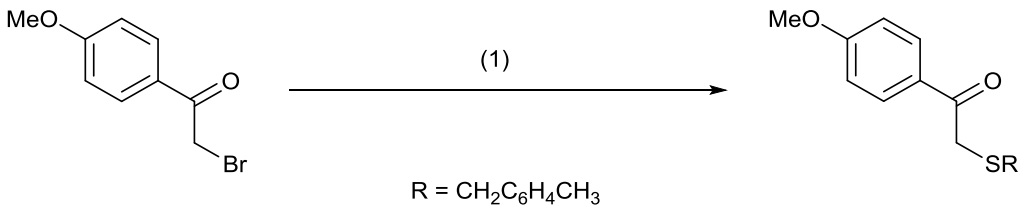
Lewis acids such as boron trifluoride and trimethyl aluminum were also tried but no ring opening was observed. At this point, this approach was abandoned.

3.6 Thiamorpholinone synthesis via amination



Scheme 3.50 Different amination route from same starting material

In the alternative route, the amino-thiol is synthesized first and to prevent the thiol group from undergoing oxidative dimerization, it needs to be protected. It was found in the literature[76] that the desired amino-thiol can be synthesized from 2-bromo-4'-methoxyacetophenone. However, we knew from our experience that the sulfur could not be introduced as a thioacetate as the thioester would be attacked by free amine. Therefore it was decided to introduce the sulfur as a thioether.



(**67**)

Scheme 3.51 Synthesis of 1-(4-methoxyphenyl)-2-(4-methylbenzylthio)ethan-1-one (67). (1), 4-Methylbenzyl mercaptan, DIEA, DMF, r.t. 15h

Firstly, 4-methylbenzyl mercaptan was dissolved in DMF and diisopropylethyl amine was added to the solution at 0 ^{o}C, followed by 2-bromo-4'-methoxyacetophenone. The reaction

was slowly warmed up to room temperature and was kept stirring for another 12 hours. The product was extracted with DCM and washed with 1 M HCl aqueous solution, followed by brine. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*, followed by recrystallization from ethanol to afford a colourless solid.

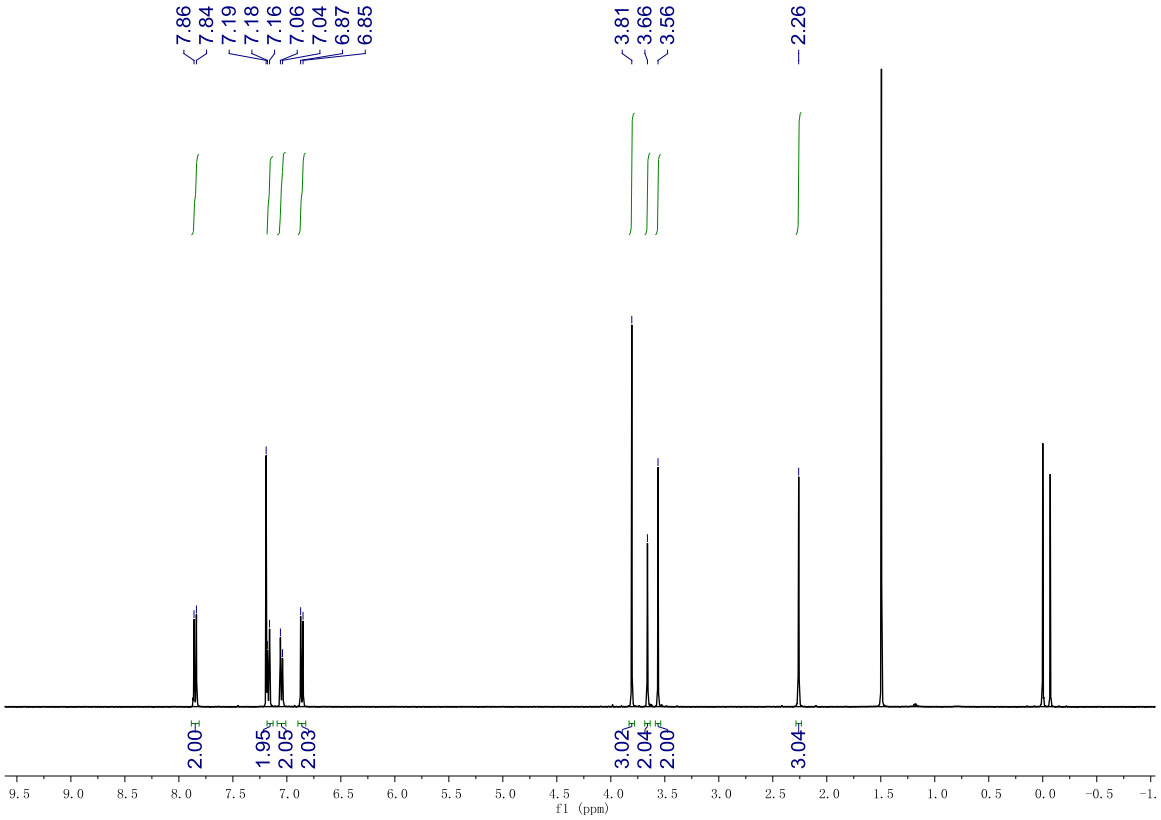
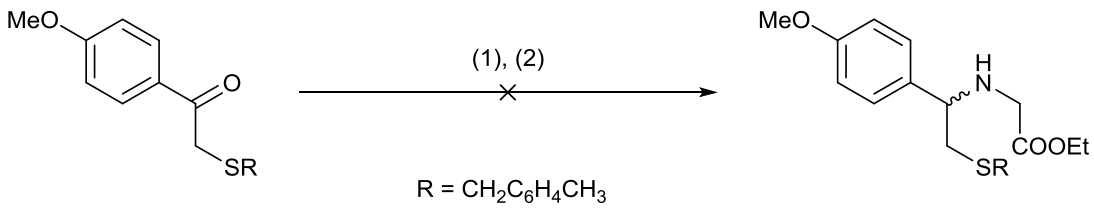


Figure 3.15 ^{1}H NMR spectrum of 1-(4-methoxyphenyl)-2-((4-methylbenzyl)thio) ethan-1-one (67).

The product was characterized by ^{1}H NMR analysis (**Figure 3.15**). Four singlets at δ 3.81, 3.66, 3.56 and 2.26 ppm corresponded to the methoxy group, two methylene groups next to sulfur and the tolyl methyl group respectively. The structure was also confirmed by mass spectrometry with a molecular ion at 287 $[M+H]^+$.

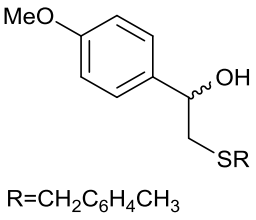


(**67**) (**68**)

Scheme 3.52 Attempt at reductive amination. (1), Glycine ethyl ester, $BF_3.Et_2O$ toluene, reflux, 8 h; (2), $NaBH_4$, MeOH, 0 ^oC

In our first effort, the resulting ketone (**67**) was subjected to an attempted reductive amination with glycine ethyl ester to obtain desired compound (**68**). Starting material (**67**) was dissolved in toluene, followed by glycine ethyl ester; then boron trifluoride etherate was added to the mixture dropwise. The resulting mixture was heated to reflux in a Dean-Stark apparatus over two days, but no product was observed by TLC analysis. Hoping this it might due to similar polarities of the two compounds, the toluene was removed *in vacuo* and the residue redissolved in methanol, followed by addition of sodium borohydride and the mixture was stirred at 0 ^oC for 4 hours. After standard work-up and purification by a column chromatography a new pure product was isolated.

Unfortunately, this product was readily identified as an alcohol (**69**) resulting from the reduction of unreacted ketone (**67**). In the ^1H NMR spectrum (**Figure 3.16),** only one ABX coupling system at δ 4.62 (J_{AB} = 3.0 Hz), 2.75 (J_{AB} = 4.0 Hz) and 2.63 (J_{AB} = 9.0 Hz) ppm, no AB coupling was observed.



(**69**)

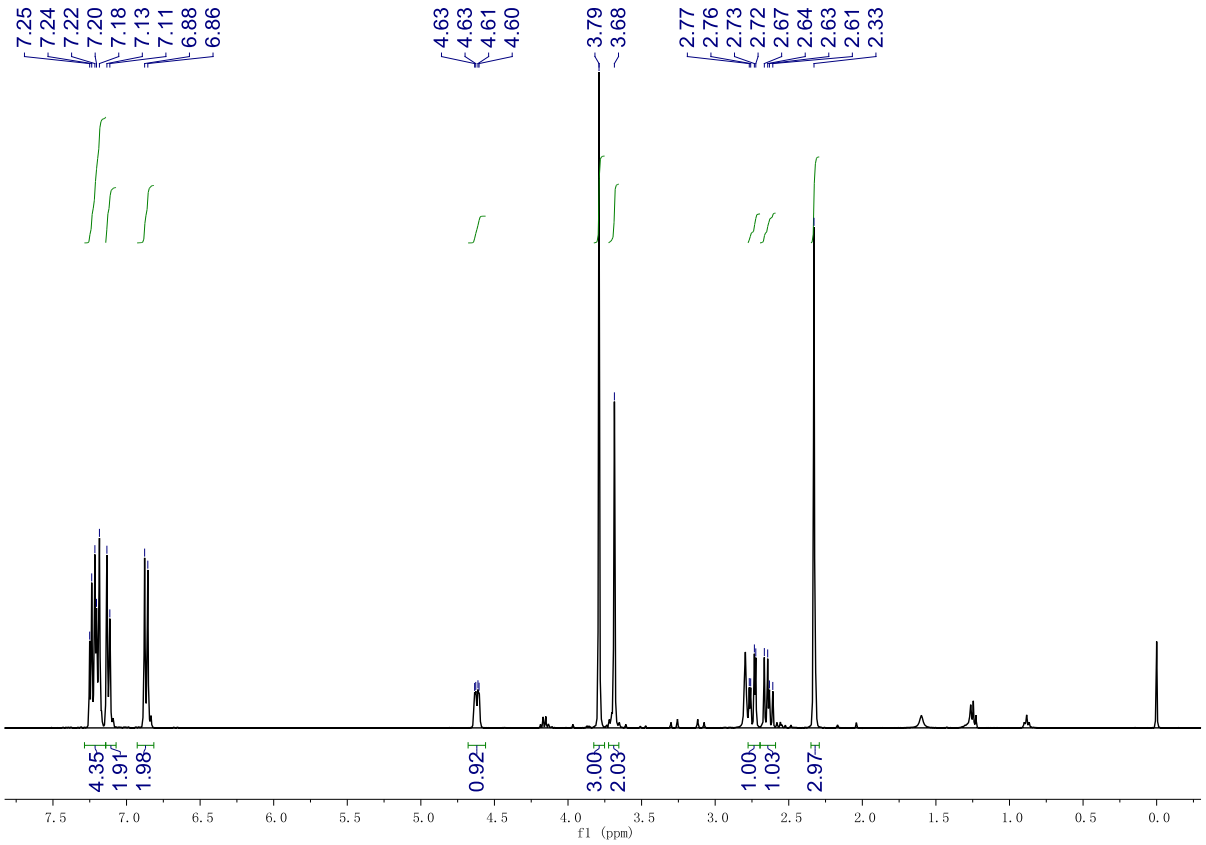
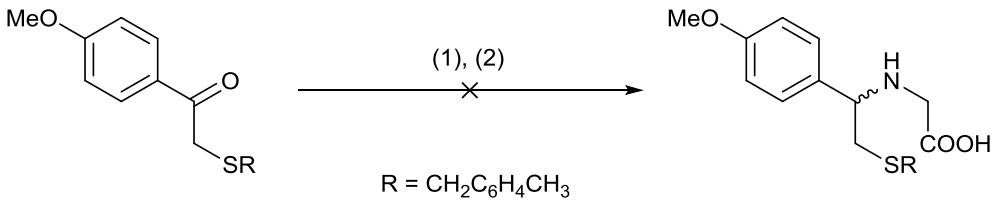


Figure 3.16 ^{1}H NMR spectrum of resulting alcohol from amination

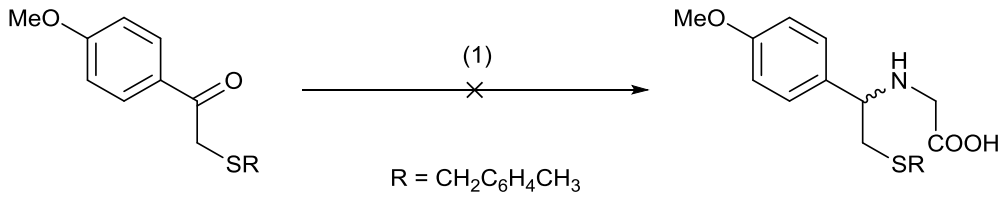
In a second attempt, the glycine ethyl ester hydrochloride was pre-treated with triethylamine in toluene and excess boron trifluoride etherate was used but unfortunately, the imine was still not formed. As it was considered that the presence of triethylamine in the system could be resulting in deactivation of the Lewis acid, disodium carbonate was also used but, again, no condensation occurred.



(67) **(66)**

Scheme 3.53 Attempt on reductive amination with glycine. (1), Glycine, $BF_3.Et_2O$ toluene:DMF, reflux; (2), $NaBH_4$, MeOH, 0 ^{o}C

The condensation was also attempted with glycine in DMF but the desired product (**66**) was still not formed.

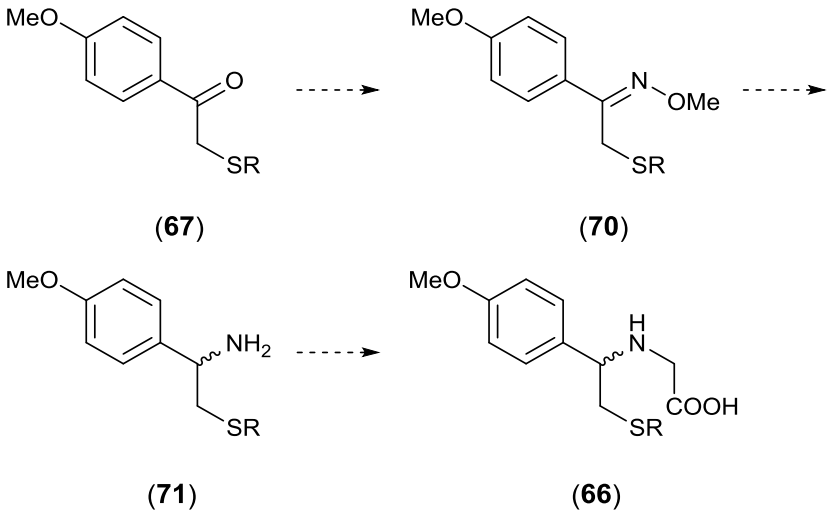


(**67**) (**66**)

Scheme 3.54 Attempted reductive amination with glycine. (1), Glycine, $NaBH(OAc)_3$, AcOH, $(CH_2Cl)_2$, r.t.

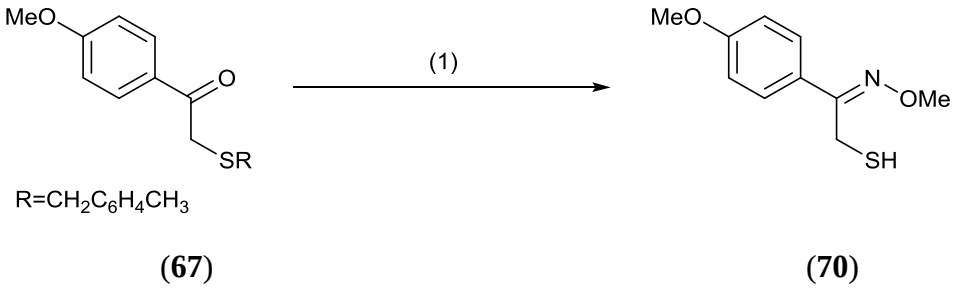
It has been reported by Abdel-Magid that glycine will condense with ketones in 1,2-dichloroethane (**Scheme 3.54)**.[77] Thus ketone (**67**) and glycine were added to 1,2-dichloroethane, followed by $NaBH(OAc)_3$ and acetic acid. The reaction was stirred at room temperature for 24 h, but only trace amounts of alcohol (**69**) was isolated and mostly starting material remained unreacted.

Despite the report of Abdel-Magid, glycine was found to be insoluble in dichloroethane, and so various solvents, such as ethyl acetate, DCM, MeOH and acetonitrile were examined but none led to the desired condensation. At this stage, rather than continue this approach, another amination method was investigated.



Scheme 3.55 Synthesis of target product (66)

In an alternative synthetic route to generate the desired compound (**66**), the ketone would first be converted into oxime (**70**) and then reduced to give a free amine (**71**) that could be then react with glyoxylic acid to give (**66**).



(**67**) (**70**)

Scheme 3.56 Synthesis of (*Z*)-1-(4-methoxyphenyl)-2-((4-methylbenzyl)thio)ethan -1-one *O*-methyl oxime. (1), Methoxylamine, pyridine, EtOH, reflux, 2 h

In the first step, compound (**67**) and pyridine were dissolved in absolute ethanol, followed by *O*-methyl hydroxylamine hydrochloride and the mixture was heated to reflux for 2 hours. Then after concentration of mixture, the residue was washed with 5 % HCl and brine, extracted with DCM and concentrated. Flash chromatography, eluting with diethyl ether and petroleum ether (1:2), afforded desired product (**70**). Minor (*E)*-oxime was also detected.

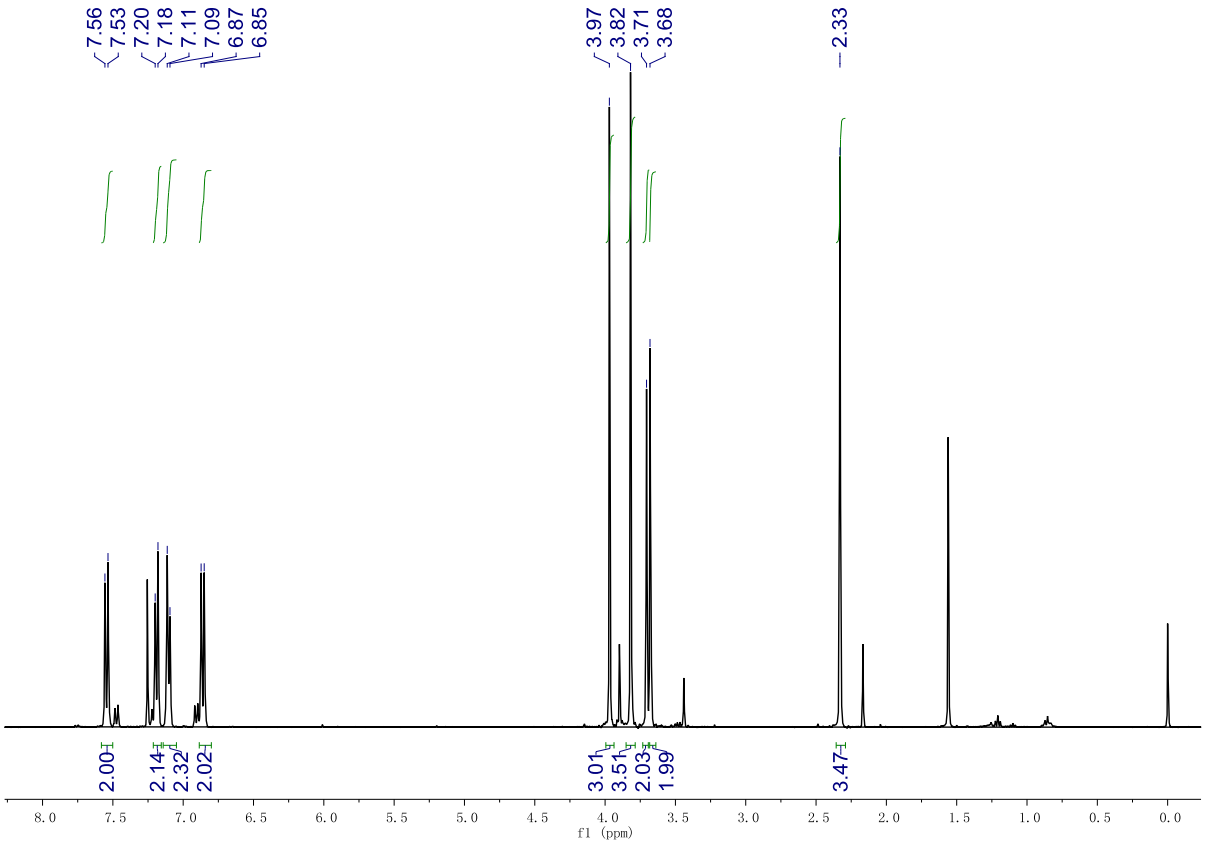
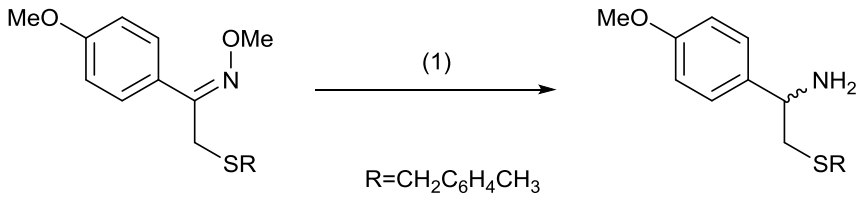


Figure 3.17 ^{1}H NMR spectrum of (*Z*)-1-(4-methoxyphenyl)-2-(4-methylbenzylthio)ethan -1-one *O*-methyl oxime (70).

In the ^{1}H NMR spectrum (**Figure 3.17**) a new singlet appeared at δ 3.97, which corresponded to the methoxy group of the oxime and mass spectrometry also confirmed the preparation of oxime (**70**) with a molecular ion at 316 $[M+H]^{+}$.



(**70**) (**71**)

Scheme 3.57 Synthesis of 1-(4-methoxyphenyl)-2-(*p*-tolylthio)ethan-1-amine (71). (1), BH_3.THF, THF, reflux, 3.5 h

In the next step, BH_3/THF solution was added to the solution of oxime (**70**) in THF at 0 ^oC, then the mixture was heated to reflux for 3.5 hours. The reaction was quenched with water and 5 M NaOH at 0 ^oC, and the mixture was stirred at room temperature for a further 30 minutes. After being acidified with 12 M HCl, the mixture was extracted with ether and the aqueous layer was basified and extracted again with DCM. After drying and concentrated *in vacuo*, column chromatography, eluting with diethyl ether and petroleum ether (1:1) led to isolation of desired product (**71**).

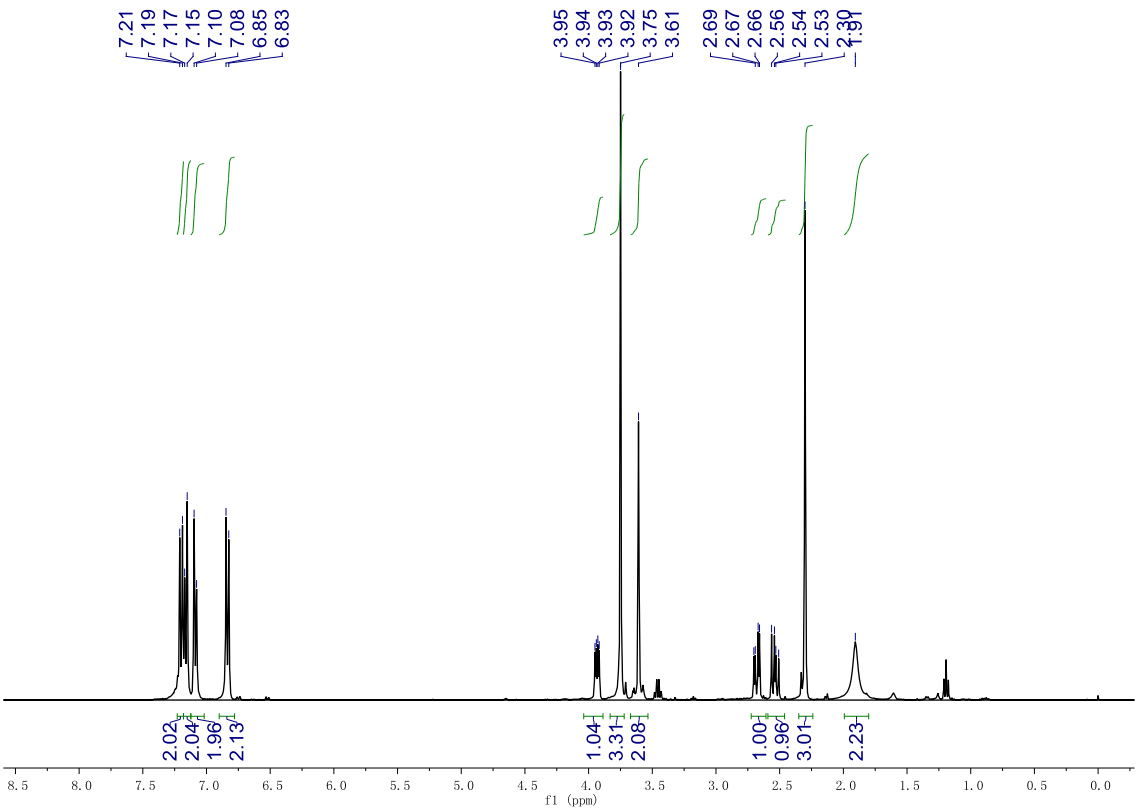
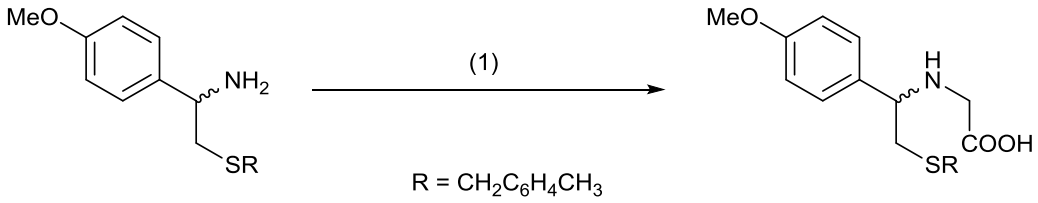


Figure 3.18 ^{1}H NMR spectrum of 1-(4-methoxyphenyl)-2-(*p*-tolylthio)ethan-1-amine (71).

Examination of the ^{1}H NMR spectrum showed that the methoxy-singlet at δ 3.97 ppm had disappeared, and an ABX system had appeared at δ 3.94 (J = 9.0, 4.5 Hz), 2.67 (J = 13.5, 4.5 Hz) and 2.54 (J_{AB} = 13.5, 9.0 Hz) ppm. Further support for the successful reduction came from mass spectrometry with a molecular ion at 288 $[M+H]^+$.



(**71**) (**66**)

Scheme 3.58 Synthesis of *N*-(1-(4-methoxyphenyl)-2-(4-methylbenzylthio)ethyl glycine (66). (1), Glyoxylic acid, $NaCNBH_3$, acid, MeOH, r.t.

Then, amine (**71**) was dissolved into methanol, followed by glyoxylic acid, a drop of concentrated hydrochloric acid, and $NaCNBH_3$. The reaction was stirred at room temperature for 24 hours, but no product was generated. As it was believed that water in concentrated HCl could be interfering with the reaction, HCl with molecular sieves and glacial acetic acid were both attempted, but both sets of conditions failed. However, when methanolic hydrochloric acid was used, white precipitate was formed after 24 hours. Work-up and purification by column chromatography furnished the desired material in a 42% yield.

The product was characterized by ^{1}H NMR analysis. The ABX coupling system appeared at δ 4.28 (J = 7.5 Hz), 3.04 (J = 14.0, 7.5 Hz) and 2.91 (J = 14.0, 7.5 Hz) ppm. The two diastereotopic protons from the methylene group of the glycine were observed at δ 3.23 ppm as an AB system (J = 16.0 Hz). The success of the reductive amination was also confirmed by mass spectrometry with a molecular ion at 346 $[M+H]^+$.

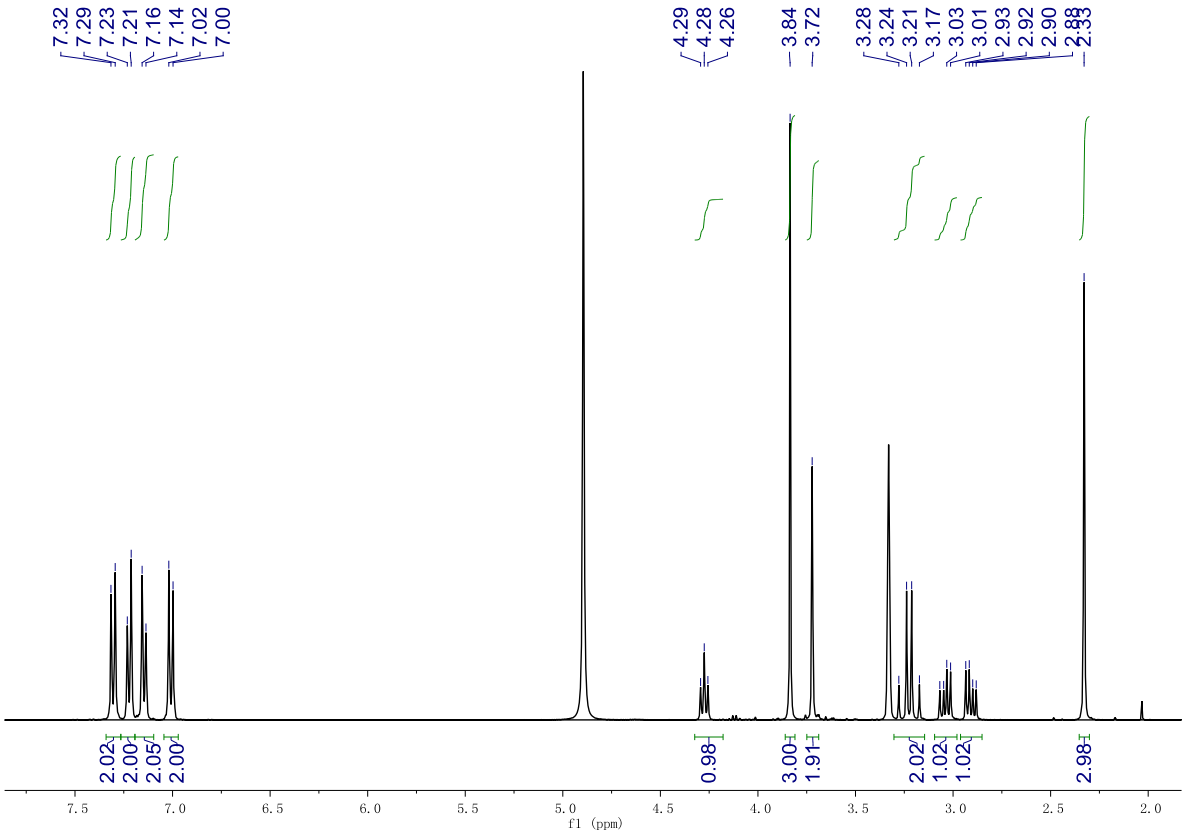
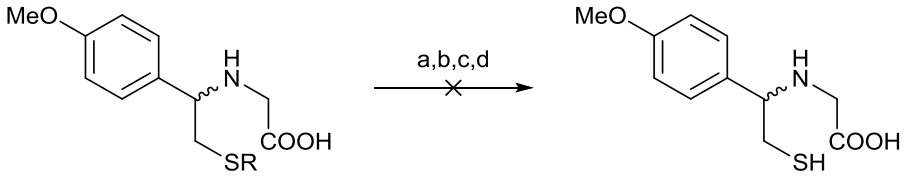


Figure 3.19 ^{1}H NMR of (1-(4-methoxyphenyl)-2-(4-methylbenzylthio)ethyl)glycine (66).



(**72**)

a, Mg, $HCOONH_4$, MeOH, r.t.;
b, $TiCl_3$, Li, THF, 50 ^{o}C;
c, Cp_2TiCl_2, *n*-Bu_2Mg, diglyme, 0 ^{o}C
d, HBF_4, thioanisole, TFA, r.t.

Scheme 3.59 Deprotection of 4-methybenzyl group.

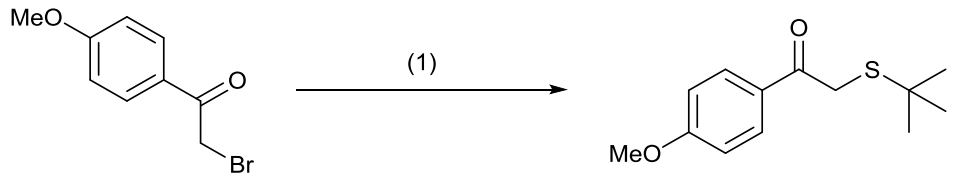
To remove the 4-methylbenzyl group we considered Mg/$HCOONH_4$ as this combination was reported by Babu *et al.* to be used for deprotection of *N*-Bn, *O*-Bn and *S*-Bn derivatives in quantitative yields.[78]

As **Scheme 3.59 (a)** shows, the reaction was carried in MeOH under N_2. After 2 hours, the crude product was characterized by ^{1}H NMR analysis and it was found that the singlet of benzyl methylene group in the starting material had become a quartet, the triplet from the ABX coupling had shifted from δ 4.28 to 3.87, the two double doublets had converged at δ 2.75 ppm and become a multiplet and the AB system had become a double doublet. Initially we thought that we had deprotected the substrate, but the mass spectrum showed the same molecular ion and fragment patterns as the starting material. Therefore, it was proposed that all the chemical shifts were due to the proton transfer from the carboxylic acid to amine.

Low valent titanium (LVT) mediated cleavage has been reported as an efficient and mild protocol for the deprotection of benzyl thioethers in good yields.[79] $TiCl_3$ and lithium metal were heated to reflux in anhydrous THF for 3 hours to prepare the LVT reagent (**Scheme 3.59 (b)**). Then compound (**66**) was added and the mixture was first stirred at room temperature for 24 hours, but no product could be observed on TLC analysis. Even when the temperature was raised to 60 ^oC for 24 hours, no product was observed on TLC analysis.

Another titanium reagent, Cp_2TiCl_2, has been reported by Akao *et al*. to be able to remove the thiobenzyl protecting group.[80] However, once again, no product was obtained from our substrate using this reagent. A solution of HBF_4 and thioanisole in TFA has found use in peptide synthesis.[81] It is able to remove thiobenzyl groups and partially remove 4-methylbenzyl groups but from mass spectroscopic analysis of the crude product from our substrate showed once again that deprotection had not occurred.

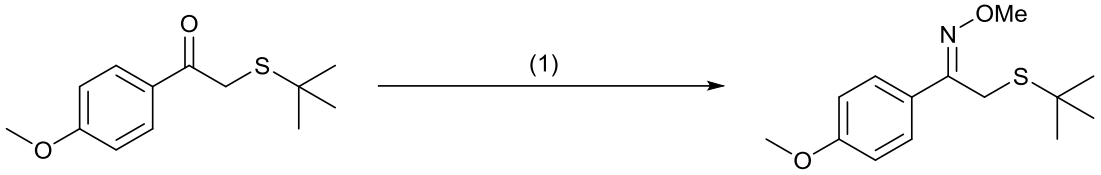
The *t*-Butyl group as another sulfur protecting group was also investigated, as it can be readily removed by strong acids such as HF,[82] HCl,[83] and combinations of $TMSiSO_3$/TFA.[84]



(**73**)

Scheme 3.60 Synthesis of 4-methoxyphenyl *tert*-butylthiomethyl ketone (73). (1), *t*-Butylthiol, DIEA, DMF, r.t. 15h

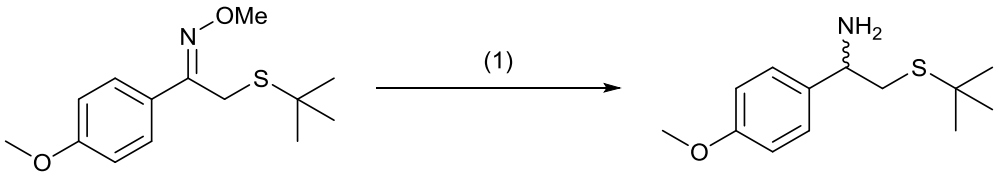
To install the *t*-butyl group onto sulfur, the procedure was followed the same as for the synthesis of 4-methoxyphenyl 4-(methylbenzyl)thiomethyl ketone (**67**). In this manner, (**73**) was synthesized successfully and characterized by ^{1}H NMR analysis. The singlet at δ 1.36 ppm confirmed the presence of the *t*-butyl group and two singlets at δ 3.87 and 3.84 ppm corresponded to the methoxy-group and methylene group respectively.



(**73**) (**74**)

Scheme 3.61 Synthesis of (*Z*)-2-(*tert*-butylthio)-1-(4-methoxyphenyl)ethan-1-one *O*-methyl oxime (74). (1), Methoxylamine, pyridine, EtOH, reflux

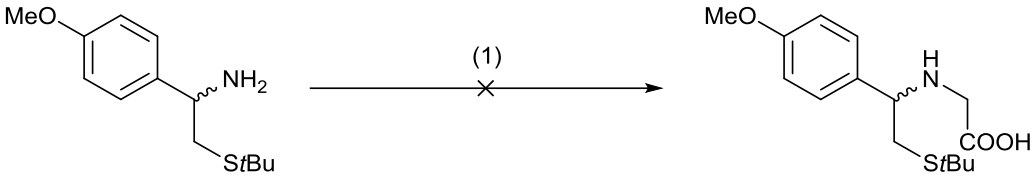
Oxime (**74**) was synthesized by heating compound (**73**) and methoxylamine in ethanol for 2 hours. After purification and characterization, a singlet appearing at 4.01 ppm confirmed the synthesis of oxime (**74**). As former experiment, (*E*)-oxime was also found in the spectrum.



(**74**) (**75**)

Scheme 3.62 Synthesis of 2-(*tert*-butylthio)-1-(4-methoxyphenyl)ethan-1-amine (75). (1), BH_3.THF, THF, reflux

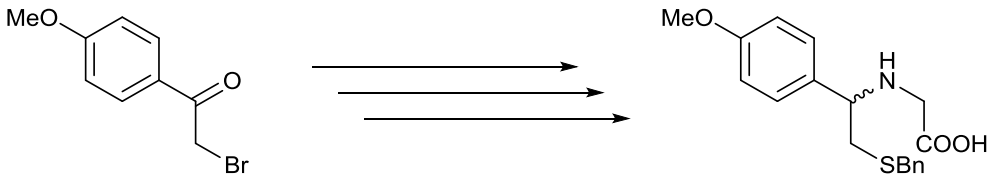
Then the oxime (**74**) was then reduced using BH_3/THF for 3.5 hours. The formation of amine (**75**) was confirmed by the presence of an ABX coupling system in the ^{1}H NMR spectrum with three double doublets appearing at δ 4.05 (*J* = 9.0, 5.0 Hz), 2.87 (*J* = 12.5, 5.0 Hz) and 2.77 (*J* = 12.5, 9.0 Hz) ppm respectively. However, when it was attempted to purify amine (**75**) by column chromatography, no product was recovered and so it was decided that the amine (**75**) would subsequently be used directly in the next step without further purification.



(**75**) (**76**)

Scheme 3.63 Amination of 2-(*tert*-butylthio)-1-(4-methoxyphenyl)ethan-1-amine (75). (1), Glyoxylic acid, $NaCNBH_3$, acid, MeOH, r.t.

Unfortunately, the reductive amination with glyoxylic acid was not successful. After more than 24 hours, no product was detected from ^{1}H NMR analysis, despite several multiple attempts to achieve this.



(**80**)

Scheme 3.64 Synthesis of (1-(4-methoxyphenyl)-2-(benzylthio)ethyl)glycine (80)

As many deprotecting methods relate to benzyl groups, benzyl mercaptan was then used to install sulfur onto 2-bromo-4'-methoxyacetophenone, followed by oxime formation, reduction and amination to furnish (1-(4-methoxyphenyl)-2-(benzylthio)ethyl)glycine (**80**) after work-up and column chromatography. In the ^{1}H NMR spectrum, an ABX system was observed with a triplet at 4.07 (J = 7.5 Hz) ppm and two double doublets at 2.92 (J = 14.0, 7.5 Hz) and 2.78 (J = 14.0, 7.0 Hz) ppm, and an AB quartet was also observed at 3.10 (J_{AB} = 16.0 Hz) ppm, which suggested the successful preparation of (**80**). However, the yield was only 17 %, which might because of its ready solubility in MeOH. At this stage there was no further time for experimentation and so, frustratingly, this chapter of the work remains incomplete.

3.7 Conclusions

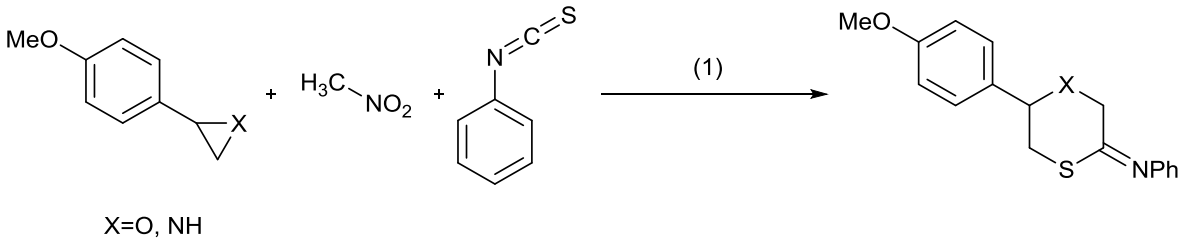
In conclusion, 5-(4-methoxyphenyl)morpholin-2-one was successfully synthesized, starting from 4-hydroxyphenylglycine. Then attempts were made to converted it to 5-(4-methoxyphenyl)thiamorpholin-2-one by Lawesson's Reagent. However, the morpholinone was not stable at elevated temperatures. After considering various retrosyntheses, the first synthetic route proposed was to use an amino-thiol to synthesize 5-(4-methoxyphenyl)thiamorpholin-2-one. The second approach was to synthesize a chlorinated thioester followed by nucleophilic substitution to generate desired the thiamorpholinone. However, a NCL related reaction was found to be favoured. An approach employing episulfide starting materials was believed to provide a means to avoid NCL but, despite literature precedent, the episulfide readily decomposed and could not be

opened with a range of Lewis acids. Finally, (1-(4-methoxyphenyl)-2-((4-methylbenzyl)thio)ethyl)glycine was successfully synthesized, starting from 2-bromo-4'-methoxyacetophenone, followed by oxime formation, reduction and amination. However, deprotection of (1-(4-methoxyphenyl)-2-((4-methylbenzyl)thio)ethyl)glycine failed after many attempts, using reagent systems such as Mg/$HCOONH_4$, TiCl3/Li, Cp_2TiCl_2, *n*-Bu_2Mg and HBF_4 in TFA. Other sulfur protecting groups were also employed, such as *t*-butyl and benzyl groups with only the benzyl pretected (1-(4-methoxyphenyl)-2-(benzylthio)ethyl)glycine being successfully prepared. Given time, in the future, it is reasonable to believe that the benzyl protecting group can be removed, followed by thioester coupling to synthesize the desired parent 5-(4-methoxyphenyl)thiamorpholin-2-one.

Chapter 4 Attempts to develop a one-pot reaction to synthesize the 1,4-thiamorpholinone template

4.1 One-pot synthesis via phenyl isothiocyanate

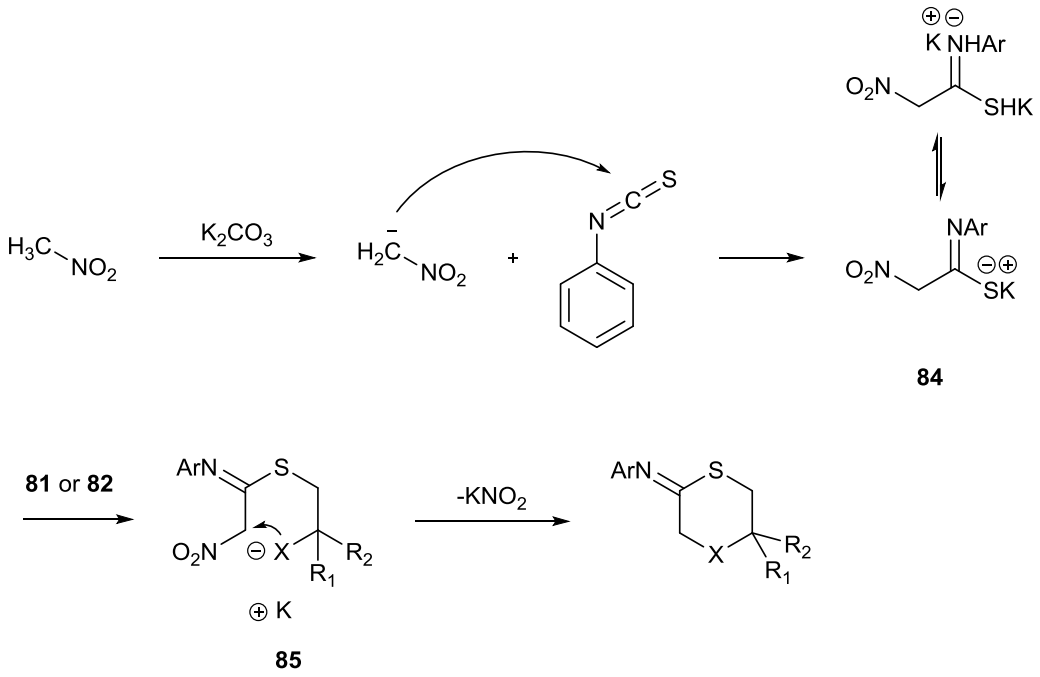
It was reported by Samzadeh-Kermani in 2014 that 1,4-oxathiane and 1,4-thiamorpholine derivatives could be prepared in a one pot reaction using nitromethane, an aromatic isothiocyanate, and epoxides or aziridines in the presence of K_2CO_3 in DMF at 60 ^oC.[85]



(**81**) X= O (**82**) X=N (**83**)

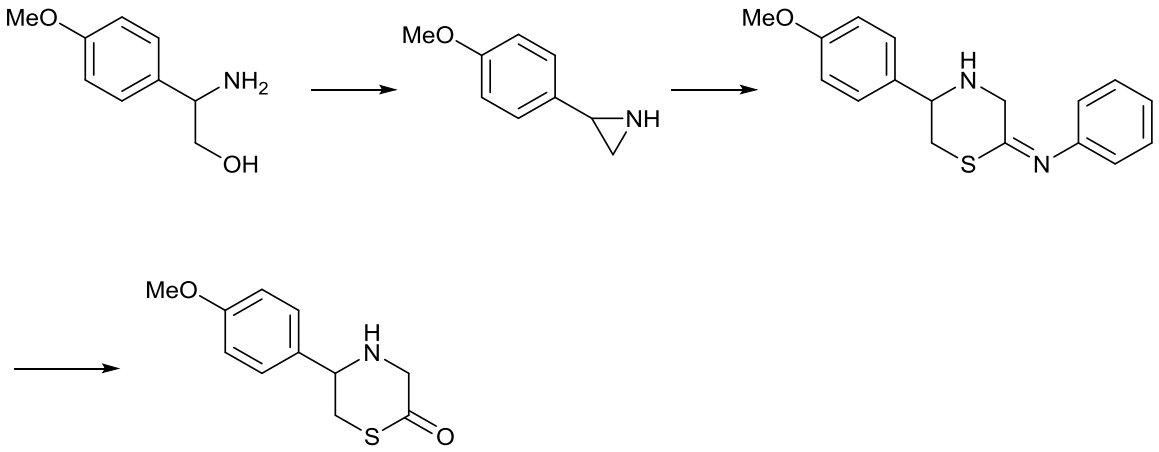
Scheme 4.1 One-pot synthesis of 1,4-thiamorpholine. (1), K_2CO_3, DMF, 60 ^oC, 8h

The reaction mechanism proposed involved deprotection of the nitromethane and nucleophilic attack on isothiocyanate to afford intermediate (**84**), followed by addition of epoxide (**81**) or aziridine (**82**) to generate open chain intermediate (**85**). Finally, the cyclization of (**85**) generates desired product (**83**) (**Scheme 4.2**).



Scheme 4.2 Proposed mechanism of one-pot synthesis of 1,4-thiamorpholine

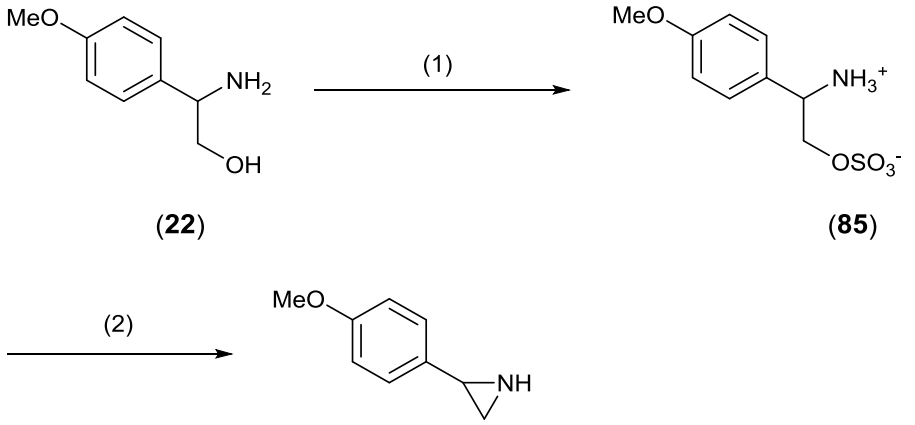
With 1,4-thiamorpholine (**83**) in hand, it would then be necessary to convert it to the desired thiamorpholinone (**22**) using acidic conditions to hydrolyse it.[86] The proposed sequence to obtain the desired product is shown in **Scheme 4.3**.



Scheme 4.3 Proposed synthetic route of synthesizing thiamorpholinone (22)

There is a range of commercially available aziridines, but the specific aziridine possessing a *para*-methoxyphenyl group is very expensive, and could only be purchased from outside the UK. According to a report by Buckley in 2012,[87] the aziridine can be synthesized in a

straightforward procedure in a yield of over 80%. Since the amino-alcohol precursor had already been made, it was decided to prepare the desired aziridine (**86**) following the sequence shown in **Scheme 4.4**.



(**22**) (**85**) (**86**)

Scheme 4.4 Synthesis of 2-(4-methoxyphenyl)aziridine (86). (1), $ClSO_3H$, MeCN, 0 ^oC; (2), NaOH(6 M) :Toluene 1:1, reflux, 18h

Amino-alcohol (**32**) was dissolved in acetonitrile, and the solution was cooled to 0 ^oC. Then chlorosulfonic acid was added dropwise to the reaction and the resulting heterogeneous solution was stirred at room temperature for 3.5 hours. After the reaction was complete, the mixture was filtered and the solvent removed *in vacuo*. The residue was washed with ethyl acetate and diethyl ether to afford (**85**) as a colourless solid.

The product was examined by ^1H NMR spectroscopy in DMSO that showed two multiplets at δ 4.65 and 4.32 ppm which coupled with each other, and a singlet at δ 3.80 ppm which proved the presence of the *para*-methoxyphenyl group. Mass spectrometry also furnished evidence for the successful synthesis of desired ammonium sulfate salt (**85**) with a molecular ion at 248 $[M+H]^+$.

The resulting ammonium sulfate (**85**) was dissolved in a 1:1 mixture of 6 M aqueous sodium hydroxide and toluene and the mixture was stirred and heated to reflux for 18 hours.

Then the organic phase separated and the aqueous phase extracted ethyl acetate and the combined organic extracts dried over $MgSO_4$. After removal of solvent, chromatographic purification eluting with diethyl ether and petrol (1:1) afforded the pure product as a pale yellow oil in a 56 % yield.

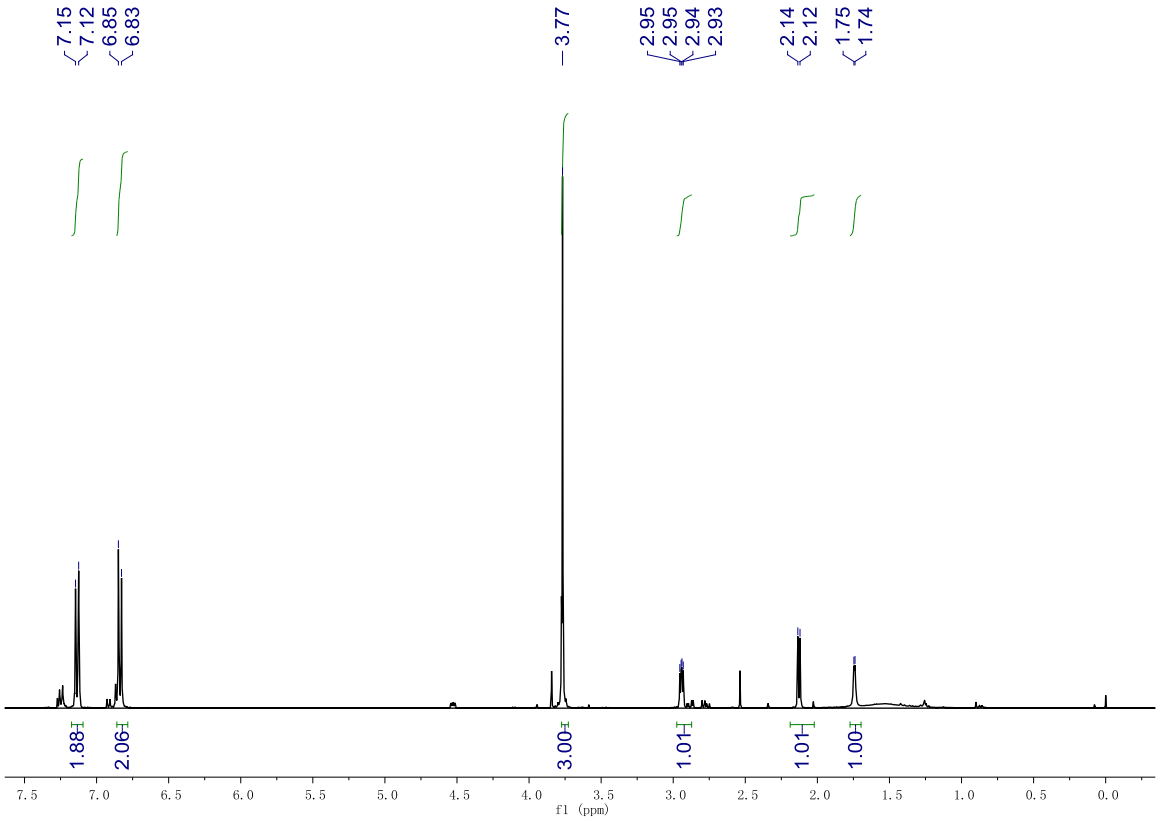
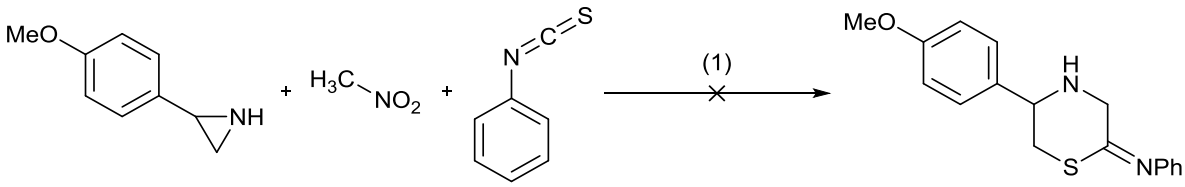


Figure 4.1 ^{1}H NMR spectrum of 2-(4-methoxyphenyl)aziridine (86)

The ^{1}H NMR spectrum showed the expected ABX system between δ 3.00 and 1.50 ppm. The double doublet at δ 2.94 ppm (J = 6.0, 3.5 Hz) was coupled with two doublets at δ 2.13 (J = 6.0 Hz) and 1.74 (J = 3.5 Hz) ppm, the latter begin the diastereotopic protons on the same carbon. Infrared analysis showed a strong absorption at 3361 cm^{-1} corresponding to the aziridine and no absorption around 1350 cm^{-1} that would have been due to the sulfate group. Finally, a molecular ion at 150 $[M+H]^+$ was observed in the mass spectrum.



(**86**) (**87**)

Scheme 4.5 Proposed synthesis of 1,4-thiamorpholine (87). (1), K_2CO_3, DMF, 60 ^oC, 8h

With the successful synthesis of the desired aziridine achieved, the next step was to make the desired 1,4-thiamorpholine (**22**). According to the literature[85], aziridine (**86**) and phenyl isothiocyanate were dissolved into DMF and K_2CO_3 was added. Then nitromethane was added to the mixture over 30 minutes. After addition, the reaction was stirred and initially heated to 60 ^oC for 8 hours. However, when no product formation was observed, the temperature was increased to 80 ^oC and the reaction time was extended to 15 hours when a new material had been formed as evidenced by TLC analysis. Work-up and purification by column chromatography, eluting with ethyl acetate and hexane (1:2) afforded the new material.

However, it rapidly became clear that this product was not our desired material. In the ^1H NMR spectrum, a 3H singlet at δ 3.79 ppm and two 2H doublets at δ 7.33 and 6.85 ppm indicated the presence of the para-methoxyphenyl group and further resonances at δ 7.26, 7.17 and 7.03 ppm indicated the phenyl isothiocyanate had also been assembled into this compound. An ABX system at δ 4.92 (J = 7.5 Hz), 4.10 (J = 11.0, 7.5 Hz) and 3.86 (J = 11.0, 7.5 Hz) ppm gave the evidence for a ring but, from the integration of these peaks, there were only three protons. Furthermore, the mass spectrum showed a molecular ion at 285 $[M+H]^+$ and thus we concluded that the product was not the desired 1,4-thioamorpholine (**87**), but in fact compound (**88**).

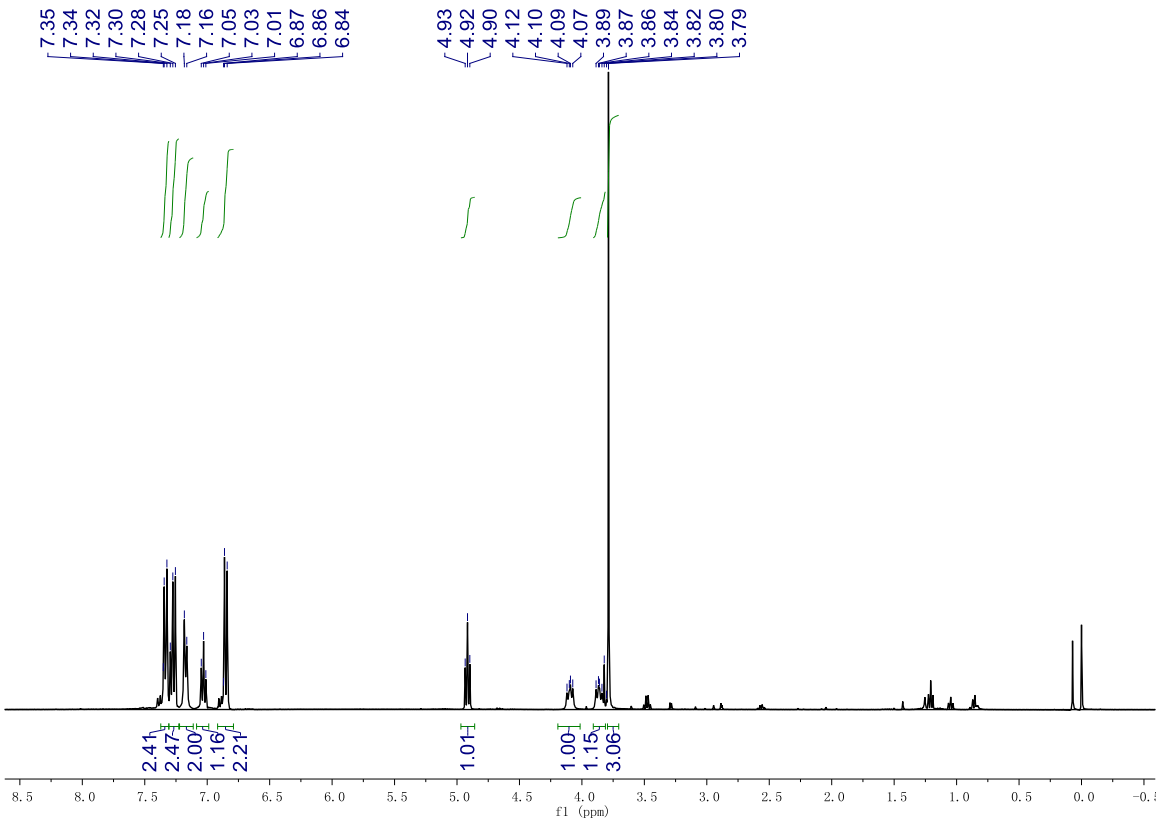
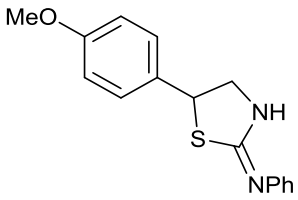
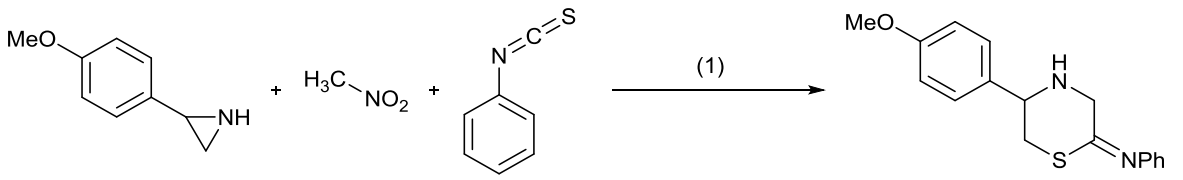


Figure 4.2 ^{1}H NMR spectrum of product (88)



Actual product 88

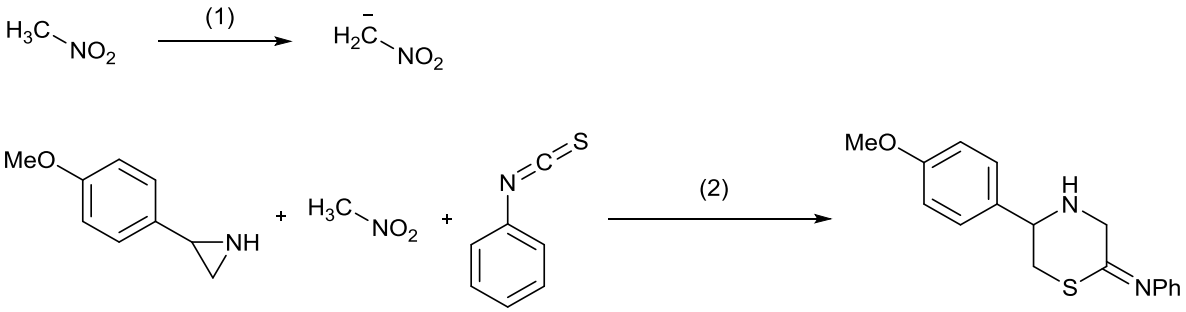
From the structure, it was clear that the nitromethane had not taken part into the reaction. As it was possible that base was not strong enough to activate the nitromethane, the next step was to use a stronger base.



(**86**) (**87**)

Scheme 4.6 Synthesis of 1,4-thiamorpholine with modified condition. (1), KOH, DMF, 60 ^{o}C, 8h

Therefore the same procedure was repeated with potassium hydroxide as inorganic base but, once again, only the five-membered ring was generated.



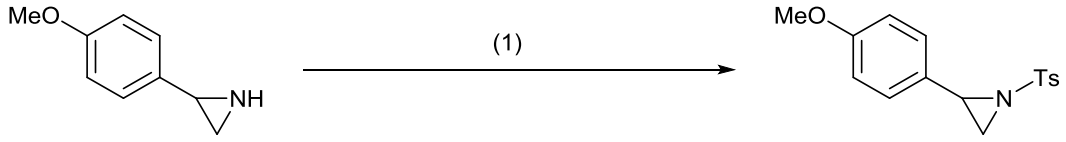
(**86**) (**87**)

Scheme 4.7 Synthesis of 1,4-thiamorpholine with sodium hydride. (1), NaH, DMF, 60 ^{o}C, 8h; (2), NaH, DMF, 60 ^{o}C, 8h

NaH, a base of wide utility in organic chemistry,[88] is capable of deprotonating a range of even weak Brønsted acids to give the corresponding sodium derivatives. Thus, sodium hydride was added to a solution of nitromethane in DMF and the mixture stirred overnight to ensure complete deprotonation. Then phenyl isothiocyanate and aziridine (**86**) were added and the reaction was stirred and heated to 60 ^{o}C for 8 hours. Once again however, the same 5-membered ring product was isolated after work-up and column chromatography.

In the original paper,[85] the *N*-protected aziridine was also used and it seemed likely therefore that the unprotected aziridine was quenching the deprotonated nitromethane. Thus

we decided prepare and use the *N*-tosylated aziridine even though removal of *N*-tosyl groups commonly requires forcing conditions.

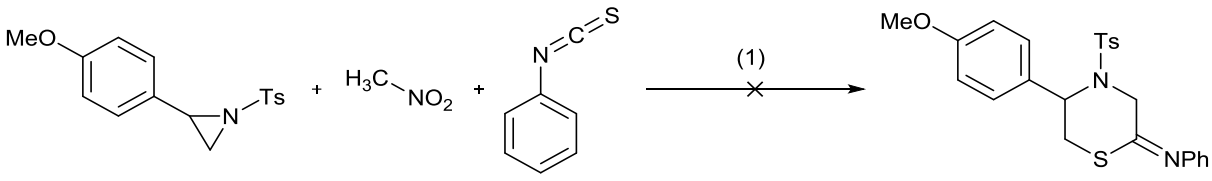


(**86**) (**89**)

Scheme 4.8 Synthesis of 2-(4-methoxyphenyl)-1-tosylaziridine (89). (1), 4-Toluenesulfonyl chloride, Et_3N, DCM, 0 ^oC-r.t.

Therefore, aziridine (**86**) was dissolved in dichloromethane containing trimethylamine, the solution cooled to 0 ^oC and *para*-toluenesulfonyl chloride was added. The reaction was stirred at this temperature for 2 hours and then it was allowed to warm to room temperature overnight. The mixture was quenched extracted with dichloromethane, washed with water, and the organic phase dried over $MgSO_4$. On removal of solvent, the residue was purified by column chromatography, eluting with diethyl ether: petrol (1:1) to afford the *N*-Ts protected aziridine.

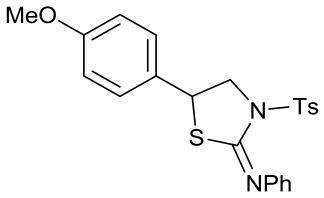
From examination of the ^1H NMR spectrum, it was clear that the *p*-toluenesulfonyl protected aziridine have been successfully synthesized with a new singlet at δ 2.43 ppm corresponding to the methyl group, and two new apparent doublets at δ 7.32 and 7.86 ppm corresponded to the para-substituted phenyl group. All three protons on the aziridine ring had shifted to lower field. The mass spectrum further supported the structure with a molecular ion at 304 $[M+H]^+$.



(**89**) (**90**)

Scheme 4.9 Synthesis of *N*-Ts protected 1,4-thiamorpholine (90). (1), Cs_2CO_3, DMF, 60 ^oC, 8h

This *N*-Ts protected aziridine was immediately used in the next reaction. Using the same conditions and procedure as reported, *N*-tosyl aziridine (**89**), phenyl isothiocyanate and nitromethane were added to DMF and the mixture was heated at 60 ^oC for 8 hours. After work-up and purification by chromatography, eluting with petroleum ether and diethyl ether (1:2) containing 1% of trimethylamine, a new product was isolated as a pale yellow oil.



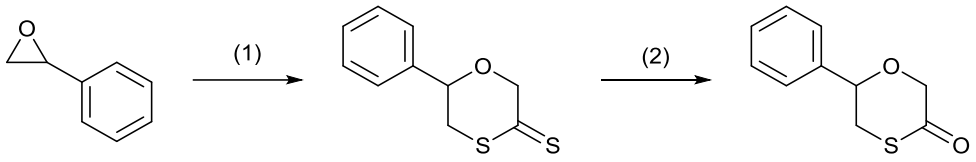
Actual product (91)

The ^1H NMR spectrum showed the presence of *N*-tosyl and methoxyphenyl groups with two singlets at δ 3.79 and 2.48 ppm. In addition, an ABX system was present similar to that observed when using unprotected aziridine and so we concluded that the five-membered ring had once again been generated and that nitromethane was still not involved in this reaction.

At this stage, at a loss as to the reasons why we could not repeat the literature reaction we decided on yet another approach.

4.2 Attempted one-pot synthesis by insertion of carbon disulfide into aziridines

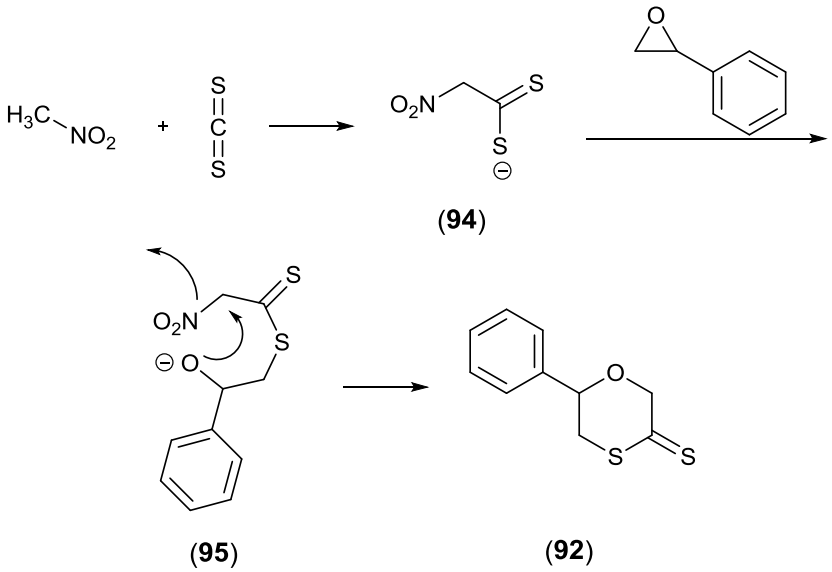
During the course of these studies, we became aware of a new method to synthesize 1,4-oxathiane-3-thiones (**92**)[89] and proposed that such 1,4-oxathiane-3-thiones could then be modified to (**93**) by a sulfur-oxygen exchange process such as was presented in the previous chapter.



(**92**) (**93**)

Scheme 4.10 Proposed synthetic route of 1,4-oxathiane-3-thiones (92). (1), CH_3NO_2, CS_2, Et_3N, H_2O, 70 ^oC, 2h; (2), $BF_3.Et_2O$

The mechanistic rationale proposed that intermediate (**94**) formed by attack of nitromethane on carbon disulfide added to the epoxide to generate (**95**). Subsequent cyclization of this intermediate would lead to compound such 1,4-oxathiane-3-thione (**92**) by loss of nitrite.



(**94**)

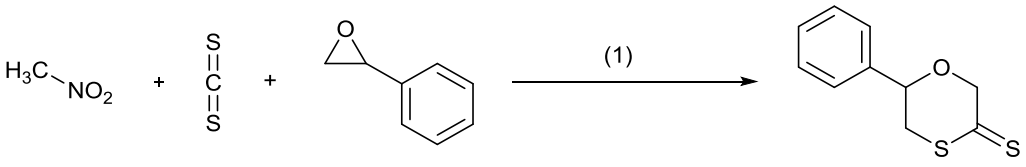
(**95**) (**92**)

Scheme 4.11 Mechanism for synthesis of 1,4-oxathiane-3-thione (92)

We proposed that an aziridine could be used in place of an epoxide to give the corresponding 1,4-thiamorpholinethione derivative that could be converted to the desired 1,4-thiamorpholinone system by reacting with BF_3 as described in the literature.[64]

In the reported work[89], the nitromethane was deprotonated under microwave condition but, as an appropriate apparatus was not available at Reading, this reaction was only carried out under standard heating.

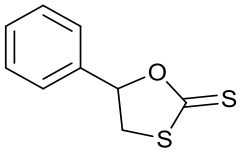
In our initial studies, we decided to use styrene oxide to prepare 1,4-oxathiane-3-thione and follow the literature protocol, with the important difference that microwave heating would not be used.

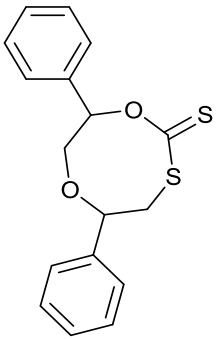


(**92**)

Scheme 4.12 Synthesis of 1,4-oxathiane-3-thione (92). (1), Et_3N, H_2O, 70 ^oC, 2h

Following the literature procedure, nitromethane and triethylamine were added to water, and the solution was heated to 70 ^oC for 30 minutes. Then styrene oxide was added, followed by CS_2, and the reaction mixture was stirred and heated to 70 ^oC for 2 hours further. After the reaction was finished a solid product was isolated and purified by recrystallization from ethyl acetate. However, analysis by ^1H NMR spectroscopy indicated that this solid consisted of two components, so it was subjected to column chromatography, eluting with ethyl acetate and hexane (1:2) and the first compound to elute was identified as the 5-membered ring product (**96**) resulting from attack of carbon disulphide directly on the epoxide.





(**96**) (**97**)

Actual product (96) and (97)

The ^{1}H NMR spectrum showed three double doublets at δ 5.64 (*J* = 10.5, 5.5 Hz), 4.18 (*J* = 12.0, 10.5 Hz) and 4.04 (*J* = 12.0, 5.5 Hz) ppm corresponded to ABX system in a ring structure corresponding to three aliphatic protons. The mass spectrum showed a molecular ion at 195, which matches formula of $C_9H_8OS_2$. As the solid before chromatography contained two components we were able to propose that they are (**96**) and (**97**). Once again, this method had given us five-membered ring without nitromethane being involved in the reaction.

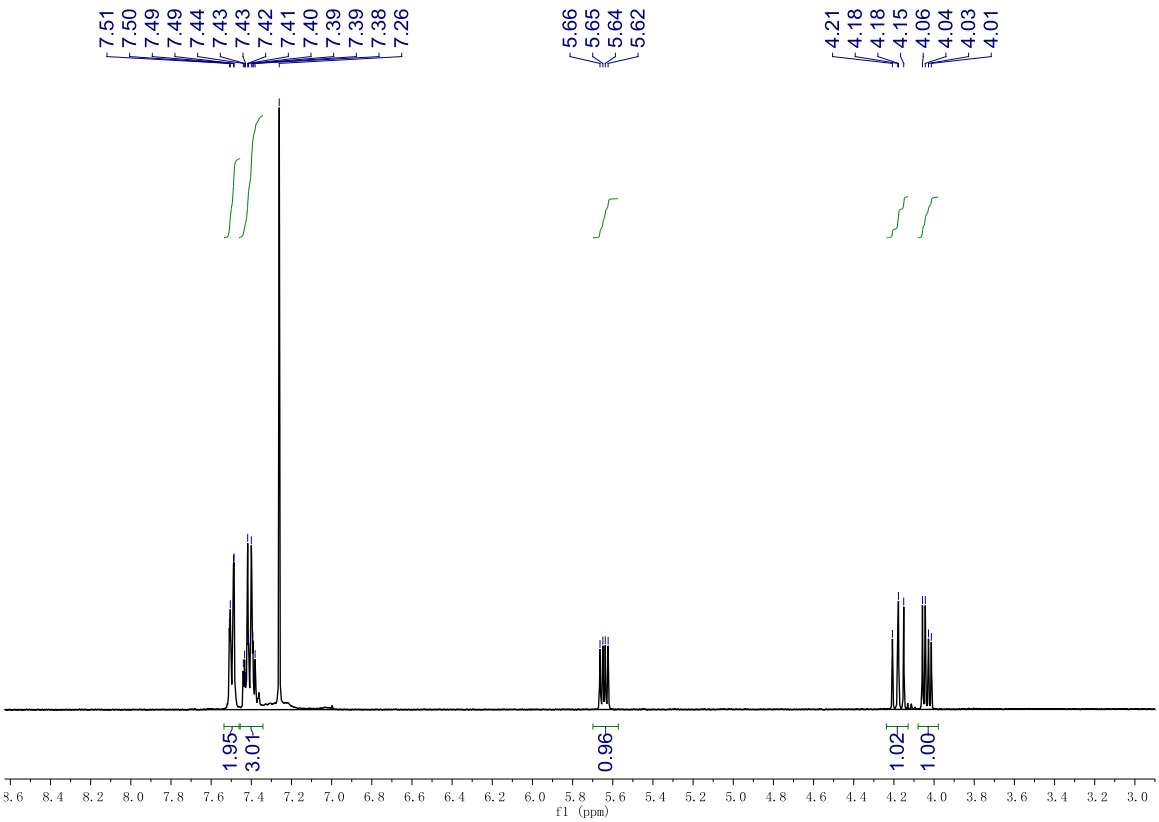


Figure 4.3 ^{1}H NMR spectrum of (96)

Presuming that application of microwave conditions was necessary for success in this reaction we abandoned this approach at this stage.

Chapter 5 Experimental section

^{1}H spectra were recorded in either chloroform-*d* ($CDCl_3$) and referenced either to residual solvent peaks or to tetramethylsilane as internal standard or in deuterated methanol (CD_3OD) using a Bruker AMX400 (400MHz), a Bruker Avance DPX400 (400MHz) and a Bruker AVIII 500 (500MHz) spectrometer. Peak positions were recorded in δ ppm with the abbreviations s, d, t, q, m, q_{AB} denoting singlet, doublet, triplet, quartet and multiplet, AB quartet respectively. *J* values are given in Hertz (Hz). ^{13}C NMR and DEPT spectra were recorded on the same spectrometers. In a few ^{13}C NMR spectra, resonances were found more than the carbon number from the structure was caused by rotamers. N.O.e experiments were carried out by Mr P. Heath and Dr R. M. Kowalczyk at the University of Reading and recorded on a Bruker AVIII 500 (500MHz) spectrometer.

Infra-red spectra were recorded on a Perkin-Elmer FT-IR spectrometer Spectrum 100. All absorption positions are quoted in cm^{-1}.

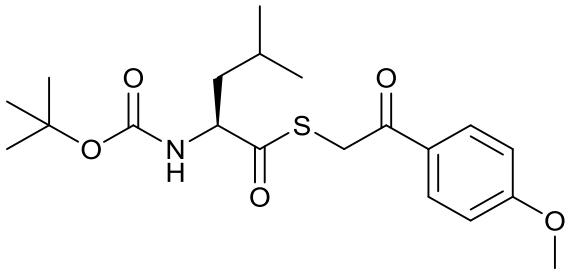
Mass-spectrometric data ($^{m}/_{z}$) and accurate mass measurements (HRMS) were recorded on a Fisons VG Autospec mass spectrometer under electron impact (E.I.) or by chemical ionisation using ammonia as the ionising source (C.I.).

Melting points were recorded using a Gallenkamp melting point apparatus and are uncorrected.

TLC analysis was obtained by using silica gel pre-coated aluminium backed plates and spots were visualised either by the quenching of fluorescence under 254 nm UV light or staining with a potassium permanganate solution, palladium (II) chloride solution, bromocresol green solution or iodine. Flash column chromatography was performed according to the method of Still[90] with silica gel 60 Å using head pressure applied by the means of hand bellows.

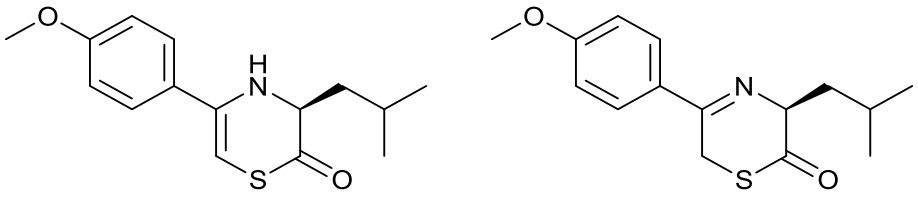
Reagents obtained from Sigma-Aldrich, Acros, Alfa Aesar, Fluka and Tokyo Chemical Industry were used as supplied. Tetrahydrofuran dried over molecular sieves or by distillation from sodium benzophenone ketyl, and dichloromethane was dried by distillation from calcium hydride under nitrogen. Petrol refers to light petroleum ether in the boiling point range 40-60 ^{o}C.

Preparation of (*S*)-2-(4-methoxyphenyl)-2-oxoethyl 2-(*tert*-butoxycarbonylamino)-4-methylpentanethioate (6)[46]



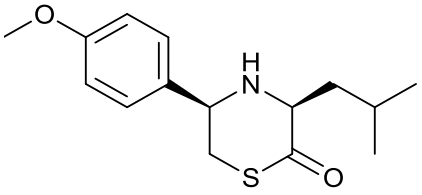
Triethylamine (1.20 mL, 8.7 mmol, 1.0 equiv) was added to a stirred solution of Boc-Leu-OH (2.00 g, 8.7 mmol) in dry THF at 0 ^{o}C. After 30 min, ethyl chloroformate (0.83 mL, 8.7 mmol, 1.0 equiv) was added to the solution, and the mixture was stirred for 10 min at 0 ^{o}C. Then sodium hydrosulfide hydrate (1.21 g, 21.6 mmol, 2.5 equiv) was added and the mixture stirred for 2 hours at 0 ^{o}C. After 2 hours, the ice bath was removed, bromomethyl(4-methoxyphenyl)ketone (1.98 g, 8.7 mmol, 1.0 equiv) was added and the mixture stirred overnight at ambient temperature. Methanol (10 mL) was added to neutralise the mixture, which was diluted with H_2O (20 mL) and extracted with DCM (3 x 20 mL), the combined organic layers were dried over $MgSO_4$, filtered, the solvent was evaporated *in vacuo* and the crude material was purified by flash column chromatography on silica, eluting with petrol and diethyl ether (2 : 1) to furnish the title product as a yellow oil (2.11 g, 58 %). m.p. 75 - 80 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (d, *J* = 9.0 Hz, 2H), 6.93 (d, *J* = 9.0 Hz, 2H), 5.18 (d, *J* = 8.5 Hz, 1H), 4.39 (d, *J* =16.0 Hz, 1H), 4.27 (d, *J* = 16.0 Hz, 1H), 3.86 (s, 3H), 1.71 - 1.68 (m, 2H), 1.53 - 1.51 (m, 1H), 1.45 (s, 9H), 0.94 (d, *J* = 6.0 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 201.42, 193.18, 163.98, 131.18, 131.18, 131.04, 128.79, 114.08, 114.06, 59.34, 55.69, 45.30, 41.37, 37.44, 36.50 28.45, 24.90, 23.21, 21.71. IR (ATR): $\acute{\upsilon}_{max}$ 2962, 2841, 2252, 1672, 1599 cm^{-1}. HRMS calculated for $C_{20}H_{29}NO_5SNa^+$: 418.1659, found 418.1659 $[M+Na]^+$. $[\alpha]_D^{20}$ -30.0. (*c* 0.5 $CHCl_3$).

Preparation of 3-*iso*butyl-5-(4-methoxyphenyl)-3,4-dihydro-2*H*-1,4-thiazin-2-one (7) and 3-*iso*butyl-5-(4-methoxyphenyl)-3,6-dihydro-2*H*-1,4-thiazin-2-one (8)[46]



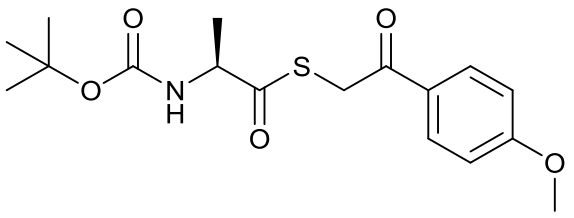
Trifluoroacetic acid (7 mL) was added to a solution of (*S*)-2-(4-methoxyphenyl)-2-oxoethyl 2'-(*tert*-butoxycarbonylamino)-4-methylpentanethioate (**6**) (1.70 g, 4.3 mmol, 1.0 equiv) in DCM (7 mL), and the mixture was stirred overnight. The solvent and excess trifluoroacetic acid were removed *in vacuo* and the residue was dissolved in DCM to which K_2CO_3 (5.00 g, excess) and 4Å molecular sieves (5.00 g) were added, and the resulting mixture was stirred for 24 hours. Filtration through a short pad of Celite®, washig with DCM and removal of solvent from the filtrate *in vacuo* gave the crude oil (1.27 g, 75 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (d, *J* = 9.0 Hz, 2H), 7.92 (d, *J* = 9.0 Hz, 2H), 7.82 (d, *J* = 9.0 Hz, 2H), 6.94 (t, *J* = 9.0 Hz, 2H), 5.23 (d, *J* = 1.5 Hz, 1H), 4.09 - 3.96 (m, 1H), 3.87 (t, *J* = 4.0 Hz, 3H), 3.61 (dd, J_1 = 9.0, J_2 = 5.0 Hz, 1H), 2.11 - 1.24 (m, 4H), 1.12 – 0.76 (m, 6H). ^{13}C (101 MHz, $CDCl_3$) δ 202.7, 193.0, 192.8, 165.7, 164.0, 163.9, 161.9, 131.2, 131.0, 129.7, 128.5, 127.5, 114.03, 113.9, 113.9, 68.3, 55.5, 45.2, 45.0, 39.5, 37.5, 29.4, 24.9, 23.4, 21.7. HRMS calculated for $C_{15}H_{20}NO_2S^+$: 278.1209, found 278.1206 $[M+H]^+$.

Preparation of 3-(*S*)-*iso*butyl-5-(*R*)-(4-methoxyphenyl)thiamorpholin-2-one (9)[46]



The mixture of 3-(*S*)-isobutyl-5-(4-methoxyphenyl)-4,5-dehydro-2*H*-1,4-thiazin-2-one (**7**) and 3-(*S*)-isobutyl-5-(4-methoxyphenyl)-5,6-dehydro-2*H*-1,4-thiazin-2-one (**8**) (1.00 g, 3.6 mmol, 1.0 equiv) was dissolved in dry THF (20 mL). Sodium cyanoborohydride (0.27 g, 4.3 mmol, 1.2 eqiuv) and acetic acid (0.30 mL, 4.3 mmol, 1.2 eqiuv) were added to the solution and the mixture stirred overnight at ambient temperature; then the excess acetic acid in the resulting mixture was neutralised by adding sodium hydrogen carbonate (2.00 g). The mixture was poured into H_2O and extracted with DCM (3 x 20 mL). The combined extracts were dried over $MgSO_4$, filtered and evaporated *in vacuo* to give the crude material, which was purified by flash column chromatography on silica, eluting with petrol and diethyl ether (1:1) to furnish the title product as a yellow oil (250 mg, 32 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 (d, J = 8.5 Hz, 2H), 6.91 (d, J = 8.5 Hz, 2H), 4.22 (dd, J_1 = 11.0 Hz, J_2 = 3.0 Hz, 1H), 3.82 (s, 3H), 3.76 (dd, J_1 = 9.0 Hz, J_2 = 4.0 Hz, 1H), 3.50 (t, J = 11.0 Hz, 1H), 3.10 (dd, J_1 = 12.0 Hz, J_2 = 3.0 Hz, 1H), 1.91 - 1.85 (m, 1H), 1.78 - 1.70 (m, 1H), 1.60 - 1.53 (m, 1H), 0.94 (d, J = 6.6, 3H), 0.88 (d, J = 6.5, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 200.75, 159.50, 134.28, 127.57, 114.27, 67.31, 58.76, 40.41, 38.09, 24.44, 23.44, 21.36. IR (ATR): υ_{max} 3329, 2963, 1671 cm^{-1}. HRMS calculated for $C_{15}H_{22}NO_2S^+$: 280.1366, found 280.1360 $[M+H]^+$. $[\alpha]_D^{20}$ -9.8. (*c* 0.5 $CHCl_3$).

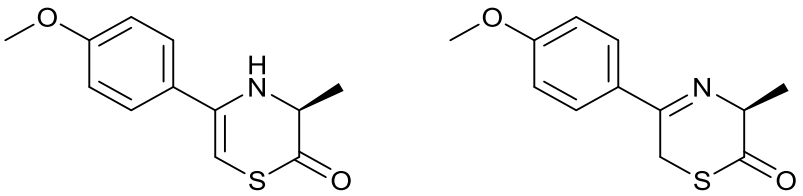
Preparation of (*S*)-2-(4-methoxyphenyl)-2-oxoethyl 2-(*tert*-butoxycarbonylamino)propanethioate (11)[46]



Triethylamine (1.46 mL, 10.6 mmol, 1.0 equiv) was added to a stirred solution of Boc-Ala-OH (2.00 g, 10.6 mmol, 1.0 equiv) in dry THF at 0 ^{o}C. After 30 min, ethyl chloroformate (1.01 mL, 10.6 mmol, 1.0 equiv) was added to the solution, and the mixture was stirred for 10 min at 0 ^{o}C. Then sodium hydrosulfide hydrate (1.48 g, 26.4 mmol, 2.5

equiv) was added and the mixture stirred for 2 hours at 0 ^{o}C. The ice bath was removed, and bromomethyl(4-methoxyphenyl)ketone (2.41 g, 10.6 mmol, 1.0 equiv) was added and the mixture stirred overnight at ambient temperature. Methanol (10 mL) was added, and the mixture was diluted with H_2O (20 mL) and extracted with DCM (3 x 20 mL). The combined organic phases were dried over $MgSO_4$, filtered and the solvent was evaporated *in vacuo* to give the crude material. The pure product (2.10 g, 56 %) was obtained as a colourless solid after recrystallization (ethyl acetate - hexane). m.p. 117- 119 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (d, *J* = 8.5 Hz, 2H), 6.95 (d, *J* = 8.5 Hz, 2H), 4.95 (s, 1H), 4.40 (d, *J* = 17.0 Hz, 2H), 4.28 (d, *J* = 17.0 Hz, 1H), 3.88 (s, 3H), 1.56 (s, 2H), 1.45 (s, 9H), 1.25 (d, *J* = 7.0 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 201.2, 191.9, 164.2, 154.8, 130.9, 128.6, 114.0, 80.5, 56.4, 55.3, 36.5, 28.3, 18.7. IR (ATR): $\acute{\upsilon}_{max}$ 2979, 2935, 1694, 1600 cm^{-1}. HRMS calculated for $C_{17}H_{23}NO_5SNa^{+}$: 376.1189, found 376.1188 $[M+Na]^{+}$. $[\alpha]_D^{20}$ -22.2. (*c* 0.5 $CHCl_3$).

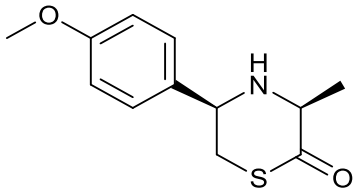
Preparation of 3-(*S*)-methyl-5-(4-methoxyphenyl)-4,5-dehydro-2*H*-1,4-thiazin-2-one (12) and 3-(*S*)-methyl-5-(4-methoxyphenyl)-5,6-dehydro-2*H*-1,4-thiazin-2-one (13)[46]



Trifluoroacetic acid (7 mL) was added to a solution of (*S*)-2-(4-methoxyphenyl)-2-oxoethyl 2'-(*tert*-butoxycarbonylamino)propanethioate (**11**) (2.10 g, 5.94 mmol, 1.0 equiv) in DCM (7 mL). The mixture was stirred for 2 hours, the solvent and trifluoroacetic acid were removed *in vacuo* and the residue was dissolved in DCM to which K_2CO_3 (5.00 g, excess) and 4 Å molecular sieves (5.00 g) were added. The resulting mixture was stirred for 24 hours. Filtration through a short pad of Celite® washig with DCM and removal of solvent from the filtrate *in vacuo* gave the crude oil (1.17 g, 84 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ

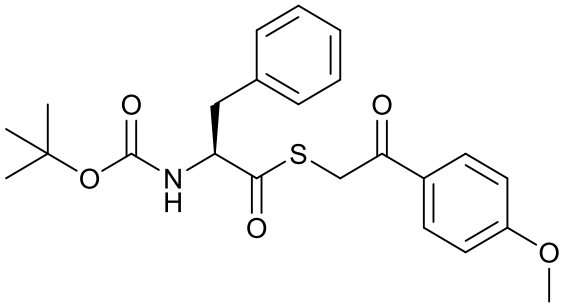
7.92 (d, *J* = 9.0 Hz, 2H), 7.80 (d, *J* = 9.0 Hz, 2H), 7.46 - 7.35 (m, 2H), 7.03 - 6.84 (m, 2H), 5.23 (d, *J* = 2.5 Hz, 1H), 4.42 - 4.23 (m, 1+1H), 4.18 – 4.00 (m, 2+1H), 3.89 - 3.84 (m, 3+3H), 3.84 (s, 1H), 1.69 (d, *J* = 6.5 Hz, 3H), 1.44 (d, *J* = 7.0 Hz, 3H). HRMS calculated for $C_{12}H_{14}NO_2S^+$: 236.0740, found 236.0743 $[M+H]^+$.

Preparation of 3-(*S*)-methyl-5-*(R)-5*-(4-methoxyphenyl)thiamorpholin-2-one (14)[46]



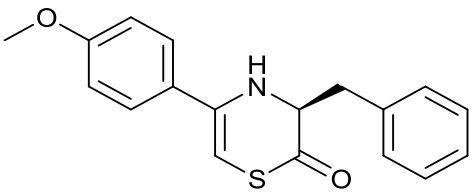
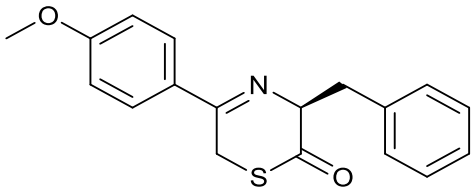
The mixture of 3-(*S*)-methyl-5-(*S*)-(4-methoxyphenyl)-4,5-dehydro-2*H*-1,4-thiazin-2-one (**12**) and 3-(*S*)-methyl-5-(*S*)-(4-methoxyphenyl)-5,6-dehydro-2*H*-1,4-thiazin-2-one (**13**) (1.40 g, 5.94 mmol, 1.0 equiv) was dissolved in dry THF (20 mL). Sodium cyanoborohydride (0.45 g, 7.13 mmol, 1.2 equiv) and acetic acid (0.40 mL, 7.13 mmol, 1.2 equiv) were added to the solution and the mixture stirred overnight at ambient temperature. The excess acetic acid in the resulting mixture was neutralised by sodium hydrogen carbonate (2.00 g), then the mixture was poured into H_2O (20 mL) and extracted with DCM (3 x 20 mL). The combined extracts were dried over $MgSO_4$, filtered and evaporated *in vacuo* to give the crude material, which was purified by flash column chromatography on silica, eluting with petrol and diethyl ether (1 : 1) to furnish the title product as a colourless oil (200 mg, 14 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 (d, *J* = 8.5 Hz, 2H), 6.90 (d, *J* = 8.5 Hz, 2H), 4.25 (dd, J_1 = 11.0 Hz, J_2 = 3.0 Hz, 1H), 3.86 (q, *J* = 6.5 Hz, 1H), 3.81 (s, 3H), 3.53 (t, *J* = 11.5 Hz, 1H), 3.09 (dd, J_1 = 11.0 Hz, J_2 = 3.0 Hz, 1H), 1.82 (s, 1H), 1.38 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 200.31, 159.54, 134.08, 127.58, 114.28, 64.93, 59.12, 55.35, 38.39, 17.80. IR (ATR): $\acute{\upsilon}_{max}$ 3315, 2976, 1685 cm^{-1}. HRMS calculated for $C_{12}H_{16}NO_2S^+$: 238.0896, found 238.0889 $[M+H]^+$. $[\alpha]_D^{20}$ -9.0. (*c* 0.5 $CHCl_3$).

Prearation of *S*-(2-(4-methoxyphenyl)-2-oxoethyl) 2'-((*tert*-butoxycarbonyl)amino)-3-phenylpropanethioate (15)[46]



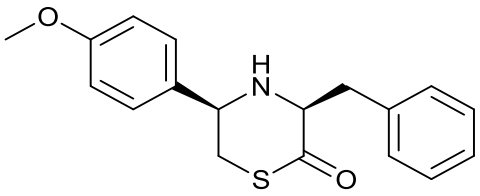
Boc-Phe-OH (3.00 g, 11.30 mmol, 1.0 equiv) and triethylamine (1.57 mL, 11.30 mmol, 1.0 equiv) were added to THF (50 mL), and the reaction was stirred on ice for 30 minutes. Ethyl chloroformate (1.07 mL, 11.30 mmol, 1.0 equiv) was then added, followed by stirring for 10 minutes. Then sodium hydrosulfide hydrate (2.62 g, 28.25 mmol, 2.5 equiv) was added and stirring continued for a further 2.5 hours. Then reaction was removed from the ice bath, 2-bromo-4'-methoxyacetophenone (3.11 g, 13.56 mmol, 1.2 equiv) was added and the reaction was stirred for 15 hours. After that, H_2O (50 mL) was added, the mixture was extracted with ethyl acetate (40 mL x3) and the combined organic extracts dried over $MgSO_4$. After filtration the solvents were removed *in vacuo*. The resulting oil was recrystallised from diethyl ether and petrol to afford the desired product as a colourless solid(3.34 g, 69%). m.p. 138- 141 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.98 (d, *J* = 9.0 Hz, 2H), 7.32- 7.20 (m, 3H), 7.18- 7.12 (m, 2H), 6.95(d, *J* = 9.0 Hz, 2H), 4.92 (d, *J* = 9.0 Hz, 1H), 4.72- 4.64 (m, 1H), 4.42 (d, *J* =16.0 Hz, 1H), 4.27 (d, *J* = 16.0 Hz, 1H), 3.88 (s, 3H), 3.18 (dd, J_1 = 14.5 Hz, J_2 = 2.0 Hz, 1H), 3.06 (dd, J_1 = 14.0 Hz, J_2 = 7.5 Hz, 1H) 1.42 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 200.36, 191.93, 164.10, 155.14, 135.62, 131.04, 129.50, 128.89, 128.75, 127.30, 114.11, 80.77, 61.12, 55.77, 38.24, 36.43, 28.42. IR (ATR): $\acute{\upsilon}_{max}$ 3353, 2973, 1680, 1599, 1172 cm^{-1}. HRMS calculated for $C_{23}H_{27}NO_5SNa^+$: 452.1502, found 452.1502 $[M+Na]^+$. $[\alpha]_D^{20}$ -20.0. (*c* 0.5 $CHCl_3$).

Preparation of 3-(*S*)-benzyl-5-(*S*)-(4-methoxyphenyl)-4,5-dehydro-2*H*-1,4-thiazin-2-one (16) and 3-(*S*)-benzyl-5-(*S*)-(4-methoxyphenyl)-5,6-dehydro-2*H*-1,4-thiazin-2-one (17)[46]

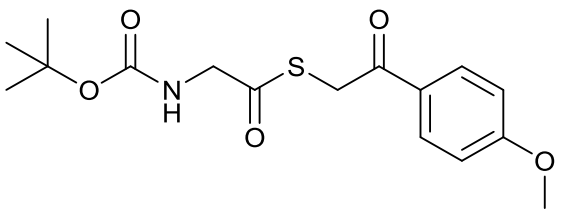
Trifluoroacetic acid (7 mL) was added to a solution of *S*-(2-(4-methoxyphenyl)-2-oxoethyl) 2'-((*tert*-butoxycarbonyl)amino)-3-phenylpropanethioate (**15**) (2.10 g, 4.89 mmol, 1.0 equiv) in DCM (7 mL). The mixture was stirred for 2 hours, the solvent and trifluoroacetic acid were removed *in vacuo* and the residue was dissolved in DCM to which K_2CO_3 (5.00 g, excess) and 4 Å molecular sieves (5.00 g) were added. The resulting mixture was stirred for 24 hours. Filtration through a short pad of Celite® washing with DCM and removal of solvent from the filtrate *in vacuo* gave the crude material (1.21 g, 80 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, J = 9.0 Hz, 2H), 7.38 - 7.18 (m, 10H), 7.00 (d, J = 9.0 Hz, 2H), 6.91 (d, J = 9.0 Hz, 2H), 6.77 (d, J = 9.0 Hz, 2H), 5.24 (d, J = 2.0 Hz, 2H), 4.93 (d, J = 15 Hz, 2H), 3.90 - 3.82 (m, 1H), 3.81 (s, 3H), 3.76 (s, 3H), 3.52 (dd, J_1 = 14.0 Hz, J_2 = 5.0 Hz, 1H), 3.28 (dd, J_1 = 14.0 Hz, J_2 = 8.5 Hz, 1H), 3.17 (dd, J_1 = 13.5 Hz, J_2 = 4.0 Hz, 1H), 3.05 (q, J = 7.5 Hz, 1H), 2.90 (dd, J_1 = 13.5 Hz, J_2 = 11.0 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 201.8, 198.5, 165.7, 163.8, 139.9 - 126.4, 114.7, 113.7, 85.4, 71.4, 62.5, 55.6, 55.4, 45.8, 37.5, 29.3. HRMS calculated for $C_{18}H_{18}NO_2S^+$: 312.1053, found 312.1056 $[M+H]^+$.

Preparation of 3-benzyl-5-(4-methoxyphenyl)thiamorpholin-2-one (18)[46]



The mixture of 3-(*S*)-benzyl-5-(*S*)-(4-methoxyphenyl)-4,5-dehydro-2*H*-1,4-thiazin-2-one (**16**) and 3-(*S*)-benzyl-5-(*S*)-(4-methoxyphenyl)-5,6-dehydro-2*H*-1,4-thiazin-2-one (**17**) (1.21 g, 3.91 mmol, 1.0 equiv) was dissolved in dry THF (20 mL). Sodium cyanoborohydride (0.30 g, 4.69 mmol, 1.2 equiv) and acetic acid (0.26 mL, 4.69 mmol, 1.2 equiv) were added to the solution and the mixture stirred overnight at ambient temperature. The excess acetic acid in the resulting mixture was neutralised by sodium hydrogen carbonate (2.00 g), then the mixture was poured into H_2O (20 mL) and extracted with DCM (20 mL x 3). The combined organic extracts were dried over $MgSO_4$, filtered and evaporated *in vacuo* to give the crude material, which was purified by flash column chromatography on silica, eluting with petrol and diethyl ether (1 : 1) to furnish the title product as a colourless oil (0.44 g, 36 %). ^{1}H NMR δ 7.30 - 7.18 (m, 7H), 6.85 (d, J = 9.0 Hz, 2H), 4.11 (dd, J_1 = 11.0 Hz, J_2 = 3.0 Hz, 1H), 3.94 (dd, J_1 = 10.0 Hz, J_2 = 3.0 Hz, 1H), 3.78 (s, 3H), 3.51 (dd, J_1 = 14.0 Hz, J_2 = 3.0 Hz, 1H), 3.44 (t, J = 11.0 Hz, 1H), 3.01 (dd, J_1 = 11.0 Hz, J_2 = 3.0 Hz, 1H), 2.82 (dd, J_1 = 14.0 Hz, J_2 = 10.0 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 199.4, 159.5, 137.4, 134.0 129.4, 129.0, 127.4, 127.0, 114.3, 69.9, 53.5, 41.0, 37.8, 30.0. IR (ATR): υ_{max} 3321, 2836, 1739 cm^{-1}. HRMS calcd for $C_{18}H_{20}NO_2S^+$: 314.1209, found 314.1203 $[M+H]^+$. $[\alpha]_D^{20}$ -5.0. (*c* 0.5 $CHCl_3$).

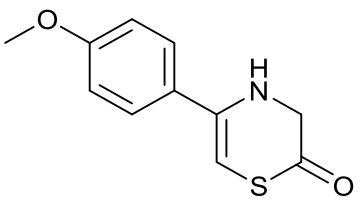
Preparation of 2-(4-methoxyphenyl)-2-oxoethyl) 2-((*tert*-butoxycarbonyl)amino)ethanethioate (19)[46]

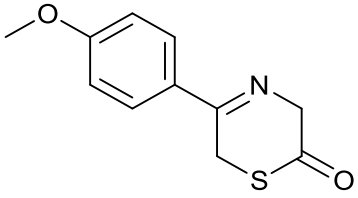


N-Boc-gly-OH (2.00 g, 11.3 mmol, 1.0 equiv) and triethylamine (1.57 mL, 11.3 mmol, 1.0 equiv) were added to THF (50 mL), and the reaction was stirred on ice for 30 minutes. Ethyl chloroformate (1.07 mL, 11.3 mmol, 1.0 equiv) was then added, followed by stirring

for 10 minutes, then sodium hydrosulfide hydrate (2.62 g, 28.25 mmol, 2.5 equiv) was added and the mixture was stirred for a further 2.5 hours. The reaction was removed from the ice bath, 2-bromo-4'-methoxyacetophenone (3.11 g, 13.56 mmol, 1.2 equiv) was added. And the reaction was stirred for a further 15 hours. After that period, the mixture was diluted with H_2O (50 mL) and extracted with ethyl acetate (3 x 40 mL). The combined organic extracts were dried over $MgSO_4$, filtered and solvent was removed *in vacuo*. The resulting oil was recrystallized from diethyl ether and petrol to afford the desired product as a colourless solid (2.15 g, 56 %). m.p. 80 - 85 ^oC. ^1H NMR (400 MHz, $CDCl_3$) δ 7.98 (d, *J* = 8.5 Hz, 2H), 6.95 (d, *J* = 8.5 Hz, 2H), 5.14 (s, 1H), 4.39 (s, 2H), 4.11 (d, *J* = 6.0 Hz, 2H), 3.88 (s, 3H), 1.47 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 197.6, 192.4, 164.1, 156.8, 130.9, 128.5, 114.0, 80.4, 55.6, 50.2, 36.0, 28.3, 27.8. IR (ATR): $\acute{\upsilon}_{max}$ 2979, 2935, 1698, 1600 cm^{-1}. HRMS calcd for $C_{16}H_{21}NO_5SNa^+$: 362.1033, found 362.1035 $[M+Na]^+$.

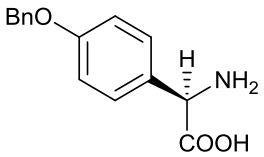
Preparation of 5-(4-methoxyphenyl)-4,5-dehydro-2*H*-1,4-thiazin-2-one (20) and 5-(4-methoxyphenyl)-5,6-dehydro-2*H*-1,4-thiazin-2-one (21)[46]





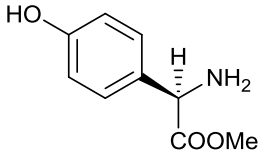
Trifluoroacetic acid (7 mL) was added to a solution of 2-(4-methoxyphenyl)-2-oxoethyl) 2-((*tert*-butoxycarbonyl)amino)ethanethioate (**19**) (2.15 g, 6.34 mmol, 1.0 equiv) in DCM (7 mL). The mixture was stirred for 2 hours, and the solvent and trifluoroacetic acid were removed *in vacuo*, the residue was dissolved in DCM to which K_2CO_3 (5.00 g, excess) and 4 Å molecular sieves (5.00 g) were added and the resulting mixture was stirred for 24 hours. Filtration through a short pad of Celite® and removal of solvent from the filtrate *in vacuo* gave the crude material (0.75 g, 54 %). ^1H NMR (400 MHz, CDCl3) δ 7.95-7.91 (m, 2H+2H), 6.93-6.85 (m, 2H+2H), 5.30 (s, 1H), 4.13 (s, 2H), 3.94 (s, 2H+2H), 3.88 (s, 3H+3H). HRMS calcd for $C_{11}H_{12}NO_2S^+$: 222.0583, found 222.00584 $[M+H]^+$.

Preparation of (*R*)-2-(4-(benzyloxyphenyl)glycine (27)[58]



A solution of $CuSO_4.5H_2O$ (3.75 g, 15.0 mmol, 1.0 equiv) in H_2O (10mL) was added to a stirred solution of (*R*)-4-hydroxyphenylglycine (2.50 g, 15.0 mmol, 1.0 equiv) in 2M NaOH (8 mL, 15.0 mmol, 1.0 equiv) at ambient temperature. The mixture was then heated to reflux for 1 h, cooled to ambient temperature and MeOH (50 mL) and 2M NaOH (8 mL) added. Benzyl bromide (3.10 g, 18.0 mmol, 1.2 equiv) was added and the mixture stirred at ambient temperature overnight. The resulting precipitate was collected and washed with H_2O (20 mL) and MeOH (20 mL), then suspended in 1M HCl (40mL) and stirred for 2 h at ambient temperature. The precipitate was filtered and washed with H_2O (50 mL), 1M NH_4OH (40 mL x 2) H_2O (50 mL) and acetone (20 mL) to give the product as a colourless solid (2.77 g, 72 %). m.p. 218 - 219 ℃ (Lit[91] 218 – 219 ^oC). ^1H NMR (400 MHz, $CDCl_3$): δ 7.36 - 7.43 (m, 5H), 7.33 (d, *J* = 8.5 Hz, 2H), 7.08 (d, *J* = 8.5 Hz, 2H), 5.24 (s, 1H), 5.12 (s, 2H). HRMS calcd for $C_{15}H_{16}NO_3^+$: 258.1125, found 258.1127 $[M+H]^+$. $[\alpha]_D^{20}$ -55.0 (*c* 0.5 $CHCl_3$).

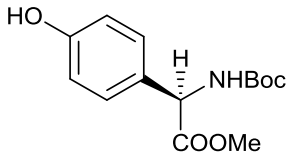
Preparation of methyl (*R*)-(4-hydroxyphenyl)glycinate (28)[92]



(*R*)-4-Hydroxyphenylglycine (2.50 g, 15.0 mmol, 1.0 equiv) was suspended in anhydrous MeOH (50 mL), and thionyl chloride (2.00 mL) was added dropwise. After the resulting mixture had been stirred at room temperature under an atmosphere of nitrogen for 12 hours,

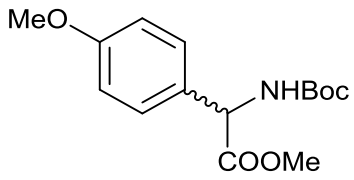
the solvent was removed *in vacuo* and the residue washed with diethyl ether (70 mL x 2) to yield the title product as a pink solid (2.77 g, 100 %), m.p. 190-191 ℃. ^{1}H NMR (400 MHz, MeOD): δ 7.27 (d, *J* = 8.5 Hz, 2H), 6.86 (d, *J* = 8.5 Hz, 2H), 5.06 (s, 1H), 3.80 (s, 3H). ^{13}C NMR (101 MHz, MeOD) δ 170.5, 160.4,130.7, 123.8, 117.2, 57.2, 53.9. IR (ATR): $\acute{\upsilon}_{max}$ 3534, 3338, 2893, 1731 cm^{-1}. HRMS calculated for $C_9H_{12}NO_3^+$: 182.0812, found 182.0811 $[M+H]^+$. $[\alpha]_D^{20}$ -134.4. (*c* 0.5 $CHCl_3$).

Preparation of methyl *N*-Boc-(*R*)-(4-hydroxyphenyl)glycinate (29)[92]



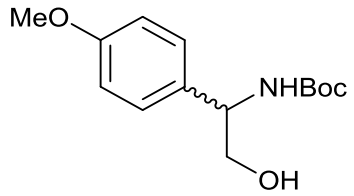
To a mixture of methyl (*R*)-(4-hydroxyphenyl)glycinate (**28**) (2.77 g, 15.0 mmol, 1.0 equiv) and sodium hydrogen carbonate (1.93 g, 22.95 mmol, 1.5 equiv) in H_2O (20 mL) was added Boc anhydride (4.00 g, 18.36 mmol, 1.2 equiv) in dioxane (20 mL). The mixture was stirred for 1 hour at 0 ^{o}C and then 15 hours at room temperature. The reaction was acidified with 1M HCl to pH 1.82 and extracted with ethyl acetate (3 x 50 mL). The organic layers were combined and washed with H_2O (100 mL) and brine (100 mL) followed by drying over Na_2SO_4. The solution was filtered and the solvent was removed *in vacuo* to furnish the title product as a white solid (3.56 g, 84 %). m.p. 138-139 ℃ (Lit.[93] 138.0-138.5 ^{o}C). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.21 (d, *J* = 8.5 Hz, 2H), 6.77 (d, *J* = 8.5 Hz, 2H), 5.50 (d, *J* = 5.5 Hz,1H), 5.23 (d, *J* = 7.0 Hz, 1H), 5.03 (s, 1H), 3.71 (s, 3H), 1.43 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.1, 156.6, 155.2, 128.4, 127.9, 115.9, 80.6, 67.0, 57.1, 52.7, 31.1, 28.3. IR (ATR): $\acute{\upsilon}_{max}$ 3534, 3338, 2893, 1731 cm^{-1}. HRMS calculated for $C_{14}H_{20}NO_5^+$: 282.1336, found 282.1338 $[M+H]^+$. $[\alpha]_D^{20}$ -181.4. (*c* 0.5 MeOH).

Preparation of methyl *N*-Boc-2-(4-methoxyphenyl)glycinate (30)



Cs_2CO_3 (20.60 g, 63.3 mmol, 5.0 equiv) was added to a stirred solution of compound (**29)** (3.56 g, 12.6 mmol, 1.0 equiv) in anhydrous THF (60 mL). The mixture was stirred at room temperature for 10 minutes under an atmosphere of nitrogen, and then iodomethane (0.94 mL, 12.6 mmol, 1.0 equiv) was added, followed by stirring the mixture for 24 hours at room temperature. The reaction was quenched by addition of H_2O (30 mL) followed by extraction with ethyl acetate (3 x 30 mL). The combined organic layers were dried over Na_2SO_4, filtered and the solvent removed *in vacuo* yielding the crude product, which was further purified by crystallisation from ethyl acetate and petrol affording the title product as colourless crystals (2.3 g, 62 %). m.p. 70-72 ^oC (Lit.[94] 66-67 ^oC). ^1H NMR (400 MHz, $CDCl_3$): δ 7.27 (d, *J* = 8.5 Hz, 2H), 6.87 (d, *J* = 8.5 Hz, 2H), 5.47 (s, 1H), 5.25 (d, *J* = 7.0 Hz, 1H), 3.79 (s, 3H), 3.72 (s, 3H), 1.43 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.9, 159.7, 154.8, 129.0, 128.4, 114.3, 80.1, 57.0, 55.3, 52.7, 28.3, 27.7. IR (ATR): $\acute{\upsilon}_{max}$ 3365, 2989, 1708, 1612 cm^{-1}. HRMS calculated for $C_{15}H_{22}NO_5^+$: 296.1492, found 296.1493 $[M+H]^+$.

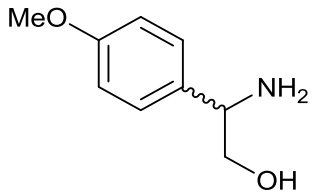
Preparation of methyl *N*-Boc-2-amino-2-(4-methoxyphenyl)ethanol (31)[62]



$NaBH_4$ (1.73 g, 46.8 mmol, 6.0 equiv) was added to a stirred solution of compound (**30)** (2.30 g, 7.7 mmol, 1.0 equiv) in THF (30 mL). The resulting suspension was stirred at 65

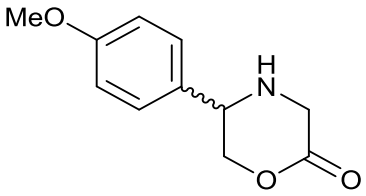
^{o}C for 15 minutes. Methanol (20 mL) was then added dropwise and the mixture was heated to reflux for 4 hours. After cooling to room temperature the reaction was quenched with 1M HCl (30 mL). The aqueous layer was basified to pH 10 using potassium hydroxide pellets, followed by extraction with ethyl acetate (3 x 50 mL). The combined organic layers were dried by $MgSO_4$, filtered and concentrated *in vacuo* to obtain the title product as a white solid (1.60 g, 78 %). m.p. 130 - 132 ^{o}C (Lit.[94] 130-131 ^{o}C). ^{1}H NMR (400 Hz, $CDCl_3$): δ 7.21 (d, *J* = 8.0 Hz, 2H), 6.88 (d, *J* = 8.0, 2H), 5.10 (s, 1H), 4.73 (s, 1H), 3.86 (s, 2H), 3.80 (s, 3H), 2.33 (s, 1H), 1.43 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 159.4, 156.3, 127.8, 127.7, 114.2, 80.1, 67.0, 56.3, 55.3, 28.4. IR (ATR): $\acute{\upsilon}_{max}$3341, 3221, 2993, 1612. HRMS calculated for $C_{14}H_{21}NO_4Na^+$: 290.1363, found 290.1364 $[M+Na]^+$.

Preparation of 2-amino-2-(4-methoxyphenyl)ethanol (32)[62]



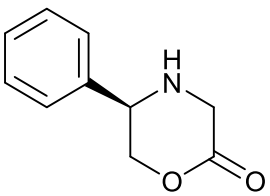
Methyl *N*-Boc-2-amino-2-(4-methoxyphenyl)ethanol **(31)** (1.60 g, 6.0 mmol, 1.0 equiv) was dissolved in trifluoroacetic acid (4 mL) in DCM (4 mL). After 60 min the reaction was shown to be complete by disappearance of the starting material by TLC. The solvent was then removed *in vacuo*, the residual oil was washed with 20 % aqueous NaOH (10 mL), extracted with ethyl acetate (3 x 10 mL) and dried over $MgSO_4$. After removal of solvent *in vacuo*, the desired product was obtained as a white solid (600 mg, 60 %). m.p. 92-93 ℃. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.25 (d, *J* = 8.5 Hz, 2H), 6.88 (d, *J* = 8.5 Hz, 2H), 4.0 (dd, J_1 = 4.5 Hz, J_2 = 3.5 Hz, 2H), 3.80 (s, 3H), 3.70 (dd, J_1 = 4.5, J_2 = 6.0 Hz, 1H), 3.52 (dd, J_1 = 8.5 Hz, J_2 = 2.5 Hz, 1H), 1.76 (s, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.2, 134.9, 127.4, 114.6, 72.8, 56.0, 55.4. IR (ATR): $\acute{\upsilon}_{max}$ 3292, 2978, 2838, 1705, 1514 cm^{-1}. HRMS calculated for $C_9H_{14}NO_2^+$: 168.1019, found 168.1018 $[M+H]^+$.

Preparation of 5-(4-methoxyphenyl)morpholin-2-one (34)[45]



To a solution of (*R*)-2-amino-2-(4-methoxyphenyl)ethanol (**32**) (200 mg, 1.19 mmol, 1.0 equiv) and *N*,*N*-diisopropylethylamine (0.25 ml, 1.43 mmol, 1.2 equiv) in anhydrous acetonitrile (15 mL) under an atmosphere of nitrogen was added, dropwise, a solution of phenyl bromoacetate (307 mg, 1.43 mmol, 1.2 equiv) in anhydrous acetonitrile (10 mL) over 10 minutes. The resulting solution was stirred at room temperature for a further 24 hours, then the solvent was removed *in vacuo* to furnish the crude product, which was immediately purified by flash column chromatography on silica, eluting initially with petrol : diethyl ether (1:2) then acetone. Recrystallization from diethyl ether and petrol yielded colourless crystals (120 mg, 48 %). m.p. 116-117 ^{o}C. ^{1}H NMR (400 Hz, $CDCl_3$): δ 7.32 (d, *J* = 8.5 Hz, 2H), 6.90 (d, *J* = 8.5 Hz, 2H), 4.36 (dd, J_1 = 4.0, J_2 = 10.5 Hz, 1H), 4.30 (t, *J* = 10.5 Hz, 1H), 4.19 (dd, J_1 = 4.0 Hz, J_2 = 10.5 Hz, 1H), 3.97 (d, *J* = 18.0 Hz, 1H), 3.89 (d, *J* = 18.0 Hz, 1H), 3.81 (s, 3H), 1.68 (s, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.0, 160.2, 129.6, 126.9, 115.4, 73.1, 55.4, 53.5, 18.6. IR (ATR): $\acute{\upsilon}_{max}$ 3355, 2984, 1742 cm^{-1}. HRMS calculated for $C_9H_{14}NO_2^+$: 208.0968 found 208.0965 $[M+H]^+$.

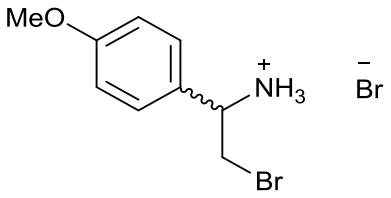
Preparation of (*R*)-5-phenylmorpholin-2-one (36)[45]



To a solution of (*R*)-(−)-2-phenylglycinol (0.50 g, 3.65 mmol, 1.0 equiv) and

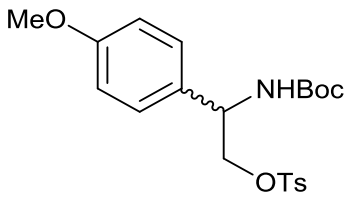
N,*N*-diisopropylethylamine (1.60 ml, 9.00 mmol, 2.5 equiv) in anhydrous acetonitrile (15 mL) under an atmosphere of nitrogen was added, dropwise, a solution of phenyl bromoacetate (0.94 g, 4.38 mmol, 1.2 equiv) in anhydrous acetonitrile (10 mL) over 10 minutes. The resulting solution was stirred at room temperature for a further 24 hours, then the solvent was removed *in vacuo* to furnish the crude product, which was immediately purified by flash column chromatography on silica, eluting initially with petrol: diethyl ether (1:2) then acetone. Recrystallization from diethyl ether and petrol yielded colourless crystals (350 mg, 54 %). m.p. 54 - 55 ℃. (Lit.[45] 53 - 54 °C). ^{1}H NMR (400 Hz, $CDCl_3$): δ 7.43-7.32 (m, 5H), 4.41 (dd, J_1 = 10.5 Hz, J_2 = 3.5 Hz, 1H), 4.30 (t, J = 10.5 Hz, 2H), 4.19 (dd, J_1 = 10.5 Hz, J_2 = 3.5 Hz, 1H), 4.00 (d, J = 18.0 Hz, 1H), 3.85 (d, J = 18.0 Hz, 1H), 1.89 (s, 1H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 168.0, 137.9, 129.3, 129.1, 127.4, 74.8, 56.9, 48.9. IR (ATR): υ_{max} 3449, 2980, 1742 cm^{-1}. HRMS calculated for $C_{10}H_{12}NO_2^+$: 178.0863 found 178.0861 $[M+H]^+$.

Preparation of 2-bromo-1-(4-methoxyphenyl)ethylammonium bromide (40)[65]



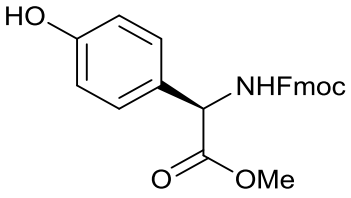
To a solution of 2-amino-2-(4-methoxyphenyl)ethanol (**32**) (1.00 g, 5.98 mmol, 1.0 equiv) of DCM (15 mL) was added $SOBr_2$ (0.43 mL, 8.97 mmol, 1.5 equiv) at 0 °C and then the mixture was allowed to warm to room temperature. The mixture was concentrated *in vacuo* and dissolved in ethanol (15 mL). Decolourizing charcoal was added and the mixture was heated to reflux for 2 hours. The mixture was allowed to cool, the charcoal was filtered off and the solvent was removed *in vacuo* to furnish the title product (1.17 g, 63.3 %). ^{1}H NMR (400 MHz, DMSO-*d*6) δ 8.22 (s, 3H), 7.37 (d, J = 9.0 Hz, 2H), 6.97 (d, J = 9.0 Hz, 2H), 4.20 (m, 1H), 3.75 (s, 3H), 3.62 (m, 2H). ^{13}C NMR (101 MHz, DMSO-*d*6) δ 159.5, 128.9, 126.7, 114.1, 114.0, 66.9, 55.2, 53.1. IR (ATR): υ_{max} 3440 cm^{-1}.

Preparation of *N*-Boc-2-amino-2-(4-methoxyphenyl)ethyl 4-methylbenzenesulfonate (42)[95]



N-Boc-2-amino-2-(4-methoxyphenyl)ethanol (**31**) (0.60 g, 2.24 mmol, 1.0 equiv) was dissolved in DCM (30 mL), and triethylamine (0.78 mL, 5.61 mmol, 2.5 equiv) was then added, followed by *p*-toluenesulfonyl chloride (0.51 g, 2.69 mmol, 1.2 equiv). The reaction was stirred at 0 ^{o}C for four hours. After filtering the resulting solid and concentrating the mixture *in vacuo*, the residual oil was purified by column chromatography, eluting with diethyl ether : petrol (1: 1) to furnish the title product as a white solid (0.60 g, 64 %). m.p. 152 - 154 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 (d, *J* = 8.0 Hz, 2H), 7.28 (d, *J* = 8.0 Hz, 2H), 7.11 (d, *J* = 8.5 Hz, 2H), 6.82 (d, *J* = 8.5 Hz, 2H), 5.04 (s, 1H), 4.84 (s, 1H), 4.19 (m, 2H), 3.79 (s, 3H), 2.44 (s, 3H), 1.40 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 158.3, 153.9, 143.9, 131.5, 128.9, 127.9, 127.1, 126.9, 126.8, 126.4, 125.0, 113.6, 113.1, 71.8, 70.6, 55.0, 54.4, 54.3, 27.3, 20.7. IR (ATR): $\acute{\upsilon}_{max}$ 3444, 2979, 2253, 1705 cm^{-1}. HRMS calculated for $C_{21}H_{28}NO_6S^+$: 422.1632, found 422.1628 $[M+H]^+$.

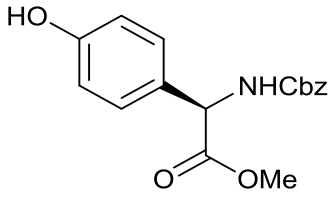
Preparation of methyl (*R*)-*N*-Fmoc-2-amino-2-(4-hydroxyphenyl)acetate (46)[92]



(*R*)-(4-Hydroxyphenyl)glycine methyl ester **15** (5.80 g, 0.032 mol, 1.0 equiv) was dissolved in H_2O (50 mL) and sodium hydrogen carbonate (3.39 g, 0.032 mol, 1.0 equiv) was added, followed by a solution of Fmoc-chloride (9.90 g, 0.042 mol, 1.2 equiv) in dioxane (50 mL)..

The mixture was stirred at 0 ^{o}C for 1 hour and then warmed up to room temperature over 2 hours. The mixture was quenched with 1 M HCl (aq.) until pH 1.82 and extracted with ethyl acetate (3 x 50 mL). The organic layers were combined and washed with H_2O (100 mL) and brine (100 mL) followed by drying over Na_2SO_4. After filtering, the solvent was removed *in vacuo* to furnish the title product as a white solid (11.4 g, 89 %). m.p. 76 - 78 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.72 (d, *J* = 7.5 Hz, 2H), 7.55 (d, *J* = 7.0 Hz, 2H), 7.36 (t, *J* = 7.5 Hz, 2H), 7.26 (t, *J* = 7.5 Hz, 2H), 7.16 (d, *J* = 8.5 Hz, 2H), 6.76 (d, *J* = 8.5 Hz, 2H), 5.97 (d, *J* = 7.0 Hz, 1H), 5.28 (d, *J* = 7.0 Hz, 1H), 4.38 (t, *J* = 7.0 Hz, 2H), 4.17 (t, *J* = 7.0 Hz, 1H), 3.69 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 171.8, 168.5, 156.1, 143.8, 143.7, 141.3, 128.6, 127.7, 127.1, 125.1, 120.0, 115.9, 67.2, 57.4, 52.9, 47.1. IR (ATR): $\acute{\upsilon}_{max}$ 3368, 3067, 2953, 2252, 1699, 1613 cm^{-1}. HRMS calculated for $C_{24}H_{22}NO_5^+$: 404.1492, found 404.1493 $[M+H]^+$. $[\alpha]_D^{20}$ -11.4. (*c* 0.5 $CHCl_3$).

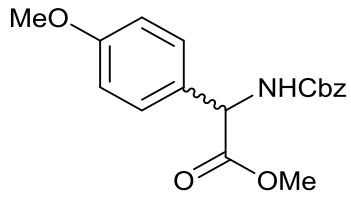
Preparation of methyl (*R*)-*N*-Cbz-2-(4-hydroxyphenyl)glycinate (49)[44]



(*R*)-(4-Hydroxyphenyl)glycine methyl ester (**28**) (2.00 g, 0.011 mol, 1.0 equiv) was dissolved in H_2O (50 mL) and sodium hydrogen carbonate (1.11 g, 0.013 mol, 1.2 equiv) was added, followed by a solution of Cbz-chloride (2.26 g, 0.013 mol, 1.2 equiv) in dioxane (50 mL). The mixture was stirred at 0 ^{o}C for 1 hour and then warmed to room temperature over 2 hours. The reaction was quenched with 1 M HCl until pH 1.82 and extracted with ethyl acetate (3 x 50 mL). The organic layers were combined and washed with H_2O (100 mL) and brine (100 mL) followed by drying over Na_2SO_4. The organic extract was filtered and the solvent was removed *in vacuo* to furnish the title product as a white solid (3.46 g, 99%). m.p. 117 - 120 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.34 (m, 5H), 7.20 (d, *J* = 8.0 Hz, 2H), 6.76 (d, *J* = 8.0 Hz, 2H), 5.82 (d, *J* = 6.0 Hz, 1H), 5.42 (s, 1H), 5.29 (d, *J* = 7.0 Hz,

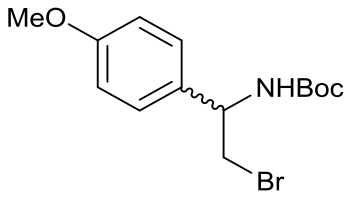
1H), 5.10 (q_{AB}, J_{AB} = 12 Hz, 2H), 3.72 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.35, 156.0, 136.1, 128.6, 128.5, 128.3, 128.2, 115.8, 101.5, 90.1, 67.0, 57.2, 53.1. IR (ATR): $\acute{\upsilon}_{max}$ 3352, 3032, 2954, 2252, 1697, 1514 cm^{-1}. HRMS calculated for $C_{17}H_{18}NO_5^+$: 316.1179, found 316.1181 $[M+H]^+$. $[\alpha]_D^{20}$ -15.8. (*c* 0.5 $CHCl_3$).

Preparation of methyl *N*-Cbz-2-(4-methoxyphenyl)glycinate (50)



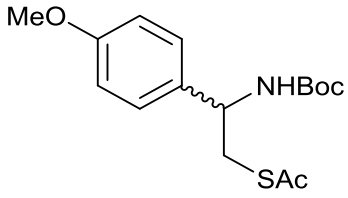
Methyl *N*-Cbz-2-(4-hydroxyphenyl)glycinate (**49**) (0.50 g, 1.50 mmol, 1.0 equiv) was dissolved in THF (50 mL), Cs_2CO_3 (2.47 g, 7.50 mmol, 5.0 equiv) was added and then, iodomethane (0.14 mL, 2.25 mmol, 1.5 equiv) was added into the mixture. The reaction was stirred under nitrogen at room temperature for 15 hours, quenched with H_2O (50 mL) and extracted with ethyl acetate (3 x 30 mL). The combined organic extracts were dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by column chromatography, eluting with diethyl ether : petrol (1:1) and was isolated as a white solid (0.23 g, 46%). m.p. 95 - 96 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 (apps, 5Hz), 7.28 (d, *J* = 8.5 Hz, 2H), 6.87 (d, *J* = 8.5 Hz, 2H), 5.82 (s, 1H), 5.31 (d, *J* = 7.0 Hz, 1H), 5.09 (q_{AB}, J_{AB} = 12.5 Hz, 2H), 3.79 (s, 3H), 3.71 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.8, 156.2, 136.2, 128.7, 128.5, 128.4, 128.2, 126.9, 114.37, 81.6, 67.2, 57.4, 55.5, 52.9. IR (ATR): $\acute{\upsilon}_{max}$ 2955, 2838, 2253, 1716, 1512. HRMS calculated for $C_{18}H_{20}NO_5^+$, 330.1336 found 330.1338 $[M+H]^+$.

Preparation of *tert*-butyl 2-bromo-1-(4-methoxyphenyl)ethylcarbamate (52)[68]



Boc-2-amino-2-(4-methoxyphenyl)ethanol (**31**) (1.00 g, 3.74 mmol, 1.0 equiv) and tetrabromomethane (1.37 g, 4.11 mmol, 1.1.0 equiv) were dissolved in THF (30 mL). Then a solution of triphenylphosphine (1.08 g, 4.11 mmol, 1.1 equiv) in THF (10 mL) was added dropwise and the reaction was stirred for 12 hours. After removal of solvent, the resulting mixture was triturated with diethyl ether (3 x 25 mL) to separate the product from the majority of the PPh_3O, followed by removal of the solvent *in vacuo* followed by purification by column chromatography, eluting with diethyl ether : petrol (1:2) to afford the desired product as an orange solid (0.46 g, 37%). m.p: 106-107 ^oC**.** ^1H NMR (400 MHz, $CDCl_3$) δ 7.22 (d, *J* = 8.5 Hz, 2H), 6.89 (d, *J* = 8.5 Hz, 2H), 5.07 (s, 1H), 4.94 (s, 1H), 3.80 (s, 3H), 3.67 (d, *J* = 5.5 Hz, 2H), 1.44 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.3, 155.1, 131.5, 127.6, 114.1, 80.3, 72.9, 56.1, 55.3, 37.5, 28.3. IR (ATR): $\acute{\upsilon}_{max}$ 2978, 2837, 1704, 1513 cm^{-1}. HRMS calculated for $C_{28}H_{41}N_2O_6{}^{79}Br_2$: 659.1326, found 659.1326 $[2M(^{79}Br_2)+H]^+$, 661.1305 $[2M(^{79}Br^{81}Br)+H]^+$ and 663.1284 $[2M(^{81}Br_2)+H]^+$ (1:2:1).

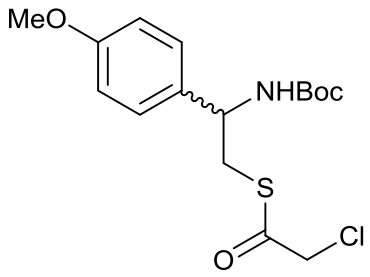
Preparation of amino-2-(4-methoxyphenyl)ethyl) ethanethioate (55)[96]



Triphenylphosphine (2.20 g, 6.72 mmol, 1.5 equiv) was dissolved in anhydrous THF (20 mL) and cooled to 0 ^oC. DIAD (1.65 mL, 6.72 mmol, 1.5 equiv) was added dropwise, and the mixture was stirred for 30 minutes. Then *N*-Boc-2-amino-2-(4-methoxyphenyl)ethanol

(**31**) (1.50 g, 5.60 mmol, 1.0 equiv) followed by thioacetic acid (0.59 mL, 6.72 mmol, 1.5 equiv) were added slowly. The reaction was stirred in an ice bath for 2 hours and then allowed to warm to room temperature. After 15 hours, solvent was removed *in vacuo*, the residue was washed with diethyl ether to separate the product from the majority of the triphenylphosphine oxide. The solvent was removed and the residue was purified by column chromatography eluting with ethyl acetate and hexane (1:9) to give the desired product as a colourless solid (0.58 g, 32 %). m.p. 94 - 96 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.22 (d, *J* = 8.5 Hz, 2H), 6.87 (d, *J* = 8.5 Hz, 2H), 5.02 (s, 1H), 4.76 (s, 1H), 3.80 (s, 3H), 3.37 - 3.05 (m, 2H), 2.34 (s, 3H), 1.41 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 195.9, 159.1, 155.2, 129.0, 128.5, 127.4, 114.2, 114.0, 55.3, 54.2, 35.1, 30.6, 28.3. IR (ATR): υ_{max} 2973, 2837, 2253, 1698, 1512 cm^{-1}. HRMS calculated for $C_{16}H_{24}NO_4S^+$: 326.1421, found 326.1425 $[M+Na]^+$.

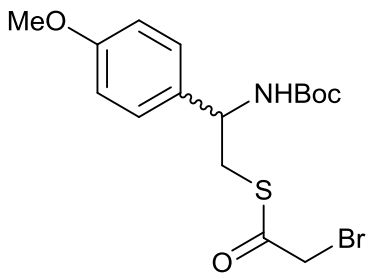
Preparation of *N*-Boc-2-amino-2-(4-methoxyphenyl)ethyl) 2-chloroethanethioate (**53**)



Methyl 2-amino-2-(4-methoxyphenyl)ethyl) ethanethioate (**55**) (1.00 g, 3.07 mmol, 1.0 equiv) was dissolved in methanol (30 mL) and 1 M NaOH aq. (10 mL) was added. The mixture was stirred for 45 minutes. The reaction was quenched with 1 M HCl aq. (12 mL) and the amino thiol was extracted with DCM (10 mL). Then triethylamine (1.20 mL, 9.21 mmol, 3.0 equiv) was added to the DCM solution, the reaction was cooled to 0 ^{o}C and chloroacetyl chloride (0.73 mL, 9.21 mmol, 3.0 equiv) was added dropwise. The reaction was stirred in an ice bath for 2 hours and at room temperature for a further 12 hours. The reaction was quenched by adding H_2O (50 mL) and extracted with DCM (3 x 30 mL) and dried over $MgSO_4$. After filtration, the solvent was removed *in vacuo* and the product was

purified by a column chromatography and obtained as a colourless solid (670 mg, 61%). m.p. 102 - 105 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23 (d, *J* = 8.5 Hz, 2H), 6.88 (d, *J* = 8.5 Hz, 2H), 4.95 (s, 1H), 4.82 (s, 1H), 4.18 (s, 2H), 3.80 (s, 3H), 3.33 (d, *J* = 8.0 Hz, 2H), 1.42 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 194.1, 177.5, 159.2, 155.1, 132.3, 132.2, 129.1, 128.6, 128.5, 127.4, 114.2, 65.9, 55.3, 48.3, 47.9, 35.5, 28.3, 15.3. IR (ATR): $\acute{\upsilon}_{max}$ 3375, 2980, 2932, 2255, 1703, 1611, 1512, 1248, 1167, 1036 cm^{-1}. HRMS calculated for $C_{16}H_{22}NO_4SClNa^+$: 382.0850, found 382.0852 [M(^{35}Cl)+Na]$^+$ and 384.0820 [M(^{37}Cl)+Na]$^+$ (3:1).

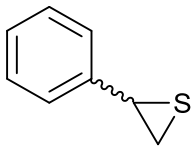
Preparation of *N*-Boc-2-amino-2-(4-methoxyphenyl)ethyl) 2-bromoethanethioate (60)



(*R*)-Methyl 2-amino-2-(4-methoxyphenyl)ethyl) ethanethioate (**55**) (1.00 g, 3.07 mmol, 1.0 equiv) was dissolved in methanol (30 mL) and 1 M NaOH aq. (10 mL) was added. The mixture was stirred for 45 minutes and then quenched with 1 M HCl aq. (12 mL). The resulting aminothiol (**56**) was extracted with DCM (10 mL) and triethylamine (1.20 mL, 9.21 mmol, 3.0 equiv) was added. The reaction was cooled to 0 ^{o}C, bromoacetyl bromide (0.80 mL, 9.21 mmol, 3.0 equiv) was added dropwise and the reaction was stirred in an ice bath for 2 hours and at room temperature for a further 12 hours. The reaction was quenched with H_2O (50 mL)**,** extracted with DCM (3 x 30 mL) and dried over $MgSO_4$. After filtration, the solvent was removed *in vacuo* and the pure product was isolated after column chromatography, eluting with ethyl acetate : hexane (1 : 2) as a colourless solid (0.45 g, 37 %). m.p. 82 - 85 ^{o}C. ^{1}H NMR δ 7.23 (d, *J* = 8.5 Hz, 2H), 6.88 (d, *J* = 8.5 Hz, 2H), 4.95 (s, 1H), 4.81 (s, 1H), 4.05 (s, 2H), 3.80 (s, 3H), 3.25 (d, *J* = 8.0 Hz, 2H), 1.42 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 190.9, 159.2, 155.3, 127.5, 114.1, 79.9, 70.3, 55.3, 52.5, 39.1,

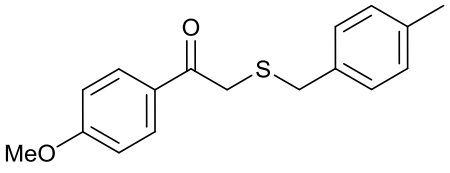
33.6, 28.4, 22.0. IR (ATR): $\acute{\upsilon}_{max}$ 2992, 2945, 2255, 1701, 1603, 1524, 1165cm^{-1}. HRMS calculated for $C_{16}H_{22}NO_4S^{79}BrNa^+$: 426.0345, found 426.0348 [M(^{79}Br)+Na]$^+$ and 428.0327 [M(^{81}Br)+Na]$^+$ (1:1).

Preparation of 2-Phenylthiirane (64)[75]



Styrene oxide (1.00 g, 8.33 mmol, 1.0 equiv) was mixed with ammonium thiocyanate (0.63 g, 8.33 mmol, 1.0 equiv) and the neat mixture was stirred and heated to 50 ^{o}C for 4 h. The mixture was extracted with DCM (30 mL) and the extract was evaporated *in vacuo*, followed by purification of the residue by a column chromatography, eluting with diethyl ether : petrol (1:1) to afford the desired product as a pale yellow oil (0.89 g, 91 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 - 7.14 (m, 5H), 3.84 (t, J = 6.0 Hz, 1H), 2.82 (dd, J_1 = 6.0, J_2 = 1.5 Hz, 1H), 2.60 (dd, J_1 = 6.0, J_2 = 1.5 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 139.2, 128.7, 127.7, 126.8, 26.3, 27.5. IR (ATR): $\acute{\upsilon}_{max}$ 3061, 3028, 2986, 1601, 1492, 1454, 1064, 1040 cm^{-1}. HRMS calculated for $C_8H_9S^+$: 137.0419, found 137.0415 [M+H]$^+$.

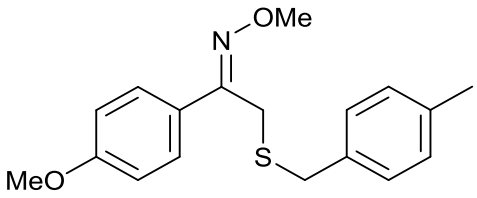
Preparation of 4-methoxyphenyl 4-(methylbenzyl)thiomethyl ketone (67)[76]



To a solution of *p*-tolylmethanethiol (1.20 mL, 8.69 mmol, 1.0 equiv) in DMF (50 mL) was added DIEA (3.00 mL, 17.37 mmol, 2.0 equiv) at 0 ^{o}C, followed by 2-bromo-4'-methoxyacetophenone (2.00 g, 8.69 mmol, 1.0 equiv) and the resulting mixture was stirred at room temperature for 4 hours. The reaction was quenched with H_2O (80 mL)

and DCM (50 mL) was added. After phase separation, the organic phase was washed with 1 M HCl solution (2 x 50 mL) and H_2O (50 mL). The organic extract was dried over $MgSO_4$, filtered and concentrated. The residual solid was recrystallized from ethanol to give a colourless solid (2.25 g, 90.4 %). m.p. 85-86 ^oC. ^1H NMR (400 MHz, $CDCl_3$) δ 7.90 (d, *J* = 9.0 Hz, 2H), 7.2 (d, *J* = 8.0 Hz, 2H), 7.11 (d, *J* = 8.0 Hz, 2H), 6.92 (d, *J* = 9.0 Hz, 2H), 3.81 (s, 3H), 3.66 (s, 2H), 3.56 (s, 2H), 2.26 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 193.3, 163.7, 136.9, 134.3, 131.1, 129.2, 129.2, 128.4, 113.8, 55.5, 35.9, 35.8, 30.9, 21.11. IR (ATR): $\acute{\upsilon}_{max}$ 3024, 2982, 1634, 1558 cm^{-1}. HRMS calculated for $C_{17}H_{19}O_2S^+$: 287.1100, found 287.1095 $[M+H]^+$.

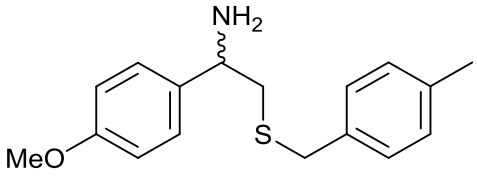
Preparation of (*Z*)-1-(4-methoxyphenyl)-2-(4-methylbenzylthio)ethan-1-one *O*-methyl oxime (70)[76]



To a mixture of 4-(methylbenzyl)thiomethyl ketone (**67**) (0.50 g, 1.75 mmol, 1.0 equiv) and pyridine (2.10 mL, 26.00 mmol, 15.0 equiv) in absolute ethanol (40 mL) was added *O*-methylhydroxylamine hydrochloride (0.44 g, 5.25 mmol, 3 equiv). The resulting mixture was heated to reflux for 2 h and then cooled to room temperature. The mixture was extracted with DCM (40 mL) and washed with 1 M HCl (50 mL x 2) and brine (50 mL). The organic phase was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude was purified by flash chromatography on silica gel with diethyl ether and petrol (1:2) to afford the product (0.43 g, 77 %). Minor (*E*)-oxime was also detected. ^1H NMR (400 MHz, $CDCl_3$) δ 7.55 (d, *J* = 9.0 Hz, 2H), 7.19 (d, *J* = 8.0 Hz, 2H), 7.10 (d, *J* = 8.0 Hz, 2H), 6.86 (d, *J* = 9.0 Hz, 2H), 3.97 (s, 3H), 3.82 (s, 4H), 3.71 (s, 2H), 3.68 (s, 2H), 2.33 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.8, 154.3, 137.1, 135.0, 129.1, 128.9, 127.8, 127.2, 113.8, 62.0, 55.3, 36.7, 24.9, 21.1. IR (ATR): $\acute{\upsilon}_{max}$ 3060, 3028, 2936, 2827, 1624, 1538, 1251,

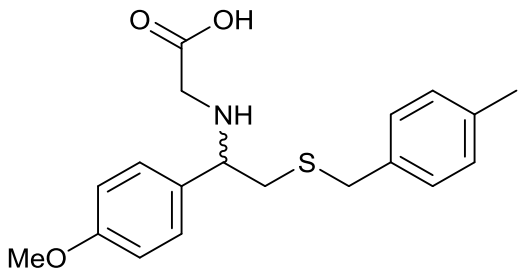
1035 cm^{-1}. HRMS calculated for $C_{18}H_{22}O_2NS^+$: 316.1366, found 316.1367 $[M+H]^+$.

Preparation of 1-(4-methoxyphenyl)-2-(4-methylbenzylthio)ethan-1-amine (71)[76]



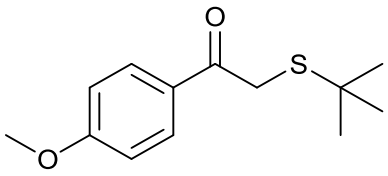
To a solution of (*Z*)-1-(4-methoxyphenyl)-2-((4-methylbenzyl)thio)ethan-1-one *O*-methyl oxime (**70**) (0.63 g, 2.00 mmol, 1.0 equiv) in THF (10 mL) at 0 ^oC was added 1 M BH3/THF (10 mL). The resulting mixture was refluxed for 3.5 h, then cooled to room temperature. Water (14 mL) and 5 M NaOH (14 mL) were then added at 0 ^oC, and the mixture was stirred for 30 min at room temperature, then acidified with 12.5 M HCl (15 mL). Aqueous layer was extracted with diethyl ether, then basified with 28% NH_4OH (15 mL), and extracted with DCM. Then combined organic layers were dried over MgSO4, filtered and concentrated *in vacuo* to give a colourless oil (0.50 g, 87 %). ^1H NMR (400 MHz, $CDCl_3$) δ 7.22 (d, J = 9.0 Hz, 2H), 7.18 (d, J = 8.0 Hz, 2H), 7.11 (d, J = 8.0 Hz, 2H), 6.86 (d, J = 9.0 Hz, 2H), 3.94 (dd, J_1 = 9.0, J_2 = 4.5 Hz, 1H), 3.79 (s, 3H), 3.64 (s, 2H), 2.67 (dd, J_1 = 13.5 Hz, J_2 = 4.5 Hz, 1H), 2.54 (dd, J_1 = 13.5 Hz, J_2 = 9.0 Hz, 1H), 2.32 (s, 3H), 1.68 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.0, 136.7, 135.2, 129.2, 128.8, 127.4, 113.9, 60.4, 55.3, 36.2, 22.5, 21.1. IR (ATR): $\acute{\upsilon}_{max}$ 3373, 3035, 2909, 2841, 1614, 1512, 1257 cm^{-1}. HRMS calculated for $C_{17}H_{22}ONS^+$: 288.1417, found 288.1417 $[M+H]^+$.

Preparation of (1-(4-methoxyphenyl)-2-(4-methylbenzylthio)ethyl)glycine (66)[76]



To a solution of *rac*-1-(4-methoxyphenyl)-2-(*p*-tolylthio)ethan-1-amine (**71**) (0.54 g, 1.88 mmol, 1.0 equiv) in methanol (15 mL) was added glyoxylic acid (0.14 mg, 1.50 mmol, 0.8 equiv), followed by sodium cyanoborohydride (0.13 g, 4.70 mmol, 2.5 equiv) and 3 M methanolic hydrochloric acid (0.6 mL), and the resulting mixture was stirred at room temperature for 24h. The reaction was quenched with H_2O (50 mL) and extracted with ethyl acetate (50 mL x 2). The organic phase was dried over $MgSO_4$ and concentrated *in vacuo*, followed by column chromatography with ethyl acetate then 10% methanol in ethyl acetate to afford a coulourless solid (215 mg, 42 %). m.p. 178-180 ^{o}C. ^{1}H NMR (400 MHz, MeOD) δ 7.30 (d, *J* = 9.0 Hz, 2H), 7.22 (d, *J* = 8.0 Hz, 2H), 7.15 (d, *J* = 8.0 Hz, 2H), 7.01 (d, *J* = 9.0 Hz, 2H), 4.28 (t, *J* = 7.5 Hz, 1H), 3.84 (*s*, 3H), 3.72 (*s*, 2H), 3.23 (q_{AB}, J_{AB} = 16.0 Hz, 2H), 3.04 (dd, J_1 = 14.0 Hz, J_2 = 7.5 Hz, 1H), 2.91 (dd, J_1 = 14.0 Hz, J_2 = 7.5 Hz, 1H), 2.33 (s, 3H). ^{13}C NMR (101 MHz, MeOD) δ 162.3, 138.2, 135.9, 130.7, 130.3, 130.1, 130.1, 127.1, 115.8, 109.5, 61.9, 55.9, 36.4, 35.1, 21.1. IR (ATR): υ_{max} 3014, 2956, 1613 cm^{-1}. HRMS calculated for $C_{19}H_{24}O_3NS^+$: 346.1471, found 346.1473 $[M+H]^+$.

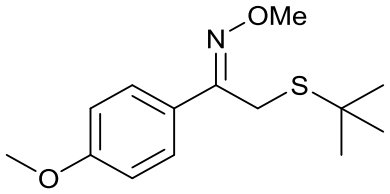
Preparation of 4-methoxyphenyl *tert*-butylthiomethyl ketone (73)[76]



To a solution of *t*-butylthiol (0.98 mL, 8.69 mmol, 1.0 equiv) in DMF (50 mL) was added

DIEA (3.00 mL, 17.37 mmol, 2.0 equiv) at 0 ^{o}C, followed by 2-bromo-4'-methoxyacetophenone (2.00 g, 8.69 mmol, 1.0 equiv) and the resulting mixture was stirred at room temperature for 4 hours. The reaction was quenched with H_2O (80 mL) and DCM (50 mL) was added. After phase separation, the organic phase was washed with 1 M HCl solution (2 x 50 mL) and H_2O (50 mL). The organic extract was dried over $MgSO_4$, filtered and concentrated, followed by a column chromatography eluting with ethyl acetate and hexane (1:2) to give a yellow oil (1.78 g, 86 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (d, *J* = 9.0 Hz, 2H), 6.94 (d, *J* = 9.0 Hz, 2H), 3.87 (s, 3H), 3.84 (s, 2H), 1.36 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 195.0, 163.6, 131.1, 128.7, 113.8, 55.5, 43.65, 35.5, 30.8. IR (ATR): $\acute{\upsilon}_{max}$ 3033, 2987, 1637, 1554 cm^{-1}. HRMS calculated for $C_{13}H_{19}O_2S^+$: 239.1100, found 239.1098 $[M+H]^+$.

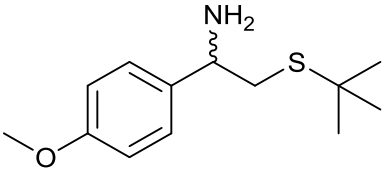
Preparation of (*Z*)-2-(*tert*-butylthio)-1-(4-methoxyphenyl)ethan-1-one *O*-methyl oxime (74)[76]



To a mixture of 4-methoxyphenyl *tert*-butylthiomethyl ketone (**73**) (0.42 g, 1.75 mmol, 1.0 equiv) and pyridine (2.10 mL, 26.00 mmol, 15.0 equiv) in absolute ethanol (40 mL) was added *O*-methylhydroxylamine hydrochloride (0.44 g, 5.25 mmol, 3.0 equiv). The resulting mixture was heated to reflux for 2 h and then cooled to room temperature. The mixture was extracted with DCM (40 mL) and washed with 1 M HCl (50 mL x 2) and brine (50 mL). The organic phase was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude was purified by flash chromatography on silica gel with diethyl ether and petrol (1:1) to afford the product (0.30 g, 65 %). Minor (*E*)-oxime was also detected. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 (d, *J* = 9.0 Hz, 2H), 6.89 (d, *J* = 9.0 Hz, 2H), 4.01 (s, 3H), 3.82 (s, 3H), 3.79 (s, 2H), 1.36 (s, 9H). ^{13}C NMR (101 MHz, CDCl3) δ 160.5, 154.5, 129.2, 127.8, 127.4,

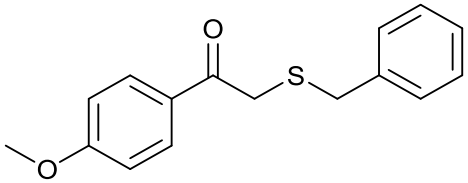
113.9, 113.4, 62.1, 55.3, 43.5, 31.5, 30.6, 22.6. IR (ATR): $\acute{\upsilon}_{max}$ 3048, 2936, 2825, 1622, 1534, 1256 cm^{-1}. HRMS calculated for $C_{14}H_{22}NO_2S^+$: 268.1366, found 268.1367 $[M+H]^+$.

Preparation of 2-(*tert*-butylthio)-1-(4-methoxyphenyl)ethan-1-amine (75)[76]



To a solution of (*Z*)-2-(*tert*-butylthio)-1-(4-methoxyphenyl)ethan-1-one *O*-methyl oxime (**74**) (0.53 g, 2.00 mmol, 1.0 equiv) in THF (5 mL) at 0 ^oC was added 1 M BH_3/THF (10 mL). The resulting mixture was refluxed for 3.5 h, then cooled to room temperature. Water (14 mL) and 5 M NaOH (14 mL) were then added at 0 ^oC, and the mixture was stirred for 30 min at room temperature, then acidified with 12.5 M HCl (15 mL). Aqueous layer was extracted with diethyl ether, then basified with 28% NH_4OH (15 mL), and extracted with DCM. Then combined organic layers were dried over MgSO4, filtered and concentrated *in vacuo* to give a colourless oil (0.20 g, 42%). ^1H NMR (400 MHz, $CDCl_3$) δ 7.30 (d, J = 8.5 Hz, 2H), 6.87 (d, J = 8.5 Hz, 2H), 4.05 (dd, J_1 = 9.0 Hz, J_2 = 5.0 Hz, 1H), 3.79 (s, 3H), 3.06 (s, 2H), 2.87 (dd, J_1 = 12.5 Hz, J_2 = 5.0 Hz, 1H), 2.77 (dd, J_1 = 12.5, J_2 = 9.0 Hz, 1H), 1.32 (s, 9H). IR (ATR): $\acute{\upsilon}_{max}$ 3375, 3031, 2926, 2845, 1626, 1518 cm^{-1}. HRMS calculated for $C_{13}H_{22}NOS^+$: 240.1417, found 240.1417 $[M+H]^+$.

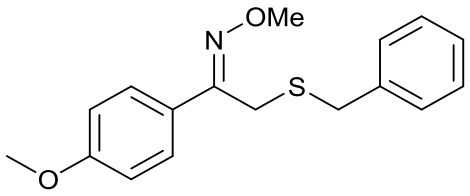
Preparation of 4-methoxyphenyl benzylthiomethyl ketone (77)[76]



To a solution of benzyl mercaptan (3.52 mL, 30.00 mmol, 1.0 equiv) in DMF (100 mL) was added DIEA (10.30 mL, 60.00 mmol, 2.0 equiv) at 0 ^oC, followed by

2-bromo-4'-methoxyacetophenone (6.87 g, 30.00 mmol, 1.0 equiv) and the resulting mixture was stirred at room temperature for 4 hours. The reaction was quenched with H_2O (50 mL) and DCM (50 mL) was added. After phase separation, the organic phase was washed with 1 M HCl solution (2 x 50 mL) and H_2O (50 mL). The organic extract was dried over $MgSO_4$, filtered and concentrated, followed by recrystallization with EtOH to afford a colourless solid (7.10 g, 87 %). m.p. 65-67 ^oC. ^1H NMR (400 MHz, $CDCl_3$) δ 7.94 (d, *J* = 9.0 Hz, 2H), 7.37-7.29 (m, 5H), 6.95 (d, *J* = 9.0 Hz, 2H), 3.90 (s, 3H), 3.79 (s, 3H), 3.66 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 137.4, 131.1, 129.3, 128.5, 127.1, 113.9, 58.4, 55.5, 36.2, 35.7, 18.4. IR (ATR): $\acute{\upsilon}_{max}$ 3061, 3027, 2937, 2838, 1661, 1596 cm^{-1}. HRMS calculated for $C_{16}H_{17}O_2S^+$: 273.0944, found 273.0945 $[M+H]^+$.

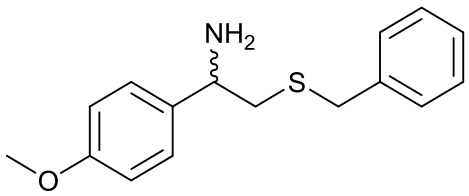
Preparation of (*Z*)-2-(phenylthio)-1-(4-methoxyphenyl)ethan-1-one *O*-methyl oxime (78)[76]



To a mixture of 4-methoxyphenyl benzylthiomethyl ketone (**77**) (0.95 g, 3.50 mmol, 1.0 equiv) and pyridine (4.20 mL, 52.00 mmol, 15.0 equiv) in absolute ethanol (40 mL) was added *O*-methylhydroxylamine hydrochloride (0.88 g, 10.50 mmol, 3.0 equiv). The resulting mixture was heated to reflux for 2 h and then cooled to room temperature. The mixture was extracted with DCM (40 mL) and washed with 1 M HCl (50 mL x 2) and brine (50 mL). The organic phase was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude was purified by flash chromatography on silica gel with diethyl ether and petrol (1:1) to afford the product (0.72 g, 69 %). Minor (*E*)-oxime was also detected. ^1H NMR (400 MHz, $CDCl_3$) δ 7.57 (d, *J* = 9.0 Hz, 2H), 7.36 – 7.25 (m, 6H), 6.89 (d, *J* = 9.0 Hz, 2H), 4.00 (s, 3H), 3.85 (s, 3H), 3.77 (s, 2H), 3.72 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.6, 154.0, 138.0, 129.0, 128.4, 127.8, 127.1, 113.9, 62.0, 55.3, 37.0, 24.9. IR (ATR): $\acute{\upsilon}_{max}$ 3060, 3028,

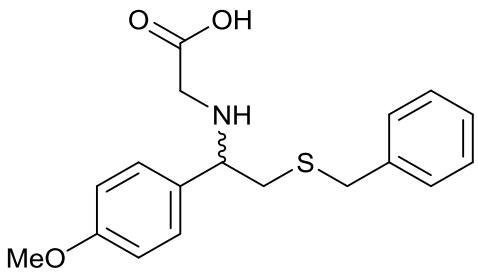
2934, 2835, 1607, 1513 cm^{-1}. HRMS calculated for $C_{17}H_{20}NO_2S^+$: 302.1209, found 302.1209 $[M+H]^+$.

Preparation of 1-(4-methoxyphenyl)-2-(benzylthio)ethan-1-amine (79)[76]



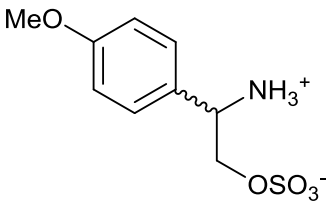
To a solution of (*Z*)-2-(benzylthio)-1-(4-methoxyphenyl)ethan-1-one *O*-methyl oxime (**78**) (0.60 g, 2.00 mmol, 1.0 equiv) in THF (5 mL) at 0 ^oC was added 1 M BH_3/THF (10 mL). The resulting mixture was refluxed for 3.5 h, then cooled to room temperature. Water (14 mL) and 5 M NaOH (14 mL) were then added at 0 ^oC, and the mixture was stirred for 30 min at room temperature, then acidified with 12.5 M HCl (15 mL). The aqueous layer was extracted with diethyl ether, then basified with 28% NH_4OH (15 mL), and extracted with DCM. Then combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo* to give a colourless oil (0.41 g, 75 %). ^1H NMR (400 MHz, $CDCl_3$) δ 7.34-7.32 (m, 5H), 7.24 (d, J = 8.5 Hz, 2H), 6.88 (d, J = 8.5 Hz, 2H), 3.99 (dd, J_1 = 9.0 Hz, J_2 = 4.5 Hz, 1H), 3.82 (s, 3H), 3.70 (s, 2H), 2.74 (dd, J_1 = 13.5 Hz, J_2 = 4.5 Hz, 1H), 2.60 (dd, J = 13.5 Hz, J_2 = 9.0 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.0, 138.3, 136.6, 128.9, 128.5, 127.4, 127.1, 113.9, 55.3, 54.2, 41.4, 36.6. IR (ATR): $\acute{\upsilon}_{max}$ 3367, 3061, 3027, 2909, 2834, 1609, 1513 cm^{-1}. HRMS calculated for $C_{16}H_{20}NOS^+$: 274.1261, found 274.1261 $[M+H]^+$.

Preparation of (1-(4-methoxyphenyl)-2-(benzylthio)ethyl)glycine (80)[76]



To a solution of *rac*-1-(4-methoxyphenyl)-2-(benzylthio)ethan-1-amine (**79**) (0.41 g, 1.50 mmol, 1.0 equiv) in methanol (15 mL) was added glyoxylic acid (0.11 mg, 1.20 mmol, 0.8 equiv), followed by sodium cyanoborohydride (0.24 g, 4.70 mmol, 2.5 equiv) and 3 M methanolic hydrochloric acid (0.5 mL), and the resulting mixture was stirred at room temperature for 24h. The reaction was quenched with H_2O (50 mL) and extracted with ethyl acetate (50 mL x 2). The organic phase was dried over $MgSO_4$ and concentrated *in vacuo*, followed by column chromatography with ethyl acetate then 10% methanol in ethyl acetate to afford a colourless oil (86 mg, 17 %). ^{1}H NMR (400 MHz, MeOD) δ 7.25 – 7.15 (m, 7H), 6.85 (d, J = 8.5 Hz, 2H), 4.07 (t, J = 7.5 Hz, 1H), 3.69 (s, 3H), 3.59 (s, 2H), 3.10 (q_{AB}, J_{AB} = 16.0 Hz, 2H), 2.92 (dd, J_1 = 14.0 Hz, J_2 = 7.5 Hz, 1H), 2.78 (dd, J_1 = 14.0 Hz, J_2 = 7.0 Hz, 1H). ^{13}C NMR (101 MHz, MeOD) δ 173.1, 161.9, 139.2, 130.7, 130.2, 129.7, 128.5, 128.3, 62.1, 55.9, 36.8, 36.1, 30.8, 23.6. IR (ATR): υ_{max} 3381, 3003, 2957, 1614 cm^{-1}. HRMS calculated for $C_{12}H_{22}O_3NS^+$: 332.1315, found 332.1317 $[M+H]^+$.

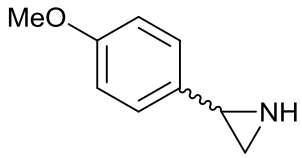
Preparation of 2-ammonium-2-(4-methoxyphenyl)ethyl sulfate (85)[87]



2-Amino-2-(4-methoxyphenyl)ethanol (**32**) (0.17 g, 1.01 mmol, 1.0 equiv) was dissolved in

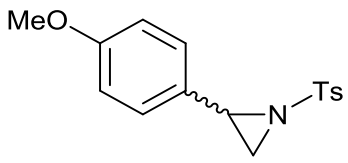
acetonitrile (10 mL), and the reaction was cooled to 0 ^{o}C, followed by addition of chlorosulfonic acid (0.12 g, 1.01 mmol, 1.0 equiv) dropwise. The resulting mixture was stirred at room temperature for 3.5 h. After that, the product was filtered off and washed with diethyl ether and ethyl acetate to give the pure product as a colourless solid (0.15 g, 60 %). m.p. 254-256 ^{o}C. ^{1}H NMR (400 MHz, DMSO-*d*6) δ 8.24 (s, 3H), 7.39 (d, *J* = 7.0 Hz, 2H), 6.99 (d, *J* = 7.0 Hz, 2H), 4.29 – 4.16 (m, 1H), 3.77 (s, 3H), 3.65 (m, 2H). ^{13}C NMR (101 MHz, DMSO-*d*6) δ 159.5, 128.8, 127.3, 114.0, 62.9, 55.4, 55.2. IR (ATR): υ_{max} 3444, 3025, 2936, 1053 cm^{-1}. HRMS calculated for $C_9H_{14}NO_5S^+$: 248.0587, found 248.0588 $[M+H]^+$.

Preparation of 2-(4-methoxyphenyl)aziridine (86)[87]



2-Ammonium-2-(4-methoxyphenyl)ethyl sulfate (**85**) (0.15 g, 0.60 mmol, 1.0 equiv) was dissolved in 6 M NaOH (10 mL), followed by the addition of toluene (10 mL). The resulting biphasic solution was stirred and heated to reflux for 18 hours. Then H_2O (10 mL) was added and the mixture was extracted with ethyl acetate (3 x 10 mL). After separation of the organic phase, it was dried over $MgSO_4$, filtered and, on removal of solvent *in vacuo*, the reside was purified by column chromatography, eluting with petrol : diethyl ether (1 : 1) and isolated as a pale yellow oil (50 mg, 56 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.14 (d, *J* = 8.5 Hz, 2H), 6.84 (d, *J* = 8.5 Hz, 2H), 3.77 (s, 3H), 2.94 (dd, J_1 = 6.0 Hz, J_2 = 3.5 Hz, 1H), 2.13 (d, *J* = 6.0 Hz, 1H), 1.74 (d, *J* = 3.5 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) 158.8, 132.4, 126.9, 113.9, 55.3, 31.7, 28.8. IR (ATR): υ_{max} 3361, 3028, 2992, 1625, 1456, 1064, 1035cm^{-1}. HRMS calculated for $C_9H_{11}NO$: 150.0913, found 150.0912 $[M+H]^+$.

Preparation of *N*-*p*-toluenesulfonyl 4-(methoxyphenyl)aziridine (89)[97]



4-(Methoxyphenyl)aziridine (89 mg, 0.60 mmol, 1.0 equiv) was dissolved in DCM (10 mL), triethylamine (0.22 mL, 1.50 mmol, 2.5 equiv) was added, followed by *p*-toluenesulfonyl chloride (0.11 g, 0.60 mmol, 1.0 equiv). The reaction was stirred at 0 ^{o}C for four hours and, after filtering the resulting solid, and concentrating the filtrate, the residue oil was purified by column chromatography, eluting with diethyl ether : petrol (1: 1) to furnish the desired prouct as a colourless solid (0.13 g, 72 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 (d, *J* = 8.0 Hz, 2H), 7.32 (d, *J* = 8.0 Hz, 2H), 7.13 (d, *J* = 8.5 Hz, 2H), 6.81 (d, *J* = 8.5 Hz, 2H), 3.77 (s, 3H), 3.73 (dd, J_1 = 7.0, J_2 = 4.5 Hz, 1H), 2.95 (d, *J* = 7.0 Hz, 1H), 2.43 (s, 3H), 2.37 (d, *J* = 4.5 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.7, 144.6, 135.1, 129.7, 127.9, 127.8, 127.0, 114.0, 55.3, 40.9, 35.8, 21.7. IR (ATR): $\acute{\upsilon}_{max}$ 3024, 2965, 2251, 1698 cm^{-1}. HRMS calculated for $C_{16}H_{18}NO_3S$: 304.1002, found 304.1002 $[M+H]^+$.

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