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Design, Synthesis and Evaluation of Cyclothialidine Analogues as DNA Gyrase Inhibitors

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Doctor of Philosophy

ASTON UNIVERSITY

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Design, Synthesis and Evaluation of Cyclothialidine Analogues as DNA Gyrase Inhibitors

A thesis submitted by Kylie Michelle Loak BSc (Hons) for the degree of Doctor of Philosophy

Absract: Cyclothialidine, a natural product isolated from *Streptomyces filipinensis* NR0484, has been proven to be a potent and selective inhibitor of the bacterial enzyme DNA gyrase. Gyrase inhibition results in cell death, the enzyme being the target of several currently used antibiotics. Cyclothialidine showed poor activity against whole bacterial cells, highlighting scope for improvement regarding cell membrane permeability in order for the full potential of this new class of antibiotics to be realised.

Structurally, cyclothialidine contains a 12-membered lactone ring which is partly integrated into a pentapeptide chain, with a substituted aromatic moiety bordering the lactone. Retrosynthetically it can be traced back to *cis*-3-hydroxyproline, 3,5-dihydroxy-2,6-dimethylbenzoic acid and four commercially available amino acids; two serine, one cysteine and one alanine.

In this work, a model of cyclothialidine was synthesised in order to establish the methodology for more complex compounds. Analogues with hydroxy, dihydroxy and dihydroxymethyl substituted aromatic moieties were then prepared to ensure successful protection methods could be performed and the pharmacophore synthesised. The key aromatic moiety, 2,6-dimethyl-3,5-dihydroxybenzoic acid was produced *via* two successive Mannich reaction/reduction steps. Acid protection using 4-nitrobenzyl bromide and TBDMS hydroxyl protection followed by bromination of one methyl afforded the desired intermediate. Reaction with a serine/cysteine dipeptide, followed by deprotection and cyclisation under Mitsunobu conditions lead to the 12-membered lactone. An amine substituted aromatic analogue and also replacement of the cysteine sulphur by oxygen were attempted but without success.

In an effort to improve cell permeability, a conjugate was synthesised between the pharmacophore and a cholesterol moiety. It was hoped the steroid fragment would serve to increase potency by escorting the molecule through the lipid environment of the cell membrane. The pharmacophore and conjugate were tested against a variety of bacterial strains but the conjugate failed to improve activity.

Keywords: Natural product, Antibiotics, Lactone, Pharmacophore, Conjugate

To Mum, Dad and Andy

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Abbreviations

ACN	acetonitrile
ADP	adenosine diphosphate
AIBN	azoisobutyronitrile
APCI	atmospheric pressure chemical ionization
ATP	adenosine triphosphate
BOP	[(benzotriazol-1-yl)oxy]tris(dimethylamino)phosphonium hexafluorophosphate
BOC_2O	di-*tert*-butyl dicarbonate
BOC-ON	(2-(*tert*-butoxycarbonyloxyimino))-2-phenyl-acetonitrile
CBZ	carbobenzyloxy
DBU	1,8-diazabicyclo[5.4.0]undec-5-ene
DCC	dicyclohexylcarbodiimide
DCM	dichloromethane
DEAD	diethylazodicarboxylate
DHU	dicyclohexylurea
DIP	direct insertion probe
DMAP	4-dimethylaminopyridine
DMF	dimethylformamide
DMSO	dimethylsulphoxide
DNA	deoxyribonucleic acid
DSC	disuccinimidyl carbonate
FTIR	fourier transform infrared
LDA	lithium diisopropylamide
MR	relative molecular mass
MRSA	methicillin-resistant *Staphylococcus aureus*
MS	mass spectrometry
MSSA	methicillin-sensitive *Staphylococcus aureus*
NBS	*N*-bromosuccinimide
NMR	nuclear magnetic resonance
PENDANT	polarisation enhancement nurtured during attached nucleus testing
PPTS	pyridinium *p*-toluene sulphonate
PyBOP	[(benzotriazol-1-yl)oxy]tripyrrolidophosphonium hexafluorophosphate
Rf	retention factor
RNA	ribonucleic acid
RT	room temperature
TBAF	tetra-butylammonium fluoride
TBDMS	*tert*-butyldimethylsilyl

TCB	2,4,6-trichlorobenzoyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
THP	tetrahydropyran
TLC	thin layer chromatography
TMS	trimethylsilyl
WSCDI. HCl	water soluble carbodiimide hydrochloride (1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride)

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Introduction to the Pharmacology of DNA Topoisomerases

1.1 INTRODUCTION

The ability of bacteria to acquire resistance to currently available antibiotics is an ever increasing problem for medicine today.[1] As we move into the twenty-first century the development of new classes of antibiotics is vital if we are to win our continuing battle against disease.

DNA gyrase, an essential bacterial enzyme, is responsible for the transcription, recombination and replication of DNA. Without it, bacterial growth is impossible and as a result the inhibition of this enzyme is an attractive target for antibiotics. Currently available DNA gyrase inhibitors need to be improved in order to reduce toxicity and increase efficacy, whilst offering a new challenge to the resistance of our bacterial enemies.

A class of natural products known as cyclothialidines, first isolated in 1992 were found to be potent and selective DNA gyrase inhibitors *in vitro*. Studies into their mode of action showed them to act by inhibition of ATP hydrolysis; a mechanism by which the bacterial enzyme manipulates DNA strands for conformational alteration. Activity, however, diminished considerably when tested against whole bacterial cells, rendering the compounds redundant as antibiotics.

The aim of the project was initially to provide a synthetic route to cyclothialidine (**Figure 1.1**), a 12 membered lactone partly integrated into a pentapeptide chain with a substituted aromatic moiety bordering the macrocycle. It was hoped the methodology developed would pave the way for the synthesis of analogues with increased cell permeability, to help realise the potential of this potent class of compounds as drugs of tomorrow.

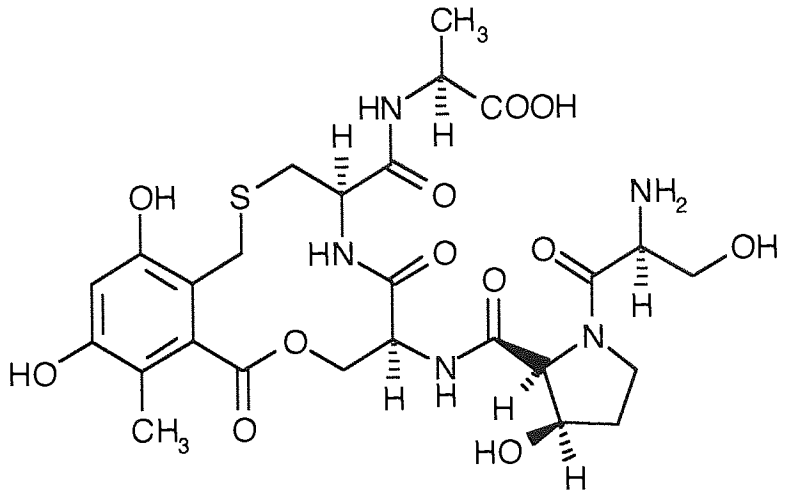

Figure 1.1 Cyclothialidine

1.2 DEOXYRIBONUCLEIC ACID (DNA)

DNA is the substance of life. Found in chromosomes within the nucleus of a cell, DNA carries the genetic code responsible for determining cell structure and function. Disruption of DNA and its activities has damning consequences for growth, health and development.

1.2.1 Structure

DNA is a polymer with a backbone of alternating sugar and phosphate residues, with an organic base attached to each sugar molecule perpendicular to the backbone. Each base-sugar-phosphate unit is known as a nucleotide. Nucleotides join together by forming a phosphate ester bond between the 5'-phosphate component of one molecule and the 3'-hydroxy on the sugar component of another molecule. One end of the polymer has a free hydroxy group at C3' (known as the 3' end) and the other end has a phosphoric acid residue at C5' (the 5' end) (**Figure 1.2**).

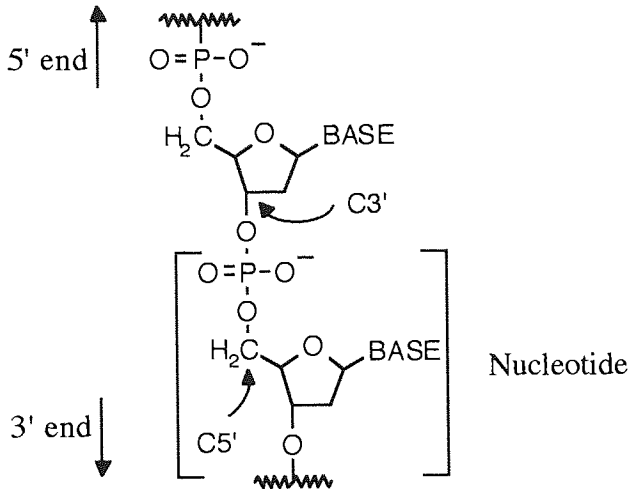

Figure 1.2 The DNA Backbone

There are four different heterocyclic bases, the order of which is critical when carrying genetic information. DNA exists as a double helix - the strands being held together by hydrogen bonding between complementary base pairs; adenine (A) in one strand pairing with thymine (T) in the other and cytosine (C) pairing with guanine (G)[2,3] (**Figure 1.3**).

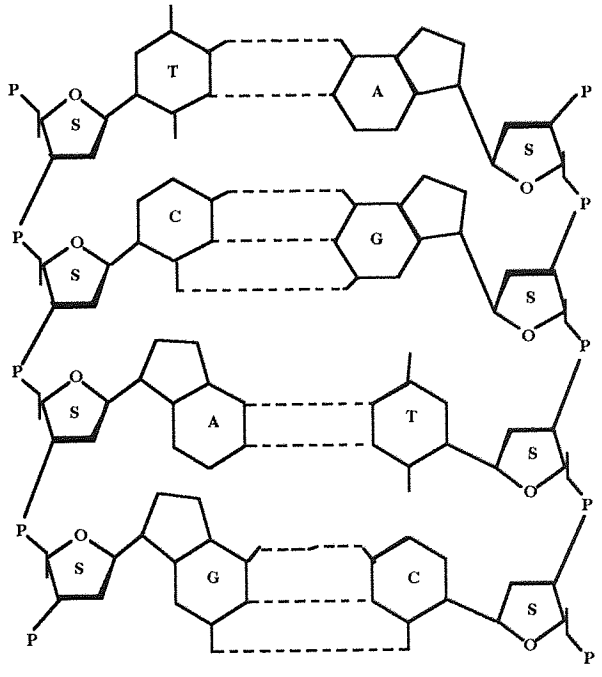

Figure 1.3 Base Pairing Between DNA Strands

1.2.2 Supercoiling: Tertiary Structure of DNA

DNA molecules have considerable length and can bend and twist in solution to form a variety of shapes. Although in eukaryotic nuclei the DNA is linear, in many bacterial cells the two ends of the duplex are covalently linked to form closed circles. Whether circular or linear, there exists a high level of conformational flexibility. Thus, while the double helix may be 'relaxed', meaning it has no twists in it other than the helical twists themselves, further twisting and coiling of the double helix is possible. This is known as supercoiling.

Circular relaxed DNA has one complete turn, *ie.* one strand crossing another, every 10.4 base pairs. If circular DNA is broken and the two ends of the resulting linear molecule are twisted in opposite directions, the double helix can be 'overwound' or 'underwound'. Overwound DNA has one turn every 9 base pairs and underwound DNA only one turn every 11 base pairs.[4] On rejoining the strands to create a circle, the molecule compensates for the change in twist by forming 'supercoils' to maintain its conformation. Overwound DNA results in positive supercoils and underwound in negative supercoils (**Figure 1.4**). Naturally occurring circular DNA is negatively supercoiled except during replication when it becomes positively supercoiled.[5]

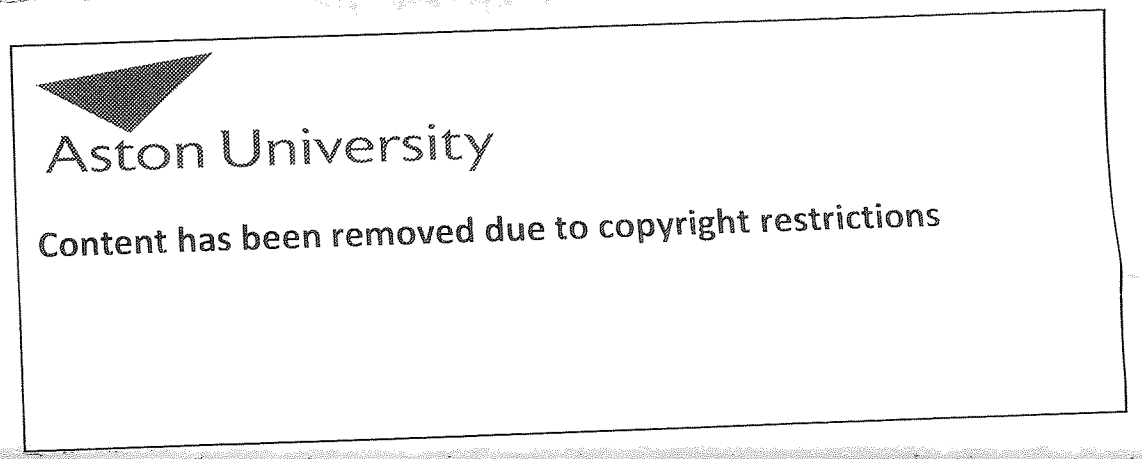

Figure 1.4 Negative (left) and Positive (right) Supercoils

(Redrawn from ref. 4)

Negative supercoiling, however, introduces torsional strain into the molecule and therefore the *elimination* of supercoils is thermodynamically favoured, *ie.* it is a spontaneous process and, as such, requires no external energy source. Supercoils can be relieved by 'local unwinding' (**Figure 1.5**) and thus synthetic activities requiring strand separation are more efficient when the DNA is negatively supercoiled.[3]

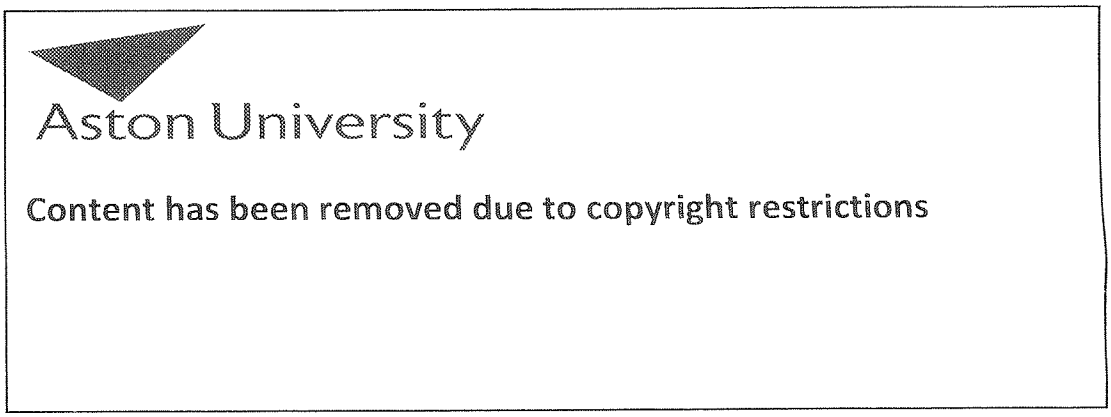

Figure 1.5 Supercoiling *versus* Local Unwinding

(Redrawn from ref. 4)

1.2.3 Action

All synthetic activities involving double stranded DNA, *ie.* recombination, replication, transcription, *etc.* require separation of the strands. For example, during replication the strands separate to allow each one to act as a template by hydrogen bonding the newly unpaired bases to complementary bases in the surrounding medium.

The helical nature of the duplex means that for separation to occur the strands must be able to rotate about each other in order to unwind. Due to the vast length of DNA we must assume the *ends* of the strands to be fixed, regardless of whether the DNA is circular or linear, and thus *unable* to rotate.[6] As a result, when two intertwined strands are pulled apart from one end, winding about each other further along the molecule is increased, *ie.* strand separation is compensated for by supercoiling (**Figure 1.6**).

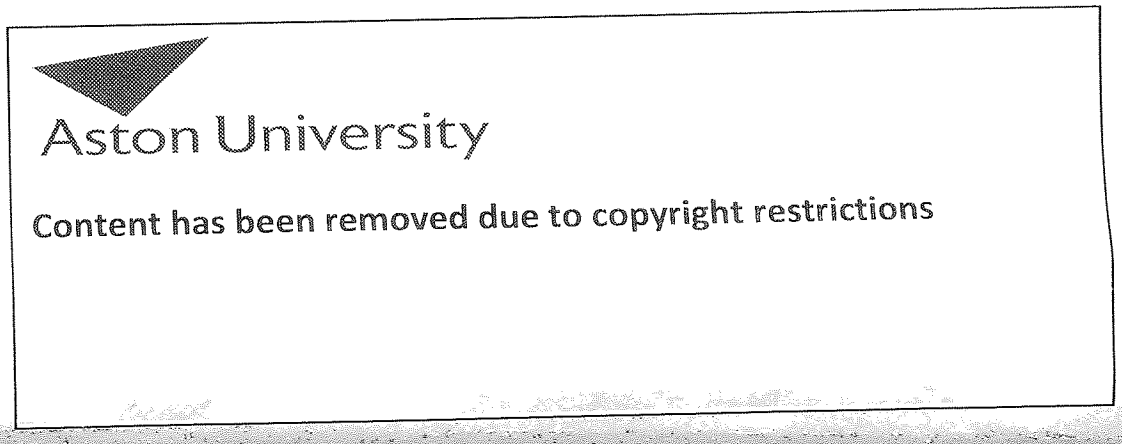

Figure 1.6 Supercoiling Resulting from Unwinding of the Double Helix

(Redrawn from ref. 6)

1.2.4 Linking Number (L)

The number of times one strand of a circular DNA molecule crosses over another when the molecule is lying flat on a plane is known as the linking number. It is constant for any covalently closed, circular molecule and is positive in right-handed helices; negative in left-handed helices. When negative supercoils are added to circular DNA the linking number decreases.

The linking number can be changed only by cleaving a phosphodiester linkage in one or both strands, rewinding the DNA and resealing the break.

1.3 TOPOISOMERASES

The problem of strand separation must be addressed if DNA is to function efficiently. Extensive rotation of the DNA strands, if possible, requires a high energy input and tangling of the DNA molecule may result. The resulting supercoils may interfere with further unwinding of the double-helix.[7]

A solution may be found in the form of a class of enzymes known as topoisomerases. Topoisomerases form enzyme-bridged strand breaks which are able to act as gateways for the passage of other DNA strands. A nick is introduced in one strand, allowing rotation about the intact strand, after which the nick can be sealed (**Figure 1.7**). Each repetition of the nicking and sealing reaction releases one superhelical turn.

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Figure 1.7 The Nicking/Resealing Reaction (Redrawn from ref. 6)

In addition to the relaxation of supercoiled DNA, topoisomerases are able to carry out other topological interconversions . These include the catenation (the linking together) and decatenation (unlinking) of DNA helices. A circular DNA nearing the end of a round of replication will generate two interlinked circles. It is likely, therefore, that topoisomerase action is necessary for the termination of replication of a circular DNA. Knotting and unknotting of strands can also be performed.

These essential nuclear enzymes are divided into two main types depending on their mechanism.

1.3.1 Type I

Topoisomerase I enzymes act by reversibly breaking one strand of the double-helix. The enzyme remains covalently attached to the 5' end of the broken strand with the 3' end moving far enough away for the unbroken strand to effectively pass through the break. The hydroxy group of the 3' end then attacks the activated, covalently bound 5' phosphate, so resealing the nick and thus relieving supercoils (**Figure 1.8**). The linking number (L) changes in units of one.

Topoisomerase enzymes do not require adenosine triphosphate (ATP) for this process as they store the energy from the phosphodiester bond they cleave and re-use it to seal the nick. Prokaryotic topoisomerase I relaxes only negative supercoils (requiring Mg^{2+}) while the eukaryotic enzyme is able to relax both positive and negative supercoils (no Mg^{2+} required). Both enzymes are monomeric and catenation of two circular DNA molecules can only occur if one is already nicked.[8]

1.3.2 Type II

Topoisomerase II enzymes function by introducing a double-stranded break in one duplex through which a section of unbroken duplex passes before the break is resealed (**Figure 1.9**). DNA wraps around the outside of the protein, which catalyses the formation of a double-stranded break in one loop. Another loop of DNA is passed through the break, the break is sealed and the DNA released.[9] As a result, two negative supercoils are introduced and the linking number (L) decreased by two.

Prokaryotic topoisomerase II relaxes positive supercoils and is able to introduce negative supercoils. The enzyme is a tetramer of the form A_2B_2 - a complex of two proteins A and B. Eukaryotic topoisomerase II is a homodimer which relaxes both positive and negative supercoils but has no supercoiling ability. Both cell types require ATP and both are able to knot/unknot DNA strands and catenate/decatenate DNA circles.

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Figure 1.8 Topoisomerase I Mechanism

(Redrawn from ref. 4)

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Figure 1.9 Topoisomerase II Mechanism

(Redrawn from ref. 4)

1.4 DNA GYRASE

1.4.1 Structure

DNA gyrase,[10,11] first discovered in 1976, is a type II prokaryotic topoisomerase, *ie.* it is found only in bacterial cells, and is essential for growth. It exists as an A_2B_2 tetramer - a complex of two proteins A and B (**Figure 1.10**).[12] Protein A, coded for by the *gyr A* gene, contains 875 amino acids and has a relative molecular mass (Mr) of 97000. The B protein, coded for by the *gyr B* gene, consists of 804 amino acids with an Mr of 90000.[13]

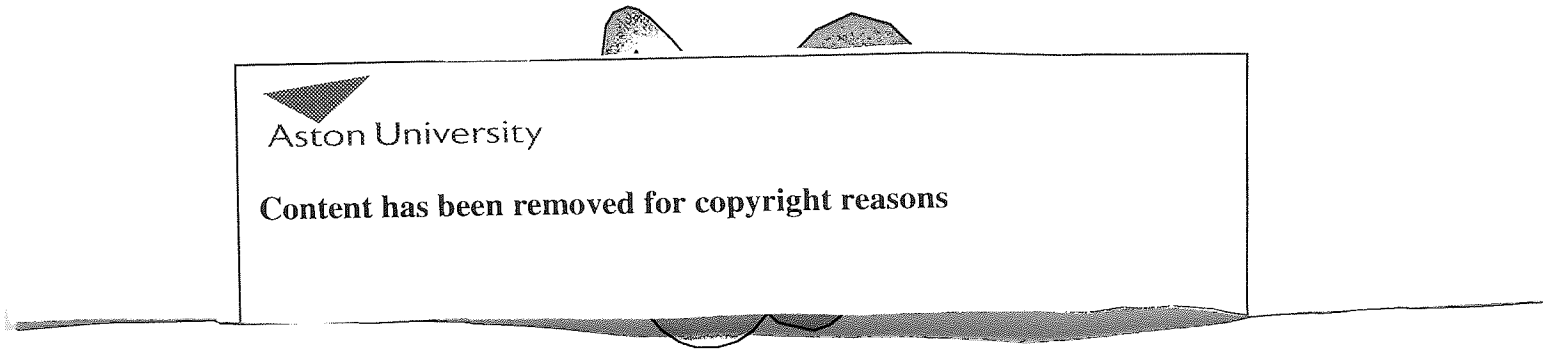

Figure 1.10 DNA Gyrase (Redrawn from ref. 13)

1.4.2 Function

Gyrase is unique in that it is able to convert relaxed circular DNA into a negative superhelix. One molecule of DNA gyrase can introduce ~100 supercoils per minute. The energy required to carry out this conversion is supplied by the hydrolysis of ATP. In the absence of ATP, gyrase relaxes negatively supercoiled DNA. The rate of this process, however, is more than 10 times slower than that of supercoil introduction.[5]

Current mechanistic models[14] of the supercoiling process involve passage of a DNA strand through a double-stranded break which is held open by the protein. DNA is thought to pass through a 'gateway' in the protein and X-ray studies (**Figure 1.11**) confirm a hole of 20 Å diameter in the protein, approximately the diameter of the DNA helix.

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Figure 1.11 Space-filling Model of the DNA Gyrase B Protein Dimer

(Redrawn from ref. 14)

Biological studies indicate that gyrase enzymes A and B work together in their introduction of supercoils (**Figure 1.12**).

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Figure 1.12 The Action of DNA Gyrase (Redrawn from ref. 6)

Firstly, gyrase A proteins bind to DNA (**1**) and the DNA strands are cleaved (**2**). A large conformational change then occurs in the protein to allow passage of another strand through the break into the interior of the protein complex (**3**). It is thought that binding energy from the association of ATP and the B protein is used to stabilise an otherwise unfavourable conformation of the protein. The break reseals (**4**) and binding energy is released when ATP is hydrolysed to ADP and inorganic phosphate, which dissociate from the protein allowing the protein to 'relax' back to the conformation at the start of the cycle (**5**).

1.4.3 Catalysis of DNA Breakage/Rejoining

The A chain of the complex is responsible for breaking and resealing DNA strands and attaches to DNA *via* the active site at tyrosine residue 122 (Tyr-122) (**Figure 1.13**).[15]

In **Figure 1.12**, step (a), the hydroxy group of a tyrosine residue at the active site of the enzyme performs a nucleophilic attack on a 5'-phosphate group within the DNA chain, forming a phosphotyrosine bond and breaking the DNA strand. The 3' end is held in place within the active site by hydrogen bonding. A portion of DNA is then able to pass through the break, step (b), before a second transesterification reaction, involving nucleophilic attack by the deoxyribose hydroxy group on the phosphotyrosine linkage, results in regeneration of the sugar-phosphate backbone, step (c),[16] so freeing the enzyme for the next cycle of reactions.

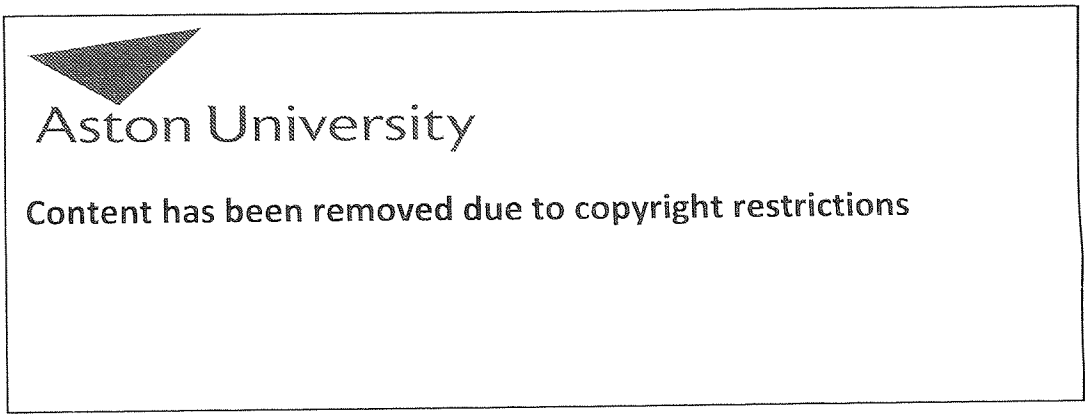

Figure 1.13 Strand Breakage/Rejoining Mechanism

(Redrawn from ref. 3)

1.4.4 Catalysis of DNA Dependant ATP Hydrolysis

All known type II topoisomerases are structurally related and ATP dependant. The B protein is responsible for ATP dependant transduction; the active sites being the lysine residues at 103 and 110 in the chain.[17]

The topoisomerase enzyme is thought to act as a molecular clamp (**Figure 1.14**), generating a 'gate' and actively transporting one DNA segment across another.[18] In the absence of ATP, the protein clamp remains open. The enzyme binds to a DNA duplex (known as the 'G-segment') and a second segment (the 'T-segment') enters the trap. ATP binding closes the clamp, trapping the T-segment, if present and transporting it through the DNA gate in the G-segment. Hydrolysis of ATP and/or the release of hydrolysed products opens the clamp and frees the conformationally altered DNA.

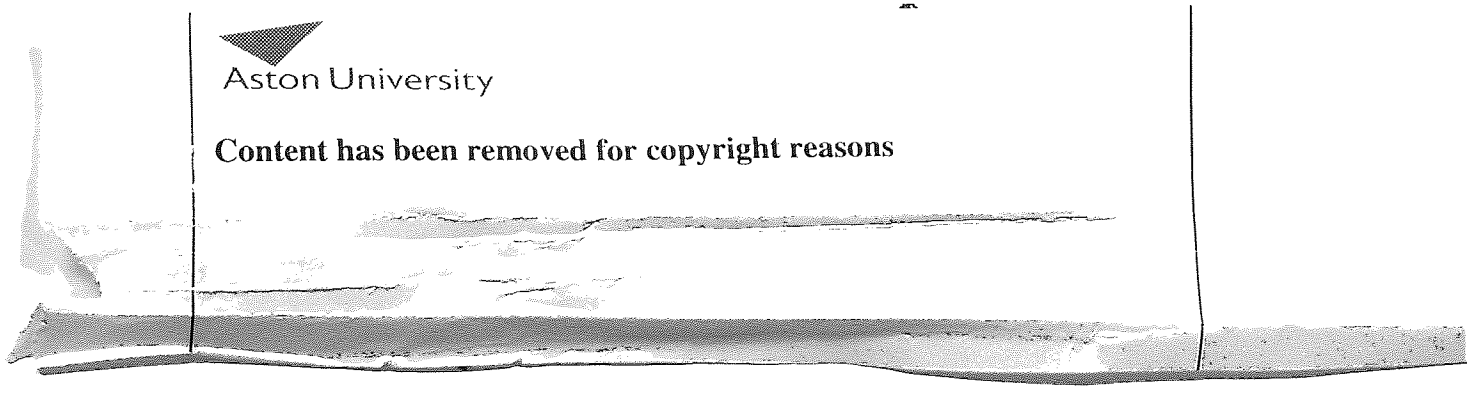

Figure 1.14 ATP Dependant Protein Clamp

(Redrawn from ref. 17)

1.4.5 Inhibition

Due to their important biological functions, topoisomerases have become interesting therapeutic targets for curing bacterial infection and cancer. Topoisomerase drugs act by converting essential enzymes into intracellular cell toxins which kill proliferating cells. Drug-induced inhibition of DNA and ribonucleic acid (RNA) synthesis rapidly leads to cell death.

Studies on the mode of action of gyrase highlight two main areas of attack for enzyme inhibitors: i) interference with reactions involving DNA strand passage by the A subunit and ii) inhibition of ATP binding to the B subunit.[19]

Currently, DNA gyrase is the target of two classes of antibiotics: the synthetic quinolones, *eg.* nalidixic acid (**Figure 1.15**) and oxolinic acid and the naturally occurring coumarins, originally isolated from *Streptomyces* species, *eg.* novobiocin (**Figure 1.16**), coumermycin and chlorobiocin.

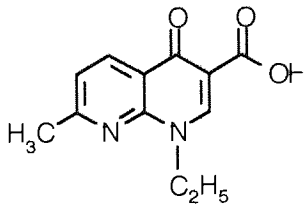

Figure 1.15 Nalidixic Acid (A Quinolone Inhibitor)

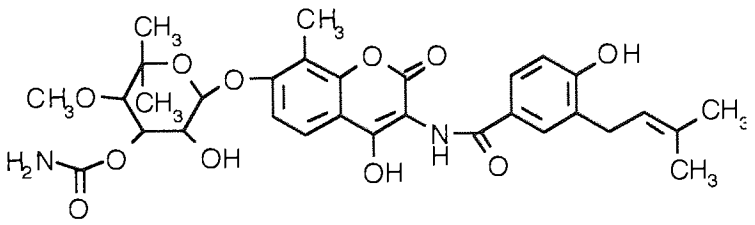

Figure 1.16 Novobiocin (A Coumarin Inhibitor)

1.4.6 Action on the A Subunit

Quinolones act on the A subunit (Gyr A) and interrupt the DNA rejoining step of the gyrase mediated strand-passing reaction by binding to the enzyme and forming a cleavable-complex, *ie.* an aborted reaction intermediate. The drug self-associates inside the enzyme-induced strand break and physically prevents the nick from resealing (**Figure 1.17**; ▬ = quinolone).[20] As a result, fragmentation of nuclear DNA is increased; RNA and DNA synthesis inhibited and the cell dies.

There are two ways in which cleavable complexes may act: either the enzyme is stabilised within the complex, rendering it catalytically inactive during a time when it is required; or the cleavable-complex induces a cellular response which results in cell death.[21] Cleavable complex formation is reversible; the complex will dissociate when the drug is removed.

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Figure 1.17 Drug Action at the A Subunit (Redrawn from ref. 12)

1.4.7 Action on B Subunit

Coumarin drugs act on the Gyr B protein and block supercoiling by interfering with the utilisation of ATP. The coumarins have been found to inhibit ATP binding and thus the ability of the enzyme to hydrolyse ATP.[22] Original studies suggested that the coumarins were simple competitive inhibitors of topoisomerase action.[23,24] More recent work, however, indicates a separate, though overlapping, drug binding site which results in the stabilisation of a conformation which is unable to bind nucleoside triphosphate, thereby inhibiting ATP hydrolysis.[25,26]

The toxicity of these drugs, especially the synthetic quinolones, necessitates a continuing search for alternative inhibitors.

1.5 CYCLOTHIALIDINE

Cyclothialidine is a potent and selective DNA gyrase inhibitor, first isolated by Arisawa *et al.*[27] in 1992 from the fermentation broth of the bacterium *Streptomyces filipinensis* NR 0484. Cyclothialidine has been shown to be twice as active against *Escherichia. coli* DNA gyrase as novobiocin and coumermycin, with an IC_{50} of 0.03μg/ml.[28,29] In addition, it showed the lowest activity, of the compounds tested, for mammalian DNA, *ie.* it has low cytotoxicity.

1.5.1 Structure

Structurally, cyclothialidine (**Figure 1.18**) contains a 12 membered lactone ring which is partly integrated into a pentapeptide chain, with a substituted aromatic moiety (resorcinol) bordering the lactone ring.[30]

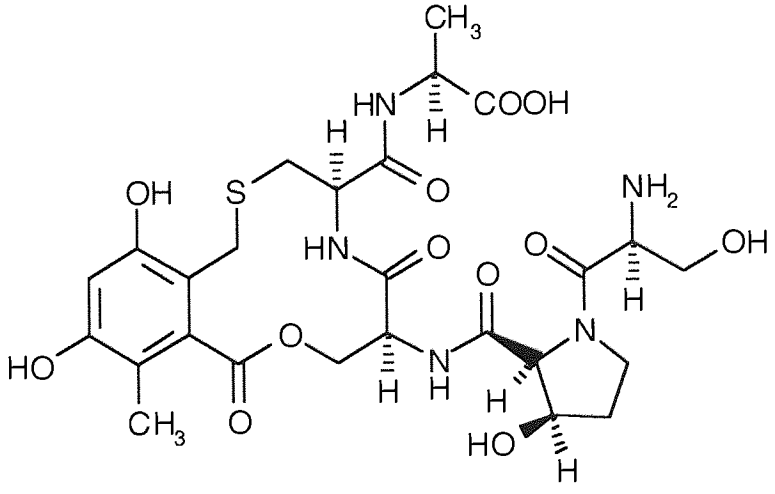


Figure 1.18 Cyclothialidine

1.5.2 Mechanism of Enzyme Inhibition

Biological studies[31] indicate that cyclothialidine does not inhibit strand cutting, strand rejoining or DNA binding. Instead, the mode of action has been shown to resemble that of the coumarins in that it inhibits ATP hydrolysis. It is not, however, a simple case of competitive inhibition. In 1995, Nakada *et al.*[32] suggested that it was likely that cyclothialidine, ATP and the coumarins shared a common, overlapping, site of action on the B subunit, though the precise active sites differed. They proposed that cyclothialidine binding occurred close to the ATP binding site of the gyrase B subunit, stabilising a conformation of the protein that is unable to bind ATP; and recognises a site different to that of the coumarins. A year later, the first crystal structures of a DNA topoisomerase/drug complex were published,[33] proving that binding was indeed competitive due to a small degree of overlap between the binding sites. The overlapping regions of the binding sites were found to be the resorcinol ring of cyclothialidine and the adenine ring of ATP. Despite the relatively small overlap, once cyclothialidine is bound, it forms a 'plug' preventing ATP binding. This explains how three very different structures can be competitive inhibitors.

1.5.3 The Pharmacophore: Essential Requirements for Activity

In 1993, Gotschi *et al.*[34] reported the minimum structural requirements (**Figure 1.19**) for the inhibition of gyrase. In a SAR study of cyclothialidines a 12-membered ring created from an aromatic moiety, cysteine and serine was identified as the pharmacophore. The phenolic hydroxyl group at position 5 in the aromatic chromophore was also shown to be essential for activity. Replacement of the group by hydrogen resulted in a reduction in gyrase inhibition of over 3 orders of magnitude.

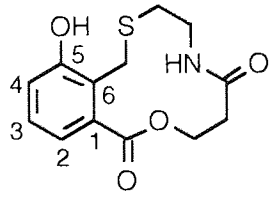


Figure 1.19 The Pharmacophore

This was later corroborated by Maxwell *et al.*[32] with the aid of crystal structures. X-ray studies of the enzyme/inhibitor complex indicated that the majority of interaction between cyclothialidine and the protein was attributed to hydrogen bonding between the enzyme and hydroxyl groups on the aromatic ring.

In 1997, Yamaji *et al.*[35] reported investigations into the physico-chemical properties of a series of cyclothialidines isolated from *Streptomyces* sp. They too discovered the 12 membered ring, created from the aromatic chromophore and serine and cysteine moieties, was essential for activity. Analogue studies indicated a two-fold increase in activity when a methyl group was present at position 2 of the aromatic ring, while varying the amino acid side chains R_1 and R_2 had little effect (**Figure 1.20**).

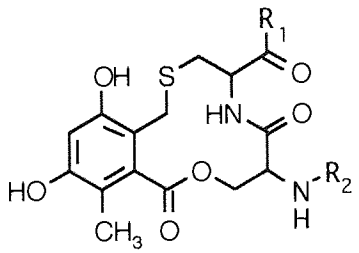


Figure 1.20 The Modified Pharmacophore

1.5.4 Analogues

The distinct structure and selective inhibition mechanism of cyclothialidine have rendered it a desirable lead for developing a new group of resistant-free drugs for curing infectious disease. In addition, although the potential of cyclothialidine as an antibiotic has been established, there remains the possiblity that it or one of its analogues may possess anticancer activity. The fact that numerous antibiotics, *eg.* the anthracycline class of compounds, have demonstrated an ability to act against tumour cells in addition to bacterial cells gives credibility to this theory.

Although the cyclothialidines investigated by Yamaji *et al.* were found to be highly potent and selective DNA gyrase inhibitors with $IC_{100's}$ ranging from 0.3 μM to 1.0 μM against *E. coli* DNA gyrase,[28,34] they exhibited little antibacterial activity against intact bacterial cells *in vitro*. Penetration through the cytoplasmic membrane of *E. coli* was negligable. Against whole bacterial cells cyclothialidine was active against only *Eubacterium* spp., a species known to be highly susceptible to antibiotics. Such poor penetration through the hydrophilic bacterial cell wall and the hydrophobic cell membrane results from the peptidic nature of cyclothialidines.

As a result, the exploitation of cyclothialidine analogues as highly active, low toxicity compounds and their improvement regarding bioactivity against bacteria, render this class of compounds extremely attractive to Medicinal Chemists.

Chapter 2:
Synthesis of the Lactone Ring

2.1 SYNTHESIS OF CYCLOTHIALIDINE

At the time the project commenced in 1995, the only source of cyclothialidine resulted from isolation of the natural product synthesised by *Streptomyces filipinensis* NR0484. The biological method of fermentation, however, results in very low yields, *eg.* 110 litres of broth filtrate afforded just 76 mg of cyclothialidine.[29] For the inhibitor to have any viable use as a potential drug, a synthetic procedure is vital and a suitable method would pave the way for further analogue synthesis.

2.1.1 Building Blocks

Retrosynthetically cyclothialidine may be traced back into four amino acid molecules: two serine, one cysteine, one alanine; *cis*-3-hydroxyproline and an aromatic moiety[29] (**Figure 2.1**).

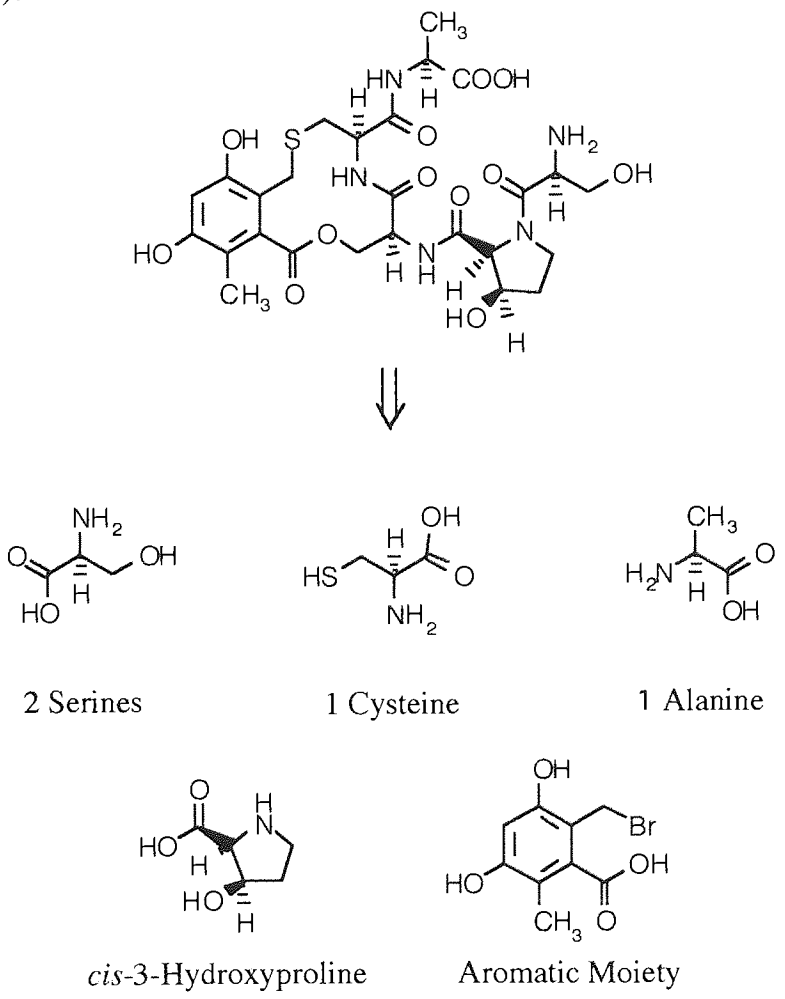


Figure 2.1 Retrosynthesis

Since serine, cysteine and alanine were commercially available, it was *cis*-3-hydroxyproline and the aromatic moiety which posed the synthetic challenge, along with the assembly of the lactone from the synthetic building blocks.

2.2 MODEL COMPOUND

Before attempting the synthesis of the complex natural product, priority was given to investigation of the formation of the 12 membered lactone. For these studies a model compound (**Figure 2.2**) was chosen as this encompassed the desired macro-lactone ring, whilst being based on the commercially available aromatic moiety, *o*-toluic acid (**Figure 2.3**).

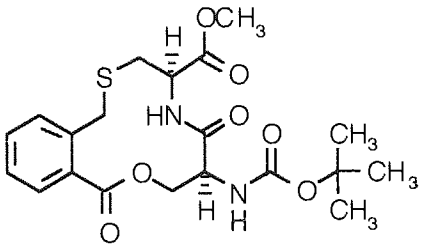


Figure 2.2 Model Compound

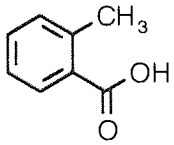
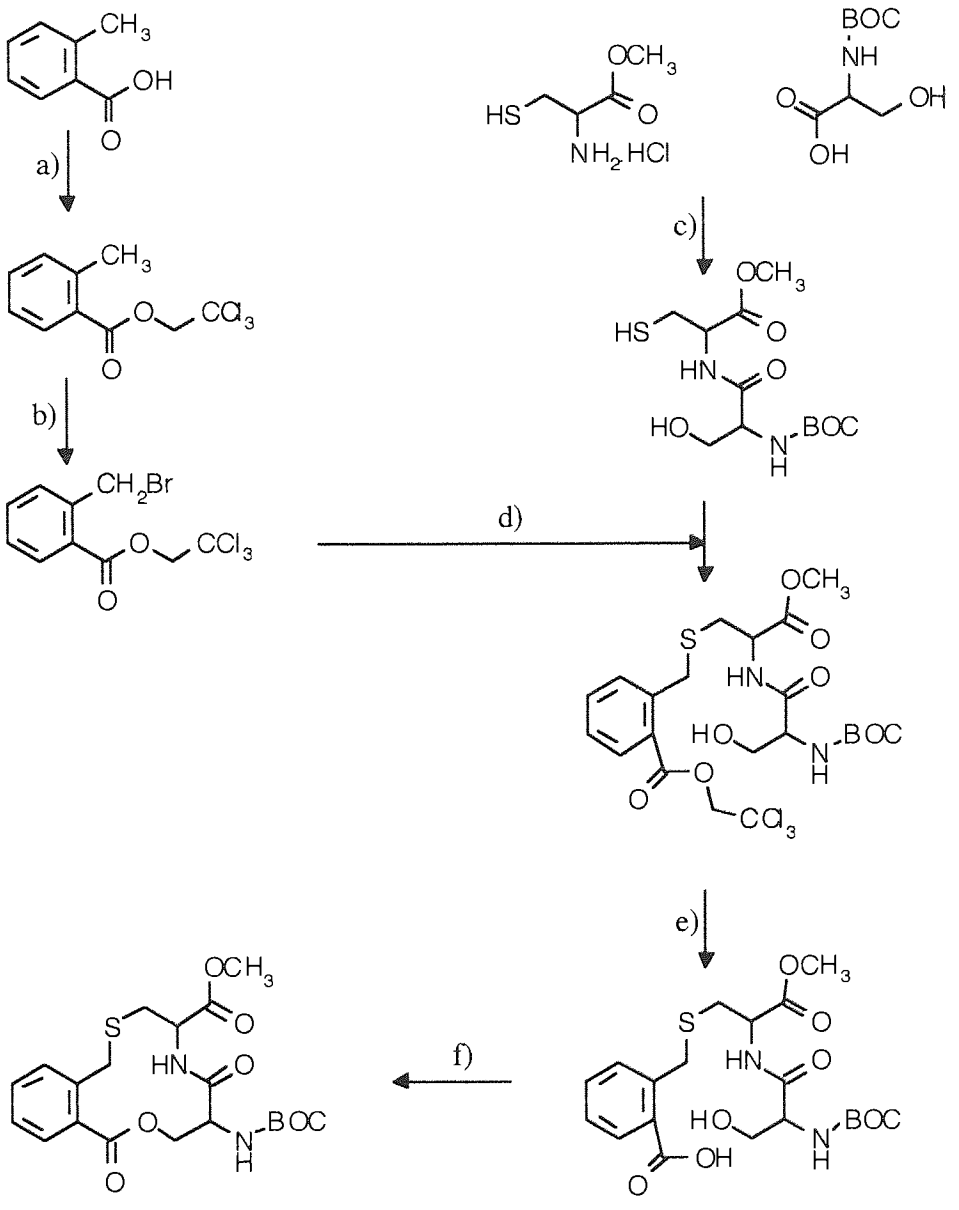


Figure 2.3 *o*-toluic acid

A patent by Arisawa *et al.*[27] cited a route to the 12-membered lactone (**Scheme 2.1**). No yields were given, so the degree of success of the methodology was unknown. The patent involved protection of the carboxylic acid with 2,2,2-trichloroethanol (**a**) and bromination of the side-chain methyl using *N*-bromosuccinimide (**b**), prior to reaction (**d**) with a dipeptide (which may be readily prepared from commercially

available amino acids (**c**).) Deprotection of the acid (**e**) followed by subsequent cyclisation (**f**) lead to formation of the 12-membered ring structure.



a) **i.** $SOCl_2$, reflux, 45 min; **ii.** CCl_3CH_2OH, CH_2Cl_2, Et_3N, RT, 3 h; **b**) NBS, CCl_4, reflux, hυ, 3 h; **c**) 4-methylmorpholine, DCC, acetonitrile, 0 °C, 3 h; **d**) CH_2Cl_2, Et_3N, 1 h, 0 °C; then 3 h, RT; **e**) Zn, THF, 1M H_3PO_4, 1M NaH_2PO_3, RT, 2.5 h; **f**) Cyclisation

Scheme 2.1 Route to the Model Compound

Over the years, the literature has outlined several procedures for the cyclisation of macro-lactones.

2.3 CYCLISATION PROCEDURES

A variety of cyclisation procedures have been utilised in natural product synthesis, incorporating numerous reagents and varying conditions. Lactone formation becomes slower as ring size increases so, unless some means can be found to activate the reacting groups, undesirably high reaction temperatures or excessively slow addition of the hydroxy acid are required. Slow addition of the hydroxy acid into the reaction mixture *via* a cryocooled syringe, known as the 'high dilution' technique, keeps the concentration of acid to a minimum. If the concentration becomes too high dimerisation can occur resulting in lower product yields.

Some of the most common methods are discussed below.

2.3.1 The 'Double Activation' Method

In 1974 Corey *et al.*[36] related the use of 2-pyridinethiol esters for the internal esterification of hydroxy acids into large ring lactones. 2,2'-Dipyridyl disulphide (**Figure 2.4**) was reacted with the hydroxy acid in the presence of triphenylphosphine to yield the 2-pyridinethiol ester, which was then added over 15 hours to refluxing xylene (138-144 °C) to form the lactone.

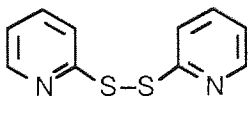
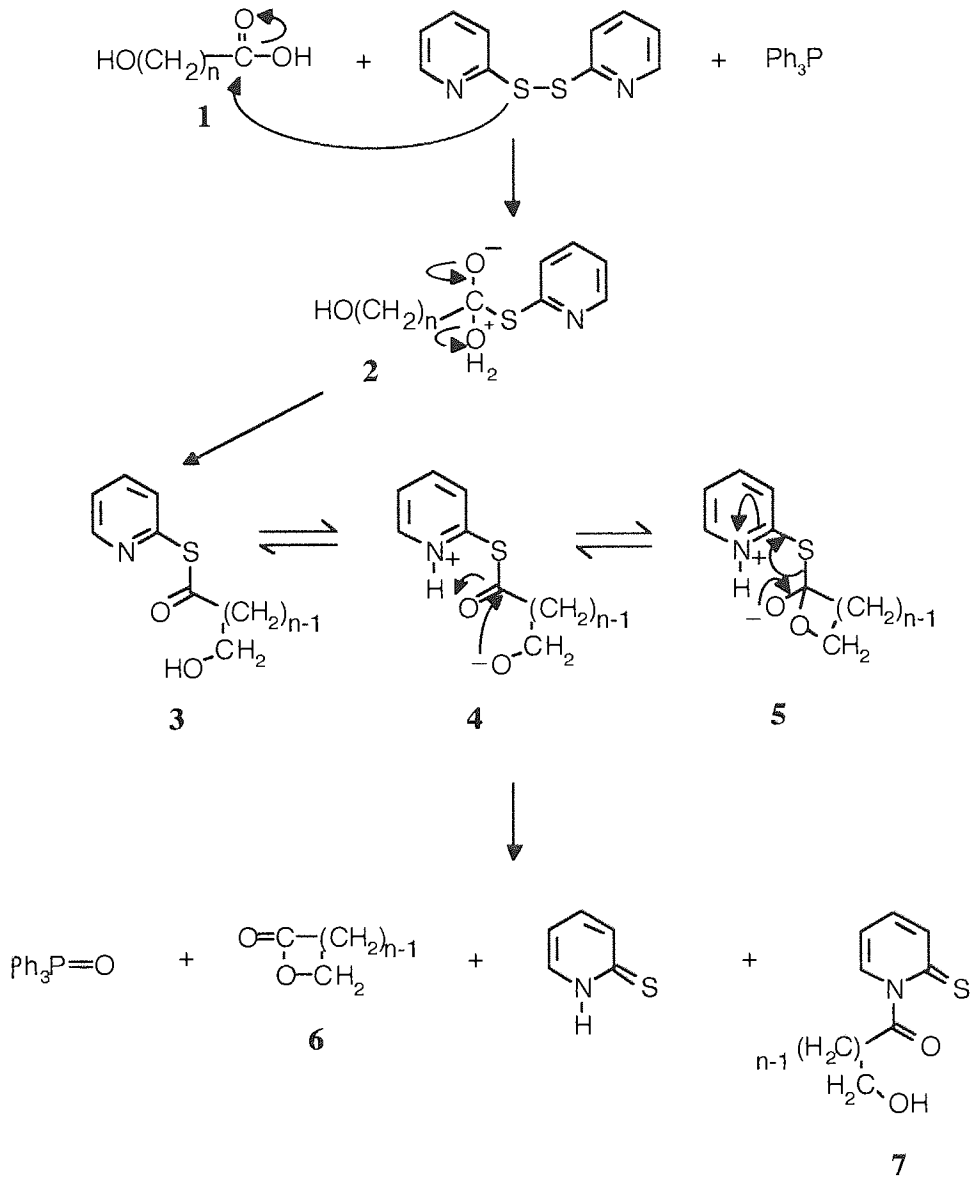


Figure 2.4 2,2'-Dipyridyl disulphide

The 2-pyridinethiol esters simultaneously activated both the carbonyl and hydroxyl groups by favouring internal proton transfer from hydroxyl to carboxylic oxygen[37] (**Scheme 2.2**).



Scheme 2.2 'Double Activation' Mechanism

Nucleophilic attack by sulphur on the carbonyl carbon of hydroxy acid (**1**) produces tetrahedral intermediate (**2**), which loses water affording 2-pyridinethiol ester (**3**). Internal proton transfer from oxygen to nitrogen, (**4**), results in a nucleophilic attack by

O^- on the carbonyl carbon leading to tetrahedral carbonyl adduct (**5**). Elimination affords lactone (**6**) along with the three by-products shown in **Scheme 2.2**.

The rearranged by-product (**7**) is formed as a result of nucleophilic attack by the nitrogen on the carbonyl carbon of the hydroxy acid (**1**). *S*-acyl (**3**) and *N*-acyl (**7**) intermediates are produced independently by the first step, though only the *S*-acyl results in lactone formation. This observation by Corey *et al.*[38] in 1976 led to the discovery of 2,2'-*bis*-(4-*t*-butyl-*N*-isopropyl)imidazolyl disulphide (**Figure 2.5**) as a superior reagent to 2,2'-dipyridyl disulphide. Formation of the *N*-acyl intermediate is effectively inhibited by the steric effect of bulky side chains. As a result lower reaction temperatures could be employed which resulted in an increase in the isolated yields.

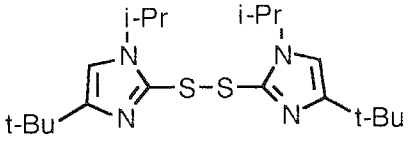


Figure 2.5 2,2'-*Bis*-(4-*t*-butyl-*N*-isopropyl)imidazolyl disulphide

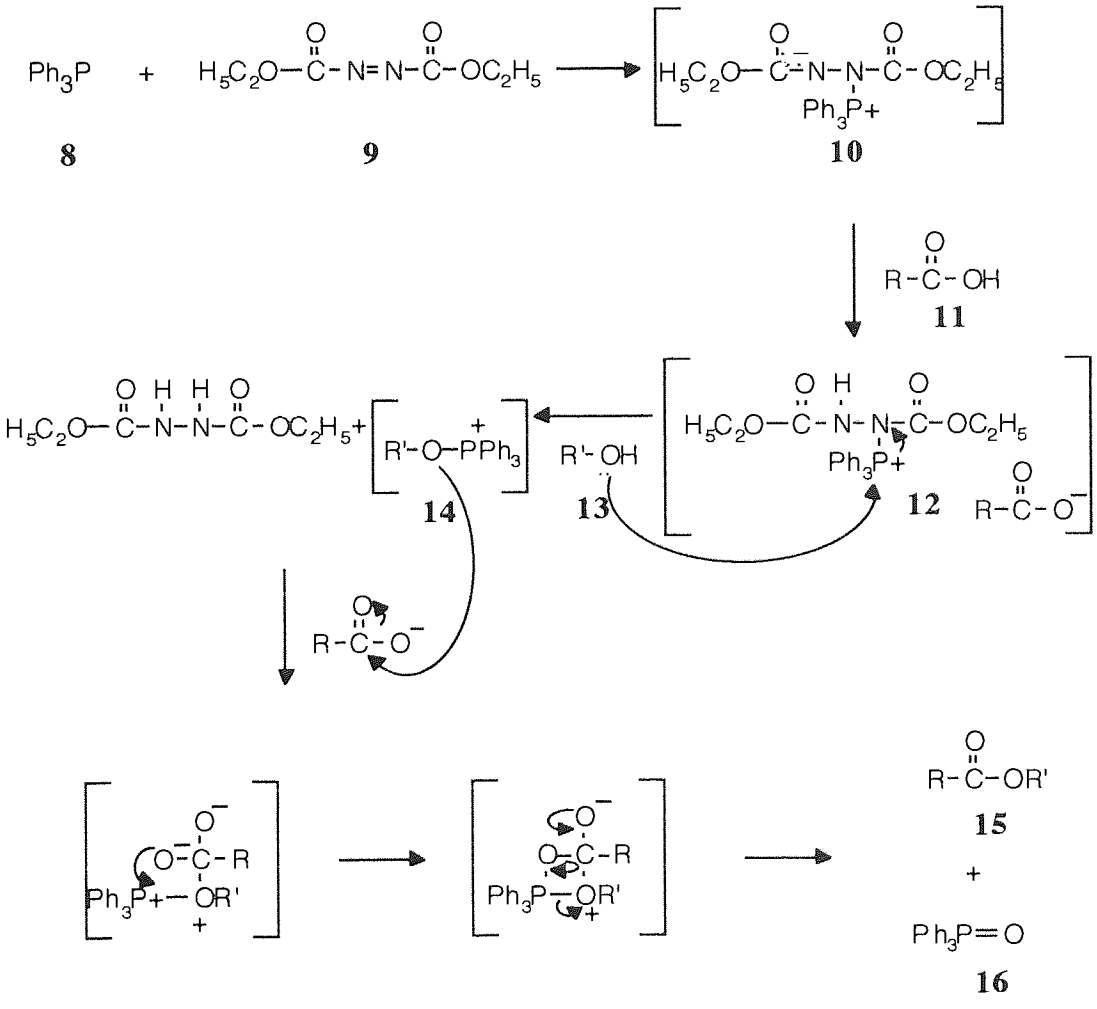
2.3.2 Mitsunobu Method

The Mitsunobu[39,40] method of macrolactonization was first published in 1971 and involves the addition of an hydroxy acid over a period of hours to a mixture of diethyl azodicarboxylate (DEAD) and triphenylphosphine (Ph_3P). In 1993 Waddell and Blizzard[41] used the procedure to synthesise a series of erythromycin derivatives varying in size from 13-16-membered rings.

Reaction of Ph_3P (**8**) with DEAD (**9**) (**Scheme 2.3**) yields quaternary phosphonium salt (**10**) which is protonated on the addition of acid (**11**) to produce intermediate (**12**). Nucleophilic attack by alcohol (**13**) on (**12**) affords the alkoxyphosphonium salt (**14**) which in a mechanism analogous to the Wittig reaction results in ester (**15**) and

triphenylphosphine oxide (**16**). When the acid and alcohol functionalities are part of the same molecule, a lactone is produced.

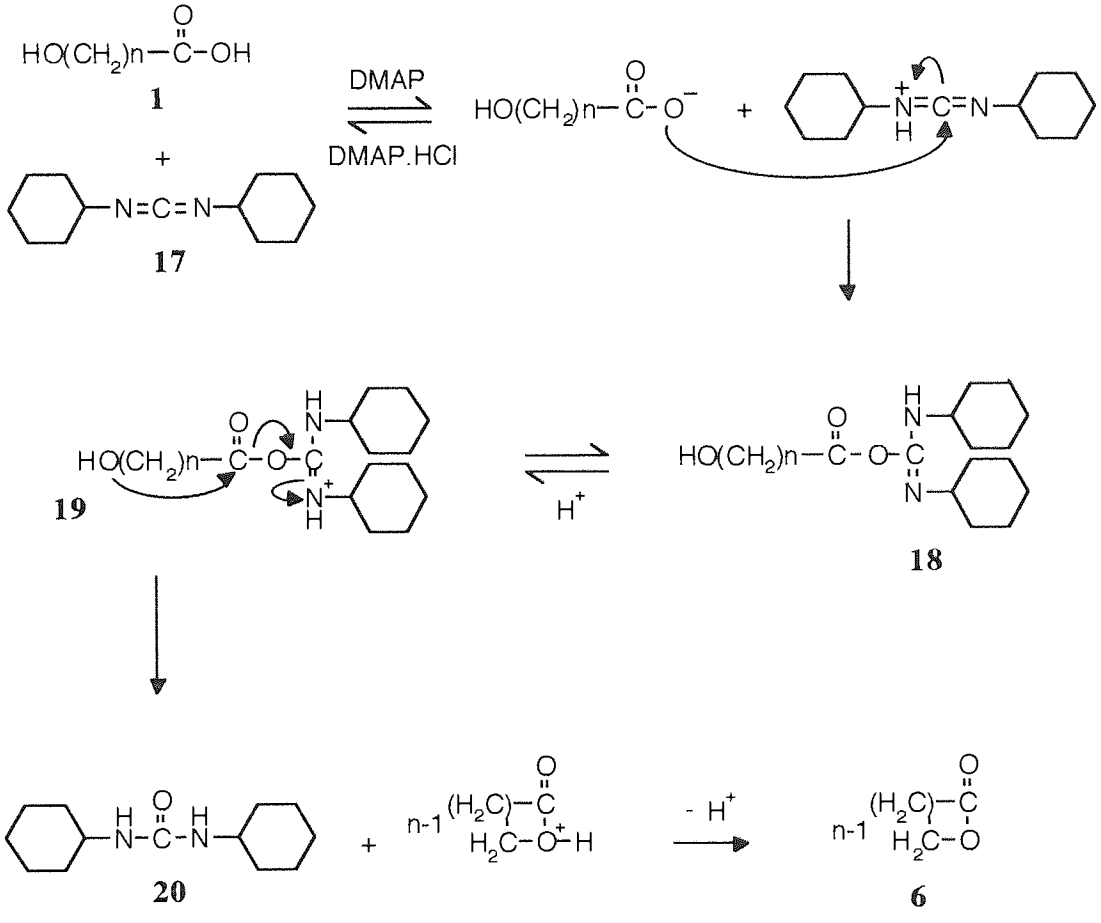
In 1991 modified Mitsunobu conditions were utilised by Justus and Steglich[42] to synthesise a 14-membered strained biaryl ether lactone in 59 % yield. They increased the equivalents of DEAD and Ph_3P used relative to those in the Mitsunobu procedure and the hydroxy acid was added over 10 hours at ambient temperature. Later the modified method was adopted by Couladouros and Soufli[43] who were able to synthesise a series of caffrane macrolactones. They discovered that dimerisation could be prevented by increasing the reaction temperature to 40-50 °C, whereupon the reaction proceeded faster and addition time could be reduced to 5 hours.



Scheme 2.3 Mitsunobu Mechanism

2.3.3 Keck Method

In 1985 Boden and Keck[44] reported the synthesis of hexadecanolide from 15-hydroxypentadecanoic acid using dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) in the presence of DMAP.HCl. Addition of carboxylic acid (**1**) to DCC (**17**) under basic conditions (**Scheme 2.4**) results in *O*-acyl urea (**18**). Cyclisation of protonated intermediate (**19**), affords lactone (**6**) and by-product dicyclohexylurea (DHU) (**20**).



Scheme 2.4 DCC Mechanism

Keck *et al.* discovered that use of the amine hydrochloride prevented formation of the undesirable *N*-acylurea (**Figure 2.6**) from DCC by acting as a proton-transfer agent; preserving the 'active ester' species under conditions of high dilution. As a result the yield of the desired ring system was increased. Keck later used similar conditions[45] to synthesise (-)-colletol, a 14-membered natural product.

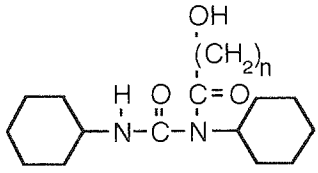
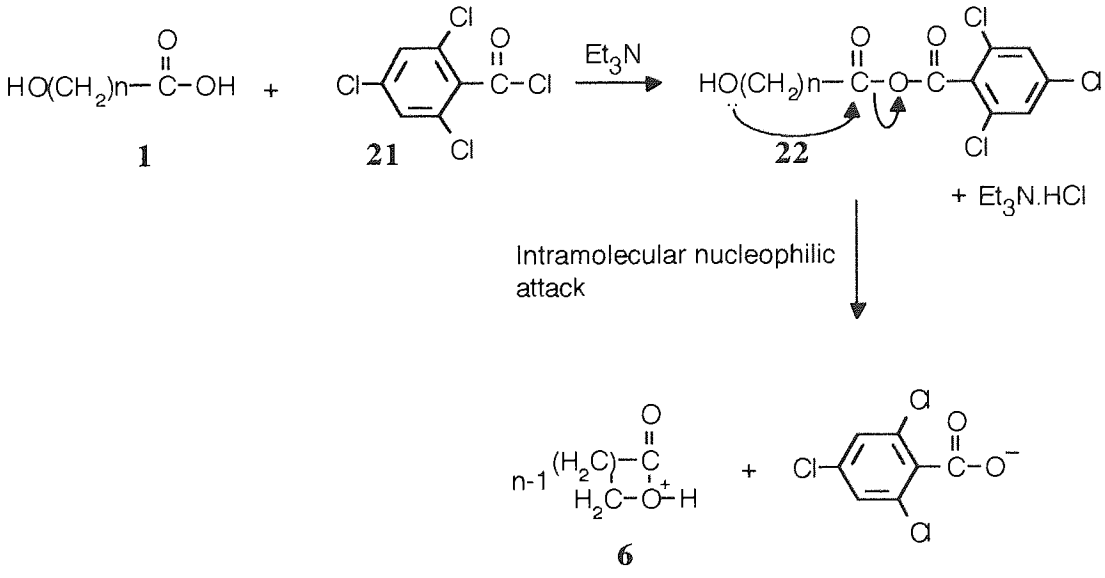


Figure 2.6 *N*-acylurea

2.3.4 Yamaguchi Method

In 1979 Yamaguchi *et al.*[46] developed a mixed anhydride (**22**), formed by reaction of 2,4,6-trichlorobenzoyl (TCB) chloride (**21**) with the desired hydroxy acid (**1**) which, in the presence of DMAP in refluxing toluene (111 °C), resulted in effective synthesis of 9-13 membered ring lactones (**6**) (**Scheme 2.5**).

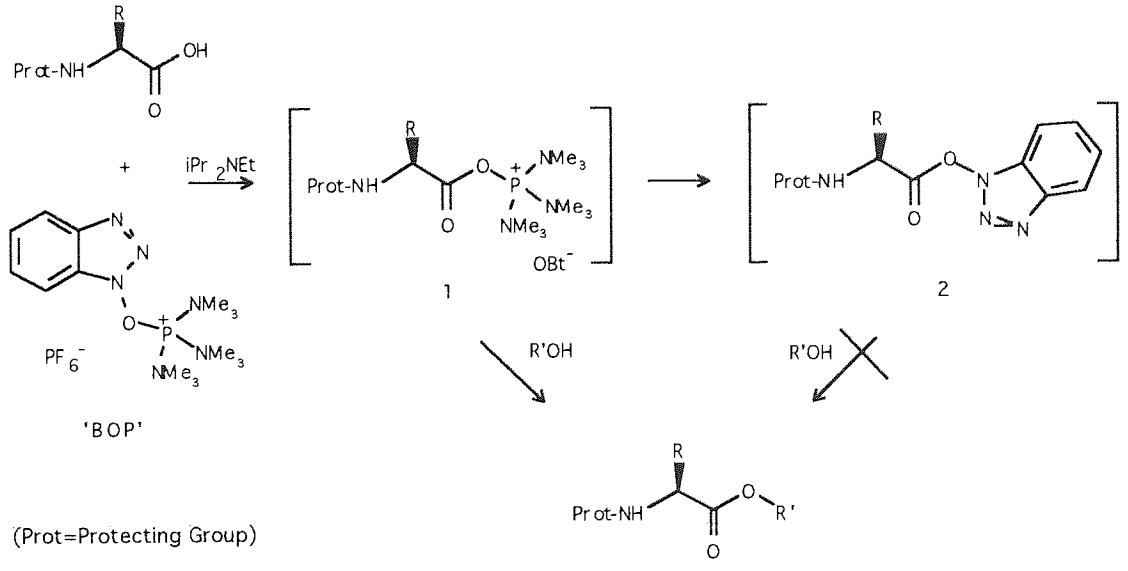


Scheme 2.5 Yamaguchi Lactonisation

Later, Yonemitsu *et al.*[47] reported that the efficiency of Yamaguchi's lactonization was highly dependent on the concentration of DMAP used. In 1990 Yonemitsu[48] described how lactonization could occur in a one pot reaction at ambient temperature, if the hydroxy acid and TCB chloride were treated with a large excess of triethylamine and a small amount of DMAP. Evans *et al.*[49] used Yomemitsu's modified conditions in 1993 to synthesise rutamycin B, a macrolide antibiotic, after the Keck, Corey and standard Yamaguchi methods had all failed.

2.3.5 BOP and PyBOP

[(Benzotriazol-1-yl)oxy]tris(dimethylamino)phosphonium hexafluorophosphate (**23**) (BOP) was first cited as a peptide coupling reagent by Castro *et al.*[50] in 1975. In 1994 Patel *et al.*[51] described the use of BOP as a reagent for the esterification of carboxylic acids, and gave an account of its mechanism (**Scheme 2.6**). According to Patel the acid reacts with BOP to generate the very reactive phosphonium intermediate (**24**), which over time transforms to the less reactive benzotriazolyl ester (**25**). Hence for ester formation to be successful, conversion of (**24**) to (**25**) needs to be slowed down, so prolonging the existence of the more electrophilic intermediate. Thus, to improve yields the reaction must be conducted at low temperatures with an excess of the alcohol.



Scheme 2.6 'BOP' Mechanism

Coste *et al.*,[52] however, have since carried out successful reactions at ambient temperature with both BOP and its analogue [(benzotriazol-1-yl)oxy]tripyrrolidophosphonium hexafluorophosphate (PyBOP) (**Figure 2.7**) casting doubt on the mechanism suggested by Patel. PyBOP was used by Griffen *et al.*[53] in the synthesis of the cyclopeptide Bacitracin A.

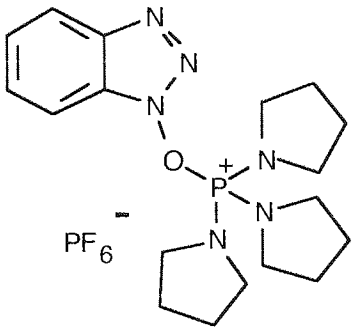


Figure 2.7 PyBOP

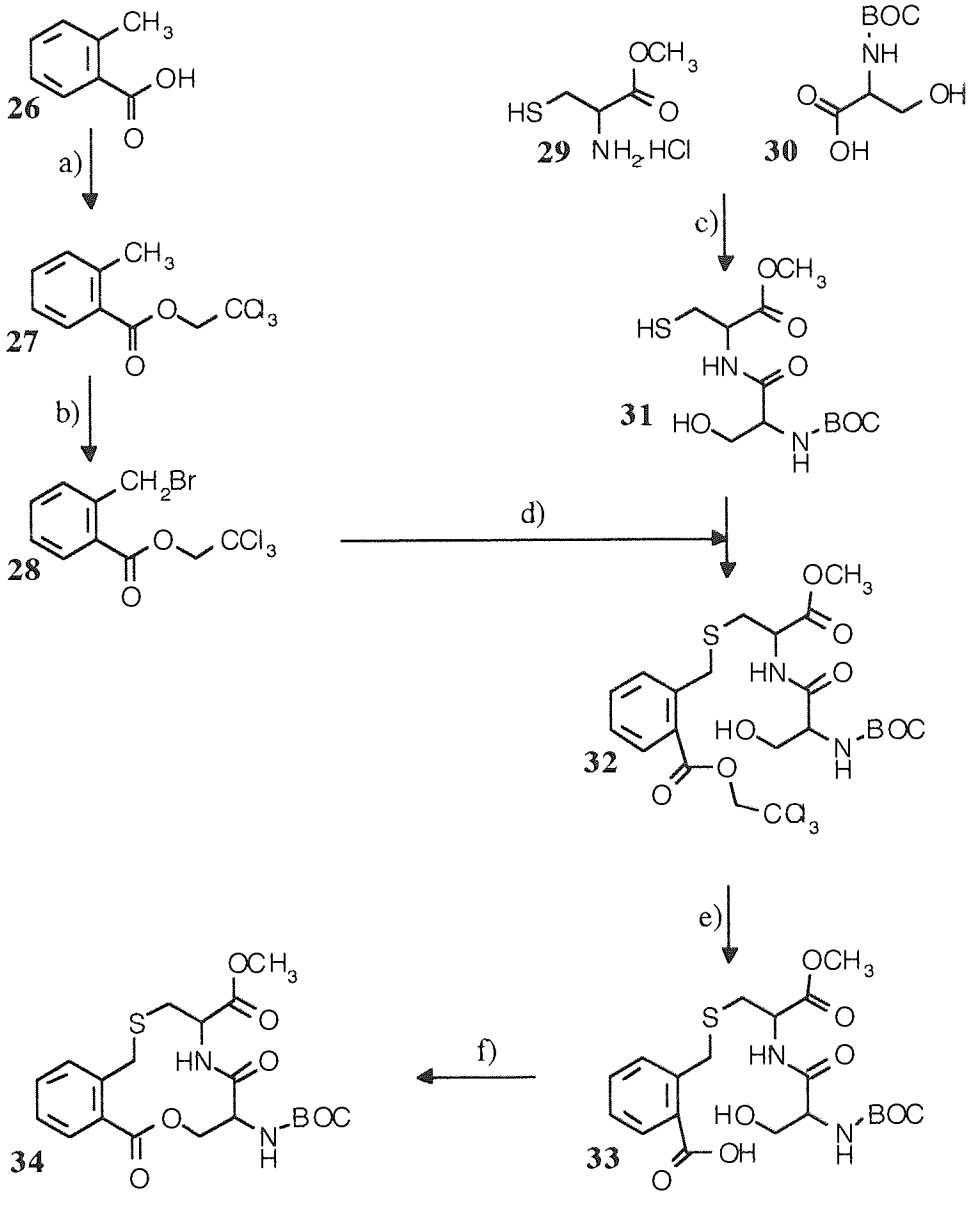
2.4 SYNTHESIS OF THE MODEL COMPOUND (SCHEME 2.7)

2.4.1 Protection of the acid

Firstly *o*-toluic acid (**26**) was converted into the reactive acid chloride derivative, by heating at reflux temperature (79 °C) with thionyl chloride for 45 minutes. Following removal of the solvent *in vacuo*, the acid chloride was then reacted, without further purification, with 2,2,2-trichloroethanol in dichloromethane in the presence of triethylamine at ambient temperature for 3 hours. Following work-up and purification by column chromatography, using ethyl acetate:hexane (1:20 v/v) as eluant, ester (**27**) was afforded as white crystals in 65 % yield.

The singlet at 4.95 ppm in the ^{1}H NMR spectrum was assigned to the methylene in the protecting group, $OC\underline{H}_2CCl_3$. ^{13}C NMR indicated new peaks at 74.3 ppm and 95.0 ppm corresponding to $O\underline{C}H_2$ and $CH_2\underline{C}Cl_3$ respectively. The FTIR absorbance at 1728 cm^{-1} showed the presence of an ester and the characteristic chlorine MS pattern,

266 (M^+, ^{35}Cl x 3, 44 %), 268 (M^+, ^{35}Cl x 2, ^{37}Cl, 41 %), 270 (M^+, ^{35}Cl, ^{37}Cl x 2, 13 %) was clearly visible.



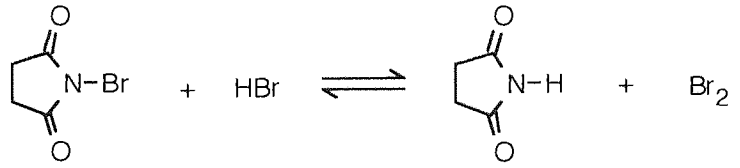
a) **i.** $SOCl_2$, reflux, 45 min; **ii.** CCl_3CH_2OH, CH_2Cl_2, Et_3N, RT, 3 h; 65%. **b**) NBS, CCl_4, reflux, hυ, 3 h; 77%. **c**) 4-methylmorpholine, DCC, acetonitrile, 0 °C, 3 h; 57%. **d**) CH_2Cl_2, Et_3N, 1 h, 0 °C; then 3 h, RT; 44%. **e**) Zn, THF, 1M H_3PO_4, 1M NaH_2PO_3, RT, 2.5 h; 61%. **f**) PyBOP, iPr_2NEt, CH_2Cl_2, 0 °C, 3.5 h; 29%.

Scheme 2.7 Synthesis of the Model Compound

2.4.2 Bromination of the Methyl Group

N-Bromosuccinimide (NBS) (**35**) is a highly selective brominating reagent which reacts with alkylbenzenes to brominate the benzylic position through a radical chain mechanism.[54,55] Reaction occurs readily at the benzylic position as the benzylic radical produced is highly stabilised by resonance. NBS may also be used to successfully halogenate olefins in the allylic position for the same reason.

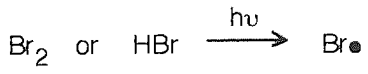
NBS effects bromination by providing a constant, though very low, concentration of Br_2, *via* a fast ionic reaction (**Scheme 2.8**). The reaction generates succinimide (**36**) as a white solid by-product which can be removed by filtration. In carbon tetrachloride, NBS sinks to the bottom of the mixture while succinimide floats, so the appearance of the insoluble by-product floating on the surface can be used as an indicator to the completion of the reaction.



35 **36**

Scheme 2.8 Bromine Formation from NBS

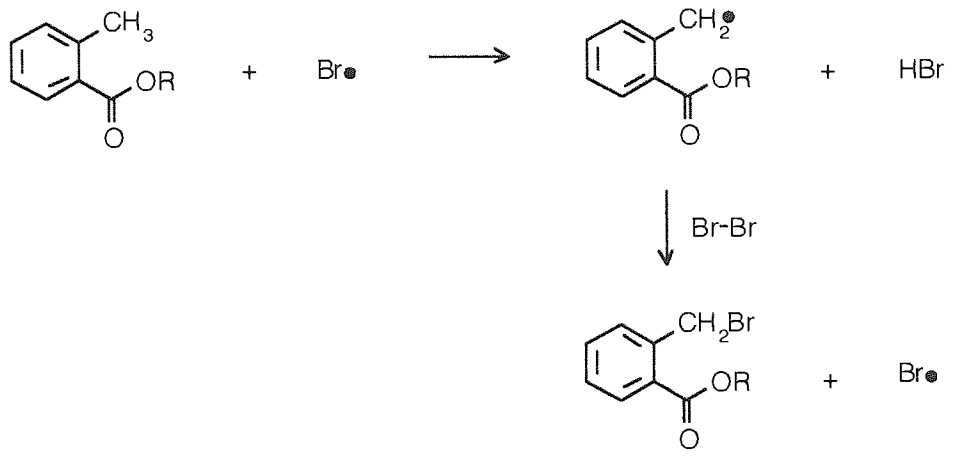
There is usually a trace of Br_2 or HBr in the NBS that can react with the initiator to generate the initial Br• to start the reaction (**Scheme 2.9**). Light irradiation may provide the source of initiation required and the radical is generated by photolytic homolysis.



Scheme 2.9 Generation of Radical

Peroxide initiators may also be used in place of UV radiation.

The mechanism of benzylic bromination (**Scheme 2.10**) involves abstraction of a benzylic hydrogen atom of the alkylbenzene to generate an intermediate benzyl radical. The stabilised radical then reacts with Br_2 to yield product and a bromine radical, which cycles back into the reaction to carry on the chain.



Scheme 2.10 Bromination of Protected *o*-Toluic acid

Ester (**27**) was brominated using NBS in refluxing carbon tetrachloride (77 °C) with light irradiation for 3 hours. 2,2,2-Trichloroethyl 2-bromomethylbenzoate (**28**) (**Scheme 2.7**) was afforded as a yellow liquid in 77 % yield.

Absence of the methyl peak at 2.64 ppm in the ^{1}H NMR and appearance of a peak at 4.99 ppm corresponding to $C\underline{H}_2Br$ indicated that bromination had occurred. A peak at 22.0 ppm in the ^{13}C NMR showed the presence of a small amount of starting material. The peak at 31.1 ppm was assigned to the newly formed $C\underline{H}_2Br$. Positive APCI MS gave the required $(M+H)^+$ ion at 345.

2.4.3 Amino Acid Coupling

Two protected amino acids, L-cysteine methyl ester hydrochloride (**29**) and *N*-(*t*-butoxycarbonyl)-L-serine (**30**) were coupled using dicyclohexylcarbodiimide (DCC) (a condensation agent) in acetonitrile at 0 °C with stirring for 3 hours, in the presence of an equimolar amount of 4-methylmorpholine (an acid trap). On purification by column chromatography, using ethyl acetate:hexane (1:1 v/v) as eluant, *N*-[*N*-(*t*-butoxycarbonyl)-L-seryl]-L-cysteine methyl ester (**31**) was afforded as a colourless oil in 57 % yield.

^{1}H NMR indicated 2 new broad NH peaks at 5.57-5.60 ppm and 7.39-7.42 ppm. Peaks at 52.9 and 53.7 ppm in the ^{13}C NMR were assigned to the methyne groups adjacent to the 2 NH's. Positive APCI MS showed an $(M+H)^+$ ion at m/z 323 as required.

DCC, already mentioned in **Section 2.3.3**, is a widely used condensation agent for the coupling of amino acids. An alternative reagent, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride,[56] also known as 'water soluble carbodiimide' (WSCDI) (**Figure 2.8**) may be used. WSCDI produces a water soluble urea as a by-product which is easier to remove than DHU.

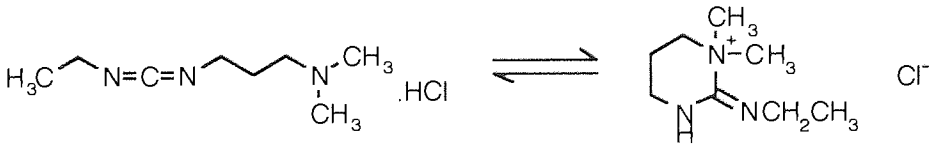


Figure 2.8 WSCDI. HCl

2.4.4 Reaction of the Two Major Components, (28) and (31)

Reaction of 2,2,2-trichloroethyl 2-bromomethylbenzoate (**28**) with *N*-[*N*-(*t*-butoxycarbonyl)-L-seryl]-L-cysteine methyl ester (**31**) in dichloromethane in the presence of triethylamine at 0 °C afforded, after column chromatography 2,2,2-trichloroethyl methyl *N*-[*N*-(*tert*-butoxycarbonyl)-L-seryl]-*S*-{2-[(2,2,2)-trichloroethyl)carbonyl] benzyl}-L-cysteinate (**32**) as a yellow oil, in 44% yield.

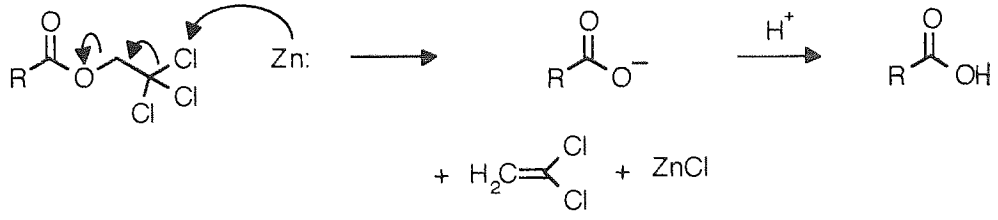
Reaction between bromomethyl derivative (**28**) and peptide (**31**) is confirmed by disappearances of the $C\underline{H}_2Br$ singlet at 4.99 ppm and the broad SH peak at 4.14-4.21 ppm in the ^1H NMR, and the appearance of a multiplet at 4.22-4.27 ppm corresponding to Ar-$C\underline{H}_2S$. The ^{13}C NMR has a peak at 34.8 ppm corresponding to the $\underline{C}H_2S$ in the product; this is downfield of the $\underline{C}H_2Br$ in the starting material which is at 31.1 ppm. The expected m/z 587 parent ion is seen in negative electrospray MS.

2.4.5 Deprotection

Deprotection of the ester group was carried out using zinc metal powder in a buffered solution of 1M phosphoric acid and 1M aqueous sodium dihydrogen phosphate solution, to avoid disturbance of the acid-sensitive *tert*-butoxycarbonyl group. The deprotected derivative (**33**) was afforded after column chromatography, as a white oil that solidified on standing, in 61% yield.

The absence of peaks at 4.97 ppm and 74.6 ppm in ^1H and ^{13}C NMR respectively, confirm successful deprotection. Positive APCI MS shows the sodium adduct, $[M+Na]^+$, at m/z 479.

Deprotection occurs by attack of the zinc metal lone pair on one chlorine atom, which results in the elimination of dichloroethene and re-formation of the carboxylic acid (**Scheme 2.11**).



Scheme 2.11 Ester Deprotection by Zinc Metal

2.4.6 Cyclisation

Cyclisation of the deprotected derivative (**33**) was attempted using several methods.

Firstly the method of Corey *et al.*[36] was attempted, as used by Arisawa *et al.*[14] 2,2-Dithio(4-*t*-butyl)-1-isopropylimidazole and triphenylphosphine were added to (**33**) in anhydrous toluene at 0 °C. This mixture was cooled to at 0 °C and added over 1 hour 15 minutes to refluxing toluene. After heating at reflux temperature for a further 3 hours only decomposed starting material was recovered.

The Yamaguchi method: addition of 2,4,6-trichlorobenzoyl chloride, triethylamine and DMAP to a solution of (**33**) in dichloromethane at ambient temperature followed by stirring for 2 hours was then attempted. TLC indicated a mixture of products, including a small amount of the desired lactone and subsequent isolation by column chromatography resulted in a poor yield.

Following the disappointing results of these two methods, cyclisation was attempted using PyBOP. To a solution of (**33**) in dichloromethane at 0 °C was added sequentially *N*,*N*-diisopropylethylamine and PyBOP, and the resulting mixture stirred at 0 °C for 4 hours. Following work-up and purification by column chromatography, using ethyl acetate:hexane (3:1 v/v) as eluant, the desired lactone (**34**) was afforded in 29 % yield as white crystals with a melting point 206-207 °C.

2.5 CONCLUSION

The model compound (**34**) (**Figure 2.9**) was successfully synthesised from commercially available *o*-toluic acid *via* a six step convergent procedure. Protection of the carboxylic acid as a 2,2,2-trichloroethyl ester and bromination of the ring methyl with NBS afforded the desired aromatic portion. Subsequent reaction with a synthesised dipeptide followed by deprotection and cyclisation using PyBOP yielded the target compound.

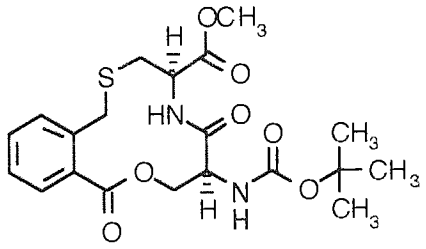


Figure 2.9 The Model Compound

In late 1996 a team from Hoffmann La-Roche in Switzerland produced a paper detailing the synthesis of the natural product, cyclothialidine. Methods were comparable except for the minor detail of using 4-nitrobenzyl bromide as the carboxylic acid protecting group instead of 2,2,2-trichloroethanol. Cyclisation was performed using 2,2'-dithiobispyridine (Corey's Double-Activation Method) and DEAD (Mitsunobu Reaction) with yields of 55 % and 73 % respectively.

The strategy for the total synthesis of cyclothialidine described by Hoffmann La-Roche was almost identical to our planned route, which was not realised ahead of them due to the fact I was working single-handed and in a less competitive environment. This kind of race occurs in any research field and on any interesting research project. Though it was disappointing that we did not get there first, the quality of the project and the significance of the work was reflected. As a result our research priority turned to the design and synthesis of analogues of cyclothialidine.

Chapter 3:
Cyclothialidine Analogues

3.1 SYNTHESIS OF THE PHARMACOPHORE

Once work to synthesise the model compound had been successfully completed, attention was turned to the pharmacophore (**Figure 3.1**), which contained the substituted aromatic ring essential for DNA gyrase inhibition.[35] R groups, consisting of peptide chains in the natural product cyclothialidine, could be added following cyclisation of the lactone.

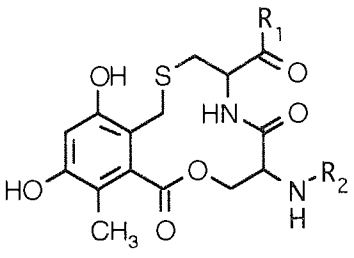


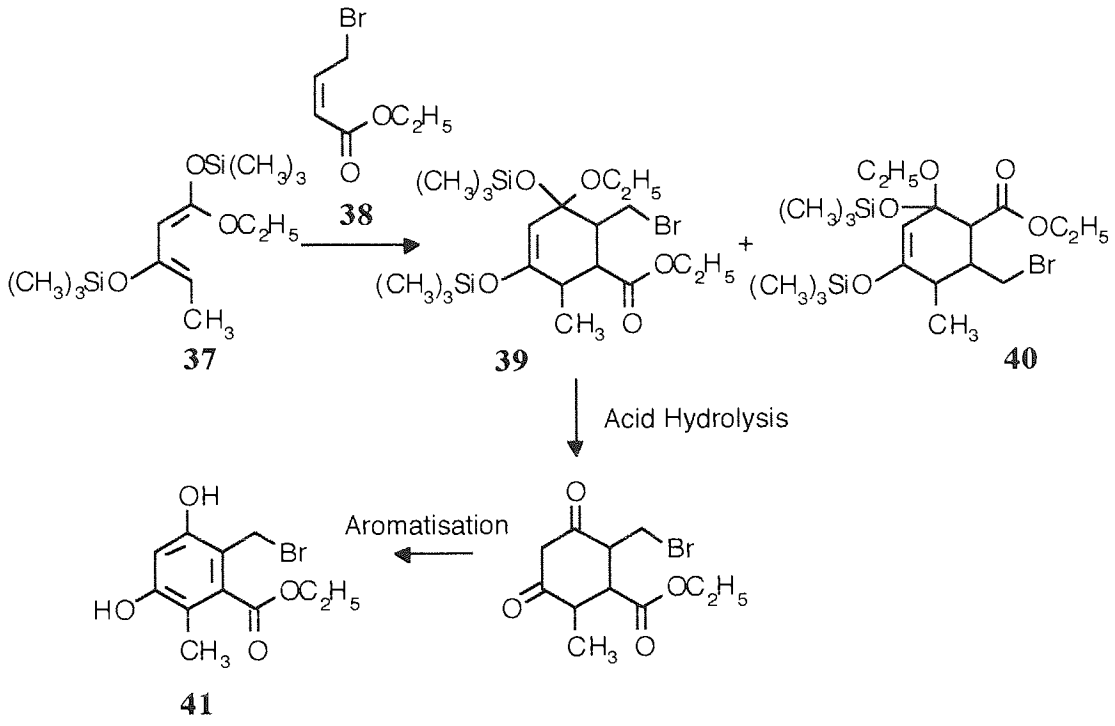
Figure 3.1 The Pharmacophore

Prior to the total synthesis of cyclothialidine being published by Hoffmann-La Roche, work on the project had long been involved in finding a successful synthesis for the aromatic moiety.

3.2 SYNTHESIS OF THE AROMATIC MOIETY

3.2.1 Attempted Synthesis *via* a Diels-Alder Reaction

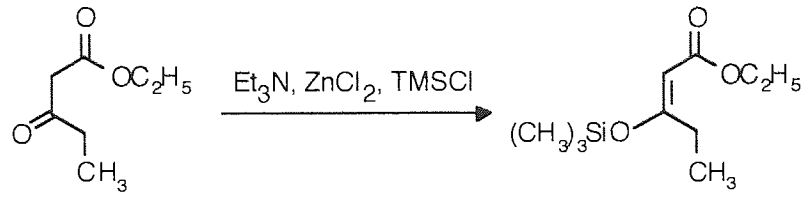
The proposed method was to synthesise a suitable diene (**37**) which when reacted with a commercially available dienophile such as ethyl 4-bromocrotonate (**38**), would produce a 6-membered ring containing intermediate **39** *via* a Diels-Alder reaction. It was hoped that though the bromine atom is fairly large, steric effects would favour synthesis of desired **39** over regioisomer **40**. Following separation of the regioisomers by column chromatography, acid hydrolysis and subsequent aromatisation was expected to result in the target substituted aromatic moiety (**41**) (**Scheme 3.1**).



Scheme 3.1 Planned Synthesis of the Aromatic Moiety (**41**) *via* a Diels-Alder Reaction

It was decided to produce the required diene by two subsequent silylation reactions.

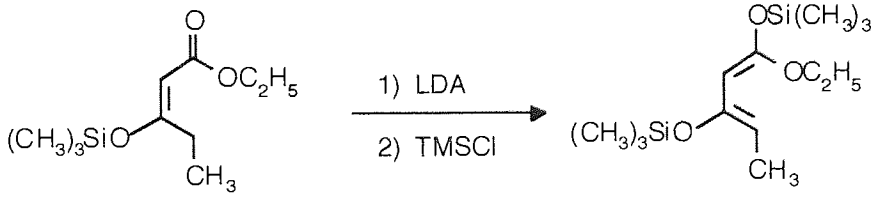
The first silylation step was carried out according to the conventional method as published by Danishefsky and Kitahara.[57] Anhydrous powdered zinc chloride was added to triethylamine and the resulting mixture stirred for 30 minutes at ambient temperature until the salt was suspended in the amine; triethylamine serving as an acid trap to 'mop up' the HCl liberated during the reaction. To this was added a solution of ethyl propionylacetate (**42**) in toluene followed by trimethylsilylchloride (TMSCl). After 1 hour the temperature was allowed to rise to 40 °C and stirring continued overnight. Work-up afforded the desired silylated derivative (**43**) as an orange liquid in 97% yield (**Scheme 3.2**), ^{1}H NMR clearly showing an olefinic proton at 4.97 ppm.



42 **43**

Scheme 3.2 Silylation of Ethyl Propionylacetate (ethyl 3-oxopentanoate)

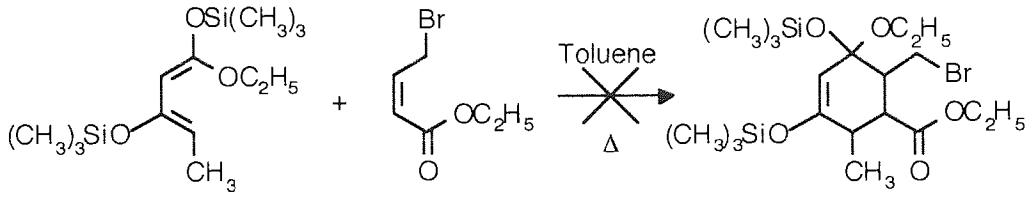
The second silylation step[58] was performed by adding a 2M solution of lithium diisopropyl amide (LDA) to silyl ether (**43**) (**Scheme 3.3**) at -78 °C and allowing the mixture to stir for 30 minutes prior to the addition of trimethylsilylchloride. The mixture was warmed to ambient temperature and stirring continued for a further 1.5 hours before work-up. ^{1}H NMR and TLC analysis of the resulting yellow liquid showed it to be a mixture of product and starting material. Purification using a Kugelrohr distillation apparatus gave diene (**37**) as a very pale yellow liquid. The product, however, was found to be highly moisture sensitive and on exposure to air readily decomposed to give the starting material, ethyl propionylacetate. During subsequent preparations stringent measures were used to ensure moisture free conditions and the compound was used immediately in the next step.



43 **37**

Scheme 3.3 Synthesis of the Diene

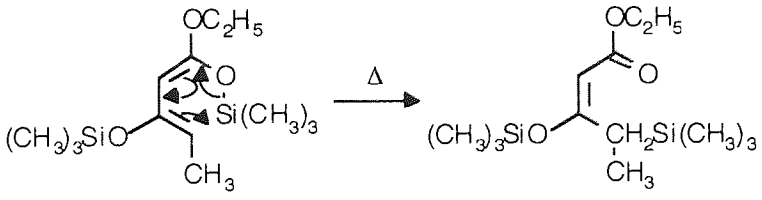
Diene (**37**) was reacted with ethyl 4-bromocrotonate (**38**) in a Diels-Alder reaction in an attempt to form six membered ring (**39**) (**Scheme 3.4**). The reaction was carried out according to the conditions of Yamamoto *et al.*[59] and all apparatus was dried in a oven overnight prior to use. To a stirred solution of freshly distilled diene (**37**) under argon in anhydrous toluene was added **38** at ambient temperature. The resulting solution was stirred at reflux temperature overnight and on cooling was hydrolysed with 0.1M HCl to yield a brown/orange liquid. Four fractions were separated by column chromatography but none contained the desired product with mainly **38** being recovered.



37 **38** **39**

Scheme 3.4 Attempted Diels-Alder Reaction

It was thought that since the reaction had been carried out at 111 °C diene (**37**) may have rearranged in the manner described by Cameron *et al.*[60,61,62] to give an α-β unsaturated ester (**44**) (**Scheme 3.5**). If this rearrangement of silicon from oxygen to carbon occurred the product would be unable to react with the dienophile to produce the required Diels-Alder adduct.

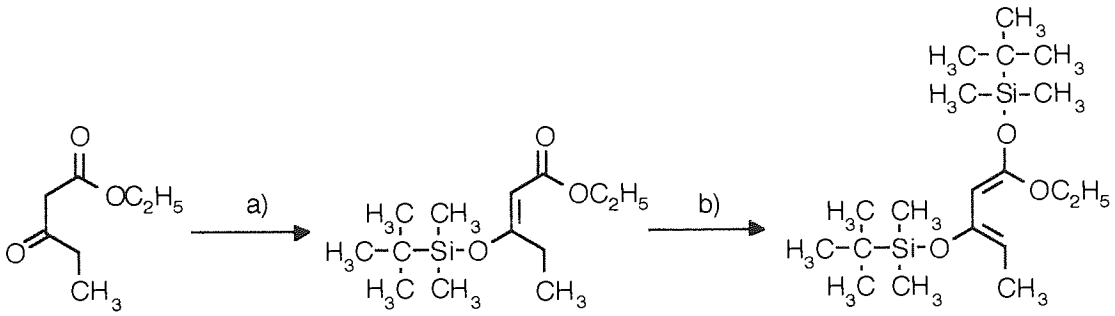


37 **44**

Scheme 3.5 1,5-Migration of Silicon to Carbon

As a result, the reaction was repeated using the conditions of Danishefsky and Kitahara.[57] This time ethyl-4-bromocrotonate (**38**) was added in neat form to a stirred solution of freshly distilled diene at ambient temperature and the resulting mixture stirred at 40 °C for 4 days. Work-up again yielded a complex mixture of products.

In an attempt to improve the stability of the diene, silylation was carried out using *tert*-butyldimethylsilyl chloride (TBDMSCl) (**Scheme 3.6**) instead of TMSCl. It was hoped that the bulky TBDMS groups would sterically hinder attack on silicon, so producing a more stable diene.



42 **45** **46**

a) **i.** ZnCl, TEA, 2 h, RT; **ii.** TBDMSCl, PhMe, 40 °C, 18 h. **b**) **i.** LDA, -78 °C, 30 min; **ii.** TBDMSCl, RT, 18 h.

Scheme 3.6 Synthesis using TBDMSCl

^{1}H NMR data for silyl ether (**45**) clearly showed singlets at 0.18 and 0.89 ppm corresponding to Si($C\underline{H}_3$)$_2$ and C($C\underline{H}_3$)$_3$ respectively. The olefinic proton could be seen at 4.99 ppm. The ^{1}H NMR for disilylated intermediate (**46**) indicated 2 additional peaks at 0.09 and 0.86 ppm, representing the second silyl group, though the reaction had not gone to completion and some mono-silylated product remained.

The Diels-Alder reaction was repeated with the TBDMS diene but yet again failed to produce any product. With purity of the diene still in question and the search for

suitable conditions proving fruitless, the method was abandoned and other approaches adopted.

3.2.2 Attempted Synthesis *via* Aromatic Sulphonation / Alkaline Fusion

It appeared sensible to begin with a commercially available substituted aromatic ring and attempt to attach the remaining groups by electrophilic substitution. Since *o*-toluic acid was in plentiful supply it was decided to add the required hydroxyl groups to this compound with the intention of repeating the reaction with the desired, but more expensive, 2,6-dimethylbenzoic acid, once conditions had been established.

Since methyl groups are activating and *ortho/para* directing and carboxylic acids are deactivating and *meta* directing, substitution was most likely to occur in the 3 and 5 positions (**Figure 3.2**). It was hypothesised the 5 position may the favoured due to the slight steric effect of the methyl group on the 3 position. In reality this was thought unimportant as it was hoped di-substitution could eventually be effected.

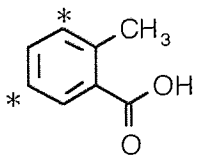
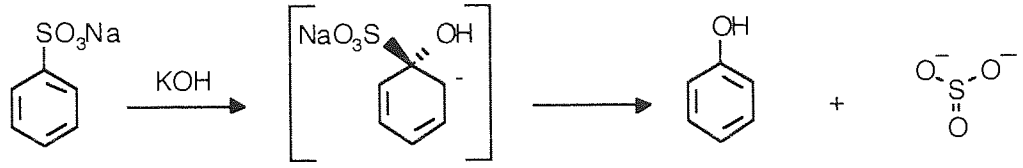


Figure 3.2 Expected Sites of Substitution

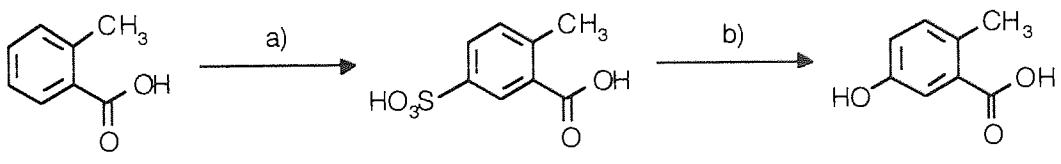
Introduction of the hydroxyl group into the ring *via* successive aromatic sulphonation and alkaline fusion reactions was the chosen method. Alkaline fusion of benzenesulphonic acid was first effected in the 1860's. Since that time it has been extensively used for the manufacture of phenol and related hydroxy compounds. Though modern methods now prevail, it is still considered one of the best methods of introducing a hydroxyl group into an aromatic ring, despite the harsh conditions required.

In 1966, Oae *et al.*[63] identified the mechanism to be one of simple nucleophilic substitution (**Scheme 3.7**), a view which remains controversial, although formation of a benzyne intermediate was clearly ruled out by isotopic labelling experiments.



Scheme 3.7 Mechanism of Alkaline Fusion

A mixture of *o*-toluic acid (**26**) and concentrated sulphuric acid (**Scheme 3.8**) was heated at 160 °C for 2.5 hours,[64] allowed to cool, then water added and the reaction allowed to stand at ambient temperature overnight. The resulting solid was recrystallised from water and saturated sodium chloride solution to afford off-white crystals. The sulphonated product (**47**) was dissolved in saturated sodium hydroxide solution, heated to 100 °C and mixed with powdered sodium hydroxide into a paste which solidified on cooling. Small pieces of this product were then added, in portions, to potassium hydroxide pellets at 180-200 °C and the resulting mixture stirred at this temperature for 4 hours. On cooling, the solid was dissolved in water and acidified with concentrated hydrochloric acid. The resulting precipitate was recrystallised from water to yield 5-hydroxy-2-methylbenzoic acid (**48**) as white needles in 70 % yield.



26 **47** **48**

a) **i.** c. H_2SO_4, 160 °C; **ii.** H_2O, sat. NaCl, 100 °C. **b)** c. NaOH, KOH, 180-200 °C.

Scheme 3.8 Synthesis of 5-Hydroxy-2-methylbenzoic Acid

^1H NMR for sulphonate (**47**) showed only 3 aromatic protons, indicating that a new substituent had been added onto the ring - information mirrored by ^{13}C NMR. Negative APCI MS gave the required $[M-H]^-$ ion at m/z 215. Following alkaline fusion, aromatic peaks in the ^1H NMR shifted upfield and in ^{13}C NMR the aromatic carbon atom attached to the newly substituted group shifted from 139.7 ppm in the sulphonate to 155.1 ppm in product (**48**). DIP MS recorded the parent ion at m/z 152.

Despite increasing the reaction time of the sulphonation reaction to 6 hours and raising the temperature, introduction of a further substituent failed. This was to be expected since the $-SO_3H$ is strongly deactivating; effectively preventing further electrophilic substitution. There is evidence[65] that di-sulphonic acids, if they form at all, do so in low yield and are very unstable.

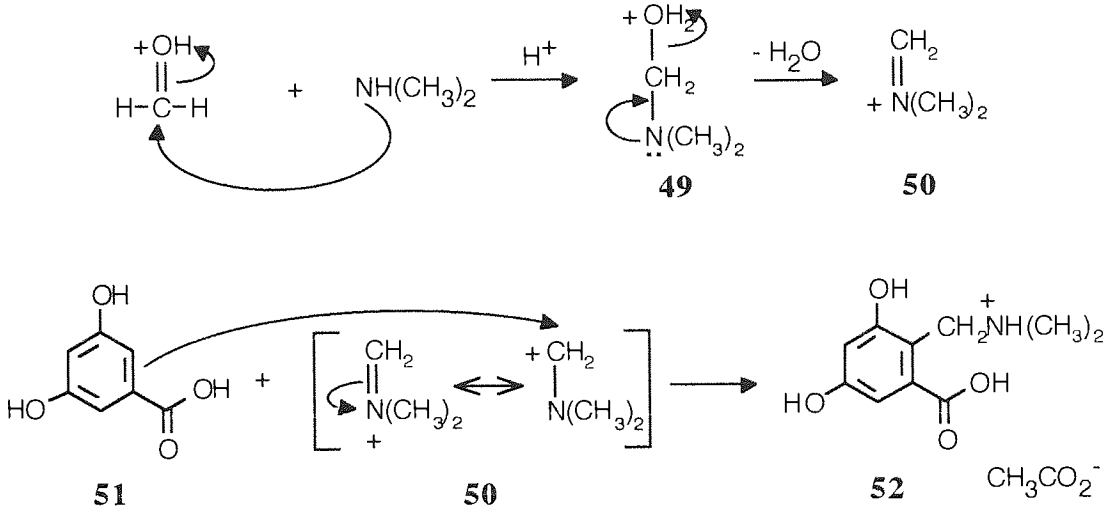
It was hoped that conversion of the $-SO_3H$ group to -OH would help to activate the ring to further sulphonation but reaction of the product with additional sulphuric acid had no effect. Even if it had been successful, however, the strongly ortho/para directing -OH would have directed further substitution to positions 4 and 6, rather than the desired 3 position.

The reaction was repeated using 2,6-dimethylbenzoic acid, in an effort to synthesise an intermediate with greater similarity to the target aromatic compound. Sulphonation occurred successfully but in very poor yield, so the method was not considered viable.

3.2.3 Synthesis *via* Two Successive Mannich Reactions

Attention turned to an alternative procedure of introducing the required methyl groups onto an aromatic ring with the hydroxyl groups already present. The activating nature of the hydroxyl groups was expected to ensure efficient electrophilic aromatic substitution. A Mannich reaction followed by reduction of the resulting Mannich base, was thought to be a promising choice.

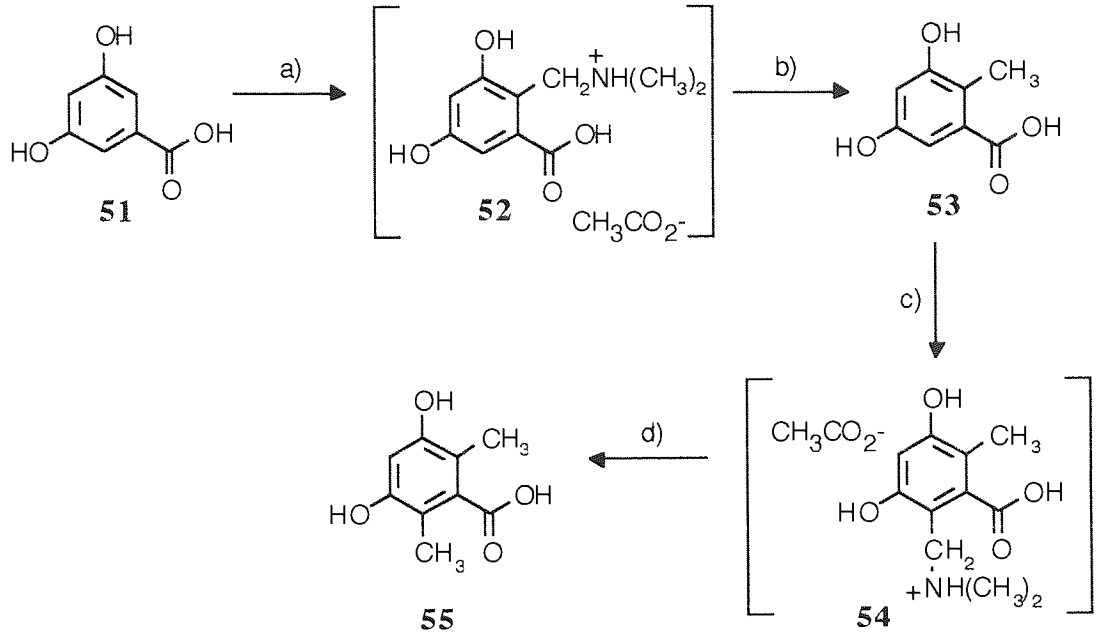
In the acid catalysed Mannich reaction (**Scheme 3.9**), an aldehyde (usually formaldehyde) is condensed with an amine (*eg.* dimethylamine) to produce an intermediate such as (**49**) which loses water to form iminium ion (**50**). A nucleophilic substitution reaction on **50** by a compound containing an active hydrogen, such as 3,5-dihydroxybenzoic acid (**51**), affords a 'Mannich base' (**52**).



Scheme 3.9 Mechanism of the Mannich Reaction

In each case the $-CH_2NMe_2$ group is found to go *ortho* to the $-CO_2H$ group rather than between the two hydroxyls. It may be hypothesised that the $-CO_2H$ acts to deliver the electrophile by neighbouring group participation.

Aqueous dimethylamine was added dropwise, with cooling, to a stirred mixture of aqueous formaldehyde, ethanol and glacial acetic acid (**Scheme 3.10**). Stirring was continued for 30 minutes, whereupon the mixture was cooled to 10 °C and **51** added. After stirring overnight at ambient temperature the resulting white precipitate was isolated by filtration to afford the desired salt (**52**) in good yield.



a) aq. CH_2O, aq. $NH(CH_3)_2$, AcOH, RT, 18 h; **b**) Pd/C, H_2, RT, 3 days; **c**) aq. CH_2O, aq. $NH(CH_3)_2$, AcOH, RT, 18 h; **d**) Pd/C, H_2, RT, 3 days.

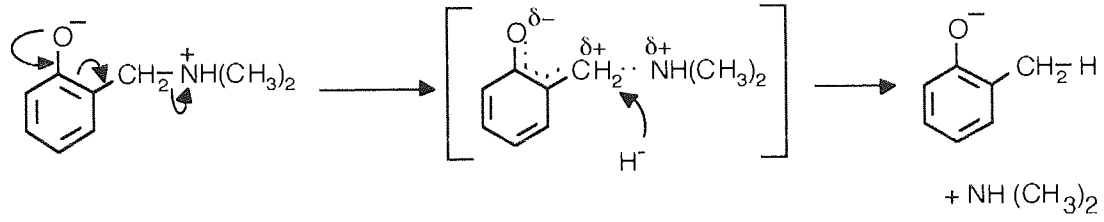
Scheme 3.10 Synthesis of 3,5-Dihydroxy-2,6-dimethylbenzoic Acid

^1H NMR of acetate salt (**52**) showed a singlet at 2.53 ppm, corresponding to the methyl protons in $N(C\underline{H}_3)_2$ and a singlet at 3.91 ppm for the methylene protons. Doublets at 6.34-6.35 ppm and 6.64-6.65 ppm were assigned to the 2 aromatic protons. The carboxylic acid proton appeared as a broad peak at 9.55 ppm. In the ^{13}C NMR, $N(C\underline{H}_3)_2$ appeared at 40.3 ppm and CH_2N at 52.3 ppm. Positive electrospray clearly gave the parent ion at m/z 212.

Yamada *et al.*[66] used sodium cyanoborohydride in hexamethylphosphoramide (HMPA) to successfully reduce quaternary ammonium salts of Mannich bases to give the corresponding methyl compounds in good yields, while Paquette and Farley[67] used lithium aluminium hydride. Both of these methods of reduction however would also reduce the carboxylic acid moiety and so are unsuitable in this case.

In order to preserve sensitive groups it was decided to perform the reduction by hydrogenation. To ensure good yields, thorough mixing of the salt, catalyst and hydrogen gas was vital. The mixture was saturated with hydrogen *via* a balloon, and a large flask was used to contain a small volume of methanol, ensuring adequate space for mixing was available. Vigorously shaking the flask on a mechanical stirrer was essential - stirring gave poor results. Surprisingly, carrying out the reaction under pressure did little to increase yields.

Under basic conditions[68] (**Scheme 3.11**), the phenolic proton is abstracted and the resulting δ+ carbon atom readily attacked by H^- to produce a methyl group and liberating the amine.



Scheme 3.11 Mechanism of Phenolic Mannich Base Reduction

Treatment of a suspension of acetate salt (**52**) in methanol with hydrogen and palladium/carbon catalyst yielded 3,5-dihydroxy-2-methylbenzoic acid (**53**) as an orange powder. The new methyl group appeared as a singlet at 2.14 ppm in ^{1}H NMR and at 17.2 ppm in the ^{13}C NMR of **53**. Negative APCI MS showed the parent ion at m/z 167.

Subsequent reaction of **53** under further Mannich/hydrogenation conditions afforded the desired 3,5-dihydroxy-2,6-dimethylbenzoic acid (**55**) as an orange solid. In 1950, Reeve and Sadle[69] documented the synthesis of a di-Mannich base in 'one pot' using harsher conditions but it was considered adequate to perform the Mannich reaction twice under more gentle conditions.

^{1}H NMR of acetate salt (**54**) showed a singlet at 2.53 ppm which corresponded to the methyl protons in N($C\underline{H}_3$)$_2$ and a singlet at 3.80 ppm attributable to the methylene protons. Peaks at 42.0 ppm and 53.8 ppm in the ^{13}H NMR could be assigned to the N($\underline{C}H_3$)$_2$ and $\underline{C}H_2$ carbons respectively. The aromatic proton appeared as a singlet at 6.31 ppm in the ^{1}H NMR and the carbon atom attached to it at 100.5 ppm in the ^{13}C NMR spectrum. An m/z 226 parent ion was seen in positive APCI MS.

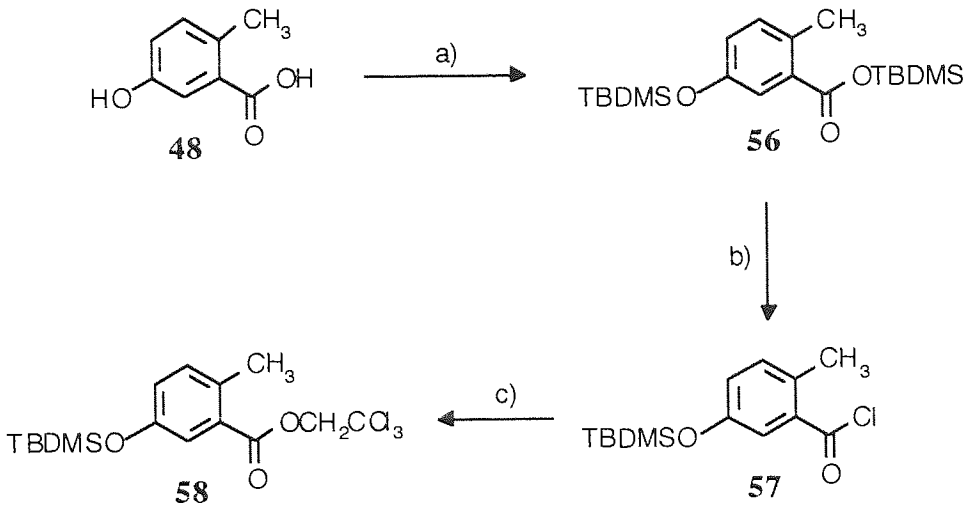
^{1}H NMR for the symmetrical substituted aromatic (**55**) indicated a singlet at 1.91 ppm corresponding to the 6 methyl protons and a peak at 12.3 ppm in the ^{13}C NMR for the two $\underline{C}H_3$ carbons. Negative APCI confirmed the parent ion as m/z 181.

3.3 SYNTHESIS OF THE 12-MEMBERED RING

Once the aromatic moiety 3,5-dihydroxy-2,6-dimethylbenzoic acid had finally been synthesised, it was necessary to investigate methods of hydroxyl and carboxylic acid group protection before 12-membered ring formation could be attempted (**Scheme 3.12, p. 71**).

3.3.1 Carboxylic Acid Protection

Esterification of a carboxylic acid cannot usually be performed directly, but requires activation, often *via* the more reactive acyl chloride. In a series of test reactions (**Scheme 3.12**) the acid and hydroxyl functionalities of 5-hydroxy-2-methylbenzoic acid (**48**) were initially protected with *tert*-butyldimethylsilyl chloride (TBDMSCl) to yield the disilylated adduct (**56**). Reaction of **56** with oxalyl chloride selectively removed the TBDMS group from the carboxylic acid affording acyl chloride (**57**). Oxalyl chloride was used as an alternative to thionyl chloride in order to preserve the highly acid labile TBDMS ether. Reaction with 2,2,2-trichloroethanol, under the usual conditions, yielded protected derivative (**58**).



a) TEA, DMAP, TBDMSCl, DCM, -78 °C, 30 min then RT, 18 h; **b)** oxalyl chloride, DMF, DCM, 30 min, 0 °C then RT, 18 h; **c)** 2,2,2-trichloroethanol, TEA, DCM, 30 min, 0 °C then RT, 18 h.

Scheme 3.12 Synthesis of the Protected Toluic Acid Derivative

This route, however, was not efficient as it wasted considerable amounts of TBDMSCl, a relatively expensive reagent. As a result, instead of protecting the carboxylic acid as a 2,2,2-trichloroethyl ester, as was the case with the model compound, attention turned to 4-nitrobenzyl bromide, a fairly cheap and commercially available reagent. Esters of 4-nitrobenzyl bromide can be readily reduced to the carboxylic acid by hydrogenation with a palladium on carbon catalyst.

1,1,4,4-Tetramethylguanidine, a strong base, was added at 0 °C to a solution of 3,5-dihydroxy-2,6-dimethylbenzoic acid (**55**) in DMF (**Scheme 3.13**). After stirring for 15 minutes at ambient temperature a pink precipitate formed, as the acid was converted into it's anion. 4-Nitrobenzyl bromide was added and stirring continued at ambient temperature overnight. Following work-up and purification, ester (**59**) was afforded as a bright yellow solid.

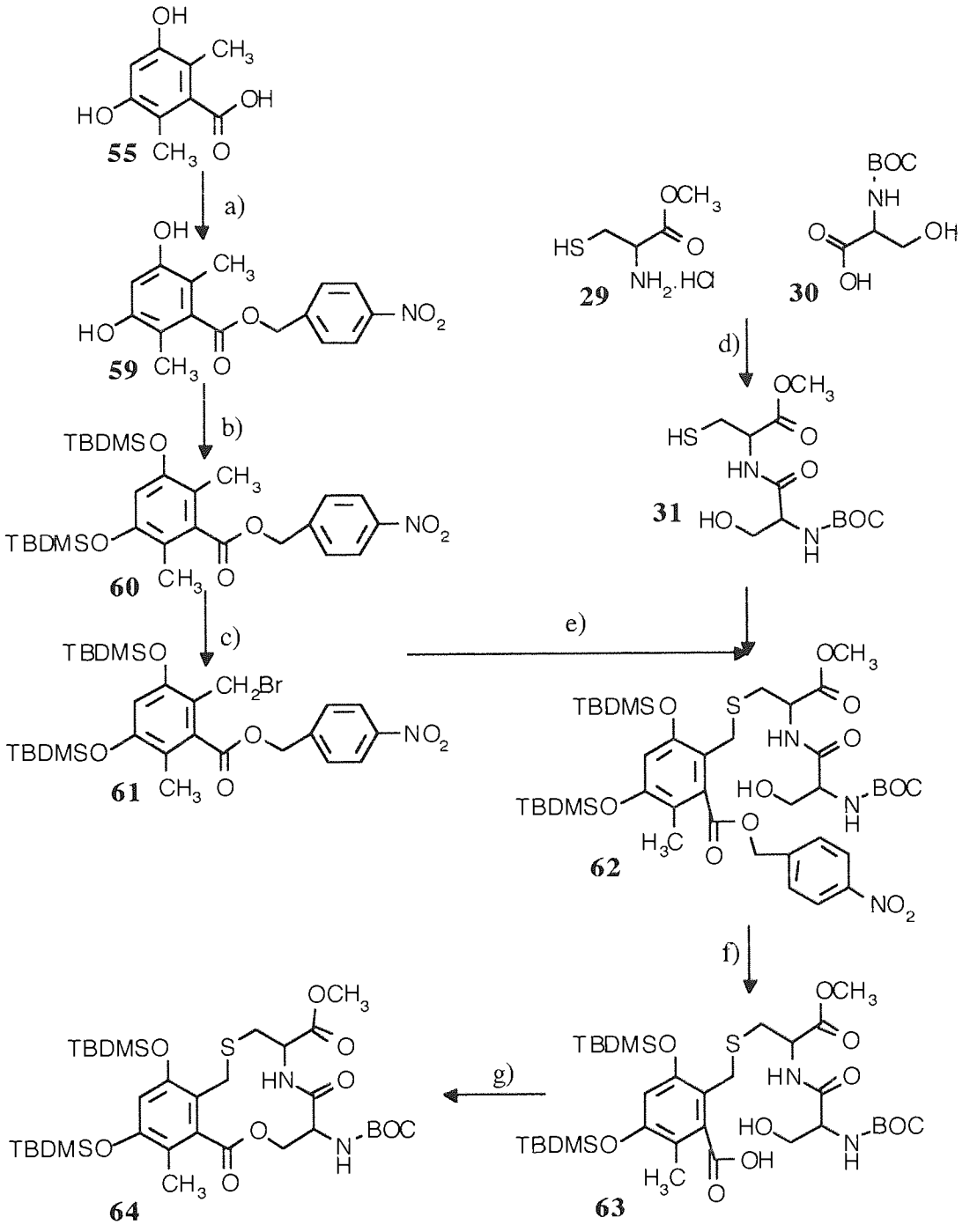
The protecting group could clearly be seen in the ^{1}H NMR spectrum; the $OC\underline{H}_2$ group was observed as a singlet at 5.44 ppm, while doublets at 7.68-7.71 ppm and 8.24-8.27 ppm corresponded to the aromatic protons in a 1,4-disubstituted benzene ring. ^{13}C NMR showed the $O\underline{C}H_2$ at 65.2 ppm, while quaternary carbons within 4-nitrobenzyl bromide appeared at 143.6 and 147.3 ppm. Aromatic CH's in the protecting group could be seen at 123.8 and 129.4 ppm. DIP MS indicated the parent ion at m/z 317.

3.3.2 Hydroxyl Group Protection

The decision was taken to protect the hydroxyl groups as *tert*-butyldimethyl silyl (TBDMS) ethers due to their ease of synthesis and deprotection. While more expensive than trimethylsilyl (TMS) chloride, *tert*-butyldimethylsilyl chloride was used in preference to the former due to the enhanced stability of the resulting ethers. The bulky *tert*-butyl groups sterically hinders attack on silicon thereby greatly reducing the risk of accidental deprotection during washing and purification stages.

Triethylamine was added to a stirred mixture of 4-nitrobenzyl 3,5-dihydroxy-2,6-dimethylbenzoate (**59**) and TBDMSCl in DMF at 0 °C. Triethylamine hydrochloride began to form as a precipitate almost immediately. The mixture was stirred at 0 °C for 4 hours before work-up and purification yielded the fully protected intermediate (**60**) (**Scheme 3.13**), as a pale yellow solid, in 72 % yield.

The $Si(C\underline{H}_3)_2$ group was assigned to the peaks at 0.18 ppm in the ^{1}H NMR and the $(C\underline{H}_3)_3$ to those at 0.98 ppm. The ^{13}C NMR was assigned as follows: $Si(\underline{C}H_3)_2$ at -4.3, $C(\underline{C}H_3)_3$ at 13.0 ppm and the quaternary carbon, $C(\underline{C}H_3)_3$ at 18.1 ppm. Positive APCI gave a parent ion at m/z 546.



a) 1,1,4,4-tetramethylguanidine, 4-nitrobenzyl bromide, DMF, RT, 18 h; 36 %. **b)** TBDMSCl, Et_3N, DMF, 0 °C, 4 h; 72 %. **c)** NBS, CCl_4, reflux, hυ, 2 h; 94 %. **d)** 4-methylmorpholine, DCC, ACN, 0 °C, 3 h; 57 %. **e)** CH_2Cl_2, Et_3N, 2 h, 0 °C; then 18 h, RT; 21 %. **f)** H_2, Pd/C, EtOAc, 4 days, RT; 68 %. **g)** DEAD, Ph_3P, 0 °C, 15 min; then 5.5 h, RT; 15%.

Scheme 3.13 Synthesis of the Pharmacophore

3.3.3 Bromination

Bromination of one of the methyl groups was performed using the conditions identified using the model compound, *ie.* *N*-bromosuccinimide (NBS) in refluxing carbon tetrachloride *via* a radical reaction. Addition of only 1.2 equivalents of NBS to the symmetrical intermediate led to predominantly mono-bromination, with only a minor amount of the di-brominated product being formed. The resulting yellow solid (**61**) was used in the next step without purification.

The main indications in ^{1}H NMR were a new $C\underline{H}_2Br$ singlet at 4.49 ppm and peaks for the silyl protecting groups which changed from singlets in **60** to two singlets at 0.18-0.28 ppm for $Si(C\underline{H}_3)_2$ and two singlets at 0.98-1.02 ppm for $(C\underline{H}_3)_3$. This occurred as a result of the compound no longer being symmetrical. In the ^{13}C NMR, $\underline{C}H_2Br$ came at 30.8 ppm. Positive APCI showed a parent ion at m/z 624.

3.3.4 Reaction with the Dipeptide

Reaction of **61** with *N*-[*N*-(t-butoxycarbonyl)-L-seryl]-L-cysteine methyl ester (**31**) in anhydrous dichloromethane, at 0 °C, in the presence of triethylamine, yielded a pale yellow oil (**62**) on purification.

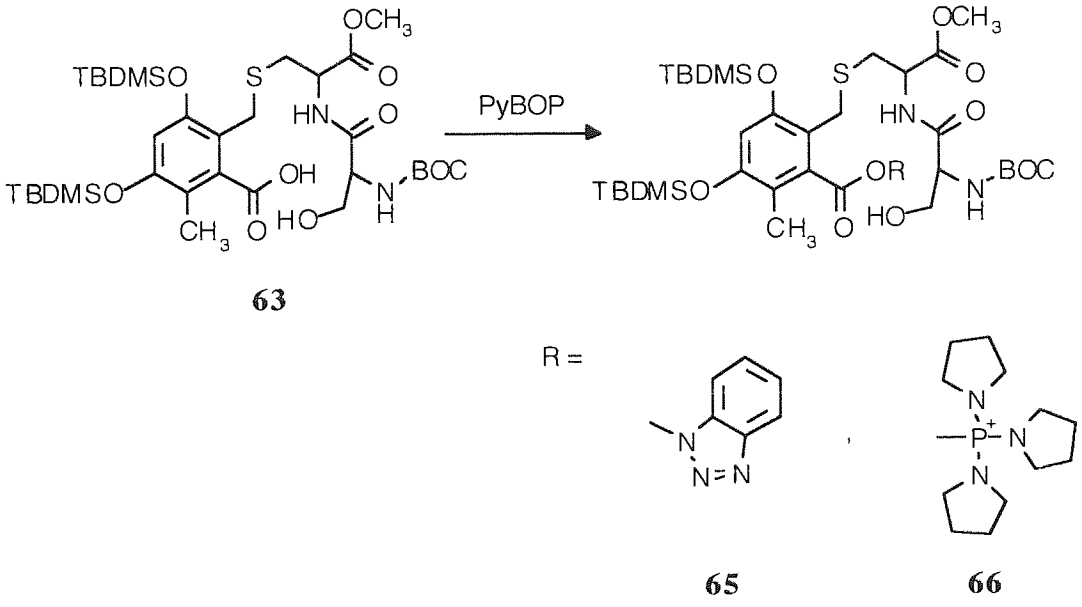
3.3.5 Deprotection

Deprotection was performed by hydrogenation of the 4-nitrobenzyl ester. Benzyl ethers and esters are cleaved by reductive hydrogenolysis, a reaction which does not affect other ethers and esters.

A mixture of hydroxy-ester (**62**) and 10% Pd/C in ethyl acetate was stirred under a hydrogen atmosphere at ambient temperature and pressure for 4 days. On work-up and purification the desired hydroxy-acid (**63**) was afforded in 68 % yield.

3.3.6 Cyclisation

An attempted cyclisation was performed using PyBOP, under the reaction conditions used previously to successfully cyclise the model compound. It was known from the literature that only phosphonium derivative (**66**) (**Scheme 3.14**) resulted in the target compound. Over time **66** was thought to transform to the less reactive benzotriazolyl ester (**65**) which, being a stable compound, would act as a poor leaving group thus preventing synthesis of the desired lactone ring.



Scheme 3.14 Two Possible Intermediates formed on Reaction of the Deprotected Derivative with PyBOP

Rearrangement of the reactive phosphonium derivative to the unreactive benzotrizole ester had not proved a problem when synthesising the model compound. On analysing the product of the most recent reaction, however, it was clear from NMR and MS analysis that the target molecule had not been produced.

The four aromatic protons in the PyBOP benzotriazole ring could clearly be seen in the proton NMR. TLC showed only one spot, with an Rf value different to that of the

starting material and positive electrospray MS gave a parent ion at m/z 847 corresponding to unreactive intermediate (**65**).

An alternative method, that of Mitsunobu, using diethylazodicarboxylate (DEAD) (**9**) and triphenylphosphine (Ph_3P) (**8**) was attempted.

Ph_3P and DEAD were added at 0 °C to a solution of deprotected intermediate (**63**) in toluene. After stirring for 5.5 hours at ambient temperature and subsequent purification, lactone (**64**) was successfully produced as a pale yellow solid.

3.4 ANALOGUE SYNTHESIS

Although the lactone ring had been successfully synthesised, a team from Hoffmann-La Roche had just published the complete synthesis[69a] of the natural product which had failed to penetrate the bacterial cell wall and membrane. As a result, our attention was turned to a cell permeable derivative as there appeared to be considerable scope for improvement.

It has been mentioned previously that cyclothialidine itself, along with a number of its analogues, display excellent activity against the DNA gyrase enzyme, but perform poorly when confronted with intact bacterial cells. To exploit their superior properties of inhibition against DNA gyrase and so convert cyclothialidines into useful antibiotics, an effective mechanism able to promote these compounds into bacterial cells is vital for the full potential to be realised.

One feasible way is to create conjugates by combining the pharmacophore with another molecule to improve the physicochemical properties of the whole. In 1994, Bodor *et al.*[70] reported the use of a lipophilic steroid moiety, which when attached to a peptide, vastly improved transport of the peptide through the blood-brain barrier.

In 1996, Regen *et al.*[71] cited the use of molecules that mimic the structure and function of umbrellas, *ie.* molecules that can cover an attached agent and shield it from an incompatible environment. For hydrophobic agents 'immersion' in water favours a shielded conformation so that intramolecular hydrophobic interactions are maximised and the external face of each wall is hydrated. Conversely in a hydrocarbon solvent, the 'umbrella' favours a fully exposed conformation where solvation and intramolecular dipole-dipole and hydrogen-bonding interactions can be optimised.

As two hydroxyl groups on the aromatic unit compose a hydrophilic 'head', combining the pharmacophore with a hydrophobic molecule could produce a conjugate with both hydrophobic and hydrophilic 'heads'. These two 'heads' may facilitate the conjugate to pass through either a hydrophilic or hydrophobic environment, acting in a similar manner to the molecules with two 'faces' cited by Regen *et al.*

Cholesterol was considered an ideal hydrophobic component for making this conjugate as it had been proven effective in conjugating with polyamines (hydrophilic) in gene delivery.[72] Regen *et al.* made use of cholic acid to act as the umbrella, due to its ease of addition, amphilicity and biocompatibility.

Further support for a cholesterol conjugate came in the form of cosalane[73,74] (**Figure 3.3**), an anti-HIV agent designed conceptually by *Cushman et al.*, linking a dichlorinated disalicylmethane unit to cholestane *via* a three carbon linker.

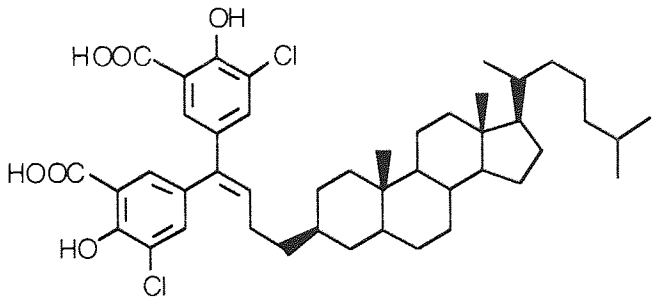


Figure 3.3 Cosalane, an anti-HIV Agent

Cushman *et al.* reasoned that the dichlorodisalicylmethane would act as the 'pharmacophore' and the cholestane fragment would serve as an accessory module to increase potency by directing the molecule to the lipid environment of the cell membrane and the viral envelope. Results did indeed indicate that the cholestane moiety functioned as a lipophilic accessory appendage to escort the pharmacophore into a lipid environment. More recent work into sterol-polyamine conjugates[75] also aims to produce new classes of antibiotics.

Thus, cholesteryl chloroformate was the derivative of choice to aid cyclothialidine permeability as it was commercially available and expected to react readily with the 12-membered, free amine derivative (**Figure 3.4**). This last point was vital since stocks of the lactone were at a premium.

It was hoped that the proposed conjugate may lead the way to new classes of antibacterial agents.

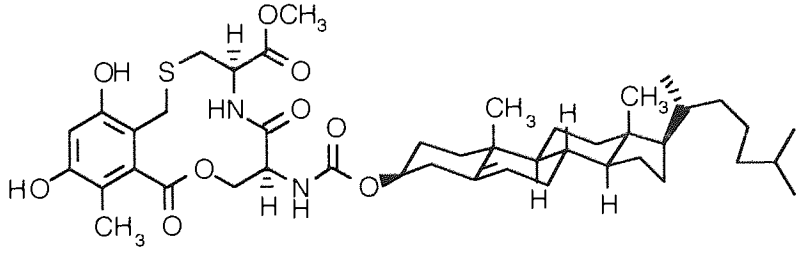
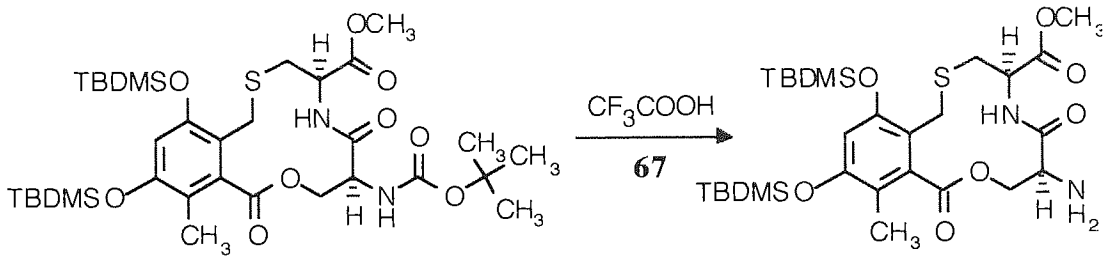


Figure 3.4 Proposed Cholesteryl Derivative

3.4.1 Amine Deprotection

The *tert*-butoxycarbonyl (BOC) group was removed from **64** (**Scheme 3.15**) using trifluoroacetic acid (TFA) (**67**) in a standard procedure. TFA was added to a stirred solution of the fully protected 12-membered lactone in anhydrous dichloromethane at ambient temperature. Stirring for 30 minutes and subsequent work-up afforded the desired free amine (**68**) as an off-white solid in 93 % yield.



64 **68**

Scheme 3.15 Amine Deprotection

The absence of peaks representative of the BOC group at 1.48 ppm and 28.1 ppm in ^{1}H NMR (**Figure 3.5**) and ^{13}C NMR (**Figure 3.6**) respectively. The ^{13}C NMR was run on PENDANT, a technique which gives improved signal to noise ratio over alternatives such as APT and shows quaternary carbons, unlike DEPT 135. CH_3 and CH's are shown as positive peaks and CH_2's and quaternaries are negative. A decrease in mass of the $[M+1]^+$ ion in positive APCI MS from m/z 713 to 613, confirmed successful deprotection of the amine.

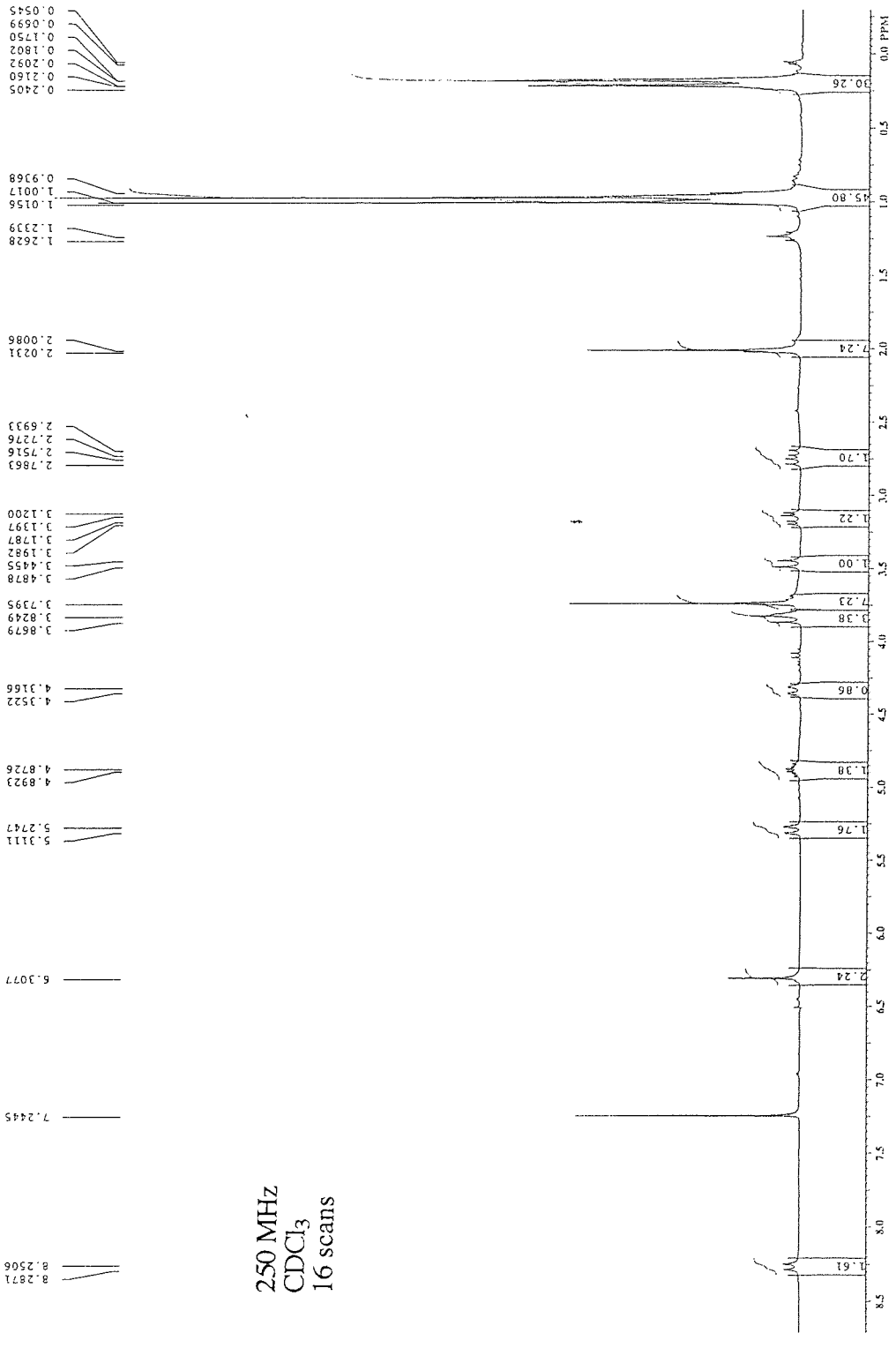


Figure 3.4 ^{1}H NMR of Free Amine (**68**)

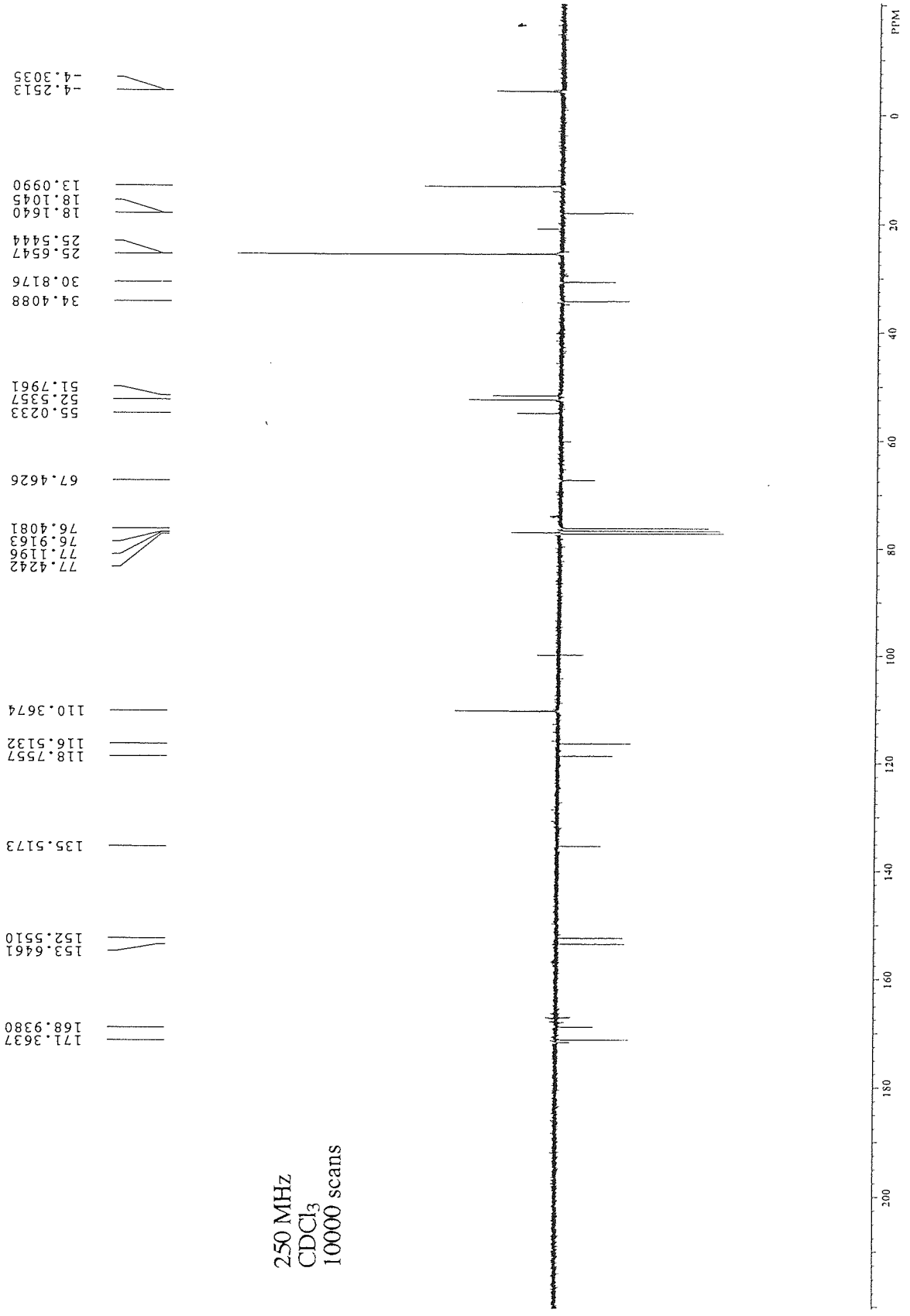
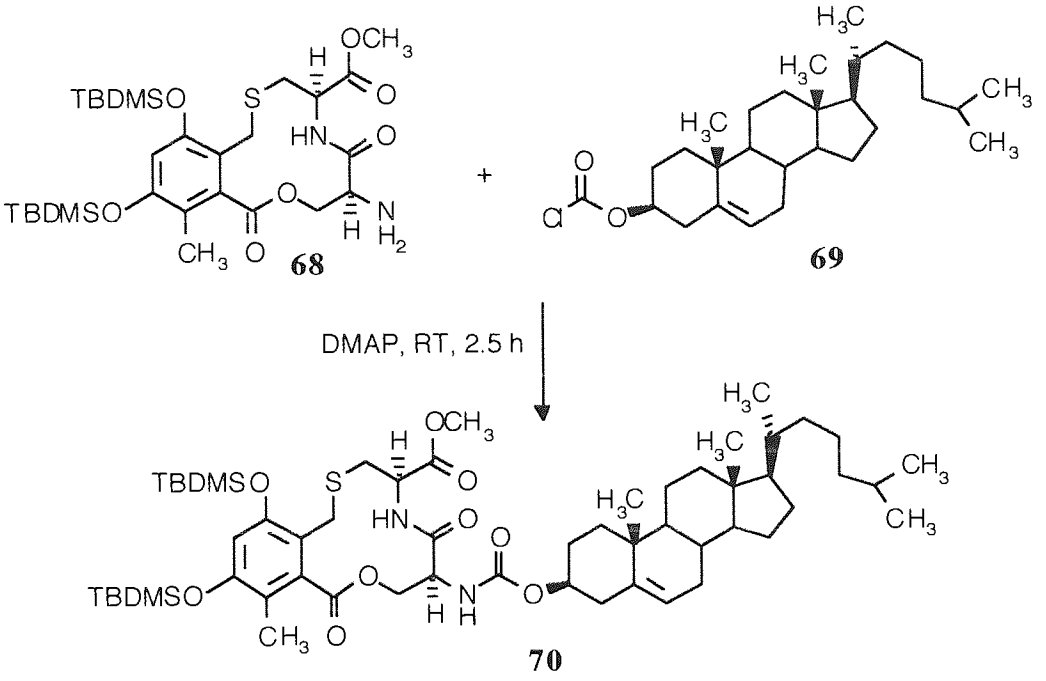


Figure 3.5 ^{13}C NMR of Free Amine (**68**)

3.4.2 Reaction with Cholesteryl Chloroformate

A solution of lactone (**68**) and cholesteryl chloroformate (**69**) (**Scheme 3.16**) was stirred at ambient temperature in the presence of 4-dimethylaminopyridine (DMAP) for 2.5 hours. After purification by column chromatography the required conjugate (**70**) was afforded as a white crystalline solid in 43 % yield.



Scheme 3.16 Synthesis of the Cholesteryl Derivative

Positive APCI MS showed an increase in mass of the $[M+1]^+$ ion to 1026 on addition of the cholesterol derivative corresponding to the desired compound.

The complex ^1H and ^{13}C NMR spectra generated are given in **Figures 3.7** and **3.8** respectively.

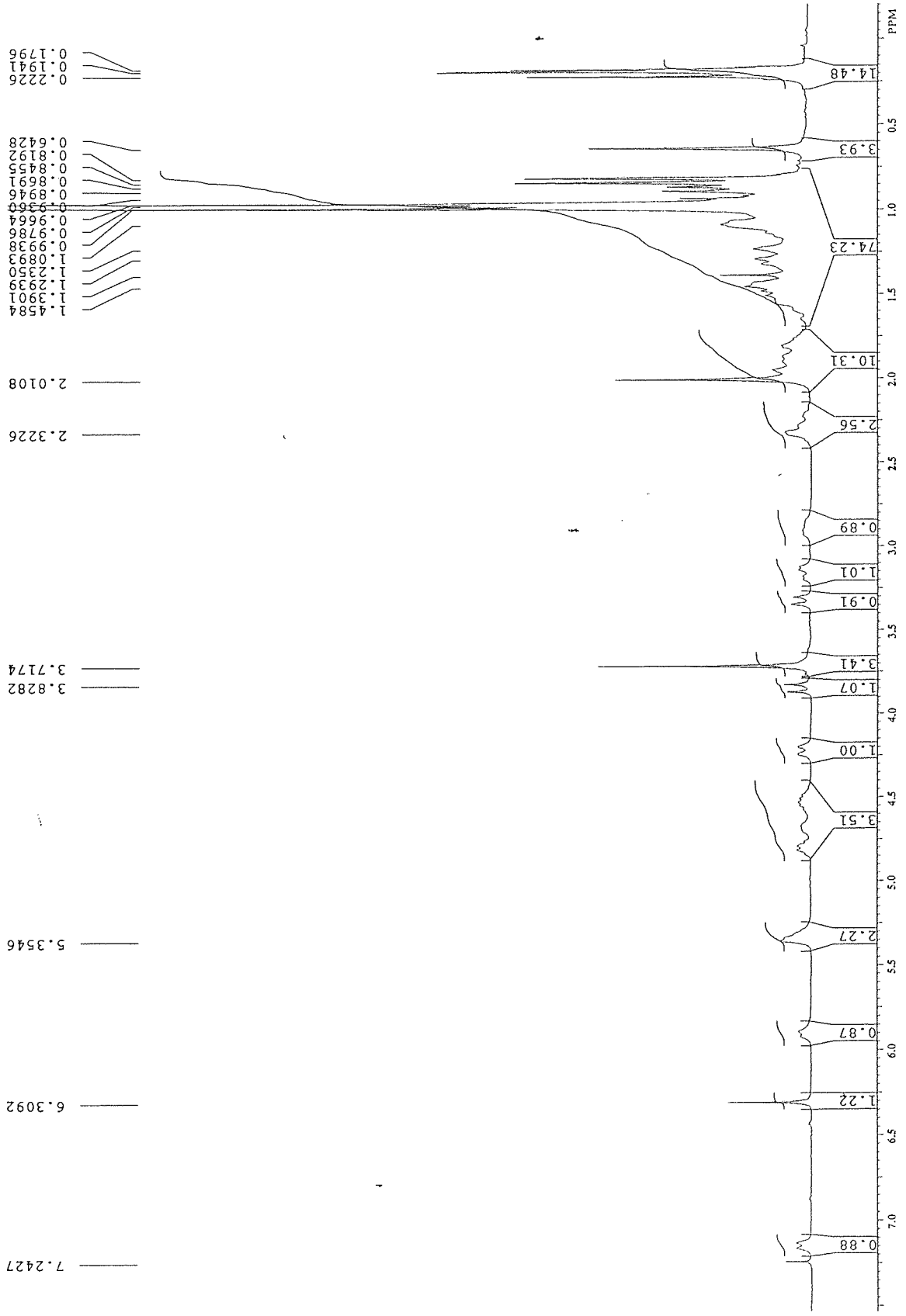


Figure 3.6 ^{1}H NMR of Silyl Protected Cholesteryl Derivative (**70**)

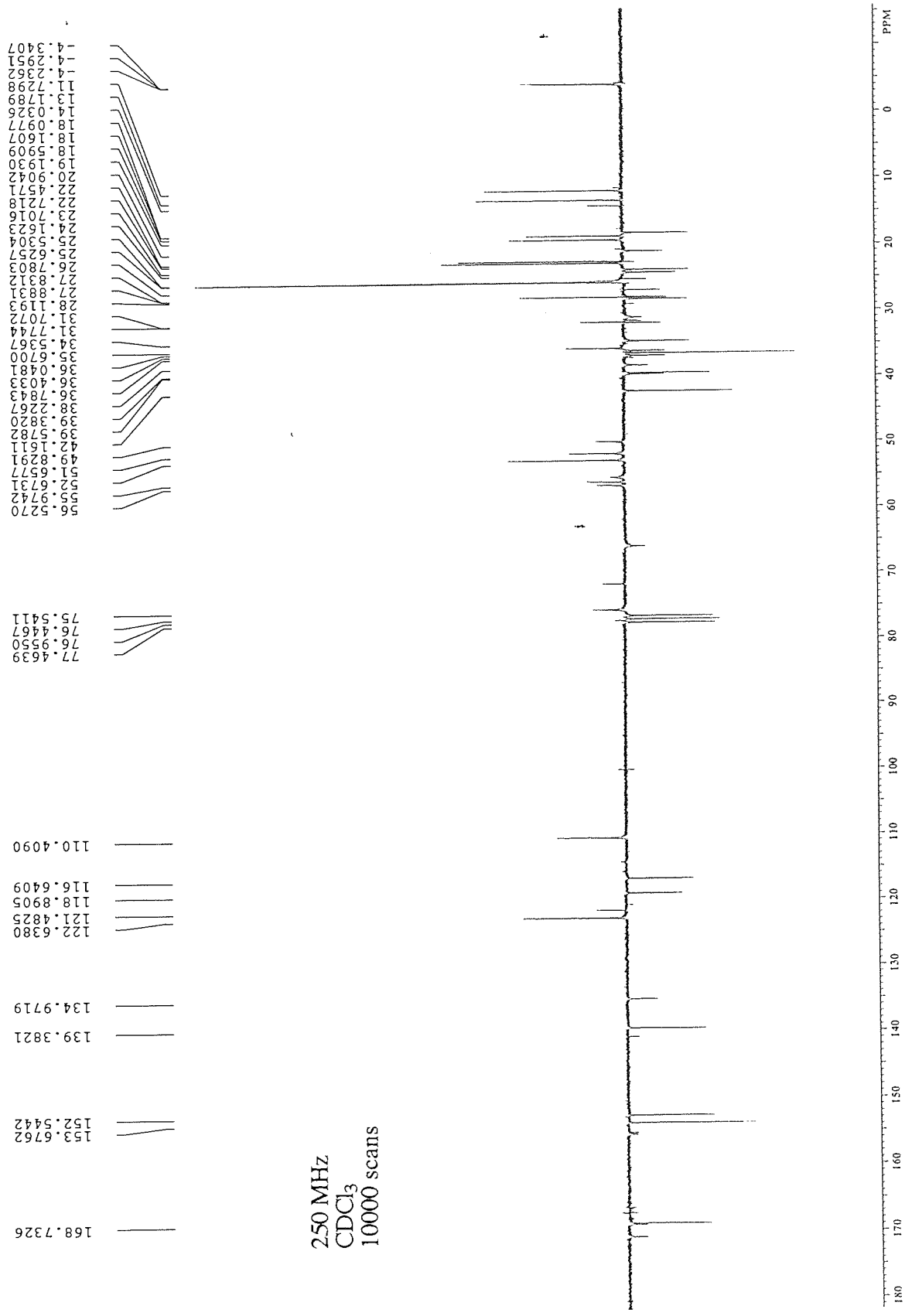
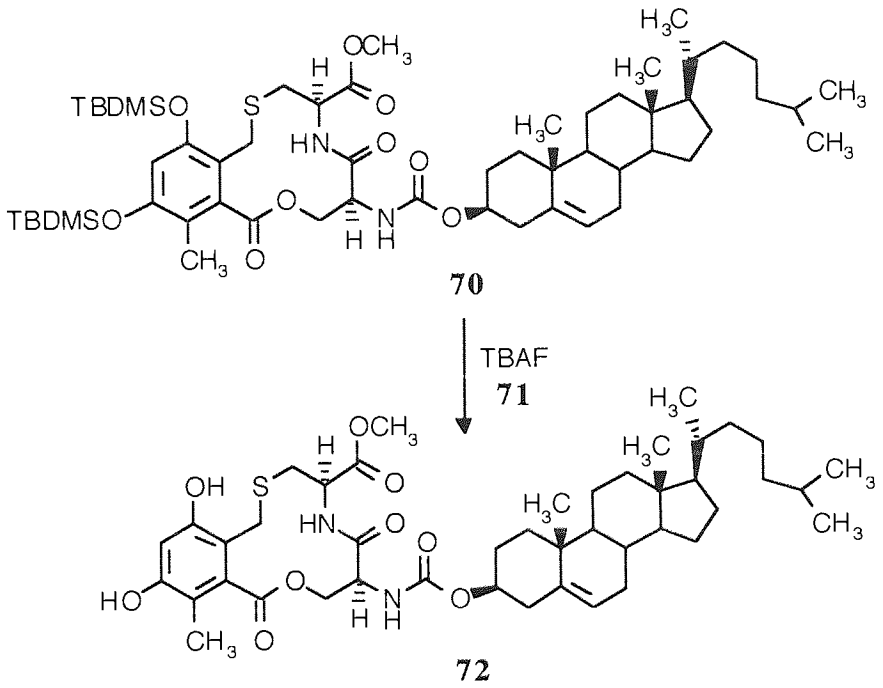


Figure 3.7 ^{13}C NMR of Silyl Protected Cholesteryl Derivative (**70**)

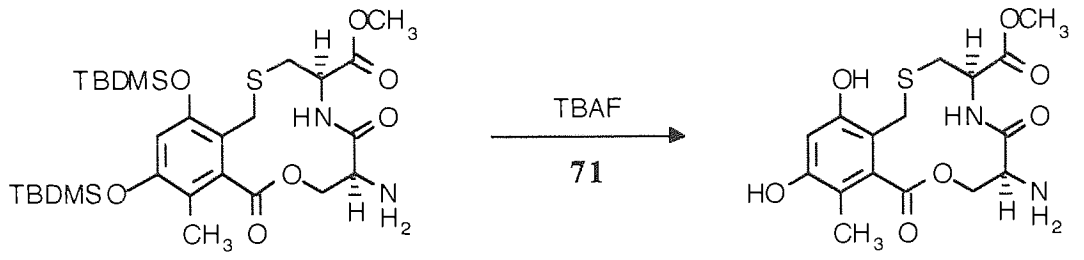
3.4.3 Hydroxyl Group Deprotection

The silyl protecting groups were readily removed by reaction of a solution of conjugate **70** in THF with 1M *tetra*-butylammonium fluoride (TBAF) (**71**) at ambient temperature (**Scheme 3.17**). After stirring for 1 hour, purification by column chromatography afforded the fully deprotected lactone (**72**) as an off-white solid. Positive APCI MS gave a $[M+1]^+$ peak at m/z 797 which was consistent with the target.



Scheme 3.17 Hydroxyl Group Deprotection of the Conjugate (**70**)

In order to discover whether or not addition of cholesteryl chloroformate was able to increase the cell permeability of the pharmacophore, the silyl group on 12-membered lactone (**68**) was also removed to give **73** (**Scheme 3.18**), to be used as a reference in anti-bacterial activity tests. Positive APCI MS gave a $[M+1]^+$ peak at m/z 385 which was consistent with the desired deprotected free amine.

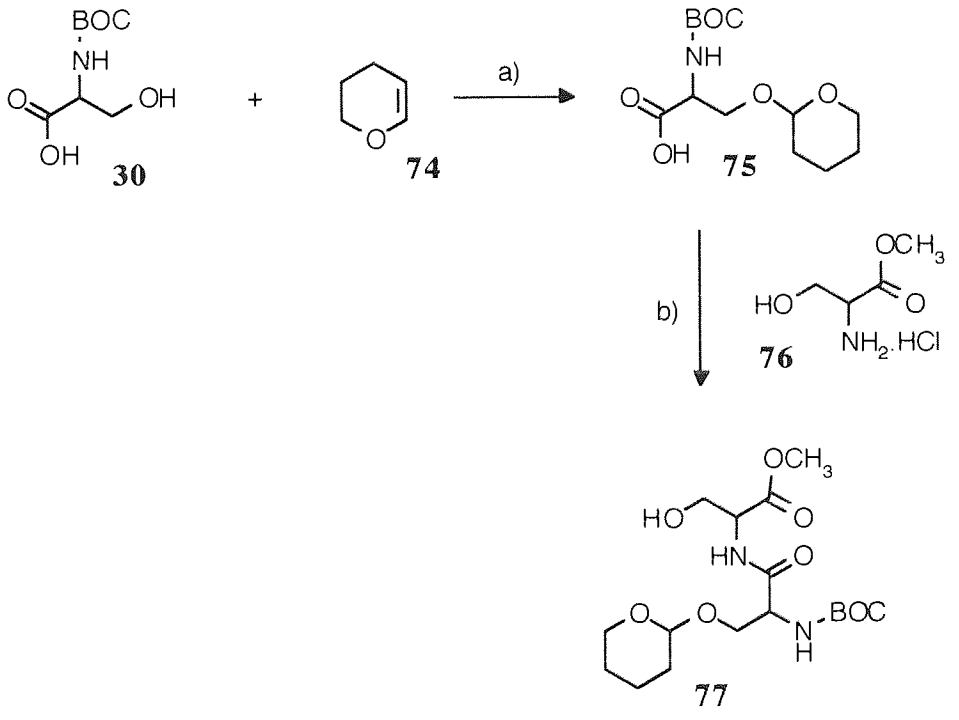


68 **73**

Scheme 3.18 Hydroxyl Group Deprotection of **68**

3.4.5 Attempted Synthesis of an Oxygen Analogue

The attempted synthesis of a 12-membered lactone containing oxygen in place of sulphur was deemed paramount in order to investigate any affect it may have on activity.



a) DCM, PPTS, RT, 4.5 h. **b)** ACN, 4-methylmorpholine, DCC, 0 °C, 3h.

Scheme 3.19 Synthesis of the THP Protected Dipeptide

Firstly, *N*-(*t*-BOC)-L-serine (**30**) (**Scheme 3.19**) was protected with 3,4-dihydropyran (**74**). This was achieved by reaction at ambient temperature in the presence of pyridinium *p*-toluene sulphonate (PPTS) in dichloromethane.[76] Work-up afforded ether (**75**) as a colourless oil in 90 % yield.

Subsequent reaction of **75** with L-serine methyl ester. HCl (**76**) in acetonitrile at 0 °C in the presence of DCC and 4-methylmorpholine afforded on purification, protected dipeptide (**77**) as a yellow solid in 66 % yield.

Reaction of dipeptide (**77**) with brominated aromatic moiety (**61**), however failed. Anticipating problems due to the decreased nucleophility of the -OH compared to -SH, triethylamine was substituted for stronger bases, such as 4-dimethylaminopyridine (DMAP) and potassium carbonate. The use of elevated reaction temperatures were also attempted. Only decomposed starting materials were isolated.

It was decided to convert the alcohol to the alkoxide, a more reactive nucleophile, in a further attempt to produce the desired ether. Alcohols are sufficiently acidic that they can easily be converted to their corresponding alkoxide by treatment with a strong base. Reaction of an alkoxide with a haloalkane, known as the Williamson synthesis, is an irreversible route to ethers. As a result the reaction was repeated using sodium hydride in an attempt to produce the ether *via* the alkoxide. Unfortunately, once again no product was isolated.

3.5 BIOLOGICAL RESULTS

The cholesterol derivative (**72**) (**Figure 3.9**) and the free amine analogue (**73**) (**Figure 3.10**) were tested against a number of bacteria using a standard procedure involving the placement of small paper discs on the surface of inoculated plates. Any inhibitory activity was shown by a zone of inhibition around the disc.

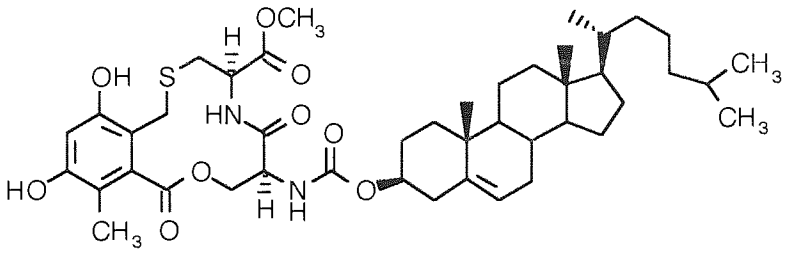


Figure 3.9 The Cholesteryl Derivative

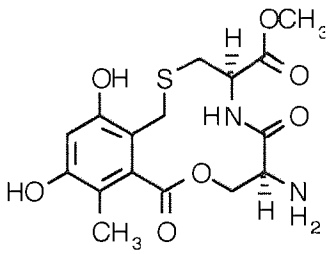


Figure 3.10 The Free Amine Analogue

The zone diameter, measured in mm, is a qualitative indication of activity. The compounds were made up into solutions of 10 mg/ml in DMSO, with 10 μl loaded onto each disc. Three antibiotics, cipofloxacin, ampicillin and novobiocin were used for comparison and a blank DMSO solution as a control. It was understood that any positive results from zones of inhibition experiments would require minimum inhibitory concentration (MIC) experiments to be carried out in order to obtain quantitative data.

The organisms tested against (**Table 3.1**) were Methicillin-sensitive *Staphylococcus aureus* (MSSA) strains NCTC 10788 and NCTC 6571; Methicillin-resistant *Staphylococcus aureus* (MRSA) strains Innsbruck and 96-7778; *Escherichia coli* DC0 and DC2 (a very sensitive strain); *Mycobacterium fortuitum* and the yeast *Candida albicans*.

Staphylococcus aureus is a Gram positive bacterium[77,78] reponsible for many severe infections, such as sepsis of wounds, endocartis (leading to heart failure) and pneumonia. Methicillin (**Figure 3.11**) was introduced into therapy as a β lactamase stable β lactam, but MRSA strains were very adaptable and responded rapidly to new treatments, to the extent that some strains became resistant to all clinically used antibiotics except vancomycin.

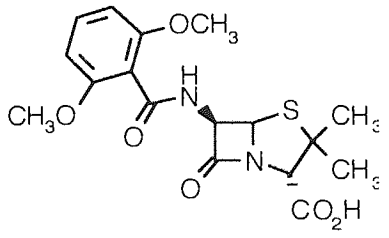


Figure 3.11 Methicillin

Escherichia coli is a Gram-negative bacterium which causes infection of the urinary and intestinal tracts. In infants it may lead to sepsis and meningitis. DC2, a permeability mutant of DC0, is a strain particularly sensitve to antibiotics, rendering it useful as a test against this new class of potential drugs. *Mycobacterium fortuitum* is found in soil and water and causes superficial and systemic disease on rare occasions. It is often resistant to antimycobacterial drugs. *Candida albicans* is a Gram-positive budding yeast responsible for a variety of conditions including thrush, endocarditis and bloodstream invasion.

	Ciprofloxacin	Ampicillin	Novobiocin	DMSO	**72**	**73**
MSSA NCTC 10788	36	52	38	0	0	0
MSSA NCTC 6571	30	48	30	0	0	7
MRSA Innsbruck	13	14	46	0	0	0
MRSA 96-7778	0	10	35	0	0	0
E. coli DC0 1850E	30	25	0	0	0	0
E. coli DC2 1852E	34	36	16	0	0	0
M. fortuitum	-	-	-	-	0	0
Candida albicans	-	-	-	-	0	0

Table 3.1 Biological Test Results (zone sizes in mm)

Contrary to expectations however, the only new activity shown was free amine **73** on MSSA NCTC 6571. Addition of the cholesterol conjugate **72** had not only failed to improve the cell permeability but the inhibitory activity had been lost.

As expected, free amine lactone **73** acted as a poor antibiotic. The results suggested a failure to penetrate cells.

3.6 CONCLUSION

The pharmacophore (**Figure 3.10**) was successfully synthesised. 3,5-Dihydroxy-2,6-dimethylbenzoic acid was produced by two successive Mannich reaction/reduction steps. Acid protection using 4-nitrobenzyl bromide and TBDMS hydroxyl protection followed by mono-bromination of the methyl afforded the key intermediate. Reaction with the dipeptide, followed by deprotection and cyclisation with DEAD/Ph_3P led to the 12 membered lactone. Deprotection of the amine using TFA and deprotection of the hydroxyls with TBAF gave the modified pharmacophore.

Reaction of TBDMS protected material with cholesteryl chloroformate and hydroxyl deprotection afforded the desired conjugate (**Figure 3.9**).

Biological testing of both compounds against a number of bacteria showed little/no activity in either compound. It is hypothesised that a linker may be required between the pharmacophore and the lipophilic accessory in order for the steriod to escort the lactone efficiently.

Chapter 4:
Intermediates Towards Amine Analogues

4.1 THE AMINE ANALOGUE

As it had been cited in the literature[33] that the hydroxyl groups played a major role in the activity of the cyclothialidines *via* hydrogen bonding, it was considered of interest to investigate the effect of substituting the hydroxyl groups for amines (**Figure 4.1**), since amines contain hydrogen atoms capable of forming hydrogen bonds with electron-donating groups also.

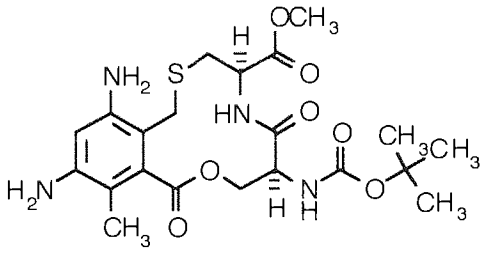
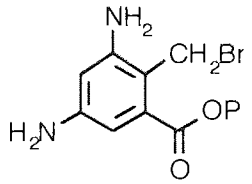


Figure 4.1 Proposed Amine Derivative

4.2 SYNTHETIC ROUTES TO INTERMEDIATE

Intermediate (**Figure 4.2**) was the primary target molecule from which the lactone could be made.



P = Protecting Group

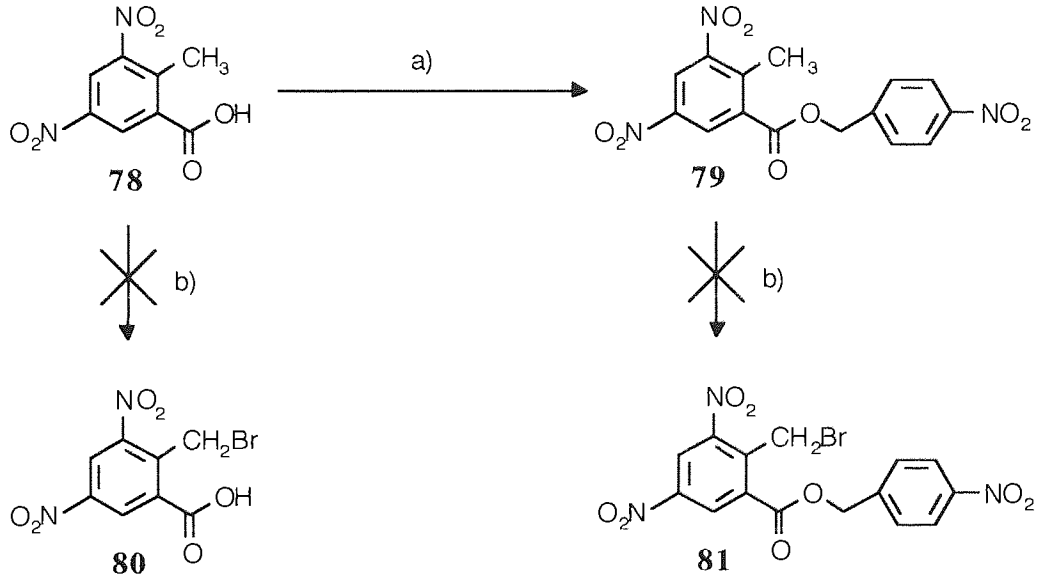
Figure 4.2 Amine Intermediate

It was envisaged that the intermediate could be synthesised *via* a series of reactions analogous to those used for the hydroxyl derivative, with the exception of the choice of amine protecting group. Following successful synthesis of the intermediate, reaction with the dipeptide, subsequent deprotection and cyclisation would result in synthesis of the required derivative.

4.2.1 First Attempt

In an initial atttempt to synthesise the intermediate, it was decided to start with commercially available 3,5-dinitro-*o*-toluic acid, protect the carboxylic acid and then carry out bromination prior to reducing the nitro groups to amines, thereby eliminating the need for amine protection.

Protection of the acid (**78**) was readily carried out using the standard method of reaction with 4-nitrobenzyl bromide (**Scheme 4.1**). On recrystallisation the required product (**79**) was isolated as a pale yellow solid, though in only 29 % yield.



a) *N,N,N,N*-tetramethylguanidine, 4-nitrobenzyl bromide, 18 h, 0 °C; **b**) NBS, CCl_4, hυ, RT.

Scheme 4.1 Attempted Synthesis *via* a Nitro-Substituted Nitrobenzyl Ester

^{1}H NMR showed a singlet at 5.55 ppm corresponding to O$\underline{C}H_2$ and doublets at 7.76-7.80 ppm and 8.25-8.29 ppm were assigned to the aromatic protons in the protecting group. The remaining aromatic protons could be seen as doublets at 8.77-8.78 ppm and 8.90-8.91 ppm. ^{13}C NMR gave O$\underline{C}H_2$ as a negative peak at 66.6 ppm while the aromatics at 122.0 and 127.5 ppm and 123.8 and 129.3 ppm were assigned to those in

the 'main' ring and protecting group respectively. Negative electrospray indicated the $[M-H]^-$ ion at 360 as required.

Direct bromination was attempted but none of the desired compound (**80**) was produced.

Bromination was then attempted using the established method of heating at reflux temperature with NBS in carbon tetrachloride under light irradiation. None of the desired compound (**81**) was produced. ^1H NMR clearly showed the presence of an unreacted methyl group, though MS indicated the presence of bromine.

The reaction was also attempted in the presence of free radical initiators, benzoyl peroxide and azoisobutyronitrile (AIBN) (**Figure 4.3**) instead of UV light, but the same results were attained.

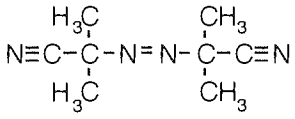
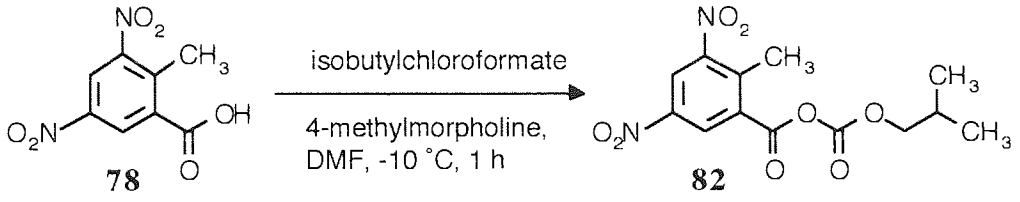


Figure 4.3 AIBN

It became apparent that bromination had occurred preferentially at the methylene of the protecting group rather than at the methyl group as required. Due to the strongly deactivating nature of the nitro substituents on the ring it is possible that the methyl group, attached to a ring containing two nitro groups, was deactivated to a greater extent than the methylene of the protecting group, which contained only one nitro group.

4.2.2 Second Attempt

As a result it was decided to use an alternative protecting group, one which was not so similar to the rest of the molecule. Isobutyl chloroformate and 4-methylmorpholine were added to 3,5-dinitro-*o*-toluic acid (**78**) (**Scheme 4.2**) at -10 °C, under argon, and the mixture stirred at -10 °C for one hour. Following purification, the protected derivative (**82**) was produced as a brown oil which solidified on standing.

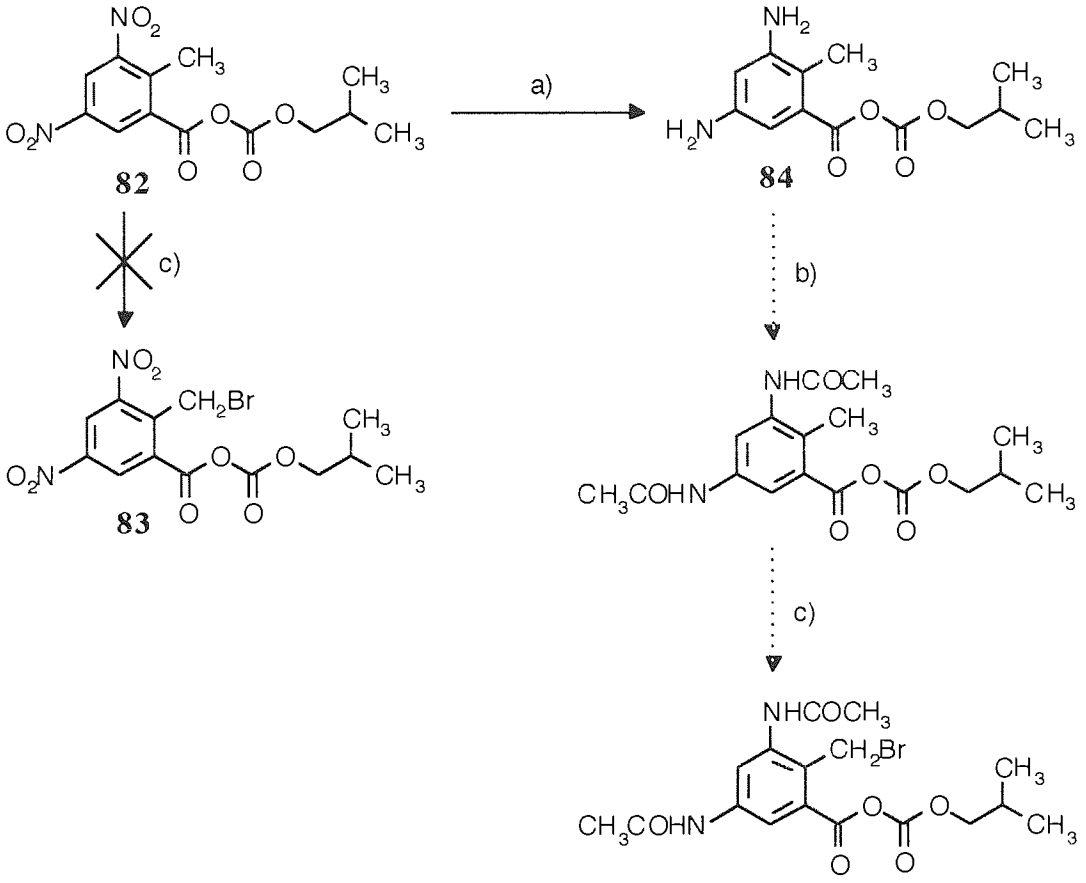


Scheme 4.2 Synthesis of the Isobutylformate Derivative

^{1}H NMR clearly indicated the presence of the protecting group; the methyl groups as a doublet at 0.95-0.98 ppm, a multiplet at 1.99-2.10 ppm corresponding to the tertiary proton and a doublet at 4.12-4.15 ppm assigned to the methylene. The methyl attached to the aromatic ring appeared as a singlet at 2.59 ppm and the aromatic protons as doublets at 8.67-8.68 ppm and 8.87-8.88 ppm, respectively.

PENDANT ^{13}C NMR gave positive peaks at 19.0 ppm and 27.3 ppm corresponding to the $(\underline{C}H_3)_2$ and $\underline{C}H$ respectively, while a negative peak at 72.0 ppm was assigned to $O\underline{C}H_2$. Positive electrospray MS showed the $(M+H)^+$ ion to be at m/z 382 as required.

Direct bromination (**Scheme 4.3**) of nitro compound (**82**), to give **83** was unsuccessful. Reduction of the nitro groups to amines, however, using hydrogen and a palladium/ carbon catalyst resulted in synthesis of amine (**84**) as a pale brown solid in quantitative yield (98 %).



a) H_2, Pd/C, RT, o/n; b) acetic anhydride, gl. AcOH, reflux, o/n; c) NBS, CCl_4, hυ, RT.

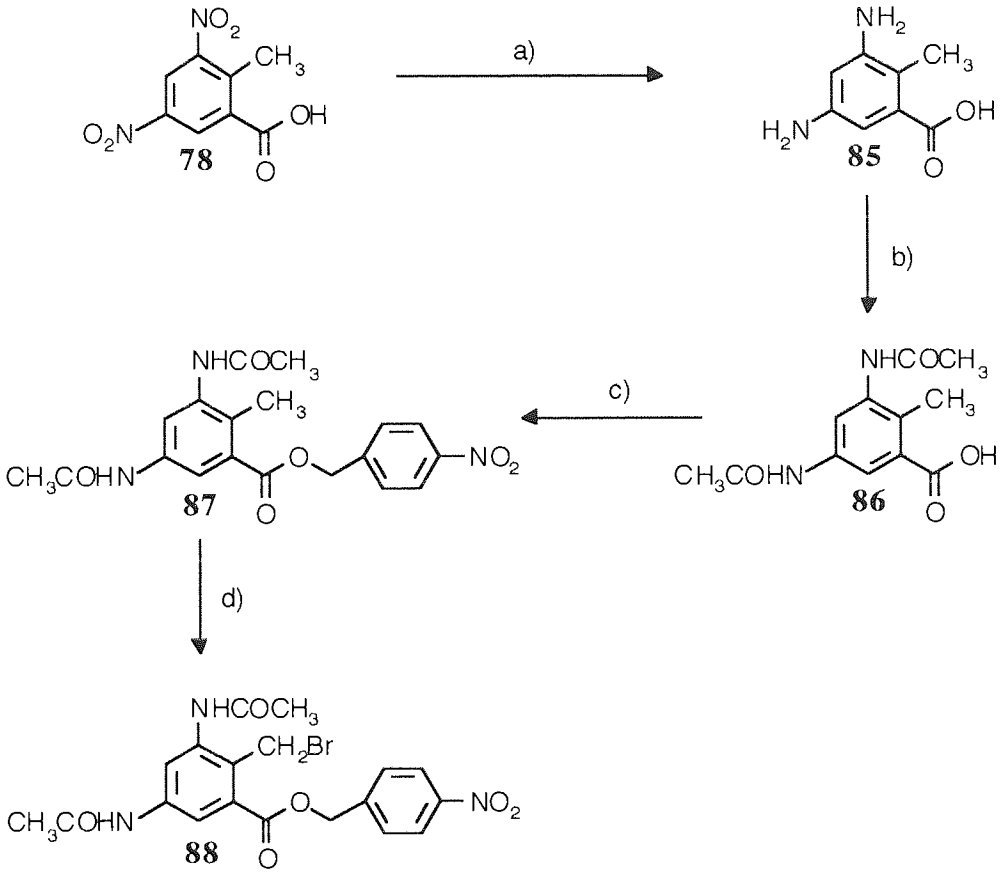
Scheme 4.3 Proposed Routes to the Target Intermediate

It was then noted, however, that the planned route, *ie.* protection of the amine substituents as amides followed by bromination of the methyl group, was not ideal. Both protecting groups, the isobutylformate and the amide would be removed under identical hydrolysis conditions. Though the amide would be expected to be less reactive than the anhydride it was most likely that both would be removed at the same time - an undesirable factor as the amines needed to remain protected until completion of the 12-membered lactone.

Thought was turned back to the original 4-nitrobenzyl bromide protecting group.

4.2.3 Third Attempt

It was decided to protect the amine functionalities prior to protection of the carboxylic acid (**Scheme 4.4**).



a) H_2, Pd/C, RT, 18 h; **b**) acetic anhydride, gl. AcOH, reflux, 18 h; **c**) *N,N,N,N*-tetramethylguanidine, 4-nitrobenzyl bromide, 18 h, 0 °C; **d**) NBS, CCl_4, hυ, RT.

Scheme 4.4 The Successful Route

3,5-Dinitro-*o*-toluic acid (**78**) was reduced by reaction for 18 hours, at ambient temperature and pressure, under a hydrogen atmosphere, in the presence of a palladium/carbon catalyst. 3,5-Diamino-*o*-toluic acid (**85**) was afforded in 91 % yield as a pale brown solid.

^{1}H NMR indicated that the aromatic protons in the product had shifted upfield considerably in comparison with those in the starting material, from doublets at 8.66-8.67 and 8.83-8.84 ppm to 6.02-6.03 and 6.21-6.22 ppm. The methyl group had shifted from 2.60 to 2.01 ppm. Positive APCI MS gave an $[M+1]^+$ at m/z 167 as required.

The free amine groups were protected as amides. 3,5-Diamino-*o*-toluic acid (**85**) was suspended in glacial acetic acid, acetic anhydride was added and the mixture heated at reflux temperature (120 °C) for 18 hours. Water was added to the resulting hot, brown suspension and the mixture allowed to stand at ambient temperature for 1 hour, followed by 4 hours at 4 °C. The resulting precipitate was collected to afford target intermediate (**86**) as an off-white solid in 69 % yield.

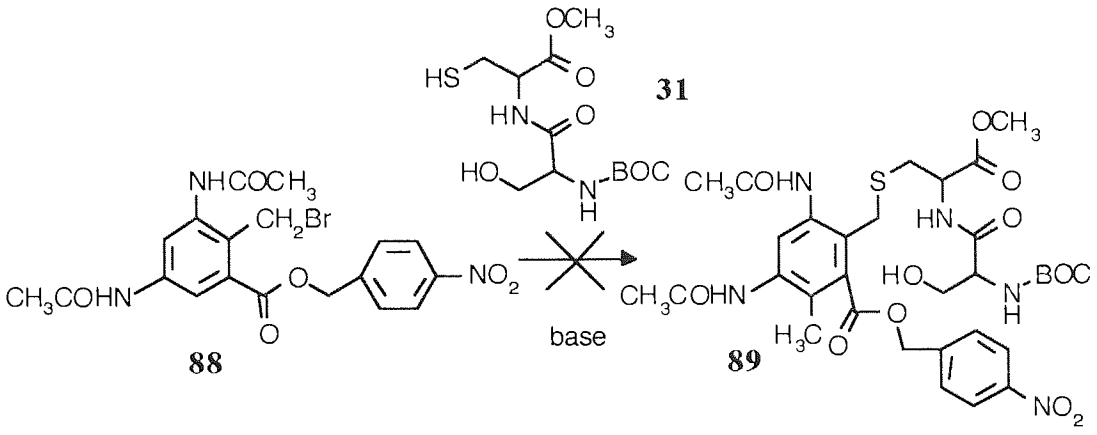
^{1}H NMR clearly showed peaks for the two methyl groups at 2.01 and 2.04 ppm in addition to singlets at 9.47 and 10.03 ppm corresponding to the NHC=O. The aromatic protons had shifted downfield to 7.74 and 7.83 ppm. Positive APCI MS gave an $[M+1]^+$ ion at m/z 251.

Protection of the carboxylic acid functionality was carried out as for the hydroxyl analogue using 4-nitrobenzyl bromide, to afford the required compound as beige solid (**87**) in 69 % yield.

Subsequent bromination of (**87**) yielded derivative (**88**) as a light brown solid in 93 % yield.

4.3 ATTEMPTED REACTION WITH DIPEPTIDE

Frustratingly, reaction of protected bromo derivative (**88**) with dipeptide (**31**) (**Scheme 4.5**) failed repeatedly to produce the desired intermediate (**89**).



Scheme 4.5 An Attempted Nucleophilic Substitution

Reaction in dichloromethane at 0 °C with triethylamine for 2 hours resulted in a yellow residue composed of unreacted starting materials. In an effort to aid formation of the sulphur anion, the reaction was carried out in dimethylformamide (DMF), a more polar solvent. Positive APCI, however, indicated the presence of unreacted brominated compound (**88**). The reaction was repeated in DMF; the temperature increased to 80 °C and stirred overnight. The resulting brown solid was, however, shown to be decomposed starting material.

4.4 CONCLUSION

After attempting several routes, the target intermediate (**Figure 4.4**) was synthesised in a four step procedure from 3,5-dinitro-*o*-toluic acid. Reduction of the nitro groups to amines *via* catalytic hydrogenation followed by amine protection then bromination of the methyl, resulted in the target compound.

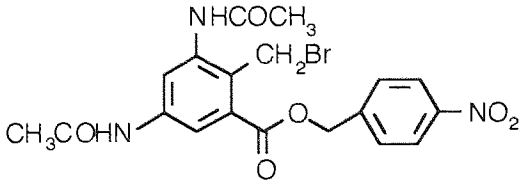


Figure 4.4 Amide Intermediate

Unfortunately, reaction of the intermediate with the required dipeptide failed repeatedly and it was decided this work should lead to another project.

Chapter 5:
cis-3-Hydroxyproline

5.1 *cis*-(2*S*,3*R*)-3-HYDROXYPROLINE

Prior to publication of the total synthesis of cyclothialidine by Hoffmann-La Roche, a great deal of literature research had been carried out into *cis*-3-hydroxyproline, the unusual amino acid required in the natural product (**Figure 5.1**).

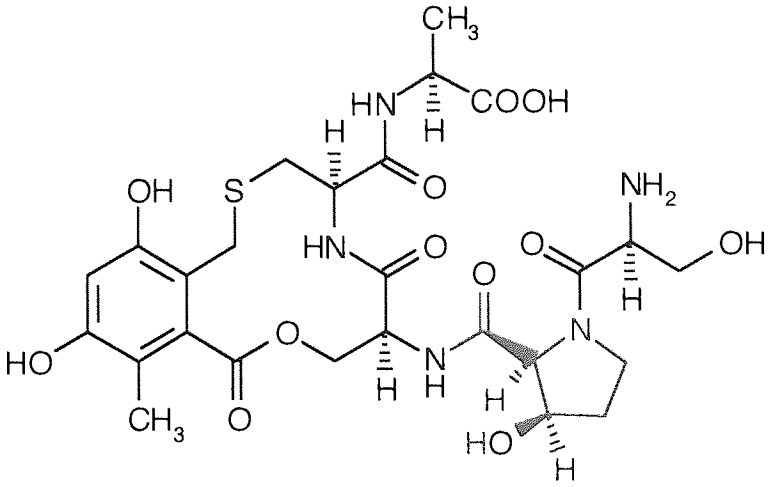


Figure 5.1 Cyclothialidine Incorporating *cis*-3-Hydroxyproline

cis-3-Hydroxyproline (**Figure 5.2**) occurs naturally as a component of the polypeptide antibiotic teleomycin,[79,80] produced by *Streptomyces sp.* *trans*-3-Hydroxy-L-proline has been isolated from Mediterranean sponge and also from teleomycin. *cis*-3-Hydroxy-L-proline was prohibitively expensive.

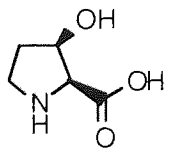


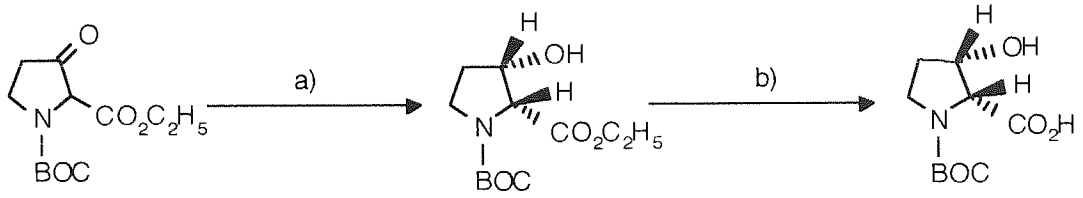
Figure 5.2 *cis*-3-Hydroxyproline

In contrast, *cis*-4-hydroxy-L-proline was found to be cheap and readily available. It appeared reasonable to find a route to convert one isomer into another but it soon became apparent the process would not be easy.

5.1.1 Enzymic Reaction

In 1988, Cooper *et al.*[81,82] investigated the possibility of using yeast reductions of 3-oxoproline derivatives as a method to access chiral hydroxyprolines.

Racemic oxo ester (**90**) (**Scheme 5.1**) was subjected to a yeast reduction, using Bakers' yeast in the presence of water and sucrose to afford derivative (**91**) which on deprotection resulted in *cis*-3-hydroxy-L-proline (**92**) in 70 % yield.



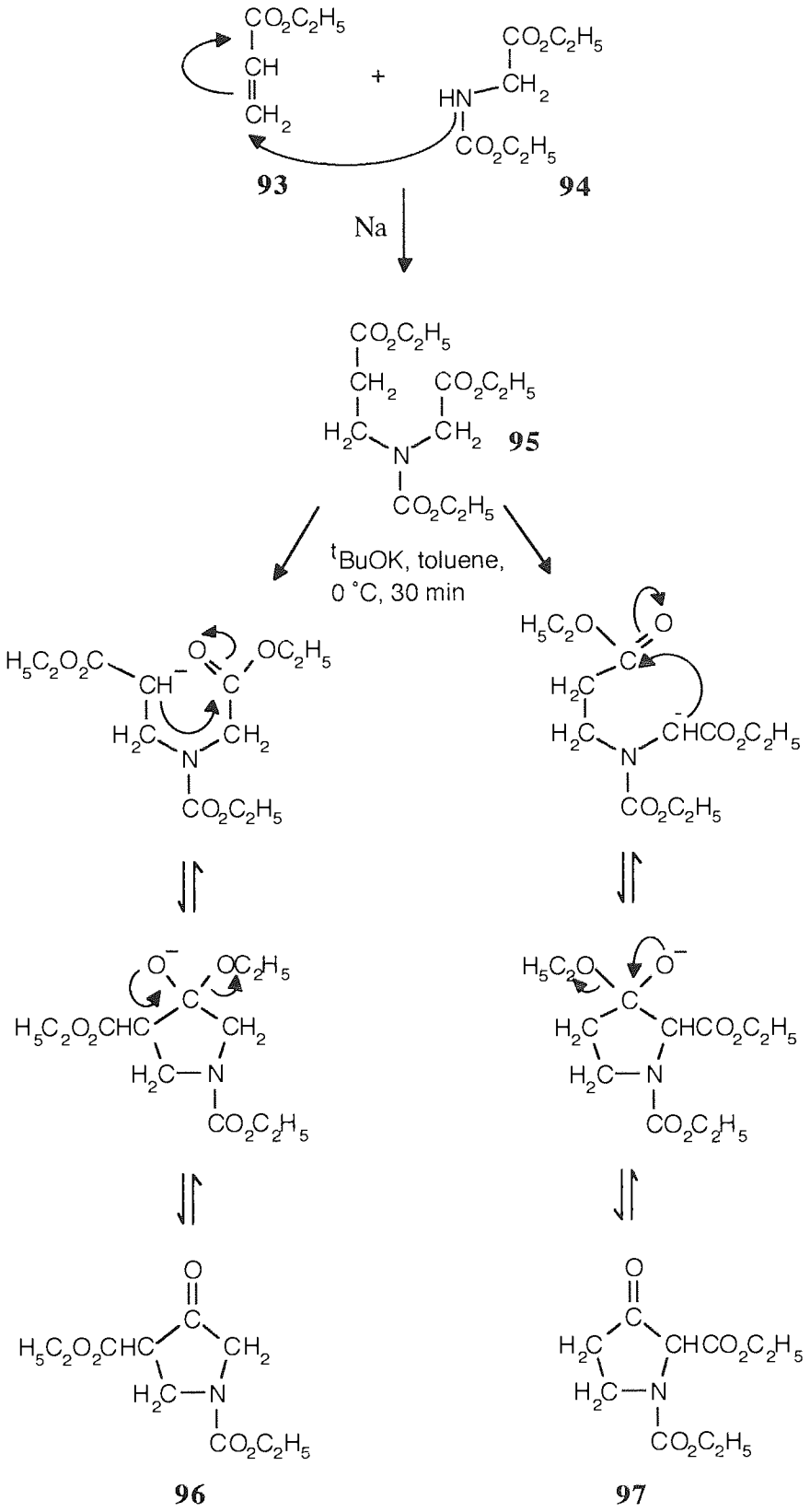
90 **91** **92**

a) H_2O, sucrose, dried Bakers yeast, 30 °C, 24 h; **b**) **i.** DCM, TFA, KOH, **ii.** Ion exchange.

Scheme 5.1 3-Hydroxyproline *via* a Bakers' Yeast Reduction

The oxo esters required were synthesised using the Dieckmann cyclisations (**Scheme 5.2**) cited by Rapoport *et al.*[83] in 1964. Contrary to the failed attempts of Morita *et al.*,[84] ethyl *N*-ethoxycarbonyl glycinate (**94**) and ethyl acrylate (**93**) were reacted in the presence of sodium to produce triester (**95**). Reaction in toluene with ^{t}BuOK resulted in a Dieckmann cyclisation to produce a 1:1 mixture of the desired regioisomer (**97**) and the by-product (**96**). Facile separation could be performed by extraction into aqueous pH 9.5 buffer to remove the undesired isomer. In 1990, Sibi *et al.*[85] reported an improvement in the chemical/optical yields of Rapoport by using the carbobenzyloxy (Cbz) group for nitrogen protection in place of *t*-butoxycarbonyl (BOC) and by immobilizing the Bakers' yeast with calcium alginate.

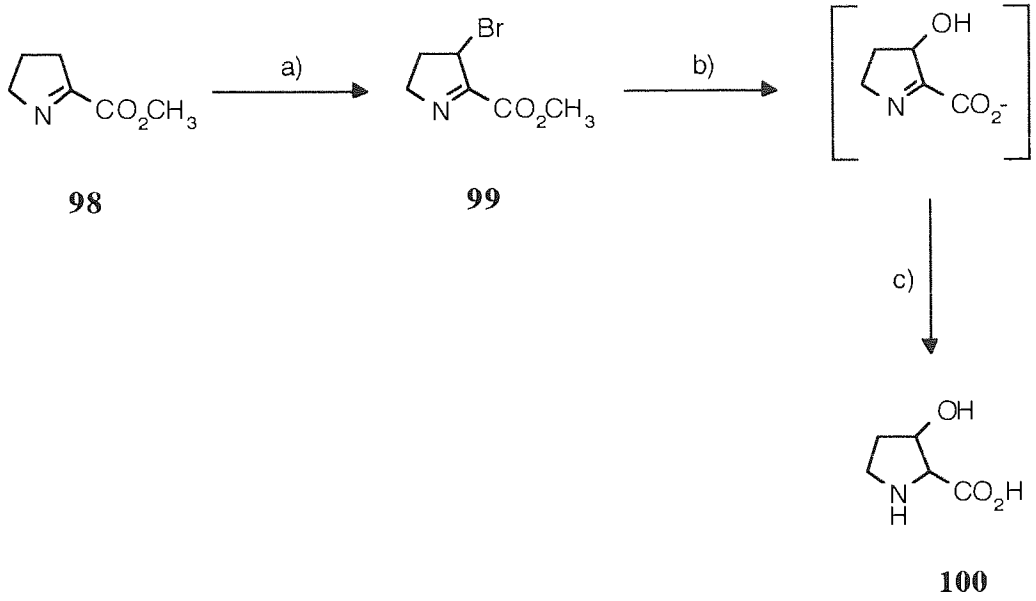
Anticipating potential problems with the use of enzymes, which require exacting conditions, it was decided to search for a more feasible, chemical synthetic method.



Scheme 5.2 Synthesis of Oxoesters *via* the Dieckmann Cyclisation

5.1.2 Synthesis *via* 1,2-dehydroproline methyl ester

In 1979, Häusler and Schmidt[86,87] reported the synthesis of 3-hydroxyproline from 1,2-dehydroproline methyl ester (**Scheme 5.3**). The method, however, resulted in four stereoisomers which would require separation.



98 **99** **100**

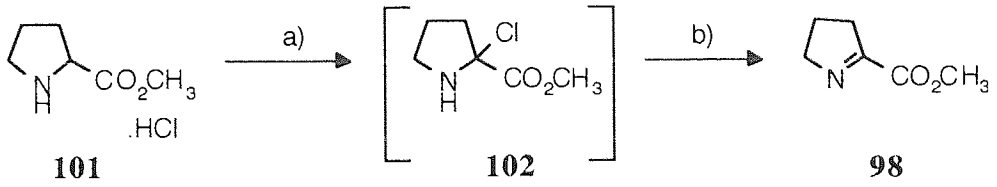
a) NBS, CCl_4, reflux; **b**) NaOH; **c**) $NaBH_4$.

Scheme 5.3 Häusler and Schmidt Synthesis of 3-Hydroxyproline

1,2-Dehydroproline methyl ester (**98**) was subjected to bromination by reaction with *N*-bromosuccinimide (NBS) at reflux temperature (77 °C) in carbon tetrachloride, affording derivative (**99**) in ~80 % yield. Nucleophilic substitution by the hydroxide ion on **99** followed by sodium borohydride reduction resulted in racemic 3-hydroxyproline (**100**).

The synthesis of 1,2-dehydroproline methyl ester (**98**) from L-proline methyl ester. hydrochloride (**101**) was cited by Poisel and Schmidt[88] (**Scheme 5.4**) in 1975 and later used successfully by Shin *et al.*[89]

Poisel halogenated L-proline methyl ester (**101**) by reaction with *tert*-butylhypochlorite, to produce intermediate (**102**). Reaction of **102** with 1,8-diazabicyclo[5.4.0]undec-5-ene (DBU) at 0 °C produced 1,2-dehydroproline methyl ester (**98**).



a) *t*-BuOCl, Et_3N, 0 °C; **b**) DBU, 0 °C.

Scheme 5.4 Synthesis of 1,2-Dehydroproline Methyl Ester

Bicyclic amidine, DBU, (**Figure 5.3**) is a strong, hindered amine base known to be a good reagent for the dehydrohalogenation of alkyl halides,[90] *ie.* the elimination of HX from an alkyl halide, especially in difficult cases.

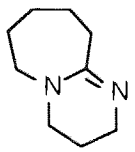
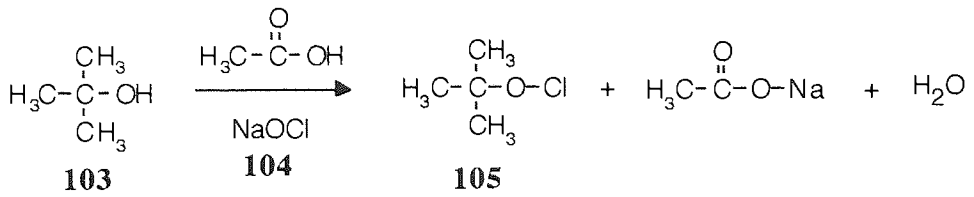


Figure 5.3 DBU

It was decided to attempt this route and so *t*-butyl hypochlorite (**105**) was synthesised according to the procedure of Mintz and Walling (**Scheme 5.5**).[91] *t*-Butyl alcohol (**103**) and glacial acetic acid were added, under subdued lighting, at 0 °C to a stirred solution of commercial household bleach (**104**). Stirring was continued for 3 minutes before separating the organic and aqueous layers and washing the organics with 10 % sodium carbonate solution. Following drying and concentration *in vacuo*, *t*-butyl hypochlorite was afforded as a yellow oil.



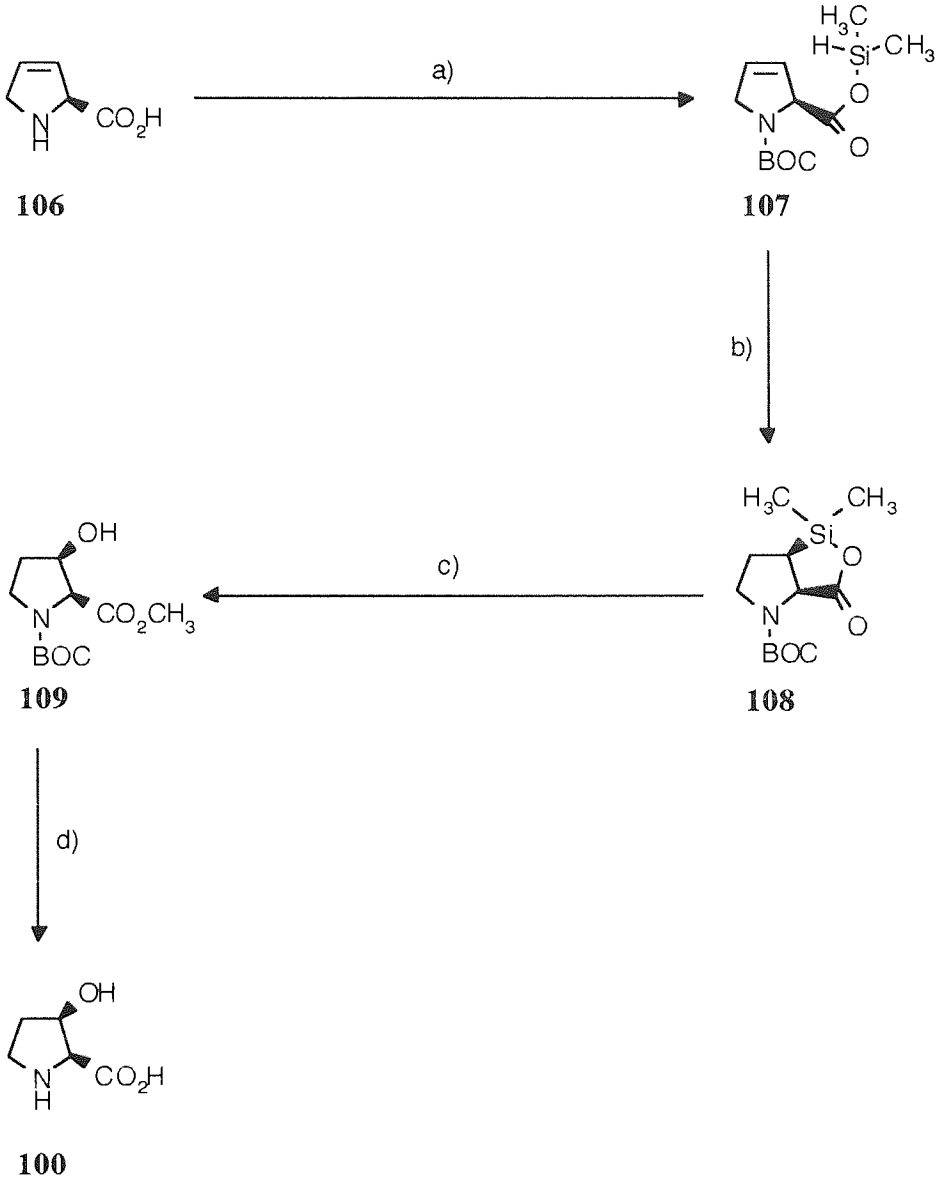
Scheme 5.5 Synthesis of *t*-Butyl Hypochlorite

Triethylamine was added to a solution of proline methyl ester. hydrochloride (**101**) (**Scheme 5.4**) in diethyl ether and stirred at ambient temperature for 2 hours. The $Et_3N.HCl$ salt which had precipitated was removed by filtration and the filtrate cooled to 0 °C. *tert*-BuOCl (**105**) was added dropwise to the resulting solution, then 1,8-diazabicyclo[5.4.0]undec-5-ene (DBU) added at 0 °C over 30 minutes. The reaction was warmed to ambient temperature and stirred for a further 40 minutes. The insoluble material was removed by filtration and the filtrate concentrated *in vacuo* to afford a brown oil.

The oil, however, was shown to be a complex mixture of products and the search to find a suitable route continued.

5.1.3 Intramolecular Hydrosilylation

In 1995, Sibi *et al.*[92] synthesised *cis*-*N*-BOC-3-hydroxyproline methyl ester (**109**) from unsaturated acyloxysilanes *via* an intramolecular hydrosilylation reaction. The method involved preparation of silyl ester (**107**) from 3,4-dehydroproline (**106**), followed by intramolecular hydrosilylation affording bicyclic intermediate (**108**). Conversion of the C-Si bond to a C-O bond with retention of configuration (**Scheme 5.6**) and deprotection of methyl ester (**109**) resulted in the required *cis*-3-hydroxyproline (**100**).



a) **i.** BOC-ON, TEA, MeOH, THF, H_2O, 2 h; **ii.** $(Me_2SiH)_2NH$, $(NH_4)_2SO_4$, reflux, 6 h; **b)** $PtCODCl_2$, DME, reflux, 30 min; **c)** **i.** H_2O_2, K_2CO_3, KHF_2, MeOH, reflux, 6 h; **ii.** CH_2N_2, Et_2O; **d)** **i.** DCM, TFA, RT, 2 h; **ii.** Ion exchange.

Scheme 5.6 Sibi Synthesis of *cis*-3-Hydroxyproline

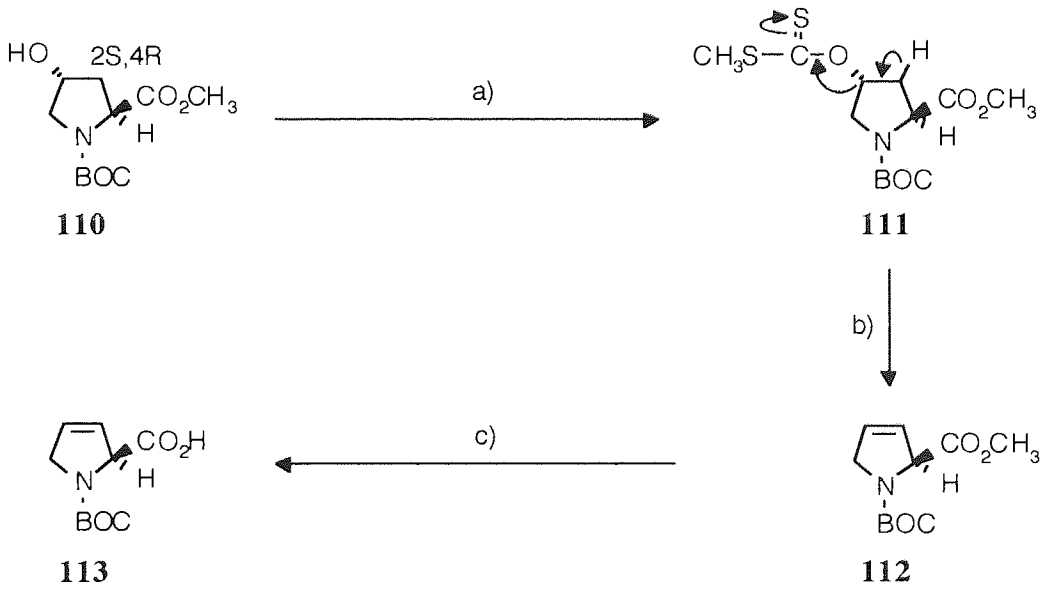
This method, however, most notably required the precursor 3,4-dehydroproline, itself a commercially available, though costly, intermediate. Attention was turned towards the synthesis of 3,4-dehydroproline.

5.2 3,4-DEHYDROPROLINE

According to the literature, synthesis of 3,4-dehydroproline was in itself a challenging task. Robertson and Witkop[93] originally synthesised 3,4-dehydroproline *via* an enzyme reaction on 3,4-dehydroproline amide but this required the undesirable resolution of racemic products. A stereospecific approach was necessary.

5.2.1 S-Methyl xanthogenate Intermediate

In 1980, Grogg *et al.*[94] succeeded in synthesising *N*-BOC-L-3,4-dehydroproline (**113**) in 70% yield *via* an S-methyl xanthogenate intermediate (**111**) (**Scheme 5.7**). Methyl xanthanates are readily prepared by reaction of alcohols with NaOH and CS_2 to afford RO-CS-SNa. Treatment with MeI results in RO-CS-SMe. *N*-BOC-L-4-hydroxyproline methyl ester (**110**) was converted into the S-methyl xanthogenate (**111**), which on Tschugaeff (Chugaev) pyrolysis gave the protected 3,4-didehydro ester (**112**). Hydrolysis of **112** afforded the required *N*-BOC-L-3,4-dehydroproline (**113**).



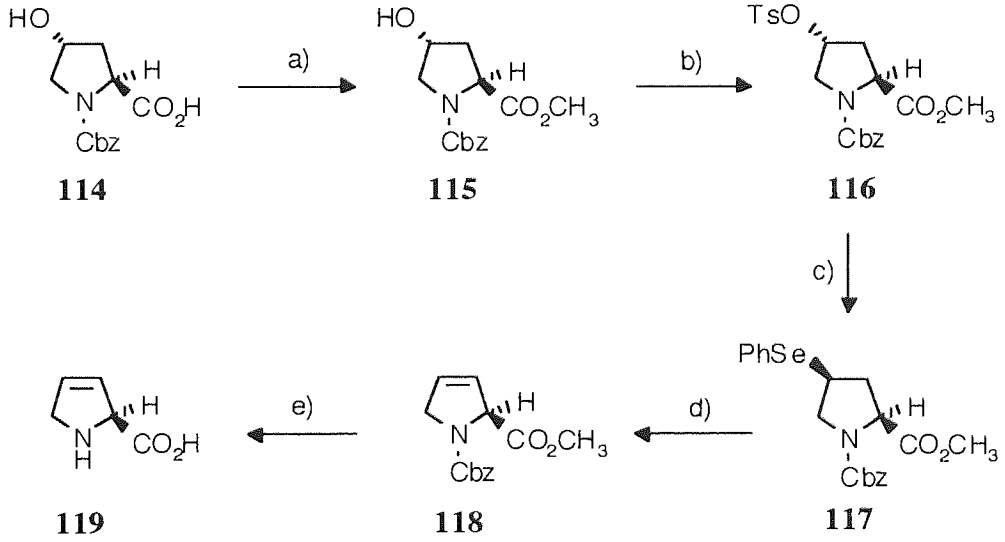
a) tetrabutylammonium hydrogen sulphate, CS_2, C_6H_6, NaOH, MeI; **b**) Chugaev Pyrolysis; **c**) H_2O/Dioxane, 50 % NaOH.

Scheme 5.7 Grogg Synthesis of *N*-BOC-L-3,4-Dehydroproline

The temperatures required to pyrolyse xanthanates are lower than for ordinary esters (100 - 250 °C as opposed to 300 - 550 °C), minimising the possibility for isomerization of the resulting alkene.

5.2.2 Selenoxide Elimination

In 1992, encouraged by the success of Grogg *et al.* in their conversion of (2*S*,4*R*)-*N*-*tert*-butoxycarbonyl-4-hydroxyproline methyl ester, *via* a Chugaev elimination, Rueger and Benn[95] decided to attempt a selenoxide elimination, hoping to increase regioselectivity from the room temperature reaction. Cbz protected 4-hydroxyproline (**114**) (**Scheme 5.8**) was esterified using diazomethane to afford methyl ester (**115**). Activation of the hydroxyl by conversion to the tosylate (**116**) facilitated introduction of the phenylselenide (**117**) which on elimination and subsequent deprotection afforded the target, (*S*)-3,4-dehydroproline (**119**).



a) EtOH, diazomethane, Et_2O; **b**) pyridine, *p*-toluenesulphonyl chloride; **c**) diphenyldiselenide, EtOH/$NaBH_4$, reflux, 2.5 h; **d**) pyridine, DCM, H_2O_2, RT, 1.5 h; **d**) CH_3CN, TMSI, reflux, 20 h.

Scheme 5.8 Rueger Synthesis of 3,4-Dehydroproline

This procedure looked promising but it was still hoped a better alternative may be found.

5.3 ATTEMPTED SYNTHESIS OF *cis*-3-HYDROXYPROLINE

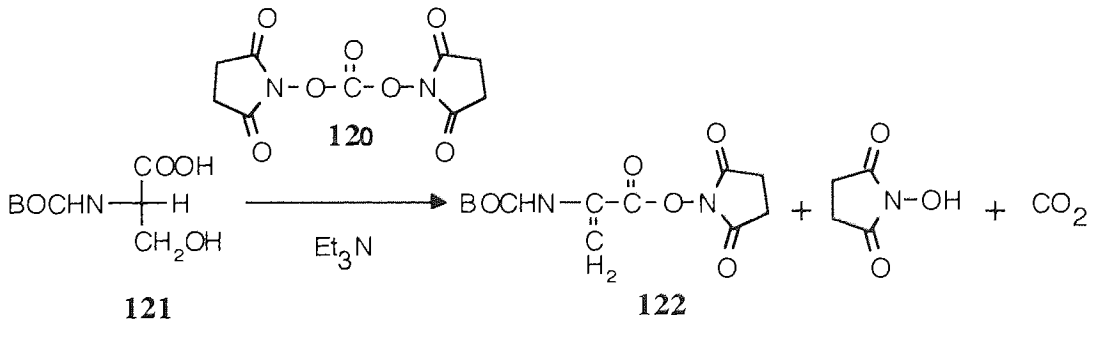
Since all published procedures for the synthesis of 3,4-dehydroproline and *cis*-3-hydroxyproline involved cumbersome multi-step routes, it was hoped a simpler and more interesting alternative may be found.

5.3.1 β-Elimination of β-Hydroxyamino Acids

Ogura *et al.*[96] reported a method of direct elimination of β-hydroxyl groups and active ester formation from β-hydroxy α-amino acids using a one step reaction with disuccinimidyl carbonate (DSC) (**120**).[96]

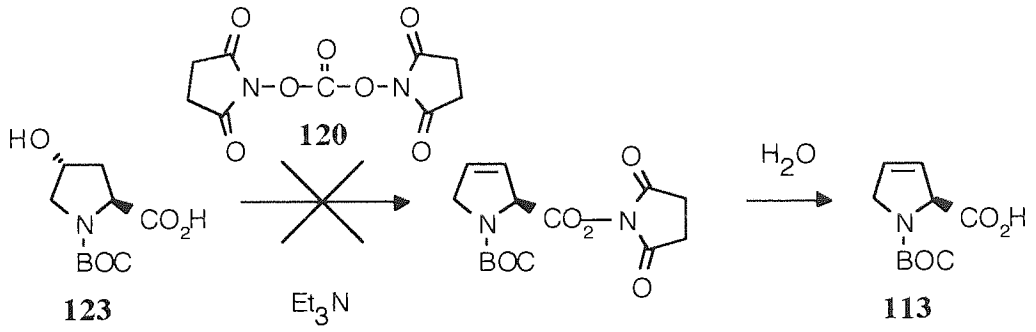
Ogura *et al.* had initially used DSC as a replacement for *N*,*N*-dicyclohexylcarbodimide (DCC) in the synthesis of optically pure active esters from which to prepare peptides. Unlike DCC, DSC was not irritating to skin and it readily decomposed to water soluble *N*-hydroxysuccinimide and carbon dioxide. They then discovered that using DSC on amino acids in the presence of equimolar triethylamine in acetonitrile resulted in β-elimination of the hydroxyl group.

Treatment of *N*-BOC-serine (**121**) with 2 equivalents of DSC in triethylamine gave the eliminated product (**122**) in quantitative yield (**Scheme 5.9**).



Scheme 5.9 Elimination using DSC

Extrapolating the result for serine to 4-hydroxyproline it was speculated that the same elimination reaction may occur to yield 3,4-dehydroproline (**113**) (**Scheme 5.10**). The resulting sp^2 hybridised carbon, however, would involve an undesirable increase in ring strain, highlighting a potential problem with the reaction.



Scheme 5.10 Attempted Elimination of 4-Hydroxyproline

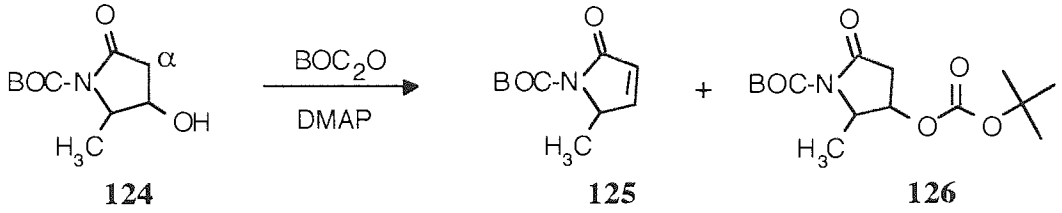
Prior to reaction, *trans*-4-hydroxyproline was protected using BOC-ON, according to a procedure cited by Itoh *et al.*[98] Dioxane and crystalline BOC-ON were added to a solution of *trans*-4-hydroxyproline and triethylamine in water at ambient temperature, and the mixture stirred for 3 hours. *tert*-BOC-*trans*-4-hydroxyproline (**123**) was afforded as a colourless oil in 28% yield. ^{1}H NMR clearly showed a singlet at 1.48 ppm corresponding to the 9 methyl protons belonging to the BOC group.

Despite numerous attempts and the presence of promising peaks in the alkene region of the proton NMR, *ie.* at 4.58 and 5.24 ppm respectively, the ^{13}C NMR spectrum failed to show any peaks between 100 and 150 ppm. Elimination had failed to occur.

5.3.2 BOC_2O Dehydration

Mattern[99] described the dehydration of 4-hydroxypyrrolidin-2-ones (**124**) using BOC_2O in the presence of DMAP at ambient temperature (**Scheme 5.11**) in THF, to afford a mixture of the desired unsaturated compound (**125**) and the intermediate

(**126**). After a reaction time of 48 hours, **125** was gained in 46 % yield. The yield could be increased to 62 % by using the carbobenzyloxy (Cbz) protecting group.

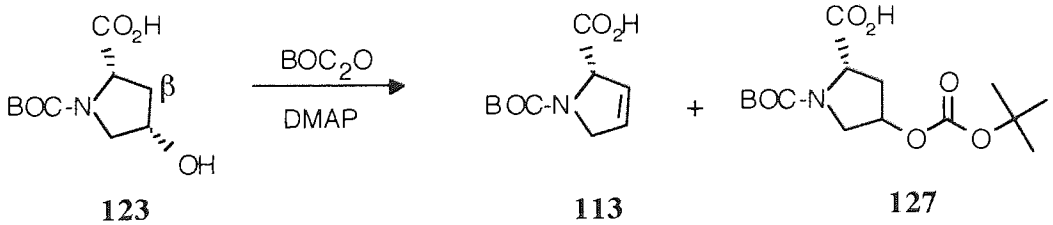


124 **125** **126**

Scheme 5.11 Synthesis of *N*-acylated Pyrrolidin-2-ones

Despite the fact that reagents used by Mattern possessed an active proton α to the carbonyl carbon and 4-hydroxyproline has a proton β to the carbonyl carbon, it was speculated that given more severe reaction conditions, some elimination may occur.

BOC_2O and DMAP were added to a stirred solution of *N*-BOC-*trans*-4-hydroxy-L-proline (**123**) in anhydrous THF (**Scheme 5.12**) and stirred at 60 °C for 6 days, followed by 19 days at ambient temperature. Following work-up, however, only intermediate (**127**) was found to be present, with none of the desired 3,4-dehydroproline (**113**) being produced.



123 **113** **127**

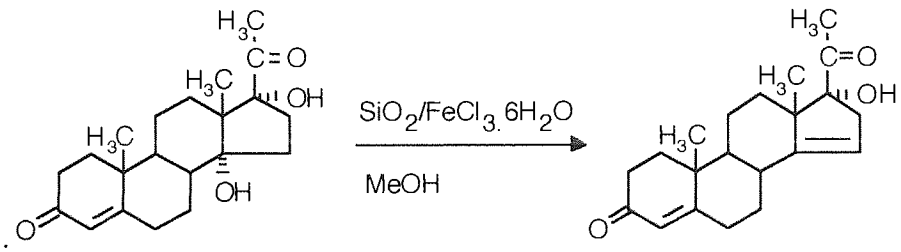
Scheme 5.12 Attempted Synthesis of 3,4-Dehydroproline using BOC_2O

5.3.3 Dehydration Reactions in Dry Media

Keinan and Mazur[100] carried out selective dehydration of alcohols using $FeCl_3$ adsorbed onto chromatographic silica gel. They discovered that when silica gel is

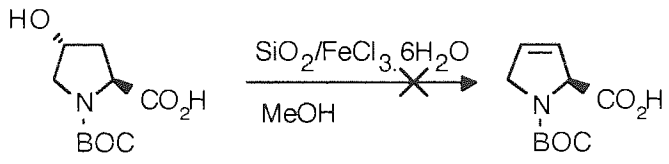
mixed with ~10% its weight of hydrated iron III chloride ($FeCl_3.6H_2O$) dissolved in a polar volatile solvent, followed by evaporation at ~50-60°C under high vacuum for 3 hours, a dry yellowish-brown powder is obtained. The powder was shown to be an efficient reagent for the dehydration of alcohols. The water content of the powder, as indicated by the colour, was shown to be vital to the success of the reaction. Too much water and the resulting bright yellow powder is inactivated, while excessive heating transforms the reagent into a dark brown powder in which the $FeCl_3$ is partly decomposed.

A series of complex compounds were successfully dehydrated using this method in >90 % yield, including steriods (**Scheme 5.13**).



Scheme 5.13 A Steriod Dehydrated by $FeCl_3.6H_2O$

Synthesis of 3,4-dehydroproline was attempted using this method (**Scheme 5.14**). Silica gel was mixed with $FeCl_3.6H_2O$ dissolved in methanol and the resulting mixture evaporated for 2 hours at 40°C under low vacuum, followed by 3 hours at 50-60 °C under high vacuum. The powder remained bright yellow. *t*-BOC-*trans*-4-hydroxyproline (**123**) in methanol was added and the solvent evaporated *in vacuo* at 50 °C. After allowing to stand for 1.5 hours, elution from the silica with methanol failed to yield any of the desired eliminated product (**113**).



123 **113**

Scheme 5.14 Attempt at Dehydrating 4-Hydroxyproline using $FeCl_3.6H_2O$

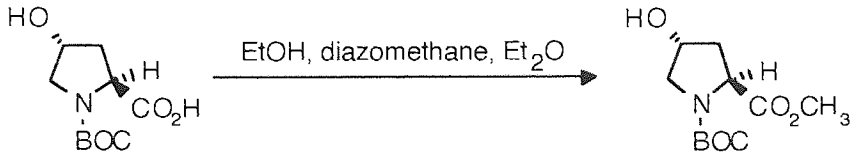
The reaction was repeated, this time heating the reagent under high vacuum at 50-60 °C for 11 hours. The powder remained bright yellow but despite allowing the substrate to react for 48 hours, no product was obtained.

In a final attempt the silica gel/$FeCl_3$/methanol mixture was distilled to try and remove all remaining water. Heating was stopped when the powder became brownish-yellow but analysis of the eluted products again failed to yield any of the desired 3,4-dehydroproline. ^{1}H NMR showed no peaks between 4 and 6 ppm, although numerous other peaks indicated that at least on this occasion the sample contained organics, though a complex mixture .

5.4 A RETURN TO THE LITERATURE

Due to the failure of the above attempted routes, it was decided that the most promising of the literature methods for the synthesis of *cis*-3-hydroxyproline was that of Sibi *et al.*,[85] who prepared *cis*-*N*-BOC-3-hydroxyproline methyl ester *via* intramolecular hydrosilylation from 3,4-dehydroproline (**Scheme 5.6**).

The first step, esterification of *N*-BOC-*trans*-4-hydroxyproline (**123**) (**Scheme 5.15**), was carried out using a 2M solution of (trimethylsilyl)diazomethane, to afford methyl ester (**110**) in 62 % yield. Proton NMR indicated a methyl peak at 3.67 ppm, while FTIR showed an absorption at 1751 cm^{-1}.



123 **110**

Scheme 5.15 Esterification of *trans-N*-BOC-4-Hydroxyproline

At this point, however, Hoffmann La-Roche published their total synthesis and attention was turned to analogues of cyclothialidine.

5.5 CONCLUSION

Due to the complex multi-step routes to *cis*-3-hydroxyproline cited in the literature, alternative methodology was applied.

In an effort to produce target intermediate, 3,4-dehydroproline, elimination of *trans*-4-hydroxyproline was attempted using DSC and dehydration attempted with BOC_2O, both without success.

Dehydration reactions using hydrated iron (III) chloride adsorbed onto silica gel also failed.

As our attention turned back to literature methods, the total synthesis of cyclothialidine was published and our efforts became focussed on a cell permeable derivative, with no necessity for the 3-hydroxyproline based side chain.

Chapter 6:
Experimental

5.1 REAGENTS

All reagents were used as supplied unless otherwise stated.

COMPOUND	RMM	SUPPLIER
acetic anhydride	102.09	Aldrich
AIBN	164.22	Aldrich
benzoyl peroxide	242.23	Aldrich
N-(*t*-BOC)-L-serine	205.2	Sigma Peptides
BOC-ON	246.27	Aldrich
N-bromosuccinimide	177.99	Aldrich
tert-butyldimethylsilyl chloride	150.73	Aldrich
cholesteryl chloroformate	449.12	Lancaster
L-cysteine methyl ester.HCl	171.6	Sigma Peptides
DCC	206.33	Aldrich
dibromotriphenylphosphorane	422.11	Aldrich
DEAD (40% in toluene)	174.16	Aldrich
3,4-dihydro-2H-pyran	84.12	Aldrich
3,5-dihydroxybenzoic acid	154.12	Aldrich
N,*N*-diisopropylethylamine	129.25	Aldrich
dimethylamine (40% aq.)	45.09	Aldrich
2,6-dimethylbenzoic acid	150.18	Aldrich
3,5-dinitro-*o*-toluic acid	226.15	Aldrich
2,2'-dithiobis[4-(*tert*-butyl)-1-isopropyl-1H-imidazole	394.65	Aldrich
DMAP	122.17	Lancaster

DSC	256.17	Aldrich
ethyl 4-bromocrotonate	193.05	Aldrich
ethyl propionylacetate	144.17	Aldrich
formaldehyde (37% aq.)	30.03	Aldrich
trans-4-hydroxy-L-proline	131.13	Aldrich
ron (III) chloride.$6H_2O$	180.36	BDH
isobutyl chloroformate	136.58	Aldrich
lithium aluminium hydride	37.95	Aldrich
LDA (2M in hexanes)	107.13	Aldrich
methyl iodide	141.94	Aldrich
4-methylmorpholine	101.15	Aldrich
4-nitrobenzyl bromide	216.04	Aldrich
oxalyl chloride	126.93	Aldrich
Pd/C (10%)	-	Aldrich
potassium carbonate	138.21	Aldrich
PyBOP	520.40	Aldrich
pyridinium *p*-toluene sulphonate	251.31	Aldrich
sodium hydride	24.00	Aldrich

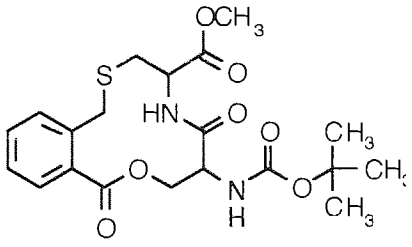
TBAF (1M in THF)	261.47	Aldrich
1,1,3,3-tetramethylguanidine	115.18	Aldrich
TFA	114.02	Aldrich
thionyl chloride	118.97	Aldrich
tin (granular)	118.69	Aldrich
o-toluic acid	136.15	Aldrich
2,4,6-trichlorobenzoyl chloride	243.91	Lancaster
2,2,2-trichloroethanol	149.40	Aldrich
triethylamine	101.19	Aldrich
trimethylsilylchloride	108.64	Aldrich
triphenylphosphine	262.29	Aldrich
zinc chloride	136.28	Aldrich
zinc powder	65.37	BDH Chemicals

5.2 GENERAL METHODS

NMR spectra were recorded on a Bruker AC250 Spectrometer at ^{1}H (250.1 MHz) and ^{13}C (62.9 MHz). ^{13}C spectra were recorded using PENDANT,[101] - an alternative NMR technique giving a better signal to noise ratio than APT and showing quaternary carbons, unlike DEPT 135. CH_3's and CH's are shown as positive signals and CH_2's and quaternaries as negative. Chemical shifts are downfield of tetramethylsilane. Mass spectroscopic analysis was carried out on a Hewlett Packard 5989B MS engine with an HP 59987A API Electrospray LC/MS interface; the LC being an HP 1100 system with autosampler. Infrared spectra were recorded on a Mattson 3000 FTIR Spectrometer. Solid samples were prepared as KBr discs and liquids as thin films between sodium chloride plates. Melting points were determined on Gallenkamp apparatus and are uncorrected. Flash column chromatography was performed using Sorbsil C60 silica gel. TLC was carried out using aluminium backed Merck Silica Gel 60 F_{254} plates and visualised under UV (254 nm). Potassium permanganate was used where appropriate to develop TLC plates.

6.3 SYNTHETIC PREPARATIONS

6.3.1 Methyl (4*S*,7*S*)-7-[(*tert*-butoxycarbonyl)amino]-1,3,4,5,6,7,8,10-octahydro-6,10-dioxo-9,2,5-benzoxathiacyclododecine-4-carboxylate 34



2,2,2-Trichloroethyl 2-methylbenzoate 27: A solution of *o*-toluic acid **26** (10 g, 73 mmol) in thionyl chloride (16 cm^3, 220 mmol) was heated at reflux temperature (79 °C) for 45 minutes. On cooling to ambient temperature excess reagent was evaporated *in vacuo*, toluene (2 x 10 cm^3) added and the mixture evaporated to dryness. The resulting solid residue was dissolved in anhydrous DCM (50 cm^3) and 2,2,2-trichloroethanol (8.4 cm^3, 88 mmol) added. The yellowish mixture was cooled to 0 °C and a solution of Et_3N (12.6 cm^3) in DCM (19 cm^3) added over 10 minutes. After stirring at 0 °C for 10 minutes and ambient temperature for 3 h, the reaction mixture was washed consecutively with 30 cm^3 portions of 3M HCl, water, saturated aqueous Na_2CO_3 solution and saturated NaCl solution. The aqueous phases were back extracted with DCM (30 cm^3). The combined organic phases were dried ($MgSO_4$) and concentrated *in vacuo* to yield a yellow oil. Purification by column chromatography using EtOAc/hexane (1:20 v/v) as eluant afforded **27** (12.78 g, 65 %) as a white crystalline solid; mp 34-37 °C; IR (KBr disc): ν_{max} 711, 738, 783, 1041, 1078, 1263, 1146, 1728 cm^{-1}; ^1H NMR [$CDCl_3$]: δ 2.65 (3H, s, $C\underline{H}_3$), 4.95 (2H, s, $OC\underline{H}_2CCl_3$), 7.25-7.32 (2H, m, 2 x Ar-C<u>H</u>), 7.42-7.48 (1H, m, Ar-C<u>H</u>), 8.04-8.08 (1H, m, Ar-C<u>H</u>); ^{13}C NMR [$CDCl_3$]: δ 21.9 ($\underline{C}H_3$), 74.3 ($O\underline{C}H_2$), 95.0 ($CH_2\underline{C}Cl_3$), 125.9 (Ar-<u>C</u>H), 127.7 (Ar-<u>C</u>COO), 131.1 (Ar-<u>C</u>H), 131.8 (Ar-<u>C</u>H), 132.8 (Ar-<u>C</u>H), 141.1 (Ar-<u>C</u>CH_3), 165.5 (<u>C</u>=O); m/z 268 [M^+].

2,2,2-Trichloroethyl 2-bromomethylbenzoate 28: A stirred mixture of 2,2,2-trichloroethyl 2-methylbenzoate **27** (10.5 g, 39.3 mmol) and NBS (8.4 g, 47.1 mmol) in CCl_4 was heated at reflux temperature (77 °C) with light irradiation for 3 h. The solution was cooled to 0 °C and the resulting white precipitate removed by filtration. The filtrate was diluted with DCM (100 cm^3), washed with water (3 x 30 cm^3), dried ($MgSO_4$) and the solvent evaporated *in vacuo* to afford **28** (10.35 g, 77 %) as a yellow syrup; IR (liquid film): ν_{max} 570, 620, 720, 792, 1054, 1116, 1249, 1731 cm^{-1}; ^1H NMR [$CDCl_3$]: δ 4.97 (2H, s, $OC\underline{H}_2CCl_3$), 4.99 (2H, s, $C\underline{H}_2Br$), 7.39-8.22 (4H, m, Ar-C<u>H</u>); ^{13}C NMR [$CDCl_3$]: δ 31.1 ($\underline{C}H_2Br$), 74.3 ($O\underline{C}H_2$), 94.8 ($CH_2\underline{C}Cl_3$), 127.2 (Ar-<u>C</u>COO), 128.7 (Ar-<u>C</u>H), 131.7 (Ar-<u>C</u>H), 132.9 (Ar-<u>C</u>H), 133.4 (Ar-<u>C</u>H), 140.0 (Ar-<u>C</u>CH_3), 164.5 (<u>C</u>=O); m/z 345 $[M+H]^+$.

***N*-[*N*-(*t*-Butoxycarbonyl)-L-seryl]-L-cysteine methyl ester 31:** A suspension of L-cysteine methyl ester.HCl **29** (1.67 g, 9.8 mmol) and *N*-(*t*-butoxycarbonyl)-L-serine **30** (2 g, 9.8 mmol) in ACN (25 cm^3) was treated at 0 °C with 4-methylmorpholine (1.1 cm^3, 9.8 mmol). To the stirred solution was added dropwise at 0 °C over 30 minutes a solution of DCC (2 g, 9.8 mmol) in ACN (15 cm^3). After stirring the reaction for 3 h at 0 °C, the resulting white precipitate was removed by filtration and the filtrate evaporated *in vacuo*. The white oily residue so formed was dissolved in EtOAc (100 cm^3) and the solution washed consecutively with 0.5M HCl, water, 5% aqueous $NaHCO_3$ solution and saturated NaCl solution in 50 cm^3 portions. The organic layer was dried ($MgSO_4$) and the solvent evaporated *in vacuo* to yield a yellow oil. Purification by column chromatography using EtOAc/hexane (1:1 v/v) as eluant afforded **31** (1.78 g, 57 %) as a colourless oil; IR (liquid film): ν_{max} 1161, 1222, 1248, 1367, 1519, 1670, 1685, 1741 cm^{-1}; ^1H NMR [$CDCl_3$]: δ 1.44 (9H, s, BOC), 2.96-3.02 (2H, m, $C\underline{H}_2SH$), 3.08 (1H, m, O<u>H</u>), 3.63-3.69 (1H, m, $C\underline{H}CH_2OH$), 3.77 (3H, s, $OC\underline{H}_3$), 4.06-4.11 (2H, m, $C\underline{H}_2OH$); 4.14-4.21 (1H, m, S<u>H</u>); 4.80-4.87 (1H, m, $C\underline{H}CH_2SH$), 5.57-5.60 (1H, m, N<u>H</u>) 7.41 (1H, m, N<u>H</u>BOC); ^{13}C NMR [$CDCl_3$]: δ 26.4 ($\underline{C}H_2SH$), 28.2 ($\underline{C}H_3$), 52.9 (<u>C</u>HNH), 53.7 (<u>C</u>HNH), 54.9 ($O\underline{C}H_3$),

62.7 ($\underline{C}H_2OH$), 81.1 ($\underline{C}(CH_3)_3$), 156.9 ($\underline{C}OO$), 170.2 ($NH\underline{C}O$), 171.3 ($NH\underline{C}O$); m/z 323 $[M+H]^+$.

Methyl *N*-[*N*-(*tert*-butoxycarbonyl)-L-seryl]-*S*-{2-[(2,2,2)-trichloroethyl)carbonyl] benzyl}-L-cysteinate 32: To a solution of *N*-[*N*-(*t*-butoxycarbonyl)-L-seryl]-L-cysteine methyl ester **31** (3.10 g, 9.6 mmol) and 2,2,2-trichloroethyl 2-bromomethylbenzoate **28** (3.33 g, 9.6 mmol) in DCM (43 cm^3) was added dropwise at 0 °C, Et_3N (1.34 cm^3, 9.6 mmol) in DCM (5 cm^3). After stirring for 1 h at 0 °C and 3 h at ambient temperature, the reaction mixture was washed with 1M HCl (2 x 5 cm^3) and saturated NaCl solution (2 x 25 cm^3). The organic layer was dried ($MgSO_4$) and the solvent evaporated *in vacuo* to afford a pale yellow oil. Purification by column chromatography using EtOAc:hexane (1:1 v/v) as eluant yielded **32** (2.47g, 44 %) as a yellow oil; IR (liquid film): ν_{max} 1119, 1164, 1245, 1359, 1506, 1650, 1750, 3350 cm^{-1}; ^1H NMR [$CDCl_3$]: δ 1.44 (9H, s, BOC), 2.88-2.94 (3H, m, $SC\underline{H}_2$+$C\underline{H}OH$), 3.62-3.70 (2H, m, $ArC\underline{H}_2SH$), 3.72 (3H, s, $OC\underline{H}_3$), 4.07-4.12 (1H, m, $NHC\underline{H}$ (ser)), 4.22-4.27 (1H, m, $C\underline{H}OH$), 4.81(1H, m, $NHC\underline{H}$ (cys)), 4.97 (2H, s, $OC\underline{H}_2CCl_3$), 5.54 (1H, m, $N\underline{H}$); 7.26 (1H, m, $N\underline{H}$), 7.34-8.09 (4H, m, Ar-$C\underline{H}$); ^{13}C NMR [$CDCl_3$]: δ 28.2 ($C(\underline{C}H_3)_3$), 33.5 ($Ar\underline{C}H_2S$), 34.8 ($\underline{C}H_2S$), 51.8 ($O\underline{C}H_3$), 52.8 ($NH\underline{C}H$), 55.0 ($NH\underline{C}H$), 63.2 ($\underline{C}H_2OH$), 74.6 ($O\underline{C}H_2CCl_3$), 80.1 ($\underline{C}(CH_3)_3$), 103.2 ($OCH_2\underline{C}Cl_3$), 127.5 (Ar-$\underline{C}CO_2$), 127.7 (Ar-$\underline{C}CH_2S$), 131.4 (Ar-$\underline{C}H$), 131.8 (Ar-$\underline{C}H$), 133.0 (Ar-$\underline{C}H$), 140.3 (Ar-$\underline{C}CO$), 165.2 ($\underline{C}OO$), 170.9 ($\underline{C}OO$), 171.2 ($\underline{C}OO$); m/z 587 $[M^+]$, 623 $[M+2Na]^+$.

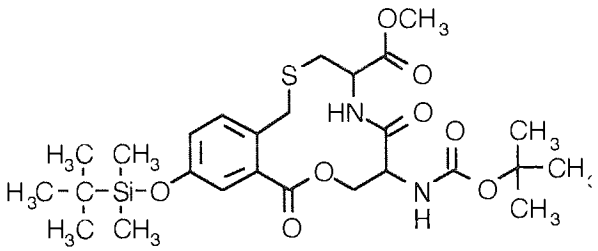
Methyl *N*-[*N*-(*tert*-butoxycarbonyl)-L-seryl]-*S*-(2-carboxybenzyl}-L-cysteinate 33: A mixture of methyl *N*-[*N*-(*tert*-butoxycarbonyl)-L-seryl]-*S*-{2-[(2,2,2)-trichloroethyl)carbonyl]benzyl}-L-cysteinate **32** (2.19 g, 3.8 mmol), THF (50 cm^3), 1M phosphoric acid (12.5 cm^3), 1M aqueous sodium dihydrogen phosphate solution (12.5 cm^3) and zinc powder (3.7 g) was stirred at ambient temperature for 2.5 h. The mixture was filtered and the insoluble material washed with EtOAc (2 x 25 cm^3) and

water (2 x 10 cm^3). The organic layer was dried ($MgSO_4$) and the solvent evaporated *in vacuo* to afford a colourless solidifying oil. Purification by column chromatography, using EtOAc/hexane (3:1 v/v) as eluant afforded **33** (1.04 g, 61 %) as a white powder; mp 120-124 °C; IR (KBr disc): ν_{max}1075, 1166, 1248, 1371, 1399, 1549, 1660, 3407 cm^{-1}; ^{1}H NMR [$CDCl_3$]: δ 1.40 (9H, s, BOC), 2.85-2.98 (2H, br, $SC\underline{H}_2$), 3.66 - 3.74 (6H, m, $OC\underline{H}_3$, Ar-$C\underline{H}_2S$, C<u>H</u>OH), 3.99-4.12 (1H, d, C<u>H</u>OH), 4.27-4.45 (2H, m, C<u>H</u>NH (ser), C<u>H</u>OH), 4.64-4.67 (1H, br, C<u>H</u>NH (cys)), 5.97 (1H, m, N<u>H</u>), 7.24-7.39 (3H, m, Ar-CH), 7.77 (1H, m, N<u>H</u>), 7.93 (1H, m, Ar-C<u>H</u>); ^{13}C NMR [$CDCl_3$]: δ 28.2 ($C(\underline{C}H_3)_3$), 33.5 ($Ph\underline{C}H_2S$), 35.8 ($\underline{C}H_2S$), 52.0 ($O\underline{C}H_3$), 52.5 (<u>C</u>HNH), 55.5 (<u>C</u>HNH), 62.4 ($\underline{C}H_2OH$), 80.6 ($\underline{C}(CH_3)_3$), 127.5 (Ar-<u>C</u>H), 129.4 (Ar-<u>C</u>CH_2S), 130.5 (Ar-<u>C</u>H), 130.7 (Ar-<u>C</u>H), 132.9 (Ar-<u>C</u>H), 139.0 (Ar-<u>C</u>CO), 156.2 (<u>C</u>OO), 170.0 (<u>C</u>OO), 170.9 (<u>C</u>OO), 171.2 (<u>C</u>OO); m/z 479 [*M*+Na]$^+$.

Methyl (4*S*,7*S*)-7-[(*tert*-butoxycarbonyl)amino]-1,3,4,5,6,7,8,10-octahydro-6,10-dioxo-9,2,5-benzoxathiacyclododecine-4-carboxylate 34: To a stirred solution of methyl *N*-[*N*-(*tert*-butoxycarbonyl)-L-seryl]-*S*-(2-carboxybenzyl}-L-cysteinate **33** (502 mg, 1.1 mmol) in DCM (10 cm^3) at 0 °C was added *N*,*N*-diisopropylethylamine (0.19 cm^3, 1.1 mmol) followed by PyBOP (572 mg, 1.1 mmol). The resulting mixture was stirred at 0 °C for 2 h, then additional *N*,*N*-diisopropylethylamine (0.19 cm^3, 1.1 mmol) was added. Stirring was continued at 0 °C for 2 h. The mixture was diluted with DCM (10 cm^3) and washed with saturated NaCl solution (2 x 25 cm^3). The solution was dried ($MgSO_4$) and the solvent evaporated *in vacuo* to afford a yellow solid. Purification by column chromatography, using EtOAc/hexane (3:1 v/v) as eluant yielded **34** (140 mg, 29 %) as a white crystalline solid; mp 206-207 °C (lit. 206 °C); IR (KBr disc): ν_{max} 1065, 1108, 1163, 1257, 1659, 1708, 1729, 3338 cm^{-1}; ^{1}H NMR [$CDCl_3$]: δ 1.44 (9H, s, BOC), 3.17-3.23 (2H, m, $C\underline{H}_2S$), 3.73 (3H, s, $OC\underline{H}_3$), 4.03 (2H, m, $PhC\underline{H}_2S$), 4.54-4.58 (2H, m, $C\underline{H}_2O$); 4.83 (1H, br, C<u>H</u>NH (ser)), 4.92-4.97 (1H, dd, C<u>H</u>NH (cys)), 5.69 (1H, br, N<u>H</u>), 7.28-7.82 (5H, m, N<u>H</u>+Ar-CH); ^{13}C NMR [$CDCl_3$]: δ 28.1 ($C(\underline{C}H_3)_3$), 34.6 ($Ph\underline{C}H_2S$), 36.3 ($\underline{C}H_2S$), 51.7 (<u>C</u>HNH),

52.8 (O<u>C</u>H$_3$), 66.5 (<u>C</u>H$_2$O), 80.5 (<u>C</u>(CH$_3$)$_3$), 127.6 (Ar-<u>C</u>H), 129.8 (Ar-<u>C</u>CH$_2$S), 131.3 (Ar-<u>C</u>H), 132.4 (Ar-<u>C</u>H), 137.2 (Ar-<u>C</u>H), 139.2 (Ar-<u>C</u>CO), 155.2 (<u>C</u>OO), 169.1 (<u>C</u>OO), 169.8 (<u>C</u>OO), 170.5 (<u>C</u>OO); m/z 439 [M^+], 457 [M+NH$_4$]$^+$.

6.3.2 Methyl (4*S*,7*S*)-7-[(*tert*-butoxycarbonyl)amino]-12[(*tert*-butyl) dimethyl silyloxy]-1,3,4,5,6,7,8,10-octahydro-6,10-dioxo-9,2,5-benzoxathiacyclododecine-4-carboxylate 131



Sodium 6-methyl-3-sulphobenzoate 47: A mixture of *o*-toluic acid **26** (50.43 g, 0.37 mol) and conc. H_2SO_4 was heated at 160 °C for 2.5 h. Following the addition of water (15 cm^3), the solution was allowed to stand at ambient temperature overnight. The resulting crystals were dissolved in water (180 cm^3) and poured into saturated NaCl solution (500 cm^3) at 100 °C. On the addition of powdered NaCl (12.5 g) **47** (78.09 g, 98 %) formed as an off-white precipitate and was collected by filtration; mp >300 °C; IR (KBr disc): ν_{max} 594, 1035, 1182, 1228, 1251, 1708, 3421, 3473 cm^{-1}; ^{1}H NMR [d_6-DMSO]: δ 2.50 (3H, s, C<u>H</u>$_3$), 7.24-7.27 (1H, d, Ar-C<u>H</u>), 7.62-7.66 (1H, dd, Ar-C<u>H</u>), 8.07-8.08 (1H, d, Ar-C<u>H</u>); ^{13}C NMR [d_6-DMSO]: δ 21.2 (<u>C</u>H$_3$), 127.7 (Ar-<u>C</u>H), 128.8 (Ar-<u>C</u>H), 129.8 (Ar-<u>C</u>CO$_2$), 131.2 (Ar-<u>C</u>H), 139.7 (Ar-<u>C</u>SO$_3$H), 145.8 (Ar-<u>C</u>CH$_3$), 168.5 (C=O); m/z 215 [M-H]$^-$.

3-Hydroxy-6-methylbenzoic acid 48: Sodium 6-methyl-3-sulphobenzoate **47** (26 g, 0.1 mol) was dissolved in conc. NaOH solution (10 cm^3) and heated to 100 °C. The solution was mixed with powdered NaOH (10 g) into a paste which solidified on cooling. Small pieces of this product were added in portions to fused KOH (26 g) at

180-200 °C and the resulting mixture stirred at 180-200 °C for 4 h. On cooling, the mass was dissolved in water (140 cm^3), insoluble material removed by filtration and the filtrate acidified with conc. HCl. The resulting precipitate was recrystallised from water and dried to yield **48** (12.79 g, 70 %) as white needles; mp >300 °C; IR (KBr disc): ν_{max} 1078, 1230, 1274, 1311, 1454, 1678, 1697, 3272 cm^{-1}; ^1H NMR [d_6-DMSO]: δ 2.37 (3H, s, $C\underline{H}_3$), 6.80-6.85 (1H, dd, Ar-C<u>H</u>), 7.04-7.07 (1H, d, Ar-C<u>H</u>), 7.21-7.23 (1H, d, Ar-C<u>H</u>), 9.54 (1H, s, O<u>H</u>), 12.75 (1H, s, $CO_2\underline{H}$); ^{13}C NMR [d_6-DMSO]: δ 20.5 ($\underline{C}H_3$), 116.7 (Ar-<u>C</u>H), 119.0 (Ar-<u>C</u>H), 129.1 (Ar-<u>C</u>CH_3), 131.1 (Ar-<u>C</u>CO_2H), 132.7 (Ar-<u>C</u>H), 155.1 (Ar-<u>C</u>OH), 168.8 (<u>C</u>=O); m/z 152 [M^+].

***tert*-Butyldimethylsilyl 6-methyl-3-*tert*-butyldimethylsiloxybenzoate 56:** To a stirred solution of 3-hydroxy-6-methylbenzoic acid **48** (1 g, 6.6 mmol), Et_3N (2.2 cm^3, 15.8 mmol) and DMAP (32 mg, 0.26 mmol) in anhydrous DCM (4.4 cm^3) at -78 °C was added dropwise a solution of *tert*-butyldimethylsilyl chloride (2.2 g, 14.7 mmol) in anhydrous DCM (3.5 cm^3). Stirring was continued at -78 °C for 30 minutes, then at ambient temperature for 18 h. Following removal of the resulting amine hydrochloride by filtration, the solvent was evaporated *in vacuo* to afford **56** (1.91g, 76 %) as a yellow oil; IR (thin film): ν_{max} 829, 871, 1301, 1228, 1257, 1703, 2952, 2929 cm^{-1}; ^1H NMR [$CDCl_3$]: δ 0.12 (6H, s, $Si(C\underline{H}_3)_2$), 0.29 (6H, s, $Si(C\underline{H}_3)_2$), 0.84 (9H, s, $C(C\underline{H}_3)_3$), 0.91–0.94 (9H, d, $C(C\underline{H}_3)_3$), 2.46 (3H, s, $C\underline{H}_3$), 6.80-6.84 (1H, dd, Ar-C<u>H</u>), 6.99-7.03 (1H, d, Ar-C<u>H</u>), 7.39-7.40 (1H, d, Ar-C<u>H</u>); m/z 381 $[M+H]^+$.

***tert*-Butyldimethylsiloxy-6-methylbenzoyl chloride 57:** To a stirred solution of *tert*-butyldimethylsilyl 6-methyl-*tert*-butyl-dimethylsiloxybenzoate **56** (5.41 g, 14.2 mmol) and 10 drops of DMF in anhydrous DCM (20 cm^3) at 0 °C, was added dropwise oxalyl chloride (2 cm^3, 22.7 mmol). Stirring was continued at 0 °C for 30 minutes, then at ambient temperature overnight. Solvent evaporated *in vacuo* to afford **57** (6.61 g, 68 %) as an off-white powder; mp 84-86 °C; IR (KBr disc): ν_{max} 802, 1033, 1477, 1768, 2487, 2679, 2736, 2971 cm^{-1}; ^1H NMR [$CDCl_3$]: δ 0.34 (6H, s, $Si(C\underline{H}_3)_2$),

0.95–0.98 (9H, d, $C(C\underline{H}_3)_3$), 2.45 (3H, s, $C\underline{H}_3$), 6.99-7.00 (1H, dd, Ar-$\underline{C}$H), 7.10-7.13 (1H, d, Ar-$\underline{C}$H), 7.66-7.67 (1H, d, Ar-$\underline{C}$H); m/z 281 $[M+H]^+$.

2,2,2-Trichloroethyl 3-(*tert*-butyldimethylsilyloxy)-6-methylbenzoate 58: To a suspension of *tert*-butyldimethylsiloxy-6-methylbenzoyl chloride **57** (6.31 g, 22.2 mmol) in anhydrous DCM (20 cm^3) was added 2,2,2-trichloroethanol (2.6 cm^3, 26.6 mmol), at ambient temperature, whereupon the solid dissolved. The solution was cooled to 0 °C and a solution of Et_3N (5 cm^3) in DCM (6 cm^3) added over 10 minutes. After stirring for 10 minutes at 0 °C and at ambient temperature for 48 h, the resulting solid was removed by filtration and the solvent evaporated *in vacuo* to afford **58** (8.17 g, 93 %) as a yellow oil; IR (thin film): ν_{max} 785, 843, 854, 1211, 1255, 1736, 2929, 2952 cm^{-1}; ^1H NMR [$CDCl_3$]: δ 0.13-0.18 (6H, d, $Si(C\underline{H}_3)_2$), 0.92-0.95 (9H, d, $C(C\underline{H}_3)_3$), 2.53 (3H, s, $C\underline{H}_3$), 4.90 (2H, s, $OC\underline{H}_2CCl_3$), 6.94-6.95 (1H, dd, Ar-C$\underline{H}$), 7.09-7.12 (1H, d, Ar-C$\underline{H}$), 7.53-7.54 (1H, d, Ar-C$\underline{H}$); m/z 398 $[M^+]$.

2,2,2-Trichloroethyl 6-bromomethyl-3-(tert-butyldimethylsiloxy) benzoate 128: A stirred mixture of 2,2,2-trichloroethyl 3-(*tert*-butyldimethylsilyloxy)-6-methylbenzoate **58** (8.11 g, 20.4 mmol) and *N*-bromosuccinimide, (4.4 g, 24.5 mmol) in CCl_4 (25 cm^3), was heated at reflux temperature (77 °C) with light irradiation for 3 h. The solution was cooled in an ice-bath and the resulting brown/black solid removed by filtration. The filtrate was diluted with DCM (75 cm^3), washed carefully with water (2 x 25 cm^3), dried ($MgSO_4$) and the solvent evaporated *in vacuo* to afford **128** (4.96 g, 51 %) as a brown oil; IR (thin film): ν_{max} 1205, 1307, 1403, 1448, 1502, 1625, 1726, 3197 cm^{-1}; ^1H NMR [$CDCl_3$]: δ 0.16 (6H, s, $Si(C\underline{H}_3)_2$), 0.90 (9H, s, $C(C\underline{H}_3)_3$), 4.87 (2H, s, $C\underline{H}_2Br$), 4.88 (2H, s, $OC\underline{H}_2CCl_3$), 6.93-6.95 (1H, dd, Ar-C$\underline{H}$), 7.27-7.30 (1H, d, Ar-C$\underline{H}$), 7.51-7.52 (1H, d, Ar-C$\underline{H}$); m/z 474 $[M^+]$.

Methyl *N*-[*N*-(*tert*-butoxycarbonyl)-L-seryl-*S*-{4-(*tert*-butyldimethylsilyloxy]-2-[2,2,2-trichloroethyl]benzyl}-L-cysteinate 129: To a solution of 2,2,2-trichloroethyl 6-

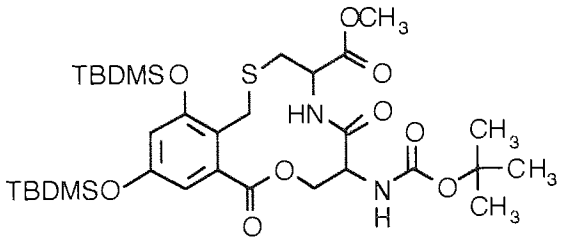
bromomethyl-3-(*tert*-butyldimethylsiloxy) benzoate **128** (1.52 g, 3.2 mmol) and *N*-[*N*-(*t*-butoxycarbonyl)-L-seryl]-L-cysteine methyl ester **31** (1 g, 3.2 mmol) in anhydrous DCM (14 cm^3) was added dropwise at 0 °C, Et_3N (0.45 cm^3, 3.2 mmol) in DCM (1.6 cm^3). After stirring for 1 h at 0 °C and overnight at ambient temperature, the reaction mixture was washed with 5% aqueous $NaHCO_3$ solution (2 x 10 cm^3) and saturated NaCl solution (2 x 10 cm^3) and the aqueous extracts back-extracted with DCM (10 cm^3). The organics were dried ($MgSO_4$) and the solvent evaporated *in vacuo* to afford a brown oil. Purification by column chromatography, using EtOAc:hexane (1:1) as eluant, yielded **129** (760 mg, 33 %) as pale yellow crystals; mp 66-69 °C; IR (KBr disc): ν_{max} 781, 837, 1209, 1498, 1683, 1737, 2929, 2952 cm^{-1}; ^1H NMR [$CDCl_3$]: δ 0.21 (6H, s, $Si(C\underline{H}_3)_2$), 0.97 (9H, s, $C(C\underline{H}_3)_3$), 1.43 (9H, s, BOC), 2.82-2.88 (3H, m, $SC\underline{H}_2$, $CH_2O\underline{H}$), 3.61-3.72 (6H, m, $OC\underline{H}_3$, Ar-$C\underline{H}_2S$, C<u>H</u>OH), 4.04-4.32 (2H, m, NHC<u>H</u> (ser), C<u>H</u>OH), 4.74-4.80 (1H, m, NHC<u>H</u> (cys)), 4.95 (2H, m, $OC\underline{H}_2CCl_3$), 5.60 (1H, br, N<u>H</u>), 6.92-7.00 (1H, dd, Ar-C<u>H</u>), 7.23-7.24 (1H, d, Ar-C<u>H</u>), 7.29-7.32 (1H, br, N<u>H</u>), 7.54-7.55 (1H, d, Ar-C<u>H</u>); m/z 719 $[M+H]^+$.

Methyl *N*-[*N*-(*tert*-butoxycarbonyl)-L-seryl-*S*-[4-(*tert*-butyldimethylsilyloxy)-2-carboxybenzyl]-L-cysteinate 130: A mixture of methyl *N*-[*N*-(*tert*-butoxycarbonyl)-L-seryl-*S*-{4-(*tert*-butyldimethylsilyloxy]-2-[2,2,2-trichloroethyl]benzyl}-L-cysteinate **129** (870 mg, 1.2 mmol), THF (15 cm^3), 1M phosphoric acid (8 cm^3), 1M aqueous sodium dihydrogen phosphate solution (8 cm^3) and zinc powder (1.2 g) was stirred at ambient temperature for 2 h. The reaction mixture was diluted with EtOAc (20 cm^3) and the insoluble material removed by filtration. The filtrate was washed with water (2 x 10 cm^3), dried ($MgSO_4$) and the solvent evaporated *in vacuo* to afford a yellow oil. Purification by column chromatography using EtOAc:hexane (2:1 v/v) as eluant, yielded **130** (270 mg, 38 %) as a white solid; mp 75-78 °C; IR (KBr disc): ν_{max} 1223, 1253, 1276, 1498, 1527, 1711, 2929, 2958 cm^{-1}; ^1H NMR [$CDCl_3$]: δ 0.18 (6H, s, $Si(C\underline{H}_3)_2$), 0.95 (9H, s, $C(C\underline{H}_3)_3$), 1.50 (9H, s, BOC), 2.92 (2H, br, $SC\underline{H}_2$), 3.70 (3H, s, $OC\underline{H}_3$), 3.84 (1H, s, C<u>H</u>OH), 4.03-4.06 (4H, m, NHC<u>H</u> (ser), $C\underline{H}_2S$),

4.79 (1H, br, NHC<u>H</u> (cys)), 4.95 (2H, m, OC<u>H</u>$_2$CCl$_3$), 5.60 (1H, br, N<u>H</u>), 6.92-7.00 (1H, dd, Ar-C<u>H</u>), 5.83 (1H, br, N<u>H</u>), 6.87-6.91 (1H, dd, Ar-C<u>H</u>), 7.13-7.16 (1H, d, Ar-C<u>H</u>), 7.40-7.42 (1H, d, Ar-C<u>H</u>), 7.86 (1H, br, N<u>H</u>) ; m/z 587 [M^+], 609 [M+Na]$^+$.

Methyl (4*S*,7*S*)-7-[(*tert*-butoxycarbonyl)amino]-12[(*tert*-butyl)dimethylsilyloxy]--1,3,4,5,6,7,8,10-octahydro-6,10-dioxo -9,2,5- benzoxathiacyclododecine -4-carboxylate 131: To a stirred solution of methyl *N*-[*N*-(*tert*-butoxycarbonyl)-L-seryl-*S*-[4-(*tert*-butyldimethylsilyloxy)-2-carboxybenzyl]-L-cysteinate **130** (100 mg, 0.17 mmol) in DCM (2 cm^3) at 0 °C was added *N*,*N*-diisopropylethylamine (0.03 cm^3, 0.17 mmol) followed by PyBOP (89 mg, 0.17 mmol). The resulting mixture was stirred at 0 °C for 2 h then at ambient temperature overnight. The mixture was diluted with DCM (10 cm^3) and washed with saturated NaCl solution (2 x 10 cm^3). The solution was dried ($MgSO_4$) and the solvent evaporated *in vacuo* to afford a pale yellow oil. Purification by column chromatography, using (EtOAc:hexane; 1:1 v/v) as eluant yielded **131** (40 mg, 41 %) as a white powder; mp 148-150 °C; IR (KBr disc): ν_{max} 839, 970, 1171, 1263, 1305, 1496, 1662, 1715 cm^{-1}; ^{1}H NMR [$CDCl_3$]: δ 0.18 (6H, s, Si(C<u>H</u>$_3$)$_2$, 0.95 (9H, s, (C<u>H</u>$_3$)$_3$), 1.45 (9H, s, BOC), 3.12-3.21 (2H, br, SC<u>H</u>$_2$), 3.73 (3H, s, OC<u>H</u>$_3$), 3.95-4.05 (2H, m, COOC<u>H</u>$_2$), 4.54-4.58 (2H, br, Ph-C<u>H</u>$_2$S), 4.81 (1H, br, NHC<u>H</u> (cys)), 4.88-4.94 (1H, dd, NHC<u>H</u> (ser), 5.67 (1H, br, N<u>H</u>), 6.86-6.91 (1H, dd, Ar-C<u>H</u>), 7.15-7.32 (3H, m, 2 x Ar-C<u>H</u>, N<u>H</u>); m/z 513 [M+H]$^+$.

6.3.3 Methyl *N*-[*N*-*tert*-butoxycarbonyl)-L-seryl]-*S*-[4,6-bis(*tert*-butyl)dimethylsilyloxy]-2-carboxybenzyl]-L-cysteinate 138



3,5-dihydroxy-2-(*N*,*N*--dimethylamino)methylbenzoic acid acetate 52: To a stirred mixture of 37% aqueous formaldehyde (26.5 cm^3, 0.32 mol), ethanol (70 cm^3) and glacial acetic acid (70 cm^3) was added dropwise with cooling, 70% aqueous dimethylamine (36.5 cm^3, 0.32 mol), keeping the temperature at ~25°C. Stirring was continued for 30 minutes, whereupon the mixture was cooled to 10 °C and 3,5-dihydroxybenzoic acid **51** (50 g, 0.32 mol) added. The cooling bath was removed and stirring continued overnight. The resulting white precipitate was isolated by filtration, and washed consecutively with ethanol and diethyl ether to yield **52** (54.29 g, 62 %) as a yellow solid; mp >300 °C; IR (KBr disc): ν_{max} 764, 1150, 1294, 1356, 1471, 1558, 1614, 3130 cm^{-1}; ^{1}H NMR [$CDCl_3$]: δ 2.53 (6H, s, N($C\underline{H}_3$)$_2$), 3.91 (2H, s, $C\underline{H}_2$N), 6.34-6.35 (1H, d, Ar-C<u>H</u>), 6.64-6.65 (1H, d, Ar-C<u>H</u>), 9.56 (1H, br, COO<u>H</u>); ^{13}C NMR [$CDCl_3$]: δ 40.3 (N($\underline{C}H_3$)$_2$), 52.3 ($\underline{C}H_2$N), 103.1 (Ar-<u>C</u>H), 107.1 ($\underline{C}CH_3$), 109.3 (Ar-<u>C</u>H), 143.6 (<u>C</u>COOH), 157.2 (<u>C</u>OH), 158.2 (<u>C</u>OH), 171.1 (<u>C</u>OOH); m/z 212 [*M*+H]$^+$.

3,5-Dihydroxy-2-methylbenzoic acid 53: A suspension of 3,5-dihydroxy-2-(*N*,*N*–dimethylamino)methylbenzoic acid acetate **52** (30 g, 0.11 mol) in methanol (300 cm^3) was treated with a suspension of 10% Pd/C in 3M NaOH solution (45 cm^3), under a hydrogen atmosphere. The resulting mixture was stirred under hydrogen at ambient temperature for 4 days. The pH of the mixture was adjusted to 1 by the addition of conc. HCl, the catalyst removed by filtration through celite and the filtrate concentrated

in vacuo. The resulting residue was diluted with water (150 cm^3) and extracted with EtOAc (4 x 50 cm^3). The organics were washed consecutively with 2M HCl (2 x 50 cm^3) and 15% aqueous NaCl solution (2 x 50 cm^3), dried ($MgSO_4$) and the solvent evaporated *in vacuo* to afford **53** (9.31 g, 50 %) as an orange powder; mp 245-248 °C; IR (KBr disc): ν_{max} 1009, 1159, 1306, 1328, 1411, 1610, 1689, 3240 cm^{-1}; ^1H NMR [d_6-DMSO]: δ 2.14 (3H, s, $C\underline{H}_3$), 6.43-6.44 (1H, d, Ar-C<u>H</u>), 6.59-6.60 (1H, d, Ar-C<u>H</u>), 9.23 (1H, s, Ar-O<u>H</u>), 9.42 (1H, s, Ar-O<u>H</u>), 12.56 (1H, s, COO<u>H</u>); ^{13}C NMR [d_6-DMSO]: δ 17.2 ($\underline{C}H_3$), 110.3 (Ar-<u>C</u>H), 112.3 (Ar-<u>C</u>H), 120.3 ($\underline{C}CH_3$), 137.8 (<u>C</u>COOH), 160.1 (<u>C</u>OH), 161.6 (<u>C</u>OH), 174.4 (<u>C</u>OOH); m/z 167 [*M*-H]$^-$.

4-Nitrobenzyl 3,5-dihydroxy-2-methylbenzoate 133: To a solution of 3,5-dihydroxy-2-methylbenzoic acid **53** (3 g, 17.8 mmol) in DMF (18 cm^3) was added at 0 °C, 1,1,3,3-tetramethylguanidine (2.2 cm^3, 17.8 mmol). After stirring for 15 minutes at ambient temperature (pink precipitate formed), 4-nitrobenzyl bromide (3.85 g, 17.8 mmol) was added and stirring continued at ambient temperature overnight. The mixture was diluted with EtOAc (80 cm^3) and washed with 1M HCl (2 x 20 cm^3) and 15% NaCl solution (3 x 30 cm^3). The organic layer was dried ($MgSO_4$) and the solvent evaporated *in vacuo* to afford an orange powder. Recrystallisation from EtOAc:hexane, yielded **133** (3.32 g, 61 %) as a pale orange solid; mp 151-156 °C; IR (KBr disc): ν_{max} 1136, 1240, 1280, 1335, 1342, 1598, 1670, 3357 cm^{-1}; ^1H NMR [d_6-DMSO]: δ 2.15 (3H, s, $C\underline{H}_3$), 5.39 (2H, s, $OC\underline{H}_2$), 6.49-6.50 (1H, d, Ar-C<u>H</u>), 6.69-6.70 (1H, d, Ar-C<u>H</u>), 7.67-7.70 (2H, d, Ar-C<u>H</u>NO_2), 8.23-8.27 (2H, d, Ar-C<u>H</u>NO_2), 9.42 (1H, s, Ar-O<u>H</u>), 9.64 (1H, s, Ar-O<u>H</u>); ^{13}C NMR [d_6-DMSO]: δ 12.2 ($\underline{C}H_3$), 64.9 ($O\underline{C}H_2$-Ar), 106.1 (Ar-<u>C</u>H), 107.5 (Ar-<u>C</u>H), 116.0 ($\underline{C}CH_3$), 123.8 (Ar-$\underline{C}HNO_2$), 128.7 (Ar-$\underline{C}HNO_2$), 130.9 (<u>C</u>COOH), 144.2 ($OCH_2\underline{C}$-Ar), 147.3 (Ar-$\underline{C}NO_2$), 155.5 (<u>C</u>OH), 156.9 (<u>C</u>OH), 167.1 (<u>C</u>OOH); m/z 302 [*M*-H]$^-$.

4-Nitrobenzyl 3,5-bis(*tert*-butyldimethylsilyloxy)-2-methylbenzoate 134: To a stirred mixture of 4-nitrobenzyl 3,5-dihydroxy-2-dimethylbenzoate **133** (4.5 g, 14.8 mmol)

and TBDMSCl (4.9 g, 32.7 mmol) in DMF (12 cm^3) was added at 0 °C Et_3N (5 cm^3, 30 mmol), a precipitate being formed immediately. The mixture was stirred at 0 °C for 3 h, then diluted with EtOAc (75 cm^3), washed with water (2 x 30 cm^3), (ppt dissolved), dried ($MgSO_4$) and the solvent evaporated *in vacuo* to afford a dark orange oil. Careful washing of the oil with cold ethanol yielded **134** (1.49 g, 19 %) as white crystals; mp 62-66 °C; IR (KBr disc): ν_{max} 779, 835, 1055, 1222, 1344, 1531, 1716, 2927 cm^{-1}; ^1H NMR [$CDCl_3$]: δ 0.17–0.19 (12H, d, 2 x $Si(C\underline{H}_3)_2$, 0.95–0.99 (18H, d, 2 x $(C\underline{H}_3)_3$), 2.30 (3H, s, $C\underline{H}_3$), 5.38 (2H, s, $OC\underline{H}_2$), 6.47-6.48 (1H, d, Ar-C<u>H</u>), 6.97-6.98 (1H, d, Ar-C<u>H</u>), 7.55-7.59 (2H, d, Ar-C<u>H</u>NO_2), 8.21-8.24 (2H, d, Ar-C<u>H</u>NO_2); ^{13}C NMR [$CDCl_3$]: δ –4.5 ($Si(CH_3)_2$), 13.1 ($\underline{C}H_3$), 18.2 ($Si\underline{C}(CH_3)_3$), 25.6 ($SiC(\underline{C}H_3)_3$), 65.0 ($O\underline{C}H_2$-Ar), 114.6 (Ar-<u>C</u>H), 114.8 (Ar-<u>C</u>H), 123.7 (Ar-<u>C</u>HNO_2), 124.0 ($\underline{C}CH_3$), 128.1 (Ar-<u>C</u>HNO_2), 130.8 (<u>C</u>COO), 143.4 ($OCH_2\underline{C}$-Ar), 147.5 (Ar-<u>C</u>NO_2), 153.3 (<u>C</u>OH), 155.0 (<u>C</u>OH), 167.1 (<u>C</u>OO); m/z 532 $[M+H]^+$.

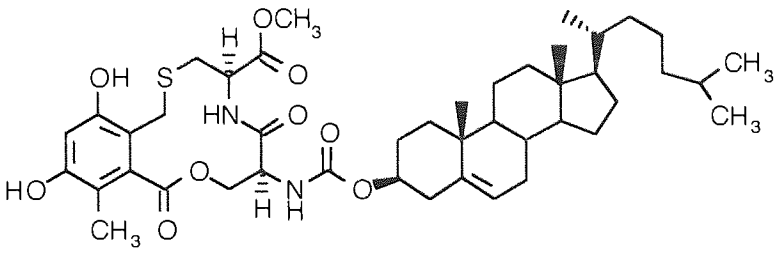
4-Nitrobenzyl 3,5-bis(*tert*-butyldimethylsilyloxy)-2-(bromomethyl) benzoate 135: A stirred mixture of 4-nitrobenzyl 3,5-bis(tert-butyldimethylsilyloxy)-2-methylbenzoate **134** (1 g, 1.9 mmol) and NBS (402 mg, 2.3 mmol) in CCl_4 (12 cm^3) was heated at reflux temperature (77 °C) with light irradiation for 2 h, until the insoluble material floated on the surface. Insoluble material removed by filtration and solvent evaporated *in vacuo* to afford **135** (904 mg, 79 %) as a brown powder; mp 95-99 °C; IR (KBr disc): ν_{max} 783, 837, 1037, 1178, 1336, 1525, 1593, 1726 cm^{-1}; ^1H NMR [$CDCl_3$]: δ 0.23 (6H, s, $Si(C\underline{H}_3)_2$), 0.29 (6H, s, $Si(C\underline{H}_3)_2$), 0.95 (9H, s, $(C\underline{H}_3)_3$), 1.03 (9H, s, $(C\underline{H}_3)_3$), 4.93 (2H, s, $C\underline{H}_2Br$), 5.44 (2H, s, $OC\underline{H}_2$), 6.49-6.50 (1H, d, Ar-C<u>H</u>), 7.04-7.05 (1H, d, Ar-C<u>H</u>), 7.59-7.62 (2H, d, Ar-C<u>H</u>NO_2), 8.21-8.25 (2H, d, Ar-C<u>H</u>NO_2); ^{13}C NMR [$CDCl_3$]: δ –4.5 ($Si(\underline{C}H_3)_2$), –4.2 ($Si(\underline{C}H_3)_2$), 18.2 ($Si\underline{C}(CH_3)_3$), 25.6 ($SiC(\underline{C}H_3)_3$), 25.7 ($SiC(\underline{C}H_3)_3$), 29.6 ($\underline{C}H_2Br$), 65.4 ($O\underline{C}H_2$-Ar), 114.3 (Ar-<u>C</u>H), 115.6 (Ar-<u>C</u>H), 122.9 ($\underline{C}CH_3$), 123.6 (Ar-<u>C</u>HNO_2), 128.4 (Ar-<u>C</u>HNO_2), 130.6 (<u>C</u>COO), 142.9 ($OCH_2\underline{C}$-Ar), 148.7 (Ar-<u>C</u>NO_2), 155.9 (<u>C</u>OSi), 156.1 (<u>C</u>OSi), 166.0 (<u>C</u>OO); m/z 610 $[M+H]^+$.

Methyl *N*-[*N*-*tert*-butoxycarbonyl)-L-seryl]-*S*-{4,6-bis[(*tert*-butyldimethylsilyloxy]-2-[(4-nitrobenzyloxy)carbonyl]benzyl}-L-cysteinate 136: To a solution of 4-nitrobenzyl 3,5-bis(*tert*-butyldimethylsilyloxy)-2-(bromomethyl) benzoate **135** (750 mg, 1.23 mmol) and *N*-[*N*-(*t*-butoxycarbonyl)-L-seryl]-L-cysteine methyl ester **31** (396 mg, 1.23 mmol) in anhydrous DCM (12 cm^3) was added dropwise at 0 °C, Et_3N (0.17 cm^3, 1.23 mmol) in DCM (0.8 cm^3). After stirring for 2 h at 0 °C and overnight at ambient temperature, the mixture was washed with 5% $NaHCO_3$ (2 x 5 cm^3) and saturated NaCl solution (2 x 5 cm^3), back-extracted with DCM (5 cm^3), dried ($MgSO_4$) and the solvent evaporated *in vacuo* to afford a yellow oil. Purification by column chromatography (EtOAc:hexane; 1:1 v/v) afforded **136** (590 mg, 56 %) as a yellow oil; IR (KBr disc): ν_{max} 841, 1176, 1227, 1251, 1345, 1465, 1527, 1718 cm^{-1}; ^1H NMR [$CDCl_3$]: δ 0.20 (6H, s, $Si(C\underline{H}_3)_2$), 0.26 (6H, s, $Si(C\underline{H}_3)_2$), 0.96 (9H, s, $(C\underline{H}_3)_3$), 1.01 (9H, s, $(C\underline{H}_3)_3$), 1.42 (9H, s, BOC), 2.86–3.02 (2H, m, $SC\underline{H}_2$), 3.69 (3H, s, $OC\underline{H}_3$), 4.08-4.22 (4H, m, Ar-$C\underline{H}_2$S+$C\underline{H}_2$OH), 4.39 (1H, br, COC<u>H</u>), 4.83 (1H, br, COC<u>H</u>), 5.48 (2H, s, $OC\underline{H}_2$), 5.62 (1H, br, N<u>H</u>), 6.49-6.50 (1H, d, Ar-C<u>H</u>), 7.02-7.03 (1H, d, Ar-C<u>H</u>), 7.43 (1H, br, N<u>H</u>), 7.61-7.64 (2H, d, Ar-C<u>H</u>NO_2), 8.23-8.26 (2H, d, Ar-C<u>H</u>NO_2); ^{13}C NMR [$CDCl_3$]: δ –4.5 ($Si(\underline{C}H_3)_2$), –4.2 ($Si(\underline{C}H_3)_2$), 18.2 ($Si\underline{C}(CH_3)_3$), 25.5 ($SiC(\underline{C}H_3)_3$), 25.6 ($SiC(\underline{C}H_3)_3$), 27.9 ($\underline{C}H_2S$), 28.2 ($C(\underline{C}H_3)_3$), 33.8 ($\underline{C}H_2S$), 52.4 (NH<u>C</u>H), 52.7 ($O\underline{C}H_3$), 55.4 (NH<u>C</u>H), 63.7 ($\underline{C}H_2OH$), 65.6 ($O\underline{C}H_2$-Ar), 114.6 (Ar-<u>C</u>H), 115.4 (Ar-<u>C</u>H), 123.2 ($\underline{C}CH_3$), 123.7 (Ar-<u>C</u>HNO_2), 128.3 (Ar-<u>C</u>HNO_2), 130.3 (<u>C</u>COO), 143.0 ($OCH_2\underline{C}$-Ar), 147.6 (Ar-<u>C</u>NO_2), 154.9 (Ar-<u>C</u>OSi), 155.2 (Ar-<u>C</u>OSi), 166.7 (<u>C</u>OO), 167.7 (<u>C</u>OO), 171.0 (<u>C</u>=O); m/z 853 M^+.

Methyl *N*-[*N*-*tert*-butoxycarbonyl)-L-seryl]-*S*-[4,6-bis{(*tert*-butyl)dimethylsilyloxy}]-2-carboxybenzyl]-L-cysteinate 137: A mixture of methyl *N*-[*N*-*tert*-butoxycarbonyl)-L-seryl]-*S*-{4,6-bis[(*tert*-butyldimethylsilyloxy]-2-[(4-nitrobenzyloxy) carbonyl] benzyl}-L-cysteinate **136** (343 mg, 0.41 mmol) and 10% Pd/C (spatula) in EtOAc (6 cm^3) was stirred under H_2 at ambient temperature for 4 days. The catalyst was

removed by filtration through celite and the filtrate concentrated *in vacuo* to afford a yellow oil. Purification by column chromatography (EtOAc:hexane; methanol) yielded **137** (207 mg, 72 %) as a pale yellow powder; mp 156-159 °C; IR (KBr disc): ν_{max} 779, 839, 1168, 1257, 1388, 1560, 2929, 2954 cm^{-1}; ^1H NMR [$CDCl_3$]: δ 0.10 (6H, s, $Si(C\underline{H}_3)_2$), 0.21 (6H, s, $Si(C\underline{H}_3)_2$), 0.89 (9H, s, $(C\underline{H}_3)_3$), 0.98 (9H, s, $(C\underline{H}_3)_3$), 1.36 (9H, s, BOC), 2.81–2.88 (2H, m, $SC\underline{H}_2$), 3.65 (3H, s, $OC\underline{H}_3$), 4.06-4.20 (4H, m, Ar-$C\underline{H}_2S$+$CH_2O\underline{H}$), 4.33 (1H, br, $COC\underline{H}$), 4.77 (1H, br, $COC\underline{H}$), 6.15 (1H, br, $N\underline{H}$), 6.29 (1H, s, Ar-$C\underline{H}$), 6.84 (1H, s, Ar-$C\underline{H}$), 7.89 (1H, br, $N\underline{H}$) ; ^{13}C NMR [$CDCl_3$]: δ –4.1 ($Si(\underline{C}H_3)_2$), –3.7 ($Si(\underline{C}H_3)_2$), 18.5 ($Si\underline{C}(CH_3)_3$), 18.7 ($Si\underline{C}(CH_3)_3$), 26.2 ($SiC(\underline{C}H_3)_3$), 26.4 ($SiC(\underline{C}H_3)_3$), 27.9 ($\underline{C}H_2S$), 28.7 ($\underline{C}(CH_3)_3$), 33.4 ($\underline{C}H_2S$), 53.1 ($O\underline{C}H_3$), 63.2 ($\underline{C}H_2OH$), 77.6 ($NH\underline{C}H$), 100.8 ($NH\underline{C}H$), 112.3 (Ar-$\underline{C}H$), 114.7 (Ar-$\underline{C}H$), 154.9 (Ar-$\underline{C}OSi$), 155.1 (Ar-$\underline{C}OSi$), 156.8 ($\underline{C}OO$), 168.2 ($\underline{C}OO$), 171.6 ($\underline{C}$=O); m/z 717 $[M+H]^+$.

6.3.4 Methyl(4*R*,7*S*)-7[(cholesteryl)amino]-12,14-dihydroxy-1,3,4,5,6,7,8,10-octahydro-11-methyl-6,10-dioxo-9,2,5-benzoxathiazacyclododecine-4-carboxylate



3,5-Dihydroxy-2-methyl-6-(*N*,*N*-dimethylaminomethyl)benzoic acid acetate 54: To a stirred mixture of 37% aqueous formaldehyde (1.9 cm^3, 23.8 mmol), ethanol (10 cm^3) and glacial acetic acid (10 cm^3) was added dropwise with cooling 40% aqueous dimethylamine (2.7 cm^3, 23.8 mmol), keeping the temperature ~25 °C. Stirring was continued for 30 minutes, whereupon the mixture was cooled to 10 °C and 3,5-dihydroxy-2-methylbenzoic acid **53** (4 g, 23.8 mmol) added. The cooling bath was removed and stirring continued overnight. The resulting white precipitate was collected by filtration and washed consecutively with ethanol and diethyl ether to afford **54** (4.61 g, 68 %) as a white powder; mp >300 °C; IR (KBr disc): ν_{max} 1301, 1369, 1398, 1465, 1568, 1596, 2730, 2873 cm^{-1}; ^1H NMR [d_6-DMSO]: δ 1.95 (3H, s, $C\underline{H}_3$), 2.53 (6H, s, $N(C\underline{H}_3)_2$), 3.80 (2H, s, $C\underline{H}_2N$), 6.31 (1H, s, Ar-C$\underline{H}$); ^{13}C NMR [d_6-DMSO]: δ 12.7 ($\underline{C}H_3$), 42.0 ($N(\underline{C}H_3)_2$), 53.8 ($\underline{C}H_2N$), 100.5 (Ar-$\underline{C}$H), 104.5 (Ar-$\underline{C}CH_3$), 111.5 (Ar-$\underline{C}CH_2NH$), 145.9 (Ar-$\underline{C}COOH$), 154.5 (Ar-$\underline{C}OH$), 156.8 (Ar-$\underline{C}OH$); m/z 226 $[M+H]^+$.

3,5-Dihydroxy-2,6-dimethylbenzoic acid 55: A suspension of 3,5-dihydroxy-2-methyl-6-(*N*,*N*-dimethylaminomethyl)benzoic acid acetate **54** (6 g, 21 mmol) in methanol (60 cm^3) was treated with a suspension of 10% Pd/C in 3M NaOH solution (9 cm^3), under a hydrogen atmosphere. The resulting mixture was stirred under hydrogen at ambient temperature for 4 days. The pH of the mixture was adjusted to 1 by the addition of conc. HCl, the catalyst removed by filtration through celite and the

yellow filtrate concentrated *in vacuo*. The resulting residue was diluted with water (25 cm^3) and extracted with EtOAc (4 x 10 cm^3). The organics were washed consecutively with 2M HCl (2 x 15 cm^3) and 15% aqueous NaCl solution (2 x 15 cm^3), dried ($MgSO_4$) and the solvent evaporated *in vacuo* to afford **55** (2.73 g, 82%) as an orange solid; mp 166-172 °C (lit. 178-179 °C); IR (KBr disc): ν_{max} 1120, 1261, 1340, 1375, 1605, 1668, 3236, 3384 cm^{-1}; ^1H NMR [d_6-DMSO]: δ 1.91 (6H, s, $C\underline{H}_3$), 6.37 (1H, s, Ar-C$\underline{H}$), 9.15 (2H, s, Ar-O$\underline{H}$); ^{13}C NMR [d_6-DMSO]: δ 12.3 ($\underline{C}H_3$), 102.4 (Ar-$\underline{C}$H), 109.7 (Ar-$\underline{C}CH_3$), 137.8 (Ar-$\underline{C}$COOH), 153.6 (Ar-$\underline{C}$OH), 171.2 ($\underline{C}$OOH); m/z 181 $[M\text{-}H]^-$.

4-Nitrobenzyl 3,5-dihydroxy-2,6-dimethylbenzoate 59: To a solution of 3,5-dihydroxy-2,6-dimethylbenzoic acid **55** (4 g, 22 mmol) in DMF (22 cm^3) was added at 0 °C, 1,1,4,4-tetramethylguanidine (2.8 cm^3, 22 mmol). After stirring for 15 minutes at ambient temperature (pink precipitate formed), 4-nitrobenzyl bromide (4.7 g, 22 mmol) was added and stirring continued at ambient temperature overnight. The mixture was diluted with EtOAc (110 cm^3), washed with 1M HCl (2 x 25 cm^3) and 15% NaCl solution (3 x 50 cm^3), dried ($MgSO_4$) and the solvent evaporated *in vacuo* to afford the crude product as an orange solid. The addition of hot hexane resulted in oiling out of the impurities to yield **59** (2.51 g, 36 %) as a bright yellow solid; mp 161-164 °C (lit. 168-170 °C); IR (KBr disc): ν_{max} 1103, 1245, 1261, 1344, 1515, 1602, 1711, 3473 cm^{-1}; ^1H NMR [d_6-DMSO]: δ 1.86 (6H, s, $C\underline{H}_3$), 5.44 (2H, s, $OC\underline{H}_2$), 6.42 (1H, s, Ar-C$\underline{H}$), 7.68-7.71 (2H, d, Ar-C$\underline{H}NO_2$), 8.24-8.27 (2H, d, Ar-C$\underline{H}NO_2$), 9.26 (1H, s, Ar-O$\underline{H}$); ^{13}C NMR [d_6-DMSO]: δ 12.3 ($\underline{C}H_3$), 65.1 ($O\underline{C}H_2$-Ar), 103.2 (Ar-$\underline{C}$H), 110.6 (Ar-$\underline{C}CH_3$), 123.8 (Ar-$\underline{C}HCNO_2$), 129.4 (Ar-$\underline{C}HCNO_2$), 135.3 (Ar-$\underline{C}$COO), 143.6 ($OCH_2\underline{C}$-Ar), 147.3 (Ar-$\underline{C}NO_2$), 153.7 (2 x Ar-$\underline{C}$OH), 169.3 ($\underline{C}$OO); m/z 317 $[M^+]$.

4-Nitrobenzyl 3,5-bis(*tert*-butyldimethylsilyloxy)-2,6-dimethylbenzoate 60: To a stirred mixture of 4-nitrobenzyl 3,5-dihydroxy-2,6-dimethylbenzoate **59** (2.4 g, 7.6

mmol) and TBDMSCl (2.5 g, 16.6 mmol) in DMF (6 cm^3) was added at 0 °C Et_3N (2.6 cm^3, 0.015 mmol), a precipitate being formed immediately. The mixture was stirred at 0 °C for 4 h, then diluted with EtOAc (40 cm^3), washed carefully with water (2 x 15 cm^3), dried ($MgSO_4$) and the solvent evaporated *in vacuo* to afford a pale orange solid. Purification by column chromatography using EtOAc:hexane (1:2 v/v) as eluant yielded **60** (2.98 g, 72 %) as a pale yellow solid; mp 116-118 °C (lit. 121-122 °C); IR (KBr disc): ν_{max} 783, 837, 1031, 1257, 1340, 1469, 1527, 1723 cm^{-1}; ^1H NMR [$CDCl_3$]: δ 0.18 (12H, d, 2 x $Si(C\underline{H}_3)_2$, 0.98 (18H, d, 2 x $(C\underline{H}_3)_3$), 2.00 (6H, s, 2 x $C\underline{H}_3$), 5.41 (2H, s, $OC\underline{H}_2$), 6.32 (1H, s, Ar-C<u>H</u>), 7.58-7.61 (2H, d, Ar-C<u>H</u>NO_2), 8.21-8.25 (2H, d, Ar-C<u>H</u>NO_2); ^{13}C NMR [$CDCl_3$]: δ –4.3 ($Si(\underline{C}H_3)_2$), 13.0 ($\underline{C}H_3$), 18.1 $Si\underline{C}(CH_3)_3$, 25.6 ($C(\underline{C}H_3)_3$), 65.1 ($O\underline{C}H_2$-Ar), 110.7 (Ar-<u>C</u>H), 117.8 (Ar-<u>C</u>CH_3), 123.7 (Ar-<u>C</u>HNO_2), 128.9 (Ar-<u>C</u>HNO_2), 135.2 (Ar-<u>C</u>COO), 142.7 ($OCH_2\underline{C}$-Ar), 147.7 (Ar-<u>C</u>NO_2), 151.8 (Ar-<u>C</u>OSi), 169.5 (<u>C</u>OO); m/z 546 $[M+H]^+$.

4-Nitrobenzyl 2-bromomethyl-3,5-bis(*tert*-butyldimethylsilyloxy)-6-methylbenzoate 61: A stirred mixture of 4-nitrobenzyl 3,5-bis(*tert*-butyldimethylsilyloxy)-2,6-dimethylbenzoate **60** (22.44 g, 41.11 mmol) and NBS (8.78 g, 49.34 mmol) in CCl_4 (140 cm^3) was heated at reflux temperature (77 °C) with light irradiation for 2 h, until the white solid was suspended in solution. On cooling, the solid was removed by filtration and the solvent evaporated *in vacuo* to afford an orange/yellow solidifying oil. The addition of hot hexane resulted in oiling out of the impurities to yield **61** (24.02 g, 94 %) as a yellow solid; mp 204-206 °C; IR (KBr disc): ν_{max} 1119, 1281, 1313, 1348, 1521, 1718, 2952, 3249 cm^{-1}; ^1H NMR [$CDCl_3$]: δ 0.18–0.28 (12H, dd, 2 x $Si(C\underline{H}_3)_2$), 0.98–1.02 (18H, 2 x s, 2 x $(C\underline{H}_3)_3$), 2.02 (3H, s, $C\underline{H}_3$), 4.49 (2H, s, $C\underline{H}_2Br$), 5.46 (2H, s, $OC\underline{H}_2$), 6.34 (1H, s, Ar-C<u>H</u>), 7.64-7.67 (2H, d, Ar-C<u>H</u>NO_2), 8.21-8.25 (2H, d, Ar-C<u>H</u>NO_2); ^{13}C NMR [$CDCl_3$]: δ –4.5 ($Si(\underline{C}H_3)_2$), –4.2 ($Si(\underline{C}H_3)_2$), 13.1 ($\underline{C}H_3$), 18.2 ($Si\underline{C}(CH_3)_3$), 25.6 ($SiC(\underline{C}H_3)_3$), 25.7 ($SiC(\underline{C}H_3)_3$), 30.8 ($\underline{C}H_2Br$), 64.9 ($O\underline{C}H_2$-Ar), 114.6 (Ar-<u>C</u>H), 122.9 (Ar-<u>C</u>CH_3), 123.6 (Ar-

CHNO$_2$), 128.1 (Ar-CHNO$_2$), 135.2 (Ar-CCOO), 143.2 (OCH$_2$C-Ar), 152.5 (Ar-COSi), 155.1 (Ar-COSi), 167.4 (COO); m/z 624 [*M*+H]$^+$.

Methyl *N*-[*N*-(*tert*-butoxycarbonyl)-L-seryl]-*S*-{4,6-bis[(*tert*-butyl)dimethylsiloxy]-3-methyl-2-[(4-nitrobenzyloxy)carbonyl]benzyl}-L-cysteinate 62: To a solution of 4-nitrobenzyl 2-bromomethyl-3,5-bis(*tert*-butyldimethylsilyloxy)-6-methylbenzoate **61** (5.73 g, 9.17 mmol) and *N*-[*N*-(*t*-butoxycarbonyl)-L-seryl]-L-cysteine methyl ester **31** (2.96 g, 9.17 mmol) in anhydrous DCM (75 cm^3) was added dropwise at 0 °C, Et_3N (1.27 cm^3 9.17 mmol) in DCM (5 cm^3). After stirring for 2 h at 0 °C and overnight at ambient temperature the reaction mixture was washed with 5% $NaHCO_3$ (2 x 10 cm^3) and saturated NaCl solution (2 x 10 cm^3), back extracted with DCM (10 cm^3), dried ($MgSO_4$) and the solvent evaporated *in vacuo* to afford an orange oil. Purification by column chromatography using EtOAc:Hexane (1:1 v/v) as eluant yielded **62** (1.65 g, 21 %) as a pale yellow oil; IR (thin film): ν_{max} 837, 1155, 1257, 1342, 1465, 1523, 1724, 1731 cm^{-1}; ^{1}H NMR [$CDCl_3$]: δ 0.22-0.24 (12H, d, 2 x Si(CH$_3$)$_2$), 0.97–0.99 (18H, s, 2 x C(CH$_3$)$_3$), 1.42 (9H, s, BOC), 2.00 (Ar–CH$_3$), 2.87–2.90 (2H, m, SCH$_2$), 3.07 (1H, br, CH$_2$OH), 3.68-3.76 (6H, m, OCH$_3$, Ar-CH$_2$S, CHOH), 4.06 (1H, br, NHCH (ser)), 4.26 (1H, br, CHOH), 4.68-4.71 (1H, m, NHCH (cys)), 5.47 (2H, s, COOCH$_2$), 5.60 (1H, br, NH), 6.34 (1H, s, Ar-CH), 7.10 (1H, br, NH), 7.63-7.66 (2H, d, Ar-CHNO$_2$), 8.22-8.25 (2H, d, Ar-CHNO$_2$); ^{13}C NMR [$CDCl_3$]: δ –4.3 (Si(CH$_3$)$_2$), –4.2 (Si(CH$_3$)$_2$), 13.2 (CH$_3$), 18.1 (SiC(CH$_3$)$_3$), 18.2 (SiC(CH$_3$)$_3$), 25.5 (SiC(CH$_3$)$_3$), 25.6 (SiC(CH$_3$)$_3$), 28.2 (C(CH$_3$)$_3$), 33.7 (CH$_2$S + Ph-CH$_2$S), 52.0 (NHCH), 52.6 (OCH$_3$), 55.3 (NHCH), 63.2 (CH$_2$OH), 65.9 (OCH$_2$-Ar), 80.1 (C(CH$_3$)$_3$), 110.5 (Ar-CH), 117.7 (Ar-CCH$_2$S), 119.3 (Ar-CCH$_3$), 123.7 (Ar-CHNO$_2$), 129.2 (Ar-CHNO$_2$), 134.7 (CCOO), 142.4 (OCH$_2$C-Ar), 147.7 (Ar-CNO$_2$), 152.2 (COSi), 153.5 (COSi), 155.6 (COO), 169.1 (COO), 171.0 (HNC=O); m/z 866 [*M*+H]$^+$.

Methyl *N*-[*N*-(*tert*-butoxycarbonyl)-L-seryl]-*S*-{4,6-bis[(*tert*-butyl)dimethylsiloxy]}-2-carboxy-3-methylbenzyl-L-cysteinate 63: A mixture of methyl *N*-[*N*-(*tert*-butoxycarbonyl)-L-seryl]-*S*-{4,6-bis[(*tert*-butyl)dimethylsiloxy]-3-methyl-2-[(4-nitrobenzyloxy)carbonyl]benzyl}-L-cysteinate **62** (630 mg, 0.73 mmol) and 10% Pd/C (spatula) in EtOAc (7 cm^3) was stirred under H_2 at ambient temperature for 4 days. The catalyst was removed by filtration through celite and the filtrate concentrated *in vacuo* to afford an orange solid. Purification by column chromatography (EtOAc:hexane; ethanol) yielded **63** (360 mg, 68 %) as a bright yellow solid; mp 164-167 °C; IR (KBr disc): ν_{max} 783, 835, 1521, 1683, 1716, 1747, 2929, 2954 cm^{-1}; ^1H NMR [$CDCl_3$]: δ 0.15–0.20 (12H, dd, 2 x Si($C\underline{H}_3$)$_2$), 0.95–0.97 (18H, d, 2 x ($C\underline{H}_3$)$_3$), 1.38 (9H, s, BOC), 2.03 (3H, s, Ar-$C\underline{H}_3$), 2.93 (2H, br, S$C\underline{H}_2$), 3.64-3.67 (6H, m, $C\underline{H}_2$S, O$C\underline{H}_3$, C<u>H</u>OH), 3.82 (1H, d, C<u>H</u>OH), 4.08 (1H, m, NHC<u>H</u> (ser)), 4.36 (1H, m, C<u>H</u>OH), 4.75 (1H, br, NHC<u>H</u> (cys)), 6.11 (1H, br, N<u>H</u>), 6.19 (1H, s, Ar-C<u>H</u>), 8.01 (1H, br, N<u>H</u>) ; ^{13}C NMR [$CDCl_3$]: δ –4.4 (Si($\underline{C}H_3$)$_2$), –4.1 (Si($\underline{C}H_3$)$_2$), 13.3 ($\underline{C}H_3$), 18.1 (Si<u>C</u>(CH$_3$)$_3$), 25.6 (SiC($\underline{C}H_3$)$_3$), 25.7 (SiC($\underline{C}H_3$)$_3$), 28.1 (C($\underline{C}H_3$)$_3$), 29.6 ($\underline{C}H_2$S), 29.1 ($\underline{C}H_2$S), 52.7 (O$\underline{C}H_3$), 54.2 (NH<u>C</u>H), 62.1 ($\underline{C}H_2$OH), 77.1 (CO<u>C</u>H), 80.5 (<u>C</u>(CH$_3$)$_3$), 108.4 (Ar-<u>C</u>H), 115.5 (Ar-<u>C</u>CH$_3$), 117.0 (Ar-<u>C</u>CH$_2$S), 152.0 (Ar-<u>C</u>OSi), 153.2 (Ar-<u>C</u>OSi), 156.2 (<u>C</u>OO), 166.5 (<u>C</u>OO), 166.6 (<u>C</u>OO), 171.2 (C=O); m/z 729 [*M*-H]$^-$.

Methyl (4*R*,7*S*)-7-[(*tert*-butoxycarbonyl)amino]-12,14-bis[(tert-butyl)dimethylsiloxy]-1,3,4,5,6,7,8,10-octahydro-11-methyl-6,10-dioxo-9,2,5-benzoxathiazacyclododecine-4-carboxylate 64: To a solution of methyl *N*-[*N*-(*tert*-butoxycarbonyl)-L-seryl]-*S*-{4,6-bis[(*tert*-butyl)dimethylsiloxy]}-2-carboxy-3-methylbenzyl-L-cysteinate **63** (219 mg, 0.3 mmol) in toluene (7.5 cm^3) were added at 0 °C Ph_3P (102 mg, 0.39 mmol) and 95% DEAD (0.064 cm^3, 0.39 mmol). The mixture was stirred at 0 °C for 15 minutes followed by 5.5 h at ambient temperature. The solvent was evaporated *in vacuo* to afford a yellow oil. Purification by column chromatography using EtOAc:hexane (1:3/1:2/1:1) as eluant yielded **64** (152 mg, 15 %) as a pale yellow solid;

mp 66-70 °C; IR (KBr disc): ν_{max} 1155, 1257, 1338, 1473, 1683, 1724, 2935, 2954 cm^{-1}; ^1H NMR [$CDCl_3$]: δ 0.19 (6H, s, $Si(C\underline{H}_3)_2$), 0.20 (6H, s, $Si(C\underline{H}_3)_2$), 0.98–1.00 (18H, d, 2 x $(C\underline{H}_3)_3$), 1.48 (9H, s, BOC), 2.02 (3H, s, $C\underline{H}_3$), 3.05 (2H, dd, $SC\underline{H}_2$), 3.29-3.34 (1H, d, Ar-C<u>H</u>S), 3.74 (3H, s, $OC\underline{H}_3$), 3.85-3.90 (1H, d, Ar-C<u>H</u>S), 4.24 (1H, d, COOC<u>H</u>), 4.60 (1H, br, COC<u>H</u>), 4.83 (1H, m, COC<u>H</u>), 5.33 (1H, d, Ar-C<u>H</u>S), 5.74 (1H, br, N<u>H</u>), 6.32 (1H, s, Ar-C<u>H</u>), 7.11-7.15 (1H, d, N<u>H</u>) ; ^{13}C NMR [$CDCl_3$]: δ –4.3 ($Si(\underline{C}H_3)_2$), –4.2 ($Si(\underline{C}H_3)_2$), 13.2 ($\underline{C}H_3$), 18.2 ($Si\underline{C}(CH_3)_3$), 25.5 ($SiC(\underline{C}H_3)_3$), 25.6 ($SiC(\underline{C}H_3)_3$), 28.1 (($C(\underline{C}H_3)_3$), 31.4 (Ph-$C\underline{H}_2S$), 34.6 ($\underline{C}H_2S$), 51.7 (NH<u>C</u>H), 52.7 $O\underline{C}H_3$, 65.8 ($\underline{C}H_2OH$), 80.5 ($OC(\underline{C}H_3)_3$, 110.4 (Ar-<u>C</u>H), 116.7 ($\underline{C}CH_2S$), 118.9 ($\underline{C}CH_3$), 135.0 (<u>C</u>COO), 152.6 (<u>C</u>OSi), 153.7 (<u>C</u>OSi), 168.8 (<u>C</u>OO), 169.0 (<u>C</u>OO), 170.8 (<u>C</u>=O); m/z 713 $[M+H]^+$.

Methyl (4*R*,7*S*)-7-amino-12,14-bis[(*tert*-butyl)dimethylsiloxy]-1,3,4,5,6,7,8,10-octahydro-11-methyl-6,10-dioxo-9,2,5-benzoxathiazacyclododecine-4-carboxylate 68: To a solution of methyl (4*R*,7*S*)-7-[(*tert*-butoxycarbonyl)amino]-12,14-bis[(*tert*-butyl)dimethylsiloxy]-1,3,4,5,6,7,8,10-octahydro-11-methyl-6,10-dioxo-9,2,5-benzoxathiazacyclododecine-4-carboxylate **64** (200 mg, 0.28 mmol) in anhydrous DCM (6 cm^3) was added at ambient temperature TFA (3 cm^3) and stirring continued for 30 minutes. Volatiles were evaporated *in vacuo* and the residue dissolved in EtOAc (12 cm^3). Organics were washed with $NaHCO_3$ (2 x 4 cm^3), saturated NaCl solution (5 cm^3), dried ($MgSO_4$) and the solvent evaporated *in vacuo* to afford **68** (160mg, 93 %) as an off-white solid; mp 74-77 °C; IR (KBr disc): ν_{max} 841, 1259, 1338, 1467, 1733, 2358, 2856, 2929 cm^{-1}; ^1H NMR [$CDCl_3$]: δ 0.18 (6H, s, $Si(C\underline{H}_3)_2$), 0.22 (6H, s, $Si(C\underline{H}_3)_2$), 0.98 (9H, s, $(C\underline{H}_3)_3$), 1.02 (9H, s, $(C\underline{H}_3)_3$), 2.01 (3H, s, $C\underline{H}_3$), 2.74 (1H, dd, J_1 = 15 Hz, J_2 = 9 Hz, C<u>H</u>S), 3.16 (1H, dd, J_1 = 15 Hz, J_2 = 5 Hz, C<u>H</u>S), 3.47 (1H, d, J = 11 Hz, Ar-C<u>H</u>S), 3.74 (3H, s, $OC\underline{H}_3$), 3.85 (1H, d, J = 11 Hz, Ar-C<u>H</u>S), 4.33 (1H, d, J = 9 Hz, C<u>H</u>OCH_2), 4.87 (1H, m, C<u>H</u>NH), 5.29 (1H, d, J = 9 Hz, C<u>H</u>OCH_2), 6.31 (1H, s, Ar-C<u>H</u>), 8.27 (1H, d, J = 9 Hz, N<u>H</u>) ; ^{13}C NMR [$CDCl_3$]: δ –4.3 ($Si(\underline{C}H_3)_2$), –4.2 ($Si(\underline{C}H_3)_2$), 13.1 ($\underline{C}H_3$), 18.2 ($Si\underline{C}(CH_3)_3$),

25.5 (SiC($\underline{C}H_3)_3$), 25.7 (SiC($\underline{C}H_3)_3$), 30.8 (Ph-$\underline{C}H_2$S), 34.4 ($\underline{C}H_2$S), 51.8 (NH$\underline{C}$H), 52.5 (O$\underline{C}H_3$), 67.5 ($\underline{C}H_2$O), 110.4 (Ar-$\underline{C}$H), 116.5 ($\underline{C}CH_2$S), 118.8 ($\underline{C}CH_3$), 135.5 ($\underline{C}$COO), 152.6 ($\underline{C}$OSi), 153.7 ($\underline{C}$OSi), 168.9 ($\underline{C}$OO), 171.4 ($\underline{C}$OO), 171.8 ($\underline{C}$=O); m/z 613 $[M+H]^+$.

Methyl (4*R*,7*S*)-7-amino-12,14-dihydroxy-1,3,4,5,6,7,8,10-octahydro-11-methyl-6,10-dioxo-9,2,5-benzoxathiazacyclododecine-4-carboxylate 73: To a solution of methyl (4*R*,7*S*)-7-amino-12,14-bis[(*tert*-butyl)dimethylsiloxy]-1,3,4,5,6,7,8,10-octahydro-11-methyl-6,10-dioxo-9,2,5-benzoxathiazacyclododecine-4-carboxylate **68** (50 mg, 0.082 mmol) in anhydrous THF (1 cm^3) at ambient temperature was added dropwise over 5 minutes a 1M solution of TBAF in THF (0.20 cm^3, 0.196 mmol). Stirring was continued at ambient temperature for 1 h then the crude reaction mixture purified by column chromatography using EtOAc:hexane (3:1 v/v) as eluant, to afford **73** (12 mg, 39 %) as an off-white solid; mp 206-208 °C; IR (KBr disc): ν_{max} 1234, 1257, 1333, 1523, 1601, 1659, 1724, 2916 cm^{-1}; ^{1}H NMR [d_6-DMSO]: δ 1.87 (3H, s, $\underline{C}H_3$), 2.87–3.15 (2H, m, $\underline{C}H_2$S), 3.45 (1H, m, Ph-$\underline{C}$HS), 3.64 (3H, s, O$\underline{C}H_3$), 3.72-3.82 (2H, m, $\underline{C}HNH_2$, Ph-$\underline{C}$HS), 4.07-4.20 (1H, m, $\underline{C}H_2$OCO), 4.55 (1H, br, $\underline{C}$HNH), 5.12 (1H, d, $\underline{C}$HOCO), 6.43 (1H, s, Ar-$\underline{C}$H), 8.54 (1H, br, N$\underline{H}$), 9.50-9.52 (2H, d, O$\underline{H}$); m/z 385 $[M+H]^+$.

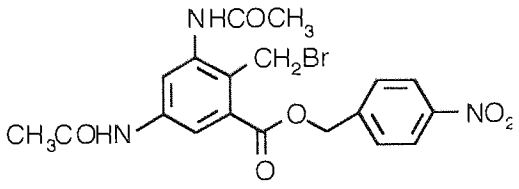
Methyl (4*R*,7*S*)-7-[(cholesteryl)amino]-12,14-bis[(*tert*-butyl)dimethylsilyloxy]-1,3,4,5,6,7,8,10-octahydro-11-methyl-6,10-dioxo-9,2,5-benzoxathiazacyclododecine-4-carboxylate 70: To a solution of methyl (4*R*,7*S*)-7-amino-12,14-dihydroxy-1,3,4,5,6,7,8,10-octahydro-11-methyl-6,10-dioxo-9,2,5-benzoxathiazacyclododecine-4-carboxylate **68** (100 mg, 0.16 mmol) and cholesteryl chloroformate **69** (73 mg, 0.16 mmol) in anhydrous DCM (2 cm^3) under argon at ambient temperature was added DMAP (20 mg, 0.16 mmol). The mixture was stirred at ambient temperature for 2.5 h and the crude mixture purified by column chromatography using EtOAc:hexane (1:3 v/v) as eluant to afford **70** (72 mg, 43 %) as a white crystalline solid solid; mp 124-127

°C; IR (KBr disc): ν_{max} 835, 1257, 1336, 1465, 1683, 1738, 2858, 2950 cm^{-1}; ^1H NMR [$CDCl_3$]: δ 0.18–0.22 (12H, d, 2 x $Si(C\underline{H}_3)_2$), 0.64 (2H, s, chol.), 0.97–0.99 (18H, d, 2 x $(C\underline{H}_3)_3$), 0.82–1.65 (m, chol.), 1.72-1.94 (4H, m, chol.), 2.01 (3H, s, $C\underline{H}_3$), 2.22–2.39 (1H, m, chol.), 2.83–2.98 (1H, br, C<u>H</u>S), 3.11-3.23 (1H, dd, C<u>H</u>S), 3.26-3.40 (1H, d, Ar-C<u>H</u>S), 3.72 (3H, s, $OC\underline{H}_3$), 3.80-3.89 (1H, d, ArC<u>H</u>S), 4.16-4.27 (1H, d, C<u>H</u>COO), 4.44-4.62 (1H, m, chol.), 4.62-4.73 (1H, br, C<u>H</u>NH (ser)), 4.75-4.88 (1H, m, C<u>H</u>NH (cys)), 4.26-5.41 (2H, m, C<u>H</u>COO + chol.), 5.86-5.96 (1H, d, N<u>H</u>), 6.31 (1H, s, Ar-CH), 7.10-7.19 (1H, d, N<u>H</u>); ^{13}C NMR [$CDCl_3$]: δ –4.35 ($Si(\underline{C}H_3)_2$), 11.7 (chol.), 13.2 ($\underline{C}H_3$), 18.1 ($Si\underline{C}(CH_3)_3$), 18.2 (chol.), 19.2 (chol.), 20.9 (chol.), 22.5 (chol.), 22.7 (chol.), 23.7 (chol.), 25.5 ($SiC(\underline{C}H_3)_3$), 27.9 (chol.), 31.0 (Ar-$\underline{C}H_2S$), 31.7 (chol.), 34.5 ($\underline{C}H_2S$), 35.6 (chol.), 36.4 (chol.), 38.2 (chol.), 39.4 (chol.), 42.2 (chol.), 49.8 (chol.), 51.7 (<u>C</u>HNH), 52.7 ($O\underline{C}H_3$), 56.0 (chol.), 56.5 (chol.), 65.8 ($\underline{C}H_2O$), 75.5 (chol.), 110.4 (Ar-<u>C</u>H), 116.6 ($\underline{C}CH_2S$), 118.9 ($\underline{C}CH_3$), 122.6 (chol.), 135.0 (<u>C</u>COO), 139.9 (chol.), 152.5 (<u>C</u>OSi), 153.7 (<u>C</u>OSi), 168.7 (<u>C</u>OO), 169.0 (<u>C</u>OO), 170.8 (<u>C</u>=O); m/z 1026 $[M+H]^+$.

Methyl (4*R*,7*S*)-7-[(cholesteryl)amino]-12,14-dihydroxy-1,3,4,5,6,7,8,10-octahydro-11-methyl-6,10-dioxo-9,2,5-benzoxathiazacyclododecine-4-carboxylate 72: To a solution of methyl (4*R*,7*S*)-7-[(cholesteryl)amino]-12,14-bis[(*tert*-butyl) dimethyl silyloxy]-1,3,4,5,6,7,8,10-octahydro-11-methyl-6,10-dioxo-9,2,5-benzoxathiazacyclo dodecine-4-carboxylate **70** (89 mg, 0.087 mmol) in anhydrous THF (1 cm^3) at ambient temperature was added dropwise over 5 minutes a 1M solution of TBAF in THF (0.21 cm^3, 0.208 mmol). Stirring was continued at ambient temperature for 1 h then the crude reaction mixture purified by column chromatography using EtOAc:Hexane (3:1 v/v) as eluant, to afford **72** (22 mg, 32 %) as an off-white solid; mp 222-225 °C; IR (KBr disc): ν_{max} 1101, 1257, 1325, 1523, 1651, 1701, 1731, 2946 cm^{-1}; ^1H NMR [$(CD_3)_2CO$]: δ 0.44 (2H, s, chol.), 0.60–1.41 (m, chol.), 1.57-1.87 (4H, m, chol.), 2.08–2.13 (5H, m, $C\underline{H}_3$ + chol.), 2.67–2.69 (1H, m, C<u>H</u>S), 2.93 (1H, dd, J_1 = 9 Hz, J_2 = 3 Hz, C<u>H</u>S), 3.16 (1H, d, J = 7 Hz, Ar-C<u>H</u>S), 3.46 (3H, s, $OC\underline{H}_3$), 3.63

(1H, d, J = 7 Hz, ArC<u>H</u>S), 4.04 (1H, d, J = 7 Hz, C<u>H</u>COO), 4.21-4.29 (2H, m, C<u>H</u>NH (ser) + chol.), 4.51-4.54 (1H, m, C<u>H</u>NH (cys)), 4.95 (1H, d, J = 6 Hz, chol.), 5.13 (1H, s, C<u>H</u>COO), 5.87 (1H, br, N<u>H</u>), 6.69 (1H, d, J = 3 Hz, Ar-CH), 7.05 (1H, d, J = 5 Hz, N<u>H</u>); ^{13}C NMR [$CDCl_3$]: δ 11.0 (chol.), 11.3 (<u>C</u>H_3), 17.8 (chol.), 18.4 (chol.), 20.3 (chol.), 21.6 (chol.), 21.8 (chol.), 23.0 (chol.), 23.5 (chol.), 27.2 (chol.), 31.1 (Ar-<u>C</u>H_2S), 33.2 (chol.), 35.0 (<u>C</u>H_2S), 35.4 (chol.), 35.8 (chol.), 38.7 (chol.), 39.0 (chol.), 41.5 (chol.), 49.2 (chol.), 51.3 (<u>C</u>HNH), 51.7 (O<u>C</u>H_3), 55.3 (chol.), 55.9 (chol.), 64.9 (<u>C</u>H_2O), 74.4 (chol.), 103.0 (chol.), 105.6 (chol.), 110.4 (Ar-<u>C</u>H), 112.8 (chol.), 115.8 (<u>C</u>CH_2S), 116.0 (<u>C</u>CH_3), 121.5 (chol.), 134.3 (<u>C</u>COO), 138.8 (chol.), 152.9 (<u>C</u>OSi), 154.0 (<u>C</u>OSi), 167.8 (<u>C</u>OO), 168.2 (<u>C</u>OO), 169.6 (<u>C</u>=O); m/z 797 $[M+H]^+$.

6.3.5 4'-Nitrobenzyl 3,5-diacetamide-2-bromomethylbenzoate 88



3,5-Diamino-o-toluic acid 85: A mixture of 3,5-dinitro-*o*-toluic acid **78** (10 g, 44.2 mmol) and 10% Pd/C (5 spatulas) in methanol (250 cm^3) was stirred under H_2 at ambient temperature for 17 h. The catalyst was removed by filtration through celite and the filtrate concentrated *in vacuo* to afford **85** (6.72 g; 91%), as a pale brown solid; mp >300 °C; IR (KBr disc): ν_{max} 1227, 1508, 1714, 2580, 2803, 2846, 3033, 3357 cm^{-1}; ^1H NMR [d_6-DMSO]: δ 2.01 (3H, s, C<u>H</u>$_3$), 6.02-6.03 (1H, d, Ar-<u>H</u>), 6.21-6.22 (1H, d, Ar-<u>H</u>); ^{13}C NMR [d_6-DMSO]: δ 14.3 (<u>C</u>H$_3$), 117.9 (Ar-<u>C</u>H), 120.1 (Ar-<u>C</u>H), 128.7 (<u>C</u>CH$_3$), 131.5 (<u>C</u>COOH), 134.2 (Ar-<u>C</u>NH), 137.2 (Ar-<u>C</u>NH), 168.0 (<u>C</u>=O); m/z 167 $[M+H]^+$.

3,5-diacetamide-*o*-toluic acid 86: 3,5-Diamino-*o*-toluic acid **85** (6.72 g, 40.4 mmol) was suspended in glacial acetic acid (75 cm^3), acetic anhydride (10 cm^3, 90.0 mmol) added and the mixture heated at reflux temperature (120 °C) for 18 h. To the resulting hot, brown suspension was added water (30 cm^3) and the mixture allowed to stand at ambient temperature for 1 h, followed by 4 h at 4 °C. The resulting precipitate was collected by filtration and washed with water to yield **86** (5.35 g, 53 %) as an off-white solid; mp 293-295 °C; IR (KBr disc): ν_{max} 1245, 1323, 1381, 1551, 1596, 1631, 1708, 3214 cm^{-1}; ^1H NMR [d_6-DMSO]: δ 2.01-2.04 (6H, d, C<u>H</u>$_3$CO), 2.24 (3H, s, C<u>H</u>$_3$), 7.74 (2H, s, Ar-<u>H</u>), 7.83 (2H, s, Ar-<u>H</u>), 9.47 (1H, s, N<u>H</u>CO), 10.03 (1H, s, N<u>H</u>CO), 12.93 (1H, s, CO$_2$<u>H</u>); ^{13}C NMR [d_6-DMSO]: δ 14.9 (<u>C</u>H$_3$), 23.3 (NHCO<u>C</u>H$_3$), 24.1, (NHCO<u>C</u>H$_3$), 117.5 (Ar-<u>C</u>H), 119.5 (Ar-<u>C</u>H), 127.4 (<u>C</u>CH$_3$), 132.6 (<u>C</u>COO), 136.8 (<u>C</u>NHCO), 137.7 (<u>C</u>NHCO), 168.5 (<u>C</u>OCH$_3$), 169.1 (<u>C</u>OCH$_3$); m/z 251 $[M+H]^+$.

4'-Nitrobenzyl 3,5-diacetamide-2-methylbenzoate 87: To a suspension of 3,5-diacetamide-*o*-toluic acid **86** (11.35 g, 45.5 mmol) in DMF (100 cm^3), was added 1,1,3,3-tetramethylguanidine (5.7 cm^3, 45.5 mmol) at 0 °C. After stirring for 25 minutes at ambient temperature, 4-nitrobenzyl bromide (9.8 g, 45.5 mmol) was added and stirring continued at ambient temperature for 18 h. Following dilution with EtOAc (500 cm^3), the solution was washed with 1M HCl (25 cm^3) and the resulting precipitate collected by filtration and dried in air to afford **87** (12.08 g, 69 %) as a beige solid; mp 190-194 °C; IR (KBr disc): ν_{max} 1218, 1371, 1527, 1604, 1676, 1695, 1727, 3386 cm^{-1}; ^1H NMR [d_6-DMSO]: δ 2.01-2.05 (6H, d, 2 x $C\underline{H}_3CO$), 2.24 (3H, s, $C\underline{H}_3$), 5.46 (2H, s, $OC\underline{H}_2$), 7.70-7.74 (2H, d, Ar-$C\underline{H}NO_2$), 7.82-7.83 (1H, d, Ar-$\underline{H}$), 7.91-7.92 (1H, d, Ar-$\underline{H}$), 8.25-8.28 (2H, d, Ar-$C\underline{H}NO_2$), 9.54 (1H, s, $N\underline{H}CO$), 10.10 (1H, s, $N\underline{H}CO$); ^{13}C NMR [d_6-DMSO]: δ 15.0 ($\underline{C}H_3$), 23.3 ($NHCO\underline{C}H_3$), 24.1, ($NHCO\underline{C}H_3$), 65.3 ($O\underline{C}H_2$-Ar), 117.5 (Ar-$\underline{C}H$), 120.1 (Ar-$\underline{C}H$), 123.8 (Ar-$\underline{C}HNO_2$), 128.0 ($\underline{C}CH_3$), 128.9 (Ar-$\underline{C}HNO_2$), 130.7 ($\underline{C}COO$), 137.0 ($\underline{C}NHCO$), 137.9 ($\underline{C}NHCO$), 143.9 ($OCH_2\underline{C}$-Ar), 147.3 (Ar-$\underline{C}NO_2$), 166.8 ($\underline{C}OCH_3$), 168.5 ($\underline{C}OCH_3$); m/z 386 $[M+H]^+$.

4'-Nitrobenzyl 3,5-diacetamide-2-bromomethylbenzoate 88: A stirred suspension of 4'-nitrobenzyl 3,5-diacetamide-2-methylbenzoate **87** (15.52 g, 40.3 mmol) and NBS (8.6 g, 48.3 mmol) in CCl_4 (50 cm^3) and DCM (50 cm^3) was heated at reflux temperature with light irradiation for 5 h. The mixture was diluted with DCM and the precipitate collected by filtration to yield **88** (17.33 g, 93%) as a light brown solid; mp 195-198 °C; IR (KBr disc): ν_{max} 1203, 1344, 1519, 1654, 1718, 1747, 3024, 3247 cm^{-1}; ^1H NMR [d_6-DMSO]: δ 2.04-2.06 (6H, d, 2 x $C\underline{H}_3CO$), 2.55 (3H, s, $C\underline{H}_3$), 5.53 (2H, s, $OC\underline{H}_2$), 5.75 (2H, s, $C\underline{H}_2Br$), 7.65 (1H, s, Ar-$\underline{H}$), 7.72-7.76 (2H, d, Ar-$C\underline{H}NO_2$), 8.23 (1H, s, Ar-$\underline{H}$), 8.24-8.28 (2H, d, Ar-$C\underline{H}NO_2$), 9.55-9.58 (2H, d, $N\underline{H}CO$); ^{13}C NMR [d_6-DMSO]: δ 15.2 ($NHCO\underline{C}H_3$), 23.3 ($NHCO\underline{C}H_3$), 29.7 ($\underline{C}H_2Br$), 66.2 ($O\underline{C}H_2$-Ar), 123.8 (Ar-$\underline{C}HNO_2$), 124.9 (Ar-$\underline{C}H$), 124.8 (Ar-$\underline{C}H$), 127.8 ($\underline{C}CH_3$), 129.6 (Ar-$\underline{C}HNO_2$), 134.8 ($\underline{C}NHCO$), 136.6 ($\underline{C}NHCO$), 142.9

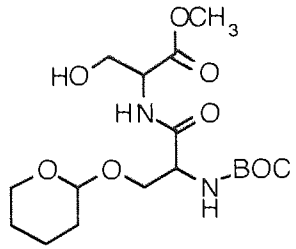
($OCH_2\underline{C}$-Ar), 147.5 (Ar-$\underline{C}NO_2$), 166.8 ($\underline{C}OCH_3$), 168.8 ($\underline{C}OCH_3$), 179.7 ($\underline{C}$=O); m/z 464 $[M+H]^+$.

Isobutyl carbonyl 3,5-dinitro-2-methylbenzoate 82: To a stirred solution of 3,5-dinitro-*o*-toluic acid **78** (5 g, 22.1 mmol) in dry DMF (100 cm^3) at -10 °C was added isobutyl chloroformate (3.2 cm^3, 24.3 mmol) and 4-methylmorpholine (2.7 cm^3, 24.3 mmol) under argon. The mixture was stirred at -10 °C for 1 h then the solvent evaporated *in vacuo* to afford a black residue. Purification by column chromatography using EtOAc:hexane:Et_3N (90:9:1) yielded the title compound **82** (1.53 g, 21 %) as a brown solidifying oil; IR (thin film): ν_{max} 734, 1261, 1346, 1467, 1539, 1728, 2956, 3088 cm^{-1}; ^1H NMR [d_6-DMSO]: δ 0.95-0.98 (6H, d, $CH(C\underline{H}_3)_2$, 1.99-2.10 (1H, m, $CH(C\underline{H}_3)_2$), 2.59 (3H, s, $C\underline{H}_3$), 4.12-4.15 (2H, d, $OC\underline{H}_2$), 8.67-8.68 (1H, d, Ar-$\underline{H}$), 8.87-8.88 (1H, d, Ar-$\underline{H}$); ^{13}C NMR [d^6-DMSO]: δ 16.3 ($\underline{C}H_3$), 19.0 ($CH(\underline{C}H_3)_2$), 27.3 ($\underline{C}H$), 72.0 (OCH_2), 121.7 (Ar-$\underline{C}H$), 127.2 (Ar-$\underline{C}H$), 134.5 ($\underline{C}CH_3$), 138.7 ($\underline{C}COO$), 145.5 ($\underline{C}NO_2$), 151.5 ($\underline{C}NO_2$), 164.4 ($\underline{C}$=O); m/z 382 $[M+H]^+$

Isobutyl carbonyl 3,5-diamino-2-methylbenzoate 84: A mixture of isobutyl carbonyl 3,5-dinitro-2-methylbenzoate **82** (250 mg, 0.766 mmol) and Pd/C (spatula) in methanol (10 cm^3) was stirred under H_2 at ambient temperature for 18 h. The catalyst was removed by filtration through celite and the filtrate concentrated *in vacuo* to yield **84** (170 mg, 98 %) as a pale brown solid; mp 116-120 °C; IR (KBr disc): ν_{max} 1051, 1230, 1348, 1606, 1697, 2958, 3340, 3403 cm^{-1}; ^1H NMR [d_6-DMSO]: δ 0.92-0.95 (6H, d, $CH(C\underline{H}_3)_2$, 1.91-1.98 (1H, m, $C\underline{H}(CH_3)_2$), 1.99 (3H, s, $C\underline{H}_3$), 3.92-3.95 (2H, d, $OC\underline{H}_2$), 4.74-4.77 (4H, d, $N\underline{H}_2$), 6.04-6.05 (1H, d, Ar-$\underline{H}$), 6.21-6.22 (1H, d, Ar-$\underline{H}$); ^{13}C NMR [d_6-DMSO]: δ 13.3 ($\underline{C}H_3$), 19.2 ($CH(\underline{C}H_3)_2$), 27.6 ($\underline{C}H$), 70.1 ($O\underline{C}H_2$), 103.3 (Ar-$\underline{C}H$), 104.5 (Ar-$\underline{C}H$), 109.3 ($\underline{C}CH_3$), 131.9 ($\underline{C}COO$), 146.5 ($\underline{C}NH_2$), 148.0 ($\underline{C}NH_2$), 167.0 ($\underline{C}$=O); m/z 223 $[M-CH_2(CH_3)_2)]^+$

4'-Nitrobenzyl 3,5-dinitro-2-methylbenzoate 79: To a solution of 3,5-dinitro-*o*-toluic acid **78** (1 g, 4.4 mmol) was added at 0 °C *N,N,N,N*-tetramethylguanidine (0.56 cm^3, 4.4 mmol). After stirring for 15 minutes at ambient temperature, 4-nitrobenzyl bromide (955 mg, 4.4 mmol) was added and stirring continued for 18 h. The mixture was diluted with EtOAc (50 cm^3), washed with 1M HCl (3 x 15 cm^3) and saturated NaCl solution (2 x 15 cm^3), dried ($MgSO_4$) and the solvent evaporated *in vacuo* to afford an orange/yellow solid. Recrystallisation using EtOAc:hexane (2:5) afforded **79** (462 mg, 29 %) as a pale yellow solid; mp 113-115 °C; IR (KBr disc): ν_{max} 837, 1160, 1265, 1346, 1512, 1533, 1544, 1726 cm^{-1}; ^1H NMR [d_6-DMSO]: δ 2.60 (3H, s, $C\underline{H}_3$), 5.55 (2H, s, $OC\underline{H}_2$), 7.76-7.80 (2H, d, Ar-$C\underline{H}NO_2$), 8.25-8.29 (1H, d, Ar-$\underline{H}$), 8.77-8.78 (1H, d, Ar-$\underline{H}$), 8.90-8.91 (2H, d, Ar-$C\underline{H}NO_2$); ^{13}C NMR [d_6-DMSO]: δ 16.4 ($\underline{C}H_3$), 66.6 ($O\underline{C}H_2$-Ar), 122.0 (Ar-$\underline{C}H$), 123.8 (Ar-$\underline{C}HNO_2$), 127.5 (Ar-$\underline{C}H$), 129.3 (Ar-$\underline{C}HNO_2$), 134.0 ($\underline{C}CH_3$), 138.9 ($\underline{C}COO$), 143.1 ($OCH_2\underline{C}$-Ar), 145.6 (Ar-$\underline{C}NO_2$), 147.5 ($\underline{C}NO_2$), 151.5 ($\underline{C}NO_2$), 164.2 ($\underline{C}OO$); m/z 360 $[M\text{-}H]^-$.

6.3.6 *N*-[*N*-(*t*-Butoxycarbonyl)-L-seryl tetrahydropyranyl]-L-serine methyl ester 77

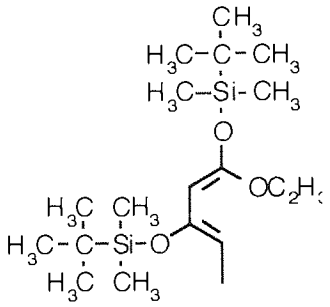


N-*t*-BOC-L-serine.THP 75: A solution of N-(*t*-BOC)-L-serine **30** (2.5 g, 12.2 mmol) and 3,4-dihydro-2H-pyran **74** (1.7 cm^3, 18.3 mmol) in anhydrous DCM (85 cm^3) containing PPTS (306 mg, 1.2 mmol) was stirred at ambient temperature for 4.5 h. The cloudy solution was diluted with diethyl ether (300 cm^3) and washed with half saturated NaCl solution (150 cm^3) to remove the catalyst. The colourless solution was dried ($MgSO_4$) and the solvent evaporated *in vacuo* to afford **75** (1.53 g, 43 %) as a yellow oil; IR (thin film): ν_{max} 1035, 1134, 1199, 1365, 1514, 1714, 2945, 3448 cm^{-1}; ^1H NMR [$CDCl_3$]: δ 1.44 (9H, s, BOC), 1.47-1.61 (6H, m, 3 x $C\underline{H}_2$), 3.40-3.43 (2H, m, $C\underline{H}_2$), 3.59-3.90 (2H, m, $C\underline{H}_2O$), 4.39-4.41 (1H, m, C<u>H</u>NH), 4.53-4.57 (1H, m, OC<u>H</u>O), 6.02 (1H, br, N<u>H</u>), 8.95 (1H, br, $CO_2\underline{H}$); m/z 290 $[M+H]^+$.

***N*-[*N*-(*t*-butoxycarbonyl)-L-seryl tetrahydropyranyl]-L-serine methyl ester 77:** A suspension of L-serine methyl ester.HCl **76** (1.88 g, 12 mmol) and **75** (3.5 g, 12 mmol) in ACN (25 cm^3) was treated at 0 °C with 4-methylmorpholine (1.3 cm^3, 12 mmol). To the stirred solution was added dropwise over 20 minutes at 8-10 °C a solution of DCC (2.5 g, 12 mmol) in ACN (12 cm^3). After stirring at 0 °C for 3 h, the resulting white ppt. was removed by filtration and the filtrate concentrated *in vacuo*. The resulting white residue was suspended in EtOAc (50 cm^3) and washed consecutively with 0.5 M HCl, water, 5% $NaHCO_3$ solution and saturated NaCl solution in 2 x 15 cm^3 portions. The organics were dried ($MgSO_4$) and the solvent evaporated *in vacuo* to afford an orange/yellow oil. Purification by column chromatography using EtOAc:hexane (1:1) as eluant afforded **77** as a colourless oil

(3.08g, 65 %); IR (thin film): ν_{max} 1171, 1252, 1365, 1502, 1705, 1753, 2943, 3349 cm^{-1}; ^1H NMR [$CDCl_3$]: δ 1.44 (9H, s, BOC), 1.50-1.74 (6H, m, 3 x $C\underline{H}_2$), 3.52-3.59 (2H, m, $C\underline{H}_2$), 3.77 (3H, s, $OC\underline{H}_3$), 3.80-4.08 (4H, m, $C\underline{H}_2O$ + CH_2OH), 4.11-4.24 (1H, m, $C\underline{H}NH$), 4.57-4.62 (2H, m, $C\underline{H}NH$ + $OC\underline{H}O$), 5.47 (1H, br, $O\underline{H}$), 5.69 (1H, br, $N\underline{H}$), 7.24 (1H, br, $N\underline{H}$); m/z 391 $[M+H]^+$.

6.3.7 1,3-di*t*(-butyldimethylsiloxy)-1-ethoxy-1,3-pentadiene



1-(*t*-Butyldimethylsiloxy)-1-ethoxypent-1-en-3-one 43: Anhydrous powdered zinc chloride (220 mg) was added to Et_3N (11 cm^3, 80 mmol) and the mixture stirred for 30 minutes at ambient temperature until the salt became suspended in the amine. A solution of ethyl propionylacetate **42** (5.46 g, 38 mmol) in toluene (16 cm^3) was added, followed by TMSCl (9.6 cm^3, 76 mmol). After 1 h the temperature was raised to 40 °C and stirring continued overnight. On cooling, diethyl ether (65 cm^3) was added and the amine hydrochloride removed by filtration to yield an orange liquid. The solvent was removed *in vacuo* to yield **43** as an orange liquid (8.45 g, 97 %); IR (thin film): ν_{max} 1026, 1163, 1247, 1309, 1353, 1718, 1751, 2979 cm^{-1}; ^1H NMR [$CDCl_3$]: δ 0.18 (6H, s, $Si(C\underline{H}_3)_2$), 0.89 (9H, s, $(C\underline{H}_3)_3$), 1.01-1.07 (3H, m, $CH_2C\underline{H}_3$), 1.18-1.24 (3H, s, $OCH_2C\underline{H}_3$), 2.63-2.72 (2H, m, $C\underline{H}_2CH_3$), 4.02-4.11 (2H, m, $OC\underline{H}_2CH_3$), 4.99 (1H, s, $\underline{H}C$=); m/z 243 $[M+H]^+$.

4-Methyl-1-ethoxy-1,3-*t*-butyldimethylsiloxy-1,3-butadiene 37: Lithium diisopropyl amide (2M solution in heptane/THF/ethylbenzene; 24.4 cm^3, 48.9 mmol) was added to a stirred sample of **43** (10.57 g, 48.9 mmol) at -78 °C over 15 minutes. The stirred solution was maintained at -78 °C for 30 minutes, before adding TMSCl (1 cm^3, 8 mmol). The mixture was allowed to warm to ambient temperature and stirring continued for a further 1.5 h. The white solid was removed by filtration and the filtrate concentrated *in vacuo* to afford a yellow liquid. Attempted purification using Kugelrohr distillation (1 mmHg; 100-150 °C) yielded **37** as a very pale yellow liquid, 650mg; m/z 373 $[M+H]^+$.

6.3.8 3-Hydroxy-2,6-dimethylbenzoic acid 139

3-Hydroxy-2,6-dimethylbenzoic acid 139: A mixture of 2,6-dimethylbenzoic acid (1 g, 6.7 mmol) and conc. H_2SO_4 (4 cm^3) was heated at 130 °C for 3 h and allowed to stand at ambient temperature for 4 days. The resulting crystalline solid was dissolved in conc. NaOH solution (10 cm^3) and heated to 100 °C. The solution was mixed with powdered NaOH (10 g) into a paste which solidified on cooling. Small pieces of this product were added in portions to fused KOH at ~185 °C and the resulting mixture heated at ~185 °C for 18 h. On cooling, the mass was dissolved in water, insolubles removed by filtration and the filtrate acidified with conc. HCl. The resulting precipitate was recrystallised from water and dried to yield **139** (720 mg, 34 %) as a colorless oil; IR (thin film): ν_{max} 1045, 1114, 1168, 1203, 1290, 1689, 1724, 3255, 3409 cm^{-1}; ^1H NMR [d_6-DMSO]: δ 2.20 (3H, s, $C\underline{H}_3$), 2.44 (3H, s, $C\underline{H}_3$), 6.99-7.02 (1H, d, Ar-$\underline{H}$), 7.63-7.67 (1H, d, Ar-$\underline{H}$); m/z 167$[M+H]^+$.

Chapter 7:
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