Synthesis of Aminosterols Structurally Related to Squalamine.

University of Leicester

Thesis submitted for the degree of Doctor of Philosophy at the University of Leicester

by

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Statement.

The accompanying thesis submitted for the degree of Doctor of Philosophy entitled “Synthesis and Activity of Aminosterols Related to Squalamine” is based on work conducted by the author in the Department of Chemistry at the University of Leicester in the period October 1997 to September 2000.

All the work in this thesis is original unless otherwise acknowledged by references. None of this work has been submitted for any other degree.

Signed... Date...

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### Abbreviations.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
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<tbody>
<tr>
<td>α</td>
<td>Below the Plane of the Molecule</td>
</tr>
<tr>
<td>β</td>
<td>Above the Plane of the Molecule</td>
</tr>
<tr>
<td>δH</td>
<td>Proton Chemical Shift</td>
</tr>
<tr>
<td>δC</td>
<td>Carbon Chemical Shift</td>
</tr>
<tr>
<td>Δ</td>
<td>Distance Between</td>
</tr>
<tr>
<td>λ</td>
<td>Wavelength</td>
</tr>
<tr>
<td>λmax</td>
<td>Wavelength of Maximum UV absorbance</td>
</tr>
<tr>
<td>μ</td>
<td>Micro (10⁻⁶)</td>
</tr>
<tr>
<td>°C</td>
<td>Degrees Celsius</td>
</tr>
<tr>
<td>ax</td>
<td>Axial</td>
</tr>
<tr>
<td>AMD</td>
<td>Age-related Macular Degeneration</td>
</tr>
<tr>
<td>BOC</td>
<td><em>tertiary</em>-Butoxycarbonyl</td>
</tr>
<tr>
<td>BOC-ON</td>
<td><em>tertiary</em>-Butoxycarbonyloxyimino-2-phenylacetonitrile</td>
</tr>
<tr>
<td>Br</td>
<td>Broad (NMR)</td>
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<tr>
<td>Bz</td>
<td>Benzoyl</td>
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<tr>
<td>dd</td>
<td>Doublet of Doublets</td>
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<tr>
<td>DEPT</td>
<td>Distortionless Enhancement by Polarisation Transfer</td>
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<td>DNA</td>
<td>Deoxyribonucleic Acid</td>
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<td>Dose of Compound Giving a 90% Reduction in Tumour Size</td>
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<td>FAB</td>
<td>Fast Atom Bombardment</td>
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<td>Fibrodysplasia Ossificans Progressiva</td>
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<td>g</td>
<td>Gram</td>
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<td>h</td>
<td>Hours</td>
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<tr>
<td>HIV</td>
<td>Human Immuno-deficiency Virus</td>
</tr>
<tr>
<td>HRMS</td>
<td>High Resolution Mass Spectroscopy</td>
</tr>
<tr>
<td>HSV</td>
<td>Herpes Simplex Virus</td>
</tr>
<tr>
<td>Hz</td>
<td>Hertz</td>
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<tr>
<td>IC₅₀</td>
<td>Concentration of Compound Necessary to Reduce Cell Growth to 50% of Control Growth After Defined Levels of Exposure</td>
</tr>
<tr>
<td>IR</td>
<td>Infrared</td>
</tr>
<tr>
<td>IT</td>
<td>Information Technology</td>
</tr>
<tr>
<td>J</td>
<td>Coupling Constant</td>
</tr>
<tr>
<td>k</td>
<td>Kilo</td>
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<td>L</td>
<td>Litre</td>
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Abstract.

Squalamine is a naturally occurring broad-spectrum antimicrobial agent isolated from the dogfish shark *Squalus acanthias*. It has been shown to have biological activity against both Gram-negative and Gram-positive bacteria, fungi, sexually transmitted diseases (STDs) and cancer. A flexible synthetic route to polyamine-steroid conjugates structurally related to squalamine has been developed.

Five different steroids and have been prepared, three of which have been combined with the protected polyamines shown below to give a large combinatorial library of aminosterols.

![Steroid A, [82] Steroid B, [85] Steroid C, [90]](image)

Reductive amination was used to combine the steroids with the protected polyamines, and putrescine.

![BocBoc BocHIBocHI](image)

Forty-four aminosterols were synthesised and are currently being tested for antimicrobial and anticancer activity.
Chapter 1.

GENERAL INTRODUCTION.
Chapter 1. General Introduction.

1.1 Background.

In the 1960s and 1970s, there were frequent pronouncements that infectious diseases had been conquered and were no longer major threats to health. The U.S. Surgeon General testified to Congress that it was time to "close the book on infectious diseases". The medical and scientific community, and the public in general, accepted this verdict and the major research effort in the 1970s were concentrated on the great "killers and cripplers", heart disease and cancer. The reason for this optimistic view was the stunning success of the introduction by the pharmaceutical industry of the sulphanilamide [1] type drugs in the 1930s, and the penicillins, e.g. [2], in the 1940s. Hundreds of new antibiotics were discovered to treat infectious diseases such as gonorrhoea, syphilis, pneumonia, tuberculosis and typhoid fever.

In fact, infectious diseases had not been eradicated and remain the largest cause of death in the world, greater than cardiovascular disease or cancer. Microbes are not idle bystanders, waiting for new opportunities offered by human mobility, ignorance, or neglect, but possess remarkable genetic versatility that enables them to develop new pathogenic vigour, to escape population immunity by acquiring new antigens, and to develop antibiotic resistance.

Over the last several years, the frequency and spectrum of antimicrobial resistant infections have increased in both the hospital and the community. Resistance has made many currently available antimicrobial drugs ineffective and in some instances is already posing very serious clinical and public health problems. Certain infections that were treatable in the past, are now essentially untreatable, and have begun to occur as epidemics both in the developing and first world. The resurgence of tuberculosis due to multi-drug resistant...
Chapter 1. General Introduction.

*Mycobacterium tuberculosis* is severely complicated by the fact that isoniazid [3] and rifampicin [4], the most effective drugs, are microbicidal and therefore resistance to both drugs may develop. The emergence of methicillin [5] resistant *Staphylococcus aureus* (MRSA) as a major problem worldwide resulted in an increased use of vancomycin, the only agent that effectively treats these bacteria, but this increased use of vancomycin created vancomycin resistance in other species such as *Enterococci*. The extensive use of antibiotics in animal feeds may explain the high antibiotic resistance of *Salmonella* species.

The increasing frequency of drug resistance has been attributed to a combination of microbial characteristics, selective pressures of antimicrobial use, and societal and technologic changes that enhance the transmission of drug resistant organisms. Antimicrobial resistance is resulting in increased morbidity, mortality, and health-care costs. Prevention and control of these infections will require prudent use of existing antimicrobial agents, new vaccines, enhanced public health efforts to reduce transmission and the development of new antimicrobial agents.

The first naturally occurring antibiotics were isolated from bacteria and fungi. Out of all described antibiotics approximately 5% come from bacteria, and all of those used in medicine are produced by the genus *Bacillus* and are non-ribosomal peptide antibiotics, for example the cyclic decapeptide gramicidin S [6]. Around 20% of antibiotics come from
fungi and these include β-lactams such as penicillins and cephalosporins, e.g. [7].

The most important antibiotic producing group are the *Antinomycetes* genera, producing 75% of described antibiotics. This extremely structurally diverse range of compounds includes amphotericin B [29], erythromycin A [8], streptomycin [9] and vancomycin.

Unfortunately, antimicrobial resistance has now built up to many of these antibiotics, particularly β-lactams, so new classes of antibiotics, ideally with novel modes of action, are urgently required to replace the current repertoire once resistance to them has compromised their effectiveness.

The search for new antimicrobial agents has led to the screening of many higher organisms for evidence of the biosynthesis and secretion of antibiotics, which has brought about the
discovery of a number of new potent antibacterial and antifungal agents. Animals require a system of host-defence against pathogens for survival. Multiple mechanisms have been described, such as the array of humoral and cellular responses of the classical vertebrate immune system, and less-specific physical and chemical barriers.

These chemical barriers include low molecular weight antibiotics that are synthesised and stored in the animal. A number of animal tissues from diverse species have been extracted and assayed. Many of the antimicrobial agents have been identified as peptides, such as defensins from mammals, magainins [10] from frogs, tachypleins from the horseshoe crab, and cecropins from insects.6 These peptides all have fewer than fifty amino acids, of which a high proportion are the basic amino acids lysine and arginine. Lipids with antimicrobial activity are represented principally by sphingolipids [11], fatty acids, and both fatty acid esters and O-alkyl ethers of polyhydric alcohols, and are present on the skins of many species of animals.7 Alkaloids form the third chemical class and are isolated from amphibian skin secretions. They include metabolites of histidine and tryptophan, such as spinaceamine [12] and bufotenine [13], and more complex molecules such as samandarine [14]. Research has shown that a number of these chemical agents are induced on wounding or introduction of bacteria into the animal’s body, supporting the view that they are host-defence mechanisms.
Michael Zasloff, an American molecular biologist, worked with the African clawed frog *Xenopus laevis*, and wondered why fresh surgical wounds rarely became infected in murky holding tanks. He quickly discovered the magainins in 1987, a family of peptides present in the frogs skin that kill bacteria, protozoa and fungi. In 1989, Zasloff heard how pregnant dogfish sharks flush their fallopian tubes with seawater to remove foetal waste, and investigated whether the sharks had any way to sterilize the microbe filled water that soaked their foetuses. Could the sharks be producing an antimicrobial compound similar to the magainins? In 1992, Zasloff and Moore discovered that the stomach extracts from the dogfish shark, *Squalus acanthias*, exhibited potent antimicrobial activity, prompting efforts to purify and identify the responsible molecule. They soon realized that the active agent was not a peptide, and following purification the chemical structure of the shark antimicrobial compound was determined by fast atom bombardment mass spectra (FAB MS) and nuclear magnetic resonance (NMR) spectra. The compound turned out to have a surprising structure, being an adduct of the polyamine spermidine with an anionic bile salt intermediate, and was named Squalamine after the shark *Squalus acanthias*. (The stereochemistry at C24 was not confirmed until 1995 by R.M. Moriarty’s stereoselective synthesis, see page 12, 1.2.3 “Total synthesis of squalamine”).

Although some antimicrobial steroids have been isolated from plants, this was the first time that one had been identified in an animal and represents a new class of antimicrobial agents.
1.2.1 Structure of squalamine.

The steroid ring structure of squalamine is similar to that of cholestanol [16]. The β-spermidine group replaces the usual hydroxyl or ketone group at C-3, and the C-7 and C-24 positions are hydroxylated, with the C-24 hydroxyl also being sulphated. The trans A-B ring junction in the molecule is commonly seen in bile alcohols from many species of fish. Hydroxylation of the C-24 position of the cholesterol side chain also occurs widely in fish, as well as reptiles and amphibians. In some of these vertebrates it is modified by sulphation.

A review of the chemical database reveals that squalamine represents the first reported example of a steroid to which spermidine is covalently coupled. Several naturally occurring, cationic aminosterols isolated from medicinal plants used in the treatment of intestinal parasitic infections have similar chemical structures to squalamine. An example is chonemorphine [17], the antiparasitic aminosterol from the Indian plant Chonemorphia macrophylla.

1.2.2 Antimicrobial activity of squalamine.

Squalamine is a broad-spectrum antimicrobial agent of similar potency to ampicillin, a widely used antibiotic. This water soluble, cationic polyamine-sterol conjugate exhibits potent antibacterial activity against Gram-negative (Escherichia coli, Pseudomonas aeruginosa) and Gram-positive (Staphylococcus aureus, Enterococcus faecalis) bacteria, is fungicidal (Candida albicans, Candida tropicalis) and induces the osmotic lysis of protozoa (Paramecium caudatum) as observed with the magainins. In addition squalamine is active in vitro against various sexually transmitted disease (STD) organisms such as
Nesseria gonorrhoea, herpes simplex virus (HSV) and the human immuno-deficiency virus (HIV),\textsuperscript{13} as well as demonstrating antiangiogenic and antitumour activity in multiple animal models.\textsuperscript{14} Squalamine has been shown to cause the haemolysis of red blood cells, but only at concentrations much higher than that required for antimicrobial activity, suggesting that there is a therapeutic window.

Table 1. Antimicrobial activity of squalamine and other related compounds. Minimum Inhibitory Concentrations (MIC's) given in µg/mL.\textsuperscript{9}

<table>
<thead>
<tr>
<th>Sample</th>
<th>E.coli</th>
<th>P.aeruginosa</th>
<th>S.aureus</th>
<th>S.faecalis</th>
<th>P.vulgaris</th>
<th>C.albicans</th>
<th>P.cadatum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squalamine</td>
<td>1-2</td>
<td>4-8</td>
<td>1-2</td>
<td>1-2</td>
<td>4-8</td>
<td>4-8</td>
<td>4-8</td>
</tr>
<tr>
<td>CHAPS</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>250-500</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;260</td>
</tr>
<tr>
<td>Taurolithocholic acid 3-sulfate</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;260</td>
</tr>
<tr>
<td>Spermidine</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>250-500</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;260</td>
</tr>
<tr>
<td>Melittin</td>
<td>8-16</td>
<td>16-31</td>
<td>8-16</td>
<td>4-16</td>
<td>16-31</td>
<td>16-31</td>
<td>2-4</td>
</tr>
<tr>
<td>Magainin-II-amide</td>
<td>31-62</td>
<td>31-62</td>
<td>&gt;250</td>
<td>&gt;250</td>
<td>125-250</td>
<td>125-250</td>
<td>33-65</td>
</tr>
<tr>
<td>CPF-amide</td>
<td>8-16</td>
<td>8-31</td>
<td>8-16</td>
<td>31-62</td>
<td>62-125</td>
<td>62-125</td>
<td>4-8</td>
</tr>
<tr>
<td>Conessine</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>31-62</td>
<td>16-33</td>
</tr>
<tr>
<td>Holothurin</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;250</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>130-260</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>2-4</td>
<td>62-125</td>
<td>&lt;1</td>
<td>&lt;0.25</td>
<td>8-16</td>
<td>&gt;125</td>
<td>&gt;65</td>
</tr>
</tbody>
</table>

The activity of squalamine was compared to that of several other related molecules: conessine [18], holothurin [19], taurolithocholic acid 3-sulfate [20], CHAPS [21], and spermidine [22], as well as against two antibiotic peptides from *Xenopus laevis*, magainin-2-amide [23] and CPF-amide [24], and a well-known antibiotic ampicillin.

Conessine is an alkaloid of plant origin used therapeutically as an antiparasitic agent.\textsuperscript{15} It exhibits antifungal and antiprotozoan activity but lacks antibacterial activity, demonstrating that potent antibiotic activity is not a trivial property of all cationic steroids. Holothurin, a steroid glycoside from the sea-cucumber exhibits modest antiprotozoan activity but shows no antibacterial or antifungal activity.\textsuperscript{16} This demonstrates that surface-active steroids of the saporin family are not comparable to squalamine in antimicrobial activity. The anionic bile salt taurolithocholic acid-3-sulfate, the synthetic zwitterionic steroidal detergent CHAPS, and spermidine are all inactive at the concentration range studied. The free
polyamine spermidine only shows growth inhibition with concentrations that exceed 500 µg/mL. Some bile acids can inhibit Gram-positive bacteria, and to a lesser extent Gram-negative organisms, but not below 100 µg/mL. This data suggests that the biological activity of squalamine results from its unique structure, with the antimicrobial activity of the whole far exceeding that of its individual components.

Figure 8. Molecules related to squalamine.

The importance of the amine substitution on the steroid ring for antibiotic activity is supported by studies carried out by Bellini and co-workers in 1983 and 1990. They synthesised new amides and amines of common bile acids and found that many of these amino-bile acids showed unexpected antibiotic activity (for structures see page 14, 1.3.1 "Bellini et al. 1983/1990"). It was also shown that the amine substitutions dramatically reduced hepatic clearance of these compounds from the bloodstream by the liver and their secretion back into bile. Amine substitutions might therefore be expected to alter the
compounds systemic bio distribution compared to bile salts. This further supports the idea
that squalamine is serving a function very different from that of an unmodified bile salt.

Subsequent evaluation of *Squalus acanthias* established that squalamine is present in many
tissues of the shark, with the highest concentrations present in the liver and gallbladder, the
sites of bile salt synthesis. It was also found, in order of decreasing concentration, in the
spleen, testes, stomach, gills, and intestine.

Seven new aminosterols related to squalamine have also since been isolated from the
sharks liver (see page 21, 1.3.5. "W.A. Kinney *et al.* 2000"). These compounds possess a
relatively invariant cholestane skeleton with a trans AB ring junction, a spermidine or
spermine attached equatorially at C3, and a steroidal side chain that may be sulphated.
Some members of this family of aminosterols exhibit a broad spectrum of antimicrobial
activity comparable to squalamine. One of these compounds, [69], has been studied
extensively *in vivo* and has been shown to induce profound appetite suppression and weight
loss in mammals, including mice, rats, dogs and monkeys. It remains to be proven whether
compound [69] is responsible for the sporadic feeding behaviour of the dogfish shark, an
animal that normally eats only once every two weeks.

Although the aminosterols possess antibiotic activity, their biological functions in the shark
are not well understood. Sharks are predatory scavengers and yet they show remarkable
resistance to bacterial and viral infections as well as an array of toxic chemicals that would
kill most mammals. The combination of the aminosterols tissue distribution and
antimicrobial activity has led to speculation that they are important host-defence
components of the non-adaptive immune system in the shark.

The biosynthetic origin of these compounds also remains to be elucidated. The structures
clearly resemble the known bile acids of the shark, which are frequently found as sulphuric
acid esters. However, the aminosterols are uniquely distinguished from bile alcohols by the
polyamine modification. Whether squalamine or related compounds are present outside this
species is not known, squalamine is made up of two ubiquitous compounds, so the chances
of it not being found elsewhere are slim. Although aminosterols of this family have not
been identified yet in mammals, it should be noted that closely related steroidal alcohols
have been discovered, which represent logical biosynthetic precursors to the aminosterol class. Human astrocytes, for example, can metabolically convert cholesterol to 24-hydroxycholesterol, 7α,25-dihydroxycholest-4-en-3-one, and other alcohols. Further conversion of 3-keto-hydroxycholestanes of this type to aminosterols of the squalamine family would require the presence of an unknown enzyme that could couple spermidine or spermine to the A ring of the steroid. In the human urinary tract incompletely defined molecules with bactericidal activity have been identified. Also, a cationic polyamine component of urine (molecular mass 500-1000) has been isolated that possesses antibacterial activity. Perhaps squalamine or an analogue is important in host resistance to infection in the urinary tract of humans.

1.2.3 Total synthesis of squalamine.

Initially the only way of obtaining squalamine was by extraction of the shark’s livers using the methods developed by Zasloff and co-workers. However, squalamine was only obtained in small amounts (0.001-0.002 weight % yield) at significant expense in both time and materials, i.e. the sharks. An efficient total synthesis of squalamine was needed, as larger quantities were required for mechanistic studies and clinical trials, as well as for confirmation of the structure and stereochemistry at C24. R. M. Moriarty and L. L. Frye published the first two total syntheses in 1994 and 1995 respectively. Both were lengthy (17 steps, 0.36% and 1.9% overall yield) and began with the expensive and rare steroids 3β-acetoxy and 3β-hydroxy-5-cholenic acid [25].

![Figure 9. Starting materials used for the synthesis of squalamine.](image)

In these reports squalamine was obtained as an equal mixture of C24-stereoisomers. This synthetic squalamine was shown to be almost identical to the natural substance in both physical and biological properties.
R. M. Moriarty published a stereoselective synthesis in 1995 using the cheap and readily available steroid stigmasterol [26] as the starting material (19 steps, 3.9% overall yield).\textsuperscript{23} The C24 stereochemistry was established as (R) by comparison of the two synthetic stereoisomers with natural squalamine. The key step was the attachment of the side chain at C22 using either (R)-2 or (S)-2 1,2-epoxy-3-methylbutane to yield the precursors, a procedure developed by Ourisson \textit{et al.} in 1983.\textsuperscript{24} Three steps were required to synthesise the epoxide from L-valine though, giving five steps overall.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{reaction_scheme_1.png}
\caption{Reaction Scheme 1. Introduction of the C24 hydroxyl group.}
\end{figure}

W.A. Kinney then developed a new, more efficient, stereoselective method for introduction of the steroidal side chain in 1997.\textsuperscript{25} This three-step approach utilised the Corey-Bakshi-Shibata (CBS) oxazaborolidine-borane reagents\textsuperscript{26} and was later applied in the most efficient total synthesis of squalamine to date.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{reaction_scheme_2.png}
\caption{Reaction Scheme 2. Introduction of the C24 hydroxyl group.}
\end{figure}

Kinney synthesised an advanced key intermediate [27] to squalamine in 10 steps, 27% yield from stigmasterol, completing the synthesis in another 5 steps with an overall yield of
11%. This stereoselective synthesis has been used to provide squalamine on a multi-gram scale for clinical trials.

An old problem in steroid chemistry is the allylic C-H oxidation at C-7 of a Δ-5-steroid to the corresponding unsaturated 7-ketone. During the scale-up to the pilot plant level, serious problems were detected with the runaway potential of the reaction using chromium reagents (PCC or PDC). H Weinmann et al. found that the ruthenium-catalysed allylic oxidation with tert-butyl hydroperoxide, recently described by Miller et al. could be successfully adjusted to accomplish the reaction on a multi-kilogram scale.

Furthermore, this environmentally friendly procedure (no high excesses of chromium) was applied successfully to several other steroids on the laboratory scale.

Several synthetic routes to squalamine have been discussed and an efficient total synthesis has now been found. However, in order to determine which structural elements are responsible for the impressive biological activity, it is necessary to examine the activity of other polyamine-sterol conjugates related to squalamine. If a wide range of analogues are synthesised and tested for activity, a pattern may develop, giving a greater insight into this group of compounds.
Chapter 1. General Introduction.

1.3 Polvamine-Sterol Conjugates Related to Squalamine.

Numerous aminosterols have been synthesised, many specifically to mimic squalamine. They are discussed here in chronological order.

1.3.1 Bellini et al. 1983/1990.17

In 1983, Bellini and co-workers synthesised 56 amide and amine derivatives of cholane compounds to investigate their antimicrobial properties.

The above compounds were tested against a variety of Gram-positive and Gram-negative strains. The following points could be made from this study:

- No activity was seen against Gram-negative strains.
- The cholic and ursodeoxycholic acid derivatives were in general not very active, whilst many of the deoxycholic acid and chenodeoxycholic acid derivatives had activity against a range of Gram-positive bacteria.
- The amines were usually more potent than the corresponding amides.
- The most active compounds were the deoxycholic amines, particularly where R2=benzylamine, N, N-diethylethlenediamine, morpholine or methylpiperazine, with MIC’s between 7 and 60 µg/mL.

Bellini followed this work with a publication in 1990 outlining the synthesis and activity of 20 new bile acid derivatives from deoxycholic acid. The compounds contained two basic functions at C24 (benzylamino, morpholino, diethanolamino, N,N-diethylethlenediamino, N-methylpiperazino) and at β-C3 (amino, methylamino, ethylamino, benzylamino). These
new compounds showed interesting antimicrobial properties (MIC’s of 0.9-31 μg/mL) against five Gram-positive and four Gram-negative strains, two fungi and one yeast. Bellini summarised the relationship between structure and antimicrobial activity of all the basic cholane derivatives and analogues synthesised.

Table 2. Structure-activity relationship for basic cholane derivatives.

<table>
<thead>
<tr>
<th>Compound</th>
<th>R₁</th>
<th>R₂</th>
<th>R₃</th>
<th>X</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lithocholyl</td>
<td>αOH</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>CH₂</td>
</tr>
<tr>
<td>Hyodeoxycholyl</td>
<td>αOH</td>
<td>αOH</td>
<td>H</td>
<td>H</td>
<td>CH₂</td>
</tr>
<tr>
<td>Ursodeoxycholyl</td>
<td>αOH</td>
<td>H</td>
<td>βOH</td>
<td>H</td>
<td>CH₂</td>
</tr>
<tr>
<td>Chenodeoxycholyl</td>
<td>αOH</td>
<td>H</td>
<td>αOH</td>
<td>H</td>
<td>CH₂</td>
</tr>
<tr>
<td>Deoxycholyl</td>
<td>αOH</td>
<td>H</td>
<td>H</td>
<td>αOH</td>
<td>CH₂</td>
</tr>
<tr>
<td>Cholyl</td>
<td>αOH</td>
<td>H</td>
<td>αOH</td>
<td>αOH</td>
<td>CH₂</td>
</tr>
<tr>
<td>Urscholyl</td>
<td>αOH</td>
<td>H</td>
<td>βOH</td>
<td>αOH</td>
<td>CH₂</td>
</tr>
<tr>
<td></td>
<td>αOH</td>
<td>H</td>
<td>H</td>
<td>αOH</td>
<td>CO</td>
</tr>
<tr>
<td></td>
<td>=O</td>
<td>H</td>
<td>H</td>
<td>=O</td>
<td>CO</td>
</tr>
<tr>
<td></td>
<td>βNR₂</td>
<td>H</td>
<td>H</td>
<td>=O</td>
<td>CO</td>
</tr>
<tr>
<td></td>
<td>βNR₂</td>
<td>H</td>
<td>H</td>
<td>αOH</td>
<td>CH₂</td>
</tr>
</tbody>
</table>

It can be seen that the addition of an amino group in the C3 position, as in squalamine, imparts the steroid with high activity.

1.3.2 S.L. Regen et al. 1995.

The first specific squalamine analogue [28] was synthesised by S.L. Regen et al. in 1995. A simple 3-step procedure was used starting from 23,24-bisnor-5-cholenic acid-3-β-ol, (20% yield overall). The design was based on the rather loose structural analogy of squalamine with amphotericin B [29], a well-known antifungal agent that is thought to be an
ionophore, owing its bacterial activity to its ability to insert into lipid bilayers. It was hypothesised that squalamine, and the polyamine-steroid analogue [28], could both owe their biological activity to their ability to adopt a cyclic conformation that resembles amphotericin B, all having a long and rigid hydrophobic unit, a flexible hydrophilic chain and a pendant polar head group. Insertion within the membrane would then lead to self assemblage into aggregates with the polyammonium moieties lining the inside of the 'channel'.

Figure 13. First squalamine analogue synthesised by Regen.

![Aminosterol](image1)

![Amphotericin B](image2)

Table 3. Antimicrobial spectrum of the polyamine analogue [28], polyether analogue and saturated analogue compared to that of squalamine. MICs are given in μg/mL.

<table>
<thead>
<tr>
<th>Sample</th>
<th><em>E. coli</em></th>
<th><em>P. aeruginosa</em></th>
<th><em>S. aureus</em></th>
<th><em>P. vulgaris</em></th>
<th><em>C. albicans</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Squalamine [28]</td>
<td>1-2</td>
<td>4-8</td>
<td>1-2</td>
<td>4-8</td>
<td>4-8</td>
</tr>
<tr>
<td>Polyether analogue</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Saturated analogue</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
</tbody>
</table>

The polyamine-sterol analogue [28] is also a potent broad-spectrum antimicrobial agent, demonstrating that substantial changes can be made to the structure of the polyamine-steroid conjugates with retention of antimicrobial activity. This synthesis makes much more accessible analogues possible. A polyether analogue was also synthesised, (structure not shown), which did not show antimicrobial activity below 100 μg/mL, showing that the pendant polyamine chain is not merely a hydrophilic structure but plays an important and more specific role in the biological activity. It was additionally shown that very small alterations to the structure could significantly affect the activity. For example, the saturated
analogue was also synthesised and showed significantly lower antimicrobial activity. This suggests that there is considerable room for fine-tuning the biological properties of such compounds.

1.3.3 S.R. Jones et al. 1996.3

The six bile acid analogues below were synthesised by S.R. Jones et al. using hyodeoxycholic acid as the starting material (6 steps, 12-36%). Although they do not contain a C24 sulphate or side-chain many still possess potent, broad-spectrum antimicrobial activity similar to that of squalamine.

![Figure 14. Squalamine analogues synthesised by Jones et al.](image)

Table 4. Antimicrobial spectrum of polyamine analogues [30] to [35] compared to that of squalamine. MIC’s are given in µg/mL.

<table>
<thead>
<tr>
<th>Sample</th>
<th>E.coli</th>
<th>P.aeruginosa</th>
<th>S.aureus</th>
<th>C.albicans</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squalamine</td>
<td>1-2</td>
<td>4-8</td>
<td>1-2</td>
<td>4-8</td>
</tr>
<tr>
<td>[30]</td>
<td>32</td>
<td>32</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>[31]</td>
<td>32</td>
<td>128</td>
<td>2-4</td>
<td>4</td>
</tr>
<tr>
<td>[32]</td>
<td>8-16</td>
<td>64</td>
<td>1</td>
<td>2-4</td>
</tr>
<tr>
<td>[33]</td>
<td>32-64</td>
<td>128</td>
<td>16</td>
<td>8</td>
</tr>
<tr>
<td>[34]</td>
<td>16</td>
<td>8</td>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td>[35]</td>
<td>&gt;256</td>
<td>128</td>
<td>&gt;256</td>
<td>&gt;256</td>
</tr>
</tbody>
</table>
Several conclusions can be drawn from the structure-activity relationships shown.

- The polyamine substituent was again shown to be crucial as none of the 3-keto-steroid intermediates had any antimicrobial activity (structures not shown).

- Changing the identity of the polyamine chain only had a small effect upon the activity, with the analogue with the longer polyamine chain usually being more active (compare [30] and [32]; [31] and [33]; [34] and [35]). This is more pronounced for the free acids.

- The stereochemistry of addition of the polyamine chain also did not have much effect upon the activity, with the 3β-analogues being slightly more effective than the 3α-analogues (compare [30] and [31]; [32] and [33]).

- Hydrolysis of the ester leaving the free acid alters the selectivity of the spermine analogue [34] compared to [30], but dramatically reduces the activity of the ethylene diamine analogue [35] compared to [32].

- The selectivity of the agents was altered by all of these changes.

With these compounds, it appears that the proposed intramolecular interaction by S.L. Regen, of the polyamine with the acid moiety giving a cyclic salt bridge, may not be necessary. A negative charge at the end of the molecule was not necessary for antimicrobial activity, and one of the most active analogues, [32], is incapable of forming a macrocyclic conformation resembling amphotericin B. The ethylene diamine is too short to reach the side chain, and there is no negatively charged group on the side chain available for electrostatic interaction with an amine. If these compounds do have the same mode of action as squalamine, then the hypothesis that a cyclic form is necessary cannot be correct. The electrostatic interactions between the positively charged amines and negatively charged lipid on the surface of bacterial cells certainly could contribute to the mechanism of antimicrobial activity. However, one would expect some relationship between activity and the length of the amine substituent. This is not shown above either.
D. Armstrong et al. prepared the aminosterols below in high yield from relatively simple, readily available cholic acids.

Several of these squalamine mimics showed activity against bacteria, including methicillin resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus faecium*, and fungi.
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The most active compound against Gram-negative rods was [45], and against Gram-positive cocci and fungi was [52]. Four of the compounds ([38], [39], [42] and [43]) were completely inactive with no MIC's less than 100 µg/mL (results not shown).

Table 5. Antimicrobial spectrum of polyamine-sterols [36] to [59] compared to that of squalamine. MIC's are given in µg/mL.

<table>
<thead>
<tr>
<th>Sample</th>
<th>E.coli</th>
<th>P.aeruginosa</th>
<th>S.aureus</th>
<th>C.albicans</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squalamine</td>
<td>1-2</td>
<td>4-8</td>
<td>1-2</td>
<td>4-8</td>
</tr>
<tr>
<td>[36]</td>
<td>12.5</td>
<td>1.56</td>
<td>6.25</td>
<td>100</td>
</tr>
<tr>
<td>[37]</td>
<td>6.25</td>
<td>3.13</td>
<td>&gt;100</td>
<td>12.5</td>
</tr>
<tr>
<td>[40]</td>
<td>100</td>
<td>25</td>
<td>100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>[41]</td>
<td>25</td>
<td>25</td>
<td>12.5</td>
<td>12.5</td>
</tr>
<tr>
<td>[44]</td>
<td>50</td>
<td>50</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>[45]</td>
<td>3.13</td>
<td>3.13</td>
<td>1.56</td>
<td>3.13</td>
</tr>
<tr>
<td>[46]</td>
<td>6.25</td>
<td>3.13</td>
<td>0.78</td>
<td>6.25</td>
</tr>
<tr>
<td>[47]</td>
<td>&gt;100</td>
<td>25</td>
<td>25</td>
<td>100</td>
</tr>
<tr>
<td>[48]</td>
<td>50</td>
<td>100</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>[50]</td>
<td>12.5</td>
<td>6.25</td>
<td>12.5</td>
<td>12.5</td>
</tr>
<tr>
<td>[51]</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>1.56</td>
</tr>
<tr>
<td>[52]</td>
<td>1.56</td>
<td>0.78</td>
<td>0.78</td>
<td>3.13</td>
</tr>
<tr>
<td>[53]</td>
<td>3.13</td>
<td>12.5</td>
<td>6.25</td>
<td>25</td>
</tr>
<tr>
<td>[54]</td>
<td>3.13</td>
<td>12.5</td>
<td>3.13</td>
<td>3.13</td>
</tr>
<tr>
<td>[55]</td>
<td>50</td>
<td>25</td>
<td>3.13</td>
<td>6.25</td>
</tr>
<tr>
<td>[56]</td>
<td>3.13</td>
<td>12.5</td>
<td>1.56</td>
<td>3.13</td>
</tr>
<tr>
<td>[57]</td>
<td>6.25</td>
<td>12.5</td>
<td>6.25</td>
<td>25</td>
</tr>
<tr>
<td>[58]</td>
<td>50</td>
<td>25</td>
<td>6.25</td>
<td>6.25</td>
</tr>
<tr>
<td>[59]</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
</tbody>
</table>

Several points can be taken from this research:

- The hydrophobicity of the sterol backbone was a critical determinant of activity. Lithocholic, chenodeoxycholic, and deoxycholic acid were all very active steroid backbones, hyodeoxycholic and cholic acid were weakly active, whilst ursodeoxycholic and urosocholic acid were inactive.

- The activity varied with the cationic charge and length of the polyamine chain with spermidine giving the most active compounds.
Addition of the SO$_3$H group reduced the analogues activity (more evidence against the formation of a cyclic salt bridged form being essential for activity).

The activity was decreased by the addition of divalent or monovalent cations. This is probably due to reduced binding of the cationic polyamine to the cell surface, again showing its importance to activity.

The activity is also reduced by horse serum, possibly because of binding to albumin. The concentration of albumin used was similar to that of normal human serum. This may limit the compounds use as systemic agents.

1.3.5 W. A. Kinney et al. 2000.

Whilst attempting to obtain large amounts of squalamine from the dogfish shark William Kinney and co-workers from Magainin Pharmaceuticals discovered, isolated and characterised a whole family of novel aminosterols. Although squalamine is the most abundant aminosterol found in the dogfish shark liver (400-800 mg, 0.001-0.002%), seven additional aminosterols ([63] to [69]) were found in amounts of 20-100 mg (0.00005-0.00025%) for a typical shark liver preparation (40 kg). Other minor aminosterols, found in trace quantities of <2 mg/40 kg shark liver, have not been fully characterised.

![Figure 16. Structures of major aminosterols isolated from dogfish shark liver.](image)
These aminosterols possess a relatively invariant steroid skeleton with a trans AB ring junction, a polyamine attached equatorially at C3, and a cholestane related side chain that may be sulphated. The structural diversity within this group of compounds is observed primarily in the side chain, although that for [64] and [69] are identical to that of squalamine.

Table 6. Antimicrobial activity of aminosterols from the dogfish shark *Squalus acanthias*. MIC’s are given in μg/mL.

<table>
<thead>
<tr>
<th>Sample</th>
<th><em>E.coli</em></th>
<th><em>P.aeruginosa</em></th>
<th><em>S.aureus</em></th>
<th><em>C.albicans</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Squalamine</td>
<td>4</td>
<td>16</td>
<td>1</td>
<td>16</td>
</tr>
<tr>
<td>[63]</td>
<td>128</td>
<td>32</td>
<td>4-8</td>
<td>16</td>
</tr>
<tr>
<td>[64]</td>
<td>16</td>
<td>16</td>
<td>8-16</td>
<td>32</td>
</tr>
<tr>
<td>[65]</td>
<td>8</td>
<td>16</td>
<td>2</td>
<td>32</td>
</tr>
<tr>
<td>[66]</td>
<td>256</td>
<td>256</td>
<td>8-16</td>
<td>128</td>
</tr>
<tr>
<td>[67]</td>
<td>128</td>
<td>128</td>
<td>8</td>
<td>32</td>
</tr>
<tr>
<td>[68]</td>
<td>16</td>
<td>16</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>[69]</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>4</td>
</tr>
</tbody>
</table>

These compounds have a broad spectrum of antimicrobial activity comparable to squalamine and, in the case of aminosterols [64], [65], [68] and [69], exhibit similar potency. Aminosterol [69] only differs from squalamine by having the longer polyamine spermine attached at C3; this increase in length and cationic charge also increases the activity. Compounds [66] and [67] show the least activity.

1.3.6 I. H. Gilbert *et al*. 2000.34

Ian Gilbert and colleagues were working as part of a programme to derive novel agents against parasitic protozoa responsible for leishmaniasis, Chagas disease and African trypanosomiasis. Squalamine and its analogues had not previously been tested against the causative parasites of these diseases, so ten simplified analogues were synthesised, using 3β-acetoxybisnor-5-cholenic acid as the starting material. Identical compounds were synthesised with or without the sulphate group, and with or without BOC protection on the polyamines, to give more information on the roles they play in activity. The literature methods used by Regen and Armstrong were modified for the synthesis of analogues,
giving the most advanced compound [77] in 6 steps, 24% yield.

These compounds were then tested against the clinically relevant forms of the parasites. Unfortunately, no squalamine could be obtained for comparison. The data is shown below in Table 7.

**Table 7.** ED$_{50}$ (µM) values for activity of compounds against trypanosomes and leishmania.

<table>
<thead>
<tr>
<th>Sample</th>
<th><em>L. donovani</em></th>
<th><em>T. cruzi</em></th>
<th><em>T. brucei</em></th>
<th>Toxicity (KB cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[70]</td>
<td>17.3</td>
<td>&gt;42</td>
<td>0.55-2.6</td>
<td>111</td>
</tr>
<tr>
<td>[71]</td>
<td>4.7</td>
<td>&gt;34(toxic)</td>
<td>0.57-1.1</td>
<td>31</td>
</tr>
<tr>
<td>[72]</td>
<td>25.7</td>
<td>&gt;15(toxic)</td>
<td>1.5-4.7</td>
<td>65</td>
</tr>
<tr>
<td>[73]</td>
<td>12.6</td>
<td>&gt;36</td>
<td>0.60</td>
<td>27</td>
</tr>
<tr>
<td>[74]</td>
<td>4.9</td>
<td>&gt;36(toxic)</td>
<td>&gt;36</td>
<td>&gt;360</td>
</tr>
<tr>
<td>[75]</td>
<td>&gt;30</td>
<td>&gt;30</td>
<td>5.4</td>
<td>1.5</td>
</tr>
<tr>
<td>[76]</td>
<td>44.7</td>
<td>&gt;63(toxic)</td>
<td>0.63-1.9</td>
<td>3.3</td>
</tr>
<tr>
<td>[77]</td>
<td>5.0</td>
<td>&gt;57(toxic)</td>
<td>0.56-1.1</td>
<td>15</td>
</tr>
<tr>
<td>[78]</td>
<td>39.9</td>
<td>13.5</td>
<td>&gt;53</td>
<td>&gt;526</td>
</tr>
<tr>
<td>[79]</td>
<td>20.5</td>
<td>&gt;48</td>
<td>&gt;48</td>
<td>&lt;0.47</td>
</tr>
</tbody>
</table>

The compounds showed the most activity against *Trypanosoma brucei*, with five compounds showing ED$_{50}$ values around 1µM. Compounds [71], [74] and [77] showed
significant activity against *Leishmania donovani*, whilst most compounds showed poor activity against *Trypanosoma cruzi*. It is unclear why there is such a marked difference between the species.

The following conclusions can be drawn from the study:

- The length and charge of the polyamine chain had little effect on the activity, the spermine series being slightly more active against *L. donovani*.
- The presence of the BOC or acetate protecting groups did not decrease activity, actually increasing it against *L. donovani*.
- The sulphated compounds were the least active, perhaps due to poor uptake into the parasites.

Compound [77] was also investigated against rodent models in *in vivo* tests. There was no effect against mice infected with *T. b. rhodesiense* (dosed at 50mg/kg for 4 days intraperitonally) and only a 16% reduction in parasite load of mice infected with *L. donovani* (dosed at 50mg/kg for 5 days intraperitonally).

### 1.3.7 Conclusions.

Although a large number of aminosterols have been synthesised and tested, no real structure activity patterns have emerged. Many of the active compounds vary immensely in structure, and yet a small change can sometimes dramatically reduce potency. Variations to the structure do lead to the spectrum of activity being altered. The above could be explained by a number of mechanisms of action being in operation for the aminosterols; different membrane components interacting with the species; a different *in vivo* metabolism for the aminosterols.

The only sound structural conclusions that one can draw from the previous studies are:

- The length and charge of the polyamine is not always important.
- The steroid structure can be extremely varied.
- The polyamine can be placed on either end of the steroid.
- The sulphate group can be replaced by a different polar group or even removed altogether.
1.4 Mode of Action Studies on Aminosterols.

The antimicrobial mode of action of squalamine and its derivatives has not yet been determined, although a number of suggestions have been made. Only a few modes of action studies have so far been published, with most of these concentrating on the disruption of cell membranes. The first squalamine mimic [28], synthesised by Regen, and squalamine itself have shown interesting yet contrasting results in these studies. Other possible modes of action include interference with ion channels, uptake via the polyamine transport system, and interaction with DNA, all of which will be discussed.

The anticancer activity shown by squalamine has been extensively researched, and is understood more fully. This will be detailed after looking at the possible antimicrobial modes of action.

1.4.1 Disruption of Cell Membranes- Ionophoric Behaviour by Regen's Mimic [28] \[31,35\]

Membrane-disrupting drugs should be ideally suited as therapeutic agents, because they circumvent the problems associated with enzymatic degradation within the cell, and export mechanisms of drug resistance.

Regen et al. designed their mimic on the analogy that squalamine, in its cyclic salt-bridge form, bears a structural and functional resemblance to amphotericin B, a well-known ionophore with the ability to insert into lipid bilayers. It was hypothesised that aminosterol [28] may also do this, hence forming ion channels and disrupting the cell membrane. If sterol-polyamine conjugates could function as ionophores, they might distinguish between
membranes based on surface charge, particularly when protonated as they would have strong interactions with negatively charged lipid. The fact that the outer surface of bacterial cell membranes is negatively charged, while that of mammalian cells is electrically neutral, suggests that they may exhibit significant cellular selectivity and thus low toxicity *in vivo*.

The ability of aminosterol [28] to discharge pH gradients across phospholipid bilayers was studied. A pH sensitive dye (pyranine) was entrapped within target vesicles with the analogue. The dye's fluorescence intensity was then used to monitor changes in intravesicular pH.

- The analogue was shown to favour the transport of ions across negatively charged egg phosphatidyl glycerol (PG) bilayers, over electrically neutral egg phosphatidyl coline (PC) bilayers.
- Greater than 95% of the dye remained entrapped within the vesicles, therefore the observed increase in fluorescence intensity clearly reflected ionophoric activity and not the release of the trapped dye by membrane lysis.

The analogue was also tested for Na⁺ and Li⁺ ion transport activity. No activity was observed for either egg PG or egg PC vesicles over a 48-hour period. This indicated that either proton and/or anion transport controlled the rate of pH discharge. Further experiments strongly indicated that ion channels are involved and that anion transport is rate limiting for pH discharge.

A proposal has been put forward for this analogue's ionophoric activity, where both the membrane selectivity and ion selectivity can be accounted for.

- If the analogue is drawn into the membrane as a 'macrocyclic salt', having its sterol moiety and polyamine chain aligned perpendicular to the membrane's surface, then its head group would be zwitterionic. Internalised aggregates of the analogue would be expected to be more stable in a 'sea' of negatively charged phospholipids (PG), rather than ones that are zwitterionic (PC), as zwitterionic head groups would favour other zwitterionic head groups as nearest neighbours. Hence the formation of channels more easily for PG vesicles.
- No Na⁺ or Li⁺ ion transport would be seen due to charge repulsion by proton- ionised channels. Only the passage of protons and anions would be permitted.

A further study was conducted where analogues of [28], in which the terminal amine group was acetylated, the 3β-hydroxyl group was left un-sulphated, and each of the secondary
Chapter 1. General Introduction.

amines were replaced with oxygen atoms, were prepared and tested as ionophores. None of these compounds discharged a pH difference across the vesicle membrane. This strongly supported Regen’s hypothesis of the mode of action of this compound.

Although this analogue may interact with target vesicles in this way, there is strong evidence that this is not the mode of action of all aminosterols. Many mimics have similar potent activity, but cannot adopt this cyclic form due to a short polyamine, or lack of sulphate group. If the mode of action for all aminosterols is the same then this proposal must be wrong. The ionophoric activity and membrane selectivity of [28] still make it a very attractive compound for the design of new antibacterial agents.

1.4.2 Disruption of Cell Membranes- Lytic Behaviour by Squalamine [15].

The ability of squalamine [15] to induce the leakage of polar fluorescent dyes from large unilamellar vesicles (LUVs) has been studied by B.S. Selinsky et al. Micromolar squalamine has been shown to cause leakage of the small molecule carboxyfluorescein (CF) from vesicles prepared from the anionic phospholipids phosphatidyl glycerol (PG), phosphatidyl serine (PS), and cordiolipin (CL). Squalamine was found to bind most tightly to PG membranes followed by PS and CL.

The leakage of CF from vesicles comprised of the zwitterionic lipid phosphatidyl choline (PC) was also studied. Leakage only occurred at much higher squalamine/lipid molar ratios and only at low residue concentrations.

From these results, (the minimal selection between anionic phospholipids and the clearly weaker binding to electrically neutral PC LUVs), it was suggested that an electrostatic interaction between the squalamine cationic polyamine and the lipid phosphate must be a primary determinant of membrane binding.
Fluorescent dye leakage was also determined to follow a graded mechanism, as opposed to an all-or-none mechanism. This suggested the formation of a discrete membrane pore rather than a generalised disruption of vesicular membranes. The size of the squalamine-induced membrane defect was measured by using fluorescently labelled dextrans of different molecular weight. Material with molecular weight \( \geq 10,000 \) g/mol was retained. The surfactant like nature of squalamine was also tested using a variety of biochemical and microscopic experiments.

A model was proposed for the interaction of squalamine with phospholipid bilayers:

- Initially squalamine could bind to the surface of phospholipid vesicles, the binding affinity being related to vesicle charge and the surface organisation of the lipid. The nature of this binding would initially be electrostatic and absolutely require the presence of phospholipid. A strong, specific interaction between squalamine’s protonated polyamine nitrogens and the phosphate would probably take place.

- After binding, squalamine would aggregate on the vesicle surface. The structure of the aggregates is unknown but probably contains both squalamine and phospholipid.

- These aggregates could then cause defects in the membrane, allowing the release of vesicular contents with molecular weight of less than 10,000 g/mol. These aggregates are not stable, and relax to a different structure.

- Squalamine complexed to lipid would then extract itself from the LUVs in the form of small vesicles.

This proposal does still not explain the lack of a relationship between the length of the polyamine chain and activity seen for some analogues. If the above proposal were correct; that there is a specific interaction between the protonated nitrogens and the phosphate; then you would expect a longer polyamine chain to bind more strongly.

This research does also not support the model proposed by Regen et al. The size of the pore generated by squalamine must be large enough to allow the passage of fluorescent dyes, for the analogue [28], a pH-sensitive fluorescent dye remained encapsulated for ion transport to be observed. When squalamine was tested in anionic and zwitterionic LUVs (as Regen had done for [28]) no ionophoric activity was observed. When squalamine was added externally to the vesicles, the dye was released. Although the analogue [28] does possess some of the structural elements of squalamine, the orientation of those elements and the
structure of the sterol ring are different. As both compounds possess significant antimicrobial activity, these results suggest that either multiple mechanisms for the antimicrobial activity of aminosterols exist; depending upon the aminosterol structure, or possibly an unrelated common mechanism for antimicrobial activity remains to be discovered.

1.4.3 Disruption of Cell Membranes- T.A. Russo and D. Mylotte. 38

More compelling evidence that squalamine does owe its antimicrobial activity to its ability to disrupt membranes came from a study by T.A. Russo and D. Mylotte in 1998. The resistance of E. coli. to host defences in animals is in part due to the K54 capsule and O4 specific antigen moiety of the lipopolysaccharide. Both the capsular polysaccharide and the O specific antigen have been shown to play important roles in resistance in a mouse model of urinary tract infection. Their roles in protecting an extraintestinal isolate of E. coli. (CP9) against the bacterial activity of squalamine was studied. The model pathogen CP9, from a clinical blood isolate of E. coli., and derivatives of CP9 devoid of the K54 capsule, the O4 specific antigen, or both, were constructed and evaluated in a variety of in vitro and in vivo systems.

The O4 specific antigen was shown to be important in E. coli. for protection against squalamine. A unique observation from the study is that not only did the K54 capsule not protect against squalamine mediated kill of E. coli., but actually enhanced it. It was postulated that the presence of squalamine in sharks, one of the oldest known species, could be an evolutionary host-defence response to encapsulated pathogens. This is the first example in which expression of a surface polysaccharide, which is an established virulence trait, may potentially put the organism at risk for increased host mediated clearance.

So there is strong evidence that these polyamine-steroid conjugates do disrupt membranes. It is strange though that the two above conjugates, squalamine [15] and the analogue [28], have very similar antimicrobial activity yet interact with LUVs in such different ways. Both of these polyamine-steroids are potent antimicrobial agents with MICs of less than 10 μg/mL against most microorganisms. In the experiments reported, they are studied at similar concentrations, but the LUVs suspensions are much more dilute than the cellular
suspensions used in determining MICs. As a result, the lytic/ionophoric behaviour observed may not relate to the antimicrobial activity, and again leaves open the possibility that a different mode of action is in operation.

1.4.4 Disruption of Cell Membranes- Detergent Behaviour.\textsuperscript{36}

Detergents have been proposed to disrupt phospholipid liposomes via a three-step mechanism. Firstly, the detergent monomers are incorporated into the phospholipid bilayers until the liposome is saturated. The second stage now begins as mixed phospholipid-detergent micelles begin to form. By the third stage, the lamellar to micellar transition is complete, there are no remaining liposomes and all of the phospholipids are found in mixed micelles.

This three-step mechanism has been used to understand the action of many detergents and could be used to explain the vesicular and cell lysis caused by polyamine-steroid conjugates. However, a simple non-specific interaction between these compounds and microbial plasma membranes does not explain the lack of relationship between polyamine length and activity observed for some analogues. Neither does it explain the lipid selectivity, nor the observed differences in specificity between microorganisms for some of the agents. This class of compounds also exhibit very low MICs. The aggregation of squalamine and its analogues is not observed at concentrations close to that of the MICs. For these reasons, a detergent-like mechanism is unlikely.

1.4.5 Interference with Ion Channels/ Pumps.\textsuperscript{39,40}

It has been suggested by A.N. Lopatin \textit{et al.} that polyamines, such as spermidine, may be responsible for the regulation of potassium channels serving as the inward rectifier of the potassium gate.\textsuperscript{39} The aminosterols polyamine moiety might be able to extend into the spermidine binding channel of bacterial cells, with the steroid component then altering the dissociation displacement kinetics, eventually leading to cell death.

M. Zasloff and M. Donowitz noticed that squalamine changes the shape of cells in model cell systems, suggesting that its potential targets might be transport proteins controlling cell
volume or cell shape. They studied the effects of squalamine on rabbit and human Na\(^+\)/H\(^+\) exchanger isoforms present in intestinal and renal brush-borders. It was found that squalamine is a species independent specific inhibitor of the brush-border NHE isoform NHE3 (no effect on NHE1 or NHE2). The inhibitory effect was concentration dependent, with 13, 47 and 57% inhibition with 3, 5, and 7 \(\mu\)g/mL. It was also time dependent, with no effect on immediate addition and maximum effect within one hour of exposure, indicating that squalamine may influence the Na\(^+\)/H\(^+\) exchanger function indirectly, perhaps through an intracellular signalling pathway or by acting as an intracellular modulator. The long time frame is consistent with the need for squalamine to be taken up by the cell until the intracellular concentration needed to inhibit the NHE3 accumulates. Squalamine had no cytotoxic effect at the concentration studied and acted in a fully reversible manner.

If squalamine is taken up into the cell, then a transport mechanism will be required, since the multiply charged nature of these polyamine-steroid conjugates would tend to prevent passive diffusion of them across the membrane.

1.4.6 Uptake via the Polyamine Transport System.

Polyamines are required by cells for growth and differentiation. Most mammalian cells have the ability to synthesise polyamines themselves, but some possess a polyamine uptake system to selectively accumulate essential polyamines, including spermidine. Bacterial cells possess a number of these polyamine transport systems, as do some tumour cells. Although little is known mechanistically about these bacterial polyamine transporters, it has been shown that a wide-range of structurally diverse polyamine analogues are extracted from the extracellular medium. This suggests that polyamine-steroid conjugates could bind to the bacterial polyamine transporter, from where there are three possible outcomes:

- They could inhibit the uptake of polyamines vital to the cell, therefore leading to cell death.
- The polyamine uptake system could inadvertently assist in inserting them into the membrane, leading to membrane disruption.
- They could be substrates for the polyamine transport system, taking them into the cell. This is of considerable significance, such that if the cellular target is within the bacteria, some transport mechanism must be implicated.

No studies of this mechanism have so far been reported.
1.4.7 DNA Binding.

If squalamine-like molecules do gain access to the cell then alternative modes of action, such as specific binding to DNA, must be considered. Polyamines are known to have a high affinity for DNA, although the nature of the interaction remains controversial. P.M. Cullis et al. have shown that chlorambucil, a well known mustard used in the treatment of cancers, has enhanced cross-linking efficiency with DNA when a spermidine side chain is attached. Although the polyamine enhanced the cross-linking efficiency, the site of cross-linking was unperturbed which is consistent with a non-specific polyamine interaction.

Burrows et al. synthesised steroidal-polyamines which also had increased DNA binding compared to simple polyamines. They concluded that DNA binding was dependent on the total number of ammonium groups, the stereo- and regio- chemistry of these groups, and also on the hydrophobic contribution of the steroid nucleus. In the case of squalamine-like structures, the non-covalent interaction made by the steroid may give rise to sequence specific binding, which may correlate with biological activity.

1.4.8 Conclusions on the Antimicrobial Mode of Action.

The antimicrobial mode of action of aminosterols is still not fully understood. If one non-specific interaction between the antimicrobial agent and the membrane were wholly responsible for cell death, (e.g. membrane lysis or ionophoric activity) it would be expected that a structural modification of the compound, such as a longer polyamine, would either increase or decrease the potency. The selective increase or decrease of potencies, shown by this class of compounds upon different organisms, suggests that the agents may be acting upon a specific site in each organism (ion channel, transport system or intracellular target). In that way, one agent may bind better to a Pseudomonas site rather that to a Candida site, giving the selectivity seen.

Otherwise, it may be that multiple mechanisms for the antimicrobial activity of aminosterols exist, which is why no structure activity relationships have developed for the class as a whole.
1.4.9 Anticancer Mode of Action.

**Angiogenesis** is the process of budding and growth of new blood vessels, from existing blood vessels, under the stimulus of a variety of growth factors. It is an essential event in many physiological processes including normal growth and development, wound healing and menstruation, but it also plays a key role in tumour formation and growth. In tumour-induced angiogenesis, endothelial cells lining blood vessels are activated by mitogens, produced by the tumour cells and surrounding stoma. These blood vessel endothelial cells eventually grow into and around the tumour, nourishing it and enabling it to thrive. Inhibitors of angiogenesis block any of several steps in the angiogenic cascade, including proliferation of endothelial cells, attachment of endothelial cells to the substratum, and migration and invasion through the tissue space that is required for the extension of capillary sprouts into the new territory.

Judah Folkman, a surgical oncologist, is widely acknowledged as the founding father of anti-angiogenesis – cutting off a tumour’s potential for growth by abrogating its growing blood vessel network. In the past he identified a number of substances with certain structural features as anti-angiogenic. A number of scientists noticed that squalamine possessed three of these structural features; a steroid, a polyamine, and a sulphate; all in one molecule, and prompted Magainin Pharmaceuticals to investigate squalamine’s anticancer properties. It was tested in several animal models with profound results, which were consistent with a mechanism by which squalamine inhibits tumour growth in vivo by interfering with tumour vascularization:

- In *in vivo* models squalamine reduced the growth of brain tumours known as gliomas, implanted in the flanks of rats, by greater than 75%.
- Squalamine demonstrated angiogenic properties in 4-day chick embryos by rapidly constricting the small capillaries and reducing blood transfer throughout the yolk sac capillary network. No damage to healthy cells or embryonic development was seen.
- Time-release capsules containing squalamine slowed the growth of new blood vessels, caused by tumours implanted in rabbits’ eyes, by up to 43% after three weeks.
Tissue culture experiments demonstrated that squalamine has specificity for endothelial cells *in vitro* by selectively inhibiting their growth. There was no evidence of toxicity on any tumour cell lines in culture, and no direct cytotoxicity was observed against endothelial cells either, the inhibitory effects only being seen in the presence of growth factors and other mitogens, and the effects being fully reversible.

Squalamine does not block the action of specific growth factor receptors because it acts in the face of a wide variety of mitogens, and does not inhibit mitogen production, as it causes no reduction in growth factor production by cultured glioma cells.

This data suggests that tumours are unlikely to develop resistance to squalamine, because it targets the growing endothelial cells, and not the genetically unstable tumour cells.

Magainin Pharmaceuticals later reported that squalamine enters endothelial cells. To identify the intracellular target fluorescently labelled squalamine analogues were synthesised, which were found to specifically bind to the protein calmodulin, an intracellular signal mediator that plays an integral role in cell growth and proliferation (angiogenesis). Under normal circumstances, calmodulin sits on the periphery of endothelial cells until it is triggered by vascular endothelial growth factors (VEGF), thrombin and other growth promoters to participate in cell proliferation. However, when squalamine binds to this protein, it physically chaperones calmodulin away from the periphery towards the cell nucleus, rendering calmodulin unable to engage in its normal angiogenic role. As a result of squalamine's intervention in the angiogenic process, a tumour is denied the nourishment of new blood vessels necessary for its growth.

Perhaps this interaction with a specific protein on the cell membrane surface, binding, then entrance into the cell leading to the disruption of cell function, is also the mechanism of action on microbial cells.
1.5 Possible Uses of Aminosterols.

1.5.1 Topical Antimicrobial Agents.

Aminosterols are a new class of broad-spectrum antimicrobial agents. The antagonism of their activities by serum and albumin and their hemolytic potential may limit their utility as systemic agents. However, because of their potencies, broad spectra of antimicrobial activity, and potential for systemic toxicity, they appear to be good candidates for development as topical antimicrobial agents. Several aminosterols are currently being investigated to discover against which antimicrobial diseases they will be most effective.

1.5.2 Cancer Therapy.

Cancer is the second most common cause of death in the Western world, exceeded only by coronary heart disease. Cancer patients are usually treated with a combination of surgery, radiation therapy and chemotherapy. Surgery and radiotherapy can be partially effective in patients in whom the disease has not yet spread to other tissue or organs. Chemotherapy is the principal treatment for tumours that have metastasised (metastatic disease is often the primary cause of death in cancer, it involves the process of tumour cell escape from the primary tumour through blood vessels to distant sites in the body). Chemotherapy involves the administration of cytotoxic drugs designed to kill cancer cells, or the administration of hormone analogues to either reduce the production of, or block the action of, certain hormones, such as estrogens and androgens, which affect the growth of tumours. Because chemotherapeutic agents generally attack rapidly dividing cells indiscriminately, damaging both normal and cancerous cells, chemotherapy patients often suffer serious side effects. Additionally, resistance to chemotherapy inevitably occurs over time.

Squalamine is an anti-angiogenic molecule with a unique mechanism of action. It works through a receptor-mediated event specific for activated endothelial cells. It acts early in the angiogenic cascade, using a multi-step process to block the effect of a variety of tumour triggered growth factors. This multi-faceted approach may allow squalamine broad application across many cancer types, resistance should also be minimal as it acts upon endothelial cells, not unstable cancer cells.
Pre-clinical data has shown squalamine to be effective in the inhibition of various cancers in animals, including lung, breast, brain and melanoma. It also enhances the effects of carboplatin and paclitaxel, a leading chemotherapeutic regime, and reduces the spread of metastases to other parts of the body. Phase I clinical trials have shown squalamine to be very well tolerated in advanced cancer patients. It is currently in Phase II clinical testing in patients with non-small cell lung cancer, ovarian cancer and other adult solid tumours, with more clinical trials planned for the near future.

Squalamine’s anti-angiogenic properties mean it may also have application in a number of disease conditions characterised by abnormal blood vessel growth.

1.5.3 Treatment of Proliferative Eye Disease.\textsuperscript{48}

Vasoproliferative retinopathy is a leading cause of blindness and occurs primarily because of over expression of VEGF.\textsuperscript{44} Retinopathy is a significant and sight threatening complication for patients with diabetes mellitus. Ninety-eight percent of patients with type I diabetes develop some degree of retinopathy. More than 700,000 patients, including adult onset diabetics, suffer from diabetic retinopathy that is caused by angiogenesis. Currently there are no effective pharmaceutical therapies for diabetic or other forms of retinopathy. Surgical techniques in use include repetitive laser therapy that is partially effective at slowing vision loss, but is expensive and causes vision loss itself. Inhibition of proliferative retinal angiogenesis, with anti-angiogenic agents such as squalamine, is a potential mode of treatment for diabetic retinopathy.

Age-related macular degeneration (AMD) is a progressive disease that damages the central vision and affects a person’s ability to read, see faces and drive. About 50 million people worldwide have AMD, and of these, about 5 million will have the more severe wet form. The wet form can manifest itself in two types: classic or occult. Over 70% of patients with wet AMD have the occult type. Presently, there is no proven treatment for preventing severe visual loss in the large majority of these patients.

Magainin Pharmaceuticals has investigated the effects of administering a single dose of squalamine on oxygen-induced retinopathy (OIR), 15 days after birth, in a mouse model. It
is at this point in time when new blood vessel formation in the eyes has started to occur. It was found that squalamine significantly inhibits abnormal blood vessel formation, but leaves normal retinal vascular development intact. Treatment did not adversely affect the general health of the neonatal mice nor alter their normal growth and development.

This strong positive result suggests that squalamine is a potentially viable candidate in the treatment of proliferative eye disease, and it is currently being defined how best to use it in the clinic.

1.5.4 Treatment of Fibrodysplasia Ossificans Progressiva (FOP)

Fibrodysplasia ossificans progressiva (FOP) is a rare genetic disorder in which there is progressive formation of new bone in the large muscles, leading to progressive immobility and disability. The disease starts in childhood, with initial painful swelling of muscles, which in days to weeks often turns to bone. The disease begins in the neck and upper spine, and progresses over a period of years to the muscles around the hips, jaw and other major joints. The swollen muscles represent a type of growth very similar in appearance to a sarcoma cancer. Similar to cancer, these growths in the swollen muscles are nourished by a network of newly formed primitive blood vessels, as a result of active angiogenesis in the lesions. It has been shown that an active angiogenesis factor, basic fibroblast growth factor, is elevated during periods of active disease in FOP. By blocking the angiogenic process, squalamine has the potential to inhibit the progression of the muscle growths seen in FOP, and prevent the muscle turning into bone.

Heterotopic ossification, in which abnormal blood vessel growth allows abnormal bone formation, also afflicts about 5% of people who receive artificial hip replacement, or other physical implants.

1.5.5 Treatment of Sexually Transmitted Diseases (STDs)

Squalamine is being developed as a vaginal microbicide and contraceptive agent. It has activity against multiple STD-causing organisms including Neisseria gonorrhoea, herpes simplex virus (HSV), chlamydia and HIV, has spermicidal activity and is well-tolerated in
rabbit and mouse vaginal irritation studies. With squalamine's characteristics it could become an important broad-spectrum microbicide with spermicidal activity, that is well tolerated in the vagina, and as such could impact on the serious and often life-threatening STDs occurring in several segments of the population today.

1.5.6 Gene Delivery.50

Gene therapy has been receiving much attention due to its promise to prevent and treat many acquired and inherited diseases. A corrective gene or genes may be identified which, if introduced into the appropriate organs and cells of the body in vivo, should correct the basic pathophysiological defect of the disease. Unfortunately, the introduction of the potentially corrective gene or genes is not straightforward. Under certain circumstances, naked DNA may be administered, but usually a delivery vehicle or vector is required.

![Figure 20. Structure of CTAP, a promising compound for gene delivery.](image)

Cationic liposomes are rapidly proving to be very effective at mediating the delivery of genes to cells in vitro. However, it became apparent that substantial improvements in the gene delivery efficiency would be needed for human lung transfection to be possible in vivo. Miller et al. synthesised the above compound CTAP [80] and other analogues. Cationic liposomes formed from CTAP and the neutral lipid DOPE have been shown to have suitable properties for use in human gene therapy approaches towards lung disorders and other clinical conditions. There are several structural similarities between CTAP and the polyamine-steroid analogues already mentioned. This could perhaps be a further use of this class of compounds.

Therapeutic opportunities for these compounds may include obesity, various malignancies, inflammatory diseases, and viral infections.
1.6 Proposal.

The aims of our work were:

- To develop a flexible synthetic route to polyamine-sterol conjugates structurally related to squalamine.

- To then use this synthesis to produce a large library of compounds with structural variety.

- To test these compounds in as wide a screen as possible, for both antimicrobial and anticancer activity.

- To analyse these results in an attempt to find any structure-activity patterns and further probe their mode of action.

We decided to choose stigmasterol as our starting material as it is cheap, readily available and has the correct structure for manipulation on both the A and D rings of the steroid.

We envisioned the synthesis of a variety of steroids, containing a carbonyl group on either the A or the D ring, allowing us to introduce a variety of polyamines via a reductive-amination reaction.

The synthesis of various protected polyamines suitable for attaching to the steroid is well established by previous researchers at the University of Leicester.⁸³
Chapter 2.
SYNTHESIS OF STEROIDS.
2.1 Introduction.

It can be seen from Chapter 1 that a large number of aminosterols have been synthesised and tested to date, with no real structure-activity patterns emerging. The steroid backbone can be extremely varied, with small changes affecting both the activity and selectivity of these compounds. This suggests that there is room for fine-tuning, and further analogues are required to further explore this class of compounds properties.

On this basis, we decided to synthesise a large combinatorial library of aminosterols, with varying steroid backbones and polyamine chains. A convergent synthesis was used, synthesising the steroids and polyamines separately and then combining them via a reductive amination reaction. Stigmasterol [26] is a useful starting material for steroid synthesis as it is cheap, readily available and has functionality on both the A- and D-rings.

2.2 Reaction Schemes.

Stigmasterol [26] has been used to synthesise three steroids containing a trans A-B ring junction and carbonyl functionality (Steroids A, B and C). These steroids have then been used to form a library of aminosterols. The synthesis of two further steroids, (Steroids D and E), containing a cis A-B ring junction and carbonyl functionality has been investigated; research is continuing in our group to complete this work.

2.2.1 Synthesis of steroid A [82].

The simplest steroid produced, Steroid A, can be made in two-steps from stigmasterol. This compound has previously been synthesised in the same manner by F. Dany et al. in the preparation of 3β-carboxysteranes.51
Hydrogenation with 5% Pd on a charcoal support, under acidic conditions, was used to reduce the double bonds giving \((R)-24\text{-ethylcholesta-3\beta-ol}\ [81]\). This gave the trans A-B ring junction seen in squalamine. Comparisons with published NMR data indicate that the chemical shifts of the two angular \(\text{CH}_3\)'s and the two quaternary carbons are consistent with alpha junctions between the rings.\(^{52}\) The stereochemistry of the hydrogenation is explained on page 47, 2.3.1 “Hydrogenation to give a cis- or trans-A-B Ring Junction”. This step also removes complications that arise from over oxidation of stigmasterol in the allylic position, and reductive amination with a double bond that could move into conjugation. The reaction was monitored by TLC (20% EtOAc in petrol), with the product [81] having an Rf of 0.24. After work-up the white solid was purified by recrystallization from EtOAc-Petrol to give white crystals in a 93% yield. \(^1\)H and \(^{13}\)C NMR, MS, and IR confirmed that the double bonds had been removed.

Compound [81] could now be oxidised cleanly using Jones' reagent giving \((R)-24\text{-ethylcholesta-3-one}\ [82].\(^{53}\) The reaction was monitored by colour change, from orange/yellow to green/blue, and is complete within 5 minutes. The product was recrystallized from ethyl acetate to give a white solid in an 89% yield. \(^1\)H and \(^{13}\)C NMR, MS, and IR confirmed that the alcohol had been oxidised.

Polyamines could now be attached in the C3-position on the A-ring of Steroid A, as in squalamine, using the reductive amination reaction. Although the aminosterols formed will lack many of the structural features present in squalamine, they will be extremely useful for comparison with the more complex compounds to be synthesised.

2.2.2 Synthesis of Steroid B [85].

Steroid B can be synthesised in three-steps from stigmasterol. The more substituted ring alkene must first be protected before the chain alkene can be cleaved via ozonolysis. The synthesis was carried out using methods previously developed in the preparation of this compound for use in steroid synthesis.

The protection was achieved by mesylating the C3-hydroxyl group to form \((R)-24\text{-ethyl-3-mesyl-5,22-cholestadiene-3\beta-ol},\) compound [83]. This method was developed by Nes and Steele.\(^{54}\) The reaction was monitored by TLC (20% ethyl acetate in petrol, \(R_f= 0.4\)). After work-up the yellow solid was purified by recrystallization from acetone to give white
crystals in 98% yield. $^1$H and $^{13}$C NMR, MS, and IR confirmed that the mesyl group had been added.

The ring alkene was then attacked with methanol, displacing the good leaving group at C3, and forming a cyclopropane ring, giving compound 3α,5α-cyclo-6β-methoxycholest-22-ene [84], and a by-product [84b] (see page 50, 2.3.4 Formation of the Cyclopropane Ring). This work was developed by Steele and Mosettig in the synthesis of house fly sterols.55 The reaction was monitored by TLC (10% ethyl acetate in petrol, [84] Rf = 0.83, [84b] Rf = 0.74), and gives approximately 75% desired product [84] to 25% by-product [84b]. It is impractical and inefficient to separate these two isomers on a large scale using flash chromatography due to their similar polarities. However, a colour change is observed as the end-point of the next ozonolysis reaction (excess ozone turns the solution blue), so the crude material was run through a column of silica to separate any coloured impurities that could obscure this change. A small amount compound was separated by flash chromatography (gradient of 0-20% ethyl acetate in petrol) for characterisation.

3α,5α-Cyclo-6β-methoxycholest-22-ene [84] was now ozonolysed to give an aldehyde on the D-ring, by the method of Slomp and Johnson.56 Trimethyl phosphite was found to be the most reliable reagent to reduce the zwitterion, as the previously used thiourea gave low yields and reproducibility.57 After work-up the compounds were separated by flash chromatography (5-20% ethyl acetate in petrol) to yield a white crystalline solid, [85], in 70% yield as a single stereoisomer. $^1$H and $^{13}$C NMR, MS, and IR confirmed that the
alkene had been cleaved. It has been reported that on prolonged standing, (several weeks at room temperature), epimerisation may occur at C-20, therefore the aldehyde was stored at 0°C and was used in as soon as possible. 

Polyamines can now be attached to Steroid B, 3α,5α-cyclo-6β-methoxypregnane-20-carboxaldehyde [85] on the D-ring, as was the case for the first squalamine analogue [28] synthesised by Regent. 

2.2.3 Synthesis of Steroid C [90].

Steroid B can be further transformed, in five reactions, to give Steroid C which has a carbonyl group in the C3 position. Polyamines can therefore be introduced to the same position as in squalamine.

Initial reactions involved the Grignard reaction was used to introduce an isopropyl group next to the hydroxyl group, as is the case in squalamine. Two diastereoisomers are formed in a 9:1
ratio after optimisation, with the major diastereoisomer [86] being the one with the hydroxyl group as in squalamine. After work-up the two isomers could be separated by flash chromatography (gradient of 0-20% ethyl acetate in petrol, [86] Rf= 0.38, [86b] Rf= 0.29 in 20%). The highest yield of desired compound, 72%, was obtained when three molar equivalents of Grignard reagent were used. $^1$H and $^{13}$C NMR, MS, and IR confirmed that the isopropyl group had been added. X-ray crystallography (provided by J. Fawcett at the University of Leicester) was used to prove the stereochemistry.

The new hydroxyl group then required protection before the ring alkene could be deprotected. It was decided to use a benzoyl group for this purpose, as it is resistant to both oxidation and hydrogenation. Benzoyl cyanide reacts slowly, giving 3α,5α-Cyclo-6β-methoxy-(S’)-22-benzoyl-23-methylcholane [87] in a high yield (95%) after flash chromatography (gradient of 0-10% ethyl acetate in petroleum ether). This was a method first employed by Havel et al. in their selective acylation of hydroxy steroids. The ring alkene was then deprotected using $p$-toluenesulphonic acid in refluxing water and dioxane, a method devised by McKennis et al. (1948). The reaction was followed by TLC (20% ethyl acetate in petrol, [88] Rf= 0.12) and was complete in six hours. After work-up, flash chromatography was used to purify the product (gradient of 0-20% ethyl acetate in petrol). All sulphur must be removed to prevent poisoning of the catalyst in the next reaction. White crystals were collected in an 85% yield. $^1$H and $^{13}$C NMR, MS, and IR confirmed that the ring alkene had been deprotected.

The deprotected compound, (S)-22-benzoyl-23-methylcholan-5-ene-3β-ol [88], was then hydrogenated as in the synthesis of Steroid A, giving (S)-22-benzoyl-23-methylcholan-3β-ol [89] in a 97% yield after recrystallization from EtOAc-petroleum ether. Comparisons with published data indicate that the chemical shifts of the two angular CH$_3$s and the two quaternary carbons are consistent with alpha junctions between the rings. This compound was then oxidised using Jones reagent giving Steroid C, (S)-22-benzoyl-23-methylcholan-3-one [90]. The reaction takes 5 minutes and gives a yield of 94% after recrystallization from ethyl acetate. $^1$H and $^{13}$C NMR, MS, and IR confirmed the compounds structure.
2.2.4 Synthesis of Steroid D [93].

Some preliminary work was also carried out in an attempt to synthesise steroids with a cis-A-B ring junction. The aminosterols from these compounds would be of interest, as the polyamine on the A-ring would be brought into closer contact with the polar head group on the D-ring. Small quantities of these compounds were synthesised, and work is continuing in our labs at present to produce them on a larger scale with improved yield.

Steroid D was synthesised by oxidation of stigmasterol using Jones’ reagent, followed by isomerization to conjugate the double bond, a method developed by C. Djerassi et al. The conjugated double bond is then hydrogenated under basic conditions.

The oxidation is not very clean as it is very easy to over oxidise in the allylic position. Flash chromatography was used to purify the compounds (gradient of 0-20% ethyl acetate in petrol) giving a white powder, (R)-24-ethyl-5,22-cholestadiene-3-one [91], in 62% yield. The carbonyl $^{13}$C signal is at $\delta 210$, indicating that the double bond has not moved into conjugation.

This compound was then refluxed in potassium hydroxide and methanol to move the double bond into conjugation giving (R)-24-ethyl-4,22-cholestadiene-3-one [92]. The reaction only takes 5 minutes, after which time no starting material remained by TLC (20% ethyl acetate in petrol, $R_f= 0.40$). The carbonyl $^{13}$C signal is now at $\delta 200$, indicating that
the double bond has moved into conjugation.

Hydrogenation gives the cis-A-B ring junction compound 5β-(R)-24-ethyl-22-cholestene-3-one [93]. Comparisons with published data indicate that the chemical shifts of the two angular CH₃s and the two quaternary carbons are consistent with a beta junction between the A-B rings. The stereochemistry of the hydrogenation is explained on page 47 2.3.1 “Hydrogenation to give a cis- or trans-A-B Ring Junction”. The lack of hydrogenation at the branched alkene and low yield are unexplained at this point, although the steric hindrance of the bond and basic conditions may play a part.

2.2.5 Synthesis of steroid E [96].

The synthesis of steroid E was attempted by carrying out the above reactions on (S)-22-benzoyl-23-methylcholan-5-ene-3β-ol [88]. The initial oxidation was once again a problem with low yield and formation of other unidentified products. The crude white powder was purified by flash chromatography (0-20% ethyl acetate in petrol, in 20% ethyl acetate Rf= 0.33) to give white foam [94] in 65% yield.

Conjugation of the double bond goes in high yield in only 5 minutes using refluxing potassium hydroxide and methanol. Flash chromatography (0-20% ethyl acetate in petrol) gave a white solid [95], which was recrystallized from methanol in 94% yield.
(S)-22-Benzoyl-23-methylcholan-4-ene-3-one [95] was then hydrogenated using the same conditions as for steroid D. An unidentified steroid was isolated in 54% yield but the NMR data was not consistent for the desired product [96]. This reaction is being further investigated in our laboratories.

2.3 Reaction Mechanisms.

2.3.1 Hydrogenation to give a cis- or trans-A-B Ring Junction. 64, 65

Alkenes react with hydrogen in the presence of a suitable catalyst to yield the corresponding saturated alkane addition products. Platinum and palladium are the two catalysts used for most alkene hydrogenations. Palladium is normally employed in a very finely divided state “supported” on an inert material such as charcoal to maximise surface area (Pd/C). Platinum is normally used as PtO₂, a reagent known as Adams’ catalyst after its discoverer, Roger Adams.

Catalytic hydrogenation, unlike most other organic reactions, is a heterogeneous process rather than a homogeneous one. That is, the hydrogenation occurs on the surface of solid catalyst particles rather than in solution, and the reaction is therefore difficult to study mechanistically. Observations have shown, however, that hydrogenation usually occurs with syn stereochemistry; both hydrogens add to the double bond from the same face.

The first step in the reaction is adsorption of hydrogen onto the catalyst surface, followed by complexation between catalyst and alkene as vacant metal orbitals overlap with the filled alkene π-orbital. In the final steps, hydrogen is inserted into the double bond, and the
saturated product diffuses away from the catalyst. It is clear from Figure 22 why the stereochemistry of hydrogenation is syn; it must be because both hydrogens add to the double bond from the same catalyst surface.

The reaction is sensitive to the steric environment around the double bond as one side of the flat alkene must be in contact with the catalyst. As a result, the catalyst often approaches only one face, giving rise to a single product. This can be used effectively to give either the trans- or cis-A-B ring junction.

![Figure 22. Hydrogenation of steroids to give a cis or trans A-B ring junction.](image)

In Case A, the double bond is in the B ring, and the C3 position is not oxidised; the steroid is therefore flat. The overriding factor here is the steric bulk of the methyl group, which blocks the top face of the steroid. Hydrogenation occurs mainly from below, giving the trans-A-B ring junction compound. In Case B, there are three \( sp^3 \) carbons in the A ring of the steroid. This bends the A-ring so that it forms a bowl with ring-B. In this case, hydrogenation occurs mainly from outside the bowl, giving a cis-A-B ring junction.

Hydrogenation in alkaline condition has been shown to favour hydrogenation in the steric sense, therefore KOH is used in case B; whilst acidic conditions have been shown to promote the formation of the cis ring junction.\(^{64}\)

### 2.3.2 Oxidation of C3 hydroxyl group

Initially, several oxidations were used on stigmasterol to discover the best reagents. These included Collins\(^{66}\), Swern\(^{67}\), TPAP and NMO\(^{68}\), Oppenauer\(^{69}\) and Jones Oxidation's\(^{53}\). It was found that Jones's reagent was best employed to avoid over oxidation of the allylic
position and conjugation of the double bond. It was also used to oxidise steroids that had previously been hydrogenated as it is quick and gives extremely good yields.

Secondary alcohols are oxidized to ketones via a two-electron process. The mechanism is complex and can be best summed up as below. The initial step is the formation of an ester of the alcohol and chromic acid. The ester then decomposes to the carbonyl compound and the chromium (IV) acid. This then reacts with chromic oxide to form chromium (V) acid, which then also oxidizes the alcohol via its ester.

![Figure 22. Hydrogenation of steroids to give a cis or trans A-B ring junction.](image)

2.3.3 Mesylation of stigmasterol.

A well-known steroid method, exemplified by Steele and Mosettig in the synthesis of house fly sterols, was used to protect the ring alkene.

![Reaction Scheme 11. Mechanism for the mesylation of stigmasterol.](image)

Initially, the C3-hydroxyl group was converted into the sulfonic ester-leaving group. Mesylation was used rather than tosylation as it had previously been established to be superior, 10 minutes vs. 12 hours, by Rawlings and James at the University of Leicester. Triethylamine was used as the base.
2.3.4 Formation of the Cyclopropane Ring.

A methoxide nucleophile was used to form the cyclopropane ring. The mechanism is concerted antiperiplanar addition to the double bond with the mesylate acting as the leaving group. The attacking π-orbital has good overlap with the anti-bonding orbital of the leaving group, and can therefore easily displace it to form an intermediate non-classical carbocation.

Unfortunately, this carbocation can now be attacked by the methoxide nucleophile at either C3, which gives an undesired by-product 3-methoxystigmasterol [84b] with retention of configuration⁵⁵,⁵⁷, or at C6 to give the desired product 3α,5α-cyclo-6β-methoxy-22-cholesten [84].

2.3.5 Ozonolysis of the side chain.⁵⁶,⁷⁰

This is a standard method for the conversion of cis or trans disubstituted alkenes into aldehydes, and was therefore used in this case. Although much has been done to understand the mechanism of ozonolysis, it is still not fully understood. Criegee has outlined a basic mechanism that involves three main steps: ⁷²

- Initial 1,3-dipolar addition of ozone across the double bond to give a molozonide.
- This first formed molozonide is very unstable so decomposes to an aldehyde plus a zwitterionic intermediate.
Chapter 2. Synthesis of Steroids.

- The zwitterionic intermediate may then undergo one of three processes;
  i) recombination with the aldehyde, which can then hydrolyse to products.
  ii) dimerisation to disperoxide, then hydrolysis to products.
  iii) dimerisation, followed by elimination of oxygen to give products.

If the reaction is carried out in a non-nucleophilic solvent, such as dichloromethane, it will stop at the above equilibrium until it is hydrolysed, oxidised or reduced. The addition of a nucleophilic solvent, such as methanol, will lead to the rapid solvolysis of these Criegee intermediates.

These compounds can then be reduced or oxidised to give products. In our case, we wished to reduce to the aldehyde. Several nucleophiles have been used, with dimethyl sulfite or thiourea being the most common. P.R. James, at the University of Leicester, found the reproducibility with thiourea poor, with yields being reported from 15% to 80%. We found trimethyl phosphite a more successful reagent, giving better yields and reproducibility. This
method was first employed by W.S. Knowles and Q.E. Thompson in 1960\textsuperscript{57}, and further followed up by W.G. Salmond in his efficient synthesis of 25-hydroxycholesterol.\textsuperscript{58}

The main by-products in the reaction are the other half of the cleaved double bond, \((R)-2\)-ethyl-3-methylbutanal \([85b]\), the ozonolysis product \([85c]\) of 3-methoxy stigmastene, the corresponding acid of the desired steroid aldehyde \([85d]\), and \(\alpha\)-sitosteryl methyl ether \([85e]\) (derived from a contaminant of commercial stigmasterol).\textsuperscript{58}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{by-products.png}
\caption{By-products in the ozonolysis reaction.}
\end{figure}

2.3.6 Grignard reaction.\textsuperscript{59, 65, 70}

Grignard reactions have been studied since the early 1900's. The organometallic reagents are prepared by the reaction of alkyl halides and certain metals, usually magnesium, the order of reactivity of the halides generally being iodine> bromine> chlorine. The mechanism for the formation of Grignard reagents has not been fully elucidated, but it is known that it involves free radicals, the proposal being via a single electron transfer (S.E.T.) mechanism.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{reaction_scheme_grignard.png}
\caption{Reaction Scheme 15. Mechanism for the formation of Grignard reagents.}
\end{figure}

Grignard reagents are very reactive with air and water so must be prepared under nitrogen. Isopropylmagnesium bromide was prepared for reaction with the aldehyde, as this is the same group as that next to the alcohol in squalamine. It is easy to prepare from readily
available starting materials. The Grignard reaction with the aldehyde forms a new chiral centre, giving two diastereoisomers as the products.

A number of models to predict stereochemical outcome in the Grignard reaction with carbonyls have been put forward, with Felkin and co-workers proposal being widely accepted.\textsuperscript{73} It is proposed that in an open chain model, it is the orientation of the largest $\alpha$-substituent in a conformation perpendicular to the carbonyl group, which is most relevant. Calculations by Anh and Einstein further refined this model to state that nucleophilic attack occurs tilted away from the carbonyl bond, with preferential attack on a course closest to the smallest substituent. It should be noted however, that the degree of stereoselectivity is affected by changes in reaction conditions such as solvent, temperature, and organometallic reagent.

![Figure 25. Felkin-Ahn models to predict stereochemistry in the Grignard reaction.](image)

In the case of steroid B both (S) [86] and (R) [86b] diastereoisomers are formed, with the major diastereoisomer having (S) stereochemistry as predicted (proven by X-ray crystallography).

![Figure 26. Stereochemistry prediction for reaction.](image)

The above reaction was carried out with three equivalents of Grignard reagent, as this was found to give the largest amount of the desired product, with typically a 9:1 ratio of (S) to
Chapter 2. Synthesis of Steroids.

(R) diastereoisomer (d.e. = 75-85%). These could be separated by flash chromatography.

2.3.7 Protection of the C-22 Hydroxyl Group

The newly formed C22 hydroxyl group requires protection from oxidation in a following step. The best way to achieve this is to replace the proton with an R group. A benzoate ester was chosen as it will survive the acidic conditions used to deprotect the ring olefin, the hydrogenation and the conditions used to oxidize the C3 hydroxyl group.

Alcohols react with benzoyl chloride (PhCOCl) in the presence of base (NEt₃) to give the benzoyl derivative (benzoate ester, OBz). Benzoate's are more stable to hydrolysis than acetates, with pH stability from 1-11, and are more stable to reactions with nucleophiles. They also resist hydrogenation, reaction with borohydrides and oxidizing agents. The group is usually cleaved by basic hydrolysis or LiAlH₄ reduction.

Benzyol cyanide and the mild tertiary base triethylamine were chosen here because of their high sensitivity to steric conditions in the neighbourhood of the hydroxyl group. M. Havel and co-workers found that C22 (R) hydroxy steroids take 24 hours to react, whilst C22 (S) hydroxy steroids take 96 hours to react. It was thought that the duration of the reaction could give clues to the stereochemistry at C22, as this was unknown at the time.

![Reaction Scheme 16. Mild benzoylation of the C22 alcohol.](image)

The reaction took 96 hours, indicating that the major diastereoisomer formed is (S), agreeing with the Felkin-Ahn model. Fortunately, this compound was also crystalline enough for X-ray crystallography, which confirmed the structure and showed both predictions to be correct.
2.3.8 Deprotection of the Ring Alkene.\textsuperscript{62}

This was achieved using \textit{p}-toluenesulfonic acid monohydrate in water. This method was devised by H. McKennis Jr and has since been used for the deprotection of many similar steroids.\textsuperscript{74}

![Reaction Scheme 17. Deprotection of ring alkene.](image)

Harsh conditions are needed to force the reaction, 80°C for 8 hours, but a good yield of about 90% is obtained. The protecting group on C22 remains intact.

\textbf{2.4 Conclusions.}

The above reactions were used successfully to synthesise a range of steroids containing a carbonyl group. Three steroids, A, B and C, have been made on a scale large enough so that they can now be condensed with a variety of polyamines to give a large library of aminosterols.

More work is currently being carried out by others in our group to produce the cis A-B ring junction steroids in larger quantities with better yields. These will then be used to produce aminosterols.
3.1 Introduction.

The naturally occurring polyamines putrescine [97], spermidine [22], and spermine [98] were first detected at the end of the 18th century in human sperm. They have since been found to be present in many mammalian cells, occupying a vital role in the functioning of a cell. Their presence plays a part in cell replication and they are intimately tied in with cell differentiation and growth. Cells obtain polyamines from three sources; the first is from other cells, which are either dying or have too many polyamines themselves; the second is from exogenous sources, such as the food we consume or from microorganisms that excrete extracellular polyamines into the intestine; the third is intracellular, via the polyamine biosynthetic pathway.

![Figure 27. Three naturally occurring polyamines.](image)

The exact mechanism of mammalian polyamine uptake is not fully understood, although an active uptake system has been studied in a variety of cells, particularly tumour cells, which have increased uptake. Cancer cells grow more rapidly than normal cells, therefore, the transformation of a normal cell to a tumour cell is characterised by an increase in polyamine biosynthesis and an enhanced capacity for polyamine uptake. This makes polyamine metabolism an extremely interesting new target for chemotherapy. Cullis and Stark have attempted to exploit the polyamine transport system to deliver and target cytotoxic agents selectively to rapidly proliferating cells.

The charged nature of polyamines at physiological pH makes passage through the cell membrane by a method other than active transport impossible. Transport is a temperature dependent process, with maximum uptake occurring at 37°C, and can be saturated, which suggests carrier-mediated transport. All of the above polyamines share a specific transport system, which is distinct from any other known.
Many modified polyamines and polyamine conjugates have been synthesised, and are still recognised by cells and taken across the membrane by this uptake system.\textsuperscript{77, 78, 79b, 80} This suggests that initially the polyamine is delivered to the cell membrane, perhaps by a transport protein, where it binds to a specific receptor on the surface. This interaction must be electrostatic in origin rather than from steric effects. The polyamine is then engulfed by a section of the cell membrane, before being released into the interior of the cell. This process is called receptor mediated endocytosis. The other option for cellular transport is passage \textit{via} a protein pore.

We wish to prepare polyamine-steroid conjugates for structure-activity studies. It was decided to synthesise three polyamines to investigate the effects of different chain length/branching and charge upon therapeutic activity. Spermidine [22], the polyamine contained in squalamine, has a three-carbon and a four-carbon chain, making it unsymmetrical. Due to this asymmetry, a protected version [101] must be synthesised masking the nitrogen on the four-carbon chain, otherwise in the reductive amination reaction it would not be incorporated regioselectively to the steroid. Methods have already been developed for this purpose by Nakanishi\textsuperscript{81} and Stroh and Volkmann\textsuperscript{82} in their synthesis of spider venoms. The same methods have then been used to prepare the longer 3,3,4-protected polyamine [104] using [101] as the starting material. A branched Boc protected polyamine [107] was also synthesised, using methods developed by Cullis at the University of Leicester.\textsuperscript{83} These polyamines will then be attached to the steroids already mentioned, giving a variety of aminosterols for study.

\textbf{3.2 Reaction Schemes.}

The protected polyamines were synthesised by repetition of a three-step sequence of Michael addition to acrylonitrile, \textit{tert}-butoxycarbonyl (Boc) protection, and then reduction of the nitrile by hydrogenation or lithium aluminium hydride.

\textbf{3.2.1 Synthesis of Protected Spermidine [101].}\textsuperscript{81, 82}

Putrescine [97] is a good starting material for polyamine synthesis as it is cheap and readily available. The initial reaction is not very good, as there is no control of the addition to both
ends of the polyamine, giving a by-product [99b]. The desired polyamine [99] is the main product (approximately 60%) if the reaction is carried out at 0°C, with one molar equivalent of acrylonitrile to putrescine. The mixture is easily separated by flash chromatography (15:5:1 CHCl₃: CH₃OH: 'PrNH₂).

![Reaction Scheme 18](image)

The nitrogen atoms were then Boc protected using two molar equivalents of di-tert-butyl dicarbonate giving compound [100]. Di-tert-butyl dicarbonate was chosen, as it will react with both primary and secondary amines at room temperature in 86% yield. The Boc groups are stable to reductive amination and can be removed easily with acid. The reaction was stirred overnight by which time it was complete by TLC, the compound then being purified by flash chromatography (100% EtOAc, Rf= 0.75).

The nitrile group was then reduced by catalytic hydrogenation using Raney Nickel. This afforded the Boc protected spermidine cleanly after work-up in 73% yield, with no need for further purification. All compounds were characterised by ¹H and ¹³C NMR, MS and IR.

3.2.2 Synthesis of Protected Thermospermine [104].

It was decided to synthesise the longer protected polyamine thermospermine [104]. The same three reactions were employed, using protected spermidine [101] as the starting material. The addition of acrylonitrile can be carried out cleanly here, as only one of the
nitrogens is unprotected. One molar equivalent of acrylonitrile reacts within 18 hours giving a 93% yield. Product [102] was obtained as yellow oil and was one spot by TLC (15: 5: 1 CH₃Cl: CH₃OH: PrNH₂, R_f = 0.89).

Two equivalents of (Boc)₂O were again used to protect the nitrogen, giving a 93% yield after work-up. This was followed by reduction of the nitrile using catalytic hydrogenation. Protected thermospermine was isolated in 88% yield after purification by flash chromatography (15: 5: 1 CH₃Cl: CH₃OH: PrNH₂, R_f = 0.43). All compounds were characterised by ¹H and ¹³C NMR, MS and IR.

3.2.3 Synthesis of Protected Branched-polyamine [107].

The Cullis group has been interested in the activity and transport of polyamines for some time. The branched polyamine shown below has been previously synthesised in the group to probe the uptake of polyamines into cells. Spermidine [22] can be used as the starting material. The aminosterols from this compound may show interesting therapeutic activity due to the branching of the polyamine.

Initially two molar equivalents of 2-(tert-butoxycarbonyloxyiminono)-2-phenylacetonitrile, Boc-ON, must be used to protect the primary amines leaving the secondary position free for reaction. Two equivalents of BOC-ON are added over 1 hour at 0°C; the reaction is then stirred for a further hour giving [105] in 83% yield after recrystallization from diethyl ether. This extremely efficient one step synthesis was devised by Cullis et al. (1992), the two previous syntheses being inefficient or involving six steps. The steric bulk of the Boc-
ON group gives the selectivity for primary over secondary amines, which react more slowly.

The previous Michael addition of acrylonitrile was carried out in a Young's tube to increase the yield, giving 90% after purification by flash chromatography (100% ethyl acetate, Rf = 0.45).

Catalytic hydrogenation with Raney nickel can then be used to complete the synthesis. After work-up the crude oil was purified by flash chromatography (Rf = 0.5, 15:5:1 CHCl₃: MeOH: NH₄OH, Rf = 0.47) to yield [107] as yellow oil in 67% yield. All compounds were characterised by ¹H and ¹³C NMR, MS and IR.

3.3 Reaction Mechanisms.

3.3.1 Michael addition to acrylonitrile. ⁶⁶, ⁷⁰, ⁷³

The addition of a nucleophile to an α,β-unsaturated carbonyl system is a well known reaction, and is usually referred to as a Michael addition. The addition is often reversible, and the relative stability of the starting material vs. the product determines the position of the equilibrium. The solvent is important and alcoholic solvents usually promote the equilibrium to the products. The nitrogen acts as the Michael donor towards the double
bond, with attack at the least substituted end so that resulting anion can be stabilised by the nitrile group.

![Reaction Scheme 21. Mechanism for the Michael addition.]

Bis-cyanoethylated products are minor (<5%), a result of the reduced nucleophilicity of cyanoethylated amines.

### 3.3.2 Boc Protection

The nitrogens must now be protected before the nitrile is reduced. The best way to do this is to delocalise the lone pairs of electrons. One of the most useful protecting groups is Boc, which generates an amide derivative in which the electron pair is completely delocalised. The Boc protecting group is easily introduced, in high yield, by reaction of the amine with two molar equivalents of di-tert-butyl dicarbonate (Boc₂O) in a nucleophilic acyl substitution reaction.

![Reaction Scheme 22. Mechanism for the Boc protection.]

In the synthesis of the branched protected polyamine, [107], 2-(tert-butoxycarbonyloxyimino)-2-phenylacetonitrile (Boc-ON) is used to selectively protect the primary amines over the secondary one. This regioselectivity is presumably due to steric influences, as under more forceful conditions it does react.
The Boc group can easily be removed, in high yield, by brief treatment with a strong acid such as trifluoroacetic acid, \(\text{CF}_3\text{COOH}\), or aqueous hydrochloric acid.

### 3.3.3 Reduction of the Nitrile Group

Reduction of nitrile groups gives a primary amine, allowing the nitrile to function as a protected (latent) aminomethylene group. Two different methods, hydrogenation and LiAlH\(_4\) reduction, were employed for this reaction. Both were equally successful, but the LiAlH\(_4\) was only used on a small scale due to safety concerns.

We have already looked at the hydrogenation reaction in Chapter 2, for the reduction of alkenes. Alkenes are much more reactive than other functional groups towards catalytic hydrogenation and therefore mild conditions can be used. To reduce a nitrile group more vigorous conditions are required. A specially prepared nickel powder known as Raney Nickel is used as the catalyst, and aqueous NaOH is added. Otherwise, the mechanism for the reaction is the same as that for alkenes.

Another method was reduction with lithium aluminium hydride, a powder that is soluble in ether and tetrahydrofuran. It is a more powerful and reactive reducing agent than NaBH\(_4\). It reacts violently with water, decomposes when heated above 120°C, and has even been known to explode when being ground with a mortar and pestle. Despite these drawbacks, it is an extremely valuable reagent that is used daily in thousands of laboratories.

The reaction proceeds via initial reduction to an iminium salt [A] shown in scheme 24, which is converted to a bis-imino aluminate [B]. The literature is rather vague concerning
the structure of this intermediate, but reduction with additional hydride leads to [C], as reported by Soffer and Katz.\textsuperscript{84} The hydrogens in the final amine product probably come from hydrolysis, although the nature and substitution pattern of the nitrogen has not been specified. The Al may be coordinated by solvent (diethyl ether was used) or a second aluminium species.

\begin{center}
\textbf{Reaction Scheme 24.} Mechanism of nitrile reduction with lithium aluminium hydride.
\end{center}

\begin{center}
\begin{align*}
\text{R} &= \text{H} \quad \text{N} \\
\text{LiAlH}_4 &\xrightarrow{\text{Li}^+} [\text{A}] \\
\text{Li}^+ &\xrightarrow{\text{H}^-} [\text{B}] \\
2 \text{R} &= \text{NH}_2 + \text{H}_2\text{O}^+ \\
\end{align*}
\end{center}

3.4 Conclusions.

Three reactions have been used to synthesise three protected polyamines, \[101\], \[104\] and \[107\], along with the natural polyamine putrescine \[97\], can now be added to the steroids previously synthesised to give a variety of aminosterols. The differences in the polyamines, i.e. chain length and branching, number of nitrogens and their distribution, should affect the behaviour and antimicrobial activity of the aminosterols.

\begin{center}
\textbf{Figure 28.} Polyamines to be used in the reductive amination.
\end{center}

- Putrescine [97]
- Protected spermidine [101]
- Protected thermospermine [104]
- Protected branched polyamine [107]
Chapter 4.

SYNTHESIS OF AMINOSTEROLS.
4.1 Introduction.

The various steroids were now combined with the protected polyamines to give a large library of aminosterols. The reductive amination reaction was used to attach the polyamines to the steroids. The aminosterol conjugates synthesised were then deprotected at the polyamine nitrogens and steroid hydroxyl groups. All compounds synthesised are currently being tested for antimicrobial and anticancer activity.\(^8^5\)

4.2 Reaction Schemes.

The reactions of a steroid with the various polyamines were run in parallel. Four flasks were set up containing the steroid in methanol, with a different polyamine then being added to each. This method was also used for the Boc and benzoyl deprotections.

4.2.1 Synthesis of aminosterols from steroid A [82].

Putrescine [97] does not require any protection, as it is symmetrical; reaction at either end of the polyamine giving the same aminosterol. Two aminosterols are formed in the reductive amination due to the carbonyl group being an unsymmetrical ketone, with attack from the top or bottom face giving different stereoisomers. The experimental procedure used by L. L. Frye was used as a guide.\(^2^2\)

![Reaction Scheme 25. Synthesis of aminosterols [108] and [109].](image)

The reaction gave a crude yield of 62% after work-up. Column chromatography, gradient of 100% chloroform to 8:2:1 chloroform: methanol: isopropylamine, can be used to separate the two diastereoisomers, compound [108] being less polar. Problems were encountered in the purification of these compounds with a poor total yield of 24% after
chromatography. The diastereoisomers were isolated in a 1:7 ratio of axial [108]:
equatorial [109]. This is not the ratio they were formed in, but unfortunately the crude
NMR could not be used to determine the crude ratio due to overlapping signals.

Once isolated, $^1$H NMR was used to determine the stereochemistry. The resonance for the
equatorial C-3 proton of compound [108] is observed downfield from the methylene
protons of the polyamine side chain at 3.0 ppm. In contrast, the resonance for the axial C-3
proton of compound [109] is not resolved from the overlapping triplets associated with the
polyamine side chain methylene protons, appearing as part of the multiplet at 2.3-2.8 ppm.
This is consistent with data reported for the C-3 proton of 3α-aminocholestane (3.2 ppm)
and 3β-aminocholestane (2.6 ppm) reported by S.-H. Ryu et al. It is also consistent with
the NMR data for squalamine and other analogues synthesised.

Reductive amination of steroid A [82] with Boc protected spermidine [101] gave the two
aminosterols [110] and [111]. The Boc protected diastereoisomers could not be separated
by flash chromatography (gradient of 100 % chloroform to 8:2:1 chloroform: methanol:
isopropylamine, Rf 0.70, 71 % yield), coming off the column at the same time.

---

**Reaction Scheme 26.** Synthesis of aminosterols [110], [111], [112] and [113].

(R)-24-Ethylcholesta-3-one [82] 
Steroid A

\[{\text{[110]}}\]

\[{\text{[111]}}\]

\[{\text{[112]}}\]

\[{\text{[113]}}\]
The standard method for Boc deprotection, trifluoroacetic acid (TFA) in \(\text{CH}_2\text{Cl}_2\) at RT, was chosen to deprotect the amines. The experimental procedure used by L. L. Frye was once again used as a guide.\(^{22}\) This gave aminosterols [112] \((R_f= 0.54, \text{31}\%\text{ yield})\) and [113] \((R_f= 0.22, \text{57}\%\text{ yield})\) after separation by flash chromatography (gradient of 100 % chloroform to 8:2:1 chloroform: methanol: isopropylamine). \(^1\text{H}\) and \(^{13}\text{C}\) NMR, MS, and IR were used to characterise all compounds.

Reductive amination of steroid A [82] with Boc protected thermospermine [104] gave the two aminosterols [114] and [115]. The Boc protected diastereoisomers could not be separated by flash chromatography (gradient of 100 % chloroform to 8:2:1 chloroform: methanol: isopropylamine, \(R_f= 0.65, \text{73}\%\text{ yield})\), both coming off the column at the same time.

---

**Reaction Scheme 27.** Synthesis of aminosterols [114], [115], [116] and [117].

(R)-24-Ethylcholesta-3-one [82]

**Reaction Conditions:**

1. **i.**
   - a) 2.5 MEQ \(\text{H}_2\text{N}\), 3A MS, MeOH, rt, 24h.
   - b) 3.0 MEQ \(\text{NaCNBH}_4\) (1M solution in THF), rt, 24h.
   - 73% total.

2. **ii.**
   - TFA, \(\text{CH}_2\text{Cl}_2\), rt, 2h, [117] : [118]. 19% : 35%.

---

TFA in \(\text{CH}_2\text{Cl}_2\) at RT was once again used to deprotect the amines giving compounds [116] \((R_f= 0.73, \text{19}\%\text{ yield})\) and [117] \((R_f= 0.44, \text{35}\%\text{ yield})\) after flash chromatography (gradient of 100 %
Chapter 4. Synthesis of Aminosterols.

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chlorofrom to 8:2:1 chloroform: methanol: isopropylamine). $^1$H and $^{13}$C NMR, MS, and IR were used to characterise all compounds.

Reductive amination of steroid A [82] with Boc protected branched spermidine [107] gave the two aminosterols [118] and [119]. The Boc protected diastereoisomers could not be separated by flash chromatography (gradient of 100 % chloroform to 8:2:1 chloroform: methanol: isopropylamine, $R_t=0.63$, 78 % yield), both coming off the column at the same time.

### Reaction Scheme 28. Synthesis of aminosterols [118], [119], [120] and [121].

(F?)-24-Ethylcholesta-3-one [82]

Steroid A

\[ \text{Reaction Scheme 28: } \text{Synthesis of aminosterols [118], [119], [120] and [121].} \]

\[ (\text{F?})-24\text{-Ethylcholesta-3-one [82]} \]

Steroid A

\[ \text{i. a) 2.5 MEQ BocHN, } \]

\[ \text{H}, \text{ 3A MS, MeOH, rt, 24h.} \]

\[ \text{b) 3.0 MEQ NaCNBH}_4 (1M solution in THF), \text{ rt, 24h.} \]

\[ 78\% \text{ total.} \]

\[ \text{ii. TFA, CH}_2\text{Cl}_2, \text{ rt, 2h, [120] : [121], 19\% : 57\%.} \]

\[ \text{TFA in CH}_2\text{Cl}_2 \text{ at RT was once again used to deprotect the amines giving compounds [120] (R$_f$= 0.70, 19 \%) and [121] (R$_f$= 0.32, 57 \%) after flash chromatography (gradient of 100 \% chloroform to 8:2:1 chloroform: methanol: isopropylamine).} \]

\[ ^1\text{H and } ^{13}\text{C NMR, MS, and IR were used to characterise all compounds.} \]

4.2.2 Synthesis of Aminosterols from Steroid B [85].

The reductive amination reaction of a polyamine with steroid B produces only a single aminosterol, as steroid B is an aldehyde. The same experimental conditions were used as for steroid A. When steroid B [85] was reacted with putrescine [97], aminosterol [122] was
isolated as an oil (43% yield, $R_f = 0.64$) after purification by flash chromatography (gradient of 100% chloroform to 8:1:1 chloroform: methanol: isopropylamine).

The ring alkene was then deprotected using para-toluenesulfonic acid ($p$-TSA) to give aminosterol [123]. This was purified by flash chromatography (8:1:1 chloroform: methanol: isopropylamine) to yield a white solid ($R_f = 0.56$, 46%). The lower yield for the deprotection reaction may be due to the presence of the basic nitrogens. $^1$H and $^{13}$C NMR, MS, and IR were used to characterise all compounds.

Reductive amination of steroid B [85] with Boc protected spermidine [101] gave aminosterol [124]. Purification by flash chromatography (gradient of 100% chloroform to 8:1:1 chloroform: methanol: isopropylamine, $R_f = 0.81$) gave a clear oil (83% yield).

Problems were encountered with selectivity when attempting to deprotect the groups separately. It was therefore decided to carry out both reactions at once with an excess of
Chapter 4. Synthesis of Aminosterols.

p-TSA. After work-up, the crude oil was purified by flash chromatography (8:1:1 chloroform: methanol: isopropylamine, Rf = 0.48) giving [125] as a solid (13 % yield). Unfortunately, this reaction gave a very low yield, which was not improved due to time constraints. 

Reductive amination of steroid B [85] with Boc protected thermospermine [104] gave aminosterol [126]. Purification by flash chromatography (gradient of 100 % chloroform to 8:2:1 chloroform: methanol: isopropylamine, Rf = 0.82) gave a clear oil (88 % yield).

An excess of p-TSA was once again used to deprotect the ring alkene and nitrogens, as all four deprotections were run in parallel. The crude yellow oil was purified by column chromatography (8:2:1 chloroform: methanol: isopropylamine, Rf = 0.78) giving [127] as clear oil (14 % yield). 

Reductive amination of steroid B [85] with Boc protected branched spermidine [107] gave aminosterol [128], which was purified by flash chromatography after work-up (gradient of 100 % chloroform to 8:2:1 chloroform: methanol: isopropylamine, Rf = 0.78). This gave the aminosterol as clear oil in high yield (91 % yield).

An excess of p-TSA was once again used to deprotect the ring alkene and nitrogens. The crude yellow oil was purified by column chromatography (8:2:1 chloroform: methanol:
isopropylamine, Rf= 0.65) giving [129] as clear oil (10 % yield). $^1$H and $^{13}$C NMR, MS, and IR were used to characterise all compounds.

### Reaction Scheme 32: Synthesis of aminosterols [128], [129].

[Diagram of Reaction Scheme 32]

- **Steroid B**
  - 3α,5α-Cyclo6b-methoxyprogastone
  - ([R]-20-carboxaldehyde [85])

**Reaction Scheme:**

1. **i.** 2.5 M EQ HNBocH₂⁺, 3 M MS, MeOH, rt, 24h
2. **ii.** pTSA, H₂O, Dioxane, reflux, 8h, 10%.

### 4.2.3 Synthesis of Aminosterols from Steroid C [90].

As was the case for steroid A, two diastereoisomers are formed in the reductive amination of steroid C [90] with a putrescine [97]. Aminosterols [130] and [131] were purified by flash chromatography (gradient of 100% chloroform to 8:2:1 chloroform: methanol: isopropylamine). The 3α-compound [130] came off the column first as a clear wax (8:1:1 chloroform: methanol: isopropylamine Rf= 0.64, 20 % yield). The 3β-compound [131] came off the column second, also as an oily wax (8:1:1 chloroform: methanol: isopropylamine Rf= 0.55, 39 % yield).

Once isolated, $^1$H NMR was used to determine the stereochemistry. The resonance for the equatorial C-3 proton of compound [130] is observed downfield from the methylene protons of the polyamine side chain at 3.1ppm. In contrast, the resonance for the axial C-3 proton of compound [131] is not resolved from the overlapping triplets associated with the polyamine side chain methylene protons, appearing as part of the multiplet at 2.6 ppm. This is consistent with data previously reported.$^9, ^{22}, ^{32}$
The C-22 alcohols were then deprotected. The sterically hindered nature of the benzoyl group made deprotection difficult. Initially, \( \text{NH}_3 : \text{MeOH} : \text{H}_2 \text{O} \) (1:5:1) was used with no success. Refluxing methanol, containing 6 MEQ NaOH, was eventually found to slowly deprotect the group, with the reaction taking 72 hours to complete.

Protected aminosterol [130] was deprotected in this way to give aminosterol [132]. The crude residue was purified by flash chromatography (gradient of 100 % chloroform to 8:1:1 chloroform: methanol: isopropylamine, \( R_f = 0.4 \)) to yield a yellow solid (91 % yield).

Protected aminosterol [131] was deprotected giving aminosterol [133]. The crude residue was purified by flash chromatography (gradient of 100 % chloroform to 8:1:1 chloroform: methanol: isopropylamine, \( R_f = 0.3 \)) to yield an opaque solid (92 % yield). \(^1\text{H}\) and \(^{13}\text{C}\) NMR, MS, and IR were used to characterise all compounds.

Reductive amination of steroid C [90] with Boc protected spermidine [101] gave the two aminosterols [134] and [135]. The Boc protected diastereoisomers could not be separated by flash chromatography (gradient of 100 % chloroform to 8:2:1 chloroform: methanol:
isopropylamine, $R_f = 0.71$), coming off the column at the same time with an impurity giving a crude yield of $>100\%$.

TFA in CH$_2$Cl$_2$ at RT was used to deprotect the amines. This gave aminosterols [136] ($R_f = 0.38$, 13 % yield) and [137] ($R_f = 0.25$, 66 % yield) after separation by flash chromatography (gradient of 100 % chloroform to 8:2:1 chloroform: methanol: isopropylamine).

Refluxing methanol, containing 6 MEQ NaOH, was then used to benzoyl deprotect aminosterol [136] to give aminosterol [138]. The crude residue was purified by preparative TLC (8:1:1 chloroform: methanol: isopropylamine, $R_f = 0.36$) to yield yellow solid (85 %
yield). Protected aminosterol [137] was deprotected giving aminosterol [139]. The crude residue was purified by flash chromatography (gradient of 100 % chloroform to 8:1:1 chloroform: methanol: isopropylamine, Rf= 0.3) to yield an opaque solid (83 % yield). $^1$H and $^{13}$C NMR, MS, and IR were used to characterise all compounds.

Reductive amination of steroid C [90] with Boc protected thermospermine [104] gave the two aminosterols [140] and [141].

Reaction Scheme 35. Synthesis of aminosterols [140] to [145].

i. a) 2.5 MEQ $\text{H}_2\text{N}$ Boc
   b) 3.0 MEQ NaCNBH$_4$ (1M solution in THF), rt, 24h, 100% crude.

ii. TFA, CH$_2$Cl$_2$, rt, [142]: [143], 35%: 42%.

iii. KOH, MeOH, reflux, 72h, 80%.
The Boc protected diastereoisomers could not be separated by flash chromatography (gradient of 100 % chloroform to 8:2:1 chloroform: methanol: isopropylamine, \( R_f = 0.69 \)), coming off the column at the same time with an impurity giving a crude yield of >100 %.

TFA in CH\(_2\)Cl\(_2\) at RT was used to deprotect the amines. This gave aminosterols [142] \( (R_f = 0.34, \text{35 \% yield}) \) and [143] \( (R_f = 0.11, \text{42 \% yield}) \) after separation by flash chromatography (gradient of 100 % chloroform to 8:2:1 chloroform: methanol: isopropylamine).

Refluxing methanol, containing 6 MEQ NaOH, was then used to benzoyl deprotect aminosterol [142] to give aminosterol [144]. The crude residue was purified by preparative TLC (8:1:1 chloroform: methanol: isopropylamine, \( R_f = 0.29 \)) to yield an opaque solid (72 % yield). Protected aminosterol [143] was deprotected giving aminosterol [145]. The crude residue was purified by flash chromatography (gradient of 100 % chloroform to 8:1:1 chloroform: methanol: isopropylamine, \( R_f = 0.26 \)) to yield an opaque solid (83 % yield). \(^1\)H and \(^{13}\)C NMR, MS, and IR were used to characterise all compounds.

Reductive amination of steroid C [90] with BOC protected branched spermidine [107] gave the two aminosterols [146] and [147]. The BOC protected diastereoisomers could not be separated by flash chromatography (gradient of 100 % chloroform to 8:2:1 chloroform: methanol: isopropylamine, \( R_f = 0.52 \)), coming off the column at the same time with an impurity giving a crude yield of >100 %.

TFA in CH\(_2\)Cl\(_2\) at RT was used to deprotect the amines. This gave aminosterols [148] \( (R_f = 0.34, \text{21 \% yield}) \) and [149] \( (R_f = 0.16, \text{43 \% yield}) \) after separation by flash chromatography (gradient of 100 % chloroform to 8:2:1 chloroform: methanol: isopropylamine).

Refluxing methanol, containing 6 MEQ NaOH, was then used to benzoyl deprotect aminosterol [148] to give aminosterol [150]. The crude residue was purified by preparative TLC (8:1:1 chloroform: methanol: isopropylamine, \( R_f = 0.4 \)) to yield an opaque solid (73 % yield). Protected aminosterol [149] was deprotected giving aminosterol [151]. The crude residue was purified by flash chromatography (gradient of 100 % chloroform to 8:1:1
chloroform: methanol: isopropylamine, Rf = 0.32) to yield opaque solid (59 % yield). $^1$H and $^{13}$C NMR, MS, and IR were used to characterise all compounds.

**Reaction Scheme 36. Synthesis of aminosterols [146] to [151].**

- i. a) 2.5 MEQ $\text{NaCNBH}_4$ (1M solution in THF), rt, 24h, 100% crude.
- ii. TFA, CH$_2$Cl$_2$, rt, [148] : [149], 21% : 43%.
- iii. KOH, MeOH, reflux, 72h, 73%
4.3 Reaction Mechanisms.

4.3.1 Reductive Amination.\textsuperscript{66, 70, 73}

Amines can be synthesised in a single step by treatment of a ketone or an aldehyde with ammonia or an amine in the presence of a reducing agent, a reaction called reductive amination. Sodium cyanoborohydride (NaBH\textsubscript{3}CN) is widely used for the selective reduction of many organic functional groups.\textsuperscript{87} In particular, the reaction of an aldehyde or ketone with ammonia, or primary, secondary and tertiary amines, via reductive amination of the carbonyl group.

![Reaction Scheme 37. Mechanism for reductive amination.](http://example.com/scheme37.png)

The amine attacks the carbonyl group in a nucleophilic addition reaction to yield an intermediate carbinolamine. This intermediate then loses water to give an imine. The imine is then reduced by sodium cyanoborohydride. The reduction of imines is rapid at pH 6-7, whilst that of aldehydes and ketones is negligible; therefore, a small amount of acid is added to the reaction. Molecular sieves are also added to the reaction to remove the water, driving imine formation forward.

4.3.2 Boc Deprotection.\textsuperscript{60, 66, 70, 73}

\textit{t}ert-Butoxycarbonyl groups can easily be removed by exposure to strong acid. Trifluoroacetic acid is the reagent of choice, the reaction working well in chloroform or dichloromethane. In some cases a cation scavenger such as triethylsilane or thiophenol is used to “mop-up” the \textit{t}-butyl cations, thus preventing alkylation of the amine. It was not found to be necessary in our case. TFA (approximately 13 molar equivs.) with
dichloromethane (32 molar equivs.) as solvent were chosen to deprotect the aminosterols, the reaction usually giving between 70-80% yield.

4.3.3 Benzoyl Deprotection.\textsuperscript{66, 70, 73}

Ester hydrolysis occurs through a typical nucleophilic acyl substitution pathway. The hydroxide ion nucleophile adds to the ester carbonyl group to give a tetrahedral intermediate. Loss of the alkoxide ion then gives a carboxylic acid, which is deprotonated to give the carboxylate salt.

Benzoyl esters are more stable to hydrolysis than acetate esters, therefore harsher conditions are required to remove the group. This group is very sterically hindered between an isopropyl group and the steroid. Three days refluxing in methanol containing 6 molar equivalents of NaOH were therefore required.

4.4 Conclusions.

In total 44 aminosterols, including those Boc, benzoyl and ring alkene protected, have been synthesised. These compounds are currently being tested for antimicrobial and anticancer activity.\textsuperscript{85} Unfortunately, the results of these tests have not yet been determined, therefore no comment can yet be made upon the activity of these compounds. Once the results of
these tests are made available, the activity, and hence structure-activity relationships of these aminosterols will be examined.

Further work is continuing in our laboratory at present, with the synthesis of aminosterols containing a cis A-B ring junction being investigated. Work has been carried out at the University of Leicester to establish polyamine uptake assays in E. coli. The synthesised polyamines will be investigated to determine whether they are substrates or inhibitors of the polyamine uptake system in bacteria. This will further the understanding of the mode of action of this class of compounds.

The aminosterols from steroid C can also be modified by the attachment of a sulphate or phosphate group on the alcohol at C-22. The role of the sulphate in squalamine is not yet understood, although it may be important for the formation of cyclic salt bridged aminosterols as proposed by Regen et al. 29 The activity of squalamine mimics has been shown to decrease with the addition of a sulphate group by Armstrong et al., and more work is required to determine its importance.
Chapter 5. Experimental.
5.1 General Conditions.

5.1.1 Materials.

Stigmasterol was supplied by Steraloids Inc., and Aldrich Chemical Company Ltd. All other chemicals and solvents were obtained from Aldrich Chemical Company Ltd, Fisons Scientific Supplies, Lancaster Synthesis or Sigma Chemical Company Ltd.

Solvents were purified following the methods of Perrin and Armarego as follows.\(^8\) Methanol was dried and distilled from magnesium methoxide, prepared \textit{in situ}, and then stored over 3A Molecular Sieves for at least 24 hours.\(^9\) Diethyl ether was distilled from lithium aluminium hydride. Chloroform was dried and purified by filtration through a column of alumina. Dichloromethane and triethylamine were distilled from calcium hydride. All other solvents were used without further purification. Petroleum ether refers to the 40-60°C fraction.

Ozone was provided by a Penwalt BA 023012 generator at 150V and 60litre/h oxygen. All reactions were carried out under an atmosphere of nitrogen unless otherwise stated.

5.1.2 Methods and Instrumentation.

Melting points were determined on a Kofler Hot Stage apparatus and are uncorrected.

Thin layer chromatography (TLC) analysis was conducted on standard commercial aluminium sheets pre-coated with a 0.2 mm layer of silica gel 60 F\(_{254}\) (Merck 5554) and were stained using an ethanolic solution of phosphomolybdic acid (PMA). Flash chromatography was carried out according to Still’s method using sorbisol C-60 silica gel, 40-60 μm.\(^9\)

Elemental analysis was carried out by Butterworth Laboratories, Teddington, Middlesex. Infrared spectra (IR) were recorded on a Perkin Elmer 298 IR spectrometer in units of cm\(^{-1}\), the samples prepared as solution cells in CH\(_2\)Cl\(_2\) unless otherwise stated. The peaks are referred to as strong (s), medium (m) or weak (w). Optical rotations were measured using a Perkin Elmer 341 polarimeter, the values given in units of 10\(^{\circ}\)degcm\(^{-2}\)g\(^{-1}\). Mass spectra were recorded on a Kratos Concept Sector double focusing mass spectrometer using
electron impact (EI) at 70eV and 170 °C or fast atom bombardment (FAB) ionisation. X-ray structure determinations were carried out by Dr J. Fawcett at the University of Leicester.

$^1$H and $^{13}$C NMR spectra were recorded on a Bruker ARX250 spectrometer (250MHz for $^1$H and 62.9MHz for $^{13}$C) unless otherwise specified. Spectra were recorded in deuterochloroform (CDCl$_3$) unless otherwise stated, with chemical shift values quoted in ppm relative to tetramethyl silane (TMS) internal standard and CDCl$_3$ ($^1$H, δ 7.27; $^{13}$C, δ 77.0), and are reported as positive when downfield from the standard. J values are given in hertz (Hz). The following abbreviations are used: (s)-singlet, (d)-doublet, (t)-triplet, (q)-quartet, (quin)-quintet, (m)-multiplet, and (br)-broad. $^{13}$C spectra were proton decoupled and multiplicities were determined by performing DEPT experiments at 90° and 135°.

All organic extracts were dried over anhydrous sodium sulphate or anhydrous magnesium sulphate, and solvents were removed in vacuo by means of a Buchi rotary evaporator.

5.2 Synthesis of steroid A and accompanying aminosterols.

5.2.1 (R)-24-ethylcholestan-3β-ol [81], Beilstein Registry Number 2564507

![Chemical Structure of Stigmasterol][1]

Stigmasterol [26] (5.00 g, 12.0 mmol) was dissolved in dry diethyl ether (500 mL) in a 1 L round-bottomed (RB) flask. Glacial acetic acid (50 mL) and 5 % Pd-C (1.2 g) were added. The stirred solution was hydrogenated at atmospheric pressure for 16 hours. TLC (20 % ethyl acetate in petrol, R$_f$ = 0.24) confirmed that no starting material remained, showing only one spot. The reaction was filtered through celite to remove the 5% Pd-C, the celite pad being washed with diethyl ether (3 x 50 mL). The ether was then washed with water (3 x 100 mL) to remove the acetic acid, dried over magnesium sulphate and the solvent removed in vacuo to yield a white solid [81]. This was recrystallized from EtOAc-
petroleum ether (4.68 g, 11.2 mmol, 93 % yield). \( m/z \) (FAB)= 417 (M\(^+\), H, 26 %), 255 (steroid skeleton, 40 %); \( v_{\text{max/cm}^{-1}} \) (CH\(_2\)Cl\(_2\)) = 3250 (s, O-H), 3000-2850 (m, C-H);

\( \delta_{\text{H}} (\text{CDCl}_3, 300 \text{ MHz}) = 0.6-2.2 \text{ ppm (51H, m)} \) [including 0.65 (3H, s, C\(^{18}\)-CH\(_3\)), 0.80 (3H, s, C\(^{19}\)-CH\(_3\)), 0.81 (3H, d, \( J = 6.4 \text{Hz} \), C\(^{26}\)-CH\(_3\)), 0.83 (3H, d, \( J = 6.7 \text{Hz} \), C\(^{27}\)-CH\(_3\)), 0.84 (3H, t, \( J = 7.0 \text{Hz} \), C\(^{29}\)-CH\(_3\)), 0.91 (3H, d, \( J = 6.4 \text{Hz} \), C\(^{21}\)-CH\(_3\))], 3.59 (1H, m, CH-OH); \( \delta_{\text{C}} (\text{CDCl}_3, 75.5 \text{MHz}) = 12.4 (\text{CH}_3), 12.5 (\text{CH}_3), 12.7 (\text{CH}_3), 19.1 (\text{CH}_3), 19.4 (\text{CH}_3), 20.2 (\text{CH}_3), 21.7 (\text{CH}_2), 23.4 (\text{CH}_2), 24.6 (\text{CH}_2), 26.4 (\text{CH}_2), 28.7 (\text{CH}_2), 29.1 (\text{CH}_2), 29.5 (\text{CH}), 31.8 (\text{CH}_2), 32.5 (\text{CH}_2), 34.3 (\text{CH}_2), 35.8 (\text{C}), 35.9 (\text{CH}), 36.6 (\text{CH}), 37.4 (\text{CH}_2), 38.5 (\text{CH}_2), 40.4 (\text{CH}_2), 43.0 (\text{C}), 45.2 (\text{CH}), 46.2 (\text{CH}), 54.7 (\text{CH}), 56.6 (\text{CH}), 56.9 (\text{CH}), 71.8 (\text{CH})]

5.2.2 \((R)-24\text{-ethylcholesta-3-one [82]}\) Beilstein Registry Number 3066565

To a cooled (10-15 °C) solution of \((R)-24\text{-ethylcholesta-3β-ol [81]}\) (4.60 g, 11.0 mmol) in dry acetone (600 mL) in a 1 L 3-necked flask was added rapidly, with stirring, from a burette Jones Reagent (5 mL, 13.4 mmol CrO\(_3\)).* Nitrogen gas was bubbled through all the solvents, reagents and reaction solution before and during the oxidation.† After 5 minutes, the solution had changed from orange/yellow to green/blue. The reaction was quenched by the addition of a saturated solution of sodium metabisulphite (100 mL). This destroyed any excess chromic acid and the solution turned brown. Water (300 mL) and ether (600 mL) were added and the solution stirred until all the Cr\(^{3+}\) salts were dissolved. The ether layer was the separated and the water layer extracted with ether (2 x 150 mL). The combined organic extracts were washed with water (2 x 100 mL), saturated potassium carbonate (2 x 100 mL) and brine (2 x 100 mL). The ether was then dried over magnesium sulphate, filtered and the solvent removed in vacuo to yield a white solid [82]. This was recrystallized from ethyl acetate, slight warming in a nitrogen atmosphere followed by rapid cooling in dry ice (4.07 g, 9.8 mmol, 89 % yield).

*Jones's Reagent was taken from a solution made up of chromium trioxide (26.72 g) in concentrated sulphuric acid (23 mL) made up to a volume of 100 mL with water. This was used throughout.

†This was done to prevent any atmospheric oxygen entering the system as it has previously been reported that \( \Delta^1 \)-3-ketones can yield hydroperoxides.
Chapter 5. Experimental.

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\[ m/z = 415 \text{ (M}^+\text{H, 47 %), 255 (steroid skeleton, 40 %); } \nu_{\text{max/cm}^{-1}}(\text{CH}_2\text{Cl}_2) = 3000-2850 \text{ (m, C-H), 1680 (s, C}=\text{O)}; \delta_{\text{H}}(\text{CDCl}_3, 250\text{MHz}) = 0.6-2.5 \text{ ppm (50H, m) [including 0.68 (3H, s, C}^{18}-\text{CH}_3), 0.80 (3H, d, } J = 5.3\text{Hz, C}^{26}-\text{CH}_3), 0.83 (3H, d, } J = 4.8\text{Hz, C}^{27}-\text{CH}_3), 0.85 (3H, t, } J = 7.1, \text{ C}^{29}-\text{CH}_3), 0.90 (3H, d, } J = 6.4, \text{ C}^{31}-\text{CH}_3) \text{, 1.0 (3H, s, C}^{19}-\text{CH}_3)); \delta_{\text{C}}(\text{CDCl}_3, 62.9\text{MHz}) = 11.8 \text{ (CH}_3), 12.4 \text{ (CH}_3), 12.4 \text{ (CH}_3), 19.1 \text{ (CH}_3), 19.4 \text{ (CH}_3), 20.2 \text{ (CH}_3), 21.8 \text{ (CH}_2), 23.5 \text{ (CH}_2), 24.6 \text{ (CH}_2), 26.5 \text{ (CH}_2), 28.6 \text{ (CH}_2), 29.4 \text{ (CH}_2), 29.6 \text{ (CH), 32.1 (CH}_2), 34.3 \text{ (CH}_2), 35.8 \text{ (CH), 36.0 (C), 36.5 (CH), 38.6 (CH}_2), 39.0 \text{ (CH}_2), 40.3 \text{ (CH}_2), 43.0 \text{ (C), 45.1 (CH}_2), 46.2 \text{ (CH), 47.1 (CH), 54.2 (CH), 56.6 (CH), 56.7 (CH), 212.4 (CO).}

5.2.3 (R)-24-ethyl-3α-[1,4-diaminobutane]-cholestane [108] and (R)-24-ethyl-3β-[1,4-diaminobutane]-cholestane [109].

All glassware was oven dried. (R)-24-ethylcholesta-3-one [82] (0.50 g, 1.2 mmol), 1,4-diaminobutane (0.265 g, 3.0 mmol), 2 drops of acetic acid and powdered 3 A molecular sieves (M/S) (0.70 g) were dissolved in dry methanol (30 mL) and sealed under a nitrogen atmosphere in a 100 mL RB flask. The solution was stirred for 24 hours, and then NaCNBH3 (3.6 mL of 1M solution in THF, 3.6 mmol) was added and the reaction stirred for a further 24 hours. At this point, a mass spectrum showed only product against starting material. The contents of the flask were then filtered through celite and the pad washed with methanol (2 x 10 mL) and dichloromethane (2 x 10 mL). The solvents were removed in vacuo and the residue dissolved in CH2Cl2 (80 mL). This was washed with water made slightly basic with 5% NaOH (2 x 40 mL) and brine (2 x 40 mL). The combined aqueous layers were back extracted with CH2Cl2 (2 x 40 mL). The organic layers were combined and dried over magnesium sulphate, filtered and the solvent removed in vacuo. This left a crude white solid (360 mg, 62 %), which was purified by flash chromatography (gradient of 100 % chloroform to 8:2:1 chloroform: methanol: isopropylamine). Compound [108]
came off the column first as clear oil (17 mg, 0.04 mmol, 3 % yield, 8:2:1 chloroform: methanol: isopropylamine R=0.80). \( m/z = 489 \) (50 %, M+2H), 488 (100 %, M+H), 414 (5%), 413 (10%, -(CH\(_2\))\(_4\)NH\(_2\)); HRMS (FAB) = calculated for (MH+) \( \text{C}_{46}\text{H}_{86}\text{N}_3\text{O}_4 \) 487.4992, found 487.4965; \( v_{\text{max}}/\text{cm}^{-1} \) (CH\(_2\)Cl\(_2\)) = 3440 (w, N-H), 3000-2850 (m, C-H); \( \delta_H \) (CD\(_3\)OD, 250MHz) = 0.6-2.5ppm (57H, m) [including 0.68 (3H, s, C\(^{18}\)-CH\(_3\)), 0.82 (3H, d, \( J = 5.0\)Hz, C\(^{26}\)-CH\(_3\)), 0.86 (3H, d, \( J = 4.9\)Hz, C\(^{27}\)-CH\(_3\)), 0.89 (3H, t, \( J = 7.2\)Hz, C\(^{29}\)-CH\(_3\)), 0.90 (3H, d, \( J = 6.4\)Hz, C\(^{21}\)-CH\(_3\)), 1.0 (3H, s, C\(^{19}\)-CH\(_3\))], 2.6-2.8 (4H, m, CH\(_2\)NH), 3.0 (1H, m, CHNH); \( \delta_C \) (CDCl\(_3\), 62.9MHz) = 11.5 (CH\(_3\)), 11.6 (CH\(_3\)), 11.7 (CH\(_3\)), 18.2 (CH\(_3\)), 18.5 (CH\(_3\)), 19.3 (CH\(_3\)), 22.0 (CH\(_2\)), 23.0 (CH\(_2\)), 24.2 (CH\(_2\)), 25.1 (CH\(_2\)), 26.0 (CH\(_2\)), 26.8 (CH\(_2\)), 27.0 (CH\(_2\)), 27.5 (CH\(_2\)), 28.2 (CH\(_2\)), 28.9 (CH\(_2\)), 29.6 (CH\(_2\)), 29.9 (CH\(_2\)), 31.4 (CH\(_2\)), 34.3 (CH\(_2\)), 35.6 (CH\(_2\)), 35.8 (CH), 36.0 (C), 36.5 (CH), 38.6 (CH\(_2\)), 39.5 (CH\(_2\)), 40.1 (CH), 43.0 (C), 45.4 (CH), 46.6 (CH), 54.3 (CH), 55.9 (CH), 56.2 (CH).

Compound [109] was second off the column as cream foam (125 mg, 0.26 mmol, 21 % yield, 8:2:1 chloroform: methanol: isopropylamine R=0.50). \( m/z = 489 \) (28 %, M+2H), 488 (100 %, M+H), 414 (5 %), 413 (10 %, -(CH\(_2\))\(_4\)NH\(_2\)); HRMS (FAB) = calculated for (MH+) \( \text{C}_{46}\text{H}_{86}\text{N}_3\text{O}_4 \) 487.4992, found 487.4979; \( v_{\text{max}}/\text{cm}^{-1} \) (CH\(_2\)Cl\(_2\)) = 3440 (w, N-H), 3000-2850 (m, C-H); \( \delta_H \) (CD\(_3\)OD, 250MHz) = 0.6-2.5ppm (57H, m) [including 0.69 (3H, s, C\(^{18}\)-CH\(_3\)), 0.82 (3H, d, \( J = 5.0\)Hz, C\(^{26}\)-CH\(_3\)), 0.86 (3H, d, \( J = 4.9\)Hz, C\(^{27}\)-CH\(_3\)), 0.89 (3H, t, \( J = 7.2\)Hz, C\(^{29}\)-CH\(_3\)), 0.90 (3H, d, \( J = 6.4\)Hz, C\(^{21}\)-CH\(_3\)), 1.0 (3H, s, C\(^{19}\)-CH\(_3\))], 2.3-2.8 (5H, m, CH\(_2\)NH & CHNH); \( \delta_C \) (CDCl\(_3\), 62.9MHz) = 12.4 (CH\(_3\)), 12.5 (CH\(_3\)), 12.8 (CH\(_3\)), 19.1 (CH\(_3\)), 19.4 (CH\(_3\)), 20.2 (CH\(_3\)), 21.2 (CH\(_2\)), 21.5 (CH\(_2\)), 24.6 (CH\(_2\)), 26.2 (CH\(_2\)), 26.46 (CH\(_2\)), 26.50 (CH\(_2\)), 28.4 (CH\(_2\)), 28.6 (CH\(_2\)), 29.2 (CH\(_2\)), 29.5 (CH), 32.5 (CH\(_2\)), 33.1 (CH\(_2\)), 33.6 (CH\(_2\)), 33.8 (CH\(_2\)), 34.3 (CH\(_2\)), 35.9 (CH), 36.3 (C), 36.5 (CH), 37.8 (CH\(_2\)), 40.1 (CH), 40.4 (CH\(_2\)), 42.9 (C), 45.7 (CH), 46.2 (CH), 56.6 (CH), 56.9 (CH), 57.8 (CH).

5.2.4 (R)-24-ethyl-3α-[N\(^1\),N\(^4\)-Di-(tert-butoxycarbonyl)-sperrmidine]-cholestane [110] and (R)-24-ethyl-3β-[N\(^1\),N\(^4\)-Di-(tert-butoxycarbonyl)-sperrmidine]-cholestane [111].
All glassware was oven dried. (R)-24-ethylcholesta-3-one [82] (0.50 g, 1.2 mmol), N1,N4-
Di-(tert-butoxycarbonyl)-spermidine [101] (1.035 g, 3.0 mmol), 2 drops of acetic acid and
powdered 3A molecular sieves (M/S) (0.70 g) were dissolved in dry methanol (40 mL) and
sealed under a nitrogen atmosphere in a 100 mL RB flask. The solution was stirred for 24
hours, and then NaCNBH3 (3.6 mL of 1M solution in THF, 3.6 mmol) was added and the
reaction stirred for a further 24 hours. At this point, a mass spectrum showed only product
and no starting material. The contents of the flask were then filtered through celite and the
pad washed with methanol (2 x 10 mL) and dichloromethane (2 x 10 mL). The solvents
were removed in vacuo and the residue dissolved in CH2Cl2 (80mL). This was washed with
water made slightly basic with 5% NaOH (2 x 40 mL) and brine (2 x 40 mL). The
combined aqueous layers were back extracted with CH2Cl2 (2 x 40 mL). The organic layers
were combined and dried over magnesium sulphate, filtered and the solvent removed in
vacuo. This left a crude oil (1.42 g), which was purified by flash chromatography (gradient
of 100% chloroform to 8:2:1 chloroform: methanol: isopropylamine). The two
diastereoisomers [110] and [111] could not be separated, both coming off the column at the
same time as a clear oil (660 mg, 0.85 mmol, 71% yield) Rf= 0.70.

m/z (FAB)= 746 (50 %, M+2H), 745 (100 %, M+H), 637 (10 %), 414 (20 %); HRMS
(FAB)= calculated for (MH+) (12C46H86N3I604) 744.6619, found 744.66178; νmax/cm−1
(CH2Cl2)= 3460 (w, N-H), 2940 & 2878 (m, C-H), 1711 & 1688 (s, N-C(O)O): δH (CDCl3,
400MHz)= 0.65 (3H, s, C18-CH3), 0.80-2.0 (71H, br m, including [0.80 (3H, d, J= 5.0Hz,
C26-CH3]), 0.82 (3H, d, J= 4.9Hz, C27-CH3), 0.84 (3H, s, C19-CH3), (3H, t, J= 7.2, C29-CH3),
0.90 (3H, d, J=6.4, C21-CH3), 1.43 (9H, s, C(CH3)3), 1.45 (9H, s, C(CH3)3), 2.8 (1H, br s,
NH), 3.0-3.5 (9H, collapsed m, CH3N & CHN), 4.9 (1H, br s, NH); δC (CDCl3, 62.9MHz)=
12.3 (CH3), 12.4 (CH3), 12.6 (CH3), 19.1 (CH3), 19.4 (CH3), 20.1 (CH3), 21.1 (CH2), 21.5
(CH2), 23.4 (CH2), 24.5 (CH2), 26.0 (CH2), 26.4 (CH2), 26.4 (CH2), 27.7 (CH2), 28.6
(CH2), 28.8 (CH3), 28.8 (CH3), 29.0 (CH2), 29.5 (CH), 32.3 (CH2), 34.3 (CH2), 35.8 (CH),
36.3 (C), 36.5 (CH), 37.4 (CH2), 40.3 (CH2), 40.3 (CH2), 40.5 (CH2), 42.5 (C), 45.5 (CH),
46.1 (CH), 47.0 (CH2), 47.1 (CH2), 47.2 (CH2), 50.6 (CH), 54.6 (CH), 56.5 (CH), 56.8
(CH), 77.2 (C), 78.7 (C), 155.3 (CO), 155.9 (CO).
Chapter 5. Experimental.

5.2.5 \((R)-24\text{-ethyl-3\text{-}[spermidine]-cholestane \([112]\) and \((R)-24\text{-ethyl-3\text{-}[spermidine]-cholestane \([113]\).}

TFA (3.0mL, 40.0mmol) was added to a solution of \([110]\) and \([111]\) (520 mg, 0.70 mmol) in CH\(_2\)Cl\(_2\) (25 mL) at RT. The reaction was stirred for approximately 2 hrs, when no starting material remained by TLC and mass spectrum. The solvent was removed in vacuo and the residue purified by flash chromatography (gradient of 100 % chloroform to 8:2:1 chloroform: methanol: isopropylamine). Compound \([112]\) came off the column first as a foam (120 mg, 0.22 mmol, 31 %) with \(R_f= 0.54. [\alpha]_D^0 = 3.3^\circ \text{ (c=3, MeOH)}; m/z = 546 (52 \%, M^+2\text{H}), 545 (100 \%, M^+\text{H}); \text{HRMS } (\text{FAB}) = \text{calculated for } (\text{MH}) ^+ (\text{C}_{36}^\text{H}_{70}^{14}\text{N}_3) 544.55703, \text{found } 544.55698; v_{\text{max}}/\text{cm}^{-1} (\text{CH}_2\text{Cl}_2) = 3460 (\text{w, N-H}), 2940 & 2878 (\text{m, C-H}); \delta_{\text{H}} (\text{CD}_3\text{OD, 300MHz}) = 0.67 (3H, s, C^{18-}\text{CH}_3), 0.8-2.0 (56H, br m, including [0.80 (3H, d, J= 5.0Hz, C^{26-}\text{CH}_3), 0.82 (3H, d, J= 4.9Hz, C^{27-}\text{CH}_3), 0.84 (3H, s, C^{19-}\text{CH}_3), 0.86 (3H, t, J= 7.2, C^{29-}\text{CH}_3), 0.90 (3H, d, J=6.4, C^{21-}\text{CH}_3)], 2.7-3.1 (8H, m, CH\text{NH}), 4.9 (1H, br s, NH); \delta_{\text{C}} (\text{CD}_3\text{Cl}, 62.9MHz) = 12.2 (\text{CH}_3), 12.8 (\text{CH}_3), 13.0 (\text{CH}_3), 19.8 (\text{CH}_3), 19.9 (\text{CH}_3), 20.7 (\text{CH}_3), 22.2 (\text{CH}_2), 24.1 (\text{CH}_2), 24.4 (\text{CH}_2), 24.5 (\text{CH}_2), 24.6 (\text{CH}_2), 25.6 (\text{CH}_2), 25.9 (\text{CH}_2), 27.7 (\text{CH}_2), 29.6 (\text{CH}_2), 29.8 (\text{CH}_2), 30.8 (\text{CH}), 30.9 (\text{CH}_2), 33.1 (\text{CH}_2), 35.5 (\text{CH}_2), 37.3 (\text{C}), 37.1 (\text{CH}), 37.2 (\text{CH}_2), 37.9 (\text{CH}), 40.4 (\text{CH}_2), 40.6 (\text{CH}), 41.7 (\text{CH}_2), 44.2 (\text{C}), 44.8 (\text{CH}_2), 46.3 (\text{CH}_2), 47.7 (\text{CH}), 48.6 (\text{CH}_2), 55.3 (\text{CH}), 57.2 (\text{CH}), 58.0 (\text{CH}), 58.2 (\text{CH}).

Compound \([113]\) came off the column second as a solid (218 mg, 0.40 mmol, 57 %) with \(R_f= 0.22. m_p = 107-109^\circ; [\alpha]_D^0 = 13.8^\circ \text{ (c=3, MeOH)}; m/z = 546 (89\%, M^+2\text{H}), 545 (100\%, M^+\text{H}), 487 (15\%); \text{HRMS } (\text{FAB}) = \text{calculated for } (\text{MH}) ^+ (\text{C}_{36}^\text{H}_{70}^{14}\text{N}_3) 544.55703, \text{found } 544.55692; v_{\text{max}}/\text{cm}^{-1} (\text{CH}_2\text{Cl}_2) = 3460 (\text{w, N-H}), 2940 & 2878 (\text{m, C-H}); \delta_{\text{H}} (\text{CD}_3\text{OD).}
300MHz) = 0.68 (3H, s, C18-CH3), 0.7-2.0 (57H, br m, including [0.81 (3H, d, J= 5.3Hz, C26-CH3), 0.82 (3H, d, J= 5.2Hz, C27-CH3), 0.84 (3H, s, C19-CH3), 0.86 (3H, t, J= 7.2Hz, C29-CH3), 0.90 (3H, d, J=6.4Hz, C31-CH3)], 2.6-3.0 (9H, m, CH2NHR & CHNHR); δc (CDCl3, 62.9MHz) = 12.9 (CH3), 13.1 (CH3), 13.1 (CH3), 19.9 (CH3), 20.1 (CH3), 20.8 (CH3), 22.7 (CH2), 24.7 (CH2), 25.7 (CH2), 26.0 (CH2), 27.5 (CH2), 27.7 (CH2), 27.8 (CH2), 28.2 (CH2), 29.8 (CH2), 30.2 (CH2), 30.7 (CH), 33.6 (CH2), 34.2 (CH2), 35.6 (CH2), 37.3 (C), 37.3 (CH), 37.9 (CH), 38.6 (CH2), 41.3 (CH2), 41.8 (CH2), 44.2 (C), 45.5 (CH2), 46.8 (CH), 47.7 (CH), 48.5 (CH2), 49.9 (CH2), 55.9 (CH), 58.0 (CH), 58.3 (CH), 58.9 (CH).

5.2.6 (R)-24-ethyl-3α-[N1,N4,N8-tri-(tert-butoxycarbonyl)]-thermospermine-cholestane [114] and (R)-24-ethyl-3β-[N1,N4,N8-tri-(tert-butoxycarbonyl)]-thermospermine-cholestane [115].

All glassware was oven dried. (R)-24-ethylcholesta-3-one [82] (0.50 g, 1.2 mmol), N1,N4,N8-Tri-(tert-butoxycarbonyl)-thermospermine [104] (1.506 g, 3.0 mmol), 2 drops of acetic acid and powdered 3 A molecular sieves (M/S) (0.70 g) were dissolved in dry methanol (40 mL) and sealed under a nitrogen atmosphere in a 100mL RB flask. The solution was stirred for 24 hours, and then NaCNBH3 (3.6 mL of 1M solution in THF, 3.6mmol) was added and the reaction stirred for a further 24 hours. At this point, a mass spectrum showed only product and no starting material. The contents of the flask were then filtered through celite and the pad washed with methanol (2 x 10 mL) and dichloromethane (2 x 10 mL). The solvents were removed in vacuo and the residue dissolved in CH2Cl2 (80 mL). This was washed with water made slightly basic with 5% NaOH (2 x 40 mL) and brine (2 x 40mL). The combined aqueous layers were back extracted with CH2Cl2 (2 x 40 mL). The organic layers were combined and dried over magnesium sulphate, filtered and the solvent removed in vacuo. This left a crude oil (1.80 g) which was purified by flash chromatography using a gradient of 100 % chloroform to 8:2:1 chloroform: methanol: isopropylamine. The two diastereoisomers [114] and [115] could not be separated, both
coming off the column at the same time as a clear oil (790 mg, 0.88 mmol, 73 % yield) $R_f=0.65$. $m/z$ (FAB) = 902 (76 %, M$^+2$H), 901 (100 %, M$^+H$), 601 (12 %), 454 (9 %), 414 (29 %); $\nu_{\text{max}}/\text{cm}^{-1}$ (CH$_2$Cl$_2$) = 3460 (w, N-H), 2960 (br m, C-H), 1690 (br s, N-C(O)O); $\delta_H$ (CDCl$_3$, 250MHz) = 0.58 (3H, s, C$_{18}$-CH$_3$), 0.6-2.0 (67H, br m, including [0.73 (3H, d, $J=$ 4.8Hz, C$_{26}$-CH$_3$), 0.74 (3H, d, $J=$ 4.9Hz, C$_{27}$-CH$_3$), 0.77 (3H, s, C$_{19}$-CH$_3$), 0.80 (3H, t, $J=$ 7.2, C$_{29}$-CH$_3$), 0.90 (3H, d, $J=$ 6.4, C$_{21}$-CH$_3$), 1.36 (9H, s, C(CH$_3$)$_3$), 1.37 (9H, s, C(CH$_3$)$_3$), 1.37 (9H, s, C(CH$_3$)$_3$), 2.9-3.4 (13H, collapsed m, CH$_2$N & CHN), 4.4 (1H, br s, NH), 4.9 (1H, br s, NH); $\delta_C$ (CDCl$_3$, 62.9MHz) = 12.3 (CH$_3$), 12.4 (CH$_3$), 12.5 (CH$_3$), 19.0 (CH$_3$), 19.3 (CH$_3$), 20.1 (CH$_3$), 21.0 (CH$_2$), 21.4 (CH$_2$), 23.3 (CH$_2$), 24.4 (CH$_2$), 25.9 (CH$_2$), 26.3 (CH$_2$), 27.6 (CH$_2$), 28.5 (CH$_2$), 28.7 (CH$_3$), 28.7 (CH$_3$), 28.7 (CH$_3$), 28.9 (CH$_2$), 29.4 (CH), 29.9 (CH$_2$), 31.8 (CH$_2$), 32.2 (CH$_2$), 32.4 (CH$_2$), 34.2 (CH$_2$), 35.7 (CH), 36.3 (C), 36.4 (CH), 37.2 (CH$_2$), 37.3 (CH$_2$), 38.6 (CH$_2$), 40.0 (CH), 40.2 (CH$_2$), 40.4 (CH$_2$), 42.7 (C), 45.0 (CH$_2$), 45.1 (CH$_2$), 46.1 (CH), 47.0 (CH$_2$), 54.4 (CH), 54.7 (CH), 56.5 (CH), 56.7 (CH), 77.9 (C), 78.9 (C), 79.5 (C), 155.6 (CO), 156.3 (CO), 156.3 (CO).

5.2.7 (R)-24-ethyl-3α-[thermospermine]-cholestan [116] and (R)-24-ethyl-3β-[thermospermine]-cholestan [117].

![Image](image-url)

TFA (3.5mL, 48.0mmol) was added to a solution of [114] and [115] (760mg, 0.86mmol) in CH$_2$Cl$_2$ (30mL) in a 50mL RB flask at RT. The reaction was stirred for approximately 2hrs, when no starting material remained by TLC and mass spectrum. The solvent was removed in vacuo and the residue purified by flash chromatography (gradient of 100% chloroform to 8:2:1 chloroform: methanol: isopropylamine). Compound [116] came off the column first as a solid (96mg, 0.16mmol, 19%) with $R_f=0.73$. $[\alpha]_D^{20}=18.6^\circ$ (c=3, MeOH); $m/z = 601$
(100%, M⁺H); HRMS (FAB) = calculated for (MH⁺) (¹²C₃⁹H₇₇¹⁴N₄) 601.6149, found 601.6178; \( \nu_{\text{max}}/\text{cm}^{-1} \) (CH₂Cl₂) = 3440 (w, N-H), 2956 & 2865 (m, C-H); δH (CD₂OD, 250MHz) = 0.66 (3H, s, C₁₈-CH₃), 0.8-2.0 (55H, br m, including [0.80 (3H, d, J= 5.4Hz, C²⁶-CH₃), 0.82 (3H, d, J= 5.2Hz, C²⁷-CH₃), 0.84 (3H, s, C¹⁹-CH₃), 0.85 (3H, t, J= 7.0, C²⁹-CH₃), 0.90 (3H, d, J=6.4, C²¹-CH₃)], 2.4-3.6 (18H, m, CH₂NHR & CHNH & NH); δC (CDCl₃, 62.9MHz) = 12.4 (CH₃), 12.9 (CH₃), 13.1 (CH₃), 19.9 (CH₃), 20.0 (CH₃), 20.8 (CH₃), 22.3 (CH₃), 24.0 (CH₂), 24.5 (CH₂), 24.6 (CH₂), 25.3 (CH₂), 25.6 (CH₂), 25.7 (CH₂), 27.6 (CH₂), 27.7 (CH₂), 28.0 (CH₂), 29.8 (CH₂), 30.9 (CH), 31.5 (CH₂), 33.5 (CH₂), 35.6 (CH₂), 37.2 (CH), 37.5 (C), 37.9 (CH), 41.0 (CH), 41.3 (CH₂), 41.8 (CH₂), 44.2 (C), 47.7 (CH), 48.0 (CH₂), 48.4 (CH₂), 53.7 (CH₂), 54.0 (CH₂), 55.0 (CH₂), 55.5 (CH), 56.3 (CH₂), 56.5 (CH), 58.1 (CH), 58.4 (CH).

Compound [117] came off the column second as a solid (180mg, 0.3mmol, 35%) with Rf = 0.44 (α) = 15.4° (c=3, MeOH); m/z = 601 (100%, M⁺H), 594 (26%, M⁺H), 582 (11%); HRMS (FAB) = calculated for (MH⁺) (¹²C₃⁹H₇₇¹⁴N₄) 601.6149, found 601.6134; \( \nu_{\text{max}}/\text{cm}^{-1} \) (CH₂Cl₂) = 3447 (w, N-H), 2950 & 2860 (m, C-H); δH (CD₂OD, 300MHz) = 0.60 (3H, s, C¹⁸-CH₃), 0.7-2.0 (57H, br m, including [0.74 (3H, d, J= 5.6Hz, C²⁶-CH₃), 0.76 (3H, d, J= 5.4Hz, C²⁷-CH₃), 0.78 (3H, s, C¹⁹-CH₃), 0.80 (3H, t, J= 7.6Hz, C²⁹-CH₃), 0.90 (3H, d, J=6.4, C²¹-CH₃)], 2.2-2.5 (6H, m, CH₃NHR), 2.6-2.9 (9H, m, CH₃NHR & CHNH & NH), 3.1 (1H, br m, NH); δC (CDCl₃, 62.9MHz) = 12.9 (CH₃), 13.1 (CH₃), 13.2 (CH₃), 19.9 (CH₃), 20.1 (CH₃), 20.8 (CH₃), 22.7 (CH₂), 24.7 (CH₂), 25.2 (CH₂), 25.6 (CH₂), 25.7 (CH₂), 27.0 (CH₂), 27.7 (CH₂), 28.2 (CH₂), 29.9 (CH₂), 30.2 (CH₂), 30.9 (CH), 33.4 (CH₂), 33.6 (CH₂), 35.6 (CH₂), 37.3 (CH), 37.5 (C), 37.9 (CH), 38.4 (CH₂), 41.4 (CH₂), 41.8 (CH₂), 44.2 (C), 45.8 (CH₂), 46.7 (CH), 47.7 (CH), 48.5 (CH₂), 53.6 (CH₂), 53.8 (CH₂), 54.4 (CH₂), 56.0 (CH), 56.1 (CH₂), 58.0 (CH), 58.3 (CH), 58.9 (CH).

5.2.8 (R)-24-ethyl-3α-[N⁴-(3-aminopropyl)-N¹,N⁸-di-(tert-butoxycarbonyl)-spermidine]-cholestane [118] and (R)-24-ethyl-3β-[N⁴-(3-aminopropyl)-N¹,N⁸-di-(tert-butoxycarbonyl)-spermidine]-cholestane [119].
All glassware was oven dried. (R)-24-ethylcholesta-3-one [82] (0.50 g, 1.2 mmol), N₄-(3-aminopropyl)-N₁,N₈-di-(tert-butoxycarbonyl)-spermidine [107] (1.209 g, 3.0 mmol), 2 drops of acetic acid and powdered 3 A molecular sieves (M/S) (0.70 g) were dissolved in dry methanol (35 mL) and sealed under a nitrogen atmosphere in a 100 mL RB flask. The solution was stirred for 24 hours, and then NaCNBH₃ (3.6 mL of 1 M solution in THF, 3.6 mmol) was added and the reaction stirred for a further 24 hours. At this point, a mass spectrum showed only product and no starting material. The contents of the flask were then filtered through celite and the pad washed with methanol (2 x 10 mL) and dichloromethane (2 x 10 mL). The solvents were removed in vacuo and the residue dissolved in CH₂Cl₂ (80 mL). This was washed with water made slightly basic with 5% NaOH (2 x 40 mL) and brine (2 x 40 mL). The combined aqueous layers were back extracted with CH₂Cl₂ (2 x 40 mL). The organic layers were combined and dried over magnesium sulphate, filtered and the solvent removed in vacuo. This left a crude oil (1.32 g), which was purified by flash chromatography (gradient of 100 % chloroform to 8:2:1 chloroform: methanol: isopropylamine). The two diastereoisomers [118] and [119] could not be separated, both coming off the column at the same time as a clear oil (743 mg, 0.93 mmol, 78 % yield, 8:2:1 chloroform: methanol: isopropylamine) Rf=0.63.

m/z (FAB)= 803 (48%, M+H), 802 (100%, M+H -1), 702 (7%); HRMS (FAB)= calculated for (MH+) (12C₃₉H₇₇N₄O₄) 801.7197, found 801.71974; v max/cm⁻¹ (CH₂Cl₂)= 3465 (w, N-H), 2945 & 2880 (br m, C-H), 1710 (br s, N-C(=O)O); δH (CDCl₃, 250MHz)= 0.65 (3H, s, C₁₈-CH₃), 0.7-2.0 (67H, br m, including [0.80 (3H, d, J= 4.6Hz, C₂₆-CH₃), 0.82 (3H, d, J= 4.6Hz, C₂₇-CH₃), 0.84 (3H, s, C₁₉-CH₃), 0.87 (3H, t, J= 7.2, C₂₉-CH₃), 0.90 (3H, d, J=6.4, C₂¹-CH₃), 1.4 (18H, s, C(CH₃)₃), 1.37 (9H, s, C(CH₃)₃)], 2.4-3.5 (14H, collapsed m, CH₂N & CHN), 4.1 (4H, br s, NH₂); δC (CDCl₃, 62.9MHz)= 12.3 (CH₃), 12.4 (CH₃), 12.6 (CH₃), 19.1 (CH₃), 19.4 (CH₃), 20.1 (CH₃), 21.1 (CH₂), 21.4 (CH₂), 23.4 (CH₂), 24.5 (CH₂), 25.6 (CH₂), 26.4 (CH₂), 27.6 (CH₂), 28.6 (CH₂), 28.8 (CH₃), 28.8 (CH₃), 29.0 (CH₂), 29.5 (CH), 29.7 (CH₂), 30.1 (CH₂), 32.6 (CH₂), 32.8 (CH₂), 32.9 (CH₂), 34.3 (CH₂), 35.8 (CH), 36.2 (C), 36.5 (CH), 37.5 (CH₂), 37.8 (CH₂), 38.5 (CH₂), 39.0 (CH₂), 40.2 (CH₂), 40.4 (CH₂), 42.9 (C), 45.1 (CH₂), 46.1 (CH), 53.4 (CH), 54.6 (CH), 56.5 (CH), 56.8 (CH), 57.7 (CH), 77.7 (C), 79.2 (C), 156.5 (CO), 156.5 (CO).
5.2.9 (R)-24-ethyl-3α-[N4-(3-aminopropyl)-spermidine]-cholestan e [120] and (R)-24-ethyl-3β-[N4-(3-aminopropyl)-spermidine]-cholestan e [121].

TFA (3.6mL, 48.0mmol) was added to a solution of [118] and [119] (713 mg, 0.89 mmol) in CH₂Cl₂ (30 mL) in a 50mL RB flask under a nitrogen atmosphere at rt. The reaction was stirred for approximately 2 hrs, when no starting material remained by TLC and mass spectrum. The solvent was removed in vacuo and the residue purified by flash chromatography (gradient of 100% chloroform to 8:2:1 chloroform: methanol: isopropylamine). Compound [120] came off the column first as a solid (96 mg, 0.16 mmol, 19%) with Rf = 0.70. mp = 147-148°; [α]D = 24.3° (c=3, MeOH); m/z = 602 (54%, M'H), 55 (100%); HRMS (FAB) = calculated for (MH+) (12C₃₉H₇₇N₄) 601.6149, found 601.61489; νmax/cm⁻¹ (CH₂Cl₂) = 3480 (w, N-H), 2960 (m, C-H); δH (CD₂OD, 250MHz) = 0.64 (3H, s, C_{18}-CH₃), 0.7-2.0 (55H, br m, including [0.78 (3H, d, J= 5.4Hz, C_{26}-CH₃), 0.80 (3H, d, J= 5.4Hz, C_{27}-CH₃), 0.82 (3H, s, C_{19}-CH₃), 0.83 (3H, t, J= 7.2, C_{29}-CH₃), 0.88 (3H, d, J= 6.4, C_{21}-CH₃), 2.3-2.9 (17H, m, CH₂NHR & NHR & NH₂), 3.2 (1H, collapsed m, CHNH); δC (CDCl₃, 62.9MHz) = 12.2 (CH₃), 12.8 (CH₃), 13.0 (CH₃), 19.8 (CH₃), 19.9 (CH₃), 20.7 (CH₃), 22.3 (CH₂), 22.4 (CH₂), 24.3 (CH₂), 24.6 (CH₂), 24.7 (CH₂), 25.6 (CH₂), 25.9 (CH₂), 26.9 (CH₂), 27.7 (CH₂), 29.7 (CH₂), 29.8 (CH₂), 30.8 (CH), 31.0 (CH₂), 33.3 (CH₂), 35.5 (CH₂), 37.1 (CH), 37.4 (C), 37.9 (CH), 39.9 (CH₂), 40.7 (CH), 41.0 (CH₂), 41.7 (CH₂), 44.2 (C), 46.6 (CH₂), 46.7 (CH₂), 47.7 (CH), 52.4 (CH₂), 52.7 (CH₂), 54.4 (CH₂), 55.4 (CH), 56.8 (CH), 58.0 (CH), 58.2 (CH).

Compound [121] came off the column second as a solid (218 mg, 0.40mmol, 57%) with Rf = 0.32. mp = 178-180°; [α]D = 11.2° (c=3, MeOH); m/z = 602 (89%, M' H), 154 (34%), 55 (100%); HRMS (FAB) = calculated for (MH+) (12C₃₉H₇₇N₄) 601.6149, found.
5.3 Synthesis of steroid B and associated aminosterols.

5.3.1 3-Mesyl-(7R,24-ethyl-5,22-cholestadien-3β-ol [83].

Stigmasterol [26] (50.00g, 95% purity, 115.1mmol, MW 412.7) was dried in an oven at 60°C then dissolved in dry chloroform (600mL) and dry triethylamine (40mL). The solution was cooled to 0°C and methane sulphonyl chloride (9.3mL, 120.0mmol) was added dropwise with stirring under a nitrogen atmosphere. The reaction was monitored by TLC (20% ethyl acetate in petrol, Rf= 0.4). After the reaction was complete, (30-60 min.) the mixture was poured into saturated brine (375mL) and the organic layer separated. This was washed with hydrochloric acid (2×125mL, 2M) and water (125mL), dried over anhydrous magnesium sulphate and filtered. The solvent was removed in vacuo to yield [83] as a yellow crystalline solid (55.95g, 114.0mmol, 99% yield). This was recrystallized from acetone to give white crystals, m.p. 127-128°C; [α]D= -42° (c=3, CH2Cl2); m/z (E.I.)= 490 (MH+, 2%), 394 (-MsO, 100%), 255 (steroid skeleton, 40%); νmax/cm⁻¹ (CH2Cl2)= 3000-2850 (m, C-H), 1460 (m, C-H), 1320 (-SO2O-), 1170 (s, C-O); δH (CDCl3, 250MHz)= 0.6-2.2ppm (41H, br m, [including 0.7 (3H, s, C18-CH3), 0.75-0.9 (9H, m, C26 (t), C28 (d) and C29 (d) CH3’s), 1.0 (3H, s, C19-CH3), 1.0 (3H, d, J= 6.6Hz, C21-CH3)]), 2.5 (2H, m), 3.0
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(3H, s, CH₃S⁻), 4.5 (1H, m, CHOS), 5.1 (2H, m, HC=CH), 5.4 (1H, m, C=CH). δ(CDCl₃, 62.9MHz)= 12.4 (CH₃), 12.6 (CH₃), 19.4 (CH₃), 19.6 (CH₃), 21.4 (CH₂), 21.5 (CH₃), 21.6 (CH₃), 24.7 (CH₂), 25.8 (CH₂), 29.3 (CH₂), 29.4 (CH₂), 32.2 (CH), 32.25 (CH₂), 32.3 (CH), 36.8 (C), 37.3 (CH₂), 39.1 (CH₃S), 39.6 (CH₂), 40.0 (CH₂), 40.9 (CH), 42.6 (C), 50.4 (CH), 51.6 (CH), 56.3 (CH), 57.1 (CH), 82.4 (CH), 124.2 (CH), 129.7 (CH), 138.6 (CH), 139.1 (C).

5.3.2 3α,5α-cyclo-6β-methoxy-22-cholestone [84] ³⁴,⁵⁵

Beilstein Registry Number 4712127

Oven dried (60°C) 3-mesyl-(R)-24-ethyl-5,22-cholestadien-3p-ol [83] (23 g, 46.9 mmol) was dissolved in dry chloroform (200 mL), dry pyridine (20 mL) and dry methanol (50 mL). Fused potassium acetate was added and the stirred solution heated at reflux for 15 hrs under a drying tube. At this point, no starting material remained by TLC (10 % ethyl acetate in petrol, [84] Rf= 0.83, [84b] Rf= 0.74). The reaction was cooled and the solvent removed in vacuo. The remaining residue was dissolved in dichloromethane (150 mL), washed with dilute hydrochloric acid (2×50 mL, 2M), water (50 mL), dried over anhydrous magnesium sulphate and filtered. The solvent was removed in vacuo to yield the crude product as yellow semisolid (19.66 g, 98 %). Approximately 75 % of this was desired product [84] by TLC, the remainder being the 3β-regioisomer [84b]. The two products have similar polarities so it is impractical to separate by flash chromatography; it is also unnecessary and inefficient to remove the by-product at this stage. However, the crude material was still run down a column of silica gel to separate any coloured impurities, as a colour change is observed as the next reaction endpoint, leaving colourless semisolid. A small amount compound was separated by flash chromatography (gradient of 100 % petroleum ether, rising to 20 % ethyl acetate: 80 % petroleum ether) for characterisation. 3α,5α-cyclo-6β-methoxy-22-cholestone [84] is the least polar compound, and was collected as colourless oil, which gave cubes when recrystallized from acetone. m.p. 54-56°C; [α]D⁰ = +35° (c=1, CH₂Cl₂); m/z (EI)= 426 (M⁺, 26%), 411 (-CH₃, 42%), 394 (-
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CH$_3$OH, 38%), 371 (79%), 351 (28%); $\nu_{\text{max/cm}^{-1}}$(CH$_2$Cl$_2$) = 3000-2800(CH), 1455(Me), 1380(CH$_3$), 1095(C-O), 970 (C=CH); $\delta_{1}$(CDCl$_3$, 250MHz) = 0.35-0.50ppm (1H, m, cyclopropyl CH), 0.65 (1H, t, $J$= 4.3Hz, CH), 0.7-2.2 (~43H, br m, [including 0.7 (3H, s, C$^{18}$-CH$_3$), 0.75-0.9 (9H, m, C$^{26}$ (t), C$^{28}$ (d) and C$^{29}$ (d) CH$_3$'s), 1.0 (3H, s, C$^{19}$-CH$_3$), 1.02 (3H, d, $J$= 7.0Hz C$^{21}$-CH$_3$)], 2.75 (1H, t, $J$= 2.5Hz, CHOCH$_3$), 3.4 (3H, s, OCH$_3$), 5.1 (2H, m. CH=CH); $\delta_{2}$(CDCl$_3$, 62.9MHz) = 11.8 (CH$_3$), 12.1 (CH$_3$), 12.3 (CH$_2$), 18.7 (CH$_3$), 19.1 (CH$_3$), 21.0 (CH$_3$), 21.1 (CH$_3$), 21.3 (CH), 22.6 (CH$_2$), 24.1 (CH$_2$), 24.8 (CH$_2$), 25.3 (CH$_2$), 28.9 (CH$_2$), 30.2 (CH), 31.8 (CH), 33.1 (CH$_2$), 35.0 (CH$_2$), 35.2 (C), 41.2 (CH$_2$), 41.6 (CH), 42.7 (C), 43.1 (C), 47.9 (CH), 51.1 (CH), 56.0 (CH), 56.5 (CH$_3$), 56.7 (CH), 82.2 (CH), 129.0 (CH), 138.2 (CH).

The 3β-methoxy regioisomer [84b] was collected as a white solid and recrystallized from acetone-hexane. m.p. 120-121°C; [$\alpha$]$_{D}$ = -55.4 (c=1, CH$_2$Cl$_2$); $m/\xi$(EI) = 426 (MH$^+$, 100%), 411 (-Me, 10%), 394 (-CH$_3$OH, 40%), 255 (steroid skeleton, 60%); $\nu_{\text{max/cm}^{-1}}$(CH$_2$Cl$_2$) = 3000-2800 (m, C-H), 1620 (w, C=C), 1460 and 1380 (m, C-H), 1120 (s, C-O-C), 970 (m, C=C-H); $\delta_{1}$(CDCl$_3$, 250MHz) = 0.8-2.6 (43H, br m, [including 0.7 (3H, s, C$^{18}$-CH$_3$), 0.75-0.9 (9H, m, C$^{26}$ (t), C$^{28}$ (d) and C$^{29}$ (d) CH$_3$'s), 1.0 (3H, s, C$^{19}$-CH$_3$), 1.02 (3H, d, $J$= 7.0Hz C$^{21}$-CH$_3$)], 2.9-3.2 (1H, m, CHOCH$_3$), 3.4 (3H, s, OCH$_3$), 4.9-5.3 (2H, m, CH=CH), 5.4 (1H, m, C=CH), $\delta_{2}$(CDCl$_3$, 62.9MHz) = 12.4 (CH$_3$), 12.6 (CH$_3$), 19.4 (CH$_3$), 19.8 (CH$_3$), 21.5 (CH$_2$), 21.58 (CH$_3$), 21.59 (CH$_3$), 24.8 (CH$_2$), 25.8 (CH$_2$), 28.4 (CH$_2$), 29.3 (CH$_2$), 32.28 (CH), 32.30 (CH), 32.33 (CH$_2$), 37.3 (C), 37.6 (CH$_2$), 39.1 (CH$_2$), 40.1 (CH$_2$), 40.9 (CH), 42.6 (C), 50.6 (CH), 51.6 (CH), 55.9 (CH), 56.4 (CH), 57.3 (CH$_3$), 80.7 (CH), 122.0 (CH), 129.7 (CH), 138.7 (CH), 141.2 (C).

5.3.3 3α,5α-cyclo-6β-methoxy-pregnane-(R)-20-carboxaldehyde [85].$^{56,58}$

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Pure recrystallized 3α,5α-cyclo-6β-methoxy-22-cholestene [84] (10.00 g, 23.4 mmol) was dissolved in a 2:1 mixture of dry dichloromethane (260 mL) and dry methanol (130 mL,) in a three-necked round-bottomed flask fitted with a gas diffuser inlet, and an outlet into a
flask containing potassium iodide (as an indicator to monitor the reaction). A trap
containing dry ice was set up between the KI solution and the reaction to avoid suck back.
The stirred solution was cooled to -78°C, and after flushing with nitrogen, oxygen
containing ozone gas was bubbled through (V = 150 v). A faint blue coloration appeared
after about 5 hours. The reaction was flushed with nitrogen, and TLC used to confirm that
no starting material remained (20% ethyl acetate in petrol, Rf = 0.53).* Trimethyl phosphite
(25 mL, 212 mmol) was added to reduce the peroxidic products and the stirred solution
allowed to warm to room temperature for one hour. The reaction mixture was then washed
with sodium sulphite solution (2 x 100 mL) to ensure complete removal of peroxides, and
then with water (2 x 100 mL). The organic layer was dried over anhydrous magnesium
sulphate and the solvent removed in vacuo (cold finger rotary evaporator). The oil still
contained some trimethyl phosphite, so was re-dissolved in toluene and this then removed
in vacuo. The crude product was separated on flash silica (5 to 20% ethyl acetate in
petroleum ether) to yield a white crystalline solid [85] (5.63 g, 16.3 mmol, 70% yield).

A small amount of material has the same Rf as starting material, but this is sitosteryl-i-ether, since sitosterol
(22,23-dihydrostigmasterol) is a usual contaminant in commercial stigmasterol.

5.3.4 3α,5α-cyclo-6β-methoxy-(R)-20-22-[1,4-diaminobutane]-pregnane [122].

All glassware was oven dried. 3α,5α-cyclo-6β-methoxy-pregnane-(R)-20-carboxaldehyde
[85] (500 mg, 1.45 mmol), 1,4-diaminobutane (320 mg, 3.63 mmol), 2 drops of acetic acid
and powdered 3A molecular sieves (M/S) (0.7 g) were dissolved in dry methanol (30 mL) and sealed under a nitrogen atmosphere in a 100 mL RB flask. The solution was stirred for 24 hours, and then NaCNBH₃ (4.35 mL of 1M solution in THF, 4.35 mmol) was added and the reaction stirred for a further 24 hours. At this point, a mass spectrum showed only product and no starting material. The contents of the flask were then filtered through celite and the pad washed with methanol (2 x 10 mL) and dichloromethane (2 x 10 mL). The solvents were removed in vacuo and the residue dissolved in CH₂Cl₂ (60 mL). This was washed with water made slightly basic with 5% NaOH (2 x 20 mL) and brine (2 x 20 mL). The combined aqueous layers were back extracted with CH₂Cl₂ (2 x 20 mL). The organic layers were combined and dried over magnesium sulphate, filtered and the solvent removed in vacuo. This left a crude oil (720 mg) which was purified by flash chromatography (gradient of 100% chloroform to 8:1:1 chloroform: methanol: isopropylamine) giving a clear oil (259 mg, 0.62mmol, 43% yield) Rf = 0.64. [α]D = 42.7° (c=3, CHCl₃); m/z (EI) = 417 (M⁺H, 100%), 398 (27%), 109 (69%); HRMS (FAB) = calculated for (MH+) (C₂₇H₄₉N₂O) 417.3846, found 417.3844; v max/cm⁻¹ (CH₂Cl₂) = 3450 (w, N-H), 2970-2800 (m, C-H), 1450 (m, C-H), 1380 (m, C-C), 1095 (s, C-O); δH (CDCl₃, 250MHz) = 0.4-0.5 ppm (1H, m, cyclopropyl CH), 0.6 (1H, t, J= 4.6Hz, CH), 0.8-2.0 (33H, br m, [including 0.75 (3H, s, C₁⁸CH₃), 1.0 (3H, d, J=6.4Hz C²¹CH₃), 1.1 (3H, s, OC₁⁹CH₃)], 2.4-2.9 (9H, m, CH₂NHR & NHR & CHOCH₃), 3.3 (3H, s, OCH₃); δC (CDCl₃, 62.9MHz) = 11.6 (CH₃), 12.8 (CH₂), 17.0 (CH₃), 18.8 (CH₃), 21.5 (CH), 22.7 (CH₂), 24.2 (CH₂), 24.7 (CH₂), 26.4 (CH₂), 28.0 (CH₂), 29.6 (CH₂), 30.6 (CH), 33.3 (CH₂), 35.0 (CH₂), 35.1 (C), 36.2 (CH), 40.4 (CH₂), 40.9 (CH₂), 43.0 (C), 43.3 (C), 48.7 (CH), 49.5 (CH₂), 54.7 (CH), 55.0 (CH₂), 55.8 (CH), 56.4 (CH₃), 82.7 (CH).

5.3.5 20(R)-22-[1,4-diaminobutane]-5-pregnene-3β-ol [123].

3α,5α-Cyclo-6β-methoxy-(R)-20-22-[1,4-diaminobutane]-pregnane [115] (200 mg, 0.48 mmol), water (2.0 mL), dioxane (7 mL), and p-toluene sulfonic acid monohydrate (12 mg) were stirred at 80°C for six hours. TLC (8:1:1 chloroform: methanol: isopropylamine)
showed no starting material after this point. After cooling, the solution was evaporated in vacuo, leaving a white residue. This was dissolved in dichloromethane (25mL), washed with aqueous sodium hydrogen carbonate solution (2x 10mL), dried over anhydrous magnesium sulphate and filtered. The solvent was evaporated in vacuo to leave a crude white solid (110mg). This was purified by flash chromatography (8:1:1 chloroform: methanol: isopropylamine) to yield [123] (90mg, 0.224mmol, 46%) as white solid Rf= 0.56. \( [\alpha]_D = -29.8^\circ \) (c=3, CH\(_2\)Cl\(_2\)); m/z (EI) = 403 (M\(^+\), 100%), 273 (36%); \( \nu_{\text{max/cm}^{-1}} \) (CH\(_2\)Cl\(_2\)) = 3610 (w, N-H), 3460 (br w, O-H), 2940, 2870 (s, C-H), 1670 (m, C=C), 1450 (m, C-H), 1380 (m, C-C), 1055 (m, C-O); \( \delta_{\text{H}} \) (CDCl\(_3\), 250MHz) = 0.6-2.5 (35H, br m, [including 0.70 (3H, s, C\(_{18}\)-CH\(_3\)), 1.0 (3H, s, C\(_{19}\)-CH\(_3\)), 1.1 (3H, d, \( J=6.4 \)Hz C\(_{21}\)-CH\(_3\))], 2.4-2.9 (9H, m, CH\(_2\)NHR & NH\(_2\)), 3.6 (1H, m, CHOH), 5.4 (1H, m, C=CH); \( \delta_{\text{C}} \) (CDCl\(_3\), 62.9MHz) = 12.3 (CH\(_3\)), 12.7 (CH\(_3\)). 19.8 (CH\(_3\)), 21.2 (CH\(_2\)), 21.4 (CH\(_2\)), 23.9 (CH), 24.6 (CH\(_2\)), 24.7 (CH\(_2\)), 26.2 (CH\(_2\)), 27.5 (CH\(_2\)), 30.0 (CH\(_2\)), 32.0 (CH\(_2\)), 32.3 (CH), 36.9 (CH\(_2\)), 37.7 (C), 39.3 (CH\(_2\)), 39.9 (CH\(_2\)), 40.0 (CH\(_2\)), 42.6 (C), 42.7 (CH\(_2\)), 50.5 (CH), 54.7 (CH), 57.0 (CH), 71.9 (CH), 121.9 (CH), 141.3 (C).

5.3.6 3α,5α-Cyclo-6β-methoxy-(R)-20-22-[N\(^1\),N\(^4\)-Di-(tert-butoxycarbonyl)-spermidine]-pregnane [124].

All glassware was oven dried. 3α,5α-cyclo-6β-methoxypregnane-(R)-20-carboxaldehyde [85] (500 mg, 1.45 mmol), N\(^1\),N\(^4\)-Di-(tert-butoxycarbonyl)-spermidine [101] (1.25 g, 3.63 mmol), 2 drops of acetic acid and powdered 3A molecular sieves (M/S) (0.7 g) were dissolved in dry methanol (35 mL) and sealed under a nitrogen atmosphere in a 100 mL RB flask. The solution was stirred for 24 hours, and then NaCNBH\(_3\) (4.35 mL of 1M solution in THF, 4.35 mmol) was added and the reaction stirred for a further 24 hours. At this point, a mass spectrum showed only product and no starting material. The contents of the flask were then filtered through celite and the pad washed with methanol (2 x 10 mL) and dichloromethane (2 x 10 mL). The solvents were removed in vacuo and the residue dissolved in CH\(_2\)Cl\(_2\) (60 mL). This was washed with water made slightly basic with 5%
NaOH (2 x 20 mL) and brine (2 x 20 mL). The combined aqueous layers were back extracted with CH₂Cl₂ (2 x 20 mL). The organic layers were combined and dried over magnesium sulphate, filtered and the solvent removed in vacuo. This left a crude oil (1.55 g) which was purified by flash chromatography (gradient of 100% chloroform to 8:1:1 chloroform: methanol: isopropylamine) giving [124] as clear oil (826 mg, 1.2 mmol, 83% yield) Rf = 0.81. [α]D = 13.6° (c=3, CHCl₃); m/z (EI) = 675 (M+H, 100%), 346 (42%); HRMS (FAB) = calculated for (MH⁺) (C₄₀H₇₂N₃O₅) 674.5472, found 674.54713; νmax/cm⁻¹ (CH₂Cl₂) = 3462 (w, N-H), 2970-2800 (m, C-H), 1710 (s, C=O); δH (CDCl₃, 300MHz) = 0.25-0.3 ppm (1H, m, cyclopropyl CH), 0.49 (1H, t, J=4.4Hz, CH), 0.5-2.0 (53H, br m, including 0.6 (3H, s, C¹⁸-CH₃)), 0.9 (3H, d, J=6.4Hz C²¹-CH₃), 0.91 (3H, s, C¹⁹-CH₃), 1.27 (9H, s, C(CH₃)₃), 1.30 (9H, s, C(CH₃)₃), 2.4-3.1 (13H, m, CH₂NHR & NHR& CHOCH₃), 3.2 (3H, s, OCH₃); δC (CDCl₃, 62.9MHz) = 12.5 (CH₃), 13.3 (CH₂), 17.4 (CH₃), 17.6 (CH₃), 19.6 (CH), 21.7 (CH), 22.9 (CH₂), 24.2 (CH₂), 24.4 (CH₂), 25.2 (CH₂), 26.0 (CH₂), 27.6 (CH₂), 28.68 (CH₃), 28.7 (CH₃), 30.7 (CH), 33.6 (CH₂), 35.2 (CH₂), 35.4 (C), 40.31 (CH₂), 40.36 (CH₂), 42.9 (C), 43.30 (C), 43.7 (CH₂), 46.2 (CH₂), 47.0 (CH₂), 47.3 (CH₂), 48.1 (CH), 54.5 (CH), 55.6 (CH), 56.4 (CH₂), 56.8 (CH₃), 79.1 (C), 80.6 (C), 82.5 (CH), 156.5 (CO), 157.3 (CO).

5,3.7 20(R)-22-spermidine-5-pregnene-3β-ol [125].

3α,5α-Cyclo-6β-methoxy-(R)-20-22-[N¹,N⁴-Di-(tert-butoxycarbonyl)-spermidine]-pregnane [124] (640 mg, 1.4 mmol), water (5.0 mL), dioxane (15 mL), and p-toluene sulfonic acid monohydrate (300 mg) were stirred at 80°C for six hours. TLC (8:1:1 chloroform: methanol: isopropylamine) showed no starting material after this point. After cooling, the solution was evaporated in vacuo. This was dissolved in dichloromethane (25 mL), washed with aqueous sodium hydrogen carbonate solution (2x 10 mL), dried over anhydrous magnesium sulphate and filtered. The solvent was evaporated in vacuo to leave a crude oil (236 mg). This was purified by flash chromatography (8:1:1 chloroform: methanol: isopropylamine) giving [125] (81 mg, 0.18 mmol, 13%) as solid Rf = 0.48. [α]D =
-32.5 (c=3, CH₂Cl₂); m/z= 461 (M=H, 100%); ν max/cm⁻¹ (CH₂Cl₂)= 3610 (w, N-H), 1670 (m, C=C), 1456 (m, C-H), 1380 (m, C-C), 1559 (m, C-O); δ H (CDCl₃, 250MHz)= 0.6-2.5 (37H, br m, [including 0.65 (3H, s, C¹⁸-CH₃)], 0.97 (3H, s, C¹⁹-CH₃), 1.2 (3H, d, J=6.4Hz C²¹-CH₃)], 2.6-3.2 (14H, m, CH₂NHR & NHR), 3.6 (1H, m, CHOH), 5.4 (1H, m, C=CH); δ C (CDCl₃, 62.9MHz)= 12.4 (CH₃), 12.8 (CH₃), 19.8 (CH₃), 21.2 (CH₂), 21.4 (CH₂), 23.9 (CH), 24.6 (CH₂), 24.7 (CH₂), 26.2 (CH₂), 27.5 (CH₂), 27.7 (CH₂), 28.8 (CH₂), 30.0 (CH₂), 32.0 (CH₂), 32.2 (CH₂), 37.6 (CH₂), 37.7 (C), 39.2 (CH₂), 39.9 (CH₂), 40.0 (CH₂), 42.6 (C), 42.6 (CH₂), 50.5 (CH), 53.2 (CH), 56.9 (CH), 58.8 (CH), 71.9 (CH), 121.9 (CH), 141.3 (C).

5.3.8 3α,5α-Cyclo-6β-methoxy-22-[N¹,N⁴,N⁸-Tri-(tert-butoxycarbonyl)-thermospermine]-\((R)\)-20-pregnan [126].

All glassware was oven dried. 3α,5α-cyclo-6β-methoxypregnane-(R)-20-carboxaldehyde [85] (0.50 g, 1.45 mmol), N¹,N⁴,N⁸-tri-(tert-butoxycarbonyl)-thermospermine [104] (1.82 g, 3.63 mmol), 2 drops of acetic acid and powdered 3A molecular sieves (M/S) (0.70 g) were dissolved in dry methanol (40 mL) and sealed under a nitrogen atmosphere in a 100 mL RB flask. The solution was stirred for 24 hours, and then NaN⁺BH₄ (4.35 mL of 1M solution in THF, 4.35 mmol) was added and the reaction stirred for a further 24 hours. At this point, a mass spectrum showed only product and no starting material. The contents of the flask were then filtered through celite and the pad washed with methanol (2 x 20 mL) and dichloromethane (2 x 20 mL). The solvents were removed in vacuo and the residue dissolved in CH₂Cl₂ (80 mL). This was washed with water made slightly basic with 5% NaOH (2 x 40 mL) and brine (1 x 40 mL). The combined aqueous layers were back extracted with CH₂Cl₂ (2 x 40 mL). The organic layers were combined and dried over magnesium sulphate, filtered and the solvent removed in vacuo. This left a crude oil (2.35 g), which was purified by flash chromatography (gradient of 100% chloroform to 8:2:1 chloroform: methanol: isopropylamine). Compound [126] came off the column as clear oil.
3α,5α-cyclo-6β-methoxy-22-[N\(^1\),N\(^8\)-tri-(tert-butoxycarbonyl)-thermospermine]-(R)-20-pregnane [126] (700 mg, 0.84 mmol), water (5.2 mL), dioxane (16 mL), and p-toluene sulfonic acid monohydrate (750 mg) were stirred at 80°c for six hours. TLC (8:2:1 chloroform: methanol: isopropylamine) showed no starting material after this point. After cooling, the solution was evaporated in vacuo. This was dissolved in dichloromethane (75 mL), washed with aqueous sodium hydrogen carbonate solution (2× 25 mL), dried over anhydrous magnesium sulphate and filtered. The solvent was evaporated in vacuo to leave crude yellow oil (420 mg). This was purified by column chromatography (8:2:1 chloroform: methanol: isopropylamine) giving [127] (60 mg, 0.12 mmol, 14%) as a foam R\(_f\) = 0.78. [\(\alpha\)\(_D\)] = -46.7° (c=3, CH\(_2\)Cl\(_2\)); \(m/z\) (EI) = 518 (M+H, 100%); HRMS (FAB) = calculated for (MH+) \([^{12}C_{32}H_{60}^{14}N_4^{16}O]\) 517.4846, found 517.4829; \(v_{\text{max/cm}^{-1}}\) (CH\(_2\)Cl\(_2\)) = 3603 (w, N-H), 3360 (br w, O-H), 3000-2800 (s, C-H); \(\delta_{\text{H}}\) (CDCl\(_3\), 250MHz) = 0.5-2.5 (38H, br m, C-H).
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[including 0.67 (3H, s, C\textsuperscript{18}-CH\textsubscript{3}), 0.99 (3H, s, C\textsuperscript{19}-CH\textsubscript{3}), 1.2 (3H, d, J=6.5Hz C\textsuperscript{21}-CH\textsubscript{3})], 2.6-3.2 (20H, m, CH\textsubscript{2}NHR & NHR), 3.6 (1H, m, CHOH), 5.4 (1H, m, C=CH); \delta\textsubscript{C} (CDCl\textsubscript{3}, 62.9MHz)= 12.4 (CH\textsubscript{3}), 12.8 (CH\textsubscript{3}), 19.8 (CH\textsubscript{3}), 21.2 (CH\textsubscript{2}), 21.4 (CH\textsubscript{2}), 23.9 (CH), 24.6 (CH\textsubscript{2}), 24.7 (CH\textsubscript{2}), 26.1 (CH\textsubscript{2}), 26.5 (CH\textsubscript{2}), 27.3 (CH\textsubscript{2}), 27.5 (CH\textsubscript{2}), 27.7 (CH\textsubscript{2}), 28.8 (CH\textsubscript{2}), 30.0 (CH\textsubscript{2}), 32.0 (CH\textsubscript{2}), 32.2 (CH\textsubscript{2}), 37.6 (CH\textsubscript{2}), 37.6 (C), 39.2 (CH\textsubscript{2}), 39.9 (CH\textsubscript{2}), 40.1 (CH\textsubscript{2}), 40.3 (CH\textsubscript{2}), 42.6 (C), 42.6 (CH\textsubscript{2}), 50.5 (CH), 53.3 (CH), 57.0 (CH), 58.8 (CH), 72.0 (CH), 121.9 (CH), 141.3 (C).

5.3.10 3α,5α-cyclo-6β-methoxy-22-[N\textsuperscript{4}-(3-aminopropyl)-N\textsuperscript{1},N\textsuperscript{8}-di-(tert-butoxycarbonyl)-spermidine]-(R)-20-pregnane [128].

![Chemical Structure](image)

All glassware was oven dried. 3α,5α-cyclo-6β-methoxy-pregnane-(R)-20-carboxaldehyde [85] (0.50 g, 1.45 mmol), N\textsuperscript{4}-(3-aminopropyl)-N\textsuperscript{1},N\textsuperscript{8}-di-(tert-butoxycarbonyl)-spermidine [107] (1.46 g, 3.63 mmol), 2 drops of acetic acid and powdered 3A molecular sieves (M/S) (0.70 g) were dissolved in dry methanol (40 mL) and sealed under a nitrogen atmosphere in a 100 mL RB flask. The solution was stirred for 24 hours, and then NaCNBH\textsubscript{3} (4.35 mL of 1M solution in THF, 4.35 mmol) was added and the reaction stirred for a further 24 hours. At this point, a mass spectrum showed only product and no starting material. The contents of the flask were then filtered through celite and the pad washed with methanol (2 x 20 mL) and dichloromethane (2 x 20 mL). The solvents were removed in vacuo and the residue dissolved in CH\textsubscript{2}Cl\textsubscript{2} (80 mL). This was washed with water made slightly basic with 5% NaOH (2 x 40 mL) and brine (1 x 40 mL). The combined aqueous layers were back extracted with CH\textsubscript{2}Cl\textsubscript{2} (2 x 40 mL). The organic layers were combined and dried over magnesium sulphate, filtered and the solvent removed in vacuo. This left a crude oil (2.35 g), which was purified by flash chromatography (gradient of 100% chloroform to 8:2:1 chloroform: methanol: isopropylamine). Compound [128] came off the column as clear oil (965 mg, 1.32 mmol, 91% yield) R\textsubscript{f} = 0.78. [α\textsubscript{D}]= 13.6\textdegree{} (c=3, CHCl\textsubscript{3}); m/z (EI)= 732 (M\textsuperscript{+}H, 48%), 632 (5%), 404 (46%), 226 (100%); HRMS (FAB)= calculated for (MH+)
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\((^{12}\text{C}_{43}^{1}\text{H}_{79}^{14}\text{N}_{1}^{6}\text{O}_{5})\) 731.6051, found 731.60501; \(\nu_{\text{max/cm}^{-1}}\) (CH\(_2\)Cl\(_2\)) = 3465 (w, N-H), 3000-2800 (m, C-H), 1710 (s, C=O); \(\delta_{\text{H}}\) (CDCl\(_3\), 300MHz) = 0.3ppm (1H, m, cyclopentyl CH), 0.57 (1H, t, \(J=4.1\text{Hz}\), CH), 0.5-2.0 (55H, br m, [including 0.66 (3H, s, C\(_{18}\)-CH\(_3\)), 0.93 (3H, d, \(J=6.9\text{Hz}\) C\(_{21}\)-CH\(_3\)), 0.95 (3H, s, C\(_{19}\)-CH\(_3\)), 1.35 (9H, s, C(CH\(_3\))\(_3\)), 1.36 (9H, s, C(CH\(_3\))\(_3\))], 2.1-3.1 (18H, m, CH\(_2\)NHR & NH\(_2\)R & CHOCH\(_3\)), 3.2 (3H, s, OCH\(_3\)); \(\delta_{\text{C}}\) (CDCl\(_3\), 62.9MHz) = 12.6 (CH\(_3\)), 13.4 (CH\(_3\)), 13.4 (CH\(_2\)), 18.0 (CH\(_1\)), 19.6 (CH), 21.7 (CH), 23.0 (CH\(_2\)), 24.31 (CH\(_2\)), 24.32 (CH\(_2\)), 24.5 (CH\(_2\)), 25.2 (CH\(_2\)), 26.6 (CH\(_2\)), 27.0 (CH\(_2\)), 27.1 (CH\(_2\)).

5.3.11 20\((R)\)-22-branched-5-pregnene-3\(\beta\)-ol [129].

\[\text{C}_{32}\text{H}_{50}\text{N}_{4}\text{O}_{6}\]

Exact Mass: 516.4767
Mol. Wt.: 516.8452
C, 74.36; H, 11.70; N, 10.84; O, 3.10

\(3\alpha,5\alpha\)-cyclo-6\(\beta\)-methoxy-22-[\(\text{N}^1,\text{N}^4,\text{N}^8\)-tri-(tert-butoxycarbonyl)-thermospermine]\-(R)-20-pregnane [128] (900 mg, 1.23 mmol), water (8 mL), dioxane (25 mL), and \(p\)-toluene sulphonatic acid monohydrate (950 mg) were stirred at 80\(^\circ\)C for six hours. TLC (8:2:1 chloroform: methanol: isopropylamine) showed no starting material after this point. After cooling, the solution was evaporated in vacuo. This was dissolved in dichloromethane (75 mL), washed with aqueous sodium hydrogen carbonate solution (2× 25 mL), dried over anhydrous magnesium sulphate and filtered. The solvent was evaporated in vacuo to leave crude yellow oil (433 mg). This was purified by column chromatography (8:2:1 chloroform: methanol: isopropylamine) giving [129] (60 mg, 0.12 mmol, 10%) as a foam \(R_f=0.65\). \([\alpha]_D= -40.6^\circ\) (c=3, CH\(_2\)Cl\(_2\)); \(m/z= 518\) (M=H, 100%); \(\nu_{\text{max/cm}^{-1}}\) (CH\(_2\)Cl\(_2\)) = 3600 (w, N-H), 3410 (br w, O-H), 3000-2800 (s, C-H); \(\delta_{\text{H}}\) (CDCl\(_3\), 250MHz) = 0.5-2.5 (38H, br m, [including 0.65 (3H, s, C\(_{18}\)-CH\(_3\)), 0.99 (3H, s, C\(_{19}\)-CH\(_3\)), 1.2 (3H, d, \(J=6.2\text{Hz}\) C\(_{21}\)-CH\(_3\))], 2.6-3.2 (20H, m, CH\(_2\)NHR & NH\(_2\)R & OH), 3.6 (1H, m, CHOH), 5.3 (1H, m, C=CH); \(\delta_{\text{C}}\)
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(CDCl₃, 62.9 MHz) = 12.3 (CH₃), 12.8 (CH₃), 19.8 (CH₃), 21.2 (CH₂), 21.4 (CH₂), 23.9 (CH), 24.6 (CH₂), 24.7 (CH₂), 24.8 (CH₂), 26.2 (CH₂), 27.5 (CH₂), 28.3 (CH₂), 30.0 (CH₂), 32.0 (CH₂), 32.2 (CH₂), 33.5 (CH₂), 35.0 (CH₂), 37.6 (CH₂), 37.7 (C), 39.2 (CH₂), 39.3 (CH₂), 39.9 (CH₂), 40.1 (CH₂), 42.8 (C), 42.6 (CH₂), 50.5 (CH), 53.3 (CH), 57.0 (CH), 58.8 (CH), 71.9 (CH), 121.8 (CH), 141.3 (C).

5.4 Synthesis of Steroid C and accompanying aminosterols.

5.4.1 3α,5α-Cyclo-6β-methoxy-23-methyl-cholan-(S)-22-ol [86] and 3α,5α-Cyclo-6β-methoxy-23-methyl-cholan-(R)-22-ol [86b].

A 500 mL 3-necked RB flask, pressure equalising dropping funnel, stoppers, condenser, magnetic bead and fine magnesium turnings (2.114 g, 87 mmol) were all placed in an oven (100°C) for one hour. The apparatus was then set up with nitrogen immediately flowing through it. The reaction performed under a very slow flow of nitrogen. The magnesium turnings (2.114 g, 87 mmol) and a crystal of iodine were placed in the three-necked RB flask fitted with the double surface condenser and pressure equalising dropping funnel, then enough dry diethyl ether (20 mL) to just cover the magnesium was added. A solution of isopropylbromide (8.2 mL, 87 mmol) in dry ether (25 mL) was prepared and placed in the dropping funnel. The mixture was magnetically stirred, and the isopropylbromide solution added slowly until an exothermic reaction occurred, then added as to continue the reflux. After all the isopropylbromide solution had been added, the mixture was refluxed on a water bath for a further twenty minutes until all the magnesium had been consumed. The solution was then allowed to cool.

3α,5α-Cyclo-6β-methoxy-pregnane-20-carboxaldehyde [85] (10.00 g, 29.0 mmol) was dissolved in dry ether (350 mL). This solution was placed in the dropping funnel and added slowly to the well-stirred solution of isopropyl-magnesium-bromide. After the addition was complete, the mixture was heated under reflux on a water bath for a further thirty minutes.
The cooled solution was then poured into water/ice mixture (200 mL), and the mixture rendered acidic by the addition of dilute sulphuric acid (10 mL). The ethereal and aqueous layers were separated, and the aqueous layer extracted with ether (3 x 100 mL). During the extraction, the RB flask was well rinsed with both layers to ensure that all of the white precipitate dissolved. The combined ether layers were dried over anhydrous sodium sulphate, filtered and the solvent removed in vacuo. The crude mixed product, a white solid (10.66 g, 95% yield), was separated by flash chromatography (gradient of 0-20% ethyl acetate in petroleum ether). Two diastereoisomers [86] and [86b] are produced as a new chiral centre is formed. The least polar compound [86] (20% ethyl acetate in petrol, Rf= 0.38) was obtained as a white powder and was recrystallized from ethanol (8.16 g, 21.0 mmol, 72% yield). m.p. = 112-114°C; \( m/z \) (EI) = 388 (80%, M⁺H), 373 (60%, -CH₃), 356 (90%, -CH₃OH), 333 (100%), 255 (20%, steroid skeleton); \( \nu_{\text{max/cm}^{-1}} \) (CH₂Cl₂) = 3620 cm⁻¹ (m br, OH), 2820-3000 (C-H stretches), 1450-1470 (C-CH₃, C-H deformations), 1095-1070 (O-CH₃); δH (CDCl₃, 300MHz)= 0.4-0.5ppm (1H, m, cyclopropyl H), 0.65 (1H, t, J= 4.4Hz, CH), 0.7-2.1 (40H br m, including [0.7 (3H, s, C₁₈-CH₃), 0.8 (3H, d, J= 6.7Hz, C₂₄-CH₃), 0.9 (3H, d, J= 6.7Hz, C₂₅-CH₃), 1.0 (3H, s, C₁₉-CH₃), 1.1 (3H, d, J= 7.3Hz, C₂¹-CH₃)], 2.8 (1H, t, J= 2.6Hz, CH-OCH₃), 3.15 (1H, dd, J= 9.35Hz, 1.17Hz, CHOH), 3.3 (3H, s, OCH₃); δC (CDCl₃, 75.8MHz)= 11.7 (CH₃), 12.6 (CH₂), 13.5 (CH₃), 19.5 (CH₃), 19.7 (CH₃), 20.8 (CH), 21.9 (CH₃), 23.2 (CH₂), 24.5 (CH₂), 25.3 (CH₂), 28.2 (CH₂), 30.9 (CH), 31.6 (CH), 33.7 (CH₂), 35.5 (CH₂), 35.6 (C), 37.6 (CH), 40.7 (CH₂), 43.1 (C), 43.8 (C), 48.4 (CH), 53.0 (CH), 56.8 (CH₃), 56.9 (CH), 79.9 (CH), 82.8 (CH).

The other diastereoisomer [86b] (20% ethyl acetate in petrol, Rf= 0.29) was also collected as a white powder and was recrystallized from ethanol (1.01 g, 2.59 mmol, 9% yield). m.p. = 120-121°C; \( m/z \) (EI) = 388 (60%, M⁺H), 373 (50%, -CH₃), 356 (90%, -CH₃OH), 333 (100%), 255 (18%, steroid skeleton); \( \nu_{\text{max/cm}^{-1}} \) (CH₂Cl₂) = 3620 cm⁻¹ (m br, OH), 2820-3000 (C-H stretches), 1450-1470 (C-CH₃, C-H deformations), 1095-1070 (O-CH₃); δH (CDCl₃, 250MHz)= 0.4-0.5ppm (1H, m, cyclopropyl H), 0.65 (1H, t, J= 4.1Hz, CH), 0.7-2.2 (40H br m, including [0.74 (3H, s, C₁₈-CH₃), 0.91 (3H, d, J= 6.7Hz, C₂₄-CH₃), 0.96 (3H, d, J= 6.7Hz, C₂₅-CH₃), 1.01 (3H, d, J= 5.2Hz, C₂¹-CH₃), 1.02 (3H, s, C₁₉-CH₃)], 2.8 (1H, t, J= 2.5Hz, CHOCH₃), 3.3 (3H, s, OCH₃), 3.4 (1H, dd, J= 9.0Hz, 1.28Hz, CHOH); δC (CDCl₃, 62.9MHz)= 12.6 (CH₃), 13.5 (CH₂), 15.2 (CH₃), 18.3 (CH₃), 19.7 (CH₃), 21.91 (CH), 21.94 (CH₃), 23.2 (CH₂), 24.8 (CH₂), 25.4 (CH₂), 28.4 (CH₂), 30.0(CH), 30.9 (CH),...
33.8 (CH$_2$), 35.4 (CH$_2$), 35.7 (C), 40.7 (CH$_2$), 42.4 (CH), 43.1 (C), 43.8 (C), 48.5 (CH), 53.9 (CH), 56.5 (CH$_3$), 56.9 (CH), 79.5 (CH), 82.8 (CH).

5.4.2 3α,5α-Cyclo-6β-methoxy-22(S)-benzoyl-23-methyl-cholane [87].

![Chemical Structure](image)

Exact Mass: 492.3603

Mol. Wt.: 492.7324

C, 80.44; H, 9.82; O, 9.74

OMe

3α,5α-cyclo-6β-methoxy-23-methylcholan-(S)-22-ol [86] (3.54 g, 9.12 mmol) was dissolved in a mixture of acetonitrile (180 mL) and triethylamine (90 mL). Benzoyl cyanide (7.00 g, 54.72 mmol) was added and the solution stirred under a nitrogen atmosphere. The reaction course was monitored by TLC (20% ethyl acetate in petroleum ether, R$_f$= 0.58). After 5 days stirring at RT. the reaction was complete. The solution had changed from light yellow to dark orange/red. Un-reacted benzoyl cyanide was hydrolysed by addition of water (10 mL) and the solvent was evaporated in vacuo. The crude residue was purified by flash chromatography (gradient of 0-10% ethyl acetate in petroleum ether).

A white powder was collected and recrystallized from ethanol (4.09 g, 8.30 mmol, 91%).

m.p. = 96-98°C; $[a]_D = -14^\circ$ (c=3, CH$_2$Cl$_2$); $m/z$ (El) = 516 (25%, M$^+$Na), 493 (20%, M$^+$H), 461 (35%, -CH$_3$OH), 453 (90%), 257 (100%); $v_{max}/cm^{-1}$ (CH$_2$Cl$_2$) = 2820-3000 (m, C-H stretches), 1720 (s, C=O, aryl ester), 1450-1470 (C-CH$_3$, C-H deformations), 1095-1070 (O-CH$_3$); $\delta_H$(CDCl$_3$, 250MHz) = 0.4 ppm (1H, m, cyclopropyl CH), 0.6 (1H, t, J= 4.1Hz, CH), 0.65-2.2 (43H, br m, including [0.76 (3H, s, C$_{18}$-CH$_3$), 0.9 (3H, d, J= 6.7Hz, C$_{24}$-CH$_3$), 0.95 (3H, d, J= 6.9Hz, C$_{25}$-CH$_3$), 1.0 (3H, s, C$_{19}$-CH$_3$), 1.1 (3H, d, J= 6.7Hz, C$_{21}$-CH$_3$)], 2.7 (1H, t, J= 2.5Hz, CHOCH$_3$), 3.3 (3H, s, OCH$_3$), 4.96 (1H, collapsed dd, J= 9.7Hz, CHOOr), 7.3-7.6 (3H, m, Ph H’s), 8.0-8.1 (2H, m, Ph H’s); $\delta_C$(CDCl$_3$, 62.9MHz) = 12.4 (CH$_3$), 13.1 (CH$_3$), 13.4 (CH$_2$), 19.69 (CH$_3$), 19.70 (CH$_3$), 20.2 (CH), 21.9 (CH$_3$), 23.2 (CH$_2$), 24.5 (CH$_2$), 25.3 (CH$_2$), 29.0 (CH$_3$), 30.3 (CH), 30.9 (CH), 33.8 (CH$_2$), 35.3 (CH$_2$), 35.7 (C), 38.2 (CH), 40.6 (CH$_2$), 43.1 (C), 43.7 (C), 48.3 (CH), 53.2 (CH), 56.8 (CH$_3$), 56.9 (CH), 82.2 (CH), 82.8 (CH), 128.7 (CH), 130.0 (CH), 131.2 (C), 133.0 (CH), 166.7 (CO); X-ray crystal structure confirmed stereochemistry (see appendix).
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5.4.3 (S)-22-Benzoyl-23-methyl-cholan-6-ene-3β-ol [88].

3α,5α-cyclo-6β-methoxy-(S)-22-benzoyl-23-methylcholane [87] (4.51 g, 9.17 mmol) was dissolved in dioxane (113 mL) and water (37 mL). p-Toluene sulfonic acid monohydrate (183 mg, 0.96 mmol) was added and the solution refluxed at 80°C for six hours. TLC (20% ethyl acetate in petrol, Rf = 0.12) showed no starting material after this point. After cooling, the solution was evaporated in vacuo, leaving a white residue. This was dissolved in dichloromethane (150 mL), washed with aqueous sodium hydrogen carbonate solution (2×70 mL), dried over anhydrous magnesium sulphate and filtered. The solvent was evaporated in vacuo to leave a crude white solid (4.61 g). Flash chromatography was used to purify the product (gradient of 0-20% ethyl acetate in petrol), as all sulphur must be removed to prevent poisoning of the catalyst in the next reaction. White crystals were collected (4.15 g, 8.7 mmol, 95%). m.p = 137-139°C; [α]D = -32° (c=3, CH2Cl2); m/z (El) = 501 (10%, M+Na), 479 (10%, M+H), 300.1 (100%), 255 (15%, steroid skeleton); νmax/cm⁻¹ (CH2Cl2) = 3320 (m br, O-H), 2820-3000 (m, C-H stretches), 1720 (s, C=O, aryl ester), 1600, 1500 (s, C=C aromatic), 1450-1470 (C-CH3, C-H deformations); δH (CDCl3, 250MHz) = 0.7-2.4ppm (36H, br m, including [0.7 (3H, s, C18-CH3), 0.89 (3H, d, J = 6.7Hz, C24-CH3), 0.95 (3H, d, J = 6.9Hz, C25-CH3), 1.0 (3H, s, C19-CH3), 1.1 (3H, d, J = 6.7Hz, C21-CH3)], 3.5 (1H, m, CH-OH), 4.9 (1H, collapsed dd, J = 9.9 CHOBz), 5.3 (1H, collapsed dd, J = 4.7, C=CH), 7.4-7.6 (3H, m, Ph H's), 8.0-8.1 (2H, m, Ph H's); δC (CDCl3, 62.9MHz) = 12.0 (CH3), 13.2 (CH3), 19.7 (CH3), 19.8 (CH3), 20.2 (CH3), 21.5 (CH3), 24.7 (CH3), 28.9 (CH2), 30.3 (CH), 32.0 (CH2), 32.2 (CH2), 32.3 (CH), 36.8 (C), 37.6 (CH2), 38.2 (CH), 40.1 (CH2), 42.6 (CH2), 42.6 (C), 50.4 (CH), 53.1 (CH), 57.0 (CH), 72.1 (CH), 82.2 (CH), 122.0 (CH), 128.8 (CH), 130.1 (CH), 131.2 (C), 133.1 (CH), 141.1 (C), 166.8 (C).
5.4.4 (S)-22-Benzoyl-23-methyl-cholan-3β-ol [89].

(S)-22-benzoyl-23-methylcholan-6-ene-3β-ol [88] (2.00 g, 4.18 mmol) was dissolved in dry diethyl ether (200 mL) in a 500 mL RB flask. Glacial acetic acid (20 mL) and 5% Pd-C (0.48 g) were added. The stirred solution was hydrogenated at atmospheric pressure for 16 hours. TLC (20% ethyl acetate in petrol. Rf= 0.25) confirmed that no starting material remained, showing only one spot. The reaction was filtered through celite to remove the 5% Pd-C, the celite pad being washed with diethyl ether (3 x 25 mL). The ether was then washed with water (3 x 50 mL) to remove the acetic acid, dried over magnesium sulphate and the solvent removed in vacuo to yield a white solid. This was recrystallized from EtOAc-petroleum ether (1.93 g, 4.01 mmol, 97% yield). m.p= 156°C; m/z (FAB)= 481 (100%, M+H), 255 (20%, steroid skeleton); HRMS (FAB)= calculated for (MH+) (12C32H48N14) 481.3682, found 481.3632; ν max/cm⁻¹ (CH2Cl2)= 3320 (m br, O-H), 2820-3000 (m, C-H stretches), 1720 (s, C=O, aryl ester), 1600, 1500 (s, C=C aromatic), 1450-1470 (C-CH3, C-H deformations); δH (CDCl3, 250MHz)= 0.7-2.2ppm (40H, br m, including [0.7 (3H, s, C18-CH3), 0.8 (3H, s, C19-CH3), 0.85 (3H, d, J= 6.7Hz, C21-CH3), 0.91 (3H, d, J= 6.7Hz, C25-CH3), 1.2 (3H, d, J= 6.7Hz, C21-CH3)], 3.5 (1H, m, CH-OH), 4.9 (1H, collapsed dd, J= 9.9Hz, CHO), 7.4-7.6 (3H, m, Ph H's), 8.0-8.1 (2H, m, Ph H's); δC (CDCl3, 62.9MHz)= 12.2 (CH3), 12.7 (CH3), 13.1 (CH3), 19.7 (CH3), 20.2 (CH3), 21.6 (CH2), 24.6 (CH2), 28.9 (CH2), 29.1 (CH2), 30.3 (CH), 31.8 (CH2), 32.3 (CH2), 35.8 (C), 35.9 (CH), 37.4 (CH2), 38.2 (CH), 38.5 (CH2), 40.4 (CH2), 42.9 (C), 45.2 (CH), 53.2 (CH), 54.6 (CH), 56.7 (CH), 71.7 (CH), 82.2 (CH), 128.7 (CH), 130.0 (CH), 131.2 (C), 133.1 (CH), 166.8 (C).
5.4.5 (S)-22-Benzoyl-23-methyl-cholan-3-one [90].

To a cooled (10-15°C) solution of (S)-22-benzoyl-23-methylcholan-3β-ol [89] (1.66 g, 3.5 mmol) in dry acetone (220 mL) in a 500 mL 3-necked RB flask was added rapidly, with stirring, from a burette Jones Reagent (1.8 mL, 4.8 mmol CrO₃). Nitrogen gas was bubbled through all the solvents, reagents and reaction solution before and during the oxidation.

After 5 minutes, the solution had changed from orange/yellow to green/blue. The reaction was quenched by the addition of a saturated solution of sodium metabisulphite (36 mL). This destroyed any excess chromic acid and the solution turned brown. Water (100 mL) and ether (200 mL) were added and the solution stirred until all the Cr³⁺ salts were dissolved. The ether layer was the separated and the water layer extracted with ether (3 x 70 mL). The combined organic extracts were washed with water (2 x 50 mL), saturated potassium carbonate (2 x 50 mL) and brine (2 x 50 mL). The ether was then dried over magnesium sulphate, filtered and the solvent removed *in vacuo* to yield a white solid. This was recrystallized from ethyl acetate (1.57 g, 3.3 mmol, 94% yield).

*Jones’s Reagent was taken from a solution made up of chromium trioxide (26.72 g) in concentrated sulphuric acid (23 mL) made up to a volume of 100 mL with water. This was used throughout.

This was done to prevent any atmospheric oxygen entering the system as it has previously been reported that Δ³-3-ketones can yield hydroperoxides.

m.p= 78-80°C; [α]D= 36° (c=3, CH₂Cl₂); m/z (FAB)= 501 (100%, M⁺Na), 478 (5%, M⁺H); HRMS (FAB)= calculated for (MH⁺) (12C₁₂H₄₆N₃) 479.3526, found 479.3591; v max/cm⁻¹ (CH₂Cl₂)= 2820-3000 (m, C-H stretches), 1720 (s, C=O, aryl ester), 1675 (CO, ketone), 1600, 1500 (s, C=C aromatic), 1450-1470 (C-CH₃, C-H deformations) δH (CDCl₃, 250MHz)= 0.7-2.4ppm (40H, br m, including [0.7 (3H, s, C¹⁸-CH₃), 0.9 (3H, d, J= 6.7Hz, C²⁴-CH₃), 0.95 (3H d, J= 6.7Hz, C²⁵-CH₃), 1.0 (3H, s, C¹⁹-CH₃), 1.1 (3H, d, J= 6.7Hz, C²¹-CH₃)], 4.9 (1H, collapsed dd, J= 9.9Hz, CHOBz), 7.4-7.6 (3H, m, Ph H’s), 8.0-8.1 (2H, m, Ph H’s); δC (CDCl₃, 125MHz)= 118 (CH₃), 12.2 (CH₃), 13.1 (CH₃), 19.7 (CH₃), 20.2 (CH₃), 21.8 (CH₂), 24.6 (CH₂), 28.9 (CH₂), 29.3 (CH₂), 30.3 (CH), 32.0 (CH₂), 35.8 (CH), 36.0 (C), 38.2 (CH), 38.5 (CH₂), 38.9 (CH₂), 40.2 (CH₂), 42.9 (C), 45.1 (CH₂), 47.0 (CH), 53.2 (CH), 54.1 (CH), 56.5 (CH), 82.1 (CH), 128.7 (CH), 130.0 (CH), 131.2 (C), 133.1 (CH), 166.7 (ester CO), 212.4 (ketone CO).
5.4.6 (S)-22-Benzoyl-23-methyl-3α-[1,4-diaminobutane]-cholane [130] and (S)-22-Benzoyl-23-methyl-3β-[1,4-diaminobutane]-cholane [131].

All glassware was oven dried. (S)-22-benzoyl-23-methylcholan-3-one [90] (0.40 g, 0.84 mmol), 1,4-diaminobutane (0.184 g, 2.09 mmol), 2 drops of acetic acid and powdered 3A molecular sieves (M/S) (0.50 g) were dissolved in dry methanol (25 mL) and sealed under a nitrogen atmosphere in a 50 mL RB flask. The solution was stirred for 24 hours, and then NaCNBH₃ (2.51 mL of 1M solution in THF, 2.51 mmol) was added and the reaction stirred for a further 24 hours. At this point, a mass spectrum showed only product and no starting material. The contents of the flask were then filtered through celite and the pad washed with methanol (2 x 7 mL) and dichloromethane (2 x 7 mL). The solvents were removed in vacuo and the residue dissolved in CH₂Cl₂ (50 mL). This was washed with water made slightly basic with 5% NaOH (2 x 20 mL) and brine (2 x 20 mL). The combined aqueous layers were back extracted with CH₂Cl₂ (2 x 20 mL). The organic layers were combined and dried over magnesium sulphate, filtered and the solvent removed in vacuo. This left a crude oil (450 mg), which was purified by flash chromatography (gradient of 100% chloroform to 8:2:1 chloroform: methanol: isopropylamine). The 3α-compound [130] came off the column first as a clear wax (92 mg, 0.17 mmol, 20% yield), (8:1:1 chloroform: methanol: isopropylamine, Rf= 0.64). [α]D = 15.6° (c=3, CH₂Cl₂); m/z = 551 (M+1, 70%), 105 (BzCO⁺, 100%); HRMS (FAB)= calculated for (M⁺H) C₃₆H₅₉N₂O₂ 551.8741, found 551.45768; vmax/cm⁻¹ (CH₂Cl₂)= 3420 (br m, N-H), 2960, 2940, 2865 (s, C-H), 1720 (s, C-O, aryl ester), 1608 (w, N-H), 1600, 1500 (w, C=C, aryl), 1275 (s, C-O), 1030 (w, C-N); δH (CDCl₃, 250MHz)= 0.5-2.4 ppm (47H, br m, including [0.68 (3H, s, C¹⁸-CH₃), 0.77 (3H, s, C¹⁹-CH₃), 0.88 (3H, d, J= 6.4Hz, C²⁴-CH₃), 0.94 (3H, d, J= 6.7Hz, C²⁵-CH₃), 1.06 (3H, d, J= 6.7Hz, C²¹-CH₃), 2.56 (2H, t, J= 6.7Hz, CH₂N), 2.70 (2H, t, J= 6.7Hz, CH₂N), 3.1...
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(1H, collapsed m, CH$_{eq}$N), 4.91 (1H, collapsed dd, $J = 9.9$Hz, CHO$_B$z), 7.3-7.6 (3H, m, Ph CH’s), 0.80 (2H, m, Ph CH’s); $\delta_{C}$ (CDCl$_3$, 62.9MHz) = 11.9 (CH$_3$), 12.2 (CH$_3$), 13.1 (CH$_3$), 19.7 (CH$_2$), 20.2 (CH$_3$), 21.1 (CH$_2$), 24.5 (CH$_2$), 26.0 (CH$_2$), 28.0 (CH$_2$), 28.9 (CH$_2$), 29.1 (CH$_2$), 30.3 (CH), 31.8 (CH$_2$), 32.3 (CH$_2$), 33.0 (CH$_2$), 33.3 (CH$_2$), 35.9 (CH), 36.5 (C), 38.2 (CH), 40.1 (CH), 40.4 (CH$_2$), 42.3 (CH$_2$), 42.9 (C), 47.5 (CH$_2$), 52.8 (CH), 53.1 (CH), 54.7 (CH), 56.8 (CH), 82.2 (CH), 128.7 (CH), 130.0 (CH), 131.2 (C), 133.1 (CH), 166.8 (CO).

The 3β-compound [131] came off the column second, also as an oily wax (180mg, 0.33mmol, 39% yield), (8:1:1 chloroform: methanol: isopropylamine, R$_f$ = 0.55). [α]$_D$ = 14.7° (c=3. CH$_2$Cl$_2$); m/z = 551 (M+1, 50%), 105 (BzCO$^+$, 100%); HRMS (FAB) = calculated for (MH$^+$) C$_{36}$H$_{59}$N$_2$O$_2$ 551.8741, found 551.45751; $\nu_{max}$/cm$^{-1}$ (CH$_2$Cl$_2$) = 3418 (br m, N-H), 2988, 2940, 2864 (s, C-H), 1720 (s, C=O, aryl ester), 1609 (w, N-H), 1590, 1495 (w. C=C. aryl), 1275 (s, C-O), 1030 (w, C-N); $\delta_{H}$ (CDCl$_3$, 250MHz) = 0.5-2.1ppm (47H, br m. including [0.67 (3H, s, C$^{18}$-CH$_3$), 0.74 (3H, s, C$^{19}$-CH$_3$), 0.81 (3H, d, $J = 6.4$Hz, C$^{24}$-CH$_3$). 0.90 (3H, d, $J = 6.7$Hz, C$^{25}$-CH$_3$), 1.06 (3H, d, $J = 6.7$Hz, C$^{31}$-CH$_3$)]. 2.38 (2H, collapsed t, CH$_2$N), 2.60 (3H, m, CH$_2$N & CH$_{ax}$N), 4.91 (1H, collapsed dd, $J = 9.6$Hz, CHOBz). 7.3-7.6 (3H, m, Ph CH’s), 0.80 (2H, m, Ph CH’s); $\delta_{C}$ (CDCl$_3$, 62.9MHz) = 12.2 (CH$_3$), 12.7 (CH$_3$), 13.1 (CH$_3$), 19.7 (CH$_3$), 20.2 (CH$_3$), 21.5 (CH$_2$), 24.5 (CH$_2$), 27.9 (CH$_2$), 28.9 (CH$_2$), 29.2 (CH$_2$), 29.3 (CH$_2$), 29.4 (CH$_2$), 30.3 (CH), 31.6 (CH$_2$), 32.3 (CH$_2$), 35.9 (CH), 36.3 (C), 37.8 (CH$_2$), 38.2 (CH), 40.4 (CH$_2$), 42.2 (CH$_2$), 42.9 (C), 45.7 (CH), 47.0 (CH$_2$), 53.2 (CH), 54.7 (CH), 56.7 (CH), 57.7 (CH), 82.2 (CH), 128.7 (CH), 130.0 (CH), 131.2 (C), 133.0 (CH), 166.7 (CO).

5.4.7 (S)-22-Hydroxy-23-methyl-3α-[1,4-diamainobutane]-cholane [132].

A stirred solution of (S)-22-Benzoyl-23-methyl-3α-[1,4-diamainobutane]-cholane [130] (58 mg, 0.11 mmol) and KOH (92 mg, 0.63 mmol) in dry methanol (5 mL) was refluxed (60°C) under a nitrogen atmosphere. After 72 hours, no starting material remained by mass
spectroscopy. The solvent was removed in vacuo and the residue dissolved in CH$_2$Cl$_2$ (15 mL), washed with water (3 x 5 mL), dried over magnesium sulphate and filtered. The solvent was again removed and the crude residue purified by flash chromatography (gradient of 100% chloroform to 8:1:1 chloroform: methanol: isopropylamine, R$_f$ = 0.4) to yield yellow solid (50 mg, 0.10 mmol, 91% yield). [a]$_D$ = 14.5$^\circ$ (c=3, CH$_2$Cl$_2$); m/z (FAB) = 447 (100%, M$^+$H), 428 (30%); HRMS (FAB) = calculated for (MH$^+$) C$_{29}$H$_{55}$N$_2$O 447.7665, found 447.43192; $\nu_{\max}$/cm$^{-1}$ (CH$_2$Cl$_2$) = 3685, 3600 (br m, N-H), 3360 (br w, OH), 2940, 2860 (s, C-H), 1600 (w, N-H), 1170 (m, C-O), 1115 (w, C-N); $\delta_H$ (CDCl$_3$, 250 MHz) = 0.5-2.1 (48H, br m. including [0.66 (3H, s, C$_{18}$-CH$_3$), 0.78 (3H, s, C$_{19}$-CH$_3$), 0.80 (3H, d, J= 6.4 Hz, C$_{24}$-CH$_3$), 0.86 (3H, d, J= 6.5 Hz, C$_{25}$-CH$_3$), 1.0 (3H, d, J= 6.2 Hz, C$_{21}$-CH$_3$)], 2.0-2.9 (4H, collapsed m, NCH$_3$'s), 3.1 (1H, collapsed dd, J= 9.2 Hz, CHO$_2$H), 3.2 (1H, m, NCH$_2$); $\delta_C$ (CDCl$_3$, 62.9 MHz) = 11.6 (CH$_3$), 11.9 (CH$_3$), 12.4 (CH$_3$), 19.4 (CH$_3$), 20.8 (CH$_3$), 21.2 (CH$_2$), 24.5 (CH$_2$), 26.3 (CH$_2$), 28.2 (CH$_2$), 29.1 (CH$_2$), 29.2 (CH$_2$), 31.6 (CH), 32.3 (CH$_2$), 32.4 (CH$_2$), 33.1 (CH$_2$), 33.7 (CH$_2$), 36.0 (CH), 36.3 (C), 37.6 (CH), 40.1 (CH), 40.5 (CH$_2$), 42.5 (CH$_2$), 42.9 (C), 47.7 (CH$_2$), 52.6 (CH), 53.0 (CH), 54.7 (CH), 56.9 (CH), 79.7 (CH).

5.4.8 (S)-22-Hydroxy-23-methyl-3β-[1,4-diamainobutane]-cholane [133].

A stirred solution of (S)-22-benzoyl-23-methyl-3β-[1,4-diamainobutane]-cholane [131] (160 mg, 0.29 mmol) and KOH (97 mg, 1.74 mmol) in dry methanol (10 mL) was refluxed (60°C) under a nitrogen atmosphere. After 72 hours, no starting material remained by mass spectroscopy. The solvent was removed in vacuo and the residue dissolved in CH$_2$Cl$_2$ (20 mL), washed with water (3 x 10 mL), dried over magnesium sulphate and filtered. The solvent was again removed and the crude residue purified by preparative TLC (8:1:1 chloroform: methanol: isopropylamine, R$_f$ = 0.3) to yield an opaque solid (130 mg, 0.29 mmol, 92% yield). m/z (FAB) = 447 (100%, M$^+$H); HRMS (FAB) = calculated for (MH$^+$) C$_{29}$H$_{55}$N$_2$O 447.7665, found 447.43147; $\nu_{\max}$/cm$^{-1}$ (CH$_2$Cl$_2$) = 3685, 3600 (br m, N-
H), 3360 (br w. OH), 2940, 2860 (s, C-H), 1600 (w, N-H), 1170 (m, C-O), 1115 (w, C-N); 
δ_H (CDCl3, 250MHz)= 0.6-2.0 (48H, br m, including [0.66 (3H, s, C18-CH3), 0.78 (3H, s, 
C19-CH3), 0.80 (3H, d, J= 6.9Hz, C24-CH3), 0.86 (3H, d, J= 6.7Hz, C25-CH3), 1.0 (3H, d, J= 
6.2Hz, C21-CH3)], 2.0-2.9 (5H, collapsed m, NCH2's & NCH3), 3.1 (1H, collapsed dd, J= 
9.2Hz, CHOCH); δ_C (CDCl3, 62.9MHz)= 11.7 (CH3), 12.4 (CH3), 12.7 (CH3), 19.5 (CH3), 
20.9 (CH3), 21.5 (CH2), 24.5 (CH2), 28.2 (CH2), 29.3 (CH2), 29.4 (CH2), 29.7 (CH2), 30.1 
(CH2), 31.6 (CH), 32.5 (CH2), 36.0 (CH), 36.1 (CH2), 36.4 (C), 37.7 (CH), 37.9 (CH2), 
38.9 (CH2), 40.5 (CH2), 42.9 (C), 45.8 (CH), 47.2 (CH2), 53.0 (CH), 54.9 (CH), 56.9 (CH), 
57.8 (CH), 79.6 (CH).

5.4.9 (S)-22-benzoyl-23-methyl-3α-[N1,N4-di-(tert-butoxycarbonyl)-spermidine]-cholane 
[134] and (S)-22-benzoyl-23-methyl-3β-[N1,N4-di-(tert-butoxycarbonyl)-spermidine]- 
cholane [135].

All glassware was oven dried. (S)-22-Benzoyl-23-methyl-cholan-3-one [90] (0.40 g, 0.84 
mmol), N1,N4-Di-(tert-butoxycarbonyl)-spermidine [101] (0.72 g, 2.09 mmol), 2 drops of 
aetic acid and powdered 3A molecular sieves (M/S) (0.50 g) were dissolved in dry 
ethanol (30 mL) and sealed under a nitrogen atmosphere in a 50 mL RB flask. The 
solution was stirred for 24 hours, and then NaCNBH₃ (2.51 mL of 1M solution in THF, 
2.51 mmol) was added and the reaction stirred for a further 24 hours. At this point, a mass 
spectrum showed only product and no starting material. The contents of the flask were then 
filtered through celite and the pad washed with methanol (2 x 7 mL) and dichloromethane 
(2 x 7 mL). The solvents were removed in vacuo and the residue dissolved in CH₂Cl₂ (50 
ml). This was washed with water made slightly basic with 5% NaOH (2 x 20 mL) and 
brine (1 x 20 mL). The combined aqueous layers were back extracted with CH₂Cl₂ (2 x 20 
ml). The organic layers were combined and dried over magnesium sulphate, filtered and 
the solvent removed in vacuo. This left a crude oil (1.02 g), which was purified by flash 
 chromatography (gradient of 100% chloroform to 8:2:1 chloroform: methanol:
Chapter 5. Experimental.

isopropylamine). The two products could not be separated both coming off the column at the same time as clear oil (845mg, >100%) $R_f = 0.71$. $m/z= 809$ (M+H, 100%); HRMS (FAB) calculated for (M$^+H$) $C_{49}H_{82}N_3O_6$ 809.2956; $v_{max}/cm^{-1}$ (CH$_2$Cl$_2$) = 3420 (br m, N-H), 2960, 2940, 2865 (s, C-H), 1720 (s, C=O, aryl ester), 1660 (br s, C=O, amide), 1618 (w, N-H), 1600, 1500 (w, C=C, aryl), 1275 (s, C-O), 1032 (w, C-N); $\delta_{n}$ (CDCl$_3$, 250MHz) = 0.5-2.4ppm (64H, br m, including [0.68 (3H, s, C$^{18}$-CH$_3$), 0.77 (3H, s, C$^{19}$-CH$_3$), 0.88 (3H, d, $J= 6.4$Hz, C$^{24}$-CH$_3$), 0.94 (3H, d, $J= 6.4$Hz, C$^{25}$-CH$_3$), 1.06 (3H, d, $J= 6.4$Hz, C$^{21}$-CH$_3$), 1.4 (9H, s, C(CH$_3$_3)), 1.5 (9H, s, C(CH$_3$_3)), 1.6 (2H, t, $J= 6.7$Hz, CH$_2$NHR), 3.0 (9H, m, CH$_2$NR(BOC), CHNHR), 4.91 (1H, collapsed dd, $J= 9.9$Hz, CHOBz), 7.3-7.6 (3H, m, Ph CH's). 2.6 (2H, m, Ph CH's); $\delta_{c}$ (CDCl$_3$, 62.9MHz) = 12.1 (CH$_3$), 12.5 (CH$_3$), 13.0 (CH$_3$), 19.6 (CH$_3$), 20.1 (CH$_3$), 21.1 (CH$_2$), 21.3 (CH$_2$), 24.4 (CH$_2$), 25.4 (CH), 25.9 (CH$_2$), 26.0 (CH$_2$), 27.7 (CH$_2$), 28.73 (CH$_3$), 28.74 (CH$_3$), 29.0 (CH$_2$), 30.2 (CH), 32.2 (CH$_2$), 33.0 (CH$_2$), 35.7 (CH), 36.5 (C), 37.2 (CH$_2$), 38.1 (CH), 40.2 (CH$_2$), 40.5 (CH$_2$), 42.9 (C), 43.2 (CH), 43.7 (CH$_2$), 44.0 (CH$_2$), 46.9 (CH$_2$), 47.0 (CH$_2$), 53.1 (CH), 56.6 (CH), 57.8 (CH), 79.2 (C), 79.7 (C), 82.1 (CH), 128.7 (CH), 129.9 (CH), 131.1 (C), 133.0 (CH), 156.3 (CO), 156.4 (CO), 166.6 (CO).

5.4.10 (S)-22-benzoyl-23-methyl-3α-[spermidine]-cholane [136] and (S)-22-benzoyl-23-methyl-3β-[spermidine]-cholane [137].

![Diagram of (S)-22-benzoyl-23-methyl-3α-[spermidine]-cholane]

TFA (4.7mL, 63mmol) was added to a stirred solution of (S)-22-benzoyl-23-methyl-3-[$N^1,N^4$-di-( tert-butoxycarbonyl)-spermidine]-cholane diastereoisomers [134] and [135]
diastereoisomers (845 mg, 1.05 mmol) in CH\textsubscript{2}Cl\textsubscript{2} (40 mL) at RT. The reaction was stirred for approximately 2 hrs, when no starting material remained by TLC (8: 2: 1 chloroform: methanol: isopropylamine) and mass spectrum. The solvent was removed in vacuo, CH\textsubscript{2}Cl\textsubscript{2} added and then removed in vacuo (2 x 20 mL) to remove any excess TFA. Ether (20 mL) was added to the cream solid and the precipitate stirred for 10 minutes, this was then filtered removing organic impurities, leaving a white solid which was purified by flash chromatography (100% chloroform to 8: 1: 1 chloroform: methanol: isopropylamine, Rf= 0.38, Rf= 0.25). (S)-22-benzoyl-23-methyl-3α-[spermidine]-cholane [136] was collected first as a white solid (85 mg, 0.14 mmol, 13% yield).

\[\text{Mp}= 87-89^{\circ}; \quad [\alpha]_D= 14.9^{\circ} \quad (c=3, \quad \text{CH}_2\text{Cl}_2); \quad m/z= 608 \quad (60\%, \quad \text{M}^+\text{H}), \quad 105 \quad (100\%); \quad \text{HRMS (FAB)= calculated for (MH+) C}_{39}\text{H}_{66}\text{N}_3\text{O}_2 \quad 608.9694, \quad \text{found} \quad 608.91543; \quad \nu_{\text{max/cm}}^{-1} (\text{CH}_2\text{Cl}_2)= 3680 \quad (w, \quad \text{N-H}), \quad 2940, \quad 2870 \quad (s, \quad \text{C-H}), \quad 1715 \quad (s, \quad \text{C}=\text{O}, \quad \text{aryl ester}), \quad 1115 \quad (m, \quad \text{C}=\text{O}); \quad \delta_{\text{H}} (\text{CDCl}_3, \quad 250\text{MHz})= \quad 0.5-1.75\text{ppm} \quad (50\text{H}, \quad \text{br m, including} \quad [0.66 \quad (3\text{H}, \quad s, \quad \text{C}^{18}\text{CH}_3), \quad 0.75 \quad (3\text{H}, \quad s, \quad \text{C}^{19}\text{-CH}_3), \quad 0.86 \quad (3\text{H}, \quad d, \quad J= 6.4\text{Hz}, \quad \text{C}^{24}\text{-CH}_3), \quad 0.91 \quad (3\text{H}, \quad d, \quad J= 6.7\text{Hz}, \quad \text{C}^{25}\text{-CH}_3), \quad 1.05 \quad (3\text{H}, \quad d, \quad J= 6.7\text{Hz}, \quad \text{C}^{21}\text{-CH}_3)], \quad 1.8 \quad (2\text{H}, \quad \text{br m}), \quad 2.62 \quad (6\text{H}, \quad \text{m}, \quad \text{CH}_2\text{N's}), \quad 2.78 \quad (1\text{H}, \quad \text{collapsed m, CH}_3\text{N}), \quad 4.90 \quad (1\text{H}, \quad \text{collapsed dd, J= 9.6Hz, CHOBz}), \quad 7.3-7.6 \quad (3\text{H}, \quad \text{m}, \quad \text{Ph CH's}), \quad 0.80 \quad (2\text{H}, \quad \text{m}, \quad \text{Ph CH's})); \quad \delta_{\text{C}} (\text{CDCl}_3, \quad 62.9\text{MHz})= \quad 11.9 \quad (\text{CH}_3), \quad 12.2 \quad (\text{CH}_3), \quad 13.1 \quad (\text{CH}_3), \quad 19.7 \quad (\text{CH}_3), \quad 20.2 \quad (\text{CH}_3), \quad 21.1 \quad (\text{CH}_2), \quad 24.50 \quad (\text{CH}_2), \quad 24.51 \quad (\text{CH}_2), \quad 26.0 \quad (\text{CH}_2), \quad 28.0 \quad (\text{CH}_2), \quad 28.9 \quad (\text{CH}_2), \quad 29.0 \quad (\text{CH}_2), \quad 30.3 \quad (\text{CH}), \quad 32.24 \quad (\text{CH}_2), \quad 32.25 \quad (\text{CH}_2), \quad 32.9 \quad (\text{CH}_2), \quad 33.0 \quad (\text{CH}_2), \quad 33.3 \quad (\text{CH}_2), \quad 35.8 \quad (\text{CH}), \quad 36.5 \quad (\text{C}), \quad 38.2 \quad (\text{CH}), \quad 39.2 \quad (\text{CH}), \quad 40.1 \quad (\text{CH}), \quad 40.4 \quad (\text{CH}_2), \quad 42.9 \quad (\text{C}), \quad 47.0 \quad (\text{CH}_2), \quad 49.3 \quad (\text{CH}_2), \quad 50.1 \quad (\text{CH}_2), \quad 53.1 \quad (\text{CH}), \quad 54.7 \quad (\text{CH}), \quad 56.8 \quad (\text{CH}), \quad 82.2 \quad (\text{CH}), \quad 128.7 \quad (\text{CH}), \quad 130.0 \quad (\text{CH}), \quad 131.2 \quad (\text{C}), \quad 133.1 \quad (\text{CH}), \quad 166.7 \quad (\text{CO}).

The more polar (S)-22-benzoyl-23-methyl-3β-[spermidine]-cholane [137] also came off the column as a white solid (420mg, 0.69mmol, 66% yield). \([\alpha]_D= 12.6^{\circ} \quad (c=3, \quad \text{CH}_2\text{Cl}_2); \quad m/z= 608 \quad (100\%); \quad \text{HRMS (FAB)= calculated for (MH+) C}_{39}\text{H}_{66}\text{N}_3\text{O}_2 \quad 608.9694, \quad \text{found} \quad 608.9047; \quad \nu_{\text{max/cm}}^{-1} (\text{CH}_2\text{Cl}_2)= 3680 \quad (w, \quad \text{N-H}), \quad 2940, \quad 2870 \quad (s, \quad \text{C-H}), \quad 1715 \quad (s, \quad \text{C}=\text{O}, \quad \text{aryl ester}), \quad 1115 \quad (m, \quad \text{C}=\text{O}); \quad \delta_{\text{H}} (\text{CDCl}_3, \quad 250\text{MHz})= \quad 0.5-2.1\text{ppm} \quad (50\text{H}, \quad \text{br m, including} \quad [0.66 \quad (3\text{H}, \quad s, \quad \text{C}^{18}\text{CH}_3), \quad 0.73 \quad (3\text{H}, \quad s, \quad \text{C}^{19}\text{-CH}_3), \quad 0.77 \quad (3\text{H}, \quad d, \quad J= 6.4\text{Hz}, \quad \text{C}^{24}\text{-CH}_3), \quad 0.86 \quad (3\text{H}, \quad d, \quad J= 6.6\text{Hz}, \quad \text{C}^{25}\text{-CH}_3), \quad 1.02 \quad (3\text{H}, \quad d, \quad J= 6.7\text{Hz}, \quad \text{C}^{21}\text{-CH}_3)], \quad 2.2-3.2 \quad (9\text{H}, \quad \text{collapsed m, CH}_2\text{N and CH}_3\text{N}), \quad 4.91 \quad (1\text{H}, \quad \text{collapsed dd, J= 9.7Hz, CHOBz}), \quad 7.3-7.6 \quad (3\text{H}, \quad \text{m}, \quad \text{Ph CH's}), \quad 0.80 \quad (2\text{H}, \quad \text{m}, \quad \text{Ph CH's})); \quad \delta_{\text{C}} (\text{CDCl}_3, \quad 62.9\text{MHz})= \quad 12.2 \quad (\text{CH}_3), \quad 12.6 \quad (\text{CH}_3), \quad 13.0 \quad (\text{CH}_3), \quad 19.6 \quad (\text{CH}_3), \quad 20.1
(CH₃), 21.4 (CH₂), 24.4 (CH₂), 25.0 (CH₂), 26.2 (CH₂), 28.8 (CH₂), 29.1 (CH₂), 29.3 (CH₂), 30.1 (CH₂), 30.2 (CH), 31.6 (CH₂), 32.1 (CH₂), 32.2 (CH₂), 35.9 (CH), 36.0 (C), 37.0 (CH₂), 37.9 (CH₂), 38.1 (CH), 39.5 (CH₂), 40.0 (CH₂), 40.2 (CH₂), 42.8 (C), 45.2 (CH), 45.8 (CH₂), 47.8 (CH₂), 53.1 (CH), 54.5 (CH), 56.6 (CH), 58.1 (CH), 82.1 (CH), 128.7 (CH), 130.0 (CH), 131.1 (C), 133.1 (CH), 166.7 (CO).

5.4.11 (S)-22-hydroxy-23-methyl-3α-[spermidine]-cholane [138].

A stirred solution of (S)-22-benzoyl-23-methyl-3α-[spermidine]-cholane [136] (55 mg, 0.09 mmol) and KOH (30 mg, 0.54 mmol) in dry methanol (10 mL) was refluxed (60 °C) under a nitrogen atmosphere. After 72 hours, no starting material remained by mass spectroscopy. The solvent was removed in vacuo and the residue dissolved in CH₂Cl₂ (20 mL), washed with water (3 x 10 mL), dried over magnesium sulphate and filtered. The solvent was again removed and the crude residue purified by preparative TLC (8:1:1 chloroform: methanol: isopropylamine, Rf= 0.36) to yield clear oil (39 mg, 0.077 mmol, 85% yield). m/z (FAB)= 504 (100%), 447 (60%), 376 (20%); νmax/cm⁻¹ (CH₂Cl₂)= 3695, 3602 (br m, N-H), 2940, 2860 (s, C-H), 1600 (w, N-H), 1175 (m, C-O), 1115 (w, C-N); δH (CDCl₃, 250MHz)= 0.5-2.1 (51H, br m, including [0.66 (3H, s, C¹⁸-CH₃), 0.78 (3H, s, C¹⁹-CH₃), 0.80 (3H, d, J= 6.0Hz, C²⁴-CH₃), 0.86 (3H, d, J= 6.7Hz, C²⁵-CH₃), 1.0 (3H, d, J= 6.4Hz, C²¹-CH₃)], 2.0-2.9 (8H, collapsed m, NCH₂⁺s), 3.2 (1H, m, NCH₂eq), 3.1 (1H, collapsed dd, J= 9.4Hz, CHOH); δC (CDCl₃, 62.9MHz)= 11.6 (CH₃), 11.9 (CH₃), 12.4 (CH₃), 19.4 (CH₃), 20.8 (CH₃), 21.2 (CH₂), 23.6 (CH₂), 24.5 (CH₂), 24.6 (CH₂), 28.2 (CH₂), 28.3 (CH₂), 29.1 (CH₂), 30.1 (CH₂), 31.6 (CH), 32.2 (CH₂), 32.3 (CH₂), 33.5 (CH₂), 35.9 (CH), 36.5 (C), 37.6 (CH), 39.3 (CH₂), 39.3 (CH), 39.8 (CH), 40.5 (CH₂), 42.9 (C), 49.6 (CH₂), 53.0 (CH), 53.5 (CH₂), 55.4 (CH), 55.8 (CH₂), 56.9 (CH), 79.8 (CH).
A stirred solution of (S)-22-benzoyl-23-methyl-3β-[spermidine]-cholane [137] (140 mg, 0.23 mmol) and KOH (73 mg, 1.3 mmol) in dry methanol (20 mL) was refluxed (60°C) under a nitrogen atmosphere. After 72 hours, no starting material remained by mass spectroscopy. The solvent was removed in vacuo and the residue dissolved in CH$_2$Cl$_2$ (20 mL), washed with water (3 x 5 mL), dried over magnesium sulphate and filtered. The solvent was again removed and the crude residue purified by flash chromatography (8:1:1 chloroform: methanol: isopropylamine, R$_f$= 0.30) to yield clear oil (97 mg, 0.19 mmol, 83% yield).

$m/z$ = 504 (100%), 447 (20%); $\nu_{max}$/cm$^{-1}$ (CH$_2$Cl$_2$)= 3695, 3602 (br m, N-H), 3330 (br w, OH), 2940, 2860 (s, C-H), 1600 (w, N-H), 1175 (m, C-O), 1115 (w, C-N); $\delta_H$(CDCl$_3$, 250MHz)= 0.6-2.9 (51H, br m, including [0.66 (3H, s, C$_{18}$-CH$_3$), 0.76 (3H, s, C$_{19}$-CH$_3$), 0.80 (3H, d, $J$= 6.5Hz, C$_{24}$-CH$_3$), 0.86 (3H, d, $J$= 6.6Hz, C$_{25}$-CH$_3$), 1.0 (3H, d, $J$= 6.6Hz, C$_{21}$-CH$_3$)], 2.0-2.9 (9H, collapsed m, NCH$_2$'s & NCH$_3$), 3.1 (1H, collapsed dd, $J$= 9.4Hz, CHOH); $\delta_C$(CDCl$_3$, 62.9MHz)= 11.6 (CH$_3$), 12.4 (CH$_3$), 12.7 (CH$_3$), 19.4 (CH$_3$), 20.8 (CH$_3$), 21.5 (CH$_2$), 24.5 (CH$_2$), 24.6 (CH$_2$), 25.4 (CH$_2$), 28.1 (CH$_2$), 28.2 (CH$_2$), 29.4 (CH$_2$), 29.7 (CH$_2$), 31.6 (CH), 31.8 (CH$_2$), 32.5 (CH$_2$), 36.0 (CH), 36.2 (C), 37.6 (CH), 38.3 (CH$_2$), 40.3 (CH$_2$), 40.5 (CH$_2$), 42.9 (C), 46.2 (CH), 48.7 (CH$_2$), 53.0 (CH), 53.4 (CH$_2$), 54.8 (CH), 55.8 (CH$_2$), 56.9 (CH), 57.8 (CH), 79.7 (CH).
5.4.13 (S)-22-benzoyl-23-methyl-3α-[N1,N4,N8-tri-(tert-butoxycarbonyl)-thermospermine]-cholane [140] and (S)-22-benzoyl-23-methyl-3β-[N1,N4,N8-tri-(tert-butoxycarbonyl)-thermospermine]-cholane [141].

All glassware was oven dried. (S)-22-benzoyl-23-methyl-cholan-3-one [90] (0.40 g, 0.84 mmol), N1,N4,N8-tri-(tert-butoxycarbonyl)-thermospermine [104] (1.05 g, 2.09 mmol), 2 drops of acetic acid and powdered 3A molecular sieves (M/S) (0.50 g) were dissolved in dry methanol (40 mL) and sealed under a nitrogen atmosphere in a 50 mL RB flask. The solution was stirred for 24 hours, and then NaCNBH3 (2.51 mL of 1M solution in THF, 2.51 mmol) was added and the reaction stirred for a further 24 hours. At this point, a mass spectrum showed only product and no starting material. The contents of the flask were then filtered through celite and the pad washed with methanol (2 x 8 mL) and dichloromethane (2 x 8 mL). The solvents were removed in vacuo and the residue dissolved in CH2Cl2 (50 mL). This was washed with water made slightly basic with 5% NaOH (2 x 20 mL) and brine (2 x 20 mL). The combined aqueous layers were back extracted with CH2Cl2 (2 x 20 mL). The organic layers were combined and dried over magnesium sulphate, filtered and the solvent removed in vacuo. This left a crude oil (1.2 g), which was purified by flash chromatography (gradient of 100% chloroform to 8:2:1 chloroform: methanol: isopropylamine, Rf = 0.69). The two compounds could not be separated coming off the column at the same time as clear oil (894 g). m/z= 967 (M+ H+, 100%), 105 (BzCO+, 36%); \( \nu_{\text{max/cm}^{-1}} \) (CH2Cl2) = 3420 (br m, N-H), 2960, 2940, 2865 (s, C-H), 1720 (s, C=O, aryl ester), 1658 (br s, C=O, amide), 1608 (w, N-H), 1600, 1500 (w, C=C, aryl), 1275 (s, C-O), 1030 (w, C-N); \( \delta_{\text{H}} \) (CDCl3, 250MHz) = 0.5-2.4 ppm (75H, br m, including [0.69 (3H, s, CH3), 0.78 (3H, s, C19-CH3), 0.88 (3H, d, J= 6.4Hz, C24-CH3), 0.94 (3H, d, J= 6.7Hz, C25-CH3), 1.0 (3H, d, J= 6.7Hz, C21-CH3), 1.43 (9H, s, C(CH3)), 1.44 (9H, s, C(CH3)), 1.45 (9H, s, C(CH3)), 2.8-3.6 (15H, m, CH2NHR & CHNHR & NH's), 4.91 (1H, collapsed dd, J= 9.9Hz, CHOBz), 7.4-7.6 (3H, m, PH CH’s), 0.80 (2H, m, PH CH’s); \( \delta_{\text{C}} \) (CDCl3, 62.9MHz) = 11.9 (CH3), 12.2 (CH3), 13.1 (CH3), 19.6 (CH3), 20.1 (CH3), 21.4 (CH2), 24.5 (CH2), 26.0 (CH2), 26.1 (CH2), 26.2 (CH2), 27.7 (CH2), 28.7 (CH3) 28.7 (CH3), 28.8 (CH3),
28.9 (CH₂), 29.1 (CH₂), 30.3 (CH), 31.8 (CH₂), 32.2 (CH₂), 35.8 (CH), 36.1 (C), 38.2 (CH), 40.0 (CH₂), 40.2 (CH₂), 40.5 (CH₂), 40.6 (CH₂), 42.9 (C), 43.3 (CH), 44.6 (CH₂), 45.2 (CH₂), 45.3 (CH₂), 45.6 (CH₂), 47.1 (CH₂), 53.1 (CH), 54.4 (CH), 56.6 (CH), 58.0 (CH), 77.7 (C), 79.4 (C), 79.7 (C), 82.2 (CH), 128.7 (CH), 130.0 (CH), 131.1 (C), 133.0 (CH), 155.9 (CO), 155.9 (CO), 156.4 (CO), 166.7 (CO).

5.4.14 22(S)-benzoyl-23-methyl-3α-[thermospermine]-cholane [142], and 22(S)-benzoyl-23-methyl-3β-[thermospermine]-cholane [143].

TFA (4.6 mL, 61.8 mmol) was added to a solution of (S)-22-benzoyl-23-methyl-3-[N¹,N⁴,N⁸-tri-(tert-butoxycarbonyl)-thermospermine]-cholane diastereoisomers [140] and [141] (890 g, 0.93 mmol) in CH₂Cl₂ (40 mL) at RT. The reaction was stirred for approximately 2 hrs, when no starting material remained by TLC (8: 2: 1 chloroform: methanol: isopropylamine) and mass spectrum. The solvent was removed in vacuo, CH₂Cl₂ added and then removed in vacuo (2 x 30 mL) to remove any excess TFA. Ether (40 mL) was added to the cream solid and the precipitate stirred for 10 minutes, this was then filtered removing organic impurities, leaving a white solid which was purified by flash chromatography (100% chloroform to 8: 1: 1 chloroform: methanol: isopropylamine, Rf= 0.34, Rf= 0.11). Aminosterol [142] was collected first as a white foam (290 mg, 0.44 mmol, 35% yield). m/z= 666 (100%, M+H); νmax/cm⁻¹ (CH₂Cl₂)= 3655 (w, N-H), 2940, 2870 (s, C-H), 1718 (s, C=O, aryl ester); δ_H(CDCl₃, 250MHz)= 0.5-1.75ppm (49H, br m, including [0.66 (3H, s, C¹⁸-CH₃), 0.75 (3H, s, C¹⁹-CH₃), 0.86 (3H, d, J= 6.4Hz, C²⁴-CH₃), 0.91 (3H, d, J= 6.7Hz, C²⁵-CH₃), 1.05 (3H, d, J= 6.7Hz, C²¹-CH₃)], 2.62 (16H, collapsed m, CH₂N’s), 3.2 (1H, m, CHₑ₈N), 4.90 (1H, collapsed dd, J= 9.6Hz, CHOBz), 7.3-7.6 (3H, m,
The more polar aminosterol [143] also came off the column as a white foam (350 mg, 0.52 mmol, 42% yield). [α]D = 16.8° (c=3, CH2Cl2); m/z = 666 (100%); νmax/cm⁻¹ (CH2Cl2) = 3420 (w, N-H), 2940, 2870 (s, C-H), 1722 (s, C=O, aryl ester), 1115 (m, C-O); δH (CDCl3, 250MHz) = 0.5-2.1 ppm (48H, br m, including [0.66 (3H, s, C18-CH3), 0.74 (3H, s, C19-CH3), 0.78 (3H, d, J= 6.5Hz, C24-CH3), 0.86 (3H, d, J= 6.6Hz, C25-CH3), 1.02 (3H, d, J= 6.6Hz, C21-CH3)], 2.2-3.2 (18H, collapsed m, CHaN & CHaxN & NH's), 4.91 (1H, collapsed dd, J= 9.7Hz, CHOβz), 7.3-7.6 (3H, m, Ph CH's), 0.80 (2H, m, Ph CH's); δC (CDCl3, 62.9MHz) = 12.1 (CH3), 12.5 (CH3), 13.0 (CH3), 19.6 (CH3), 20.1 (CH3), 21.3 (CH2), 23.5 (CH2), 23.9 (CH2), 24.4 (CH2), 25.2 (CH2), 27.2 (CH2), 28.7 (CH2), 30.2 (CH), 31.3 (CH2), 32.0 (CH2), 32.5 (CH2), 35.6 (CH), 36.1 (C), 37.0 (CH2), 37.9 (CH2), 38.0 (CH), 39.5 (CH2), 40.1 (CH2), 40.2 (CH2), 42.8 (C), 43.6 (CH2), 45.2 (CH), 52.2 (CH2), 52.3 (CH2), 52.8 (CH2), 53.0 (CH), 54.1 (CH), 54.8 (CH2), 56.5 (CH), 57.4 (CH), 82.0 (CH), 128.6 (CH), 129.9 (CH), 131.0 (C), 133.0 (CH), 166.6 (CO).

5.4.15 22(S)-hydroxy-23-methyl-3α-[thermospermine]-cholane [144].

A stirred solution of 22(S)-benzoyl-23-methyl-3α-[thermospermine]-cholane [142] (200 mg, 0.3 mmol) and KOH (68 mg, 1.2 mmol) in dry methanol (5 mL) was refluxed (60 °C) under a nitrogen atmosphere. After 72 hours, no starting material remained by mass spectroscopy. The solvent was removed in vacuo and the residue dissolved in CH2Cl2 (20 mL), washed with water (3 x 10 mL), dried over magnesium sulphate and filtered. The
solvent was again removed and the crude residue purified by preparative TLC (8:1:1 chloroform: methanol: isopropylamine, Rf= 0.29) to yield an opaque solid (121 mg, 0.216 mmol, 72% yield). m/z (FAB)= 562 (100%); HRMS (FAB)= calculated for (MH+)
$C_{35}H_{68}N_4O$ 561.5472, found 561.5501; $\nu_{\text{max/cm}^{-1}}$ ($\text{CH}_2\text{Cl}_2$)= 3611 (br m, N-H), 3325 (br w, OH), 2940, 2860 (s, C-H), 1600 (w, N-H), 1175 (m, C-O), 1115 (w, C-N); $\delta$$_\text{H}$ (CDCl$_3$, 250MHz)= 0.5-2.1 (58H, br m, including [0.66 (3H, s, C$_{18}$-CH$_3$), 0.78 (3H, s, C$_{19}$-CH$_3$), 0.80 (3H, d, J= 6.4Hz, C$_{24}$-CH$_3$), 0.86 (3H, d, J= 6.6Hz, C$_{25}$-CH$_3$), 1.0 (3H, d, J= 6.4Hz, C$_{21}$-CH$_3$)], 2.0-2.9 (8H, collapsed m, NCH$_3$'s), 3.2 (1H, m, NCH$_3$), 3.1 (1H, collapsed dd, J= 9.4Hz, CHOH); $\delta$$_\text{C}$ (CDCl$_3$, 62.9MHz)= 11.6 (CH$_3$), 11.9 (CH$_3$), 12.4 (CH$_3$), 19.4 (CH$_3$), 20.8 (CH$_3$), 21.2 (CH$_2$), 21.5 (CH$_2$), 23.9 (CH$_2$), 24.5 (CH$_2$), 26.2 (CH$_2$), 28.2 (CH$_2$), 28.8 (CH$_2$), 29.1 (CH$_2$), 29.2 (CH$_2$), 31.6 (CH), 32.3 (CH$_2$), 32.4 (CH$_2$), 33.0 (CH$_2$), 35.9 (CH), 36.5 (CH), 37.6 (CH), 39.3 (CH$_2$), 39.3 (CH), 39.8 (CH), 40.4 (CH$_2$), 40.5 (CH$_2$), 42.9 (C), 46.3 (CH$_2$), 53.0 (CH), 52.8 (CH$_2$), 54.0 (CH$_2$), 55.4 (CH), 55.4 (CH$_2$), 56.9 (CH), 79.8 (CH).

5.4.16 22(S)-hydroxy-23-methyl-3$\beta$-[thermospermine]-cholane [145].

A stirred solution of 22(S)-benzoyl-23-methyl-3$\beta$-[thermospermine]-cholane [143] (193 mg, 0.29 mmol) and KOH (90 mg, 1.6 mmol) in dry methanol (8 mL) was refluxed (60 °C) under a nitrogen atmosphere. After 72 hours, no starting material remained by mass spectroscopy. The solvent was removed in vacuo and the residue dissolved in CH$_2$Cl$_2$ (20 mL), washed with water (3 x 10 mL), dried over magnesium sulphate and filtered. The solvent was again removed and the crude residue purified by preparative TLC (8:1:1 chloroform: methanol: isopropylamine, Rf= 0.26) to yield opaque solid (160 mg, 0.285 mmol, 83% yield). m/z = 562 (100%); HRMS (FAB)= calculated for (MH+)
$C_{35}H_{68}N_4O$ 561.5472, found 561.5467; $\nu_{\text{max/cm}^{-1}}$ ($\text{CH}_2\text{Cl}_2$)= 3695, 3602 (br m, N-H), 3330 (br w, OH), 2940, 2860 (s, C-H), 1600 (w, N-H), 1175 (m, C-O), 1115 (w, C-N); $\delta$$_\text{H}$ (CDCl$_3$, 250MHz)= 0.6-2.9 (49H, br m, including [0.65 (3H, s, C$_{18}$-CH$_3$), 0.76 (3H, s, C$_{19}$-CH$_3$), 0.81 (3H, d, J= 6.4Hz, C$_{24}$-CH$_3$), 0.86 (3H, d, J= 6.5Hz, C$_{25}$-CH$_3$), 1.0 (3H, d, J= 6.6Hz, C$_{21}$-CH$_3$), 2.0-2.9 (8H, collapsed m, NCH$_3$'s), 3.2 (1H, m, NCH$_3$), 3.1 (1H, collapsed dd, J= 9.4Hz, CHOH); $\delta$$_\text{C}$ (CDCl$_3$, 62.9MHz)= 11.6 (CH$_3$), 11.9 (CH$_3$), 12.4 (CH$_3$), 20.8 (CH$_3$), 21.2 (CH$_2$), 21.5 (CH$_2$), 23.9 (CH$_2$), 24.5 (CH$_2$), 26.2 (CH$_2$), 28.2 (CH$_2$), 28.8 (CH$_2$), 29.1 (CH$_2$), 29.2 (CH$_2$), 31.6 (CH), 32.3 (CH$_2$), 32.4 (CH$_2$), 33.0 (CH$_2$), 35.9 (CH), 36.5 (CH), 37.6 (CH), 39.3 (CH$_2$), 39.3 (CH), 39.8 (CH), 40.4 (CH$_2$), 40.5 (CH$_2$), 42.9 (C), 46.3 (CH$_2$), 53.0 (CH), 52.8 (CH$_2$), 54.0 (CH$_2$), 55.4 (CH), 55.4 (CH$_2$), 56.9 (CH), 79.8 (CH).
6.6Hz, C^{21}-CH_3]), 2.0-2.9 (18H, collapsed m, NCH_3's & NCH_{ax} & NH's), 3.1 (1H, collapsed dd. J = 9.5Hz, CHO), 6_2_3, 6.29 (18H, collapsed m, NCH_3's & NCH_2's & NH's), 3.1 (1H, collapsed dd. J = 9.5Hz, CHOH), 62.9 (CH_3), 12.9 (CH_3), 12.4 (CH_3), 12.7 (CH_3), 19.4 (CH_3), 20.8 (CH_3), 21.5 (CH_2), 23.8 (CH_2), 24.4 (CH_2), 24.9 (CH_2), 28.1 (CH_2), 28.8 (CH_2), 29.6 (CH_2), 30.0 (CH_2), 30.1 (CH), 32.4 (CH_2), 35.9 (CH), 36.3 (CH_2), 36.3 (C), 37.6 (CH), 37.8 (CH_2), 40.4 (CH_2), 42.4 (CH_2), 42.8 (C), 45.7 (CH), 46.0 (CH_2), 48.8 (CH_2), 50.2 (CH_2), 52.8 (CH_2), 52.9 (CH), 54.0 (CH_2), 54.8 (CH), 55.4 (CH_2), 56.9 (CH), 57.8 (CH), 79.4 (CH).

5.4.17 22(S)-benzoyl-23-methyl-3α-[N^4-(3-aminopropyl)-N^1,N^8-di-(tert-butoxycarbonyl)-spermidine]-cholane [146] and 22(S)-benzoyl-23-methyl-3β-[N^4-(3-aminopropyl)-N^1,N^8-di-(tert-butoxycarbonyl)-spermidine]-cholane [147].

All glassware was oven dried. 22(S)-benzoyl-23-methylcholan-3-one [90] (0.40 g, 0.84 mmol), N^4-(3-aminopropyl)-N^1,N^8-di-(tert-butoxycarbonyl)-spermidine [107] (0.84 g, 2.09 mmol), 2 drops of acetic acid and powdered 3A molecular sieves (M/S) (0.50 g) were dissolved in dry methanol (40 mL) and sealed under a nitrogen atmosphere in a 50 mL RB flask. The solution was stirred for 24 hours, and then NaCNBH₃ (2.51 mL of 1M solution in THF, 2.51 mmol) was added and the reaction stirred for a further 24 hours. At this point, a mass spectrum showed only product and no starting material. The contents of the flask were then filtered through celite and the pad washed with methanol (2 x 8 mL) and dichloromethane (2 x 8 mL). The solvents were removed in vacuo and the residue dissolved in CH_2Cl_2 (50 mL). This was washed with water made slightly basic with 5% NaOH (2 x 20 mL) and brine (2 x 20 mL). The combined aqueous layers were back extracted with CH_2Cl_2 (2 x 20 mL). The organic layers were combined and dried over magnesium sulphate, filtered and the solvent removed in vacuo. This left a crude oil (1.42 g), which was purified by flash chromatography (gradient of 100% chloroform to 8:2:1 chloroform: methanol: isopropylamine). The two compounds could not be separated coming off the column at the same time as clear oil (894 mg). m/z = 866 (M⁺H, 100%), 403 (26%), 105
(BzCO⁺, 46%); \( \nu_{\text{max/cm}^{-1}}(\text{CH}_2\text{Cl}_2) = 3420 \) (br m, N-H), 2960, 2940, 2865 (s, C-H), 1720 (s, C=O, aryl ester), 1660 (br s, C=O, amide), 1608 (w, N-H), 1600, 1500 (w, C=O, aryl), 1275 (s, C=O), 1030 (w, C-N); \( \delta_H (\text{CDCl}_3, 250\text{MHz}) = 0.5-2.4 \text{ppm} \) (66H, br m, including [0.69 (3H, s, C\text{\textsuperscript{18}}-\text{CH}_3), 0.77 (3H, s, C\text{\textsuperscript{19}}-\text{CH}_3), 0.88 (3H, d, J = 6.4 Hz, C\text{\textsuperscript{24}}-\text{CH}_3), 0.94 (3H, d, J = 6.6 Hz, C\text{\textsuperscript{25}}-\text{CH}_3), 1.07 (3H, d, J = 6.6 Hz, C\text{\textsuperscript{21}}-\text{CH}_3), 1.43 (9H, s, C(CH\text{\textsubscript{3}})_3), 1.44 (9H, s, C(CH\text{\textsubscript{3}})_3)], 2.2-3.2 (14H, br m, CH\text{\textsubscript{2}}N, CH\text{\textsubscript{2}}N & NH), 4.93 (1H, collapsed dd, J = 9.8 Hz, CHOBz), 5.2 (1H, br s, NHBOC), 5.7 (1H, br s, NHBOC), 7.4-7.6 (3H, m, Ph CH's), 0.80 (2H, m, Ph CH's); \( \delta_C (\text{CDCl}_3, 62.9\text{MHz}) = 12.1 \) (CH\text{\textsubscript{3}}), 12.6 (CH\text{\textsubscript{3}}), 13.0 (CH\text{\textsubscript{3}}), 19.6 (CH\text{\textsubscript{3}}), 20.1 (CH\text{\textsubscript{3}}), 21.0 (CH\text{\textsubscript{2}}), 21.4 (CH\text{\textsubscript{2}}), 24.4 (CH\text{\textsubscript{2}}), 24.5 (CH\text{\textsubscript{2}}), 27.1 (CH\text{\textsubscript{2}}), 27.2 (CH\text{\textsubscript{2}}), 28.2 (CH\text{\textsubscript{2}}), 29.0 (CH\text{\textsubscript{2}}). 28.7 (CH\text{\textsubscript{3}}), 28.7 (CH\text{\textsubscript{3}}), 32.2 (CH\text{\textsubscript{2}}), 32.8 (CH\text{\textsubscript{2}}), 30.2 (CH), 33.4 (CH\text{\textsubscript{2}}), 35.6 (CH\text{\textsubscript{2}}), 35.7 (CH), 36.4 (C), 37.6 (CH\text{\textsubscript{2}}), 38.1 (CH), 40.3 (CH\text{\textsubscript{2}}), 40.7 (CH), 42.8 (C), 45.5 (CH), 45.9 (CH\text{\textsubscript{2}}), 52.4 (CH\text{\textsubscript{2}}), 52.8 (CH\text{\textsubscript{2}}), 53.1 (CH), 53.8 (CH\text{\textsubscript{2}}), 54.6 (CH), 56.6 (CH), 57.8 (CH), 77.9 (C), 79.0 (C), 82.0 (CH), 128.6 (CH), 129.9 (CH), 131.0 (C), 133.0 (CH), 156.4 (CO), 156.4 (CO), 166.8 (CO).

**5.4.18** (S)-22-benzoyl-23-methyl-3α-[N\text{\textsuperscript{4}}-(3-aminopropyl)]-spermidine]-cholane [148] and (S)-22-benzoyl-23-methyl-3β-[N\text{\textsuperscript{4}}-(3-aminopropyl)]-spermidine]-cholane [149].

TFA (4.6 mL, 61.8 mmol) was added to a solution of [146] and [147] (800 mg, 0.92 mmol) in CH\text{\textsubscript{2}}Cl\text{\textsubscript{2}} (35 mL) at RT. The reaction was stirred for approximately 2 hrs, when no starting material remained by TLC and mass spectrum. The solvent was removed in vacuo and the residue purified by flash chromatography (100% chloroform to 8: 1: 1 chloroform:
Aminosterol [148] came off the column first as a solid $R_f = 0.34$ (130 mg, 0.195 mmol, 21%).

\[\alpha_l = 19.3^\circ \text{ (c=3, CH}_2\text{Cl}_2) ; m/z = 666 \text{ (100\%), } M^{+}H, 480 \text{ (29\%)}; \text{ HRMS (FAB)= calculated for (M}^+H \text{) } C_{42}H_{73}N_{4}O_{2} \text{, found 665.5734; } v_{\text{max/cm}^{-1}} \text{ (CH}_2\text{Cl}_2) = 3390 \text{ (w, N-H), } 2940, 2870 \text{ (s, C-H), } 1712 \text{ (s, C=O, aryl ester); } \delta_{\text{H}} \text{(CDCl}_3, 250MHz)= 0.5-1.75ppm \text{ (48H, br m, including [0.67 (3H, s, C}_{18}-\text{CH}_3), 0.76 (3H, s, C}_{19}-\text{CH}_3), 0.87 (3H, d, J= 6.5Hz, C}_{24}-\text{CH}_3), 0.92 (3H, d, J= 6.6Hz, C}_{25}-\text{CH}_3), 1.05 (3H. d, J= 6.7Hz, C}_{21}-\text{CH}_3), 2.62 (17H, collapsed m, CH}_{2}\text{N's & NH's), 3.2 (1H, m, } CH_{eq}\text{N), 4.9 (1H, collapsed dd, J= 9.6Hz, CHO\text{Bz), 7.4-7.6 (3H, m, Ph CH'}\text{s), 8.0 (2H. m, Ph CH'}\text{s); } \delta_{\text{C}} \text{(CDCl}_3, 62.9MHz) = 11.9 \text{ (CH}_3), 12.2 \text{ (CH}_3), 13.1 \text{ (CH}_3), 19.6 \text{ (CH}_3), 20.1 \text{ (CH}_3), 21.1 \text{ (CH}_2), 24.0 \text{ (CH}_2), 24.5 \text{ (CH}_2), 28.7 \text{ (CH}_2), 28.8 \text{ (CH}_2), 28.9 \text{ (CH}_2), 29.0 \text{ (CH}_2), 29.9 \text{ (CH}_2), 30.3 \text{ (CH), 32.30 \text{ (CH}_2), 32.33 \text{ (CH}_2), 32.9 \text{ (CH}_2), 35.8 \text{ (CH), 36.5 (C), 37.0 (CH}_2), 37.7 \text{ (CH}_2), 38.2 \text{ (CH), 39.3 \text{ (CH), 39.9 (CH), 40.3 \text{ (CH}_2), 42.9 (C), 43.5 \text{ (CH}_2), 43.7 \text{ (CH}_2), 46.0 \text{ (CH}_2), 48.5 \text{ (CH}_2), 53.1 \text{ (CH), 54.7 (CH), 56.8 (CH), 57.7 (CH), 82.2 \text{ (CH), 128.7 \text{ (CH), 130.0 \text{ (CH), 131.2 (C), 133.0 \text{ (CH), 166.7 \text{ (CO).}}

Aminosterol [149] came off the column second as a white solid $R_f = 0.16$ (265 mg, 0.398 mmol, 43%).

\[m/z = 666 \text{ (100\%); HRMS (FAB)= calculated for (M}^+H \text{) } C_{42}H_{73}N_{4}O_{2} \text{, found 665.57119; } v_{\text{max/cm}^{-1}} \text{ (CH}_2\text{Cl}_2) = 3420 \text{ (w, N-H), } 2940, 2870 \text{ (s, C-H), 1712 \text{ (s, C=O, aryl ester), 1115 \text{ (m, C-O); } \delta_{\text{H}} \text{(CD}_3\text{OD, 250MHz)= 0.5-2.1ppm \text{ (48H, br m, including [0.64 \text{ (3H, s, C}_{18}-\text{CH}_3), 0.75 (3H, s, C}_{19}-\text{CH}_3), 0.79 (3H. d, J= 6.5Hz, C}_{24}-\text{CH}_3), 0.86 (3H, d, J= 6.5Hz, C}_{25}-\text{CH}_3), 1.0 (3H. d, J= 6.6Hz, C}_{21}-\text{CH}_3)), 2.2-3.2 \text{ (18H, collapsed m, CH}_{2}\text{N & CH}_{ax\text{N & NH's), 4.92 (1H, collapsed dd, J= 9.8Hz, CHO\text{Bz), 7.3-7.6 (3H, m, Ph CH'}\text{s), 8.0 (2H. m, Ph CH'}\text{s); } \delta_{\text{C}} \text{(CD}_3\text{OD, 62.9MHz) = 12.1 \text{ (CH}_3), 12.5 \text{ (CH}_3), 13.0 \text{ (CH}_3), 19.6 \text{ (CH}_3), 20.0 \text{ (CH}_3), 21.6 \text{ (CH}_2), 23.9 \text{ (CH}_2), 24.0 \text{ (CH}_2), 24.1 \text{ (CH}_2), 24.3 \text{ (CH}_2), 27.9 \text{ (CH}_2), 28.7 \text{ (CH}_2), 30.2 \text{ (CH), 31.1 \text{ (CH}_2), 31.7 \text{ (CH}_2), 32.5 \text{ (CH}_2), 35.6 \text{ (CH), 35.9 (C), 36.7 \text{ (CH}_2), 36.8 \text{ (CH}_2), 37.0 \text{ (CH}_2), 38.0 \text{ (CH), 39.6 \text{ (CH}_2), 40.1 \text{ (CH}_2), 40.2 \text{ (CH}_2), 40.9 \text{ (CH}_2), 42.0 \text{ (CH}_2), 42.7 (C), 43.4 \text{ (CH), 45.1 \text{ (CH), 53.0 \text{ (CH), 54.1 \text{ (CH), 56.5 \text{ (CH), 57.7 \text{ (CH), 82.0 (CH), 128.6 (CH), 129.9 (CH), 131.0 (C), 133.0 (CH), 166.6 (CO).}}

Chapter 5. Experimental.
5.4.19 (S)-22-hydroxyl-23-methyl-3α-[N⁴-(3-aminopropyl)-spermidine]-cholane [150].

A stirred solution of (S)-22-benzoyl-23-methyl-3α-[N⁴-(3-aminopropyl)-spermidine]-cholane [148] (100 mg, 0.15 mmol) and KOH (83 mg, 1.5 mmol) in dry methanol (8 mL) was refluxed (60 °C) under a nitrogen atmosphere. After 72 hours, no starting material remained by mass spectroscopy. The solvent was removed in vacuo and the residue dissolved in CH₂Cl₂ (20 mL), washed with water (3 x 10 mL), dried over magnesium sulphate and filtered. The solvent was again removed and the crude residue purified by preparative TLC (8:1:1 chloroform: methanol: isopropylamine, Rf= 0.4) to yield an opaque solid (60 mg, 0.11 mmol, 73% yield).

m/z (FAB)= 562 (100%); ν max/cm⁻¹ (CH₂Cl₂)= 3611 (br m, N-H), 3325 (br w, OH), 2940, 2860 (s, C-H), 1600 (w, N-H), 1175 (m, C-O), 1115 (w, C-N); δH(CDCl₃, 250MHz)= 0.5-2.1 (58H, br m, including [0.66 (3H, s, C₁₈-CH₃), 0.78 (3H, s, C₁⁹-CH₃), 0.80 (3H, d, J= 6.5Hz, C₂⁴-CH₃), 0.85 (3H, d, J= 6.5Hz, C₂⁵-CH₃)], 1.1 (3H, d, J= 6.4Hz, C₂¹-CH₃)], 2.0-2.9 (17H, collapsed m, NCH₂'s & NH's), 3.2 (1H, m, NCH₂eq), 3.1 (1H, collapsed dd, J= 9.4Hz, CHO); δC (CDCl₃, 62.9MHz)= 11.9 (CH₃), 12.2 (CH₃), 13.1 (CH₃), 19.4 (CH₃), 19.7 (CH₃), 21.2 (CH₂), 23.9 (CH₂), 24.5 (CH₂), 26.2 (CH₂), 28.2 (CH₂), 28.9 (CH₂), 29.1 (CH₂), 30.1 (CH₂), 31.6 (CH), 32.3 (CH₂), 32.4 (CH₂), 33.0 (CH₂), 35.9 (CH), 36.5 (C), 37.0 (CH₂), 37.6 (CH), 37.7 (CH₂), 39.3 (CH), 39.8 (CH), 40.4 (CH₂), 42.9 (C), 43.5 (CH₂), 43.7 (CH₂), 46.3 (CH₂), 49.0 (CH₂), 53.1 (CH), 55.4 (CH), 56.9 (CH), 82.2 (CH).
5.4.20 (S)-22-hydroxyl-23-methyl-3β-[N⁴-(3-aminopropyl)-spermidine]-cholane [151].

A stirred solution of (S)-22-benzoyl-23-methyl-3β-[N⁴-(3-aminopropyl)-spermidine]-cholane [149] (158 mg, 0.237 mmol) and KOH (132 mg, 2.4 mmol) in dry methanol (10 mL) was refluxed (60 °C) under a nitrogen atmosphere. After 72 hours, no starting material remained by mass spectroscopy. The solvent was removed in vacuo and the residue dissolved in CH₂Cl₂ (20 mL), washed with water (3 x 10 mL), dried over magnesium sulphate and filtered. The solvent was again removed and the crude residue purified by preparative TLC (8:1:1 chloroform: methanol: isopropylamine, Rf = 0.32) to yield an opaque solid (80 mg, 0.14 mmol, 59% yield).

\[ m/z = 562 \text{(100%); } v_{\text{max}}/\text{cm}^{-1} \text{ (CH}_2\text{Cl}_2) = 3695, 3602 \text{ (br m, N-H), 3330 (br w, OH), 2940, 2860 (s, C-H), 1600 (w, N-H), 1175 (m, C-O), 1115 (w, C-N); } \delta_H \text{ (CDCl}_3, 250 \text{MHz}) = 0.6-2.9 \text{ (49H, br m, including [0.66 (3H, s, C}^{18}\text{-CH}_3), 0.76 (3H, s, C}^{19}\text{-CH}_3), 0.82 (3H, d, J = 6.5Hz, C}^{24}\text{-CH}_3), 0.86 (3H, d, J = 6.6Hz, C}^{25}\text{-CH}_3), 1.0 (3H, d, J = 6.6Hz, C}^{21}\text{-CH}_3)], 2.0-2.9 \text{ (18H, collapsed m, NCH}_2\text{'s & NCH}_ax \text{ & NH's), 3.1 (1H, collapsed dd, J = 9.5Hz, CHOH); } \delta_C \text{ (CDCl}_3, 62.9 \text{MHz}) = 11.6 (\text{CH}_3), 12.3 (\text{CH}_3), 12.7 (\text{CH}_3), 19.4 (\text{CH}_3), 20.0 (\text{CH}_3), 21.5 (\text{CH}_2), 23.8 (\text{CH}_2), 24.0 (\text{CH}_2), 24.1 (\text{CH}_2), 24.5 (\text{CH}_2), 28.2 (\text{CH}_2), 28.9 (\text{CH}_2), 31.1 (\text{CH}_2), 31.6 (\text{CH}), 31.7 (\text{CH}_2), 32.4 (\text{CH}_2), 35.9 (\text{CH}), 36.3 (\text{CH}_2), 36.4 (\text{CH}_2), 36.3 (\text{C}), 37.0 (\text{CH}_2), 37.6 (\text{CH}), 37.8 (\text{CH}_2), 40.2 (\text{CH}_2), 41.0 (\text{CH}_2), 42.4 (\text{CH}_2), 42.8 (\text{C}), 43.4 (\text{CH}_2), 45.7 (\text{CH}), 46.0 (\text{CH}_2), 53.0 (\text{CH}), 54.8 (\text{CH}), 56.9 (\text{CH}), 57.9 (\text{CH}), 79.7 (\text{CH}).]
5.5 Synthesis of Steroids with a CIS A-B Ring Junction.

5.5.1 (R)-24-ethyl-5.22-cholestadien-3-one [91].

To a cooled (10-15°C) solution of stigmasterol [26] (200 mg, 95% purity, 0.46 mmol) in dry acetone (25 mL) in a 50 mL 3-necked flask was added rapidly, with stirring, from a burette Jones Reagent (0.17 mL, 0.46 mmol CrO3). Nitrogen gas was bubbled through all the solvents, reagents and reaction solution before and during the oxidation. After 4 minutes, the solution had changed from orange/yellow to green/blue. The reaction was quenched by the addition of water (127 mL) giving a cream precipitate, which was filtered and washed well with water to give a white solid. TLC (20% ethyl acetate in petrol, Rf= 0.46) showed a mixture of products. Flash chromatography was used to purify the compounds (gradient of 0-20% ethyl acetate in petrol) giving a white powder (111 mg, 0.27 mmol, 62% yield).

*Jones’s Reagent was taken from a solution made up of chromium trioxide (26.72 g) in concentrated sulphuric acid (23 mL) made up to a volume of 100 mL with water. This was used throughout.

This was done to prevent any atmospheric oxygen entering the system as it has previously been reported that Δ^3-ketones can yield hydroperoxides.

\[ \delta_H (CDCl_3, 301 MHz) = \begin{array}{c} 0.7 \ (3H, s, C^{18}-CH_3), \\
0.7-2.1 \ (39H, br m, including [0.76 \ (3H, d, J=5.8Hz, C^{26}-CH_3), \\
0.78 \ (3H, t, J=7.3Hz, C^{29}-CH_3), \\
0.80 \ (3H, d, J=6.4Hz, C^{27}-CH_3), \\
1.0 \ (3H, d, J=6.7Hz, C^{21}-CH_3), \\
1.5 \ (3H, s, C^{19}-CH_3) \end{array} ] \]

\[ \begin{array}{c} 2.27 \ (1H, m, CH), \\
2.48 \ (1H, m, CH), \\
2.8 \ (1H, dd, J=16.4Hz, 1.85Hz, CH), \\
3.3 \ (1H, dd, J=18.7Hz, 2.34Hz, CH), \\
4.9-5.2 \ (2H, m, CH=CH) \end{array} ] \]

\[ \delta_C (CDCl_3, 75.8 MHz) = \begin{array}{c} 12.5 \ (CH_3), \\
12.7 \ (CH_3), \\
19.4 \ (CH_3), \\
19.6 \ (CH_3), \\
21.5 \ (CH_3), \\
21.6 \ (CH_3), \\
21.7 \ (CH_2), \\
24.7 \ (CH_2), \\
25.8 \ (CH_2), \\
29.3 \ (CH_2), \\
30.1 \ (C), \\
32.2 \ (CH_2), \\
32.3 \ (CH), \\
37.3 \ (CH), \\
37.3 \ (CH_2), \\
38.0 \ (CH_2), \\
40.0 \ (CH_2), \\
40.9 \ (CH), \\
42.6 \ (C), \\
48.7 \ (CH_2), \\
49.6 \ (CH), \\
51.6 \ (CH), \\
56.3 \ (CH), \\
57.1 \ (CH), \\
123.3 \ (CH), \\
129.7 \ (CH), \\
138.6 \ (CH), \\
138.9 \ (C), \\
210.6 \ (CO) \end{array} ] \]
5.5.2 (R)-24-ethyl-4,22-cholestadien-3-one [92].

(R)-24-ethyl-5,22-cholestadien-3-one [91] (100 mg, 0.24 mmol) was dissolved in warm methanol (20 mL). Three drops of 10% potassium hydroxide were added and the solution heated on a steam bath for 5 minutes, after which time no starting material remained by TLC (20% ethyl acetate in petrol, R<sub>t</sub> = 0.40). The reaction was neutralised with acetic acid, the solvent removed in vacuo and the crude product purified by flash chromatography (0-20% ethyl acetate in petrol) to give white solid which was recrystallized from methanol (86 g, 0.21 mmol, 87% yield). δ<sub>H</sub> (CDCl<sub>3</sub>, 250 MHz) = 0.6-2.5 (43H, br m, including 0.7 (3H, s, C<sub>18</sub>-CH<sub>3</sub>), 0.71-0.85 (9H, m, C<sup>26</sup>-d), C<sup>27</sup>-d), C<sup>28</sup>-t) CH<sub>3</sub>'s), 0.98 (3H, d, J = 6.7 Hz, C<sub>21</sub>-CH<sub>3</sub>), 1.2 (3H, s, C<sup>19</sup>-CH<sub>3</sub>), 4.9-5.2 (2H, m, CH=CH), 5.7 (1H, s, CH=C); δ<sub>C</sub> (CDCl<sub>3</sub>, 62.9 MHz) = 12.5 (CH<sub>3</sub>), 12.6 (CH<sub>3</sub>), 17.7 (CH<sub>3</sub>), 19.4 (CH<sub>3</sub>), 21.4 (CH<sub>2</sub>), 21.5 (CH<sub>3</sub>), 21.5 (CH<sub>3</sub>), 24.6 (CH<sub>2</sub>), 25.8 (CH<sub>2</sub>), 29.2 (CH<sub>2</sub>), 32.2 (CH), 32.4 (CH<sub>2</sub>), 33.3 (CH<sub>2</sub>), 34.3 (CH<sub>2</sub>), 36.0 (CH), 36.1 (CH<sub>2</sub>), 39.0 (CH<sub>2</sub>), 39.9 (C), 40.8 (CH), 42.6 (C), 51.6 (CH), 54.2 (CH), 56.3 (CH), 56.4(CH), 124.1(CH), 129.8 (CH), 138.5 (CH), 172.0 (C), 199.9 (CO).

5.5.3 (R)-24-ethyl-βS5H-cholest-22-ene-3-one [93].

A solution of (R)-24-ethyl-4,22-cholestadien-3-one [92] (86 mg, 0.21 mmol), KOH (17.5 mg) and 5% Pd-C catalyst (5 mg) in isopropyl alcohol (5 mL) was hydrogenated at atmospheric pressure for 16 hours. By TLC it appeared that the reaction would not go to completion (20% ethyl acetate in ether, R<sub>t</sub> = 0.76 and 0.41). The solution was neutralised with acetic acid, then filtered through a pad of celite to remove the catalyst. The solvent
was removed in vacuo and the crude yellow solid purified by flash chromatography (0-20% ethyl acetate in petrol) to yield the product as white crystals (37 mg, 0.09 mmol, 41% yield). The more polar compound was collected (5 mg) but could not be identified, it was not starting material.

\[ \text{m.p.} = 99-101^\circ\text{C}; \delta_{\text{H}}(\text{CDCl}_3, 250\text{MHz})= 0.6-2.4 (\text{H, br m, including [0.7 (3H, s, C}^{18}\text{-CH}_3), 0.75-0.85 (9H, m, C}^{26}\text{ (d), C}^{27}\text{ (d) and C}^{29}\text{ (t) CH}_3\text{'s), 1.01 (3H, d, J= 6.7Hz, C}^{21}\text{-CH}_3), 1.03 (3H, s, C}^{19}\text{-CH}_3), 2.7 (1H, t, 13.8Hz, CH), 4.9-5.2 (2H, m, CH=CH); }\]

\[ \delta_{\text{C}}(\text{CDCl}_3, 62.9\text{MHz})= 12.6 (\text{CH}_3), 19.4 (\text{CH}_3), 21.5 (\text{CH}_3), 21.59 (\text{CH}_2), 21.6 (\text{CH}_3), 23.1 (\text{CH}_3), 24.7 (\text{CH}_2), 25.8 (\text{CH}_2), 26.2 (\text{CH}_2), 27.0 (\text{CH}_2), 29.4 (\text{CH}_2), 32.3 (\text{CH}_3), 33.3 (\text{C}), 35.9 (\text{CH}), 37.4 (\text{CH}_2), 37.6 (\text{CH}_2), 40.4 (\text{CH}_2), 40.9 (\text{CH}), 41.2 (\text{CH}), 42.7 (\text{CH}_2), 43.0 (\text{C}), 44.7 (\text{CH}), 51.6 (\text{CH}), 56.5 (\text{CH}), 57.0 (\text{CH}), 129.8 (\text{CH}), 138.6 (\text{CH}), 213.5 (\text{CO}).\]

5.5.4 (S)-22-benzoyl-23-methylcholan-5-ene-3-one [94].

To a cooled (10-15°C) solution of (S)-22-benzoyl-23-methylcholan-5-ene-3β-ol [88] (250 mg, 0.52 mmol) in dry acetone (30 mL) in a 50 mL 3-necked flask was added rapidly, with stirring, from a burette Jones Reagent (0.22 mL, 0.57 mmol CrO₃).* Nitrogen gas was bubbled through all the solvents, reagents and reaction solution before and during the oxidation. After 3 minutes, the solution had changed from orange/ yellow to green/ blue. The reaction was quenched by the addition of water (160 mL) to give a cream precipitate. The solution was filtered and the solid washed well with water yielding a crude white powder (240 mg). This was purified by flash chromatography (0-20% ethyl acetate in petrol, in 20% ethyl acetate Rf= 0.33) to give white foam (163 mg, 0.34 mmol, 65% yield).

*Jones's Reagent was taken from a solution made up of chromium trioxide (26.72 g) in concentrated sulphuric acid (23 mL) made up to a volume of 100 mL with water. This was used throughout.

*This was done to prevent any atmospheric oxygen entering the system as it has previously been reported that Δ²-3-ketones can yield hydroperoxides.
\[ \delta_H (\text{CDCl}_3, 250\text{MHz}) = 0.6-2.5 \ (\text{H, br m, including} [0.7 \ (3\text{H, s, C}^{18}-\text{CH}_3), 0.81 \ (3\text{H, d, } J=6.7\text{Hz, C}^{24}-\text{CH}_3), 0.89 \ (3\text{H, d, } J=6.7\text{Hz, C}^{25}-\text{CH}_3), 1.05 \ (3\text{H, d, } J=6.7\text{Hz, C}^{21}-\text{CH}_3), 1.1 \ (3\text{H, s, C}^{19}-\text{CH}_3)], 2.7 \ (1\text{H, collapsed dd, } J=16.1\text{Hz, CH}), 3.2 \ (1\text{H, collapsed dd, } J=15.6\text{Hz, CH}), 4.9 \ (1\text{H, collapsed dd, } J=9.6\text{Hz, CHO}Bz), 5.2 \ (1\text{H, m, C=CH}), 7.4 \ (3\text{H, m, Ph CH's}), 8.0 \ (2\text{H, m, Ph CH's}); \delta_C (\text{CDCl}_3, 62.9\text{MHz}) = 12.0 \ (\text{CH}_3), 13.1 \ (\text{CH}_3), 19.4 \ (\text{CH}_3), 19.6 \ (\text{CH}_3), 20.1 \ (\text{CH}_3), 21.6 \ (\text{CH}_2), 24.5 \ (\text{CH}_2), 28.7 \ (\text{CH}_2), 30.2 \ (\text{CH}), 32.0 \ (\text{CH}_2), 32.2 \ (\text{CH}), 37.1 \ (\text{CH}_2), 37.2 \ (\text{C}), 37.8 \ (\text{CH}_2), 38.1 \ (\text{CH}), 39.9 \ (\text{CH}_2), 42.6 \ (\text{C}), 48.5 \ (\text{CH}_2), 49.3 \ (\text{CH}), 53.0 \ (\text{CH}), 56.7 \ (\text{CH}), 81.9 \ (\text{CH}), 123.0 \ (\text{CH}), 128.7 \ (\text{CH}), 129.9 \ (\text{CH}), 131.0 \ (\text{C}), 133.0 \ (\text{CH}), 138.8 \ (\text{C}), 166.4 \ (\text{CO}), 209.7 \ (\text{CO}). \]

5.5.5 (S)-22-benzoyl-23-methylcholan-4-ene-3-one [95].

Compound [94] (100 mg, 0.21 mmol) was dissolved in warm methanol (20 mL). Three drops of 10% potassium hydroxide were added and the solution heated on a steam bath for 5 minutes. It was then neutralised with acetic acid and the solvent removed in vacuo. This was purified by flash chromatography (0-20% ethyl acetate in petrol) to give white solid, which was recrystallized from methanol (95 mg, 0.20 mmol, 94% yield). \( \delta_H (\text{CDCl}_3, 250\text{MHz}) = 0.6-2.5 \ (36\text{H, br m, including} [0.77 \ (3\text{H, s, C}^{18}-\text{CH}_3), 0.90 \ (3\text{H, d, } J=6.6\text{Hz, C}^{24}-\text{CH}_3), 0.96 \ (3\text{H, d, } J=6.7\text{Hz, C}^{25}-\text{CH}_3), 1.13 \ (3\text{H, d, } J=6.6\text{Hz, C}^{21}-\text{CH}_3), 1.1 \ (3\text{H, s, C}^{19}-\text{CH}_3)], 2.8 \ (1\text{H, collapsed dd, } J=16.1\text{Hz, CH}), 3.3 \ (1\text{H, collapsed dd, } J=15.5\text{Hz, CH}), 4.95 \ (1\text{H, collapsed dd, } J=9.5\text{Hz, CHO}Bz), 6.15 \ (1\text{H, s, CH=C}), 7.5 \ (3\text{H, m, Ph CH's}), 8.1 \ (2\text{H, m, Ph CH's}); \delta_C (\text{CDCl}_3, 62.9\text{MHz}) = 12.0 \ (\text{CH}_3), 13.1 \ (\text{CH}_3), 17.9 \ (\text{CH}_3), 19.7 \ (\text{CH}_3), 20.1 \ (\text{CH}_3), 21.2 \ (\text{CH}_2), 24.3 \ (\text{CH}_2), 28.6 \ (\text{CH}_2), 30.3 \ (\text{CH}), 34.3 \ (\text{CH}_2), 34.5 \ (\text{CH}), 35.8 \ (\text{CH}_2), 38.1 \ (\text{CH}), 39.4 \ (\text{CH}_2), 39.9 \ (\text{CH}_2), 40.1 \ (\text{C}), 42.8 \ (\text{C}), 47.0 \ (\text{CH}_2), 51.2 \ (\text{CH}), 52.9 \ (\text{CH}), 56.7 \ (\text{CH}), 81.9 \ (\text{CH}), 125.8 \ (\text{CH}), 128.8 \ (\text{CH}), 130.0 \ (\text{CH}), 131.0 \ (\text{C}), 133.2 \ (\text{CH}), 161.3 \ (\text{C}), 166.7 \ (\text{CO}), 199.7 \ (\text{CO}). \)
5.5.6 Unidentified compound isolated. (S)-22-benzoyl-23-methylcholan-3-one [96].

A solution of (S)-22-benzoyl-23-methylcholan-4-ene-3-one [95] (80 mg, 0.17 mmol), KOH (16 mg) and 5% Pd-C catalyst (5 mg) in isopropyl alcohol (5 mL) was hydrogenated at atmospheric pressure for 16 hours. The solution was neutralised with acetic acid, then filtered through a pad of celite to remove the catalyst. The solvent was removed *in vacuo* and the crude yellow solid purified by flash chromatography (0-20% ethyl acetate in petrol) to yield the product as white solid (43 mg, 0.09 mmol, 54% yield). A major product was formed but could not be identified to satisfaction. Data is below.

$$\delta_H (\text{CDCl}_3, 250 \text{MHz}) = 0.6-2.5 \text{ (38H, br m, including [0.69 (3H, s, C}^{18}\text{-CH}_3), 0.85 (3H, d, J= 6.7 \text{Hz, C}^{24}\text{-CH}_3), 0.94 (3H, d, J= 6.7 \text{Hz, C}^{25}\text{-CH}_3), 1.03 (3H, d, J= 6.6 \text{Hz, C}^{21}\text{-CH}_3), 1.1 \text{ (3H, s, C}^{19}\text{-CH}_3)], 4.85 \text{ (1H, collapsed dd, J= 9.6 Hz, CHOBz), 7.4 \text{ (3H, m, Ph CH's), 8.0 \text{ (2H, m, Ph CH's); } \delta_C (\text{CDCl}_3, 62.9 \text{MHz}) = 12.1 \text{ (CH}_3), 13.1 \text{ (CH}_3), 19.6 \text{ (CH}_3), 19.8 \text{ (CH}_3), 20.1 \text{ (CH}_3), 21.6 \text{ (CH}_2), 24.2 \text{ (CH}_2), 28.6 \text{ (CH}_2), 30.2 \text{ (CH), 32.0 (C), 37.2 (CH}_2), 37.6 \text{ (CH}_2), 38.1 \text{ (CH), 38.3 (CH), 39.0 (CH}_2), 39.7 \text{ (CH}_2), 41.4 \text{ (CH}_2), 43.2 \text{ (C), 46.7 (CH}_2), 53.0 \text{ (CH), 53.6 (CH), 56.7 (CH), 57.6 (CH), 81.9 (CH), 128.8 (CH), 130.0 (CH), 131.0 \text{ (C), 133.1 (CH), 166.6 (CO), 209.2 (CO).}}$$
5.6 Synthesis of Protected Polyamines.

5.6.1 $N$-(cyanoethyl)-1,4-butylldiamine [99] and $N^1,N^4$-(dicyanoethyl)-1,4-butylldiamine [99b]. Beilstein Number [99] 1854797, Beilstein Number [99b] 1705938.

A solution of acrylonitrile (11.0 g, 208 mmol) and dry methanol (6.0 mL) was slowly added to a solution of 1,4-diaminobutane (18.27 g, 208 mmol) in methanol (6.0 mL) at 0°C. The reaction was kept at 0°C and magnetically stirred for 12 hours. The solvent was evaporated in vacuo leaving crude yellow oil (30.36 g), three spots by TLC. This was separated by flash chromatography (15: 5: 1 CH$_3$Cl: CH$_3$OH: PrNH$_2$). The reaction mass was split into three for chromatography due to column size.

The undesired by-product [99b] (Rf= 0.81 in 15: 5: 1 CH$_3$Cl: CH$_3$OH: PrNH$_2$) where both amines have reacted was collected first as clear oil (12.12 g, 62.4 mmol, 30%). m/z (C.I.)= 195 (M$^+$H, 100%), 154 (10%), 142 (55%), 125 (13%); $\nu_{\text{max}}$/cm$^{-1}$ (CH$_2$Cl$_2$)= 3446 (w, N-H), 2949 (m, C-H), 1500 (m, C-N); $\delta_{\text{H}}$ (250MHz)= 1.1 ppm (2H, quin, J= 2.8Hz, CH$_2$CH$_2$CH$_2$), 1.3 (1H, br s, NH), 2.1 (2H, J= 6.8Hz, CH$_2$NH), 2.2 (2H, J= 6.3Hz, CH$_2$NH), 2.5 (2H, J= 6.6Hz, CH$_2$CN); $\delta_{\text{C}}$ (62.9MHz)= 18.1 (CH$_2$), 27.6 (CH$_2$), 45.1 (CH$_2$), 48.9 (CH$_2$), 119.5 (CN).

The desired product [99] (Rf= 0.38 in 15:5:1 CH$_3$Cl: CH$_3$OH: PrNH$_2$) was collected as clear oil (16.66 g, 117.97 mmol, 57%). m/z (C.I.)= 142 (M$^+$, 100%), 125 (10%), 101 (7%), 84 (4%), 72 (8%), 56 (3%); $\nu_{\text{max}}$/cm$^{-1}$ (CH$_2$Cl$_2$)= 3446 (w, N-H), 2949 (m, C-H), 1500 (m, C-N); $\delta_{\text{H}}$ (250MHz)= 0.79 (4H, m, CH$_2$CH$_2$CH$_2$CH$_2$), 1.85 (6H, m, CH$_3$NHR), 2.15 (2H, J= 6.6Hz, CH$_2$CN), 2.25 (3H, s, NH$_3$); $\delta_{\text{C}}$ (62.9MHz)= 17.5 (CH$_2$), 27.0 (CH$_2$), 30.6 (CH$_2$), 41.4 (CH$_2$), 45.0 (CH$_2$), 48.8 (CH$_2$), 119.4 (CN).

The last compound off the column is unreacted 1,4-diaminobutane.
5.6.2 \(N^1\)-cyanoethyl-\(N^1, N^4\)-Di-(tert-butoxycarbonyl)-1,4-butylidiamine [100].

Beilstein Number 2005430.

To a solution of [99] (12.00 g, 85.0 mmol) in dichloromethane (500 mL) was added di-tert-butyl dicarbonate (37.10 g, 170 mmol, Mr= 218.25) dissolved in dichloromethane (30 mL). The reaction mixture was stirred at room temperature for 24 hours, by which time no starting material remained by TLC (100% EtOAc). The reaction was concentrated in vacuo giving crude yellow oil (26.86 g), which was purified by flash chromatography (100% EtOAc. Rf= 0.75). Yellow oil [100] (24.81 g, 72.8 mmol, 86%) was collected. \(m/z\) (E.I.)= 364 (M+Na, 100%), 342 (M+H, 10%), 241 (15%), 168 (50%), 123 (50%), 85 (98%), 57 (90%); \(\nu_{\text{max/cm}}^\text{1} (\text{CH}_2\text{Cl}_2)= 3446 (\text{w, N-H}), 2949 (\text{m, C-H}), 1690 (\text{s, C=O}), 1500 (\text{m, C-N}); \delta_H (250MHz. CD_2OD)= 1.3ppm (9H, s, C(Me)_3), 1.4 (9H, s, C(Me)_3), 1.42 (4H, m, CH_2CH_2CH_2CH_2), 2.4 (2H, t, J= 6.6Hz, CH_2CN) 2.8 (2H, t, J= 6.6Hz, CH_2N), 3.2 (2H, t, J= 6.9Hz, CH_2N), 3.4 (2H, t, J= 6.9Hz, CH_2N). 4.9 (1H, s, NH); \delta_C (62.9MHz, CD_2OD)= 18.9 (CH_2), 27.5 (CH_2), 27.6 (CH_2), 28.0 (CH_2), 28.6 (Me), 28.7 (Me), 45.3 (CH_2), 49.0 (CH_2), 119.1 (CN), 156.8 (C=O), 157.2 (C=O).

5.6.3 \(N^1, N^4\)-Di-(tert-butoxycarbonyl)-spermidine [101].

Beilstein Number 2059741.

To a solution of nitrile [100] (24.5 g, 71.8 mmol) in ethanol (600 mL, 95%) was added Raney nickel (5 g) and sodium hydroxide (8.0 g). The mixture was hydrogenated at room temperature and atmospheric pressure for 24 hours. At this time, no starting material remained by TLC (100% EtOAc). The catalyst was removed by filtration through celite, the cake being washed with ethanol. The filtrate was then reduced in vacuo to yield a crude oil. This was dissolved in dichloromethane (300 mL) and washed with NaOH (2x100 mL, 2M) followed by brine (100 mL). The organic layer was then dried over anhydrous magnesium sulphate, and the solvent removed in vacuo. Compound [101], yellow oil (18.06 g, 52.3 mmol, 73%), showed only one spot Rf= 0.56 by TLC (15: 5: 1 CH_3Cl: CH_3OH: 'PrNH_2), and was therefore used in the next reaction without further purification.
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$m/z$ (E.I.) = 346 (M$^+$H, 100%), 159 (20%), 114 (30%), 82 (25%), 57 (84%); $\nu_{\text{max}}$/cm$^{-1}$ (CH$_2$Cl$_2$) = 3440 (w, N-H), 2910 (m, C-H), 1695 (s, C=O), 1490 (m, C-N); $\delta_{\text{H}}$ (250MHz) = 1.40 ppm (9H, s, C(Me)$_3$), 1.42 (9H, s, C(Me)$_3$), 1.43 (6H, m, CH$_2$CH$_2$CH$_2$ CH$_2$ and CH$_2$CH$_2$CH$_2$), 1.7 (2H, t, $J$ = 6.6Hz, CH$_2$NH$_2$), 2.7 (2H, t, $J$ = 6.6Hz, CH$_2$N), 3.2 (6H, m, CH$_2$NRBOC), 4.4 (2H, s, NH$_2$), 5.4 (1H, s, NH); $\delta_{\text{C}}$ (62.9MHz, CD$_2$OD) = 25.9 (CH$_2$), 27.5 (CH$_2$), 28.6 (Me), 28.7 (Me), 29.8 (CH$_2$), 38.5 (CH$_2$), 40.3 (CH$_2$), 44.2 (CH$_2$), 46.9 (CH$_2$), 78.8 (C), 79.6 (C), 155.9 (C=O), 156.8 (C=O).

5.6.4 N$^8$-cyanoethyl-N$^1$, N$^4$-Di-(tert-butoxycarbonyl)-spermidine [102].

Beilstein Number 4205681.

To a solution of [101] (12.11 g, 35.1 mmol) in methanol (300 mL) was added acrylonitrile (1.855 g, 2.30 mL, 35.1 mmol). The reaction was stirred at room temperature for 18 hours, after which no starting material remained by TLC (15: 5: 1 CH$_3$Cl: CH$_3$OH: PrNH$_2$). The solvent was then removed in vacuo and the crude oil dissolved in dichloromethane, (3 x 100 mL), which was also then removed in vacuo with any excess acrylonitrile. The desired product [102] was obtained as yellow oil (12.94 g, 32.5 mmol, 93%) and was one spot $R_f$ = 0.89 by TLC (15: 5: 1 CH$_3$Cl: CH$_3$OH: PrNH$_2$). $m/z$ (C.I.) = 421 (M$^+$Na, 11%), 399 (M$^+$H, 60%), 346 (100%); $\nu_{\text{max}}$/cm$^{-1}$ (CH$_2$Cl$_2$) = 3450 (w, N-H), 2950 (m, C-H), 1700 (s, C=O), 1500 (m, C-N); $\delta_{\text{H}}$ (250MHz) = 1.44 ppm (9H, s, C(Me)$_3$), 1.45 (9H, s, C(Me)$_3$), 1.46 (4H, m, CH$_2$CH$_2$CH$_2$CH$_2$), 1.69 (2H, quin, $J$ = 6.9Hz, CH$_2$CH$_2$CH$_2$), 1.92 (1H, br s, NH), 2.5 (2H, t, $J$ = 6.7Hz, CH$_2$CN), 2.6 (2H, t, $J$ = 6.7Hz, CH$_2$N), 2.9 (2H, t, $J$ = 6.6Hz, CH$_2$N), 3.2 (6H, m, CH$_3$NRBOC), 4.7 (1H, br s, NH); $\delta_{\text{C}}$ (62.9MHz, CD$_2$OD) = 19.0 (CH$_2$), 26.0 (CH$_2$), 27.7 (CH$_2$), 28.75 (Me), 28.79 (Me), 39.8 (CH$_2$), 40.5 (CH$_2$), 44.5 (CH$_2$), 45.5 (CH$_2$), 46.5 (CH$_2$), 47.0 (CH$_2$), 79.3 (C), 79.7 (C), 119.1 (CN), 156.0 (C=O), 156.4 (C=O).

5.6.5 N$^8$-cyanoethyl-N$^1$, N$^4$, N$^8$-Tri-(tert-butoxycarbonyl)-spermidine [103].

Beilstein Number 4216562.
To a solution of nitrile [102] (12.94 g, 32.5 mmol) in dichloromethane (300 mL) was added di-tert-butyl dicarbonate (14.17 g, 65 mmol). The mixture was stirred at room temperature for 24 hours after which time no starting material remained by TLC (15: 5: 1 CH₃Cl: CH₃OH: 'PrNH₂). The mixture was then concentrated in vacuo; the oil dissolved in diethyl ether (200 mL), washed with NaOH (2 x 75 mL, 2M), brine (75 mL) and dried over sodium sulphate. The desired product [103] (14.14 g, 28.3 mmol, 87%) was collected as colourless oil and gave one spot Rf= 0.35 by TLC (20% EtOAc in petrol), m/z (C.I.) = 522 (M⁻Na, 100%), 499 (M⁺H, 5%), 475 (50%), 312 (48%); ν max/cm⁻¹ (CH₂Cl₂) = 3450 (w, N-H), 2950 (m, C-H), 1700 (s, C=O), 1500 (m, C-N); δH (250Hz) = 1.44 ppm (9H, s, C(Me)₃), 1.50 (9H, s, C(Me)₃), 1.56 (9H, s, C(Me)₃), 1.60 (4H, m, CH₂CH₂CH₂CH₂), 1.75 (2H, quin, J= 6.9Hz, CH₂CH₂CH₂), 2.6 (2H, t, J= 6.6Hz, CH₃CN), 3.15 (6H, m, CH₂NRBOC), 3.25 (2H, t, J= 6.6Hz, CH₃NHBOC), 4.7 (1H, br s, NH); δC (62.9MHz, CD₃OD) = 19.8 (CH₂), 26.1 (CH₂), 27.7 (Me), 28.0 (CH₂), 28.75 (Me), 28.80 (Me), 40.1 (CH₂), 40.5 (CH₂), 41.7 (CH₂), 42.3 (CH₂), 45.0 (CH₂), 47.1 (CH₂), 79.3 (C), 79.8 (C), 80.9 (C), 119.0 (CN), 147.1 (C=O), 155.8 (C=O), 156.4 (C=O).

5.6.6 N¹,N⁴,N⁸ - Tri-(tert-butoxycarbonyl)-thermospermine [104].

Beilstein Number 4215033.

To a solution of nitrile [103] (13.64g, 27.4mmol) in ethanol (300mL, 95%), was added Raney nickel (5g) and sodium hydroxide (6.0g). The mixture was hydrogenated at room temperature and atmospheric pressure for 24 hours. At this time, no starting material remained by TLC (20% EtOAc in petrol). The catalyst was removed by filtration through celite, the cake being washed with ethanol. The filtrate was then reduced in vacuo to yield a crude oil. This was dissolved in dichloromethane (150mL) and washed with water (2x75mL, 2M) followed by brine (75mL). The organic layer was then dried over anhydrous magnesium sulphate, and the solvent removed in vacuo. Compound [104] was purified by flash chromatography (15: 5: 1 CH₃Cl: CH₃OH: 'PrNH₂, Rf = 0.43) to yield yellow oil (12.12g, 24.1mmol, 88%). m/z (C.I.) = 526 (M⁻Na, 5%), 503.8 (M⁺H, 100%), 403 (-BOC, 7%), 303 (-2BOC, 5%); ν max/cm⁻¹ (CH₂Cl₂) = 3445 (w, N-H), 2916 (m, C-H), 1694 (s, C=O), 1490 (m, C-N); δH (250Hz) = 1.10ppm (9H, s, C(Me)₃), 1.12 (9H, s, C(Me)₃), 1.13
Chapter 5. Experimental.

(9H, s, C(Me)₃), 1.50 (6H, m, CH₃CH₂CH₂CH₂ and CH₂CH₃CH₂), 2.4 (2H, t, J = 6.6 Hz, CH₂NH₂), 2.6-3.3 (10H, m, CH₃NRBOC), 4.4 (2H, br s, NH₂), 5.1 (1H, br s, NH); δc (62.9 MHz, CD₂OD) = 25.7 (CH₂), 27.5 (CH₂), 28.0 (Me), 28.50 (Me), 28.80 (Me), 30.8 (CH₂), 32.0 (CH₂), 38.6 (CH₂), 39.0 (CH₂), 40.2 (CH₂), 43.8 (CH₂), 44.9 (CH₂), 46.9 (CH₂), 78.6 (C), 79.2 (C), 79.5 (C), 155.5 (C=O), 156.3 (C=O), 156.4 (C=O).

5.6.7 N¹, N⁸-Di-(tert-butoxycarbonyl) spermidine [105].

Beilstein Number 4701579.

All glassware was oven dried. A solution of Boc-ON (17.5 g, 70 mmol) in dry THF (14 mL) was added over 1 hour to a stirred solution of spermidine [22] (5.00 g, 34.5 mmol) in dry THF (25 mL) at 0°C under a nitrogen atmosphere. This solution was stirred for another hour, at which point no starting material remained by TLC (MeOH: EtOAc, 1:1, developed with PMA). The solvent was removed in vacuo, and the slightly yellow resin then dissolved in diethyl ether (300 mL). This was washed with NaOH solution (near saturated, 5 g in 400 mL) until the entire coloration was removed (4 x 100 mL), dried over magnesium sulphate, filtered and the solvent removed in vacuo. This was recrystallized from diethyl ether to give [105] as white solid (9.94 g, 28.8 mmol, 83% yield), which was one spot on TLC (1:1, MeOH: EtOAc). m/z (ES) = 346 (MH⁺, 100%), 290 (70%), 234 (75%), 146 (80%); v in cm⁻¹ (CH₂Cl₂): 3440 (w, N-H), 2910 (m, C-H), 1695 (s, C=O), 1490 (m, C-N); δH (CDCl₃, 250 MHz) = 1.24 ppm (18H, s, 2 x C(Me)₃), 1.26 (1H, s, NH) 1.32 (4H, m, CH₂CH₂CH₂CH₂), 1.45 (2H, quin, J = 6.5 Hz, CH₂CH₂CH₂), 2.39 (4H, m, CH₂NH), 3.00 (4H, m, CH₂NHBOC), 4.6 (1H, s, NH), 4.9 (1H, s, NH); δC (CDCl₃, 62.9 MHz) = 27.7 (CH₂), 28.2 (CH₂), 28.82 (Me), 28.84 (Me), 30.2 (CH₂), 39.5 (CH₂), 40.7 (CH₂), 48.0 (CH₂), 49.8 (CH₂), 79.16 (C), 79.19 (C), 156.4 (CO), 156.5 (CO).

5.6.8 N¹, N⁸-Di-(tert-butoxycarbonyl)-N⁴-(2-cyanoethyl) spermidine [106].

Beilstein Number 4884179.
N^1, N^8-Di-(tert-butoxycarbonyl) spermidine [105] (5.84 g, 16.9 mmol) was transferred to an oven dried Young’s tube containing a magnetic bead. Acrylonitrile (17 mL, 254 mmol) was added and the solid dissolved with gentle heating under a nitrogen atmosphere. The tube was then sealed under nitrogen and heated at 90°C for 24 hours with stirring. TLC showed only product (R_f= 0.5, ethyl acetate). The solution was transferred to a 50 mL RB flask and the excess acrylonitrile removed in vacuo leaving a yellow oil. This was placed on an oil pump overnight to remove any remaining acrylonitrile and then purified by flash chromatography (100% ethyl acetate) to give [106] as colourless oil (6.34 g, 15.9 mmol, 90% yield). m/z (ES)= 421.0 (M^+Na, 100%), 399.0 (M^+H, 50%); v_max/cm\(^{-1}\) (CH\(_2\)Cl\(_2\))= 3450 (w, N-H), 2950 (m, C-H), 1700 (s, C=O), 1500 (m, C-N); \(\delta\)\(_H\) (CDCl\(_3\), 250MHz)= 1.45-1.51 (22H, m, [1.49 (18H, s, C(CH\(_3\))\(_3\)], 1.55 (4H, m, CH\(_2\)CH\(_2\)CH\(_2\)CH\(_2\))), 1.7 (2H, quin, J= 6.7Hz, CH\(_2\)CH\(_3\)CH\(_3\)), 2.4-2.7 (6H, m, CH\(_2\)N), 2.85 (2H, t, J= 6.6Hz, CH\(_3\)CN), 3.1-3.4 (4H, m, CH\(_3\)NHBOC), 4.8 (1H, s, NH), 5.1 (1H, s, NH); \(\delta\)\(_C\) (CDCl\(_3\), 62.9MHz)= 16.6 (CH\(_2\)), 24.6 (CH\(_2\)), 27.8 (CH\(_2\)), 28.0 (CH\(_2\)), 28.82 (Me), 28.86 (Me), 39.4 (CH\(_3\)), 40.5 (CH\(_2\)), 52.0 (CH\(_2\)), 53.4 (CH\(_2\)), 53.9 (CH\(_2\)), 79.29 (C), 79.3 (C), 119.5 (CN), 156.54 (CO), 156.58 (CO).

5.6.9 N^4-(3-aminopropyl)-N^1, N^8-Di-(tert-butoxycarbonyl)-spermidine [107].

Beilstein Number 4881694.

A solution of sodium hydroxide (10 g) dissolved in water (12 mL) was added to a 500 mL RB flask containing N^1,N^8-Di-(tert-butoxycarbonyl)-N^4-(2-cyanoethyl) spermidine [106] (6.34 g, 15.9 mmol) dissolved in ethanol (250 mL). Raney nickel (5 g) was added and the stirred suspension hydrogenated at atmospheric pressure for 16 hours. TLC showed no starting material at this point (15:5:1 CHCl\(_3\): MeOH: NH\(_4\)OH). The catalyst was removed by filtration through celite, the pad being washed with EtOH (2 x 50 mL). The catalyst was kept wet at all times, and was placed in a residue bottle containing water. The solvent was then removed in vacuo leaving a white residue, which was made up to 150 mL with water. This was extracted with CH\(_2\)Cl\(_2\) (4 x 100 mL), the organic extracts dried over magnesium sulphate, filtered and the solvent removed in vacuo. The crude oil was purified by flash chromatography (R_f= 0.5, 15:5:1 CHCl\(_3\): MeOH: NH\(_4\)OH) to yield [107] as yellow oil.
(4.29 g, 10.6 mmol, 67%). \( m/z \) (EI) = 403 (M+H, 100%), 303 (50%, -BOC), 203 (20%, -2BOC); \( \nu_{\text{max/cm}^{-1}} \) \( (\text{CH}_2\text{Cl}_2) = 3300 \) (br m, N-H), 2900 (m, C-H), 1690 (s, C=O), 1520 (m, C-N); \( \delta_{\text{H}} (\text{CDCl}_3, 250\text{MHz}) = 1.4-1.51 \) (22H, m, [1.44 (18H, s, C(CH$_3$)$_3$), 1.48 (4H, m, \( \text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2 \))], 1.6 (4H, m, 2 x \( \text{CH}_2\text{CH}_2\text{CH}_2 \)), 1.8 (2H, s, NH$_2$), 2.3-2.5 (6H, m, \( \text{CH}_2\text{N} \)), 2.7 (2H, t, \( J = 6.6\text{Hz} \), \( \text{CH}_2\text{NH}_2 \)), 3.0-3.2 (4H, m, \( \text{CH}_2\text{NHBOC} \)), 5.1 (1H, s, NH), 5.6 (1H, s, NH); \( \delta_{C} (\text{CDCl}_3, 62.9\text{MHz}) = 24.7 \) (CH$_2$), 27.7 (CH$_2$), 28.3 (CH$_2$), 28.82 (Me), 28.86 (Me), 31.0 (CH$_2$), 40.0 (CH$_2$), 40.6 (CH$_2$), 40.9 (CH$_2$), 52.1 (CH$_2$), 53.0 (CH$_2$), 54.0 (CH$_2$), 79.29 (C), 79.32 (C), 156.34 (CO), 156.45 (CO).
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References.


85. Professor Workman, Cancer Research Centre for Cancer Therapeutics, Institute of Cancer Research, 15 Cotswold Road, Belmont, Sutton, Surrey, SM2 5NG, UK.


Appendix.
Appendix.

**Postgraduate Activities.**

Postgraduate Module CH5Q1: Research Techniques.

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Additional Modules Taken During Postgraduate Training.

CH300-Structure, Reactivity and Selectivity. Dr Malpass, Dr Harger, Dr Atkinson

CH308-Bioorganic Chemistry. Prof Cullis, Dr Rawlings.

CH205-Bifunctional Molecules. Prof Cullis, Dr Jenkins.

Lectures and Seminars Attended.

- **07-10-97** Prof N. Simpkins- New Asymmetric Chemistry With Chiral Bases.
- **14-10-97** Dr M. Wills- Recent Developments in Asymmetric Catalysis.
- **15-10-97** Dr S. Handa, Dr A Lightfoot, Dr B. Linclau, Dr L. Twyman- Lectures by Organic Academic Interviewies.
- **17-10-97** Dr T. Gallagher- A Way to Make β-lactams.
- **04-11-97** Dr N. Walshe- Current Research at Pfizer.
- **12-11-97** Prof Ghosh- Terpene Synthesis.
Appendix.

14-11-97  Prof Ghosh- A New Stereocontrolled Approach to Substituted Cyclopentanones.
20-11-97  Prof Ghosh- An Expeditious Approach to Taxanes.
20-11-97  Dr J.F. Eccleston- Ras and Rho: Gaps in Mechanism.
10-12-97  Prof B. Cox- Bigger and Better Reactions.
11-12-97  Prof D. Crout- Carbohydrates in Biological Systems; More than Simply Sweet.
11-12-97  Dr D. Ager- Large Scale Synthesis of Amino Acids.
19-01-98  Prof C. Moberg- Chiral Pyridine Ligands in Asymmetric Catalysis.
12-02-98  Dr P. F. Knowles- Self Assembled Peptide and Membrane Systems.
02-03-98  Prof S. Davies- Asymmetric Synthesis of Aldehydes and Ketones.
13-03-98  Prof S. Laschat- Stereoselective Synthesis of Piperidines via Iminocyclisations.
06-05-98  Dr D. O'Hagen- Fluorinated Natural Products.
27-05-98  Dr G. Lloyd-Jones- Isotopic Desymmetrization.
05-10-98  Prof Hawthorne- UCLA- Boron Chemistry in Neutron Capture Therapy.
06-10-98  Prof Mann- Reading- The Elusive Magic Bullet and Attempts to Find It.
13-10-98  Dr Haigh/Dr King- SB- PPAR Agonists.
26-10-98  Prof Johnson- Cambridge- The Shape of Things To Come.
10-12-98  Dr Schofield- Oxford- Stereoelectronics of Enzyme Catalysis.
13-01-99  Prof Stoodly- UMIST- Stereocommunications Through Glycosidic Bonds.
25-01-99  Dr Winter- Sheffield- Chemistry on the WWW.
17-01-99  Dr Abraham- UCL- Hydrogen Bonding.
22-04-99  Prof Katsuki- Kyushu- Asymmetric Catalysis.
19-05-99  Prof Murphy- Strathclyde- New Rxns and Complex Mols.
19-10-99  Dr S Marsden- Imperial-New Synthetic Methods in Main Group Chemistry.
26-10-99  Prof J Boukouvalas- Laval- Total Synthesis of Architecturally Novel Natural Products of Biomedical Importance.
25-11-99  Prof T Hudlicky- Florida- Recent Advances in Chemoenzymatic Synthesis in Natural Products.
29-01-0  Prof R Grigg- Leeds- Recent Advances in Catalytic Cascade Reactions.
22-03-0  Chem Talks.
03-04-0  Prof D Leigh- Warwick- Molecules with Moving Parts.
03-04-0  Prof J-P Sauvage- Louis Pasteur- Porphyrin-Stoppered Rotaxanes.
17-05-0  Dr S Clark- Nottingham- Synthesis of Terpene Derived Polycyclic Natural Products.
Appendix.

10-07-00  Prof M Murata- Osaka- Determination of Stereochemistry on the Basis of Carbon-Proton Coupling Constants.
24-07-00  Prof Nakai- Tokyo- Classic Wittig.
05-09-00  Prof Katsuki- Kyushu- Asymmetric Catalysis with Salen Ruthenium Compounds.

Conferences Attended.


16-12-97  Modern Aspects of Stereochemistry (31st Symposium)- Sheffield.

31-03-98  Sheffield University.
           Prof Sir J. Baldwin, Dr K. Brooker-Milburn, Prof G. Stork.

           -Prof Kocienski, Glasgow, Synthesis of Biologically Active Marine Natural Products.
           -Prof Phaltz, Planck Inst., Design of Chiral Ligands for Asymmetric Catalysis.
           -Dr Hodgson, Oxford, Asymmetric Carbenoid Chemistry.

07-12-98  Stereochemistry at Sheffield University.
           -Prof Hegedus, Colorado, Stereoselectivity in the Synthesis of Azapenams, Dioxocyclams and Capped Dioxocyclams.
           -Dr James, Pfizer, The Proteinase Gene Family as Drug Discovery Targets.
           -Prof Meijer, Amplification of Chirality In Supramolecular Assemblies.
           -Prof Parsons, Sussex, Cascade Reactions in Synthesis.
           -Prof Barton, California, DNA-Mediated ET-Chemistry at a Distance.

06-01-99  Bioorganic Postgraduate Symposium at Leicester.

14-04-99  RSC Perkin Division, East Midlands Meeting, Leicester.

Presented
Poster

- Ms Cox, Leic., Epibatidine.
- Dr Matharu, Trent, Fluoroaromatic and Thiophene LCD’s.
- Dr Skabara, Sheff., Electroactive Heterocycles and Macromolecules.
- Dr Hayes, Nott., alpha, alpha-Dialkyl a.a.’s.
- Dr Spivey, Sheff., Aryl Germaines for Solid Phase Synthesis.
- Dr Potter, De. Montfort, Anticancer agent Abiraterone Acetate.
- Dr Christie, Loughborough, Asymmetric Control in Synthesis.

- Prof Vasella, ETH, Carbohydrate Mimics.
Appendix.

14-12-99

**Stereochemistry at Sheffield University.**
- Prof EM Carreira, ETH, Studies in Asymmetric Synthesis.
- Dr P Leeson, AstraZeneca, Design of Combinatorial Medicinal Libraries.
- Prof DA Tirrell, Architectural Control in Macromolecular Chemistry.
- Prof JKM Sanders, Cambridge, Adventures in Molecular Recognition.
- Prof MT Reetz, Max Planck, Evolution in the Test Tube as a Means to Create Enantioselective Enzymes for Organic Synthesis.

14-12-99

**Stereochemistry at Sheffield University.**
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- Prof MT Reetz, Max Planck, Evolution in the Test Tube as a Means to Create Enantioselective Enzymes for Organic Synthesis.

16-12-00

**RSC Perkin Division, Bioorganic Group, University of Oxford.**
- Presented
  - L Mciver, S Fowler, A Rullay, S Furrukh, S Rafiq, A Appleyard, C Fauroux, M Tavasli, M. Fandrich, A Siskos, J Westcott.
- Poster

03-01-00 to 07-01-00

**Mona Symposium 2000, Natural Products and Medicinal Chemistry, University of the West Indies.**
- Gave Lecture
  - R Taylor, J Cha, V Rawal, J Rosazza, G Ashley, A Kinghorn, S Clark, W Fenical, M Yamaguchi, G Cragg, M Tori, C Moody.

31-05-00

**RSC Perkin Division, East Midlands Meeting, Loughborough.**
- Won AZ
  - Poster
    - Dr G Weaver, Dr A Westwell, Dr J Harrity, P Thorne, J Kempson, Dr S Handa, Prof H Hiemstra.
- Prize

06-07-00

**RSC Bioorganic & Carbohydrate Groups, University of Warwick.**
- J-M Beau, R Cosstick, B Davis, M Rohmer, P Smith, JB Spencer, C Unverzagt, Prof A Vasella.

**Other Postgraduate Activities.**

**Courses:**
- Dr P. Jenkins- Organometallic Chemistry.
- Prof S. Laschat- Organometallic Reagents in Organic Synthesis.
- Dr P. Jenkins- Heterocyclic Chemistry.

**Group Meetings:**
- Prof P. Cullis.

**Problem Sessions:**
- Dr P. Jenkins, Dr S. Handa.
Appendix.

X-Ray Crystallography.
Table 1. Crystal data and structure refinement for 2.

<table>
<thead>
<tr>
<th>Identification code</th>
<th>9958</th>
</tr>
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<tbody>
<tr>
<td>Empirical formula</td>
<td>C$<em>{33}$H$</em>{48}$O$_{3}$</td>
</tr>
<tr>
<td>Formula weight</td>
<td>492.71</td>
</tr>
<tr>
<td>Temperature</td>
<td>190(2) K</td>
</tr>
<tr>
<td>Wavelength</td>
<td>0.71073 Å</td>
</tr>
<tr>
<td>Crystal system</td>
<td>Orthorhombic</td>
</tr>
<tr>
<td>Space group</td>
<td>P$_2_1$P$_2_1$P$_2_1$</td>
</tr>
<tr>
<td>Unit cell dimensions</td>
<td>$a = 11.379(2)$ Å, $alpha = 90^\circ$ $b = 13.079(2)$ Å, $beta = 90^\circ$ $c = 19.356(1)$ Å, $gamma = 90^\circ$</td>
</tr>
<tr>
<td>Volume, Z</td>
<td>2880.7(7) Å$^3$, 4</td>
</tr>
<tr>
<td>Density (calculated)</td>
<td>1.136 Mg/m$^3$</td>
</tr>
<tr>
<td>Absorption coefficient</td>
<td>0.070 mm$^{-1}$</td>
</tr>
<tr>
<td>F(000)</td>
<td>1080</td>
</tr>
<tr>
<td>Crystal size</td>
<td>0.38 x 0.27 x 0.25 mm</td>
</tr>
<tr>
<td>$\theta$ range for data collection</td>
<td>1.88 to 25.00$^\circ$</td>
</tr>
<tr>
<td>Limiting indices</td>
<td>$-13 \leq h \leq 0$, $-15 \leq k \leq 1$, $-23 \leq l \leq 1$</td>
</tr>
<tr>
<td>Reflections collected</td>
<td>3310</td>
</tr>
<tr>
<td>Independent reflections</td>
<td>3224 ($R_{int} = 0.0234$)</td>
</tr>
<tr>
<td>Completeness to $\theta = 25.00^\circ$</td>
<td>99.7 %</td>
</tr>
<tr>
<td>Absorption correction</td>
<td>Not applied</td>
</tr>
<tr>
<td>Refinement method</td>
<td>Full-matrix least-squares on F$^2$</td>
</tr>
<tr>
<td>Data / restraints / parameters</td>
<td>3224 / 0 / 330</td>
</tr>
<tr>
<td>Goodness-of-fit on F$^2$</td>
<td>1.008</td>
</tr>
<tr>
<td>Final R indices [I&gt;2\sigma(I)]</td>
<td>$R_1 = 0.0597$, $wR_2 = 0.1145$</td>
</tr>
<tr>
<td>R indices (all data)</td>
<td>$R_1 = 0.1074$, $wR_2 = 0.1340$</td>
</tr>
<tr>
<td>Absolute structure parameter</td>
<td>-2(3)</td>
</tr>
<tr>
<td>Extinction coefficient</td>
<td>0.0056(8)</td>
</tr>
<tr>
<td>Largest diff. peak and hole</td>
<td>0.188 and -0.169 eÅ$^{-3}$</td>
</tr>
</tbody>
</table>
Table 2. Atomic coordinates [ x 10^4] and equivalent isotropic displacement parameters [Å^2 x 10^3] for O. U(eq) is defined as one third of the trace of the orthogonalized $U_{ij}$ tensor.

<table>
<thead>
<tr>
<th></th>
<th>x</th>
<th>y</th>
<th>z</th>
<th>U(eq)</th>
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<tbody>
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<td>O(1)</td>
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<td>8725(3)</td>
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<td>5036(3)</td>
<td>4136(2)</td>
<td>1086(2)</td>
<td>34(1)</td>
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<tr>
<td>O(3)</td>
<td>3138(3)</td>
<td>3645(3)</td>
<td>972(2)</td>
<td>46(1)</td>
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<td>C(1)</td>
<td>6751(4)</td>
<td>7393(4)</td>
<td>-3023(3)</td>
<td>46(1)</td>
</tr>
<tr>
<td>C(2)</td>
<td>6217(4)</td>
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<td>-3461(3)</td>
<td>51(2)</td>
</tr>
<tr>
<td>C(3)</td>
<td>4957(5)</td>
<td>6899(4)</td>
<td>-3591(3)</td>
<td>45(1)</td>
</tr>
<tr>
<td>C(4)</td>
<td>4819(5)</td>
<td>7981(4)</td>
<td>-3811(3)</td>
<td>51(2)</td>
</tr>
<tr>
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<td>4665(4)</td>
<td>7694(4)</td>
<td>-3060(2)</td>
<td>36(1)</td>
</tr>
<tr>
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<td>3442(4)</td>
<td>7735(4)</td>
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<td>7022(4)</td>
<td>-2125(2)</td>
<td>35(1)</td>
</tr>
<tr>
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<td>4326(3)</td>
<td>7241(4)</td>
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<tr>
<td>C(9)</td>
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<td>7840(4)</td>
<td>-2582(3)</td>
<td>38(1)</td>
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<tr>
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<td>6516(3)</td>
<td>7333(4)</td>
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<td>C(16)</td>
<td>3452(3)</td>
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<td>92(2)</td>
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<td>C(17)</td>
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<td>63(2)</td>
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<td>924(2)</td>
<td>33(1)</td>
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<td>4670(4)</td>
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<td>9481(4)</td>
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<td>48(2)</td>
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Table 3. Bond lengths [Å] and angles [°] for 2.

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<th>Bond</th>
<th>Length [Å]</th>
<th>Angle [°]</th>
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<td>O(1) - C(33)</td>
<td>1.432(5)</td>
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<tr>
<td>O(2) - C(26)</td>
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<td>C(1) - C(10)</td>
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<td></td>
</tr>
<tr>
<td>C(1) - C(2)</td>
<td>1.538(7)</td>
<td></td>
</tr>
<tr>
<td>C(2) - C(3)</td>
<td>1.533(5)</td>
<td></td>
</tr>
<tr>
<td>C(2) - C(4)</td>
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<td>1.520(6)</td>
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<td></td>
</tr>
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<td></td>
</tr>
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</tr>
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<td>(16) - C(17) - C(18)</td>
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Table 4. Anisotropic displacement parameters [Å$^2 \times 10^3$] for 2.

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