Peptide Mimics Targeting Bacterial Membranes

By

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Little do ye know your own blessedness; for to travel hopefully is a better thing than to arrive, and the true success is to labour.

Robert Louis Stevenson

Virginibus Puerisque, 1881.
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<th>Definition</th>
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</thead>
<tbody>
<tr>
<td>°C</td>
<td>Degrees Celsius</td>
</tr>
<tr>
<td>μL</td>
<td>Micro litre</td>
</tr>
<tr>
<td>μλ</td>
<td>Microwave</td>
</tr>
<tr>
<td>δ (ppm)</td>
<td>Chemical shift</td>
</tr>
<tr>
<td>ABC transporters</td>
<td>Bacterial cellular peptides</td>
</tr>
<tr>
<td>CAMP</td>
<td>Cationic Antimicrobial Peptide</td>
</tr>
<tr>
<td>CMC</td>
<td>Critical Micelle Concentration</td>
</tr>
<tr>
<td>CMCs</td>
<td>Critical Micelle Concentrations</td>
</tr>
<tr>
<td>Dab</td>
<td>dianinobutyric</td>
</tr>
<tr>
<td>DAIIB</td>
<td>diacetoxyiodobenzene</td>
</tr>
<tr>
<td>DCC</td>
<td>dicyclohexylcarbodiimide</td>
</tr>
<tr>
<td>DLS</td>
<td>Dynamic Light Scattering</td>
</tr>
<tr>
<td>DOSPER</td>
<td>1,3-dioleyl-oxy-2-(6-carboxyspermyl)-propylamide</td>
</tr>
<tr>
<td>E. coli</td>
<td>Escherichia coli</td>
</tr>
<tr>
<td>eq.</td>
<td>Equivalents</td>
</tr>
<tr>
<td>FQRP</td>
<td>Fluoroquinolone-Resistant Pseudomonas Aeruginosa</td>
</tr>
<tr>
<td>g</td>
<td>grams</td>
</tr>
<tr>
<td>h</td>
<td>hours</td>
</tr>
<tr>
<td>HNP</td>
<td>Human Neutrophil Peptides</td>
</tr>
<tr>
<td>HOMO</td>
<td>Highest Occupied Molecular Orbital</td>
</tr>
<tr>
<td>IR</td>
<td>Infrared</td>
</tr>
<tr>
<td>ITC</td>
<td>Isothermal Titration Calorimetry</td>
</tr>
<tr>
<td>IBX</td>
<td>2-iodoxybenzoic acid</td>
</tr>
<tr>
<td>KDO</td>
<td>2-keto-3-deoxyoctonoic acid</td>
</tr>
<tr>
<td>Leu</td>
<td>Leucine</td>
</tr>
<tr>
<td>LPS</td>
<td>Lipopolysaccharide</td>
</tr>
<tr>
<td>LPSs</td>
<td>Lipopolysaccharides</td>
</tr>
<tr>
<td>LUMO</td>
<td>Lowest Unoccupied Molecular Orbital</td>
</tr>
<tr>
<td>LUVs</td>
<td>Large Unilamellar Vesicles</td>
</tr>
<tr>
<td>MDR</td>
<td>Multi-Drug Resistant</td>
</tr>
<tr>
<td>mg</td>
<td>milligram</td>
</tr>
<tr>
<td>mins</td>
<td>minutes</td>
</tr>
<tr>
<td>mL</td>
<td>millilitre</td>
</tr>
<tr>
<td>mmol</td>
<td>millimole</td>
</tr>
<tr>
<td>MRSA</td>
<td>Methicillin-Resistant Staphylococcus Aureus</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear Magnetic Resonance</td>
</tr>
<tr>
<td>PG</td>
<td>Protecting Group</td>
</tr>
<tr>
<td>pH</td>
<td>Potential of hydrogen</td>
</tr>
<tr>
<td>Phe</td>
<td>Phenylalanine</td>
</tr>
<tr>
<td>PMBN</td>
<td>Polymyxin B Nonapeptide</td>
</tr>
<tr>
<td>poly(Gro-P)</td>
<td>Polyglycerol phosphate</td>
</tr>
<tr>
<td>poly(Rbo-P)</td>
<td>Polyribitol phosphate</td>
</tr>
<tr>
<td>r.t.</td>
<td>room temperature</td>
</tr>
<tr>
<td>Rf</td>
<td>Retention factor</td>
</tr>
<tr>
<td>RP-HPLC</td>
<td>Reverse Phase High Performance Liquid Chromatography</td>
</tr>
<tr>
<td>SUVs</td>
<td>Small Unilamellar Vesicles</td>
</tr>
<tr>
<td>Thr</td>
<td>Threonine</td>
</tr>
<tr>
<td>USA</td>
<td>United States of America</td>
</tr>
<tr>
<td>VRE</td>
<td>Vancomycin Resistant Enterococci</td>
</tr>
</tbody>
</table>
Abstract

With the rise of multi-drug resistant bacteria, there has been an increasing need for the development of novel antibacterials. A family of molecules that have consistently proved effective against bacteria are Cationic Antimicrobial Peptides. These peptides are found extensively in nature, unfortunately, except a few like Polymyxin B, are unsuitable for mass production and commercial use. Polymyxin B, one of the few CAMPs administered commercially is exceedingly toxic causing as much damage as the principal infection.

This research project involved the design of small molecules that mimicked the essential characteristics of Cationic Antimicrobial Peptides. Previous research showed that the norbornane scaffold was an excellent motif as the rigidity of the structure ensured the segregation of hydrophobic and hydrophilic residues, a characteristic necessary for the amphiphilicity of a molecule. Furthermore, the norbornane scaffold proved easy to functionalise. The acetal was used to attach different hydrophobic residues and amides links the necessary hydrophilic functionality.

![Figure 1 - General structure of norbornane based CAMP mimics.](image)

The main goal of the current project was to synthesise two libraries of norbornane based mimics and have them tested for antibacterial activity. This was completed successfully and a selection of compounds was further evaluated using fluorescence spectroscopy, dynamic light scattering and isothermal titration calorimetry to elucidate a potential mode of action.
Plain English Summary

In recent years, society has been increasingly concerned with bacteria that are no longer susceptible to commercial antibiotics. Faced with a lack of tools, medical practitioners today are forced to prescribe medicines that, although effective, cause as much harm to the patient as the principal infection. The purpose of this research project is to develop novel antibacterials that remain potent against bacterial infections without being toxic to the patient.
Chapter 1
Introduction

In this chapter, there is a brief discussion regarding the concerning issue of antibiotic resistance. The bacterial membrane is selected as an appropriate antibiotic target and a description of its important molecular features is provided. Cationic Antimicrobial Peptides (CAMPs), their different classifications and modes of action are elaborated. Polymyxin B is utilised as a case study to convey how CAMPs potentially act when targeting the bacterial membrane. From there, a suitable pharmacophore is identified and examples of synthetically derived CAMP mimics highlighted. Finally, the aims of the current project are outlined.

1.1 Antibiotic pipeline

When antibiotics were first discovered, they were heralded as ‘miracle drugs’.\(^1\)

However as Alexander Fleming himself warned

\hspace{1cm}“The time may come when penicillin can be bought by anyone in the shops. Then, there is the danger that the ignorant man may easily underdose himself and by exposing his microbes to non-lethal quantities of the drug make them resistant” (Alexander Fleming, Nobel lecture, 1945).\(^2\)

With uncanny foresight, he predicted a scenario that has now come to fruition. Decades of overuse and misuse have caused a global epidemic where bacteria have become resistant to most antibiotics.\(^1,3,4\)
Antibacterial resistance typically occurs within hospitals where antibiotics are administered as a preventative measure against post-surgery bacterial infections.\(^4,7,8\) Such medical practices have led to the evolution of Multiple Drug Resistant (MDR) bacteria, also known as ‘superbugs’. Examples of MDR bacteria and their growing resistance are documented in Figure 2. The prevalence of Methicillin-Resistant Staphylococcus Aureus (MRSA) has grown to more than 60% of clinical isolates. Hospital patients who contract infections from MDR bacteria (nosocomial infections) are difficult to treat and consequently, there has been a rise in morbidity.\(^5,9-15\) In 1995, an estimated two million people in the United States acquired nosocomial infections and ninety thousand died as a result.\(^4,16-18\) MDR bacterial infections are no longer confined to hospitals as incidences are now being detected within the wider community.\(^16\)
Contrary to the current health emergency and the obvious need for new antibiotics, pharmaceutical companies have decreased their efforts in the search for novel antibacterials. The number of companies researching antibiotics has decreased from eighteen in 1993 to four in 2010 (Figure 2). There has also been a high attrition rate of compounds undergoing clinical evaluation (Figure 3). In 2011, there were twenty-two compounds in phase II, five compounds in Phase III and only one compound where a New Drug Application was filed (last step before commercialisation).

With a dwindling number of therapeutic options, medical practitioners are being forced to reconsider the viability of drugs such as Polymyxin B and Colistin (Polymyxin E). Although effective, these fell out of use decades ago due to toxicity concerns. Both Polymyxin B and Colistin have been known to cause neurotoxicity, nephrotoxicity and respiratory failure. Unfortunately, medical practitioners have to ignore side effects and follow strict guidelines for administering these antibiotics in an effort to treat patients suffering from life threatening bacterial infections.
In summary, there is a very real need for the development of novel antibacterial agents.20, 24, 25 A more detailed examination of antibiotics and the current antibiotic ‘crisis’ is needed before elaborating on the goal of the current project.

### 1.2 Antibiotics

#### 1.2.1 Definitions

Antimicrobial is a general term for any substance that either kills or slow the growth of microbes.12 An antibiotic is a type of antimicrobial that is effective against bacteria and fungi but not against viruses.12 If classified as bacteriostatic, the antibiotic stops bacteria from growing. If classified as bactericidal, it causes bacteria death.24 Antibiotics can be natural products or the result of synthesis. More commonly, they are produced through the synthetic alterations of natural products.12

#### 1.2.2 History

World War One had a profound influence on the field of medicinal chemistry. With bacterial infections a cause of large numbers of fatalities amongst wounded soldiers, the search for treatment was accelerated.

![Figure 4](image-url) – (a) Prontosil (1). (b) Penicillin G (2).26, 27
In 1932, Gerhard Johannes Paul Domagk, Fritz Mietzsch and Josef Klarer were credited for the discovery of Prontosil 1, a synthetic antibacterial developed by a pharmaceutical research program under the Friedrich Bayer Company (Figure 4a).26

Alexander Fleming’s discovery of penicillin was somewhat serendipitous. In 1928, he noticed that a mould growing on a laboratory plate was surrounded by a large zone of inhibition repelling staphylococcal bacteria growing alongside.2, 12 Fleming found that the mould was of the genus Penicillium. He proceeded to then test it against other bacterial strains such as streptococcus, Bacillus diphtherice and Bacillus typhosus and the mould repelled some bacteria but not all. His most important deduction was the mould was effective against microbes that were responsible for many of the common infections in society back then. Ten years after publishing his findings, Fleming provided his strain of Penicillium notatum to Ernst Chain and Howard Florey who succeeded in isolating the substance known as penicillin G (2) from the mould (Figure 4b).2 The discovery of these antibiotics (Prontosil and penicillin) is widely accepted as one of modern medicine’s greatest achievements.1, 12

1.2.3 Resistance

When a new antimicrobial begins clinical use, the manifestation of resistance is imminent. Cases of resistance against antibiotics such Erythromycin and Linezolid were reported after only one year of being prescribed in clinics (Table 1).4, 28 Vancomycin was used commercially for almost fifteen years before the first case of resistance emerged. Although the effective life time of antibiotics
may vary, one thing is certain: once used in clinics, bacterial isolates exhibiting resistance against the antibiotic will emerge.4

Table 1 – A comparison of when an antibiotic is discovered, introduced into clinical circulation and when resistant bacteria are first detected.4

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Discovered</th>
<th>Introduced in clinic</th>
<th>Emergence of resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin</td>
<td>1940</td>
<td>1943</td>
<td>1940 (methicillin, 1965)</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>1944</td>
<td>1947</td>
<td>1947, 1956</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>1947</td>
<td>1949</td>
<td>1970</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>1948</td>
<td>1952</td>
<td>1956</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>1952</td>
<td>1955</td>
<td>1956</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>1963</td>
<td>1967</td>
<td>1970</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>1956</td>
<td>1972</td>
<td>1987</td>
</tr>
<tr>
<td>TMP/SMX</td>
<td>1969</td>
<td>1973</td>
<td>1979</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>1980</td>
<td>1987</td>
<td>1992</td>
</tr>
<tr>
<td>Linezolid</td>
<td>1990</td>
<td>2000</td>
<td>2001</td>
</tr>
<tr>
<td>Telithromycin</td>
<td>1997</td>
<td>2001</td>
<td>2003</td>
</tr>
<tr>
<td>Quinupristin/Dalfopristin</td>
<td>1999</td>
<td>2000</td>
<td>2000</td>
</tr>
<tr>
<td>Daptomycin</td>
<td>1980</td>
<td>2003</td>
<td>2004</td>
</tr>
</tbody>
</table>

Antibiotic resistance exhibited by Gram-positive bacteria began in the 1950s when penicillin G (2) proved ineffective against some *Staphylococcus aureus* infections.29 Since then, penicillin-resistant *Staphylococcus aureus* has become the most common Gram-positive pathogen in clinics and is the leading cause of skin, bloodstream and bone and joint infections (Figure 5).29, 30 Each year in the USA, four hundred thousand people are admitted to hospital for *Staphylococcus aureus* infections with nineteen thousand people dying in hospital.29, 31

![Figure 5](image)

Figure 5 – Number of antibiotic resistant bacterial isolates collected from clinical specimens from January 1998 to June 2003.5, 30

Although receiving much less attention than Gram-positive bacteria, experts now agree that Gram-negative bacteria are of more concern being responsible
for more than half of all bacterial infections and acquiring resistance at much faster rates. The faster rate at which Gram-negative bacteria develop resistance is demonstrated by the percentage of cephalosporin-resistant *Klebsiella pneumonia* isolates increasing dramatically from 3% in 1989 to 22% in 1991, a period of only two years. Figure 5 shows that the number of antibiotic resistant Gram-negative bacterial isolates is almost equal to that of Gram-positive bacteria. According to a report released by the European Centre for Disease Prevention and Control, Gram-negative bacterial infections were responsible for 67% of twenty-five thousand deaths in 2009.

The scale of antibiotic resistance emphasises the need to develop novel antimicrobials targeting Gram-positive and Gram-negative bacteria.

### 1.3 Antibiotic target

When developing novel antibiotics, it is necessary to first select a target. Most current antibiotics fall within four classes: cell wall biosynthesis inhibitors, ribosome inhibitors, DNA and RNA synthesis inhibitors and membrane disruptors. The first three classes refer to compounds that interfere with critical functions within the bacteria cell inhibiting cell growth and eventually leading to cell death.

Membrane disruptors however are membrane-damaging agents; they disrupt the stability of the cell membrane immediately leading to cell death. As membrane disruptors cause rapid cell death, instances of bacteria developing resistance to these compounds is rare. Developing a novel antibiotic that targets bacterial membranes in such a way would therefore be ideal. Before
discussing how a compound may be tailored to ‘attack’ the bacterial membrane, an examination of the composition and structure of bacterial membranes is necessary.

1.4 Bacteria

1.4.1 Classifications of bacteria

Bacteria are prokaryotic pathogenic cells which are further classified into Gram-positive and Gram-negative bacteria based on the structural differences in their cell walls. Gram-positive bacteria have cell membranes encased in a thick peptidoglycan layer (Figure 6a). Gram-negative bacteria have a much thinner peptidoglycan layer encasing the cell membrane which is further surrounded by an outer membrane (Figure 6b).

Bacterial membranes contain one-third of the proteins found in the cell and are important in many processes, such as transport of nutrients, necessary for survival.
1.4.2 Gram-positive bacteria outer membrane

Bacteria often live in harsh environments like the digestive systems of humans. In order to withstand such conditions, the outer membranes of Gram-positive bacteria can be up to 100 nm thick. Although there are variations in the structure of the outer membrane across all the species of Gram-positive bacteria, there are common entities present in most: the peptidoglycan layer, teichoic acids and lipoteichoic acids.

The peptidoglycan layer is comprised of a series of glycan chains cross-linked by peptides which form the mesh-like framework seen in Figure 7. The basic disaccharide consists of a β-1,4-linked N-acetylglucosamine and N-acetylmuramic acid units (Figure 8). The carboxyl group of the N-acetylmuramic acid unit is always substituted with a peptide. However, not all of these peptides are cross linked. The proportion of cross-linkages in peptidoglycan differs for each species of bacteria. Starting the scale is peptidoglycan from *Escherichia coli* which only have 50% of the peptides cross-linked. The other extreme is the peptidoglycan from Staphylococci in which all peptides are cross-linked.
Threaded through the peptidoglycan layer are teichoic acids and lipoteichoic acids. A difference between the two acids is that teichoic acids are covalently bonded to the peptidoglycan layer whereas lipoteichoic acids are anchored in the cell membrane with large portions extending through the peptidoglycan (Figure 9a).41, 44

Common to both teichoic acids and lipoteichoic acids are repeating units of anionic polymers known as poly(Gro-P) 3 and poly(Rbo-P) 4 (Figure 9b). Covalently linked phosphate anions are a feature in the anionic polymers and
contribute to a ‘continuum of anionic charge’ that pervades the outer membrane of Gram-positive bacteria.44

1.4.3 Gram-negative bacteria outer membrane

The outer membrane in Gram-negative bacteria is an asymmetric lipid bilayer interspersed with porins (protein channels) (Figure 10). The inner monolayer is composed of phospholipids and lipoproteins whilst the outer monolayer is a mixture of phospholipids and lipopolysaccharide (LPS) molecules.50 As lipopolysaccharides (LPSs) comprise about 45% of the outer monolayer and is the major constituent, the layer is often referred to as the LPS layer.51, 52

![Figure 10 - Schematic representation of outer membrane of Gram-negative bacteria.43](image)

Lipopolysaccharides are known as the endotoxin of Gram-negative bacteria and are released whenever cells grow, divide or lyses.53 When LPS is released, it causes a cascade of responses from the host body which can result in sepsis and in some cases, death.54-58 The LPS molecule has three main sections (Figure 11): the O-antigen, the core oligosaccharide and the lipid A (5) moiety.51, 59-62 Of the three sections, it is the lipid A (5) moiety that is the most conserved across the different species of Gram-negative bacteria.
The O-antigen section of the LPS molecule varies considerably between the different strains of bacteria and is composed of repeating units of five to eight monosaccharides.\textsuperscript{20, 51, 54, 62} The core oligosaccharide is divided into an inner core and an outer core. The structure of the outer core differs but the inner core is always composed of 2-keto-3-deoxyoctonoic acid (KDO).\textsuperscript{20, 61} Studies have shown that in order for a bacterial cell to live, at a minimum there must be a single molecule of KDO present in the LPS molecules.\textsuperscript{51}

The Lipid A (5) is a hydrophobic and lipid rich moiety comprised of a $\beta$-(2'-4')-linked $\delta$-glucosamine disaccharide phosphorylated at the 1- and 4'-positions respectively. Six acyl chains are connected to the disaccharide, four $\beta$-hydroxyacyl chains are directly attached to the glucosamine sugars (5', 6', 5'- and 6' positions) with two secondary chains attached to the $\beta$-hydroxy group.\textsuperscript{20, 58, 59, 66, 67} The amide-linked chains are both 3-hydroxymyristate whilst the ester-linked chains vary between myristate, laurate or palmitate.\textsuperscript{51} The Lipid A (5) molecule anchors the LPS within the outermost layer of the outer membrane forming ionic bridges with alternating calcium and magnesium cations (Figure 11 - General structure of LPS found in \textit{E. coli}. Structure of Lipid A (5) has been elaborated.\textsuperscript{63-65}
11). The ionic bridges contribute significantly to the stability of the outer membrane.

Table 2 – Chemical composition of lipid A (5) derived from different bacterial groups.65

<table>
<thead>
<tr>
<th>Lipid A</th>
<th>Disaccharide backbone</th>
<th>Phosphates</th>
<th>Fatty Acids</th>
<th>AraN*</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td><em>Salmonella</em></td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td><em>Chromobacterium violaceum</em></td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td><em>Rhodospirillum tenue</em></td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td><em>Rhodopseudomonas viridis</em></td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

*AraN* = 4-amino-L-arabinose; *Ara* = D-arabinose; *FA* = fatty acids; *EtN* = ethanolamine; *P* = phosphate

Although it is the most conserved section of LPS, the chemical composition of Lipid A (5) can still vary. Table 2 features eight species of Gram-negative bacteria and highlights the differences between the chemical structures of their respective lipid A (5). Common to most bacteria is the D-glucosamine disaccharide that forms the backbone. In *Rhodopseudomonas viridis*, there is no disaccharide; instead there is a single unit of 2,3-diamino-D-glucose that forms the backbone. The presence of phosphate on the lipid A (5) backbone is not guaranteed either with *Rhodopseudomonas viridis* also not having any. Another common variation is the substituents on the D-glucosamine disaccharide. These may vary between 4-amino-L-arabinosa, D-arabinose and ethanolamine (Table 2).

In the current project, cationic antimicrobial peptides (CAMPs), natural products already deemed effective against the bacterial membrane, were closely examined.36 Using natural products as lead compounds is an established approach in medicinal chemistry with 50% of current commercial medicines originating from natural products isolated from plant, animal or fungal
Furthermore, CAMPs have broad spectrum activity against bacteria, fungi and some viruses and as such are investigated extensively in the pursuit of novel antibacterials. A detailed examination of CAMPs follows.

1.5 Cationic Antimicrobial Peptides

Cationic Antimicrobial Peptides (CAMPs) are found widely distributed amongst plants and animals and form part of nature’s defence mechanisms against bacteria serving a fundamental protective role in evolution. Despite being present in nature for such a long time, CAMPs have remained effective. Their potency is attributed to their mode of action: disruption of the bacterial outer membrane. Cationic Antimicrobial Peptides cause rapid disorganisation of the bacterial outer membrane which quickly facilitates the death of the cell.

A common feature, regardless of classification is the amphipathic structure CAMPs adopt before or during the interaction with outer membranes of bacteria. Amphipathicity is the three-dimensional shape in which hydrophobic and hydrophilic residues are positioned on opposite sides of the molecule.

CAMPs vary extensively in composition. They contain a number of amino acids and have different positively charged residues (e.g. arginine, lysine). The peptides can be classified into three broad structural groups: β-sheet peptides with disulfide bridges, α-helical peptides and extended peptides. Besides the main groups, there are a number of other smaller families of CAMPs, one of which are the lipopeptides. These classes are discussed in some detail below.
1.5.1 β-sheet CAMPs

The β-sheet CAMPs are peptides with two to four disulfide bridges of which an example are the defensins. Defensins are CAMPs found in rabbits, guinea pigs, rats and humans. They exhibit activity against both Gram-positive and Gram-negative bacteria as well as fungi and viruses. There are currently fifty known defensins.

Defensins found in humans are called Human Neutrophil Peptides (HNP). The three human defensins identified are named HNP1, HNP2 and HNP3 (Figure 12) and exhibit activity against bacteria, fungi and viruses. Although they are classified as small amphipathic peptides, HNPs still contain up to thirty amino acids. The human defensins are rich in arginine and cysteine and possess a net charge of either +3 or +2. Each human defensin contains a triple-stranded β-sheet and its amphipathicity is ensured with three disulfide linkages between cysteine residues ensuring a high degree of conformational rigidity.
1.5.2 α-helical CAMPs

One of the most famous examples of the α-helical peptides is magainin (7) (Figure 13). Magainin (7) was the first CAMP isolated from the skin of the African clawed frog *Xenopus laevis.* Magainin (7) is comprised of twenty-three amino acids with a highly basic with a net charge of +4 at a pH of 7.84

![Figure 13 - Active conformation of magainin (7). Hydrophobic amino acids are green, cationic residues are blue and anionic residues are red.](image)

Generally, α-helical CAMPs are flexible and will only assume the characteristic α-helix upon contact with bacterial membranes.83 Hence, to determine the α-helical structure of magainin (7), Opella *et. al.* observed the interactions of the peptide (7) with anionic micelles in solution using two-dimensional NMR.83, 84 By interpreting interactions, the α-helix of magainin (7) was constructed clearly showing the amphipathicity of the peptide. From the structure detailed in Figure 13, amphipathicity is clear; hydrophobic residues (coloured in green) occupy one side of the helix and cationic residues (coloured in blue) the other.83

1.5.3 Extended CAMPs

Extended CAMPs are a group of antimicrobial peptides that do not form a regular secondary structure when interacting with the bacterial membrane.85, 86

Extended peptides commonly comprise of high percentages of arginine,
tryptophan, proline and histidine and the most common example is indolicidin \( 8 \) (Figure 14).\(^{72}\).

![Structure of Indolicidin](image)

Figure 14 – Structure of Indolicidin (8) solved by solution NMR spectroscopy in the presence of detergent micelles. Cationic residues are highlighted blue and hydrophobic residues are highlighted grey.\(^{72}\)

Indolicidin (8) is found in cows, is comprised of thirteen amino acids and has the highest fraction of tryptophan (39%) observed in a natural peptide.\(^{87-89}\) Indolicidin (8) has a net charge of +4 and exhibits broad spectrum activity against Gram-positive and Gram-negative bacteria as well as protozoa, fungi and viruses.\(^{86, 89}\)

### 1.5.4 Lipopeptides

The last group of CAMPs to be discussed are lipopeptides. Lipopeptides feature a peptide with a fatty acid chain. A well studied and clinically used example are the Polymyxins. The polymyxins are a family of five related compounds: Polymyxin A to E.\(^{21, 86, 88}\) They were discovered in the late 1940s and are secondary metabolite nonribosomal peptides isolated from \textit{Bacillus polymyxa}.\(^{20, 21, 90, 91}\) All exhibit potent activity against Gram-negative and, to a lesser extent, against Gram-positive bacteria.\(^{92, 93}\) The Polymyxin family share the same general structure: a polypeptide ring with a fatty acid chain extending from it
Only Polymyxin B (9) and E have been commercially marketed as antibiotics for humans.21,97,98

Polymyxin B (9) was discovered in 1950s and is a mixture of Polymyxin B1 and B2 differing in the fatty acid side chain (Figure 15a).9,90 When Polymyxin B (9) is bound to LPS, it assumes an amphipathic structure where the hydrophobic residues occupy one side of the structure and the cationic residues occupy the other (Figure 15b).

In all examples of CAMPs, there is the common motif of amphipathicity. The amphipathic characteristic is integral in the mode of action of CAMPs leading to the rapid disorganisation of the outer membrane of bacteria.
1.6 CAMP Mode of Action

The detergent-like mechanisms by which CAMPs attack bacterial membranes rely on a combination of hydrophobic and electrostatic interactions.\textsuperscript{38, 71, 100} There are numerous theories as to the mode by which CAMPs completely disorganise the outer membranes of Gram-positive and Gram-negative bacteria. Each theory shares a common beginning; the initial electrostatic attraction between the anionic outer membranes of bacteria and the positively charged CAMPs which result in the adsorption of the peptide to the membrane (Figure 16).

![Figure 16](image)

Figure 16 – Events occurring after the initial electrostatic interaction between the negative outer membrane of bacteria and CAMPs.\textsuperscript{72, 101} The three main theories are highlighted with an orange border.

The many theories by which CAMPs disorganise the outer membranes of bacteria are illustrated in Figure 16. The exact mechanism of a particular CAMP may involves aspects of these. The three main theories are the Barrel-Stave model, the Carpet-Like model and the Membrane-thinning model and details of each follow.
1.6.1 The Barrel-Stave Model

Figure 17 - Cartoon illustrating the ‘Barrel-Stave’ model. (a) Peptides first assemble on the surface of the outer membrane. (b) Peptides insert into membrane. (c) Structure of ceratoxin 1 (10).

In the Barrel-Stave model, CAMPs aggregate to form ‘bundles’ of peptides before interaction and adsorption to the outer membrane. These aggregates proceed to embed themselves into the membrane forming transmembrane channels (Figure 16). The channels facilitate the leaking of cellular contents and the entry of molecules otherwise excluded by a stable membrane. Most importantly, the transmembrane channels promote the entry of CAMPs themselves where they may elicit further antibacterial action. With the destabilisation of the outer membrane, cell lysis soon follows. Ceratotoxins are an example of a family of CAMPs that have been proven to disrupt the membranes of bacteria according to the Barrel-Stave model (Figure 17c). Ceratotoxins are α-helical peptides found in insects and spiders.
### 1.6.2 The Carpet-Like Model

![Figure 18](image)

Figure 18 – Cartoon illustrating ‘carpet’ model. (a) Peptides ‘carpet’ the surface of the membrane. (b and c) When a critical local concentration is reached, destabilisation and disintegration of membrane occurs.\(^{102}\) (d) Structure of Indolicidin (8)\(^{72}\)

The Carpet-like Model proposes that individual CAMP molecules interact with the LPS. When enough peptides have ‘carpeted’ the surface of the outer membrane and a critical local concentration has been reached, the hydrophobic residues of CAMPs penetrate the membrane. The resulting destabilisation and disintegration of the membrane eventually leads to cell lysis (Figure 16).\(^{103}\)

Indolicidin (8) is an example of a CAMP that is proposed to act via the Carpet-Like model.\(^{108}\)

### 1.6.3 The Membrane-Thinning Model

The Membrane-Thinning model is a derivative of the Carpet-like model and similarly proposes that CAMP molecules ‘carpet’ the membrane. However, a localised electrostatic disturbance of the outer membrane is proposed. The LPS molecules in the outer membrane then rearrange to accommodate the electrostatic disturbance and a thinning of the membrane develops (Figure 16). The CAMPs are believed to then aggregate on the surface of the outer
membrane. With a thinned membrane and a reduced local surface tension, the CAMP aggregates insert into the membrane which becomes further disordered and cell lysis ensues. Magainin (7) is an example of a CAMP that disorganises bacterial membranes via the Membrane-Thinning model.\textsuperscript{109, 110}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{membrane-thinning.png}
\caption{Cartoon illustrating ‘membrane-thinning’ model. (a) Peptides ‘carpet’ membrane surface. (b) Membrane rearranges to accommodate electrostatic disturbance and thinning of membrane results. (c) Peptides insert into membrane which disrupts further causing cell lysis. (d) Structure of magainin (7).}
\end{figure}

The theories of membrane disruption provide a brief explanation of how CAMPs may act against bacteria, many theories are idiosyncratic to a particular family of peptides.\textsuperscript{112} To further illustrate how CAMPs interact with the outer membranes of bacteria, a case study using the well studied Polymyxin against Gram-negative bacteria is presented in the following section.

\subsection{1.6.4 Polymyxin against Gram-negative bacteria}

NMR spectroscopy and molecular modelling studies on the solution structures of Polymyxin B (9) show a three-dimensional conformation which is structurally amphipathic.\textsuperscript{9, 20, 54} In this structure, the fatty acid chain and hydrophobic residues ( Phenylalanine-6 and Leucine-7) are distinctly separated from the Diaminobutyric residues 1, 4, 5, 8 and 9 (Figure 20).\textsuperscript{54} When interacting with the Lipid A (5) molecule, the Diaminobutyric residues displace
the calcium and magnesium ions and bind to the phosphate anions.\cite{71,113,114} The hydrophobic residues then insert into the LPS layer further destabilising the membrane. Cell lysis then follows.

Extensive studies using structurally modified Polymyxins have proven that the cyclic peptide ring is integral in the binding of Lipid A (5) and the neutralisation of LPS. The presence of the fatty acid chain however is necessary for antibacterial activity.\cite{94} The group of Sakura synthesised various analogues of Polymyxin B (9) testing the role of each amino acid by systematically substituting each residue (Figure 21).\cite{115}
Figure 21 - Synthetic Polymyxin B analogues used in study by Sakura et. al.\textsuperscript{115} (a) Analogue 11 where Diaminobutyric-3 replaced by alanine. (b) Analogue 12 where Diaminobutyric-5 replaced by alanine. (c) Analogue 13 where Phenylalanine-6 and Leucine-7 replaced by glycine. Replacements highlighted in orange.

Tested by Sakura et. al., Polymyxin B (9) exhibited MIC values of 0.5-1 nmol/ml against various Gram-negative bacteria. The absence of Diaminobutyric-3 in analogue 11 (Figure 21a, MIC: 1-2 nmol/ml) had no noticeable effect however without Diaminobutyric-5 in analogue 12, (Figure 21b, MIC: 16 nmol/ml), there was was a substantial decrease of potency. Variation of the hydrophobic chains,
Phenylalanine-6 and Leucine-7, had little effect on activity, however when both were replaced in analogue 13 (Figure 21c), a loss of activity was observed.

Polymyxin B Nonapeptide (14) (PMBN) clearly illustrates the importance of the fatty acid chain for the antibacterial activity of the Polymyxin family (Figure 22). With reduced hydrophobicity, PMBN 14 has a lower potency than Polymyxin B (9) and requires higher concentrations to be effective. Although antibacterial activity is markedly less than Polymyxin B (9), PMBN 14 has the advantage of being 150 times less neurotoxic than either Polymyxin B (9). However, PMBN 14 still causes nephrotoxicity and as higher doses are needed, it can cause as much damage as the parent molecule. Polymyxin B Nonapeptide 14 still binds to Lipid A (5) and disorganises the LPS layer rendering Gram-negative bacteria sensitive to other hydrophobic antibiotics, such as Daptomycin, that would otherwise be excluded by a stable outer membrane.
1.7 Resistance against CAMPs

1.7.1 Resistance in Gram-positive bacteria

A thorough discussion of CAMPs is not complete without mentioning resistance. One of the most common resistance mechanisms seen in Gram-positive bacteria is the incorporation of D-Alanine into the teichoic acids of the cell wall.\cite{68,120,121} By esterifying the carbohydrate polymers with D-Alanine, the positive charges from the free amines work to neutralise the ‘continuum of anionic charge’ associated with the outer membrane of Gram-positive bacteria (Figure 23).\cite{120,121} A decrease of anionic charge results in the consequential decrease of the initial electrostatic interactions between CAMPs and the bacterial outer membrane.

![Figure 23 - Esterification carbohydrate groups with D-Alanine which results in the introduction of more positive charges thereby neutralising the overall negative teichoic acids.\cite{120}](image)

Other Gram-positive antibiotic resistance mechanisms involve the use of ABC transporters and protection proteins (bacterial cellular peptides). ABC transporters are designed to expel CAMPs once they have inserted into the cell and protection proteins are proposed to repel CAMPs from the surface of the cell membrane.\cite{121}
1.7.2 Resistance in Gram-negative bacteria

The most common form of resistance in Gram-negative bacteria against CAMPs is the replacement of phosphate groups on the Lipid A moiety. A substitution with 4-amino-arabinose (Figure 24a) or phosphoethanolamine (Figure 24b) is effective in reducing the initial electrostatic interaction between CAMPs and the outer membrane.\textsuperscript{9, 13, 62, 68, 96, 97, 122-125} As the substitutions contain free amino groups, there is also a repulsion of the CAMP molecule. Another form of Lipid A alteration involves the addition of a fatty acid chain (Figure 24c) which reduces the permeability of the outer membrane by decreasing fluidity of the LPS layer.\textsuperscript{67, 123, 124}

![Figure 24 - Examples of modifications to Lipid A that result in antibiotic resistant bacteria. (a) Substitution with 4-amino-arabinose (L-Ara4N). (b) Substitution with phosphoethanolamine. (c) Addition of fatty acid chain.\textsuperscript{123, 126}]

Besides altering the chemical structure of Lipid A, Gram-negative bacteria have evolved a resistance mechanism which involves the production of a polysaccharide capsule (also known as a cell envelope) which encases the entire cell. This prevents the approach of CAMPs. \textsuperscript{9, 92, 122, 124}
1.7.3 Resistance is rare

Although a number of resistance mechanisms were outlined above, the incidence of resistance against CAMPs is still comparatively low. A reason for the lower rate of resistance is the target chosen by CAMPs: the bacterial membrane. For bacteria to develop resistance, a cell must change the structure, composition and organisation of its outer membrane which is a ‘costly solution’.\textsuperscript{38, 106} Also, CAMPs are composed of amino acids and have no unique sequence that bacteria can recognise and specifically target.\textsuperscript{38} Most importantly, there is difficulty in developing resistance against a mode of action (detailed above) based on hydrophobic and ionic interactions that causes cell death so quickly.\textsuperscript{90, 105}

1.8 Peptidomimetics

1.8.1 Why develop a CAMP mimic

The previous discussion emphasises the effectiveness of CAMPs in targeting bacteria. However, outside a select few (e.g. Polymyxin B), these peptides are not suitable for clinical use.\textsuperscript{127} There are toxicities associated with current medicinal peptides.\textsuperscript{101} Patients injected intravenously with Polymyxins suffer many side effects such as dizziness, weakness, numbing, peripheral neuropathy and vertigo. Extreme effects like respiratory failure, nephrotoxicity (toxic to kidneys) and neurotoxicity (toxic to the human brain) have been reported.\textsuperscript{9, 20-22, 122, 128, 129} Other peptides such as magainin (7) require high doses to be effective in animal models of infection and unfortunately, the effective concentration of
*m*againin (7) is close to the toxic dose of the peptide making it unsuitable for therapeutic purposes.\textsuperscript{130}

Another reason CAMPs are not suited for clinical use is the high cost of production due to their structural complexity. For example, the synthesis of Polymyxin B (9) has twenty-four synthetic steps. Each synthetic step is efficient (>95% yields) however the overall yield is only 20% (Scheme 1).\textsuperscript{131} As such, fermentation is the method of manufacture. Fermentation itself has many disadvantages. It requires large amounts of biological material which does not directly translate into the production of equal amounts of the desired product. Extensive processing involving multiple extraction and filtration steps is required to remove all traces of unwanted biomaterial. The complex process of

\begin{center}
\textbf{Scheme 1 - Solid phase synthesis of Polymyxin B (9).}\textsuperscript{131}
\end{center}
fermentation results in products that typically cost ten to twenty times the amount of synthetically produced pharmaceuticals.\textsuperscript{132}

Unfortunately, CAMPs also suffer sensitivity to physiological pH and salt concentrations. Human defensins, for example, lose all activity against \textit{E. coli} at physiological salt levels.\textsuperscript{133, 134} The antibacterial activity of \( \beta \)-sheet CAMPs decreases drastically when subject to physiologic concentrations of sodium and potassium.\textsuperscript{135} Effects of pH are idiosyncratic to a particular class of peptides. For example, \( \beta \)-sheet CAMPs perform well in neutral or slightly basic environments however \( \alpha \)-helical CAMPs perform better in acidic media.\textsuperscript{135}

The last reason why CAMPs are unsuitable for clinical use is due to the degradation faced when subjected proteolysis (digestion by enzymes). Often CAMPs suffer a reduction of activity ranging from 50\% to 94\% (10 min to 24 hours) with proteases capable of rendering CAMPs completely useless.\textsuperscript{136-138}

\subsection{1.8.2 Pharmacophore of CAMPs}

A CAMP mimic would be based on a simpler scaffold whilst still embodying the essential characteristics which are: cationic functional groups, hydrophobic residues and a scaffold that ensures structural amphipilicity (Figure 25).

\begin{center}
\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure25}
\caption{Essential characteristics of CAMPS.}
\end{figure}
\end{center}
Mimics should be cheaper to synthesis and be tailored to combat the disadvantages CAMPs currently suffer (toxicity, salt and pH sensitivity, proteolysis). Developing CAMP mimics using a simple scaffold is not a novel concept and the following section introduces some synthetic CAMP mimics.

1.9 CAMP mimics

1.9.1 Mimics based on Cholic Acid

The CAMP mimics based on a cholic acid scaffold have been synthesised by the group of Savage et. al. (Figure 26). Each cholic acid based mimic had a hydrophobic residue, multiple cationic groups and a scaffold (cholic acid) that ensured structural amphiphilicity.

Ceragenin CSA-13 (22) is currently in pre-clinical testing. It has comparable activity to Polymyxin B (9) with respect to Gram-negative bacteria. However, Ceragenin CSA-13 (22) is much more potent against Gram-positive bacteria. For example against *Staphylococcus aureus*, it has an MIC of only 0.40 μg/mL whereas Polymyxin B (9) has an MIC of 26 μg/mL. In the course of developing Ceragenin CSA-13 (22), Savage explored the effect that different cationic groups could have an antibacterial activity by synthesising mimics containing either amines or guanidines. Determinations of minimum inhibitory concentrations
showed that guanidine functionalised cholic acid based CAMP mimics were effective at lower concentrations than amine functionalised counterparts.

1.9.2 Mimics based on a Triaryl Scaffold

![Figure 27 - Structure of triaryl based mimics.](image)

In 2011, Tew et al. synthesised triaryl based CAMP mimics (Figure 27) that exhibited comparable antibacterial activity with magainin (7). The triaryl based CAMP mimics contained two cationic groups and a triaryl scaffold. The analogues showed that the segregation of hydrophilic and hydrophobic groups were necessary for antibacterial activity. Moreover, Tew showed that the use of a rigid scaffold, here the triaryl scaffold, would ensure that hydrophilic and hydrophobic sections remained separated from each other, i.e. structural amphiphilicity. For the triaryl scaffold, there were no differences in MIC values between either guanidine 24 or amine 23 functionalised compounds against *Escherichia coli* and *Klebsiella pneumoniae* (6.25 μg/mL and 12.5 μg/mL respectively).

1.9.3 Mimics based on Calixarene

Mayo et al. was responsible for the development of calixarene based CAMP mimics (Figure 28). Amphipathicity of mimics were maintained by ensuring
large groups composed the lower section of the molecule thereby preventing rotation and isomerisation.

![Calixarene CAMP mimics structure](image)

**Figure 28 – Structures of calixarene CAMP mimics.**

Once again, the key requirements are present in the calixarene based mimics; the hydrophobic groups, multiple cationic groups and a scaffold ensuring structural amphiphilicity. Guanidine 26 performed as well as amine 25 showing excellent activity against several bacterial strains such as *E. coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* with MICs ranging from 0.1 to 1.5 μg/mL.

### 1.9.4 Lipopolyamines

![Lipopolyamine structure](image)

**Figure 29 – Structure of lipopolyamine: 1,3-dioleoyl-oxy-2-(6-carboxyspermyl)-propylamide (DOSPER) 27.**

Lipopolyamines were originally designed to facilitate the transfection of DNA into eukaryotic cells. They proved to be of interest as antibacterials because of low toxicity to humans and approval by the FDA for other medicinal uses. The structure of 1,3-dioleoyl-oxy-2-(6-carboxyspermyl)-propylamide (DOSPER) (27) has the essential characteristics of a CAMP mimic; namely a hydrophobic
section and multiple cationic groups. DOSPER 27 was found to bind to LPS at only one-tenth of the affinity of polymyxins. Nonetheless, lipopolyamine based CAMP mimics show great promise in the development of mimics with low toxicity to humans.

1.9.5 Mimics based on Norbornanes

Research into anion recognition by Pfeffer et. al. led to anion host 28, a compound proven to bind to two phosphate anions (Figure 30a). As Lipid A (5) contains two phosphate groups, anion host 28 was used for the development of norbornane based CAMP mimics (Figure 30b). The norbornane scaffold was ideal due to a high degree of structural rigidity, ease of synthesis by the well known Diels-Alder cycloaddition and easily functionalised at multiple points.

Pfeffer et. al. incorporated an alkyl chain onto the norbornane scaffold using an acetal and substituted guanidines in place of urea as the cationic groups. Whilst a di-ether was initially more attractive than an acetal, extensive attempts using the Williamson ether approach failed entirely. Furthermore, the synthesised
norbornanes were synthesised as racemates and tested as such. This led to di-
guanidine TFA salt 30 which had activity comparable to Polymyxin E.\textsuperscript{145} The
synthesis and evaluation of future generations of norbornane based CAMP mimics form the basis of the current project.

1.10 Project Aims

The norbornane based CAMP mimics proved that novel antimicrobials could be
developed using the norbornane scaffold. However, in the original study, only
two hydrophobic regions and guanidines were explored. Therefore, the overall
purpose of this project was to conduct a systematic exploration of the effect that
variations in (i) hydrophobic and (ii) cationic groups would have on
antibacterial activity.

![General structure of norbornane based CAMP mimics.](image)

Many researchers have demonstrated that the potency of an antibiotic may be
significantly influenced by the composition of the molecule's hydrophobic
region or lack thereof.\textsuperscript{99-101, 104, 115, 146} As such, the first aim of this project was to
generate a library of functionalised norbornane based CAMP mimics with a
range of hydrophobic ‘regions’ (Chapters 2 and 3). A number of hydrophobic
groups comprising of alkyl, aryl and aryl substituted moieties were chosen (Figure 31b).

The second aim of this project was to investigate the influence that different cationic groups exert on antibacterial activity. The two cationic residues employed were primary amines and guanidines (Chapter 2 and 3).

The general structure in Figure 31 was similar to compound 30 synthesised by Pfeffer et al. as a product of a ‘convergent’ methodology. The next aim of this project therefore was to optimise the ‘convergent’ methodology to allow for the easy variation of compound 30 (Chapter 4).

The libraries of norbornane based CAMP mimics will be in the form of chloride salts which is more biologically relevant form than the TFA salt 30. The salts produced as a result of this project will be subject to antibacterial and physicochemical testing (Chapters 5 and 6).
In Chapter 2, first the linear synthesis of amine functionalised norbornane based CAMP mimics was attempted. Inefficiencies and purification problems associated with this approach are highlighted. New methodology explored the late introduction of hydrophobic regions and allowed for the successfully synthesis of a library of protected amines. Problems with deprotection and salt formation identified for certain compounds were discussed.

2.1 Retrosynthesis

2.1.1 Disconnections

In the synthesis of the target compound, key disconnections were identified (Figure 32): acetal formation, Diels-Alder cycloaddition, amide coupling, and the formation of the amine salt which was automatically selected as the final step.

![Figure 32](image_url) - Target amine with key disconnections identified.

The acetal link allows for the easy attachment of a hydrophobic moiety to a cis diol. The acetal functional group was selected in light of many previous failed attempts at di-ether synthesis using the Williamson method. The Diels-Alder
cycloaddition forms the bicyclic norbornane scaffold. Amide couplings can be used to attach the hydrophilic regions to the main scaffold. Salt formation prepares the molecules in a pharmacologically relevant form ready for further assessment.\textsuperscript{148, 149}

### 2.1.2 Retrosynthetic analysis

Salt formation and deprotection would be conducted last. Before that, di-amide couplings would attach the hydrophilic region to the main scaffold. Thus, the scaffold required di-carboxylic acids which can be prepared from esters. Aldehydes and ketones were to be attached to the main scaffold through the formation of an acetal; hence a diol must be present. The diol can be formed through the dihydroxylation of the unsaturated norbornene obtained from the Diels-Alder cycloaddition. Scheme 2 details the retrosynthesis.

Based on the retrosynthesis stated in Scheme 2, the sequence of planned reactions were as follows:

- Diels-Alder cycloaddition between cyclopentadiene \text{31} and dimethyl fumarate \text{32}.
- Upjohn dihydroxylation of the unsaturated di-ester \text{33}.
- Synthesis of any necessary aldehydes.
The formation of acetals between the diol 34 and any number of aldehydes or ketones.

Saponification to form the library of di-carboxylic acids.

Synthesis of mono-protected ethylene di-amine (EDA).

Di-amide couplings between di-carboxylic acids and mono-protected EDA.

The deprotection of protecting groups and the formation of salts.

Having determined a course of action, attention turned to the Diels-Alder cycloaddition.

2.2 Diels-Alder cycloaddition

Otto Diels and Kurt Alder first published their findings on cycloadditions in 1929.\textsuperscript{150} Their work is considered as one of the most useful carbon-carbon bond forming reactions.\textsuperscript{151-153} For such a significant contribution, both chemists shared the Nobel Prize for Chemistry in 1950.

“It is perhaps surprising that this reaction, which is extremely elegant from the chemical point of view, should not have been discovered earlier, for cyclic structures had in fact been known since the sixties of last century. Individual observations had been made but they had been misunderstood or overlooked. The correct interpretation was so simple and yet – a mere twenty years ago – so bold, so like a chemist’s utopian dream, that it was beyond reach.”\textsuperscript{152} (by Professor A. Fredga in the Presentation Speech for the Nobel Prize in Chemistry 1950)
The Diels-Alder cycloaddition involves a reaction between two molecules, a diene and dienophile (Figure 33). The diene, as the name suggests, has a set of conjugated double bonds. The dienophile (diene-loving) has one double bond\textsuperscript{152,153} Figure 33 shows an example of a diene, cyclopentadiene (31) and a dienophile, maleic anhydride (35).

\textbf{Figure 33} – Structures of cyclopentadiene (32) and maleic anhydride (35).

The Diels-Alder cycloaddition is a concerted pericyclic reaction\textsuperscript{153,154} In the transition state, the Highest Occupied Molecular Orbital of diene 34 interacts with the Lowest Unoccupied Molecular Orbital of dienophile 35 resulting in the unsaturated polycyclic compound 36 (Scheme 3). The Diels-Alder cycloaddition forms multiple rings in one step with defined stereochemistry\textsuperscript{154}

\textbf{Scheme 3} – The Diels-Alder cycloaddition between cyclopentadiene (31) and maleic anhydride (35).\textsuperscript{153}

Scheme 3 details a classic example of a diene and dienophile undergoing the Diels-Alder cycloaddition where the \textit{endo} adduct 36 is formed. Although less stable than the \textit{exo} version, compound 36 is preferred due to the interactions between the carbonyl groups in maleic anhydride (35) and the π bond that forms. Even after more than eighty years, the Diels-Alder cycloaddition remains relevant to the modern chemist. One recent example (in 2013) was the total synthesis of protoilludane aryl ester (+)-armillarivin (40) where a Diels-Alder cycloaddition began the assembly of the framework (Scheme 4).\textsuperscript{155} High
pressures promoted the cycloaddition of cis-1,2-dihydrocatechol (37) and cyclopent-2-en-1-one (38) to form Diels-Alder adduct 39 which were further elaborated to obtain the desired natural product 40.

![Scheme 4](image_url)

**Scheme 4 – Synthesis of protoilludane aryl ester (+)-armillarivin (40) which begins with a Diels-Alder cycloaddition.**

In the current project, the Diels-Alder cycloaddition was used to synthesise norbornene 33 (Scheme 5). Freshly cracked cyclopentadiene (31) was added to a solution of dimethyl fumarate (32) in dioxane and stirred for sixteen hours at room temperature. Removal of the solvent *in vacuo* led to the desired norbornene di-ester 33 in near quantitative yields. The reaction was robust and repeated multiple times on a scale of up to sixty grams.

![Scheme 5](image_url)

**Scheme 5 – Synthesis of norbornene di-ester 33.**

The norbornene di-ester 33 is a known compound and its structure was confirmed by the comparison of NMR spectra with literature spectra.156

### 2.3 Upjohn Dihydroxylation

Catalytic use of OsO₄ (41) was first employed by Hofmann for the hydroxylation of alkenes in the presence of secondary oxidants sodium/potassium chlorate.157-159 Secondary oxidants regenerate the active OsO₄ (VII) 41 by oxidising spent OsO₃ 44. Unfortunately, some oxidants led to the over-oxidation of the
unsaturated compound producing unwanted keto or carboxylic acid functionalities.\textsuperscript{158, 160}

Criegee then proved that OsO\textsubscript{4} \textbf{41} could be used stoichiometrically without any secondary oxidants.\textsuperscript{158, 161, 162} Although efficient, the stoichiometric use of OsO\textsubscript{4} \textbf{41} had many disadvantages. There was the high cost and toxicity of OsO\textsubscript{4} \textbf{41}, the difficult reaction conditions with low temperatures of -78 °C and a work up that involved treating the reaction mixture with acid for twenty-four hours. The disadvantages made the use of OsO\textsubscript{4} \textbf{41} unsuitable for large scale reactions.\textsuperscript{160, 163, 164} To combat limitations, new conditions for OsO\textsubscript{4} \textbf{41} mediated dihydroxylations were devised by researchers at the Upjohn Chemical company.\textsuperscript{160}

\begin{center}
\textbf{Scheme 6 – Mechanism of the Upjohn dihydroxylation with cyclopent-1-ene (42).}\textsuperscript{165}
\end{center}

The Upjohn dihydroxylation is now a well known reaction (Scheme 6).\textsuperscript{166, 167} First published in 1976, by VanRheenen, Kelly and Cha (from the Upjohn Chemical company), they observed that using \textit{N}-methylmorpholine \textit{N}-oxide (\textbf{45}) (NMO) stoichiometrically oxidised OsO\textsubscript{3} \textbf{44} to OsO\textsubscript{4} \textbf{41}.\textsuperscript{160} The process provided high yields required only catalytic amount of OsO\textsubscript{4} \textbf{41} while
maintaining low costs and avoiding work up problems requiring only a simple aqueous wash.

The catalytic cycle for the Upjohn dihydroxylation commences with the [3+2] addition of alkene 42 to the OsO₄ (VII) 41 species. Water hydrolyses the cyclic di-ester releasing the desired cis-diol 43 and the spent OsO₃ (VI) 44. Then NMO 45, acting as the secondary oxidant, regenerates OsO₄ (VII) 41 (Scheme 6).¹⁶⁵

Much debate surrounded the [3+2] addition of alkenes to the osmium catalyst. Criegee first proposed a concerted [3+2] route (Scheme 7a).¹⁶², ¹⁶⁸ Sharpless et al. more recently proposed a stepwise [2+2] addition through a metallaoxetane intermediate (Scheme 7b).¹⁶⁸, ¹⁶⁹ However by using density functional theory, a number of groups have showed that there is a higher energy barrier for the [2+2] pathway than the [3+2] pathway.¹⁷⁰-¹⁷² Hence, the first step most likely proceeds by way of the [3+2] concerted route.

In the current project, the Upjohn dihydroxylation was used to form diol 34 (Scheme 8). A solution of norbornene 33, NMO 45 and OsO₄ 41 in a H₂O/acetone solution was allowed to stir for sixteen hours at room temperature. After an aqueous work up, the crude oil was purified using column chromatography affording diol 34 with a yield of 79% (Scheme 8). The
structure of diol 34 was confirmed by comparison with literature reports. It is noted that the cis-diol in 34 was formed exclusively at the exo position as steric interferences disfavour endo approach of OsO4.145, 173

With the successful installation of the diol, attention now turned to the selection of suitable hydrophobic residues. The choice of appropriate aldehydes and ketones as well as the design and synthesis of 'boutique' aldehydes is detailed in the following section.

### 2.4 Hydrophobic component

#### 2.4.1 Selected aldehyde and ketones

A number of aldehydes and ketones were selected for hydrophobic regions in the final norbornane based CAMP mimics. The aldehydes and ketones are shown in Figure 34 and are either alkyl, aryl or a combination thereof.
The aldehydes highlighted in orange (Figure 34) represent compounds not commercially available. These ‘boutique’ aldehydes were chosen to explore the following factors: hydrophobicity (hexadecanal 46); combination of alkyl and aryl functionality (octynyl benzaldehyde 47, octyl benzaldehyde 48 and octyloxy benzaldehyde 49); flexibility of alkyl chain (octynyl benzaldehyde 47 and octyl benzaldehyde 48); polarity (octyl benzaldehyde 48 and octyloxy benzaldehyde 49). The synthesis of each aldehyde using known methods is elaborated in the following sections.

2.4.2 Hexadecanal 46

Hexadecanal 46 was synthesised through oxidation of commercially available hexadecanol 52 using pyridinium chlorochromate (PCC) (51) as the oxidant (Figure 35). In 1977, Corey reported PCC 51 as an excellent, mild and stable alternative to the Collins reagent 50 (known to be unstable and capricious). Corey showed that PCC 51 could successfully oxidise a variety of alcohols to the corresponding carbonyl group.167, 174-176

![figure 35](image.png)

Figure 35 – (a) Structure of the Collins reagent 50 (a solution of chromium trioxide and pyridine in methylene chloride; (b) Structure of pyridinium chlorochromate (51).

The mechanism by which PCC 51 oxidises an alcohol is first order and most textbooks propose that chromium (IV) first attacks the alcohol to form the chromate ester.165, 177 However, in the original publications by Banerji, it is the hydride transfer that is proposed to occur first.172, 175
Banerji et al. proposed two pathways whereby chromium (IV) is reduced to chromium (III). In the first proposed mechanism, a hydride transfer occurs directly leading to the formation of a chromate ester (Scheme 9a). In the second proposed mechanism, the hydride transfer occurs prior to the formation of the chromate ester (Scheme 9b). Banerji concedes that there is no kinetic evidence confirming the proposed PCC 51 oxidation steps. However, the reactions show similar kinetics to chromic acid oxidations where chromate ester formation is well established.

In the current project, PCC 51 was used to convert hexadecanol 52 to hexadecanal 46 (Scheme 10). A solution of hexadecanol 52 and PCC 51 was dissolved in DCM and stirred for sixteen hours at room temperature. After filtration through silica and Celite®, the filtrate was collected and the solvent removed in vacuo resulting in a solid with an excellent yield of 94%. Hexadecanal 46 is a known compound and its structure was confirmed by comparison of proton NMR spectra to published data.
2.4.3 Octynyl benzaldehyde 47

A literature method for the synthesis of octynyl benzaldehyde 47 involved the Sonogashira-Hagihara cross coupling reaction. The first example of this cross coupling was published in 1975 by Sonogashira and Hagihara where they explored the application of copper-catalysed alkynylation of palladium complexes as an extension of the Stephens-Castro reaction.

![Scheme 11 – Sonogashira-Hagihara cross coupling catalytic cycle](image)

The accepted mechanism for the Sonogashira-Hagihara cross coupling involves two catalytic cycles (Cycles 1 and 2, Scheme 11). In Cycle 1, oxidative addition occurs between the palladium catalyst (0) and the R-X species (oxidative addition, Scheme 11). Next, transmetalation of the copper acetylide formed by Cycle 2 occurs (transmetalation, Scheme 11). Finally reductive elimination produces the desired alkyne and regenerates the palladium catalyst (reductive elimination, Scheme 11). In Cycle 2, a proposed π-alkyne copper complex forms which increases the acidity of the alkyne proton allowing the base greater ease with deprotonation (Scheme 11).
There are many reasons why the Sonogashira-Hagihara cross coupling has become a favoured tool of synthetic chemists. Most couplings proceed at either room temperature or slightly above. The use of shock sensitive and explosive copper acetylides are avoided with the catalytic use of copper (I) salt which is commercially available and stable. Solvents and reagents do not need to be anhydrous and often the base acts as the solvent. The cross coupling works well (yields of 80%-90%) on small and large (>100 g) scales tolerating a wide variety of functional groups.\textsuperscript{153, 154} Many groups have sought to extend and evolve the scope of this reaction however, the conditions first stated by Sonogashira and Hagihara in 1975 remain most popular to this day.\textsuperscript{184}

In this project, the Sonogashira-Hagihara cross coupling was used to couple \textit{para}-bromobenzaldehyde (53) and 1-octyne (54) to synthesise octynyl benzaldehyde 47. A mixture of \textit{para}-bromobenzaldehyde (53), 1-octyne (54), bis(triphenylphosphine)palladium(II) dichloride and copper iodide in Et\textsubscript{3}N was stirred at 50 °C for sixteen hours.\textsuperscript{181} After work up, the desired octynyl benzaldehyde 47 was obtained in an excellent yield of 97% without further purification (> 95% purity as determined by \textsuperscript{1}H NMR spectroscopy).

\begin{center}
\textbf{Scheme 12 – Sonogashira-Hagihara cross coupling between \textit{para}-bromobenzaldehyde (53) and 1-octyne (54) producing octynyl benzaldehyde 47.}\textsuperscript{181}
\end{center}

The structure of octynyl benzaldehyde 47 was confirmed by comparison of spectral data with that in the literature.\textsuperscript{181}
2.4.4 Octyl benzaldehyde 48

To synthesise octyl benzaldehyde 48, catalytic hydrogenation using Pd/C was selected to reduce octynyl benzaldehyde 47. Catalytic hydrogenation was chosen as it is chemoselective for alkenes and alkynes over carbonyl groups.153

A study conducted by Bond and Wells investigated the reductive activity of alumina-supported metal catalysts.186, 187 The metals examined in the study were palladium, rhodium, platinum, iridium and osmium. Bond and Wells found that palladium performed the best when conducting hydrogenations.

In the current project, a suspension of Pd/C in a solution of octynyl benzaldehyde 47 in EtOAc was stirred for sixteen hours at room temperature under a hydrogen at 1 atm (Scheme 13).181 The crude brown oil obtained after filtration was analysed using proton NMR spectroscopy and was identical to starting material.

The first use of Pearlman’s catalyst [Pd(OH)$_2$/C] (palladium hydroxide on carbon) was reported by Pearlman in 1967 and it is considered more successful in conditions where Pd/C fails.188, 189 A suspension of Pearlman’s catalyst in a solution of octynyl benzaldehyde 47 in EtOAc was stirred for sixteen hours at room temperature under a hydrogen atmosphere (Scheme 13). The reaction
mixture was filtered through Celite®, the filtrate collected and the solvent removed in vacuo. Octyl benzaldehyde 48 was obtained in an excellent yield of 93%. The structure of octyl benzaldehyde 48 was confirmed by comparison with literature.181

2.4.5 Octyloxy benzaldehyde 49

Williamson ether synthesis

The Williamson ether synthesis was first published in 1852 by Alexander W. Williamson where he accurately identified the formula of diethyl ether from a reaction using sodium ethoxide and ethyl chloride.190 The Williamson ether synthesis is when alkoxides react (SN2) with alkyl, allyl or benzyl halides to produce ethers.154, 190

![Scheme 14 - General mechanism of the Williamson ether synthesis.](image)

In the current project, ethyl 4-hydroxybenzoate (56), iodoctane (55) and K2CO3 in a solution of DMF was stirred at room temperature for sixteen hours.191 After an aqueous work up, the crude oil was purified using column chromatography with a gradient elution system. The desired benzyl ester 58 was isolated in an excellent yield of 85% (Scheme 15). The structure of compound 57 was confirmed by comparison with literature.192

![Scheme 15 - Williamson ether synthesis between ethyl 4-hydroxybenzoate (56) and iodoctane (55).](image)
**Reduction**

The reduction of an ester to the corresponding alcohol is commonplace and there are a variety of reducing agents. One example is sodium bis(2-methoxyethoxy)aluminium hydride (58) which is commercially available as a solution in toluene, known as Vitride® or Red-Al® (Figure 36).

![Figure 36 - Structure of sodium bis(2-methoxyethoxy)aluminium hydride](image)

In the current project, Red-Al® 58 was employed to reduce octyloxy benzyl ester 57 to the desired benzyl alcohol 59. Due to the exothermic nature of the reaction, the solution of ester 57 and Red-Al® 58 in toluene was stirred at 0 °C for two hours and then allowed to warm to room temperature and stirred for a further thirty-two hours. After quenching and an aqueous work up, the desired benzyl alcohol 59 was obtained in a yield of 89% with no requirement for further purification (>95% purity as determined from ¹H NMR spectroscopy). The structure of benzyl alcohol 59 was confirmed by comparison with literature.¹⁹⁴

![Scheme 16 - Reduction of ester 56 to alcohol 59 using Red-Al® 58](image)

**Oxidation**

The benzyl alcohol 59 was subjected to oxidation by PCC 51 to obtain the desired aldehyde 49 (Scheme 17). The principles of oxidation by PCC 51 have been covered in detail on page 45.
Scheme 17 – Oxidation of benzyl alcohol 59 to obtain desired aldehyde 49.

A solution of benzyl alcohol 59 and PCC 51 was dissolved in DCM and stirred for sixteen hours. The reaction mixture was filtered through a frit of silica and Celite©. The filtrate was collected and the solvent removed in vacuo to give the desired 'boutique' aldehyde 49 in a yield of 95%. The structure of aldehyde 49 was confirmed by comparison with literature.196

The successful synthesis of octyloxy benzaldehyde 49 meant that all desired aldehydes were available. Focus now turned to the acetal formation.

### 2.5 Acetal formation

Cyclic acetals are most commonly used as protecting groups for aldehyde and ketones.167, 197 The acetal functional group is useful being easily introduced by an acid catalyst. When formed, the acetal is stable to bases, nucleophiles and most oxidants.197 The mechanism for the formation of a cyclic acetal is well established (Scheme 18). With a catalytic amount of acid present, the aldehyde is protonated. The resulting oxonium ion is then attacked by an alcohol group. Deprotonation furnishes the hemiacetal intermediate 62. The tertiary alcohol is then protonated which eventually generates water as a by-product (Scheme 18). The unstable oxonium ion 63 is then attacked by the remaining alcohol group. Deprotonation furnishes the desired acetal. Either a drying agent or Dean Stark apparatus is used to remove water ensuring the equilibrium favours the products.
In the first attempt of the current project, a suspension of MgSO₄ in a solution of diol 34, hexadecanal 46 and a catalytic quantity (0.1 eq.) of TFA in CHCl₃ was stirred for sixteen hours at room temperature. After filtration, the filtrate was collected and the solvent removed in vacuo. The crude brown oil was purified using column chromatography which afforded the desired di-ester 64 in a yield of 30% (Scheme 19). Confirmation of the structure of di-ester 64 was accomplished using proton and carbon NMR spectroscopy, IR and mass spectrometry.

The low yield was unacceptable and so steps were taken to increase the efficiency of the reaction. Many research groups have demonstrated that microwave irradiation has a positive effect on the efficiency of acetal formations. To that effect, a number of time based optimisation reactions were conducted. Ultimately, the optimised reaction conditions were to subject two equivalents of aldehyde, diol 34 and TFA (0.1 eq.) in CHCl₃ to microwave irradiation for twenty minutes at 80 °C. Using the microwave procedure, yields of di-esters ranged from 58% - 94% (Table 3). The structure of each di-ester was confirmed by proton and carbon NMR spectroscopy, IR and mass spectrometry.
Table 3 – Yields of di-esters 64 to 75.

<table>
<thead>
<tr>
<th>No.</th>
<th>Yield</th>
<th>No.</th>
<th>Yield</th>
</tr>
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<tbody>
<tr>
<td>64</td>
<td>93%</td>
<td>70</td>
<td>58%</td>
</tr>
<tr>
<td>65</td>
<td>94%</td>
<td>71</td>
<td>74%</td>
</tr>
<tr>
<td>66</td>
<td>84%</td>
<td>72</td>
<td>83%</td>
</tr>
<tr>
<td>67</td>
<td>75%</td>
<td>73</td>
<td>67%</td>
</tr>
<tr>
<td>68</td>
<td>79%</td>
<td>74</td>
<td>87%*</td>
</tr>
<tr>
<td>69</td>
<td>71%</td>
<td>75</td>
<td>65%</td>
</tr>
</tbody>
</table>

* Reactions conditions: diol 34, TFA (0.1 eq.) and MgSO₄ in neat acetone for twenty minutes at 60 °C

Figure 37 has an example of NMR spectra obtained in the confirmation of the structures of di-ester 64 - 75. As a result of the formation of the acetal, there is a distinctive signal in both proton and carbon spectrum that indicates the presence of the acetal proton and carbon (highlighted in Figure 37). Generally, the acetal proton appears around δ6.0 to δ5.0 and the acetal carbon around δ100.0 to δ110.0. Having confirmed the successful synthesis of each of the di-esters, attention now turned to saponification.
In a process known as saponification, esters are hydrolysed under alkaline conditions to make carboxylic acids.\(^\text{153}\) The process is so named as it is the method traditionally used to make soap. Treating oils with sodium hydroxide produces the principal component of soap: sodium stearate.\(^\text{153}\) The mechanism of saponification is well known and described in Scheme 20.

In this project, saponification was used to convert di-esters 64 - 75 to dicarboxylic acids 76-86 (Table 4). Each di-ester was treated with NaOH in EtOH for sixteen hours. After removing EtOH in vacuo and an aqueous acidic work up,
Each di-carboxylic acid was obtained as a solid with yields between 70% - 96% (Table 4).

<table>
<thead>
<tr>
<th>No.</th>
<th>Yield</th>
<th>No.</th>
<th>Yield</th>
</tr>
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<tbody>
<tr>
<td>76</td>
<td>87%</td>
<td>82</td>
<td>89%</td>
</tr>
<tr>
<td>77</td>
<td>88%</td>
<td>83</td>
<td>96%</td>
</tr>
<tr>
<td>78</td>
<td>70%</td>
<td>84</td>
<td>89%</td>
</tr>
<tr>
<td>79</td>
<td>88%</td>
<td>85</td>
<td>96%</td>
</tr>
<tr>
<td>80</td>
<td>89%</td>
<td>86</td>
<td>75%</td>
</tr>
<tr>
<td>81</td>
<td>92%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Each di-carboxylic acid was fully characterised using proton and carbon NMR spectroscopy, IR, mass spectrometry and melting point. Figure 38 contains an example of proton spectra collected. No signals between δ3.25 and δ4.0 ppm indicated saponification was successful in cleaving the ester groups. Furthermore, the acetal at δ5.54 integrating for one hydrogen was still intact.
Having attached the hydrophobic residues and prepared the scaffold for the di-amide couplings, preparation of mono-protected EDA commenced.

2.7 Mono-protected EDA

For the synthesis of mono-protected EDA, two protecting groups were chosen; the benzyl carbamate (Cbz) and *tert*-butyl carbamate (Boc). These are popular amino protecting groups and are likely to be cleaved in high yields using acid (Boc) or hydrogenolysis (Cbz). A brief description and the process by which these groups are attached follows.

2.7.1 Mono-protection of EDA (Cbz)

The use of Cbz as a protecting group for amines was first performed in 1932 by Bergmann and Zervas. Since then, Cbz has been used extensively; being easy to install using readily available Cbz-Cl, stable in both aqueous base and acid and easily cleaved in high yield through catalytic hydrogenolysis.

In the current project, Cbz was used to mono-protect EDA. Replicating literature conditions, a concentrated solution of benzyl chloroformate in DCM was added drop wise to a dilute solution of EDA cooled to 0 °C over ninety minutes (Scheme 21). With a yield of 93%, Cbz-EDA was
isolated as a pale yellow solid. No further purification was conducted and the structure of Cbz-EDA 89 was confirmed by comparison with literature spectra.204

Scheme 21 – The mono-protection of EDA 87 using benzyl chloroformate 88.204

2.7.2 Mono-protection of EDA (Boc)

The Boc protecting group is also used extensively in peptide synthesis with stability to bases, most nucleophiles and catalytic hydrogenation/hydrogenolysis.197, 203, 205 The Boc group is easily cleaved by acid and the deprotected product generally forms a salt with the acid counter-ion (The importance of salt formation is elaborated later in this thesis.).197 The protection of a free amine with the Boc protecting group occurs by the reaction of a free amine with commercially available di-tert-butyl dicarbonate (90) (Boc₂O).

In the current project, Boc₂O 90 was used to mono-protect EDA 87 (Scheme 22). Replicating literature conditions, a dilute solution of Boc₂O 90 in DCM was added drop wise to a concentrated solution of EDA 87 in DCM stirring at 0 °C. The desired compound 91 was obtained in a yield of 90% (Scheme 22).
The structure of Boc-EDA 91 was confirmed by comparison with literature spectra.206

2.8 Di-amide coupling

2.8.1 Trial di-amide couplings

The choice to use an amide bond to connect the hydrophilic sections to the norbornane scaffold was simple. Amide bonds are one of the most important chemical connections in nature being the bond that links amino acid building blocks to construct proteins.153, 206

An amide bond is built from a carboxylic acid and an amine. Although the reaction can occur spontaneously at high temperatures (over 200 °C and water is eliminated), the conditions have the potential to degrade other functional groups.207 To facilitate amide coupling at milder temperatures, a coupling agent was used: \( N\)-(3-dimethylaminopropyl)-\(N\)'-ethylcarbodiimide hydrochloride (EDCI) (92) (Figure 39). Coupling agent 92 acts on the carboxylic segment converting the 'hydroxyl' moiety into a better leaving group.207
In peptide chemistry, EDCI 92 is a popular carbodiimide coupling agent.\textsuperscript{167} The initial coupling agents of the carbodiimide class, such as dicyclohexylcarbodiimide (DCC), were first synthesised in 1955 and are still used today.\textsuperscript{207} Use of early DCC sometimes led to purification problems due to the dicyclohexylurea by-product.\textsuperscript{207} Sheehan \textit{et. al.} developed water soluble carbodiimides EDCI 92 with a rationale that an aqueous work up would remove any by-products formed.\textsuperscript{167, 208, 209} The ease of work up has resulted in EDCI 92 becoming one of the most popular carbodiimide coupling agents today.

When coupling a carboxylic acid and amine using carbodiimides, the first step involves the formation of the $O$-acylurea 93 (Scheme 24).\textsuperscript{167, 207} The reaction then potentially diverges into three pathways. In the first pathway, the $O$-acylurea 93 is attacked by a carboxylic acid and results in carboxylic acid anhydride 96. The carboxylic acid anhydride 96 is susceptible to attack by the desired amine affording the required amide and soluble urea by-product 95.
(Pathway 1, Scheme 24). The second pathway results in the desired amide as well as the soluble urea by-product 95 (Pathway 2, Scheme 24). Finally, the third pathway results in the formation of an undesired N-acylurea product 94.

To avoid the disadvantage of N-acylurea formation, additives such as 1-hydroxy-1H-benzotriazole (97) (HOBt) were introduced (Scheme 25). In an amide coupling involving EDCI 92 and HOBt 97, HOBt 97 reacts with the O-acylurea 93 to give the O-benzotriazole 'active' ester 98. Nucleophilic attack by the amine regenerates the HOBt 97 catalyst and furnishes the desired amine (Scheme 25).

Since their development, there have been some interesting applications of EDCI 92 and HOBt 97. One such example was in the synthesis of oxadiazoles. Das and Evans et. al. sought to integrate the oxadiazole moiety into Combretastatin derivatives. Combretastatin A4 (99) is a broad spectrum anti-cancer drug that unfortunately loses potency when the cis double bond isomerises to the trans-configuration (Scheme 26a). Das and Evans determined that by substituting the double bond in Combretastatin A4 (99) with an oxadiazole, the derivatives would not only maintain biological activity but likely increase in potency. To install the oxadiazole moiety, substrate 101 was coupled to carboxylic acid 100 using EDCI 92 and HOBt 97 (Scheme 26). After thirty
minutes, when the O-acylated product 102 had formed, the reaction was heated to reflux prompting cyclisation and affording the desired oxadiazole 103.213

Scheme 26 – (a) Structure of Combretastatin A4 99. (b) Synthesis of Combretastatin analog 103.213

Use of microwaves with coupling agents has been shown to give good yields.214 In the current project the trial amide coupling reactions using Cbz-EDA 89 were subjected to microwave irradiation. All trial reactions were conducted at 30 minutes, using MeCN, CHCl₃ or DMF as solvent and temperatures ranging from 50 °C to 100 °C. Other coupling agents gave lower yields and mono-coupled products. EDCI 92 produced the cleanest samples.

Scheme 27 – Reactions conditions in trial amide couplings that obtained the best yield.

After conducting a number of trial reactions, the best conditions obtained used microwave irradiation of di-carboxylic acid 77, Cbz-EDA 89, EDCI 92 and HOBt
in DMF for 30 minutes at 50 °C which gave the desired di-amide 104 in a yield of 41%. Although the yield was modest, it was determined sufficient for the synthesis of the amine functionalised norbornanes. These reaction conditions were applied to a selection of di-carboxylic acids.

2.8.2 Synthesis of Cbz-protected Di-amines

To synthesise the Cbz-protected di-amines, Cbz-EDA 89 was added to a solution of the relevant di-carboxylic acid, EDCI 92 and HOBT 97 in DMF. The reaction vessel was irradiated for 30 minutes at 50 °C. The Cbz-protected amines were isolated following purification by column chromatography and the yields are listed in Table 5.

Table 5 – Yields of Cbz-protected amines.

<table>
<thead>
<tr>
<th>No.</th>
<th>Isolated Yield</th>
<th>No.</th>
<th>Isolated Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>104</td>
<td>41%</td>
<td>106</td>
<td>43%</td>
</tr>
<tr>
<td>105</td>
<td>65%</td>
<td></td>
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</tbody>
</table>

Only modest yields were obtained in the synthesis of Cbz-protected amines (41% - 65%). It should be noted that when the reactions were repeated, the methodology lacked robustness, there were times yields were very low and purification proved difficult. Each compound in Table 5 was novel and characterised by proton and carbon NMR spectroscopy, IR, mass spectrometry and melting point.
2.8.3 Synthesis of Boc-protected Di-amines

In the synthesis of Boc-protected amines, di-carboxylic acids 81 and 84 were selected for coupling with Boc-EDA 91. Di-carboxylic acid 84 was chosen specifically as the hydrophobic moiety present was suspected to be not orthogonal to the Cbz functional group. If a Cbz group were to be attached to di-carboxylic acid 84, when faced with deprotection, the benzyl ether section of the hydrophobic residue would be cleaved in addition to the Cbz protecting groups. Hence, the Boc protecting group was the only option for this analogue.

Figure 40 – Structure of di-carboxylic acids 81 and 84.

For the amide couplings using Boc-EDA 91, each di-carboxylic acid was reacted according to the previously used conditions, i.e. solutions of the relevant di-carboxylic acid, Boc-EDA 91, EDCI 92 and HOBt 97 in DMF were subjected to microwave irradiation for 30 minutes at 50 °C. Once again, only modest yields were obtained and the trend of reaction unpredictability continued.

Table 6 – Yields of Boc-protected amines.

<table>
<thead>
<tr>
<th>No.</th>
<th>Yield</th>
<th>No.</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>107</td>
<td>57%</td>
<td>108</td>
<td>36%</td>
</tr>
</tbody>
</table>
2.9 New Strategy

Whilst successful, the lack of reproducibility was concerning and the synthetic methodology was re-evaluated in light of several disadvantages. The first was the low yields obtained from amide couplings however, the biggest disadvantage was the introduction of hydrophobicity early in the synthetic methodology (Figure 41). Acetal formation was the trigger point for the diverse set of compounds most of which required column chromatography for purification in subsequent steps.

[Diagram of compound pathways]

Figure 41 – Cartoon depicting trigger point for the diverse set of compounds.

Ideally, a new approach would have variation introduced as late in the synthetic pathway as possible (Scheme 28). As with the previous methodology, the scaffold would be assembled with a Diels-Alder cycloaddition. Different to the initial approach, norbornene di-ester 33 would be saponified and the di-amide would then be formed. Upjohn dihydroxylation would give the diol 34. Variation would then be introduced by the formation of an acetal with the aldehydes or ketones. The library of protected amines would then be ready for the final step of de-protection and salt formation.
To construct the desired di-amines, the sequence of planned reactions was as follows (Scheme 28):

- Diels-Alder cycloaddition to give norbornene di-ester 33.
- Saponification to obtain norbornene di-carboxylic acid 109.
- Di-amide coupling with mono-protected EDA.
- Upjohn Dihydroxylation.
- Formation of the acetal with either aldehydes or ketones.
- The protected amines are then subjected to de-protection and salt formation to finally obtain the library of amines.

The first reaction of the alternative approach involved the Diels-Alder cycloaddition between cyclopentadiene 31 and dimethyl fumarate 32. The cycloaddition to form di-ester 33 has already been discussed on page 39.

2.10 Saponification

Saponification was successful to form norbornene di-carboxylic acid 109 as a brittle white solid with a yield of 89% (Scheme 29). Confirmation of the structure of norbornene di-carboxylic 109 by comparison of spectral data.
2.11 Di-amide couplings

For multi-gram di-amide couplings, di-carboxylic acid 109, Boc-EDA 91, EDCI 92 and HOBt 97 in DMF was stirred at 50 °C for sixteen hours using conventional heating. (Scheme 30).

After slow precipitation and collection, norbornene 110 was isolated as a white solid in excellent yield of 87%. Already, the new approach appeared more effective. The proton NMR spectrum of norbornene 110 clearly indicated the successful formation of amide bonds evidenced by the presence of the N-H protons at δ7.02 and δ6.84 (Figure 42). The peaks at δ6.20 assigned to the alkene were unaffected by the di-amide coupling.

Figure 42 – Proton NMR spectrum of norbornene 110 in CDCl₃.
Further characterisation was conducted through analysis carbon NMR spectroscopy, IR, mass spectrometry and melting point.

### 2.12 Upjohn Dihydroxylation

As the earlier dihydroxylation reaction conditions were conducted on a multi-gram scale with excellent success, the same conditions were used for the dihydroxylation of norbornene di-amide **110** (Scheme 31).

![Scheme 31 – The dihydroxylation of norbornene 139.](image)

Compound **110** and NMO **45** were dissolved in a solution of H₂O/acetone. Then OsO₄ **41** was added and the solution allowed to stir at room temperature for sixteen hours. After an aqueous work up, diol **111** was isolated in an excellent yield of 88% as beige solid. Analysis of the new compound using proton NMR spectroscopy confirmed the lack of alkene functionality (Figure 43). Further characterisation of diol **111** was performed using carbon NMR spectroscopy, IR, mass spectrometry and melting point.

![Figure 43 – Proton NMR spectrum of diol 111 in CD₂OD.](image)
The synthesis of diol 111 represented the last step in the alternative synthetic strategy before the introduction of hydrophobicity. So far this methodology had performed very well; in the four steps thus far, there had been an overall yield of 68%. Each of the steps had been conducted on at least a five gram scale with excellent yields at each point. Very important was the lack of column chromatography for the purification of compounds 109 to 111. Hence, the formation of the acetal and the attachment of the hydrophobic moieties commenced.

## 2.13 Acetal formation

### 2.13.1 With tosic acid

One of the important aspects of the acetal formation is that it typically occurs using acidic conditions.\(^\text{167}\) Unfortunately, when forming an acetal with diol 111, treatment with acid may lead to the deprotection of Boc groups. Trifluoroacetic acid is an acid regularly chosen for the deprotection of Boc, hence the previously optimised reaction conditions for the formation of an acetal (which employed TFA) were not ideal.\(^\text{197}\) It was essential therefore to select an acid that could catalyse the formation of the acetal but still be weak enough to preserve the Boc protecting groups. Consequently, \(p\)-toluenesulfonic acid (tosic acid or TsOH) 112 was selected (Figure 44).

![Figure 44 - Structure of \(p\)-toluenesulfonic acid mono-hydrate (112).](image-url)
Considered a weaker acid than TFA, Tosic acid 112 has been used for the formation of acetals.\textsuperscript{153} Trifluoroacetic acid has a $pK_a$ range of 0.23 in water at 25 °C.\textsuperscript{216–218} However, Tosic acid 112 has $pK_a$ values of 6.20 and 8.5 (in MeCN and acetic acid respectively).\textsuperscript{219, 220} With a milder acid in hand, trials of acetal formations were performed (Table 7).

**Table 7 – Acetal formations with tosic acid 111.**

<table>
<thead>
<tr>
<th>No.</th>
<th>Yield</th>
<th>No.</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>113</td>
<td>0%</td>
<td>114</td>
<td>86%</td>
</tr>
<tr>
<td>108</td>
<td>0%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The reaction solvent of choice here was CHCl$_3$ having worked well in previous acetal formation reactions. The reactions were conducted using a conventional hotplate and not in a laboratory microwave. In each of the three trials listed in Table 7, a suspension of MgSO$_4$ in a solution of the relevant aldehyde or ketone, tosic acid 112 and the diol 111 in CHCl$_3$ was stirred for sixteen hours at 40 °C. Unfortunately, in only one instance was the desired product obtained, namely acetal 114. The reaction conditions were unsuccessful for products 113 and 108 therefore further optimisation was conducted.

### 2.13.2 Optimisation

In the current set of optimisation reactions, there was a decision to concentrate on two solvent/acid combinations: toluene/tosic acid 112 and MeCN/oxalic acid. These solvent/acid combinations were the preferred conditions in
literature for the formation of acetals in the presence of Boc-protected amines.221-223 One example was published by Pandey et. al.221 In their efforts at devising a total synthesis of Maritidine (115), it was necessary to form an acetal in the presence of a boc protected amine on compound 116. Formation of the acetal was achieved successfully using tosic acid 112 in benzene at reflux for ten hours with a yield of 80% (Scheme 32).221

![Scheme 32](image)

In the current project, a number of reactions were attempted using CHCl₃ as the co-solvent at different temperatures. The optimal reactions conditions were to stir the diol 111, aldehyde and oxalic acid in MeCN/CHCl₃ for sixteen hours at 50 °C which achieved a yield of 52%. Parallel to conducting the optimisation reactions, a gradient elution system was devised that allowed for the efficient purification of the desired product in under an hour. With the last of the issues surrounding the formation of the acetal solved, the synthesis of the library of Boc-protected amines began.
2.14 Synthesis of Boc-protected amines

In this section of the project, each aldehyde or ketone was added to a suspension of MgSO₄ in a solution of diol 111 and oxalic acid (1 eq.) in MeCN/CHCl₃. The reaction mixture was allowed to stir for sixteen hours at 50 °C. After filtration and removal of solvent, the crude solids were purified using column chromatography with gradient elution. All the boc-protected amines were obtained as solids (Table 8).

Table 8 – Yields from the acetal formation in the synthesis of the Boc-protected amines.

<table>
<thead>
<tr>
<th>No.</th>
<th>Yield</th>
<th>No.</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>113</td>
<td>40%</td>
<td>122</td>
<td>73%</td>
</tr>
<tr>
<td>118</td>
<td>91%</td>
<td>123</td>
<td>72%</td>
</tr>
<tr>
<td>119</td>
<td>52%</td>
<td>108</td>
<td>85%</td>
</tr>
<tr>
<td>120</td>
<td>51%</td>
<td>124</td>
<td>75%</td>
</tr>
<tr>
<td>121</td>
<td>91%</td>
<td>114</td>
<td>59%</td>
</tr>
<tr>
<td>107</td>
<td>25%</td>
<td>125</td>
<td>61%</td>
</tr>
</tbody>
</table>

The yields (Table 8) vary from 25% to 91%. Generally, the larger the hydrophobic region, the lower the yields as demonstrated by protected amines.
113 and 107 whose hydrophobic moieties are a pentadeca carbon chain and a pyrene moiety respectively. Characterisation of each product was performed using proton and carbon NMR spectroscopy, IR, mass spectrometry and melting point. Protected di-amine 125 was selected and subjected to a thorough NMR analysis. The information gleaned was used for characterising the rest of the Boc-protected amines.

2.15 NMR analysis of 125

The Boc-protected amine 125 had a hydrophobic moiety comprised of fluorine para-substituted on the aromatic ring. The following NMR experiments were conducted: proton, COSY, carbon, HSQC and HMBC NMR spectroscopy and each are discussed in detail.

2.15.1 Proton NMR (1D)
The proton NMR spectrum of protected amine 125 was conducted in CDCl$_3$ at 500 MHz. To assist with preliminary assignments of the molecule, a publication by Bakkeren et. al. provided some hints. Bakkeren synthesised and characterised Diethyl exo,exo-5,6-dihydroxy[2.2.1]heptane-trans-2,3-dicarboxylate (126).

Based on the information sourced from norbornane 126 characterised by Bakkeren et. al., the doublets at $\delta$1.91 and $\delta$1.51 correspond to the bridge head protons $H_j$ and $H_k$. The doublets at $\delta$4.29 and $\delta$4.09 correspond to the protons $H_e$ and $H_i$ which are at a substantially higher chemical shift to the rest of the norbornane scaffold due to the deshielding by oxygen atoms forming the acetal. The rest of the norbornane scaffold corresponds to the signals in the range $\delta$2.86 – $\delta$3.8. Finally, the eight ethyl protons correspond to the overlapping signals from $\delta$3.37 – $\delta$3.23. Figure 48 contains the definite assignments of the protons in amine 125.

Figure 47 – Structure of diethyl exo,exo-5,6-dihydroxy[2.2.1]heptanes-trans-2,3-dicarboxylate (126).

Figure 48 – Structure of amine 125 with some proton assignments.
2.15.2 Correlation Spectroscopy NMR (COSY 2D)

The COSY NMR was collected in CDCl$_3$ at 500 MHz. In the two-dimensional COSY NMR, both axes and the diagonal correspond to one-dimensional proton NMR spectra. The mirrored cross peaks (off the diagonal) indicate couplings between protons that are two and three bonds apart. Long range couplings may be seen from interactions through π-electrons or from W-couplings.$^{224}$

Cross peaks were observed between signals δ7.40 and δ5.47. The interaction indicates $3J_{H-H}$ coupling between $H_l$ and $H_g$ (Figure 49). No interaction was seen between δ6.99 and δ5.47 confirming that δ6.99 corresponds to $H_m$ which is too far from $H_g$ to exhibit cross peaks.

Cross peaks were observed between δ1.91 and δ1.51 which confirms the bridge protons are responsible for these signals. The bridge protons are geminal protons in different chemical environments. $H_j$ experiences greater deshielding from the acetal functional group and would therefore be assigned to the signal δ1.91. The proton $H_k$ is assigned to δ1.51 which experiences less deshielding from the single exo amide. Using the geminal Karplus correlation, the $2J_{H-H}$ coupling of 10 Hz indicates that $H_k$ and $H_j$ exist at angle of approximately 100° - 110° to each other (acceptable for tetrahedral carbon $\cong$ 109.5°).$^{225}$

![Figure 49](image-url)
Cross peaks were observed between $\delta_{1.51}$ and both signals $\delta_{4.30}$ and $\delta_{4.09}$. The interaction confirms that $H_k$ is a bridge head proton exhibiting $W$-coupling with both $H_e$ and $H_i$ (Figure 51a). Furthermore, cross peaks indicating more $W$-coupling were observed between $\delta_{1.91}$ and $\delta_{2.60}$ but not $\delta_{2.86}$ proving that $\delta_{2.60}$ is $H_c$. Therefore $H_b$ would be $\delta_{2.86}$ which would not show an interaction with $H_j$ being in the $exo$- position (Figure 51b).

Looking at $\delta_{2.86}$, cross peaks were observed with $\delta_{2.60}$ and $\delta_{2.39}$ but not with $\delta_{2.65}$. The interactions therefore are $3^J_{H-H}$ coupling of $H_b$ with $H_c$ and $H_a$. Previously assigning $\delta_{2.60}$ to $H_c$, means $H_a$ assigned to $\delta_{2.39}$ (Figure 52a). This would therefore leave $H_d$ to be assigned to $\delta_{2.65}$. Cross peaks were expected between $\delta_{2.60}$ and $\delta_{2.65}$. However, none were observed indicating that the vicinal protons $H_c$ and $H_d$ are at approximately 90 ° angle to each other (using the vicinal Karplus correlation) which is as expected for the norbornane framework.\textsuperscript{225}

---

Figure 50 – Cross peaks observed between $\delta_{1.91}$ and $\delta_{1.51}$.

Cross peaks were observed between $\delta_{1.51}$ and both signals $\delta_{4.30}$ and $\delta_{4.09}$.
Cross peaks were observed between $\delta_{2.65}$ and $\delta_{4.09}$ therefore indicating that $H_e$ is responsible for the signal at $\delta_{4.09}$. There were no cross peaks between $\delta_{4.30}$ and $\delta_{2.39}$ confirming the absence of symmetry across the norbornane scaffold due to the endo-, exo- orientation of the hydrophilic arms. There were also no cross peaks to allow for the assignment of the $-NH$ protons. Figure 53 details the assignments accomplished after analysis of the COSY NMR.

**2.15.3 Carbon NMR and Heteronuclear Single Quantum Coherence NMR (HMQC)**

The carbon NMR and HMQC spectra were collected in CDCl$_3$ at 500 MHz. The HMQC NMR spectrum allows for the identification of carbons directly bonded to hydrogens. Figure 54 details the assignments that were made based on the analysis of the carbon and HMQC NMR spectra.
The HSQC spectrum did not allow for assignments of quaternary carbons. To assign those carbons, analysis of the HMBC spectrum was required.

2.15.4 Heteronuclear Multiple Bond Coherence NMR (HMBC)

The HMBC spectrum was collected in CDCl₃ at 500 MHz. The HMBC shows long range C-H coupling between two and three bonds and allows for quaternary carbons to be assigned. The first peaks considered were the carbon peaks $\delta_{173.9}$ and $\delta_{172.3}$. These showed interactions with proton signals $\delta_{2.86}$ and $\delta_{2.60}$ indicating that they were the amide carbons (Figure 55a). The next set of carbon peaks, $\delta_{164.4}$ and $\delta_{162.5}$ showed interactions with the aromatic protons, $\delta_{7.40}$ and $\delta_{6.99}$, but not with the acetal proton $\delta_{5.47}$. The peaks were therefore assigned to the quarternary carbon directly coupled to the fluorine atom and characterised as a doublet due to the fluorine splitting ($J_F = 247$ Hz)
(Figure 55b). Interactions between carbon δ131.9 and protons δ7.40, δ6.99 and δ5.47 identified the other quaternary carbon (Figure 55c).

\[ \text{Figure 55} \quad (a) \text{ Cross coupling between protons (δ2.86 and δ2.60) and carbons (δ173.9 and δ172.3) (b) Cross coupling between doublet carbon δ163.5 (J_F = 247 Hz) and protons (δ7.40 and δ6.99).} \]

The next interaction was between carbon δ156.8 and protons δ3.37 to δ3.23. This was the $^3J_{C-H}$ coupling between the carbamate carbon and the ethyl protons. Another interaction between carbon δ80.0 and protons in signal δ1.37 assigned the tert-butyl quaternary carbon.

\[ \text{Figure 56} \quad \text{Fully assigned carbon skeleton of protected amine 125.} \]

Having fully assigned all carbons and hydrogens in di-protected di-amine 125, the deprotection of the Boc-protected amine analogues could now be considered.

## 2.16 Salt formation

### 2.16.1 Pharmaceutically useful salts

It is necessary that new bioactive compounds are tested in a form consistent with that of established pharmaceuticals.$^{148}$ Amines are commonly marketed in the form of a salt to aid; bioavailability, stability, hygroscopicity and most
importantly, improvement in aqueous solubility.\textsuperscript{148, 149} It is important therefore to conduct tests on a relevant salt form of a compound as early in the development process as possible.\textsuperscript{148}

![Structure of compound 30 with TFA counterion.\textsuperscript{145}](image)

In the original publication by Pfeffer et al., the compounds (for example 30) was tested as TFA salts (Figure 57).\textsuperscript{145} Table 9 lists the most commonly used anionic counterions in pharmaceutical salts – note the absence of TFA salts. Hence, compounds developed in the course of this project were to be produced and tested in the form of the more relevant hydrochloride salt.\textsuperscript{148}

<table>
<thead>
<tr>
<th>Salt Form</th>
<th>Approximate frequency %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrochloride/chloride</td>
<td>49</td>
</tr>
<tr>
<td>Sulfate</td>
<td>6</td>
</tr>
<tr>
<td>Hydrobromide/bromide</td>
<td>5</td>
</tr>
<tr>
<td>Tartrate</td>
<td>3</td>
</tr>
<tr>
<td>Mesylate</td>
<td>3</td>
</tr>
<tr>
<td>Citrate</td>
<td>3</td>
</tr>
<tr>
<td>Phosphate</td>
<td>2.5</td>
</tr>
<tr>
<td>Acetate</td>
<td>2</td>
</tr>
<tr>
<td>Embonate</td>
<td>1.5</td>
</tr>
<tr>
<td>Hydroiodide/iodide</td>
<td>1</td>
</tr>
<tr>
<td>Nitrate</td>
<td>1</td>
</tr>
<tr>
<td>Lactate</td>
<td>1</td>
</tr>
<tr>
<td>Methylsulphate</td>
<td>1</td>
</tr>
<tr>
<td>Fumarate</td>
<td>1</td>
</tr>
</tbody>
</table>

Chloride salts represent the most common salt form of drugs approved by the United States Food and Drug Administration.\textsuperscript{148, 226} To form chloride salts of the Boc-protected amines in the current project, deprotection measures that result directly in the HCl salt were investigated.
2.16.2 The potential for acetal degradation

To deprotect the Boc groups, the protected compound must be treated with acid.\textsuperscript{197} Examples of acids used are TFA, HCl and acetyl chloride in MeOH (creating anhydrous HCl in situ).\textsuperscript{197} Unfortunately, some conditions for the deprotection of Boc are similar to those required for the decomposition of an acetal. Hence, in this project it was necessary to devise a suitable method for deprotecting Boc groups while still preserving the acetal functional group.

Previous work published by Pfeffer et al. studied the stability of the acetal by dissolving di-ester 64 in 10% aqueous DMSO media acidified to pH 1 with HCl. The solution was analysed hourly by proton NMR spectroscopy for eighteen hours and the final proton NMR spectrum was so similar to the initial spectrum that no degradation of the acetal was presumed to have occurred.\textsuperscript{145} As such, Pfeffer et al. proceeded to treat their protected compounds with a solution of 20% TFA in DCM for sixteen hours at room temperature, a common procedure for the deprotection of boc-protected substrates to form TFA salts.\textsuperscript{227, 228}

![Figure 58 – Di-ester 64 subjected to studies of the stability of the acetal.\textsuperscript{145}](image)

![Figure 59 – Structures of di-esters selected for acid trials. (a) Di-ester 68. (b) Diester 75. (c) Di-ester 66. (d) Di-ester 64.](image)
To devise appropriate deprotection reaction conditions in the current project, a selection of acetal di-esters (64, 66, 68 and 75) were selected (Figure 59). The compounds contained the following hydrophobic functionalities: purely hydrocarbon (di-ester 64); aromatic rings with either no or different substituents (Di-esters 68, 66 and 75). Next, treatment by HCl in Dioxane was selected as an appropriate method for deprotecting Boc groups in the presence of acetals.\(^{223}\)

![Scheme 33 - Deprotection of Boc group in the presence of an acetal.\(^{222}\)](image)

A successful example of this was employed by GlaxoSmithKline in the synthesis of novel pharmaceuticals.\(^{222}\) In the course of synthesising compound 129, it was necessary to deprotected the Boc group in 127 in the presence of an acetal. Deprotection resulted in the chloride salt 128 (Scheme 33). Hence, treatment by HCl in Dioxane was the first protocol attempted at the deprotection of the Boc groups on the di-protected di-amines.

**Table 10 – Percentage degradation of the acetal functional group.**

<table>
<thead>
<tr>
<th>No.</th>
<th>Degradation</th>
<th>No.</th>
<th>Degradation</th>
</tr>
</thead>
<tbody>
<tr>
<td>64</td>
<td>0%</td>
<td>63</td>
<td>14%</td>
</tr>
<tr>
<td>75</td>
<td>12%</td>
<td>68</td>
<td>13%</td>
</tr>
</tbody>
</table>

The di-ester 64 with a purely aliphatic region exhibited no degradation of the acetal was noted confirming tests conducted by Pfeffer *et al.*\(^{145}\). The other di-
esters 66, 68 and 75 all showed slight degradation. An example of degradation is illustrated in Figure 60.

![Proton NMR spectrum of (a) di-ester 69. (b) di-ester 69 subjected to acid treatment.](image)

Figure 60 - Proton NMR spectrum of (a) di-ester 69. (b) di-ester 69 subjected to acid treatment.

It is possible that the acetal substituents played an important role in the extent (if any) of degradation. For aryl acetals, protonation may ultimately result in carbocation 130. The cationic charge would be stabilised over the aromatic ring as well as oxonium and this may eventually lead to the diol 31. This additional stability does not exist when the substituent of the acetal is alkyl (Scheme 34).

![Scheme 34 - Stabilised carbocation 130 species leading to diol 34.](image)

To determine which would be the least detrimental to the acetal, a number of deprotection protocols were trialled (Table 11). In order to calculate
degradation, the ratios of the acetal proton (δ5.56) were compared to peaks corresponding to degradation as determined from proton NMR spectra (Figure 60).

Table 11 – Percentage degradation of acetal as a result of different deprotection conditions.145, 197, 229

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Solvent</th>
<th>Acid</th>
<th>Time</th>
<th>Temp (°C)</th>
<th>Degradation (%)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DCM</td>
<td>TFA</td>
<td>16 h</td>
<td>r.t.</td>
<td>17%</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>MeOH</td>
<td>AcCl</td>
<td>16 h</td>
<td>r.t.</td>
<td>17%</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Et₂O</td>
<td>1M HCl</td>
<td>16 h</td>
<td>r.t.</td>
<td>21%</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Dioxane</td>
<td>HCl</td>
<td>16 h</td>
<td>r.t.</td>
<td>16%</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Dioxane</td>
<td>HCl</td>
<td>16 h</td>
<td>r.t.</td>
<td>14%</td>
<td>MgSO₄⁺</td>
</tr>
<tr>
<td>6</td>
<td>Dioxane</td>
<td>HCl</td>
<td>7 hrs</td>
<td>r.t.</td>
<td>15%</td>
<td>Anhydrous**</td>
</tr>
</tbody>
</table>

* MgSO₄ added to reaction vessel.
** Anhydrous solvent used.

The use of a drying agent in the reaction vessel (Entry 5, Table 11) or anhydrous solvent with a reduced time (Entry 6, Table 11) resulted in less degradation.

From the above experiments, two deprotection conditions were selected for further investigation: acetyl chloride in MeOH and HCl in anhydrous dioxane.

### 2.17 Case Study: Protected amine

The mechanism by which a Boc deprotection occurs is well established (Scheme 35).165 Firstly, the carbonyl oxygen, the most basic oxygen, is protonated. Heterolysis of the oxygen and tert-butyl bond results in carbamic acid 133 and a tert-butyl cation 132. In the final step carbamic acid 133 decarboxylates, expelling carbon dioxide and free amine. Parallel to carbamic acid decomposition, the tert-butyl cation 132 decomposes to isobutene.
Anhydrous HCl in Dioxane solution was prepared 24 hours prior to use and stored in a refrigerator over 4Å molecular sieves. To MeOH, acetyl chloride was added and allowed to stir for thirty minutes prior to addition of amine 120. In both instances, amine 120 was added and the reaction stirred vigorously for approximately four hours with progress monitored by TLC (Scheme 36).

Once the nominated treatment time was complete, the reaction solvents were removed in vacuo and the resulting residues analysed using proton NMR spectroscopy. Figure 61 shows the proton NMR spectra obtained. Comparison with the spectrum of the protected amine 120, there was an absence of the Boc peaks at δ1.30 signifying successful deprotection. Nevertheless, there were new signals from δ6.25 to δ5.25. In the region highlighted, where the acetal protons commonly appear, there were up to four different peaks indicating a mixture of products. The peak at δ5.53 was assigned to the fully formed acetal peak using the acetal peak in the proton spectrum of protected amine 120.
Figure 61 – Proton NMR spectrum of (a) protected amine 122; (b) crude product following treatment with HCl in Dioxane; (c) crude product following treatment with acetyl chloride in MeOH.

Comparison of the spectra indicated that the acetyl chloride in MeOH protocol resulted in fewer by-products (Figure 61). Each crude residue was then dissolved in water, washed with EtOAc and water removed by sublimation (freeze drying). The resulting solids resembled a ‘floss’, and were hydroscopic and became viscous oils when exposed to normal atmosphere. The solids were again analysed using proton NMR spectroscopy (Figure 62).
Following purification, Proton NMR of compound 134 after (a) treatment with HCl in Dioxane (b) treatment with acetyl chloride in MeOH. In both instances, purification served to remove most by-products, making the fully formed acetal the major product. Comparing the two methods of treatment at this point, it was determined that the acetyl chloride in MeOH and extractive purification protocol produced a sample that had the highest purity.

Having selected a deprotection and purification protocol, attention now turned to the characterisation of the purified amine salts. In the circumstances where degradation occurred, small amounts of by-products were sometimes observed present even in the purified salt. A strategy was devised to estimate the purity of the amine salts obtained. First, a pure amine salt was studied to determine characteristic peaks (Figure 63).
Figure 63 – Proton NMR spectrum in CD$_3$OD of (a) diol 135. (b) di-amine 137.

Figure 63 shows the proton spectrum of a pure amine salt (amine 137) and the diol 135. Key characteristic peaks are highlighted in both spectra. The protons forming the norbornane scaffold were chosen as ‘landmarks’ in the spectra of mixed products. The acetal is highlighted as that is characteristic of the presence of the intact hydrophobic region of the molecule (Figure 64).

Figure 64 – Proton NMR spectrum of amine salt 136 containing a mixture of products.
Percentage purity was estimated through integrations of the relevant proton and any adjacent by-products (Figure 64). Initially, the peaks in the region of $\delta 4.3$ were selected. The dominant peak ($\delta 4.29$, int.=1.00) was calculated as percentage of the total of the dominant peak ($\delta 4.29$, int.=1.00) and the degradation ($\delta 4.18$, int.=0.14) to achieve the estimated purity of 87%. Unfortunately, for many amine salts in the library, this region contained many resonances and it was difficult to select peaks to estimate purity. Hence, the acetal was selected to calculate estimated purity as this region remained the least cluttered.

The acetal integration ($\delta 5.66$, int.=0.83) was measured against the sum of integrations of both the acetal and degradation ($\delta 6.29$, int.=0.17, total = 1.00) to obtain a percentage purity of 83%. As the purity calculated using the acetal region was within a close range to the purity calculated using the protons from the norbornane scaffold, the integrations of the peaks within the acetal region (clearly visible in all amine salts) were used to estimate purities of all amine salts.

2.18 Deprotection and salt formation

Each protected amine was treated with acetyl chloride in MeOH. Once the reaction was judged complete by TLC, the reaction solvent was removed in vacuo leaving behind crude residues. The residues were re-dissolved in EtOAc and extracted using H$_2$O. The aqueous phases were combined and water removed by sublimation. The purity of each amine salt is indicated in Table 12.
Table 12 – Yields of the Boc deprotection by acetyl chloride in MeOH.

<table>
<thead>
<tr>
<th>No.</th>
<th>Mass*</th>
<th>Purity</th>
<th>No.</th>
<th>Mass*</th>
<th>Purity</th>
</tr>
</thead>
<tbody>
<tr>
<td>137</td>
<td>66%</td>
<td>100%</td>
<td>141</td>
<td>72%</td>
<td>65%</td>
</tr>
<tr>
<td>138</td>
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<td>98%</td>
<td>142</td>
<td>91%</td>
<td>79%</td>
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<td>83%</td>
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<td>100%</td>
</tr>
<tr>
<td>139</td>
<td>99%</td>
<td>67%</td>
<td>145</td>
<td></td>
<td>99%</td>
</tr>
<tr>
<td>140</td>
<td>73%</td>
<td>86%</td>
<td>146</td>
<td></td>
<td>80%</td>
</tr>
</tbody>
</table>

*Mass recovered after freeze drying.

For compounds with purities higher than 85%, proton and carbon NMR spectroscopy, mass spectrometry and IR were further conducted to confirm identity of compounds.

2.19 Conclusion

The aim of the current chapter was to devise a methodology to synthesise amphiphilic di-amines containing differing hydrophobic moieties. A revised synthetic pathway allowed for a robust method for the synthesis of the amine functionalised norbornane based CAMP mimics. The new synthetic methodology was suitable for large scale reactions (up to sixty grams) and its
most attractive features was that only once in a seven step synthetic pathway was purification by column chromatography required.

Unfortunately, deprotection of the Boc groups was problematic. The acetal functional group proved somewhat sensitive to acid treatment. A deprotection protocol and purification procedure was identified that gave highly pure di-amine di-hydrochlorides. The acetyl chloride in MeOH method produced salts with purities ranging from 56% - 100% (twelve compounds with 8 > 85% purity). For the samples in the lower range of purities, the presence of the fully degraded product, diol 134, was noted.

The collection of amine salts were sent for antibacterial testing the results of which are discussed in chapter 5. The completion of the amine library marked the point where focus could be turned to the synthesis of the di-guanidine functionalised norbornane based CAMP mimics.

2.20 Experimental

2.20.1 General Experimental

Microwave reactions were conducted using a CM Discover S-Class Explorer 48 Microwave Reactor, operating on a frequency of 50/60 Hz and continuous irradiation power from 0 to 200 W. All reactions were performed in either 10mL or 30 mL septa vials with snap caps, with the following conditions: pressure (17 bar); power max (on); and stirring (high).

All $^1$H and $^{13}$C NMR spectra were recorded on a Jeol JNM-EX 270 MHz, Jeol JNM-EX 400 MHz or Bruker Advance III 500 MHz FT-NMR as indicated. Samples were dissolved in either deuterated chloroform (CDCl$_3$), deuterated methanol
(CD$_3$OD) or deuterated water (D$_2$O) with the residual solvent peak used as an internal reference ($\delta = 7.26$ ppm for CDCl$_3$, 3.31 ppm for CD$_3$OD, 4.71 ppm for D$_2$O). Proton spectra are reported as follows: chemical shift $\delta$ (ppm), [integral, multiplicity (s = singlet, br s = broad singlet, d = doublet, dd = doublet of doublets, t = triplet, br t = broad triplet, q = quartet, m = multiplet), coupling constant J (Hz), assignment]. There is the presence of an apparent triplet which is a true doublet of doublets in the $^1$H NMR spectrum of norbornane based compounds. Carbon spectra are reported as chemical shift $\delta$ (ppm).

Mass Spectra (MS), including High Resolution Mass Spectra (HRMS), were recorded on a 6210 MSD TOF mass spectrometer (Agilent Technologies, Australia) or Agilent MS 6520 TOF with dual electrospray ionisation source with the following conditions: drying gas nitrogen (7.0 L/min, 325 °C); nebuliser gas nitrogen (15 psi); capillary voltage 3.0 kV; vaporiser temperature 29 °C; and cone voltage 40 V.

Fourier transform infrared (FT-IR) spectra were recorded on a Bruker ALPHA Platinum ATR FT-IR Spectrometer. The following abbreviations apply to peak intensity: w = weak, m = medium, s = strong, br = broad. All melting points were obtained using a Bibby Stuart Scientific SMP3 melting point apparatus, version 5.0.

Thin Layer Chromatography (TLC) was performed using aluminium-backed Merck TLC Silica gel 60 F254 plates, and samples were visualised using 254 nm ultraviolet (UV) light, potassium permanganate/potassium carbonate oxidising dip (1:1:100 KMnO$_4$:K$_2$CO$_3$:H$_2$O w/w) or ceric ammonium molybdate stain. Column Chromatography was performed using silica gel 60 (70-230 mesh).
All solvents used were AR grade. Petroleum spirits refers to the fraction boiling between 40-60 °C. THF, CHCl₃ and DMF were dried using a Pure Solv (Innovative Technologies) solvent drying system. Solvents are degassed, and passed through two drying chambers of alumina and collected under a positive pressure of nitrogen gas.

All general reagents were analytical grade and used as supplied unless otherwise stated. Purification and/or drying of general reagents were performed according to Perrin et. al. Specialist reagents such as peptide coupling reagents were supplied by Aldrich Chemical Co. or A.K Scientific Inc. and used without further purification.

2.2.0.2 Known compounds

The following compounds are known compounds and were prepared using literature conditions with no modifications:

- Dimethyl bicyclo[2.2.1]hept-5-ene-2-endo,3-exo-dicarboxylate (norbornene di-ester) 33\textsuperscript{156}
- Dimethyl 5,6-dihydroxy bicyclo[2.2.1]heptane-2-endo,3-exo-dicarboxylate (norbornane diol) 34\textsuperscript{145}
- 1-Hexadecanal 46\textsuperscript{145,231}
- \textit{para}-Oct-1’-ynyl benzaldehyde 47\textsuperscript{181}
- \textit{para}-Octyl benzaldehyde 48\textsuperscript{181}
Section 2.5 Hydrophobic component

Ethyl 4-(octyloxy)benzoate (57)\textsuperscript{192}

Ethyl 4-hydroxybenzoate 56 (1.11 g, 6.67 mmol), iodoctane 55 (4.8 g, 19.9 mmol) and K\textsubscript{2}CO\textsubscript{3} (2.76 g, 19.9 mmol) were added to DMF (10 mL) and the reaction mixture stirred for 16 hours at room temperature. The reaction mix was diluted with EtOAc (50 mL), transferred to a separatory funnel and washed with H\textsubscript{2}O (2 × 50 mL) and brine (50 mL). The organic phase was separated, dried (MgSO\textsubscript{4}) and filtered and solvent was removed \textit{in vacuo} resulting in clear oil. This crude oil was purified using column chromatography (1% EtOH in Pet 40-60). Fractions containing the desired compound (R\textsubscript{f} 0.17) were combined and the solvent removed \textit{in vacuo} to give the desired compound as viscous oil. (1.57 g, 85%); \textit{δ}H NMR (270 MHz, CDCl\textsubscript{3}): 7.97 (2H, dd, J\textsubscript{AB'} = 2.7 Hz, J\textsubscript{AB} = 8.1 Hz, H3) 6.88 (2H, dd, J\textsubscript{A'B'} = 2.7 Hz, J\textsubscript{A'B} = 8.1 Hz, H4) 4.33 (2H, q, J = 5.9 Hz, H2) 3.98 (3H, t, J = 6.4 Hz, H1) 1.83-1.27 (14H, m, H5-11) 0.87 (3H, t, J = 5.9 Hz, H12).

4-(octyloxy)benzyl alcohol (59)\textsuperscript{194}

Red-Al® 58 (1.25 g, 6.17 mmol) was added to a cooled to (0 °C) solution of ethyl 4-(octyloxy)benzoate 57 (343 mg, 1.23 mmol) in toluene (5 mL) and then allowed to stir for 16 hours at room temperature. Saturated potassium sodium tartrate solution (6 mL) was added followed by H\textsubscript{2}O (10 mL), transferred to a separatory flask and extracted with EtOAc (3 × 10 mL). The organic phases were combined, dried (MgSO\textsubscript{4}) and filtered. Solvent removed \textit{in vacuo} resulting in white viscous oil. (261 mg, 89%); \textit{δ}H NMR (270 MHz, CDCl\textsubscript{3}): 7.26 (2H, dd, J\textsubscript{AB} = 2.7 Hz, J\textsubscript{AB} =
5.4 Hz, H3) 6.87 (2H, dd, $J_{AB} = 2.7$ Hz, $J_{AB'} = 5.4$ Hz, H2) 4.60 (2H, s, H1) 3.95 (2H, t, $J = 4.5$ Hz, H4) 1.49-1.24 (12H, m, H5-10) 0.89 (3H, t, $J = 4.5$ Hz, H11).

**p-Octyloxy benzaldehyde (49)**

A solution of para-octyloxy benzyl alcohol 59 (1.14 g, 4.82 mmol) and PCC 51 (1.25 g, 5.78 mmol) in DCM (25 mL) was stirred for 16 hours at room temperature. The reaction mixture was then filtered through a silica/Celite® frit. The frit was washed with DCM (150 mL). The filtrate was collected and the solvent removed *in vacuo* resulting in yellow viscous oil. The oil was determined to be 98% pure by NMR and was used in the next step without further purification. (1.01 g, 90%); δ$_H$ NMR (270 MHz, CDCl$_3$): 9.86 (1H, s, H1) 7.81 (2H, dd, $J_{AB'} = 2.7$ Hz, $J_{AB} = 8.1$ Hz, H3) 6.97 (2H, dd, $J_{AB} = 2.7$ Hz, $J_{AB'} = 8.1$ Hz, H2) 4.02 (2H, t, $J = 6.7$ Hz, H4) 1.47-1.27 (12H, m H5-10) 0.87 (3H, t, $J = 6.4$ Hz, H11).

**Section 2.6 Acetal formation**

General methodology: A solution of aldehyde/ketone (2 eq.), norbornane diol 35 (approx. 300 mg), TFA (approx. 222 mg, 1 mmol) and MgSO$_4$ in CHCl$_3$ was irradiated in a laboratory microwave for 20 minutes at 80 °C. The reaction mixture was then filtered and the solvent removed *in vacuo*. The crude oils were purified using column chromatography [EtOAc/Pet 40-60]. Fractions containing the desired compound were combined and the solvent removed *in vacuo*. 

95
Dimethyl 7-[pentadecyl]-6,8-dioxatricyclo[5.2.1.0^6,8]decane-2-endo,3-exo-dicarboxylate (64)

Hexadecanal 46 (0.88 g, 3.63 mmol); norbornane diol 34 (0.60 g, 2.45 mmol); column eluent [EtoAc/Pet 40-60 (1:19)]; Rf 0.23; brown viscous oil (1.07 g, 93%); \( \nu_{\text{max}} \) (thin film/cm\(^{-1} \)) 2926 (s) (methyl C-H), 2855 (s) (methyl C-H), 1712 (s) (ester C=O), 1362 (br) (methylene C-H), 1120 (m) (acetal C-O); \( \delta \) H NMR (270, CDCl\(_3\)): 4.64 (1H, t, \( J = 4.9 \) Hz, H7), 4.01 (1H, d, \( J = 5.7 \) Hz, H5) 3.89 (1H, d, \( J = 5.7 \) Hz, H9) 3.69 (3H, s, \(-\text{OCH}_3\)) 3.67 (3H, s, \(-\text{OCH}_3\)) 3.21 (1H, dd, \( J = 4.9, 4.9 \) Hz, H2) 2.69 (1H, d, \( J = 5.4 \) Hz, H4) 2.62 (2H, m, H1, H3) 1.76 (1H, d, \( J = 9.6 \) Hz, H10a) 1.63-1.58 (3H, m, H11, H10b) 1.35-1.23 (26H, m, H12-H24) 0.86 (1H, t, \( J = 6.4 \) Hz, H25); \( \delta \) C NMR (67.5, CDCl\(_3\)): 174.1, 172.8, 104.3, 81.3, 78.9, 52.4, 52.2, 45.3, 45.1, 43.7, 43.32, 43.3, 34.1, 32.8, 32.0, 31.7, 29.7, 29.6, 29.5, 29.4, 29.3, 29.1, 24.8, 24.6, 24.3, 22.8, 14.2; HRMS (ESI m/z): calculated for \([C_{27}H_{46}O_6+Na]^+\) 489.3192, found 489.31607.

Dimethyl 7-[([p-phenyl]-phenyl]-6,8-dioxatricyclo[5.2.1.0^6,8]decane-2-endo,3-exo-dicarboxylate (65)

\( \text{para-Phenyl benzaldehyde} \) (280 mg, 1.54 mmol); norbornane diol 34 (250 mg, 1.02 mmol); column eluent [EtOAc/Pet 40-60 (1:10)]; Rf 0.18; white viscous oil (395 mg, 94%); \( \nu_{\text{max}} \) (thin film/cm\(^{-1} \)) 2955 (m) (methyl C-H) 2851 (w) (methyl C-H) 1647 (s) (aromatic ring C=C) 1726 (m) (ester C=O) 834 (m) (\text{para substitution on aromatic C-H}); \( \delta \) H NMR (270 MHz,
CDCl$_3$): 7.62 – 7.33 (9H, m, H11-19), 5.63 (1H, s, H7), 4.26 (1H, d, $J = 5.4$ Hz, H5), 4.12 (1H, d, $J = 5.4$ Hz, H9), 3.74 (3H, s, -OCH$_3$), 3.73 (3H, s, -OCH$_3$), 3.30 (1H, dd, $J = 4.9$, 5.2 Hz, H2), 2.86 (1H, d, $J = 2.7$ Hz, H4) 2.80 (1H, s, H3) 2.72 (1H, dd, $J = 2.7$, 5.4 Hz, H1) 2.01 (1H, dd, $J = 1.49$, 9.6 Hz, H10a) 1.46 (1H, dd, $J = 1.49$, 9.6 Hz, H10b); $\delta$C NMR (67.5 MHz, CDCl$_3$): 174.0, 172.8, 142.7, 140.8, 134.7, 128.9 (2 carbons), 127.6, 127.4 (2 carbons), 127.3 (2 carbons), 127.2 (2 carbons), 103.2, 81.8, 79.4, 52.5, 52.3, 45.4, 45.1, 43.8, 43.5, 32.0; HRMS (ESI m/z): calculated for [C$_{24}$H$_{24}$O$_6$+Na]$^+$ 431.4335, found 431.4353.

**Dimethyl 7-[p-bromo-phenyl]-6,8-dioxatricyclo[5.2.1.0$^{6,8}$]decane-2-endo,3-exo-dicarboxylate (66)**

Bromo benzaldehyde (156 mg, 844 µmol); norbornane diol 34 (103 mg, 422 µmol); column eluent [EtOAc/Pet 40-60 (1:10)]; $R_f$ 0.07; brown oil (145 mg, 84%); $\nu_{\max }$ (thin film/cm$^{-1}$) 1725 (vs) (ester C=O) 1111 (vs) (acetal C-O) 1012 (s) (aromatic C-H) 664 (m) (bromine C-Br); $\delta$H NMR (270 MHz, CDCl$_3$): 7.49 (2H, dd, $J_{AB'} = 2.7$ Hz, $J_{AB} = 5.4$ Hz, H12) 7.34 (2H, dd, $J_{A'B'} = 2.7$ Hz, $J_{A'B'} = 5.4$ Hz, H11) 5.51 (1H, s, H7) 4.21 (1H, d, $J = 3.5$ Hz, H5) 4.09 (1H, d, $J = 3.5$ Hz, H9) 3.72 (3H, s, -OCH$_3$) 3.70 (3H, s, -OCH$_3$) 3.27 (1H, dd, $J = 3.5$, 3.5 Hz, H2) 2.82 (1H, d, $J = 2.7$ Hz, H4) 2.76 (1H, s, H3) 2.70 (1H, d, $J = 2.7$ Hz, H1) 1.89 (1H, dd, $J = 0.9$, 5.7 Hz, H10a) 1.42 (1H, dd, $J = 0.9$, 5.7 Hz, H10b); $\delta$C NMR (67.5 MHz, CDCl$_3$): 173.9, 172.7, 134.9, 131.9 (2 carbons), 128.4 (2 carbons), 123.8, 102.6, 81.81, 79.4, 52.5, 52.3, 45.3, 45.2, 43.8, 43.4, 31.9; HRMS (ESI m/z): calculated for [C$_{18}$H$_{19}$BrO$_6$+Na]$^+$ 433.0263 and 435.0245, found 433.0300 and 435.0294.
Dimethyl 7-[(p-octynyl)-phenyl]-6,8-dioxatricyclo[5.2.1.0^6,8]decane-2-endo,3-exo-dicarboxylate (67)

Octynyl benzaldehyde 47 (551 mg, 2.57 mmol); norbornane diol 34 (314 mg, 1.29 mmol); column eluent [EtOAc/Pet 40-60 (1:9)]; Rf 0.23; brown viscous oil (404 mg, 75%); \( \nu_{\text{max}} \) (thin film/cm\(^{-1}\)) 2932 (m) (methyl C-H) 2856 (m) (methylene C-H) 1733 (vs) (ester C=O) 1070 (s) (acetal C-O) 832 (s) (para substitution of aromatic C-H); \( \delta \)\( _{\text{H}} \) NMR (270 MHz, CDCl\(_3\)): 7.38 (4H, s, H11-12) 5.53 (1H, s, H7) 4.21 (1H, d, \( J = 3.7 \) Hz, H5) 4.09 (1H, d, \( J = 3.7 \) Hz, H9) 3.72 (3H, s, -OCH\(_3\)) 3.71 (3H, s, -OCH\(_3\)) 3.27 (1H, dd, \( J = 3.2, 3.2 \) Hz, H2) 2.83 (1H, 2H, J = 2.7 Hz, H4) 2.77 (1H, s, H3) 2.71 (1H, dd, \( J = 3.2, 3.2 \) Hz, H2) 2.38 (2H, t, \( J = 4.7 \) Hz, H13) 1.94 (1H, dd, \( J = 0.9, 6.5 \) Hz, H10a) 1.62-1.25 (9H, m, H10b, H14-17) 0.89 (3H, t, \( J = 4.7 \) Hz, H18); \( \delta \)\( _{\text{C}} \) NMR (67.5 MHz, CDCl\(_3\)): 173.85, 172.70, 134.75, 131.58 (2 carbons), 126.53 (2 carbons), 125.52, 102.94, 91.40, 81.70, 80.23, 79.29, 52.42, 52.23, 45.30, 45.15, 43.72, 43.38, 31.35, 29.70, 28.66, 28.60, 22.56, 19.44, 14.06; HRMS (ESI \( m/z \)): calculated for [C\(_{26}\)H\(_{32}\)O\(_{6}\)+H]\(^+\) 441.22717, found 441.22741.

Dimethyl 7-phenyl-6,8-dioxatricyclo[5.2.1.0^6,8]decane-2-endo,3-exo-dicarboxylate (68)

Benzaldehyde (821 \( \mu \)L, 8.13 mmol ); norbornane diol 34 (992.3 mg, 4.06 mmol); column eluent [EtOAc/Pet 40-60 (1:3)]; Rf 0.68; brown viscous oil (1.13 g, 79%); \( \nu_{\text{max}} \) (thin film/cm\(^{-1}\)) 1731 (s) (ester C=O), 1436 (w) (aromatic C=C), 1089 (w) (acetal C-O); \( \delta \)\( _{\text{H}} \) NMR (270, CDCl\(_3\)): 7.50-7.31 (5H, m, H11-13) 5.56 (1H, s, H7) 4.21 (1H, d, \( J = 5.8 \) Hz, H5) 4.09 (1H, d, \( J = 5.8 \) Hz, H9) 3.71 (3H, s, -OCH\(_3\)) 3.70
(3H, s, -OCH₃) 3.27 (1H, d, J = 5.2 Hz, H2) 2.83 (1H, d, J = 5.4 Hz, H4) 2.77 (1H, s, H3) 2.71 (1H, d, J = 2.7 Hz, H1) 1.98 (1H, dd, J = 1.5, 9.4 Hz, H10a) 1.43 (1H, dd, J = 1.5, 9.4 Hz, H10b); δC NMR (67.5, CDCl₃): 173.9, 172.8, 135.8, 129.7, 128.6 (2 carbons), 126.8 (2 carbons), 103.2, 81.7, 79.3, 52.8, 52.3, 45.4, 45.2, 43.8, 43.5, 31.9; (ESI m/z): calculated for [C₁₈H₂₆O₆+Na]⁺ 355.33, found 355.30.

**Dimethyl 7-pyrenyl-6,8-dioxatricyclo[5.2.1.0⁶,₈]decane-2-endo,3-exo-dicarboxylate (69)**

Pyrenecarboxaldehyde (243.6 mg, 1.06 mmol); norbornane diol 34 (129.2 mg, 529 μmol); column eluent [EtOAc/Pet 40-60 (1:3)]; Rf 0.71; yellow viscous oil (170.4 mg, 71%); υmax(thin film/cm⁻¹) 1739 (w) (ester C=O) 1647 (vs) (aromatic C=C) 1015 (s) (acetal C-O); δH NMR (270 MHz, CDCl₃): 8.38-8.03 (9H, m, H11-19) 6.59 (1H, s, H7) 4.47 (1H, d, J = 2.7 Hz, H5) 4.36 (1H, d, J = 2.7 Hz, H9) 3.78 (3H, s, -OCH₃) 3.73 (3H, s, -OCH₃) 3.33 (1H, d, J = 2.7 Hz, H2) 2.94 (1H, d, J = 2.7 Hz, H4) 2.90 (1H, s, H3) 2.83 (1H, d, J = 2.7 Hz, H1) 2.03 (1H, dd, J = 1.5, 9.4 Hz, H10a) 1.47(1H, dd, J = 1.5, 9.4 Hz, H10b); δC NMR (67.5 MHz, CDCl₃): 173.9, 172.8, 132.0, 131.1, 128.8, 128.5, 128.2, 127.9, 127.3, 125.9, 125.5, 125.4, 124.6 (2 carbons), 122.8, 122.6, 122.5, 122.4, 100.8, 81.9, 79.5, 52.4, 52.3, 45.3, 45.2, 43.7, 43.5, 31.9; (ESI m/z): calculated for [C₂₈H₂₄O₆+H] 457.165, found 457.1721.
Dimethyl 7,7-dipropyl-6,8-dioxatricyclo[5.2.1.06,8]decane-2-endo,3-exo-dicarboxylate (70)

4-Heptanone (475.3 mg, 4.16 mmol); norbornane diol 34 (508 mg, 2.08 mmol); column eluent [EtOAc/Pet 40-60 (1:9)]; Rf 0.38; clear viscous oil (408 mg, 58%); \( \nu_{\text{max}}(\text{thin film/cm}^{-1}) \) 1658 (w) (ester C=O) 1022 (vs) (acetal C-O); \( \delta_{\text{H}} \) NMR (270 MHz, CDCl\(_3\)): 4.07 (1H, d, \( J = 3.5 \) Hz, H5) 3.92 (1H, d, \( J = 3.5 \) Hz, H9) 3.68 (3H, s, -OCH\(_3\)) 3.66 (3H, s, -OCH\(_3\)) 3.18 (1H, dd, \( J = 2.7, 2.7 \) Hz, H2) 2.64-2.57 (3H, m, H1, H3-4) 1.80 (1H, d, \( J = 7.2 \) Hz, H10a) 1.61-1.22 (9H, m, H10b, H11-12, H14-15) 0.89-0.84 (6H, m, H13, H16); \( \delta_{\text{C}} \) NMR (67.5 MHz, CDCl\(_3\)): 174.1, 172.9, 113.0, 80.9, 78.2, 52.4, 52.2, 45.4, 45.3, 43.6, 43.5, 38.0, 37.3, 31.7, 17.9, 17.1, 14.43, 14.40; (ESI m/z): calculated for [C\(_{17}\)H\(_{26}\)O\(_6\)+Na\(^+\)] 349.16, found 349.19.

Dimethyl 7-(p-octyl phenyl)-6,8-dioxatricyclo[5.2.1.06,8]decane-2-endo,3-exo-dicarboxylate (71)

para-Octyl benzaldehyde 48 (309 mg, 1.41 mmol); norbornane diol 34 (173 mg, 707 \( \mu \)mol); column eluent [EtOAc/Pet 40-60 (1:9)]; Rf 0.37; brown viscous oil (232 mg, 74%); \( \nu_{\text{max}}(\text{thin film/cm}^{-1}) \) 2925 (vs) (methylene C-H) 2856 (s) (methyl C-H) 1697 (vs) (aromatic C=C) 1465 (m) (ester C=O) 1104 (m) (acetal C-O); \( \delta_{\text{H}} \) NMR (270 MHz, CDCl\(_3\)): 7.43 (2H, d, \( J_{AB} = 5.4 \) Hz, H11) 7.27 (2H, d, \( J_{AB'} = 5.4 \) Hz, H12) 5.53 (1H, s, H7) 4.21 (1H, d, \( J = 3.7 \) Hz, H5) 4.09 (1H, d, \( J = 3.7 \) Hz, H9) 3.73 (3H, s, -OCH\(_3\)) 3.71 (3H, s, -OCH\(_3\)) 3.27 (1H, d, \( J = 2.7 \) Hz, H2) 2.83 (1H, d, \( J = 2.7 \) Hz, H4) 2.77 (1H, s, H3) 2.71 (1H, d, \( J = 2.7 \) Hz, H1) 1.94 (1H, dd, \( J = 0.7, 6.7 \) Hz, H10a) 1.62-1.22 (14H, m,
H13-19) 0.88 (3H, t, J = 4.5 Hz, H20); δc NMR (67.5 MHz, CDCl3): 173.89, 172.7, 134.8, 131.6 (2 carbons), 126.6 (2 carbons), 125.7, 102.9, 81.7, 79.3, 52.5, 52.3, 45.5, 45.3, 45.2, 43.8, 43.6, 43.4, 31.9, 31.4, 29.1, 28.6, 22.6, 19.5, 14.1; (ESI m/z): calculated for [C26H36O6+H]+ 446.26, found 446.29.

**Dimethyl 7-[3-(benzyloxy)phenyl]-6,8-dioxatricyclo[5.2.1.06,8]decane-2-endo,3-exo-dicarboxylate (72)**

3-Benzylxobenzaldehyde (922 mg, 4.34 mmol); norbornane diol 34 (531 mg, 2.17 mmol); column eluent [EtOAc/Pet 40-60 (1:1)]; Rf 0.46; white viscous oil (765 mg, 83%); \( \nu \) max(thin film/cm\(^{-1}\)) 1718, (m) (ester C=O) 1624 (m) (aromatic C=C) 1213 (w) (aromatic C-H) 1105 (w) (acetal C-O); δ\( \text{H} \) NMR (270 MHz, CDCl3): 7.42-6.90 (9H, m, H11-14, H16-20) 5.55 (2H, s, H15) 5.06 (1H, s, H7) 4.22 (1H, d, \( J = 3.9 \) Hz, H5) 4.10 (1H, d, \( J = 3.9 \) Hz, H9) 3.73 (3H, s, -OCH\(_3\)) 3.71 (3H, s, -OCH\(_3\)) 3.28 (1H, dd, \( J = 2.7, 2.7 \) Hz, H2) 2.83 (1H, d, \( J = 2.7 \) Hz, H4) 2.77 (1H, s, H3) 2.72 (1H, d, \( J = 2.7 \) Hz, H1) 1.94 (1H, d, \( J = 7.4 \) Hz, H10a) 1.42 (1H, d, \( J = 6.9 \) Hz, H10b); δ\( \text{C} \) NMR (67.5 MHz, CDCl3): 173.9, 172.8, 158.9, 137.3, 136.9, 129.7, 128.7, 128.1 (2 carbons), 127.6, 119.4, 116.2 (2 carbons), 113.0, 103.1, 81.7, 79.3, 70.1, 52.5, 52.3, 45.4, 45.2, 43.8, 43.5, 31.9; (ESI m/z): calculated for [C\(_{25}\)H\(_{27}\)O\(_7\)+Na]+ 462.16, 462.11.
Dimethyl 7-heptyl-6,8-dioxatricyclo[5.2.1.0^6,8]decane-2-endo,3-exo-dicarboxylate (73)

Octanal (734 mg, 5.72 mmol); norbornane diol 34 (699 mg, 2.86 mmol); column eluent [EtOAc/Pet 40-60 (1:9)]; Rf 0.44; clear viscous oil (678 mg, 67%); \( \nu_{\text{max}} \) (thin film/cm\(^{-1}\)) 2970 (s) (methyl C-H) 1408 (m) (ester C=O) 1128 (s) (acetal C-O); \( \delta_H \) NMR (270 MHz, CDCl\(_3\)): 4.64 (1H, t, \( J = 3.2 \) Hz, H7) 4.02 (1H, d, \( J = 3.7 \) Hz, H5) 3.89 (1H, d, \( J = 3.7 \) Hz, H9) 3.71 (3H, s, -OCH\(_3\)) 3.69 (3H, s, -OCH\(_3\)) 3.21 (1H, d, \( J = 2.7 \) Hz, H2) 2.71-2.31 (3H, m, H1, H3-4) 1.77 (1H, d, \( J = 7.4 \) Hz, H10a) 1.67-1.59 (3H, m, H10b, H11) 1.35-1.22 (10H, m, H12-16) 0.85 (3H, t, \( J = 4.5 \) Hz, H17); \( \delta_C \) NMR (67.5 MHz, CDCl\(_3\)): 174.08, 172.8, 104.3, 81.3, 75.9, 52.4, 52.2, 45.3, 45.1, 43.7, 43.3, 32.8, 31.8, 31.7, 29.6, 29.2, 24.3, 22.7, 14.1; (ESI \( m/z \)): calculated for \([C_{19}H_{30}O_6+H]^+\) 355.21, found 355.25.

Dimethyl 7-(dimethyl)-6,8-dioxatricyclo[5.2.1.0^6,8]decane-2-endo,3-exo-dicarboxylate (74)

A suspension of MgSO\(_4\) (250 mg) in a solution of norbornane diol 34 (301 mg, 1.23 \( \mu \)mol) and TFA (3 drops) in acetone (2 mL) was irradiated in a laboratory microwave for 20 mins at 60 °C. The reaction mixture was then filtered and the solvent removed in vacuo. The crude brown oil was purified using column chromatography with an eluent of (1:1) EtOAc/Pet 40-60. Fractions containing the desired compound (Rf 0.64) were combined and the solvent removed in vacuo resulting in clear viscous oil. (303 mg, 87%); \( \nu_{\text{max}} \) (thin film/cm\(^{-1}\)) 2923 (w) (methyl C-H) 1732 (vs) (ester C=O) 1069 (m) (acetal C-O); \( \delta_H \) NMR (270 MHz, CDCl\(_3\)): 4.64 (1H, t, \( J = 3.2 \) Hz, H7) 4.02 (1H, d, \( J = 3.7 \) Hz, H5) 3.89 (1H, d, \( J = 3.7 \) Hz, H9) 3.71 (3H, s, -OCH\(_3\)) 3.69 (3H, s, -OCH\(_3\)) 3.21 (1H, d, \( J = 2.7 \) Hz, H2) 2.71-2.31 (3H, m, H1, H3-4) 1.77 (1H, d, \( J = 7.4 \) Hz, H10a) 1.67-1.59 (3H, m, H10b, H11) 1.35-1.22 (10H, m, H12-16) 0.85 (3H, t, \( J = 4.5 \) Hz, H17); \( \delta_C \) NMR (67.5 MHz, CDCl\(_3\)): 174.08, 172.8, 104.3, 81.3, 75.9, 52.4, 52.2, 45.3, 45.1, 43.7, 43.3, 32.8, 31.8, 31.7, 29.6, 29.2, 24.3, 22.7, 14.1; (ESI \( m/z \)): calculated for \([C_{19}H_{30}O_6+H]^+\) 355.21, found 355.25.
MHz, CDCl₃): 4.12 (1H, d, J = 5.4 Hz, H5) 3.99 (1H, d, J = 5.4 Hz, H9) 3.69 (3H, s, -OCH₃) 3.68 (3H, s, -OCH₃) 3.21 (1H, d, J = 5.4 Hz, H2) 2.65 (1H, d, J = 1.62 Hz, H4) 2.63 (1H, d, J = 1.62 Hz, H3) 2.57 (1H, s, H1) 1.77 (1H, dd, J = 1.5, 9.2 Hz, H10a) 1.41 (3H, s, methyl C-H) 1.35 (1H, dd, J = 1.5, 7.9 Hz, H10b) 1.25 (3H, s, methyl C-H); δc NMR (67.5 MHz, CDCl₃): 174.1, 172.9, 109.5, 81.0, 78.4, 52.4, 52.3, 45.3, 45.2, 43.6, 43.4, 41.2, 31.5, 25.4; (ESI m/z): calculated for [C₁₄H₂₀O₆+H]⁺ 285.11, found 285.15.

Dimethyl 7-(p-fluorophenyl)6,8-dioxatricyclo[5.2.1.0⁶,₈]decane-2-endo,3-exo-dicarboxylate (75)

para-Fluorobenzaldehyde (106 mg, 856 μmol); norbornane diol 34 (105 mg, 428 μmol); column eluent [EtOAc/Pet 40-60 (1:9)]; Rf 0.14; yellow viscous oil (98 mg, 65%); υmax(thin film/cm⁻¹) 1729 (vs) (ester C=O) 1117 (vs) (acetal C-O) 1075 (s) (fluorine C-F); δh NMR (270 MHz, CDCl₃): 7.46 (2H, dd, 3JF = 2.7, 13.5 Hz, H12) 7.04 (2H, dd, 4JF = 2.7, 16.2 Hz, H11) 5.53 (1H, s, H7) 4.20 (1H, d, J = 5.7 Hz, H5) 4.08 (1H, d, J = 5.7 Hz, H9) 3.71 (3H, s, -OCH₃) 3.70 (3H, s, -OCH₃) 3.27 (1H, dd, J = 2.7, 2.7 Hz, H2) 2.82 (1H, d, J = 2.7 Hz, H4) 2.76 (1H, s, H3) 2.70 (1H, d, J = 2.7 Hz, H1) 1.93 (1H, dd, J = 1.5, 9.7 Hz, H10a) 1.43 (1H, dd, J = 1.5, 9.7 Hz, H10b); δc NMR (67.5 MHz, CDCl₃): 173.8, 172.7, 163.5 (1C, d, 3JF = 134 Hz) 131.6 (1C, d, 4JF = 2 Hz) 128.6 (2C, d, 2JF = 4 Hz) 115.4 (2C, d, 3JF = 11 Hz), 102.6, 81.7, 79.3, 52.4, 52.3, 45.3, 45.1, 43.7, 43.4, 31.9; HRMS (ESI m/z): calculated for [C₁₈H₁₉FO₆+H]⁺ 351.12384, found 351.12426.
Section 2.7 Di-carboxylic acids

General method: To a stirring solution of di-ester (100 mg to 1 g) in EtOH (5 mL) was added 1 M NaOH (20 mL) and the reaction was stirred at room temperature for 16 hours. The reaction mixture was then concentrated under vacuum to a quarter of the volume and then acidified to a pH of 1. The diluted reaction mixture was transferred to a separatory flask and extracted with EtOAc (3 × 20 mL). The organic phases were separated, dried using MgSO₄ and filtered. The filtrate was collected and the solvent removed \textit{in vacuo}. Each product was checked by NMR, confirmed as pure and used in the next step without further purification.

7-(pentadecyl)-6,8-dioxatricyclo[5.2.1.0²⁶,⁸]decane-2-endo, 3-exo-dicarboxylic acid (76)\textsuperscript{145}

Di-ester 64 (1.39 g, 2.98 mmol); white solid (1.15 g, 87%); mp 61.2 - 64.3 °C; \(\nu_{\text{max}}\) (thin film/cm\(^{-1}\)) 2977 (m) (methyl C-H) 1685 (vs) (carboxylic acid C=O) 1458 (m) (methylene C-H) 1087 (s) (acetal C-O); \(\delta_H\) NMR (270 MHz, CDCl\(_3\)): \(\delta_H 4.67 (1H, t, J = 4.9 Hz, H7) 4.04 (2H, m, H5, H9) 3.27 (1H, dd, J = 2.7, 2.7 Hz, H2) 2.78 (1H, d, J = 2.7 Hz, H4) 2.72 (1H, s, H3) 2.64 (1H, d, J = 5.4 Hz, H1) 1.82 (1H, d, J = 10.2 Hz, H10a) 1.62 (6H, m, H12-14) 1.35-1.23 (21H, m, H10b, H15-24) 0.86 (3H, t, J = 6.7 Hz, H25); \(\delta_C\) NMR (67.5 MHz, CDCl\(_3\)): 178.84, 177.64, 104.43, 81.28, 78.32, 45.19, 43.62, 34.07,
32.00, 29.76-29.13 (12 carbons), 24.75, 24.72, 22.78, 14.20; MS (ESI m/z): calculated for [C\textsubscript{25}H\textsubscript{42}O\textsubscript{6}-H]- 437.29, found 437.29.

7-[(p-phenyl)phenyl]-6,8-dioxatricyclo[5.2.1.0\textsuperscript{6,8}]decane-2-endo, 3-exo-dicarboxylic acid (77)

Di-ester 65 (703 mg, 1.72 mmol); white solid (578 mg, 88%); mp 217.5 – 219.6 °C; \(\nu_{\text{max}}\) (thin film/cm\(^{-1}\)) 1693 (s) (aromatic C=C) 1451 (s) (aromatic C=C) 1382 (m) (carboxylic acid C=O) 1089 (s) (acetal C-O); \(\delta\)\textsubscript{H} NMR (270 MHz, CD\textsubscript{3}OD): 7.50 (9H, m, H11-19) 5.62 (1H, s, H7) 4.22 (2H, m, H5, H9) 3.24 (1H, dd, \(J = 5.4, 5.4\) Hz, H2) 2.78 (1H, d, \(J = 2.7\) Hz, H4) 2.72 (1H, d, H3) 2.64 (1H, d, \(J = 5.4\) Hz, H1) 2.00 (1H, dd, \(J = 1.2, 7.7\) Hz, H10a) 1.44 (1H, dd, \(J = 1.2, 7.7\) Hz, H10b); \(\delta\)\textsubscript{C} NMR (67.5 MHz, CD\textsubscript{3}OD): 175.5, 174.2, 142.4, 140.5, 135.1, 128.6 (2 carbons), 127.3, 127.2 (2 carbons), 126.7 (2 carbons), 126.6 (2 carbons), 102.9, 81.8, 79.4, 45.4, 45.3, 43.8, 43.3, 31.4; MS (ESI m/z): calculated for [C\textsubscript{22}H\textsubscript{20}O\textsubscript{6}+Cl]- 415.09, found 415.13.

7-[(p-bromo phenyl)-6,8-dioxatricyclo[5.2.1.0\textsuperscript{6,8}]decane-2-endo, 3-exo-dicarboxylic acid (78)

Di-ester 66 (1.21 g, 2.94 mmol); white solid (975.1 mg, 87%); mp 201.2-204.6 °C; \(\nu_{\text{max}}\) (thin film/cm\(^{-1}\)) 1712 (vs) (aromatic C=C) 1701 (vs) (aromatic C=C) 1410 (s) (carboxylic acid C=O) 1070 (m) (acetal C-O) 700 (m) (bromine C-BR); \(\delta\)\textsubscript{H} NMR (270 MHz, CD\textsubscript{3}OD): 7.54 (2H, d, \(J_{AB} = 5.7\) Hz, H12) 7.40 (2H, d, \(J_{AB} = 5.7\) Hz, H11) 5.55 (1H, s, H7) 4.20 (2H, m, H5, H9) 3.22 (1H, dd, \(J = 2.7, 2.7\) Hz, H2) 2.75 (1H,
d, \( J = 2.7 \) Hz, H4) 2.70 (1H, s, H3) 2.62 (1H, d, \( J = 2.7 \) Hz, H1) 1.90 (1H, d, \( J = 6.9 \) Hz, H10a) 1.41 (1H, d, \( J = 6.9 \) Hz, H10b); \( \delta_c \) NMR (67.5 MHz, CD\(_3\)OD): 175.4, 174.1, 135.5, 131.2 (2 carbons), 128.5 (2 carbons), 123.2, 102.3, 81.8, 79.4, 45.3, 45.2, 43.7, 43.2, 31.4; MS (ESI m/z): calculated for [C\(_{16}\)H\(_{13}\)BrO\(_6\)+Na]- 402.97, found 402.98.

7-[(\( p \)-octynyl)-phenyl]-6,8-dioxatricyclo[5.2.1.0\( ^6,8 \)]decane-2-endo,3-exo-dicarboxylic acid (79)

Di-ester 67 (404 mg, 960 \( \mu \)mol); brown solid (348 mg, 88%); mp 168.3 – 173.1 °C; \( \nu_{\text{max}} \) (thin film/cm\(^{-1}\)) 1739 (vs) (alkyne C≡C) 1691 (vs) (aromatic C=C) 1421 (m) (carboxylic acid C=O); \( \delta_h \) NMR (270 MHz, CD\(_3\)OD): 7.39 (4H, s, H11-14) 5.57 (1H, s, H7) 4.23 (2H, m, H5, H9) 3.32 (1H, d, \( J = 2.7 \) Hz, H2) 2.91 (1H, d, \( J = 2.7 \) Hz, H4) 2.86 (1H, s, H3) 2.72 (1H, d, \( J = 2.7 \) Hz, H1) 2.39 (2H, t, \( J = 4.7 \) Hz, H15) 2.00 (1H, d, \( J = 6.9 \) Hz, H10a) 1.63-1.25 (9H, m, H10b, H16-19) 0.89 (3H, t, \( J = 4.7 \) Hz, H20); \( \delta_c \) NMR (67.5 MHz, CD\(_3\)OD): 175.5, 174.2, 135.4, 131.0 (2 carbons), 126.6 (2 carbons), 125.5, 102.7, 90.6, 81.8, 79.9, 79.4, 45.4, 45.2, 43.8, 43.3, 31.4, 31.2, 28.5, 28.4, 22.3, 18.7, 13.1; MS (ESI m/z): calculated for [C\(_{24}\)H\(_{28}\)O\(_6\)+H]\(^+\) 413.20, found 413.20.
7-phenyl-6,8-dioxatricyclo[5.2.1.0^{6,8}]decane-2-endo, 3-exo-dicarboxylic acid (80)

Di-ester 69 (495 mg, 1.49 mmol); white solid (403 mg, 89%); mp 81.5 – 82.9 °C; $\nu_{\text{max}}$(thin film/cm$^{-1}$) 1541 (m) (carboxylic acid C=O) 1456 (m) (aromatic C=C) 1073 (w) (acetal C-O); $\delta_H$ NMR (270 MHz, CD$_3$OD): 8.03-7.37 (5H, m, H11-15) 5.56 (1H, s, H7) 4.19 (2H, m, H5, H9) 3.22 (1H, dd, $J = 2.7, 2.7$ Hz, H2) 2.76 (1H, d, $J = 2.7$ Hz, H4) 2.71 (1H, s, H3) 2.76 (1H, d, $J = 2.7$ Hz, H1) 1.96 (1H, dd, $J = 0.9, 6.5$ Hz, H10a) 1.41 (1H, dd, $J = 0.9, 6.5$ Hz, H10b); $\delta_C$ NMR (67.5 MHz, CD$_3$OD): 176.9, 175.5, 130.7, 130.6, 129.4, 129.3, 127.9, 126.7, 104.4, 82.9, 80.6, 46.7, 46.5, 45.1, 44.6, 32.7; MS (ESI m/z): calculated for [C$_{16}$H$_{16}$O$_6$+Cl]$^-$ 339.06, found 339.03.

7-pyrenyl-6,8-dioxatricyclo[5.2.1.0^{6,8}]decane-2-endo,3-exo-dicarboxylic acid (81)

Di-ester 69 (117 mg, 256 μmol); yellow solid (101 mg, 92%); mp 272.1 – 274.1 °C; $\nu_{\text{max}}$(thin film/cm$^{-1}$) 3044 (m) (aromatic C-H) 1728 (vs) (aromatic C=C) 1408 (m) (carboxylic acid C=O); $\delta_H$ NMR (270 MHz, CD$_3$OD): 8.38-8.11 (9H, m, H11-19) 6.62 (1H, s, H7) 4.45 (2H, m, H5, H9) 3.26 (1H, dd, $J = 5.4, 5.4$ Hz, H2) 2.87 (1H, d, $J = 5.4$ Hz, H4) 2.79 (1H, s, H3) 2.72 (1H, dd, $J = 2.7, 5.4$ Hz, H1) 1.99 (1H, d, $J = 9.1$ Hz, H10a) 1.45 (1H, d, $J = 9.1$ Hz, H10b); $\delta_C$ NMR (67.5 MHz, CD$_3$OD): 175.4, 174.2, 131.9, 131.2, 130.6, 128.9, 127.6, 127.6, 127.4, 127.2, 127.0, 125.8, 125.2, 125.1, 125.0, 124.3, 124.2, 100.3, 45.4, 45.3, 45.2, 43.8, 43.7, 43.3, 31.4; MS (ESI m/z): calculated for [C$_{26}$H$_{20}$O$_6$+2H]$^{2+}$ 215.0, found 215.0.
7-[(p-octyl)-phenyl]-6,8-dioxatricyclo[5.2.1.06,8]decane-2-endo,3-exo-dicarboxylic acid (83)

Di-ester 71 (232 mg, 521 μmol); brown solid (208 mg, 96%); mp 162.3 – 165.40 °C; \( \nu_{\text{max}} \) (thin film/cm\(^{-1}\)) 2957 (s) (methyl C-H) 1708 (vs) (aromatic C=C) 1551 (s) (carboxylic acid C=O) 1131 (s) (acetal C-O) 831 (1,4-disubstitution of aromatic C-H); \( \delta \)\textsubscript{H} NMR (270 MHz, CD\textsubscript{3}OD): 7.35 (2H, d, \( J_{AB} = 5.4 \) Hz, H11) 7.32 (2H, d, \( J_{AB'} = 5.4 \) Hz, H12) 5.55 (1H, s, H7) 4.19-4.17 (2H, m, H5, H9) 3.22 (1H, dd, \( J = 2.7, 2.7 \) Hz, H2) 2.75 (1H, d, \( J = 2.7 \) Hz, H4) 2.7 (1H, s, H3) 2.62 (1H, d, \( J = 6.9 \) Hz, H10a) 1.62-1.28 (13H, m, H10b, H16-21) 0.91 (3H, t, \( J = 4.7 \) Hz, H22); \( \delta \)\textsubscript{C} NMR (67.5 MHz, CD\textsubscript{3}OD): 175.5, 174.2, 135.3, 130.9 (2 carbons), 126.5 (2 carbons), 125.4, 102.6, 90.5, 81.6, 79.8, 79.3, 47.8, 47.6, 47.4, 47.3, 31.3, 31.1, 28.4, 28.3, 28.2, 22.2, 13.0; MS (ESI m/z): calculated for [C\(_{24}\)H\(_{32}\)O\(_6\)+H]\(^+\) 417.22, found 417.22.

7-[(3-benzyloxy)phenyl]-6,8-dioxatricyclo[5.2.1.06,8]decane-2-endo,3-exo-dicarboxylic acid (84)

Di-ester 72 (120 mg, 273 μmol); white solid (96.4 mg, 89%); mp 246.0-247.1 °C; \( \nu_{\text{max}} \) (thin film/cm\(^{-1}\)) 3136 (br w) (aromatic C-H) 1645 (vs) (carboxylic acid C=O) 1217 (w) (oxy-phenyl C-O) 1015 (m) (acetal C-O); \( \delta \)\textsubscript{H} NMR (270 MHz, CD\textsubscript{3}OD): 7.44-7.00 (9H, m, H11-14, H16-20) 5.54 (1H, s, H7) 5.09 (2H, s, H15) 4.18 (2H, m, H5, H9) 3.21 (1H, dd, \( J = 5.4, 5.4 \) Hz, H2) 2.74 (1H, d, \( J = 2.7 \) Hz, H4) 2.69 (1H, s, H3) 2.61 (1H, d, \( J = 2.7 \) Hz, H1) 1.88 (1H, d, \( J = 8.1 \) Hz, H10a)
1.39 (1H, d, J = 10.8 Hz, H10a); δ H NMR (67.5 MHz, CD3OD): 174.24, 173.9, 158.9, 137.6, 137.3, 129.3, 129.1, 128.2 (2 carbons), 127.6, 127.3, 127.2, 119.2, 115.8 (2 carbons), 112.9, 102.9, 81.6, 79.3, 69.7, 45.0, 43.2, 31.4; MS (ESI m/z): calculated for [C23H22O7-2H]2- 204.06, found 204.06.

7-heptyl-6,8-dioxatricyclo[5.2.1.0^6,8]decane-2-endo,3-exo-dicarboxylic acid (85)

Di-ester 73 (678 mg, 1.91 mmol); white solid (601 mg, 96%); mp 134.4-138.0 °C; \( \nu_{\text{max}} \) (thin film/cm\(^{-1}\)) 2925 (m) (methyl C-H) 1751 (vs) (carboxylic acid C=O) 1134 (s) (acetal C-O); δ H NMR (270 MHz, CD3OD): 4.68 (1H, t, J = 2.7 Hz, H7) 4.04 (2H, br m, H5, H9) 3.26 (1H, dd, J = 2.7, 5.4 Hz, H2) 2.79 (1H, d, J = 2.7 Hz, H4) 2.73 (1H, s, H3) 2.65 (1H, d, J = 2.7 Hz, H1) 1.82 (1H, d, J = 10.4 Hz, H10a) 1.64-1.59 (2H, m, H11) 1.38-1.22 (11H, m, H10b, H12-16) 0.86 (3H, t, J = 4.7 Hz, H17); δ C NMR (67.5 MHz, CD3OD): 179.1, 177.9, 104.5, 81.3, 78.7, 45.2, 45.1, 43.6, 43.3, 32.8, 31.8, 29.8, 29.6, 29.2, 24.3, 22.7, 14.1; MS (ESI m/z): calculated for [C17H26O6-H]- 325.16, found 325.16.

7,7-dipropyl-6,8-dioxatricyclo[5.2.1.0^6,8]decane-2-endo,3-exo-dicarboxylic acid (82)

Di-ester 70 (506 mg, 1.49 mmol); white solid (411 mg, 89%); mp 196.7-200.1 °C; \( \nu_{\text{max}} \) (thin film/cm\(^{-1}\)) 2957 (w) (methyl C-H) 1697 (vs) (carboxylic acid C=O) 1140 (s) (acetal C-O); δ H NMR (270 MHz, CD3OD): 4.13-4.08 (2H, m, H5, H9) 3.25 (1H, dd, J = 2.7, 2.7 Hz, H2) 2.74 (1H, d, J = 2.7 Hz, H4) 2.69 (1H, s, H3) 2.64 (1H, d, J = 2.7 Hz, H1) 1.85 (1H,
d, $J = 10.4$ Hz, H10a) 1.61-1.2 (9H, m, H10b, H11-12, H14-15) 0.88 (6H, t, $J = 6.4$ Hz, H13, H16); $\delta$ NMR (67.5 MHz, CD$_3$OD): 178.9, 177.6, 113.2, 80.8, 78.1, 60.6, 45.3, 43.6, 37.9, 37.3, 31.8, 21.2, 20.8, 17.9, 17.2, 14.4; MS (ESI m/z): calculated for [C$_{16}$H$_{24}$O$_6$-H$^-$] 311.15, found 311.15.

**7,7-dimethyl-6,8-dioxatricyclo[5.2.1.0$^6,8$]decane-2-endo,3-exo-dicarboxylic acid (86)**

Di-ester 74 (414 mg, 1.46 mmol); white solid (280 mg, 75%); mp 197.5-197.8 °C; $\nu$ max (thin film/cm$^{-1}$) 2984 (m) (methyl C-H) 2854 (m) (methyl C-H) 1701 (vs) (carboxylic acid C=O); $\delta$H NMR (270 MHz, CD$_3$OD): 4.15 (2H, m, H5, H9) 3.26 (1H, dd, $J = 5.4$, 5.4 Hz, H2) 2.73 (1H, d, $J = 5.4$ Hz, H4) 2.67-2.64 (2H, m, H1, H3) 1.83 (1H, d, $J = 10.6$ Hz, H10a) 1.43-1.36 (4H, m, H11, H10b) 1.28 (3H, s, H12); $\delta$C NMR (67.5 MHz, CD$_3$OD): 175.6, 174.3, 109.3, 80.99, 78.42, 45.3, 45.2, 43.6, 43.2, 30.8, 24.3, 22.9; MS (ESI m/z): calculated for [C$_{12}$H$_{16}$O$_6$-H$^-$] 255.09, found 255.09.

**Section 2.8 Mono-protected EDA**

The following compounds are known compounds and were prepared using literature conditions:

- Benzyl 2-aminoethylcarbamate 89$^{,232}$
- $N$-Boc-Ethylenediamine 91$^{,206}$
Section 2.9 Di-amide coupling

General methodology: A solution of di-carboxylic acid (approx. 100 mg), mono-protected EDA (3 eq.) and EDCI 92 (3 eq.) and HOBt 97 (0.1 eq) and CHCl₃ (3 mL) was irradiated in a microwave for 30 minutes at 50 °C. The reaction mixture was transferred to a separatory flask and washed with H₂O (2 × 10 mL) and brine (10 mL). The aqueous layers were combined and extracted with CHCl₃ (10 mL). The organic layers were combined, dried (MgSO₄), filtered and the solvent removed in vacuo. The resultant crude products were purified using column chromatography with gradient elution [(1:1)EtOAc/Pet 40-60 → 5% EtOH in (1:1) EtOAc/Pet 40-60]. Fractions containing the desired compound were combined and the solvent removed in vacuo.

7-[(p-phenyl)phenyl]-6,8-dioxatricyclo[5.2.1.0⁶,8]decane-2-endo,3-exo-\(\text{N}^2,\text{N}^3\)-bis(benzyl 2-ethyl carbamate) dicarboxamide (104)

Di-carboxylic acid 77 (214 mg, 562 μmol); Cbz-EDA 89 (327 mg, 1.68 mmol); EDCI 92 (323 mg, 1.68mmol); HOBt 97 (20 mg, 148 μmol); R₇ 0.24; white oil (167 mg, 41%); \(\nu\)max(thin film/cm⁻¹) 3306 (br m) (secondary amines N-h) 1703 (s) (aromatic C=C) 1648 (s) (amide C=O); \(\delta\)H NMR (270 MHz, CDCl₃): 7.57-7.28 (19H, m, H11-19, H25-27, H33-35) 6.93 (1H, br t, -NH) 6.85 (1H, br t, -NH) 5.64 (2H, t, \(J = 3.2\) Hz, H20, H28) 5.55 (1H, s, H7) 5.07 (4H, s, H24, H32) 4.28 (1H, d, \(J = 3.7\) Hz, H5) 4.16 (1H,
d, \( J = 3.7 \) Hz, H9) 3.33-3.25 (8H, m, H21-22, H29-30) 2.99 (1H, d, \( J = 2.7 \) Hz, H2) 2.65 – 2.63 (2H, m, H3, H4) 2.54 (1H, d, \( J = 2.7 \) Hz, H1) 2.00 (1H, d, \( J = 6.9 \) Hz, H10a) 1.58 (1H, d, \( J = 6.9 \) Hz, H10b); \( \delta_{\text{C NMR}} \) (67.5 MHz, CDCl3): 174.5, 172.6, 157.4, 157.2, 142.6, 140.7, 136.5, 134.9, 128.86*, 128.61*, 128.59*, 128.22*, 128.11*, 128.07*, 127.58*, 127.26*, 126.2, 103.0, 82.1, 79.3, 67.0, 66.9, 46.7, 46.3, 44.2, 44.0, 40.9, 40.7, 40.2, 39.9, 32.6 (Figure 65); HRMS (ESI \( m/z \)): calculated for [C42H44N4O8+Na]+ 755.3057, found 755.30820.

Figure 65 – Carbon NMR spectrum of protected amine 104. \( \delta_{128.8} – \delta_{127.3} \) is magnified.

\* \( \delta_{128.86} – \delta_{127.26} \) signifies the region in the Carbon NMR spectrum where there are 19 carbons. Unfortunately, there are multiple carbons associated with each peak and it is difficult to assign a specific number of carbons for each peak.

7-[(\( p \)-bromo)-phenyl]-6,8-dioxatricyclo[5.2.1.0\( 6,8 \)]decane-2-endo,3-exo-\( N^2,N^3 \)-bis(benzyl 2-ethyl carbamate) dicarboxamide (105)

Di-carboxylic acid 78 (77.5 mg, 202 \( \mu \)mol); Cbz-EDA 89 (168 mg, 607 \( \mu \)mol); EDCI 92 (116 mg, 607 \( \mu \)mol); HOBt 97 (7 mg, 51 \( \mu \)mol); \( R_f \) 0.29; white oil (96.7 mg, 65%); \( \nu_{\text{max}} \) (thin film/cm\(^{-1}\)) 3056 (m) (aromatic C-H) 1639 (vs) (amide C=O) 1452 (vs) (aromatic C=C) 1068 (vs) (acetal C-O) 814 (vs) (para substitution of aromatic C-H) 653 (vs) (bromine C-
Br); \(\delta^H\) NMR (270 MHz, CDCl\(_3\)): 7.46 (2H, d, \(J_{AB} = 5.7\) Hz, H12) 7.27 (12H, br m, H11, H18-20, H26-28) 6.99 (1H, br t, -NH) 6.89 (1H, br t, -NH) 5.65 (1H, br t, -NH) 5.58 (1H, br t, -NH) 5.43 (1H, s, H7) 5.05-4.94 (4H, s, H17, H25) 4.24 (1H, br d, H5) 4.12 (1H, d, \(J = 1.7\) Hz, H9) 3.32-3.25 (8H, m, H14-15, H22-23) 2.82 (1H, d, \(J = 5.4\) Hz, H2) 2.63 (1H, s, H4) 2.56 (1H, d, \(J = 5.4\) Hz, H3) 2.43 (1H, d, \(J = 5.4\) Hz, H1) 1.88 (1H, d, \(J = 6.7\) Hz, H10a) 1.54 (1H, d, \(J = 5.4\) Hz, H10b); \(\delta^C\) NMR (67.5 MHz, CDCl\(_3\)): 174.14, 172.35, 157.46, 157.21, 136.37, 134.99, 131.66 (2 carbons), 128.66 (2 carbons), 128.49 (2 carbons), 128.33 (2 carbons), 128.32, 128.20 (2 carbons), 128.16 (2 carbons), 128.07 123.75, 102.43, 82.02, 79.27, 77.31 67.11, 66.89, 47.69, 44.67, 44.32, 43.49, 41.05, 40.64, 40.03, 36.24, 32.7; HRMS (ESI m/z): calculated for \([C_{36}H_{39}BrN_4O_8+H]^+\) 735.20295 and 737.20165, found 735.20313 and 737.20120.

7-[(p-octyl)-phenyl]-,6,8-dioxatricyclo[5.2.1.0\(^6,8\)]decane-2-endo,3-exo-\(N^2,N^3\)-bis(benzyl 2-ethyl carbamate) dicarboxamide (106)

Octyl phenyl di-carboxylic acid 83 (33.5 mg, 80.4 \(\mu\)mol); Cbz-EDA 89 (46.9 mg, 241 \(\mu\)mol); EDCI 92 (46.3 mg, 241 \(\mu\)mol); HOBt 97 (10 mg, 74 \(\mu\)mol); \(R_f\) 0.48; brown oil (26.3 mg, 43%); \(\nu_{\text{max}}\) (thin film/cm\(^{-1}\)) 2971 (s) (methyl C-H) 1644 (vs) (amide C=O) 1531 (s) (aromatic C=C) 1487 (m) (methylene C-H) 1263 (m) (aromatic C-H) 1073 (s) (acetal C-O) 834 (m) (para-substitution of aromatic C-H); \(\delta^H\) NMR (270 MHz, CDCl\(_3\)): 7.43-7.30 (14H, m, H11-14, H28-30, H36-38) 6.47 (2H, br m, -NH) 5.47 (1H, s, H7) 5.41 (1H, br t, -NH) 5.28 (1H, br t, -NH) 5.10-5.04 (4H, m, H27, H33) 4.25 (1H, d,
$J = 3.7$ Hz, H5) 4.10 (1H, d, $J = 3.7$ Hz, H9) 3.42-3.21 (9H, m, H2, H24-25, H32-33)
2.76 (1H, d, $J = 5.4$ Hz, H4) 2.66 (1H, s, H3) 2.56 (1H, d, $J = 2.7$ Hz, H1) 2.39 (2H, t, $J = 4.7$ Hz, H15) 1.95 (1H, d, $J = 6.9$ Hz, H10a) 1.63-1.25 (13H, m, H10b, H16-21)
0.89 (3H, t, $J = 4.7$ Hz, H22); $\delta$C NMR (67.5 MHz, CDCl₃): 174.15, 172.38, 157.48, 157.18, 136.58, 136.37, 134.98, 131.63 (2 carbons), 128.85, 128.65 (2 carbons), 128.33 (2 carbons), 128.19 (2 carbons), 128.07 (2 carbons), 126.68 (2 carbons), 125.53, 102.86, 91.44, 81.95, 80.32, 79.16, 77.29, 67.15, 66.89, 47.99, 44.40, 43.43, 41.13, 40.82, 40.65, 40.00, 32.84, 31.42, 29.77, 28.73, 28.69, 22.64, 19.52, 14.14; MS (ESI m/z): calculated for [C₄₄H₅₆N₄O₈+ 2K]²⁺ 423.17, found 423.16.

7-pyrenyl-6,8-dioxatricyclo[5.2.1.0⁶,8]decane-2-endo,3-exo-di-tert-butyl(carboxamido ethyl)carbamate (107)

Pyrenyl di-carboxylic acid 81 (262 mg, 612 μmol); Boc-EDA 91 (294 mg, 1.84 mmol); EDCI 92 (352 mg, 1.84 mmol); HOBt 97 (100 mg, 740 μmol); $R_f$ 0.02; yellow solid (248 mg, 57%); mp 225.1 – 225.9 °C; $\nu_{max}$ (thin film/cm⁻¹) 3307 (m) (secondary amine N-H) 1691 (vs) (aromatic C=C) 1641 (vs) (amide C=O); $\delta$H NMR (270 MHz, CDCl₃): 8.42-8.01 (9H, m, H11-19) 6.86 (1H, br t, -NH) 6.67 (1H, br t, -NH) 6.58 (1H, s, H7) 5.05 (1H, br t, -NH) 4.93 (1H, br t, -NH) 4.61 (1H, d, $J = 3.7$ Hz, H5) 4.40 (1H, d, $J = 3.7$ Hz, H9) 3.50-3.28 (8H, m, H21-22, H26-27) 2.95 (1H, br d, H2) 2.81 (1H, s, H4) 2.73 (1H, d, $J = 5.4$ Hz, H3) 2.54 (1H, d, $J = 5.4$ Hz, H1) 2.06 (1H, d, $J = 7.4$ Hz, H10a) 1.34-1.24 (19H, m, H10b, H24, H29); $\delta$C NMR (67.5 MHz, CDCl₃): 174.0, 172.4, 157.11 (2 carbons), 131.9, 131.2, 130.6, 128.9, 128.1 (2 carbons), 127.9 (2 carbons), 127.4 (2 carbons) 126.0 (2 carbons) 125.4 (2
carbons) 124.7 (2 carbons), 100.6, 82.1, 79.4, 77.6 (2 carbons), 47.9 43.4, 40.5 (4 carbons), 32.8, 31.9, 29.7, 28.4 (6 carbons); HRMS (ESI m/z): calculated for [C_{40}H_{48}N_{4}O_{8}+Na]^+ 735.33644, found 735.33724.

7-{[(3-benzyloxy)-phenyl]-6,8-dioxatricyclo[5.2.1.0^{6,8}]decane-2-endo,3-exo-di-tert-butyl(carboxamido ethyl)carbamate (108)

Benzylxy phenyl di-carboxylic acid 84 (68.5 mg, 167 μmol); Boc-EDA 91 (80.2 mg, 501 μmol); EDCI 92 (96 mg, 501 μmol); HOBt 97 (50 mg, 370 μmol); R f 0.02; white solid (42.2 mg, 36%); mp 145.3 – 147.5 °C; $\nu_{\text{max}}$ (thin film/cm$^{-1}$) 2925 (m) (methyl C-H) 1688 (vs) (aromatic C=C) 1642 (vs) (amide C=O) 1251 (vs) (aryl ether C-O) 1165 (vs) (acetal C-O); $\delta_{\text{H NMR}}$ (270 MHz, CDCl$_3$): 7.39-6.93 (11H, m, H11-14, H16-20 H24, H29) 5.51 (1H, s, H7) 5.40 (2H, br m, H21, H26) 5.01 (2H, s, H15) 4.29 (1H, br d, H5) 4.18 (1H, br d, H9) 3.35-3.16 (9H, m, H2, H22-23, H27-28) 2.68-2.64 (3H, m, H1, H3-4) 1.92 (1H, d, J = 6.7 Hz, H10a) 1.57 (1H, d, J = 6.7 Hz, H10b) 1.42 (18H, m, H25, H30); $\delta_{\text{C NMR}}$ (67.5 MHz, CDCl$_3$): 174.2, 172.4, 158.9, 156.8, 137.6, 136.9, 129.6, 128.6 (2 carbons), 128.1, 127.6 (2 carbons), 119.4, 116.1, 113.0, 102.9, 82.0, 79.8, 79.7, 79.2, 70.1, 47.1, 45.6, 44.4, 43.7, 40.8 (2 carbons), 40.4 (2 carbons), 32.6, 29.8, 28.5 (6 carbons); HRMS (ESI m/z): calculated for [C_{37}H_{50}N_{4}O_{9}+H]^+ 695.36506, found 695.36623.
Section 2.10 Saponification

Bicyclo[2.2.1]hept-5-ene-2,3-dicarboxylic acid (109)²¹⁵

To a stirring solution of norbornene di-ester 33 (57.5 g, 273 mmol) in EtOH (100 mL), 1M NaOH solution (300 mL) was added. The reaction mixture was stirred for 16 hours at room temperature, concentrated under vacuum to a quarter of the volume and acidified to pH 1. The reaction mixture was transferred to a separatory flask and extracted with EtOAc (3 x 100 mL). The organic phases were combined, dried with MgSO₄ and the solvent removed \textit{in vacuo}. The resulting crude solid was washed with CHCl₃ and dried under vacuum. The di-carboxylic acid was obtained as a white solid and determined pure by NMR spectroscopy. (44.5 g, 89%); mp 147.8 – 150.1 °C; $\nu_{\text{max}}$(thin film/cm⁻¹) 1647 (w) (alkene C=C) 1541 (w) (carboxylic acid C=O); $\delta_H$ NMR (270 MHz, CD₃OD): 6.28 (1H, m, H₅) 6.10 (1H, m, H₆) 3.37-3.11 (3H, m, H₂, H₃-4) 2.59 (1H, dd, $J = 2.7$, 2.7 Hz, H1) 1.61 (1H, dd, $J = 1.5$, 7.2 Hz, H₇a) 1.44 (1H, dd, $J = 1.5$, 7.2 Hz, H₇b); $\delta_C$ NMR (67.5 MHz, CD₃OD): 177.8, 176.8, 138.7, 136.2, 61.7, 52.3, 46.8, 30.7, 14.5; MS (ESI $m/z$): calculated for [C₉H₁₀O₄+Na]$^+$ 205.04, found 205.05.
Section 2.11 Di-amide coupling

Bicyclo [2.2.1]hept-5-ene-2-endo,3-exo-di-tertbutyl(carboxamido ethyl)carbamate (110)

A solution of norbornene di-carboxylic acid 109 (2.19 g, 12.03 mmol), EDCI 92 (6.92 mg, 36.1 mmol) and HOBt 97 (300 mg, 2.2 mmol) in DMF (55 mL) was stirred for 15 minutes at room temperature. Then Boc-EDA 91 (5.78 mg, 36.1 mmol) was added and the reaction mixture was stirred for 16 hours at room temperature. The reaction solvent was then removed in vacuo. A small quantity of H2O was added and the reaction mixture was allowed to stand for several hours till a thick yellow precipitate had formed. The slurry was transferred to a Hirsch funnel, washed with copious amounts of H2O (500 mL) and allowed to dry under vacuum to give the desired norbornene 110 as a white solid. (4.86 g, 87%); \( \nu_{\text{max}} \) (thin film/cm\(^{-1}\)) 1683 (vs) (amide C=O); \( \delta \) \( ^1 \text{H} \) NMR (270 MHz, CDCl\(_3\)): 7.02 (1H, br t, H11) 6.85 (1H, br t, H15) 6.23-6.19 (2H, m, H5-6) 5.02 (1H, br t, H8) 4.96 (1H, br t, H12) 3.37-3.28 (8H, m, H9-10, H13-14) 3.09 (1H, br d, H2) 3.04 (1H, br d, H4) 2.95 (1H, dd, \( J = 2.7, 5.4 \) Hz, H3) 2.32 (1H, dd, \( J = 2.7, 5.4 \) Hz, H1) 2.33 (1H, d, \( J = 2.2 \) Hz, H7a) 1.53 (1H, d, \( J = 2.2 \) Hz, H7b) 1.44 (9H, s, H13) 1.43 (9H, s, H18); \( \delta \) \( ^13 \text{C} \) NMR (67.5 MHz, CDCl\(_3\)): 175.5, 174.3, 157.2, 156.4, 137.6, 135.1, 79.9, 79.5, 51.2, 49.1, 48.4, 45.3, 44.2, 43.1, 41.2, 40.7, 40.2, 28.4 (6 carbons); HRMS (ESI m/z): calculated for [C\(_{23}\)H\(_{38}\)N\(_4\)O\(_6\)+H\(^+\)] 467.28641, found 467.28616.
Section 2.12 Upjohn Dihydroxylation

6,8-dihydroxybicyclo[2.2.1]hept-5-ene-2-endo,3-exo-di-tertbutyl(carboxamido ethyl)carbamate (111)

To a solution of H₂O: acetone (1:4) (20 mL), norbornene 110 (2.23 g, 4.78 mmol) and NMO 45 (672 mg, 5.74 mmol) were added and allowed to stir until the solution was clear. Osmium tetroxide 41 (250 μL, 5% in t-BuOH, 49 μmol) was then added and the mixture stirred at room temperature for 16 hours. Sodium metabisulphite solution (8 mL, 0.53 M) was added and the reaction mixture allowed stirring for a further ten minutes. The mixture was then transferred into a separatory funnel and extracted using EtOAc (3 × 20 mL). The combined organic phases were dried (MgSO₄) and the solvent removed in vacuo resulting in a yellow solid. No further purification was performed and the sample was determined to be pure by NMR spectroscopy. (2.10 g, 88%); mp 171.3 – 175.2 °C; \( \nu_{\text{max}} \) (thin film/cm\(^{-1} \)) 3305 (m) (hydroxyl O-H) 2974 (m) (methyl C-H) 1640 (vs) (amide C=O); \( \delta_H \) NMR (270 MHz, CD₃OD):

- 8.10 (1H, br t, -NH)
- 7.97 (1H, br t, -NH)
- 6.64 (1H, br t, -NH)
- 6.59 (1H, br t, -NH)
- 3.75 (2H, m, H5, H9)
- 3.34 – 3.07 (9H, m, H2, H9-10, H14-15)
- 2.61 (1H, d, \( J = 2.7 \) Hz, H4)
- 2.37 (1H, s, H3)
- 2.24 (1H, s, H1)
- 1.83 (1H, d, \( J = 5.4 \) Hz, H7a)
- 1.51 (1H, d, \( J = 5.4 \) Hz, H7b)

\( \delta_C \) NMR (67.5 MHz, CD₃OD): 175.16, 173.21, 157.22 (2 carbons), 78.81, 73.32, 69.44, 66.05, 54.88, 49.56, 47.13, 44.73, 39.99, 39.68, 39.54, 39.40, 31.56, 27.45 (6 carbons); MS (ESI \( m/z \)):

calculated for \([C_{23}H_{40}N_4O_8+H]^+\) 501.29, found 501.29.
Section 2.14 Synthesis of Boc-protected amines

General methodology: A solution of diol 111, aldehyde/ketone (2 eq.), oxalic acid (1 eq.) and MgSO$_4$ in CHCl$_3$/MeCN (20 mL) was stirred vigorously for 16 hours at 50 °C. The reaction mixture was cooled to room temperature, filtered and the solvent removed in vacuo resulting in brown solids. The crude solid was purified using column chromatography [(1:9) EtOAc/Pet 40-60 → 5% EtOH in (1:1) EtOAc/Pet 40-60]. Fractions containing the desired compound were combined and the solvent removed in vacuo resulting in solids.

7-pentadecyl-6,8-dioxatricyclo[5.2.1.0$^{6,8}$]decane-2-endo,3-exo-di-tert-butyl(carboxamido ethyl)carbamate (113)

Diol 111 (168.1 mg, 335.8 μmol); hexadecanal 46 (161.5 mg, 672 μmol); oxalic acid (42.3 mg, 335.8 μmol); R$_f$ 0.76 (in 5% EtOH in (1:1) EtOAc/Pet 40-60); brown solid (95 mg, 40%); mp 120.4 – 125.1 °C; $\nu_{\text{max}}$(thin film/cm$^{-1}$) 2916 (vs) (methyl C-H) 2848 (s) (methylene C-H) 1693 (vs) (amide C=O) 1129 (m) (acetal C-O); $\delta_{\text{H}}$ NMR (270 MHz, CDCl$_3$): 7.05 (1H, br t, -NH) 6.86 (1H, br t, -NH) 5.19 (2H, br m, H$_{26}$, H$_{31}$) 4.62 (1H, t, $J$ = 3.2 Hz, H$_7$) 4.10 (1H, d, $J$ = 3.5 Hz, H$_5$) 3.96 (1H, d, $J$ = 3.7 Hz, H$_9$) 3.32-3.24 (8H, m, H$_{27-28}$, H$_{32-33}$) 2.98 (1H, dd, $J$ = 2.7, 2.7 Hz, H$_2$) 2.55-2.54 (2H, m, H$_{3-4}$) 2.46 (1H, d, $J$ = 2.7 Hz, H$_1$) 1.78 (1H, d, $J$ = 6.9 Hz, H$_{10a}$) 1.47-1.23 (47H, m, H$_{10b}$,
H11-24, H30, H35) 0.86 (3H, t, J = 4.5 Hz, H25); δ\textsubscript{C} NMR (67.5 MHz, CDCl\textsubscript{3}): 174.60, 172.70, 156.89, 156.80, 103.99, 81.56, 79.72, 79.57, 78.68, 77.32, 46.76, 45.83, 44.15, 43.72, 40.26 (4 carbons), 34.16, 32.91, 32.26, 31.99, 29.76 (6 carbons), 29.54, 29.44, 29.35, 29.19, 28.47 (2 carbons), 24.85, 24.30, 22.76 (2 carbons), 14.18; HRMS (ESI m/z): calculated for [C\textsubscript{39}H\textsubscript{70}N\textsubscript{4}O\textsubscript{8}+H\textsuperscript{+}] 723.52664, found 723.52681.

7-[(p-phenyl)-phenyl]-6,8-dioxatricyclo[5.2.1.0\textsuperscript{6,8}]decane-2-endo,3-exo-di-\textit{tert}-butyl(carboxamido ethyl)carbamate (118)

Diol 111 (291 mg, 582 μmol); para-phenyl benzaldehyde (212 mg, 1.16 mmol); oxalic acid (73.3 mg, 582 μmol); Rf 0.76 (in 5% EtOH in (1:1) EtOAc/Pet 40-60); white solid (354 mg, 91%); mp 142.1 – 144.2 °C; \(\nu\textsubscript{max}\) (thin film/cm\textsuperscript{-1}) 2928 (m) (methyl C-H) 1684 (vs) (aromatic C=C) 1637 (vs) (amide C=O) 1072 (s) (acetal C-O) 835 (m) (aromatic 1,4-disubstitution C-H); δ\textsubscript{H} NMR (270 MHz, CDCl\textsubscript{3}): 7.60-7.33 (9H, m, H11-19) 6.86 (1H, br t, -NH) 6.68 (1H, br t, -NH) 5.61 (1H, s, H7) 5.04 (1H, br t, -NH) 4.96 (1H, br t, -NH) 4.38 (1H, br d, H5) 4.18 (1H, d, J = 3.5 Hz, H9) 3.45-3.30 (8H, m, H21-22, H26-27) 3.01 (1H, dd, J = 2.7, 2.7 Hz, H2) 2.71 (1H, s, H4) 2.70 (1H, d, J = 2.7 Hz, H3) 2.52 (1H, d, J = 5.4 Hz, H1) 2.04 (1H, d, J = 7.2 Hz, H10a) 1.60-1.44 (19H, m, H10b, H24, H29); δ\textsubscript{C} NMR (67.5 MHz, CDCl\textsubscript{3}): 174.1, 172.4, 157.1, 156.9, 140.8, 135.0, 128.8 (2 carbons), 127.5 (2 carbons), 127.3 (2 carbons), 127.2 (2 carbons), 124.8, 122.8 103.0, 82.0, 79.2, 77.3 (2 carbons), 49.9, 47.9, 44.5, 41.3, 40.6 (2 carbons), 40.4 (2 carbons), 32.0,
28.5 (6 carbons); HRMS (ESI m/z): calculated for [C\textsubscript{36}H\textsubscript{48}N\textsubscript{4}O\textsubscript{8}+Na\textsuperscript{+}] 687.33644, found 687.33712.

7-dimethyl-6,8-dioxatricyclo[5.2.1.0\textsubscript{6,8}]decane-2-endo,3-exo-di-tert-butyl(carboxamido ethyl)carbamate (119)

Diol 111 (300 mg, 599 μmol); para-bromo benzaldehyde (222 mg, 1.2 mmol); oxalic acid (75.6 mg, 599 μmol); R\textsubscript{f} 0.43 (in 5% EtOH in (1:1) EtOAc/Pet 40-60); white solid (209 mg, 52%); mp 141.1 – 141.8 °C; \(v\textsubscript{max}(\text{thin film/cm}^{-1})\) 2928 (m) (methyl C-H) 1690 (s) (aromatic C=C) 1638 (vs) (amide C=O) 1071 (s) (acetal C-O) 821 (m) (1,4-disubstitution of aromatic C-H) 629 (m) (bromine C-Br); \(\delta\text{H NMR (270 MHz, CDCl}_3\)): 7.49 (2H, d, \(J\textsubscript{AB} = 5.4\) Hz, H12) 7.34 (2H, d, \(J\textsubscript{A'B'} = 5.4\) Hz, H11) 6.90 (1H, br t, -NH) 6.69 (1H, br t, -NH) 6.50 (1H, s, H7) 5.03 (1H, br t, -NH) 4.97 (1H, br t, -NH) 4.36 (1H, br d, H5) 4.16 (1H, br d, H9) 3.42-3.29 (8H, m, H 14-15, H19-20) 2.96 (1H, dd, \(J = 2.7, 2.7\) Hz, H2) 2.68 (1H, s, H4) 2.65 (1H, d, \(J = 2.7\) Hz, H3) 2.47 (1H, d, \(J = 5.4\) Hz, H1) 1.92 (1H, d, \(J = 7.4\) Hz, H10a) 1.63-1.43 (19H, m, H10b, H17, H22); \(\delta\text{C NMR (67.5 MHz, CDCl}_3\)): 174.1, 172.3, 157.1, 156.9, 135.1, 131.6 (2 carbons) 128.5 (2 carbons) 123.7, 102.4, 82.0, 79.3, 77.3 (2 carbons), 47.5, 44.5, 43.5, 40.3 (4 carbons), 32.7, 32.0, 28.5 (6 carbons); HRMS (ESI m/z): calculated for [C\textsubscript{36}H\textsubscript{43}BrN\textsubscript{4}O\textsubscript{8}+Na\textsuperscript{+}] 689.2162 and 691.21471, found 689.21629 and 691.21492.
7-[(p-octynyl)-phenyl]-6,8-dioxatricyclo[5.2.1.0^6,8]decane-2-endo,3-exo-di-tert-butyl(carboxamido ethyl)carbamate (120)

Diol 111 (218 mg, 436 μmol);
octynyl benzaldehyde 47 (93.4 mg, 872 μmol);
oxalic acid (55 mg, 436 μmol); Rf 0.43 (in 5% EtOH in 1:1 EtOAc/Pet 40-60); brown solid (156 mg, 51%); mp 89.7 – 91.2 °C;

υ_{max}(\text{thin film}/\text{cm}^{-1}) 2927 (m) (methyl C-H) 1683 (vs) (aromatic C=C) 1637 (vs) (amide C=O) 830 (m) (aromatic 1,4-disubstitution C-H) 693 (m) (alkyne C-H);

δ_{H} NMR (270 MHz, CDCl₃): 7.36 (4H, s, H11-12) 7.05 (1H, br t, -NH) 6.83 (1H, br t, -NH) 5.51 (1H, s, H7) 5.15 (1H, br t, -NH) 5.09 (1H, br t, -NH) 4.32 (1H, br d, H5) 4.16 (1H, br d, H9) 3.32-3.27 (8H, m, H20-21, H25-26) 3.00 (1H, br d, H2) 2.67 (1H, m, H3-4) 2.51 (1H, d, J = 2.7 Hz, H1) 2.38 (2H, t, J = 4.5 Hz, H13) 1.95 (1H, d, J = 6.9 Hz, H10a) 1.77-1.24 (27H, m, H10b, H14-17, H23, H28) 0.89 (3H, t, J = 4.5 Hz, H18); δ_{C} NMR (67.5 MHz, CDCl₃): 174.2, 172.4, 156.9, 156.8, 135.1, 131.6 (2 carbons) 126.6 (2 carbons), 125.4, 102.8, 91.4, 81.9, 80.3, 79.8, 79.7, 79.3, 47.1, 45.6, 44.4, 43.7, 40.8 (2 carbons), 40.4 (2 carbons), 32.6, 31.4, 29.8, 28.7, 28.6, 28.5 (6 carbons), 22.8, 22.6; HRMS (ESI m/z): calculated for [C_{38}H_{56}N_{4}O_{8}+H]^{+} 697.41709, found 697.41755.
7-dimethyl-6,8-dioxatricyclo[5.2.1.0^6,8]decane-2-endo,3-exo-di-tert-butyl(carboxamido ethyl)carbamate (121)

Diol 111 (207 mg, 414 μmol); benzaldehyde (83.7 μL, 828 μmol); oxalic acid (52.2 mg, 414 μmol); Rf 0.40 (in 5% EtOH in (1:1) EtOAc/Pet 40-60); white solid (222 mg, 91%); mp 110.5 – 110.6 °C; \( \nu_{\text{max}} \) (thin film/cm\(^{-1}\)) 2929 (m) (methyl C-H) 1684 (vs) (aromatic C=C) 1638 (vs) (aromatic C=C) 1069 (m) (acetal C-O);

\( \delta^H \) NMR (270 MHz, CDCl\(_3\)): 7.45-7.34 (5H, m, H11-15) 7.14 (2H, br m, H19, H24) 5.53 (1H, s, H7) 5.36-5.34 (2H, br m, H16, H21) 4.30 (1H, d, J = 2.2 Hz, H5) 4.18 (1H, br d, H9) 3.35-3.14 (9H, m, H2 H17-18, H22-23) 2.69 (1H, br d, H4) 2.65 (1H, s, H3) 2.50 (1H, d, J = 5.4 Hz, H1) 1.97 (1H, d, J = 6.9 Hz, H10a) 1.59-1.41 (19H, m, H10a, H20, H26); \( \delta^C \) NMR (67.5 MHz, CDCl\(_3\)): 174.1, 172.3, 156.9, 156.8, 135.9, 129.5, 128.4 (2 carbons), 126.7 (2 carbons), 103.1, 81.9, 79.87, 79.68, 79.1, 47.4, 44.9, 44.5, 43.5, 40.9, 40.4, 40.3, 32.6, 29.7, 28.4 (6 carbons); HRMS (ESI m/z): calculated for [C\(_{30}\)H\(_{44}\)N\(_4\)O\(_8\)+H]\(^+\) 589.32319, found 589.32377.

7-pyrenyl-6,8-dioxatricyclo[5.2.1.0^6,8]decane-2-endo,3-exo-di-tert-butyl(carboxamido ethyl)carbamate (107)

Diol 111 (310 mg, 618 μmol); pyrenecarboxaldehyde (285 mg, 1.24 mmol); oxalic acid (77.9 mg, 618 μmol); Rf 0.57 (in 5% EtOH in (1:1) EtOAc/Pet 40-60); yellow solid (109.6 mg, 25%); mp 215.1 – 215.9 °C; \( \nu_{\text{max}} \) (thin film/cm\(^{-1}\)) 3307
(m) (secondary amine N-H) 1691 (vs) (aromatic C=C) 1641 (vs) (amide C=O); $\delta^H$ NMR (270 MHz, CDCl$_3$): 8.42-8.01 (9H, m, H11-19) 6.86 (1H, br t, -NH) 6.67 (1H, br t, -NH) 6.58 (1H, s, H7) 5.05 (1H, br t, -NH) 4.93 (1H, br t, -NH) 4.61 (1H, d, $J$ = 3.7 Hz, H5) 4.40 (1H, d, $J$ = 3.7 Hz, H9) 3.50-3.28 (8H, m, H21-22, H26-27) 2.95 (1H, br d, H2) 2.81 (1H, s, H4) 2.73 (1H, d, $J$ = 5.4 Hz, H3) 2.54 (1H, d, $J$ = 5.4 Hz, H1) 2.06 (1H, d, $J$ = 7.4 Hz, H10a) 1.34-1.24 (19H, m, H10b, H24, H29); $\delta^C$ NMR (67.5 MHz, CDCl$_3$): 174.0, 172.4, 157.1 (2 carbons), 131.9, 131.2, 130.6, 128.9, 128.1 (2 carbons), 127.9 (2 carbons), 127.4 (2 carbons) 126.0 (2 carbons) 125.4 (2 carbons) 124.7 (2 carbons), 100.6, 82.1, 79.4, 77.6 (2 carbons), 47.9 43.4, 40.5 (4 carbons), 32.8, 31.9, 29.7, 28.4 (6 carbons); HRMS (ESI $m/z$): calculated for [C$_{40}$H$_{48}$N$_4$O$_8$+Na]$^+$ 735.33644, found 735.33724.

7-[(p-octyl)-phenyl]-6,8-dioxatricyclo[5.2.1.0$^{6,8}$]decane-2-endo,3-exo-di-tert-butyl(carboxamido ethyl)carbamate (122)

Diol 111 (153.7 mg, 307 μmol); octyloxy benzaldehyde 49 (143.9 mg, 614μmol); oxalic acid (38.7 mg, 307 μmol); Rf 0.31 (in 5% EtOH in (1:1) EtOAc/Pet 40-60); white solid (160.6 mg, 73%); mp 97.0 – 101.3 °C; $\nu_{max}$(thin film/cm$^{-1}$) 2927 (m) (methylene C-H) 2857 (methyl C-H) 1637 (vs) (amide C=O) 1616 (vs) (aromatic C=C) 1450 (vs) (aromatic C=C) 1248 (vs) (aryl ether C-O) 1168 (vs) (alkyl ether C-O) 1072 (vs) (acetal C-O) 829 (s) (para substitution C-H); $\delta^H$ NMR (270 MHz, CDCl$_3$): 7.35 (2H, d, $J_{AB}$ = 8.6 Hz, H12) 7.17 (1H, br t, -NH) 6.97 (1H, br t, -NH) 6.84 (2H, d, $J_{AB'}$ = 8.6 Hz, H11) 5.48 (1H, s, H7) 5.25 (2H, br m, H21, H26) 4.27 (1H, d, $J$ = 5.5 Hz,
H5) 4.13 (1H, d, J = 5.5 Hz, H9) 3.91 (2H, t, J = 6.7 Hz, H13) 3.33-3.24 (8H, m, H22-23, H27-28) 3.00 (1H, dd, J = 2.7, 2.7 Hz, H2) 2.79 (1H, d, J = 2.7 Hz, H4) 2.66 (1H, s, H3) 2.51 (1H, d, J = 5.4 Hz, H1) 1.99 (1H, d, J = 9.6 Hz, H10a) 1.79-1.23 (31H, m, H10b, H14-19, H25, H30) 0.86 (3H, t, J = 6.2 Hz, H20); δc NMR (67.5 MHz, CDCl3): 174.1, 171.9, 163.3, 162.8, 157.0, 153.1, 128.1 (2 carbons), 114.3 (2 carbons), 103.1, 86.4, 81.8, 79.7, 79.1, 68.1, 47.54, 45.79, 44.31, 43.76, 40.56 (2 carbons), 40.26 (2 carbons), 33.82, 32.49, 31.92, 31.81, 29.70, 29.35 (3 carbons), 29.23, 29.21 (3 carbons), 28.79, 28.44; HRMS (ESI m/z): calculated for [C38H60N4O9+H]+ 717.44331, found 717.44631.

7-[(p-octyl)-phenyl]-6,8-dioxatricyclo[5.2.1.06,8]decane-2-endo,3-exo-di-tert-butyl(carboxamido ethyl)carbamate (123)

Diol 111 (298 mg, 595 μmol); octyl benzaldehyde 48 (260 mg, 1.19 mmol); oxalic acid (75.0 mg, 595 μmol); Rf 0.29 (in 5% EtOH in (1:1) EtOAc/Pet 40-60); brown solid (302 mg, 72%); mp 121.8 – 121.5 °C; νmax(thin film/cm⁻¹) 3066 (s) (aromatic C-H) 2952 (m) (methyl C-H) 2930 (methylene C-H) 1636 (vs) (amide C=O) 1606 (vs) (aromatic C=C) 1456 (vs) (aromatic C=C) 1076 (vs) (acetal C-O) 858 (m) (para substitution of aromatic C-H); δH NMR (270 MHz, CDCl3): 7.51 (2H, 3JAB = 5.4 Hz, H12, H14) 7.46 (2H, 3JAB′ = 5.4 Hz, H11, H13) 7.03 (1H, br t, H26) 6.82 (1H, br t, H31) 5.50 (1H, s, H7) 5.14-5.09 (2H, br m, H23, H28) 4.31 (1H, d, J = 5.2 Hz, H5) 4.15 (1H, d, J = 5.2 Hz, H9) 3.33-3.25 (8H, m, H24-25, H29-30) 3.00 (1H, dd, J = 4.7, 4.7 Hz, H2) 2.67 (2H, m, H3-4) 2.53 (1H, d, J = 2.7 Hz, H1) 2.38 (2H, t, J = 6.7 Hz, H15) 1.94 (1H, d, J = 9.9
Hz, H10a) 1.65-1.20 (31H, m, H10b, H16-21, H27, H32) 0.88 (3H, t, \( J = 4.9 \text{ Hz}, \text{ H22}) \); \( \delta_C \) NMR (67.5 MHz, CDCl\(_3\)): 174.33, 172.52, 156.88, 156.80, 131.52, 131.42 (2 carbons), 129.81 (2 carbons), 129.44, 107.05, 81.93, 80.27, 79.59, 79.19, 57.35, 45.20, 43.73, 40.26 (4 carbons), 32.46, 31.34, 28.67, 28.61, 28.60, 28.56 (3 carbons), 28.41 (3 carbons), 22.55, 19.52, 19.44, 15.93, 14.05; HRMS (ESI m/z): calculated for \([C_{38}H_{60}N_4O_8+K]^+\) 739.40427, found 739.40561.

7-[(3-benzyloxy)-pheynyl]-6,8-dioxatricyclo[5.2.1.0\(^6,8\)]decane-2-endo,3-exo-di-tert-butyl(carboxamido ethyl)carbamate (108)

![Chemical Structure](image)

Diol 111 (207 mg, 413 \( \mu \text{mol}\)); benzyloxy benzaldehyde (175 mg, 826 \( \mu \text{mol}\)); oxalic acid (104.1 mg, 826 \( \mu \text{mol}\)); R\(_f\) 0.37 (in 5% EtOH in (1:1) EtOAc/Pet 40-60); white solid (243 mg, 85%); mp 142.3 – 147.5 °C; \( \nu_{max}(\text{thin film/cm}^{-1}) \) 2925 (m) (methyl C-H) 1688 (vs) (aromatic C-C) 1642 (vs) (amide C=O) 1251 (vs) (aryl ether C-O) 1165 (vs) (acetal C-O); \( \delta_H \) NMR (270 MHz, CDCl\(_3\)): 7.39-6.93 (11H, m, H11-14, H16-20 H24, H29) 5.51 (1H, s, H7) 5.40 (2H, br m, H21, H26) 5.01 (2H, s, H15) 4.29 (1H, br d, H5) 4.18 (1H, br d, H9) 3.35-3.16 (9H, m, H2, H22-23, H27-28) 2.68-2.64 (3H, m, H1, H3-4) 1.92 (1H, d, \( J = 6.7 \text{ Hz}, \text{ H10a}) 1.57 (1H, d, \( J = 6.7 \text{ Hz}, \text{ H10b}) 1.42 (18H, m, H25, H30); \( \delta_C \) NMR (67.5 MHz, CDCl\(_3\)): 174.2, 172.4, 158.9, 156.8, 137.6, 136.9, 129.6, 128.6 (2 carbons), 128.1, 127.6 (2 carbons), 119.4, 116.1, 113.0, 102.9, 82.0, 79.8, 79.7, 79.2, 70.1, 47.1, 45.6, 44.4, 43.7, 40.8 (2 carbons), 40.4 (2 carbons), 32.6, 29.8, 28.5 (6 carbons); HRMS (ESI m/z): calculated for \([C_{37}H_{50}N_4O_9+H]^+\) 695.36506, found 695.36623.
7-hexyl-6,8-dioxatricyclo[5.2.1.06,8]decane-2-endo,3-exo-di-tert-butyl(carboxamido ethyl)carbamate (124)

Diol 111 (73.9 mg, 148 μmol); octanal (37.8 mg, 295 μmol); oxalic acid (14.0 mg, 73.8 μmol); Rf 0.43 (in 5% EtOH in (1:1) EtOAc/Pet 40-60); white solid (67.8 mg, 75%); mp 123.8 – 125.3 °C; \( \nu_{\text{max}}(\text{thin film/cm}^{-1}) \) 2928 (m) (methyl C-H) 1685 and 1638 (vs) (amide C=O) 1172 (vs) (acetal C-O); \( \delta_{\text{H}} \) NMR (270 MHz, CDCl₃): 6.87 (1H, br t, -NH) 6.70 (1H, br t, -NH) 5.08-5.02 (2H, br m, H18, H23) 4.62 (1H, t, \( J = 4.7 \) Hz, H7) 4.11 (1H, d, \( J = 5.4 \) Hz, H5) 3.95 (1H, d, \( J = 5.4 \) Hz, H9) 3.38-3.25 (8H, m, H19-20, H24-25) 2.91 (1H, dd, \( J = 4.7, 4.7 \) Hz, H2) 2.55-2.50 (2H, m, H3-4), 2.31 (1H, dd, \( J = 2.7, 5.4 \) Hz, H1) 1.79 (1H, d, \( J = 10.4 \) Hz, H10a) 1.62-1.24 (32H, m, H10b, H11-16, H22, H27) 0.85 (3H, t, \( J = 6.2 \) Hz, H17); \( \delta_{\text{C}} \) NMR (67.5 MHz, CDCl₃): 177.1, 174.2, 156.9, 156.7, 104.0, 81.5, 78.6, 77.2 (2 carbons), 44.3, 40.2 (4 carbons), 32.8, 31.7, 31.6, 29.7, 29.5, 29.2, 29.1, 28.9, 28.4 (6 carbons) 24.8, 24.2, 22.7; HRMS (ESI \( m/z \)): calculated for \([\text{C}_{31}\text{H}_{54}\text{N}_4\text{O}_8+\text{H}]^+\) 611.40144, found 611.40129.

7-dimethyl-6,8-dioxatricyclo[5.2.1.06,8]decane-2-endo,3-exo-di-tert-butyl(carboxamido ethyl)carbamate (114)

Diol 111 (620 mg, 639 μmol); acetone (1 mL); oxalic acid (80.5 mg, 639 μmol); Rf 0.26 (in 5% EtOH in (1:1) EtOAc/Pet 40-60); white solid (203 mg, 59%); mp 120.9 – 123.6 °C; \( \nu_{\text{max}}(\text{thin film/cm}^{-1}) \)
1) 2977 (m) (methyl C-H) 1639 (vs) (amide C=O) 1122 (s) (acetal C-O); δH NMR (270 MHz, CDCl3): 7.04 (1H, br t, -NH) 6.80 (1H, br t, -NH) 5.19-5.16 (2H, br m, H13, H18) 4.22 (1H, d, J = 3.2 Hz, H5) 4.08 (1H, d, J = 3.2 Hz, H9) 3.39-3.23 (8H, m, H14-15, H19-20) 2.97 (1H, br d, H2) 2.56-2.48 (3H, m, H1, H3 -4) 1.79 (1H, d, J = 6.9 Hz, H10a) 1.50-1.40 (19H, m, H10b, H17, H22) 1.24 (6H, t, J = 5.7 Hz, H11-12); δC NMR (67.5 MHz, CDCl3): 174.4, 172.6, 156.9, 156.8, 109.3, 81.2, 79.9, 79.7, 78.2, 47.3, 45.2, 44.3, 43.6, 40.8 (2 carbons), 40.3 (2 carbons), 32.2, 29.8, 28.5 (6 carbons) 25.4; HRMS (ESI m/z): calculated for [C26H44N4O8+Na]+ 563.30514, found 563.30589.

7-[(p-fluoro)-phenyl]-6,8-dioxatricyclo[5.2.1.06,8]decane-2-endo,3-exo-di-tert-butyl(carboxamido ethyl)carbamate (125)

Diol 111 (205.4 mg, 410 μmol); para-fluoro benzaldehyde (101.9 mg, 821 μmol); oxalic acid (51.7 mg, 410 μmol); Rf 0.11 (in 5% EtOH in (1:1) EtOAc/Pet 40-60); white solid (153 mg, 61%); mp 172.2 – 174.5 °C; υmax(thin film/cm⁻¹) 3094 (m) (aromatic C-H) 2977 (m) (methyl C-H) 1640 (vs) (amide C=O) 1610 (vs) (amide C= O) 1451 (vs) (aryl C=C) 1392 (vs) (t-butyl C-H) 1366 (t-butyl C-H) 1122 (vs) (acetal C-O) 1100 (vs) (fluoro C-F) 834 (para substitution of aromatic C-H); δH NMR (500 MHz, CDCl3): 7.40 (2H, d, 3JAB = 5 Hz, H12) 6.99 (2H, d, 3JAB = 5 Hz, H11) 6.80 (1H, br t, -NH) 6.60 (1H, br t, -NH) 5.47 (1H, s, H7) 4.96 (1H, br t, -NH) 4.89 (1H, br t, -NH) 4.30 (1H, d, J = 5 Hz, H5) 4.09 (1H, d, J = 5 Hz, H9) 3.37 – 3.23 (8H, m, H14-15, H19-20) 2.86 (1H, dd, J = 5, 5 Hz, H2) 2.65 (1H, s, H4) 2.60 (1H, d, J = 5 Hz, H3) 2.39 (1H, d, J = 5 Hz, H1) 1.91 (1H, d, J = 10 Hz, H10a) 1.51 (1H, d, J = 10 Hz, H10a) 1.51
Hz, H10b) 1.37 (18H, s, H17, H22); δC NMR (67.5 MHz, CDCl3): 173.95, 172.26, 163.46 (1C, d, JF = 247 Hz), 156.77 (2 carbons), 131.90 (1C, d, JF = 3.75 Hz), 128.62 (2C, d, JF = 8.58 Hz), 115.36 (2C, d, JF = 21.55 Hz) 102.44, 81.87, 80.04 (2 carbons), 79.14, 47.86, 44.54, 43.30, 40.51 (4 carbons), 32.74, 28.40 (6 carbons); HRMS (ESI m/z): calculated for [C30H43FNaO8+H]+ 607.31377, found 607.31318.

Section 2.18 Deprotection and salt formation

To a 2 M acetyl chloride in MeOH solution (2 mL) was added the protected di-amine (approx. 100 mg). The reaction mixture was allowed to stir for 16 hours at room temperature. The reaction solvent was removed in vacuo, re-dissolved in EtOAc (10 mL) and transferred to a separatory flask. Extraction was performed using H2O (3 × 5 mL). The aqueous phases were combined and the water removed through sublimation.

7-pentadecyl-6,8-dioxatricyclo[5.2.1.06,8]decane-2-endo,3-exo-dicarboxamido ethanamium chloride (137)

Protected di-amine 113 (127 mg, 175 μmol); white solid (90 mg, yield: 66%, purity: 100%); mp 186 – 189 °C; v max (thin film/cm⁻¹) 2924 (m) (methyl C-H) 1640 (vs) (amide C=O) 1074 (s) (acetal C-O); δH NMR (270 MHz, D2O): 4.7 (2H, t, J = 5.4 Hz, H7),
4.14 (1H, d, J = 5.4 Hz, H9) 4.09 (1H, d, J = 5.4 Hz, H5) 3.42 – 3.18 (9H, m, H2, H27-28, H31-32) 2.70 – 2.54 (3H, m, H1, H3-4) 1.71 (1H, d, J = 5.4 Hz, H10a) 1.52 (1H, d, J = 5.4 Hz, H10b) 1.38-1.24 (28H, m, H11-24) 0.84 (3H, t, J = 2.7 Hz, H25); δc NMR (67.5 MHz, CD3OD): 175.3, 173.5, 103.8, 81.1, 78.6, 62.7, 54.4, 52.6, 48.13, 48.0, 46.3, 45.7, 43.7, 43.6, 41.1, 40.6, 38.4, 37.6, 32.5, 31.7, 31.4, 29.4, 29.3, 29.1, 26.9, 23.8, 22.4, 13.1, 6.7; HRMS (ESI m/z): calculated for [C29H56N4O4-H]+ 523.42178, found 523.42229.

7-[(p-phenyl)-phenyl]-6,8-dioxatricyclo[5.2.1.06,8]decane-2-endo,3-exo-dicarboxamido ethanamium chloride (138)

Protected di-amine 118 (89.7 mg, 134.9 μmol); white hydroscopic solid (64.8 mg, yield: 89%, purity: 98%); v_max(thin film/cm-1) 1644 (vs) (amide C=O) 1221 (vs) (aromatic C=C) 1071 (s) (acetal C-O) 835 (s) (para substitution C-H); δH NMR (270 MHz, CD3OD): 7.64-7.30 (9H, m, H11-19) 5.61 (1H, s, H7) 4.26 (1H, d, J = 5.4 Hz, H9) 4.23 (1H, d, J = 5.4 Hz, H5) 3.65-3.13 (9H, m, H2, H21-22, H25-26) 2.85-2.68 (3H, m, H1, H3-4) 2.02 (1H, d, J = 8.1 H, H10a) 1.63 (1H, d, J = 8.1 Hz, H10b); δc NMR (67.5 MHz, CD3OD): 175.5, 173.7, 142.4, 140.4, 135.1, 128.51 (2 carbons), 127.07 (2 carbons), 126.6 (2 carbons), 126.5 (2 carbons), 125.93, 102.8, 81.83, 79.1, 48.06, 47.9, 46.8, 45.8, 44.8, 43.8, 39.4, 37.2, 31.6; HRMS (ESI m/z): calculated for [C26H34N4O4-H]+ 465.24963, found 465.24921.
7-\((p\text{-bromo-phenyl})\)-6,8-dioxatricyclo[5.2.1.0\textsuperscript{6,8}\textsuperscript{6,8}]decane-2-\textit{endo},3-\textit{exo}-dicarboxamido ethanamium chloride (136)

Protected di-amine 119 (109.9 mg, 164.6 μmol); white hydroscopic solid (74.9 mg, yield: 84%, purity: 96%); \(\nu_{\text{max}}\) (thin film/cm\textsuperscript{-1}) 3285 (br s) (primary amine N-H) 1643 (vs) (amide C=O) 1597 (vs) (aromatic C=C) 1072 (vs) (acetal C-O) 820 (s) (para substitution C-H) 627 (vs) (bromine C-Br); \(\delta_H\) NMR (270 MHz, D\textsubscript{2}O): 7.65 (2H, dd, \(J_{AB} = 2.7\) Hz, \(J_{AB'} = 5.4\) Hz, H12) 7.46, (2H, dd, \(J_{A'B'} = 2.7\) Hz, \(J_{A'B} = 5.4\) Hz, H11) 5.66 (1H, s, H7) 4.36 (1H, d, \(J = 2.7\) Hz, H9) 4.29 (1H, d, \(J = 2.7\) Hz, H5) 3.66 – 3.43 (4H, m, H14, H18) 3.27 (1H, dd, \(J = 2.7, 2.7\) Hz, H2) 3.18-3.14 (4H, m, H15, H19) 1.95 (1H, d, \(J = 8.1\) Hz, H10a) 1.60 (1H, d, \(J = 8.1\) Hz, H10b); \(\delta_C\) NMR (67.5 MHz, CD\textsubscript{3}OD): 175.5, 173.6, 135.5, 131.1 (2 carbons), 128.4 (2 carbons), 123.1, 102.2, 81.88, 79.17, 48.1, 47.9 46.1, 45.8, 43.7, 39.4, 37.2, 37.1, 31.6; HRMS (ESI m/z): calculated for \([C\text{\textsubscript{20}}H\text{\textsubscript{29}}BrN\text{\textsubscript{4}}O\text{\textsubscript{4}}-H]^+\) 467.12884, found 467.12989.

7-\((p\text{-octynyl})\)phenyl\)-6,8-dioxatricyclo[5.2.1.0\textsuperscript{6,8}\textsuperscript{6,8}]decane-2-\textit{endo},3-\textit{exo}-dicarboxamido ethanamium chloride (134)

Protected di-amine 120 (106 mg, 152 μmol); brown hydroscopic solid (90 mg, yield: 99%, purity: 94%); \(\nu_{\text{max}}\) (thin film/cm\textsuperscript{-1}) 2929 (m) (methylene C-H) 2858 (methyl C-H) 1638 (vs) (amide C=O) 1539 (vs) (primary amine N-H) 1124 (vs) (acetal C-O) 830 (s) (aromatic C-H) 774 (s) (para substitution C-H); \(\delta_H\) NMR (270 MHz, CD\textsubscript{3}OD): 7.51 – 7.27 (4H, m, H11-12) 5.54 (1H, s, H7) 4.25-4.19 (2H, m, H5, H9) 3.62-3.06 (9H,
m, H2, H20-21, H24-25) 2.77-2.62 (3H, m, H1, H3-4) 1.92 (1H, d, J = 10.8 Hz, H10a) 1.62 – 1.27 (H11, m, H10b, H13-17) 0.92 (3H, t, J = 5.4 Hz, H18); δ\textsubscript{c} NMR (67.5 MHz, CD\textsubscript{3}OD): 175.5, 173.7, 131.0 (2 carbons), 129.3, 126.5 (2 carbons), 125.4, 102.5, 81.8, 79.1, 46.8, 46.7, 46.1, 45.8, 43.7, 39.5, 37.1, 37.1, 31.6, 31.1, 28.4, 28.3, 22.2, 18.6, 13.0, 2 carbons under solvent peak from 48.14 – 47.12; HRMS (ESI m/z): calculated for [C\textsubscript{28}H\textsubscript{42}N\textsubscript{4}O\textsubscript{4}-H]+ 497.31223, found 497.31397.

7-phenyl-6,8-dioxatricyclo[5.2.1.0\textsuperscript{6,8}]decane-2-endo,3-exo-dicarboxamido ethanamium chloride (139)

Protected di-amine 121 (103.8 mg, 176.3 μmol); white hydroscopic solid (81.8 mg, yield: 99%, purity: 67%); υ\textsubscript{max}(thin film/cm\textsuperscript{-1}) 3357 (br vs) (primary amine N-H) 1649 (vs) (amide C=O) 1071 (m) (acetal C-O); Characteristic peaks in the proton NMR: 5.68 (1H, s, H7) 4.34 (1H, d, J = 2.47 Hz, H9) 4.27 (1H, d, J = 2.7 Hz, H5) (2H, dd, J = 2.7, 2.7 Hz, H2) 1.98 (1H, d, J = 8.1 Hz, H10a) 1.59 (1H, d, J = 8.1 Hz, H10b); MS (ESI m/z): calculated for [C\textsubscript{20}H\textsubscript{30}N\textsubscript{4}O\textsubscript{4}-H]+ 389.22, found 389.22.

7-pyrenyl-6,8-dioxatricyclo[5.2.1.0\textsuperscript{6,8}]decane-2-endo,3-exo-dicarboxamido ethanamium chloride (140)

Protected di-amine 107 (76.5 mg, 107 μmol); yellow solid (46 mg, yield: 73%, purity: 86%); m.p 217 – 220 °C; υ\textsubscript{max}(thin film/cm\textsuperscript{-1}) 2970 (br s) (aromatic C-H) 1647 (vs) (amide C=O); δ\textsubscript{H} NMR (270 MHz, CD\textsubscript{3}OD): 8.40 – 8.01 (9H, m, H11-19) 6.61 (1H, s, H7) 4.49 (1H,
d, $J = 5.4$ Hz, H9) 4.45 (1H, d, $J = 5.4$ Hz, H5) 3.76 – 3.05 (9H, m, H2, H21-22, H25-26) 2.86 – 2.62 (3H, m, H1, H3-4) 2.00 (1H, d, $J = 10.8$ Hz, H10a) 1.59 (1H, d, $J = 10.8$ Hz, H10b); $\delta$ C NMR (67.5 MHz, CD$_3$OD): 175.6, 173.7, 132.0, 131.2, 130.6, 128.9, 128.8, 127.6, 127.5, 127.0, 125.9, 125.3, 125.1, 124.3, 124.2, 124.1, 122.7, 122.5, 100.22, 82.1, 79.3, 49.1, 48.5, 46.8, 45.8, 44.8, 39.5, 37.1, 31.7; MS (ESI m/z): calculated for [C$_{30}$H$_{34}$N$_4$O$_4$-H]$^+$ 513.24963, found 513.24993.

7-[(p-octyloxy)-phenyl]-6,8-dioxatricyclo[5.2.1.0$^{6,8}$]decane-2-endo,3-exo-dicarboxamido ethanum chloride (141)

Protected di-mine 122 (74.2 mg, 104 μmol); white hydroscopic solid (44 mg, yield: 72%, purity: 65%); $\nu$ max (thin film/cm$^{-1}$) 2964 (s) (methyl C-H) 1647 (s) (amide C=O) 1544 (s) (primary amine N-H) 1459 (m) (methylene C-H) 1270 (s) (aryl ether C-O) 1076 (m) (aceta C-Ol) 1039 (m) (aromatic C-H) 798 (m) (para substitution C-H); Characteristic peaks in the proton NMR: 5.50 (1H, s, H7) 4.21 (1H, d, $J = 5.4$ Hz, H9) 4.16 (1H, d, $J = 5.4$ Hz, H5) 1.98 (1H, d, $J = 10.8$ Hz, H10a) 1.56 (1H, d, $J = 10.8$ Hz, H10b); MS (ESI m/z): calculated for [C$_{28}$H$_{44}$N$_4$O$_5$+2K]$^{2+}$ 297.13, found 297.12.
7-[(p-octyl)phenyl]-6,8-dioxatricyclo[5.2.1.0^6,8]decane-2-endo,3-exo-dicarboxamido ethanamium chloride (142)

Protected di-amine 123 (105.0 mg, 149.8 μmol); brown hydrosopic solid (78.5 mg, yield: 91%, purity: 79%); υ_{max}(thin film/cm\(^{-1}\)) 3307 (br m) (primary amine N-H) 2955 (vs) (methyl C-H) 2923 (vs) (methylene C-H) 1688 (s) (aromatic C=C) 1639 (vs) (amide C=O) 1252 (s) (aromatic C-H) 1094 (s) (acetal C-O) 778 (m) (para substitution C-H);

δ\textsubscript{H} NMR (270 MHz, CD\textsubscript{3}OD): 7.42 – 7.32 (4H, m, H11-14) 5.54 (1H, s, H7) 4.24 – 4.18 (2H, m, H5, H9) 2.77 – 2.62 (3H, m, H1, H3-4) 1.92 (1H, d, J = 10.8 Hz, H10a) 0.92 (3H, t, J = 5.4 Hz, H22); MS (ESI m/z): calculated for [C\(_{28}\)H\(_{44}\)N\(_{4}\)O\(_{4}\) +2(NH\(_{4}\))]\(^{2+}\) 268.20, found 268.20.

7-[(3-benzyloxy)phenyl]-6,8-dioxatricyclo[5.2.1.0^6,8]decane-2-endo,3-exo-dicarboxamido ethanamium chloride (143)

Protected di-amine 108 (101 mg, 145 μmol); white hydrosopic solid (72.9 mg, yield: 88%, purity: 56%); υ_{max}(thin film/cm\(^{-1}\)) 3307 (br vs) (primary amine N-H) 1651 (vs) (amide C=O) 1615 (vs) (aromatic C=C) 1272 (vs) (benzyl ether C-O) 1084 (vs) (acetal C-O) 778 (vs) (meta substitution C-H);

δ\textsubscript{H} NMR (270 MHz, CD\textsubscript{3}OD): 7.43 – 6.92 (H9, m, H11-14, H16-20) 5.52 (1H, s, H7) 5.08 (2H, s, H15) 3.80 – 3.73 (2H, d, H5, H9) 1.88 (2H, d, J = 10.8 Hz, H10a) 1.49 (1H, d, J = 10.8 Hz, H10b); MS (ESI m/z): calculated for [C\(_{27}\)H\(_{36}\)N\(_{4}\)O\(_{5}\)-H]\(^{+}\) 495.26, found 495.26.
7-octyl-6,8-dioxatricyclo[5.2.1.0^6,8]decane-2-endo,3-exo-dicarboxamido ethanamium chloride (144)

Protected di-amine 124 (105 mg, 172 μmol); white hydroscopic solid (79 mg, yield: 95%, purity: 100%); \( \nu_{\text{max}} \) (thin film/cm\(^{-1} \)) 3292 (br s) (primary amine N-H) 2924 (vs) (methylene C-H) 2856 (vs) (methyl C-H) 1641 (vs) (amide C=O) 1116 (s) (acetal C-O); \( \delta \) \(_{\text{H}}\) NMR (270 MHz, CD\(_3\)OD): 4.70 (2H, t, \( J = 5.4 \) Hz, H7) 4.14 (1H, d, \( J = 2.7 \) Hz, H5) 4.09 (1H, d, \( J = 2.7 \) Hz, H9) 3.79 – 3.69 (1H, m, H2) 3.42-3.16 (8H, m, H20-21, H24-25) 2.70 (1H, d, \( J = 2.7 \) Hz, H4) 2.67 (1H, d, \( J = 2.7 \) Hz, H3) 2.54 (1H, s, H1) 1.74 (1H, d, \( J = 10.8 \) Hz, H10a) 1.46 (1H, d, \( J = 10.8 \) Hz, H10b) 1.28 (H10, m, H12-17) 0.89 (3H, t, \( J = 8.1 \) Hz, H18); \( \delta \) \(_{\text{C}}\) NMR (67.5 MHz, CD\(_3\)OD): 175.6, 173.7, 103.7, 81.4, 78.6, 45.9, 45.8, 43.7, 43.6, 39.5, 39.4, 37.1, 37.1, 32.5, 31.5, 29.2, 28.93, 23.8, 22.3, 13.0, 2 carbon under solvent peak from 48.13 – 47.11; HRMS (ESI m/z): calculated for \([\text{C}_{22}\text{H}_{40}\text{N}_{4}\text{O}_{4}+2\text{(NH}_{4}\text{)}]^{2+}\) 230.18630, found 230.18916.

7,7-dimethyl-6,8-dioxatricyclo[5.2.1.0^6,8]decane-2-endo,3-exo-dicarboxamido ethyl guanidinium chloride (145)

 Protected di-amine 114 (94.7 mg, 175 μmol); white solid (74 mg, yield: 99%, purity: 95%); mp 143 – 146 °C; \( \nu_{\text{max}} \) (thin film/cm\(^{-1} \)) 2970 (br s) (methyl C-H) 1648 (vs) (amide C=O) 1067 (vs) (acetal C-O); \( \delta \) \(_{\text{H}}\) NMR (270 MHz, CDCl\(_3\)): 3.84 (1H, d, \( J = 5.4 \) Hz, H9) 3.76 (1H, d, \( J = 5.4 \) Hz, H5) 3.64 – 3.42 (5, m, H2, H14, H18) 3.14 (4H, m, H15, H19) 2.39 – 2.39 (3H, m, H1, H3-4) 1.86 (1H, d, \( J = 5.4 \) Hz, H10a)
1.53 (1H, d, $J = 5.4$ Hz, H10b) 1.46 (3H, s, H11) 1.32 (3H, s, H12); $\delta$ C NMR (67.5 MHz, CD$_3$OD): 175.9, 174.1, 109.1, 81.1, 78.1, 46.8, 45.7, 43.7, 39.4, 37.1, 31.5, 31.1, 24.2, 22.9, 2 carbons under solvent peak from 48.1 – 47.1; HRMS (ESI m/z): calculated for [C$_{16}$H$_{30}$N$_4$O$_4$-H]+ 341.21833, found 341.21681.

7-($p$-fluorophenyl)-6,8-dioxatricyclo[5.2.1.0$^{6,8}$]decane-2-endo,3-exo-dicarboxamido ethanamium chloride (146)

Protected di-amine 125 (100 mg, 165 μmol); white hydroscopic solid (63.2 mg, yield: 80%, purity: 70%); $\nu_{\text{max}}$(thin film/cm$^{-1}$) 3309 (br vs) (primary amine N-H) 1649 (vs) (amide C=O) 1071 (s) (acetal C-O) 1012 (s) (fluorine-aryl C-F) 821 (m) (para substitution C-H); Characteristic peaks in proton NMR: 5.56 (1H, s, H7) 4.23 (1H, d, $J = 5.4$ Hz, H9) 4.19 (1H, d, $J = 5.4$ Hz, H5) 2.77 – 2.62 (3H, m, H1, H3-4) 1.93 (1H, d, $J = 10.8$ Hz, H10a) 1.56 (1H, d, $J = 10.8$ Hz, H10b); MS (ESI m/z): calculated for [C$_{20}$H$_{27}$FN$_4$O$_4$+NH$_4$]$^+$ 424.24, found 424.23.
Chapter 3
Amines to Guanidines

In the current chapter, the aim was to synthesise a library of guanidine functionalised norbornane based CAMP mimics. Three guanylating agents were explored. Once the library of protected guanidines was produced, the deprotection protocol was re-evaluated. A number of guanidine salts were successfully formed and sent for antibacterial testing.

3.1 One Step Protocol

Scheme 37 illustrates how guanidines can be synthesised from amines. In this project therefore, in theory the protected guanidines may be obtained in just one step from the amine functionalised compounds synthesised in Chapter 2.

Scheme 37 – One step from amines to protected guanidines.

The removal of Boc groups and salt formation protocols have already been discussed and applied in Chapter 2, as such the step to be explored in detail in this chapter is the guanylation of amines.

3.2 Guanylating agents

A variety of guanylating agents are available. A selected few are shown in Scheme 38: di-Boc methyl isothiourca 147, di-Boc thiourea 148 and di-Boc
triflylguanidine 149 (each of these will convert amines to Boc-protected guanidines). A detailed discussion of each guanylating agent, their synthesis and application to the current project is provided in the following sections.

![Scheme 38 - Guanylating agents](image)

The following sections are not intended to be an authoritative review of all guanylating agents. For additional detail, please refer to the review by Katritzky and Rogovoy.

### 3.3 Di-Boc methylisothiourea 147

![Figure 66 - Structure of di-Boc methylisothiourea 147](image)

Di-Boc methylisothiourea 147 is a popular guanylating agent (Figure 66) which is used either individually or with an ‘activating’ agent. An example of the individual use of di-Boc methylisothiourea 147 was published by König et. al. in the synthesis of receptors to selectively recognise amino acids and small peptides. Reacting the amino crown ether 150 with di-Boc methylisothiourea 147 afforded the protected guanidine 151 in an excellent yield of 78% (Scheme 39).
Di-Boc methylisothiourea 147 has been also used with an ‘activating’ agent, the most common being HgCl₂. Cammidge et al. added HgCl₂ to a solution of an amine and di-Boc methylisothiourea 147 (Scheme 40) which produced the desired protected guanidines within four hours. Three of the guanidines synthesised by Cammidge are featured in Scheme 40. Guanidine 152 was sterically hindered yet a high yield of 77% was obtained. Anilines were also successfully guanylated, for example 153 and 154. These examples illustrate that with HgCl₂, 147 was an efficient guanylating agent in the presence of other functional groups.

The mechanism by which di-Boc methylisothiourea 147 and an ‘activating’ agent converts amines to guanidines is not precisely known. There are proposals that the ‘activating’ agent coordinates to di-Boc methylisothiourea
and facilitates conversion to the reactive carbodiimide 155 (Scheme 41).\textsuperscript{237} The amine nucleophile attack on the reactive carbodiimide 155 to afford the guanidine is believed to be the rate determining step.\textsuperscript{237}

\begin{center}
\includegraphics[width=0.8\textwidth]{scheme41.png}
\end{center}

Scheme 41 – Proposed mechanism for guanylations using di-Boc methylisothiourea 147 and an ‘activating’ agent.\textsuperscript{237, 239}

The method for the synthesis of di-Boc methylisothiourea 147 is robust and rapid and involves two steps: methylation of thiourea 156 and protection of thiourea 157 (Scheme 42).\textsuperscript{236, 240, 241}

\begin{center}
\includegraphics[width=0.8\textwidth]{scheme42.png}
\end{center}

Scheme 42 – Two step synthesis of di-Boc methylisothiourea 147.\textsuperscript{240, 241}

3.3.1 Synthesis of di-Boc methylisothiourea 147

In the current project, methyl iodide was chosen for the methylation of thiourea 156. A solution of thiourea 156 and methyl iodide in MeOH was stirred at 65 °C for ninety minutes (Scheme 43).\textsuperscript{240, 242, 243} A white ‘crumbly’ solid was isolated in quantitative yield and identified as methyl isothiourea 157 by comparison of proton NMR spectra with literature data.\textsuperscript{243}

\begin{center}
\includegraphics[width=0.8\textwidth]{scheme43.png}
\end{center}

Scheme 43 – The methylation of thiourea 156 using methyl iodide to synthesise methyl isothiourea 157.\textsuperscript{243}

The protection of amines using Boc\textsubscript{2}O 90 has been discussed on page 58. In the current section of the project, Boc\textsubscript{2}O 90 was employed to protect both amines of methyl isothiourea 157 (Scheme 44).
A bi-phasic mixture of methyl isothiourea 157 in saturated bicarbonate solution and Boc₂O 90 in DCM was stirred vigorously for sixteen hours at room temperature. After an aqueous extraction, a crude yellow solid was obtained. The crude solid was precipitated from hot EtOH using H₂O to give di-Boc methylisothiourea 147 with a yield of 94% (Scheme 44).

3.3.2 Synthesis of guanidines using di-Boc methylisothiourea 146

In the current project, the reaction conditions first trialled was to stir amine 139 in a solution of di-Boc methylisothiourea 147 and HgCl₂ (activating agent) in THF at 50 °C for 3 hours. Unfortunately, guanylation was not successfully (Scheme 45). An alternative method of heating was considered since guanylations using di-Boc methylisothiourea 147 and conventional heating may take up to two days (Scheme 39). 235
A microwave mediated method by Sun et al., albeit using different guanylating agent 160, showed the guanylating reaction between the PEG supported amine 159 and di-Boc benzotriazole carboxamidine (160) was reduced from six hours to just seven minutes (Scheme 46). In the current project therefore, the guanylations with di-Boc methylisothiourea 147 were also trialled using microwave irradiation (Table 13).

Table 13 - Amines subjected to guanylation using di-Boc methylisothiourea 147.

<table>
<thead>
<tr>
<th>No.</th>
<th>Yield</th>
<th>No.</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>162</td>
<td>N/A</td>
<td>163</td>
<td>N/A</td>
</tr>
<tr>
<td>158</td>
<td>N/A</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Each di-amine was dissolved in a THF solution containing di-Boc methylisothiourea 147, HgCl2 and Et3N. The reaction mixture was irradiated in a microwave laboratory for fifteen minutes at 80 °C. After aqueous work up, the crude oils were analysed by proton NMR spectroscopy. Unfortunately, none of the guanylations using di-Boc methylisothiourea 147 were successful. As such, it was deemed an unsuitable agent for the synthesis of guanidine-functionalised norbornanes. Hence, another guanylating agent, di-Boc thiourea 148 was trialled.
3.4 Di-Boc thiourea 148

Another popular guanylation agent is di-Boc thiourea 148 (Figure 67).234, 245, 246 An example of its use was in the total synthesis of peptidic minalemine A 164 (Scheme 47a). Munoz et al. utilised di-Boc thiourea 148 to install the guanidine moieties that ‘bookend’ the peptide. Both cadaverine and putrescine were guanylated using di-Boc thiourea 148 in DMF at room temperature in thirty minutes. Both guanylated products, 165 and 166 were obtained in excellent yields (92% and 91% respectively).

Like di-Boc methylisothiourea 147, di-Boc thiourea 148 was sometimes used with an ‘activating’ agent such as HgCl2. Mercury salts however are extremely toxic. Other examples of less toxic ‘activating’ agents are carbodiimides, 1-methyl-2-chloropyridinium iodide 167 (Mukaiyama’s reagent) and N-iodosuccinimide 168 (NIS) (Figure 68).
Again, the exact mechanism of di-Boc thiourea 148 guanylations is still unknown.239 However, there are a series of proposed steps similar to the proposed guanylation mechanism of di-Boc methylisothiourea 147. Firstly, the ‘activating’ agents either react or coordinate to di-boc thiourea 148.247 After the addition of Et3N, there is a proposed electrophilic di-Boc carbodiimide intermediate 155.239, 248 The amine then attacks the intermediate 155 resulting in the desired protected guanidine (Scheme 48).

\[
\text{Scheme 48} \quad \text{Mechanism of guanylation using di-Boc thiourea 148 and an ‘activating’ agent.}^{239, 247, 248}
\]

A recently discovered ‘activating’ agent of di-Boc thiourea 148 is NIS 168. Vasseur and Smietana et. al. were interested in replacing agents such as the mercury salts and also Mukaiyama’s reagent 167 as there were solubility problems in most stand organic solvents.247 They discovered that NIS 168 was an excellent replacement. By using di-Boc thiourea 148 and NIS 168, guanylation of a large number of amines at room temperature in DCM in 2 to 22 hours was achieved in yields of up to 85%.247

\[
\text{Scheme 49} \quad \text{Guanidine products from the use of di-Boc thiourea 148 and NIS 168.}^{247}
\]
In this project therefore, NIS \textbf{168} was selected as the ‘activating’ agent to be used with di-Boc thiourea \textbf{148}. First however, di-Boc thiourea \textbf{148} was required.\textsuperscript{245,246}

\subsection*{3.4.1 Synthesis of di-Boc thiourea \textbf{148}}

Following literature conditions for the synthesis of di-Boc thiourea \textbf{148}, a solution of thiourea \textbf{156} in 150 mL of THF was cooled to 0 °C. Then NaH was added and allowed to stir for five minutes at room temperature to ensure complete deprotonation. Then, the reaction mixture was cooled back down to 0 °C at which Boc\textsubscript{2}O \textbf{90} was added. The resulting slurry was stirred at room temperature for two hours and then quenched with saturated bicarbonate solution. The reaction mixture was then subjected to an aqueous extraction which furnished the desired di-Boc thiourea \textbf{148}, with no requirement for further purification, in a yield of 53\% (yields of up 86\% have been reported in the literature).\textsuperscript{246}

\begin{center}
\textbf{Scheme 50} – Synthesis of di-Boc thiourea \textbf{148}.
\end{center}

The structure of di-Boc thiourea \textbf{148} was confirmed by comparing proton NMR spectra with literature data.\textsuperscript{246}

\subsection*{3.4.2 Synthesis of guanidines using di-Boc thiourea \textbf{148}}

The reaction of Vasseur and Smietana were used (Scheme 49).\textsuperscript{247} Each amine was added to a solution of di-Boc thiourea \textbf{148} and Et\textsubscript{3}N in DCM/MeOH and once fully dissolved, NIS \textbf{168} was added and the reaction stirred for sixteen
hours. After aqueous work up, the crude brown oils obtained were purified using column chromatography. The resulting clear viscous oils were characterised using proton and carbon NMR spectroscopy, IR and mass spectrometry. The yields of all reactions attempted are listed in Table 14.

Table 14 – Yields of guanylations using di-Boc thiourea 147 and NIS 168.

<table>
<thead>
<tr>
<th>No.</th>
<th>Yield</th>
<th>No.</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>162</td>
<td>34%</td>
<td>163</td>
<td>26%</td>
</tr>
<tr>
<td>171</td>
<td>18%</td>
<td>175</td>
<td>21%</td>
</tr>
<tr>
<td>172</td>
<td>9%</td>
<td>176</td>
<td>20%</td>
</tr>
<tr>
<td>173</td>
<td>N/A</td>
<td>177</td>
<td>28%</td>
</tr>
<tr>
<td>158</td>
<td>6%</td>
<td>178</td>
<td>11%</td>
</tr>
<tr>
<td>174</td>
<td>12%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

While successful, the use of NIS 168 and di-Boc thiourea 148 gave low yields (all less than 35%) and in the case of guanidine 173 unsuccessful. It became clear that di-Boc thiourea 148 would not be a suitable guanylation agent for this project. Attention now turned to the evaluation of the last guanylation agent, di-Boc triflylguanidine 149.
3.5 Di-Boc triflylguanidine 149

Goodman et al. had certain criteria in mind when developing their guanylation agent.\textsuperscript{249-251} Firstly, guanidines should be produced in high yields using mild conditions. Secondly, guanylation should be efficient with complex amines particularly during solid phase synthesis. Lastly, the reagent should be synthesised from relatively inexpensive starting materials. The result of their endeavours was di-Boc triflylguanidine 149 (Figure 69).

![Figure 69 – Structure of di-Boc triflylguanidine 149.](image)

Goodman et al. demonstrated the efficiency of Di-Boc triflylguanidine 149 guanylation of primary amines (yields from 75\% to 100\%).\textsuperscript{249} Impressive outcomes considering the reactions were preliminary studies. Subsequent publications showed the ease at which di-Boc triflylguanidine 149 guanylated hindered secondary amines.\textsuperscript{250} Other benefits of guanylation agent 149 were its stability (it can be stored at room temperature for three months). Consequently, Goodman et al. reasoned that if the compound was refrigerated, the chemical would remain stable indefinitely.

Since, Goodman et al. first reported di-Boc triflylguanidine 149 as an efficient guanylation agent in 1998, reagent 149 has proved to be the most popular and efficient guanylation agent and is consistently used for the late stage introduction of the guanidine moiety to complex molecules.\textsuperscript{227, 228, 233, 238, 249-258} One of the best examples of how efficient di-Boc triflylguanidine 149 can be was provided by Hamm and Harth et al.
Hamm and Harth functionalised dendrimers with guanidines to target and control cellular delivery of bioactive cargo across the membrane of mammalian cells.\textsuperscript{257} With di-Boc triflylguanidine 149, installation of the guanidine moiety occurred at the second to last step on the already complex and hindered dendrimer 179. Stirring at room temperature, Hamm and Harth were able to install nine guanidines to form the protected guanylated dendrimer 180 in an excellent yield of 90\% (Scheme 51).

![Scheme 51 - Guanylation of dendrimer using di-Boc triflylguanidine 149 (n = 1 or 5).\textsuperscript{257}]

The mechanism by which guanylation occurs with di-Boc triflylguanidine 148 is not specifically addressed in the literature. In order to propose a plausible mechanism, there must be an examination of the trifluoromethylsulphonamide (triflyl) functional group 181. The triflyl group 181 was first discussed in 1957 by Gramstad and Hazeldine (Figure 70a).\textsuperscript{259} They used trifluoromethanesulphonic anhydride 183 for efficient installation of the triflyl group.
Many years later in 1973, Hendrickson and Bergeron employed the triflyl group as a protecting group for amines and found that trifluoromethanesulfonamides could be synthesised quantitatively from trifluoromethanesulfonic anhydride and the products were generally stable and crystalline (Figure 70d). Furthermore, Hendrickson and Bergeron discovered that in cleaving the triflyl group, two kinds of fragmentation were observed (Figure 71). The first type of fragmentation of the triflyl group observed is the $S_N2$ displacement of triflamide, a stabilised leaving group (Figure 71a). The second type of fragmentation results in a trifluoromethanesulfinate anion (Figure 71b).

The first type of fragmentation of the triflyl group observed is the $S_N2$ displacement of triflamide, a stabilised leaving group (Figure 71a). The second type of fragmentation results in a trifluoromethanesulfinate anion (Figure 71b). According to studies conducted by Goodman et al., during the process of guanylation by di-Boc triflylguanidine, triflamide is a by-product. Hence, the first fragmentation between carbon and nitrogen observed by Hendrickson and Bergeron most likely occurs in a guanylation by di-Boc triflylguanidine. Further studies by Glass confirmed that other nucleophiles, such as halogens, nitrogens and cyanide, attack...
trifluoromethanesulfonamides at the expense of the carbon-nitrogen bond for the preferential displacement of triflamides 184.262

By incorporating all of the information above, a proposed mechanism for the guanylations of amines by di-Boc triflylguanidine 149 is shown in Scheme 52. The nucleophile, which is the free amine 185, attacks the ‘imine’ carbon on di-Boc triflylguanidine 148. Following a proton shift, intermediate 186 may result. It is argued that since triflamide 184 would be the stable and preferred leaving group, the base would preferentially attack the hydrogen on a carbamate of intermediate 186 thereby leading to the displacement of triflamide 184 and the formation of the desired protected guanidine 187.

3.5.1 Synthesis of di-Boc triflylguanidine 149

Goodman et. al. outlined a two step methodology by which di-Boc triflylguanidine 149 could be synthesised. The first step was the protection of guanidine hydrochloride 188 using Boc₂O 90 forming di-Boc guanidine 189. From there triflylation using triflic anhydride 183 occurs whereby di-Boc triflylguanidine 149 is obtained.263, 264
3.5.2 Protection of guanidine hydrochloride 188

\[\text{NH}_2 \quad \text{NH}_2 \quad \text{Boc}_2\text{O} \quad 39\% \quad \text{Boc}_2\text{NH} \quad \text{Boc}_2\text{NH} \quad \text{Boc}\]

Scheme 54 – The synthesis of di-Boc guanidine 189 from guanidine hydrochloride 188.250

In the current project, the method of Goodman was followed (Scheme 54) to give after column chromatography the desired di-Boc guanidine 189 in a yield of 39%. The structure of di-Boc guanidine 189 was confirmed by comparison of proton NMR spectra with literature.250

Figure 72 – Structures of mono-Boc guanidine 190, di-Boc guanidine 189 and tri-Boc guanidine 191.

Yields of the di-Boc protection of guanidine hydrochloride 188 stated in literature range from 43% to 60%.249, 250, 264, 265 Therefore, a yield of 39% is not considered poor. One of the disadvantages highlighted by Goodman et. al. of his method of protection for guanidine hydrochloride 188 is that the reaction results in a mixture of products: mono-Boc guanidine 190, di-Boc guanidine 189 and tri-Boc guanidine 191 (Figure 72). With competing products, high yields are not likely. Furthermore, it was found that in the current project purification by column chromatography was very problematic sometimes requiring two or three attempts. With so many difficulties, a different method of synthesising di-Boc guanidine 189 was explored.

3.5.3 Amination of di-Boc methylisothiourea 147

Whilst developing the Mitsunobu reaction for the conversion of alcohols to protected guanidines, Hammerschmidt and Kvaternik developed an alternative
method for the synthesis of di-Boc guanidine 189. By treating di-Boc methylisothiourea 147 with ammonia, they synthesised di-Boc guanidine 189 in an excellent yield of 95%. The methodology developed by Hammerschmidt and Kvaternik was replicated in this project to synthesise di-Boc guanidine 189 (Scheme 55) in 70% yield. The method using di-Boc methylisothiourea 147 had the advantage of higher yields and no further purification needed beyond an aqueous extraction during work up.

![Scheme 55 - Synthesis of di-Boc guanidine 189 using di-Boc methylisothiourea 147.](image)

### 3.5.4 Synthesis of di-Boc triflylguanidine 149

To install the triflyl group 181, di-Boc guanidine 189 was treated with triflic anhydride 183. It is assumed that the imine 193 acts as a nucleophile in reacting with triflic anhydride 183. Trifluoromethanesulphonic acid 182 is eliminated leaving the protected imine 194 (Scheme 56).

![Scheme 56 - Proposed mechanism for the protection of an imine using triflic anhydride 183.](image)

In the current project, a solution of di-Boc guanidine 189 and Et3N in DCM was cooled to -78 °C. Then triflic anhydride 183 was added drop wise over twenty minutes. The reaction solution was allowed to warm to -20 °C over four hours. After quenching with sodium bisulphite solution and column chromatography,
the desired di-Boc triflylguanidine 149 was isolated in a yield of 61% (Scheme 57). The structure of guanylation agent 149 was confirmed by comparison with published data.250

\[
\begin{align*}
\text{Scheme 57} & \quad \text{Synthesis of di-Boc triflylguanidine 149 from di-Boc guanidine 189.}
\end{align*}
\]

3.5.5 Synthesis of guanidines using di-Boc triflylguanidine 149

In the reaction to install the guanidine moiety, guanylation agent 149 and the amine are stirred in DCM at room temperature until the reaction has reached completion.227, 249, 250, 254, 257 Accordingly, each amine was stirred in a solution of di-Boc triflylguanidine 149 and Et₃N in DCM at room temperature for 16 hours monitoring by TLC. After an aqueous work up, each crude product was purified using column chromatography (Table 15). The structure of each protected guanidine was confirmed by analysis of proton and carbon NMR spectroscopy, FT-IR and HRMS. The use of di-Boc triflylguanidine 149 successfully produced the entire library of protected guanidines. Although the lowest yield was 15% with respect to guanidine 178, yields ranged from 30% to 83%. The differences in yields are attributed to the differences in hydrophobic region; a trend also observed in the synthesis of the amine functionalised norbornanes (Chapter 2).
Table 15 – Yields of guanylation using di-Boc triflylguanidine 173.

<table>
<thead>
<tr>
<th>No.</th>
<th>Yield</th>
<th>No.</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>162</td>
<td>28%</td>
<td>195</td>
<td>62%</td>
</tr>
<tr>
<td>171</td>
<td>59%</td>
<td>163</td>
<td>26%</td>
</tr>
<tr>
<td>172</td>
<td>75%</td>
<td>175</td>
<td>83%</td>
</tr>
<tr>
<td>173</td>
<td>59%</td>
<td>176</td>
<td>54%</td>
</tr>
<tr>
<td>158</td>
<td>30%</td>
<td>177</td>
<td>29%</td>
</tr>
<tr>
<td>174</td>
<td>35%</td>
<td>178</td>
<td>15%</td>
</tr>
</tbody>
</table>

The proton NMR spectrum of protected guanidine 175 as an example is provided (Figure 73a). The six N-H protons are present in the proton spectrum ($\delta$11.5, $\delta$8.6, $\delta$8.5, $\delta$8.1 and $\delta$6.8). Furthermore, the acetal ($\delta$5.5) and benzyl ($\delta$5.0) peaks associated with the hydrophobic moiety are present indicating that both functionalities are intact. Using the previous NMR analysis from Chapter 2, the full proton assignments for protected guanidine 175 are detailed in Figure 73. A complete analysis of protected guanidine 175 is elaborated in the experimental section at the end of the current chapter.
Having successfully synthesised the protected guanidine library, attention now turned to the deprotection and salt formation step.

3.6 Case Study with Guanidine 171

In this section, once again two deprotection protocols were compared (HCl/Dioxane and acetyl chloride/MeOH) (Scheme 58).

Once judged complete by TLC (typically 24 hours), the reaction solvents were removed in vacuo and the resulting residues analysed using proton NMR spectroscopy (Figure 74).
Once again, there were a number of signals in both spectra from δ6.25 to δ5.25, the region the acetal protons commonly appear (Figure 74). The acetal proton was assigned to peak δ5.61 based on the acetal peaks present in the spectra of di-carboxylic acid 77 and protected guanidine 171. Comparison of the spectra indicated that as a consequence of much longer treatment times, the HCl in dioxane protocol did not cause as much degradation as the acetyl chloride in MeOH protocol. Comparison of the peaks in the acetal region showed that for the HCl in dioxane method, the fully formed acetal remained the dominant species. Unfortunately, for the acetyl chloride in MeOH method, degradation was extensive.

As per previous Boc deprotections, aqueous extraction was used to purify the mixtures of guanidine salts. The aqueous phases were combined and water
removed by sublimation leaving behind a white solid that were then analysed using proton NMR spectroscopy (Figure 75).

This extraction protocol was again successful in purifying the mixtures of guanidine salts however based on the comparison of the two deprotection protocols, it was decided that treatment with HCl in Dioxane and subsequent aqueous extraction was the protocol to be followed. As with the previous deprotections, the strategy was to use analysis by proton NMR spectroscopy to determine sample purity.
The same key characteristic peaks were chosen to estimate the purity of the desired guanidine. The acetal peak (δ5.61, int.=1.00) was calculated as a percentage of the acetal (δ5.61, int.=1.00) and the degradation (δ6.22, int.=0.19; δ5.42, int.=0.08) to obtain an estimated purity of 79% (Figure 76). Having settled on a method for deprotection and estimating purity, the formation of the library of guanidine salts began.

### 3.7 Deprotection and Salt Formation

Table 16 shows the yields and purities for the products obtained from the deprotection/salt formation of the guanidines using the HCl in Dioxane protocol. Each protected guanidine was dissolved in a solution of HCl/dioxane and stirred at room temperature for forty-eight hours. Once judged complete by TLC, the reaction solvent was removed *in vacuo*. The viscous residue was re-dissolved in EtOAc and extracted with H₂O. The aqueous phases were combined and water removed through sublimation leaving behind hygroscopic solids.
Unfortunately there were a number of guanidine salt mixtures that had less than 70% of the desired guanidine present. This may be attributed to the treatment times required for the deprotection of all four of the Boc protecting groups (up to forty-eight hours). In some cases, the degradation was particularly noticeable with five salts from the guanidine library being less than 50% pure.

### 3.8 Conclusion

The aim for the current chapter was to evaluate a number of guanylation agents in the synthesis of a library of protected guanidines. The three guanylation agents chosen were di-Boc methylisothiourea 147, di-Boc thiourea 148 and di-
Boc triflylguanidine 149. Di-Boc triflylguanidine 149 was clearly the best and allowed for the synthesis of the entire guanidine library. Hence, di-Boc triflylguanidine 149 would be the guanylation agent of choice for any future studies.

Once again, deprotection resulted in degradation of the acetal functional group. Unfortunately, in the current instance, the length of time required for the deprotection of the guanidines meant that only six out of twelve guanidine salts had purities higher than 70%. Nonetheless, all the guanidine functionalised norbornane based CAMP mimics were sent for antibacterial testing the results of which are discussed in Chapter 5.
3.9 Experimental

Guanylating agents.

The following compounds are known compounds and were prepared using literature conditions:

- Methyl isothiourea 157.243
- Di-Boc methylisothiourea 147.240
- Di-Boc thiourea 148.246
- Di-Boc guanidine 189.266
- Di-Boc triflylguanidine 149.249

Synthesis of protected guanidines

Method A using N-iodosuccinimide: NIS 168 was added to a solution of amine, di-Boc thiourea 148 and Et3N in DCM/MeOH (1:4) (5 mL) and allowed to stir for 16 hours at room temperature. 1 M sodium thiosulphate solution (3 mL) was added and the reaction mixture diluted with H2O (20 mL), transferred to a separatory flask and extracted using EtOAc (4 × 10 mL). The organic phases were combined and dried using MgSO4. The MgSO4 suspension was filtered and the solvent removed in vacuo resulting in crude brown oils. The oils were purified with column chromatography using gradient elution [(1:9) EtOAc/Pet 40-60 → 20% EtOH in (1:1) EtOAc/Pet 40-60]. Fractions containing the desired compound were combined and the solvent removed in vacuo resulting in a clear viscous oils.
Method B using di-Boc triflylguanidine: A solution of amine, triflylguanidine 149 and Et₃N in DCM (5 mL) was allowed to stir for 16 hours at room temperature. The reaction solution was diluted with DCM (10 mL), transferred to a separatory flask and washed with saturated bicarbonate solution (10 mL), saturated brine solution (10 mL) and the organic phase separated, dried with MgSO₄, filtered and the solvent removed in vacuo. The crude brown oil was purified using column chromatography using gradient elution [(1:3) DCM/ Pet 40-60 → 5% in (1:1) EtOAc/Pet 40-60]. Fractions containing the desired compound were combined and the solvent removed in vacuo resulting in clear viscous oils.

\[
\text{7-(pentadecyl)-6,8-dioxatricyclo[5.2.1.0^{6,8}]decane-2-endo,3-exo-di-\{N-[2-(N',N''-di-tert-butoxy carbonyl)-guanidino]-carboxamide} \ (162)\]

Method A: hexadeca amine 137 (35.3 mg, 59.2 μmol); dibocthiourea 148 (35.9 mg, 130 μmol); Et₃N (1 mL); NIS 168 (29.3 mg, 130 μmol); R f 0.36; brown viscous oil (20.5 mg, 34%); Method B: hexadeca amine 137 (86.6 mg, 145 μmol); di-boc triflylguanidine 149 (125.2 mg, 320 μmol); Et₃N (0.1 mL); R f 0.65; brown viscous oil (62.2 mg, 28%); δH NMR (270 MHz, CDCl₃): 11.46 (1H, br s, -NH) 11.44 (1H, br s, -NH) 8.62 (1H, t, J = 5.9 Hz, -NH) 8.49 (1H, t, J = 5.4 Hz, -NH) 7.99 (1H, t, J = 4.2 Hz, -NH) 6.84 (1H, t, J = 5.5 Hz, -NH) 4.59 (1H, t, J = 4.7 Hz, H7) 4.02 (1H, d, J = 5.9 Hz, H5) 3.94 (1H, d, J = 5.5 Hz, H9) 3.57-3.36 (8H, m, H27-28, H32-33) 2.93 (1H, dd, J = 2.7, 5.4 Hz, H2) 2.69 (1H, d, J = 2.7 Hz, H4)
2.56 (1H, s, H3) 2.43 (1H, d, J = 5.47 Hz, H1) 1.77 (1H, d, J = 9.4 Hz, H10a) 1.60-1.23 (63H, m, H10b, H11-24, H36-39) 0.86 (3H, t, J = 6.2 Hz, H25).

7-{4′-biphenyl}-6,8-dioxatricyclo[5.2.1.06,8] decane-2-endo,3-exo-di-{N-[2-(N′,N′'-di-tert-butoxy carbonyl)-guanidino]-carboxamide} (171)

Method A: Di-phenyl amine 138 (64.8 mg, 121 μmol); dibothiourea 148 (73.3 mg, 265 μmol); Et3N (27 mg, 265 mmol); NIS 168 (59.7 mg, 265 μmol); Rf 0.36; white viscous oil (21.1 mg, 18%);

Method B: Di-phenyl amine 138 (89.8 mg, 167 μmol); di-boc triflylguanidine 149 (143.9 mg, 368 μmol); Et3N 0.1 mL); Rf 0.51; white viscous (92.8 mg, 59%);

υmax(thin film/cm⁻¹) 1977 (m) (aromatic C=C) 2926 (m) (methyl C-H) 1638 (vs) (amide C=O) 1563 (m) (aromatic C=C) 1131 (vs) (tertiary amine C-N) 1074 (s) (acetal C-O) 833 (m) (para substitution of aromatic C-H); δH NMR (270 MHz, CDCl3): 11.47 (1H, br s, -NH) 11.45 (1H, br s, -NH) 8.64 (1H, br t, -NH) 8.51 (1H, br t, -NH) 8.08 (1H, br t, -NH) 7.56-7.34 (9H, m, H11-19) 6.90 (1H, br t, -NH) 5.58 (1H, s, H7) 4.25 (1H, d, J = 4.8 Hz, H5) 4.18 (1H, d, J = 4.8 Hz, H9) 3.58-3.39 (8H, m, H21-22, H26-27) 3.02 (1H, dd, J = 5.4, 5.4 Hz, H2) 2.82 (1H, d, J = 2.7 Hz, H4) 2.69 (1H, s, H3) 2.54 (1H, d, J = 8.1 Hz, H1) 1.99 (1H, d, J = 10.2 Hz, H10a) 1.64 (1H, d, J = 10.2 Hz, H10b) 1.48 (36H, m, H30-33); δC NMR (67.5 MHz, CDCl3): 173.92, 173.64, 163.33, 162.85, 157.75, 156.97, 153.11, 141.38, 140.85, 140.79, 135.01, 128.79 (2 carbons), 127.19 (2 carbons), 127.14 (2 carbons), 127.08 (2 carbons), 102.87, 83.61, 83.33, 79.82, 79.55, 47.49, 44.74, 44.15, 43.21, 40.10 (3 carbons), 39.99, 29.70, 29.36, 29.09, 28.40 (3 carbons), 28.26 (3
carbons), 28.047 (3 carbons), 28.05 (3 carbons); HRMS (ESI m/z): calculated for [C_{48}H_{68}N_{8}O_{12}+2Na]^{2+} 497.23706, found 497.23705.

7-(para-bromophenyl)-6,8-dioxatricyclo[5.2.1.0^{6,8}]decane-2-endo,3-exo-di-\{N-[2-(N',N''-di-tert-butoxy carbonyl)-guanidino]-carboxamide\} (172)

Method A: Bromo phenyl amine 136 (74.9 mg, 16.46 μmol); dibocthiourea 148 (100.1 mg, 36.22 μmol); Et$_3$N (36.9 mg, 36.22 mmol); NIS 168 (81.5 mg, 36.22 μmol); R$_f$ 0.74; white viscous oil (14.6 mg, 9%); Method B: Bromo phenyl amine 136 (82.4 mg, 153 μmol); di-boc triflylguanidine 149 (131 mg, 336 μmol); Et$_3$N (0.1 mL); R$_f$ 0.65; white viscous oil (108 mg, 75%); $\upsilon_{\text{max}}$(thin film/cm$^{-1}$) 1730 (vs) (aromatic C=C) 1642 (m) (amide C=O) 1173 (s) (tertiary amine C-N) 583 (m) (bromine C-Br); $\delta$$_H$ NMR (270 MHz, CDCl$_3$): 11.47 (1H, br s, -NH) 11.45 (1H, br s, -NH) 8.65 (1H, br t, $J$ = 3.9 Hz, -NH) 8.51 (1H, br t, $J$ = 3.9 Hz, -NH) 8.08 (1H, br t, $J$ = 3.2 Hz, -NH) 7.48 (2H, d, $J_{AB} = 5.7$ Hz, H12) 7.34 (2H, d, $J_{AB} = 5.4$ Hz, H11) 6.86 (1H, br t, $J$ = 3.7 Hz, -NH) 5.48 (1H, s, H7) 4.23 (1H, d, $J$ = 3.7 Hz, H5) 4.15 (1H, d, $J$ = 3.7 Hz, H9) 3.60-3.37 (8H, m, H14-15, H19-20) 3.00 (1H, dd, $J$ = 2.7, 5.4 Hz, H2) 2.80 (1H, d, $J$ = 2.7 Hz, H4) 2.67 (1H, s, H3) 2.52 (1H, d, $J$ = 2.7 Hz, H1) 1.88 (1H, d, $J$ = 7.2 Hz, H10a) 1.57-1.25 (37H, m, H10b, H23-26); $\delta$$_C$ NMR (67.5 MHz, CDCl$_3$): 173.9, 171.8, 163.6, 162.9, 159.9, 157.8, 157.6, 157.0, 153.1, 135.2, 131.5 (2 carbons), 128.5 (2 carbons), 102.3, 83.7, 83.7, 83.4, 82.1, 80.3, 79.9, 47.5, 44.7, 43.2, 41.8, 40.4 (2 carbons), 40.1 (2 carbons), 32.5, 29.8 (3
carbons), 28.3 (3 carbons), 28.2 (3 carbons), 28.1 (3 carbons); HRMS (ESI m/z): calculated for \([C_{42}H_{63}BrN_8O_{12}+2(NH_4)]^{2+}\) 493.22127, found 493.22395.

7-\{(para-octynyl-phenyl)-6,8-dioxatricyclo[5.2.1.0^{6,8}]decane-2-endo,3-exo-
di-\{N-[2-\{N',N''-di-\text{tert-butoxy carbonyl}\}-guanidino]-carboxamide\}\ (173)

Method B: Octynyl phenyl amine

134 (85.3 mg, 150 \(\mu\)mol); di-boc triflylguanidine 149 (128.9 mg, 329 \(\mu\)mol); \(Et_3N\) (0.1 mL); \(R_f\) 0.39; brown solid (87 mg, 59%); mp 90.2 – 93.2 \(^\circ\)C; \(\nu_{\text{max}}\) (thin film/cm\(^{-1}\)) 3314 (m) (alkyne) 2957 (m) (methyl C-H) 2924 (m) (methylene C-H) 1638 (vs) (amide C=O) 1453 (vs) (aromatic C=C) 1154 (vs) (tertiary amine C-N) 1070 (vs) (acetal C-O) 808 (vs) (para substitution C-H); \(\delta_H\) NMR (270 MHz, CDCl\(_3\)): 11.45 (2H, br m, H25, H30) 8.65 (1H, t, \(J = 5.5\) Hz, H24) 8.52 (1H, t, \(J = 5.7\) Hz, H29) 8.07 (1H, t, \(J = 4.5\) Hz, H21) 7.36 (4H, s, H11-14) 6.87 (1H, t, \(J = 4.47\) Hz, H26) 5.49 (1H, s, H7) 4.21 (1H, d, \(J = 5.5\) Hz, H5) 4.13 (1H, d, \(J = 5.5\) Hz, H9) 3.61-3.40 (8H, m, H22-23, H27-28) 2.99 (1H, dd, \(J = 5.4, 5.4\) Hz, H2) 2.79 (1H, d, \(J = 2.7\) Hz, H4) 2.67 (1H, s, H3) 2.50 (1H, d, \(J = 5.4\) Hz, H1) 2.38 (2H, t, \(J = 6.9\) Hz, H15) 1.92 (1H, d, \(J = 9.6\) Hz, H10a) 1.60-1.20 (45H, m, H10b, H16-19, H31-34) 0.88 (3H, t, \(J = 4.5\) Hz, H20); \(\delta_C\) NMR (67.5 MHz, CDCl\(_3\)): 173.95, 171.85, 163.29, 162.83, 157.77, 156.99, 153.10, 135.12, 131.48, 131.42 (2 carbons), 128.60, 128.50, 126.58 (2 carbons), 125.22, 102.70, 91.21, 83.62, 83.36, 81.95, 47.49, 44.63, 44.14, 43.12, 42.06, 40.04, 40.00, 39.93, 32.49, 31.35, 29.70, 28.67, 28.60, 28.25 (3 carbons), 28.33 (3 carbons), 28.06 (3
carbons), 28.04 (3 carbons), 27.83, 22.55, 19.44, 14.05; HRMS (ESI m/z): calculated for [C₅₀H₇₆N₈O₁₂+H]⁺ 981.56555, found 981.56897.

7-phenyl-6,8-dioxatricyclo[5.2.1.0⁶,⁸]decane-2-endo,3-exo-di-{N-[2-(N',N''-di-tert-butoxy carbonyl)-guanidino]-carboxamide} (158)

**Method A:** Phenyl amine 139 (115 mg, 249 μmol); dibocthiourea 148 (165 mg, 597 μmol); Et₃N (61 mg, 597 mmol); NIS 168 (134 mg, 597 μmol); Rf 0.74; white viscous oil (121.2 mg, 6%); Method B: Phenyl amine 139 (61.9 mg, 134.2 μmol); di-boc triflylguanidine 149 (115.5 mg, 295 μmol); Et₃N (0.1 mL); Rf 0.57; white viscous oil (35 mg, 30%); v_max(thin film/cm⁻¹) 2853 (m) (methyl C-H) 1715 (m) (aromatic C=C) 1639 (s) (amide C=O) 1229 (s) (tertiary amine C-N) 1132 (vs) (acetal C-O); δ_H NMR (270 MHz, CDCl₃): 11.47 (1H, br s, H20) 11.45 (1H, br s, H25) 8.64 (1H, br t, H19) 8.51 (1H, br t, H24) 8.05 (1H, br t, H16) 7.46-7.36 (5H, m, H11-15) 6.85 (1H, br t, H21) 5.52 (1H, s, H7) 4.23 (1H, d, J = 4.0 Hz, H5) 4.16 (1H, d, J = 4.0 Hz, H9) 3.62-3.35 (8H, m, H17-18, H22-23) 3.00 (1H, br d, J = 5.4 Hz, H2) 2.81 (1H, d, J = 2.7 Hz, H4) 2.68 (1H, s, H3) 2.52 (1H, d, J = 5.4 Hz, H1) 1.97 (1H, d, J = 10.2 Hz, H10a) 1.63-1.43 (37H, m, H10b, H26-29); δ_C NMR (67.5 MHz, CDCl₃): 177.4, 174.0, 171.9, 163.4, 162.9, 157.8, 157.0, 153.2, 136.1, 129.5, 128.3 (2 carbons), 126.8 (2 carbons), 103.1, 83.7, 83.4, 79.9, 79.4, 47.6, 44.8, 44.2, 43.3, 42.0, 40.2 (2 carbons), 40.0 (2 carbons), 32.6, 29.8, 29.8 (3 carbons), 29.7 (3 carbons), 28.5 (3 carbons), 28.3 (3 carbons); HRMS (ESI m/z): calculated for [C₄₂H₆₄N₈O₁₂+H]⁺ 873.4716, found 873.47608.
7-pyrenyl-6,8-dioxatricyclo[5.2.1.0^6,8]decane-2-endo,3-exo-di-\{N-[2-(N',N''-di-tert-butoxy carbonyl)-guanidino]-carboxamide\} (174)

**Method A:** Pyrenyl amine 140 (46 mg, 78.6 μmol); dibocthiourea 148 (47.8 mg, 173 μmol); Et₃N (17.6 mg, 173 mmol); NIS 168 (38.9 mg, 173 μmol); Rₚ 0.25; yellow oil (9.5 mg, 12%); **Method B:** Pyrenyl amine 140 (83.0 mg, 141.8 μmol); di-boc triflylguanidine 149 (122.1 mg, 311.9 μmol); Et₃N (0.1 mL); Rₚ 0.83; yellow oil (50.1 mg, 35%); \( \nu_{\text{max}} \) (thin film/cm⁻¹) 1718 (s) (aromatic C=C) 1640 (s) (amide C=O) 1230 (vs) (tertiary amine C-N) 1079 (s) (acetal C-O); \( \delta \) H NMR (270 MHz, CDCl₃): 11.48 (2H, br m, H24, H29) 8.66 (1H, br t, \( J = 5.9 \) Hz, H23) 8.53 (1H, br t, \( J = 5.9 \) Hz, H28) 8.40-7.96 (10H, m, H11-20) 6.96 (1H, br t, \( J = 4.9 \) Hz, H25) 6.55 (1H, s, H7) 4.48 (1H, d, \( J = 5.9 \) Hz, H5) 4.40 (1H, d, \( J = 5.9 \) Hz, H9) 3.60-3.39 (8H, m, H21-22, H26-27) 3.10 (1H, dd, \( J = 2.7, 5.4 \) Hz, H2) 2.89 (1H, d, \( J = 2.7 \) Hz, H4) 2.76 (1H, s, H3) 2.64 (1H, d, \( J = 2.7 \) Hz, H1) 1.99 (1H, d, \( J = 9.88 \) Hz, H10a) 1.49-1.43 (H37, m, H10b, H30-33); \( \delta \) C NMR (67.5 MHz, CDCl₃): 177.64, 174.02, 171.98, 163.38, 162.94, 157.80, 157.13, 153.20, 131.95, 131.23, 130.67, 129.06, 129.00, 128.15, 128.07, 127.87, 127.46, 126.01, 125.50, 125.44, 124.68, 123.03, 122.92, 122.80, 100.61, 83.68, 83.42, 82.31, 79.88, 79.69, 47.32, 45.15, 44.26, 43.48, 42.06, 40.29 (2 carbons), 40.10 (2 carbons), 32.57, 32.00, 30.29, 29.77, 29.59, 29.44, 29.24, 29.03, 28.63, 28.49, 28.31, 28.12, 27.91; HRMS (ESI m/z): calculated for [C₅₂H₆₉N₈O₁₂+H]⁺ 997.50295, found 997.50329.
7-(para-octyloxy phenyl)-6,8-dioxatricyclo[5.2.1.0^6,8]decane-2-endo,3-exo-di-{N-[2-(N',N''-di-tert-butoxy carbonyl)-guanidino]-carboxamide} (195)

Method B: Octyloxy phenyl amine 141 (78.6 mg, 134 μmol); di-boc triflylguanidine 149 (115.0 mg, 294 μmol); Et₃N (0.1 mL); Rf 0.83; white solid (82.6 mg, 62%); mp 64.0 – 67.2 °C; ν_max(thin film/cm⁻¹) 3310 (m) (secondary amine N-H) 2977 (m) (methyl C-H) 2856 (m) (methylene C-H) 1640 (vs) (amide C=O) 1614 (vs) (aromatic C=C) 1250 (s) (aryl ether C-O); δ_H NMR (270 MHz, CDCl₃): 11.46 (1H, br s, H27) 11.43 (1H, br s, H32) 8.64 (1H, t, J = 5.9 Hz, H26) 8.51 (1H, t, J = 5.7 Hz, H31) 8.07 (1H, t, J = 3.9 Hz, H23) 7.36 (2H, d, J_AB = 10.8 Hz, H11-12) 6.86-6.79 (3H, m, H13-14, H28) 5.47 (1H, s, H7) 4.19 (1H, d, J = 5.9 Hz, H5) 4.11 (1H, d, J = 5.9 Hz, H9) 3.92 (2H, t, J = 6.4 Hz, H15) 3.57-3.31 (8H, m, H24-25, H29-30) 3.00 (1H, dd, J = 2.7, 5.4 Hz, H2) 2.79 (1H, d, J = 2.7 Hz, H4) 2.66 (1H, s, H3) 2.51 (1H, d, J = 5.4 Hz, H1) 1.98 (1H, d, J = 10.4 Hz, H10a) 1.61 (1H, d, J = 10.4 Hz, H10b) 1.49-1.20 (48H, m, H16-21, H33-36) 0.86 (3H, t, J = 7.2 Hz, H22); δ_C NMR (67.5 MHz, CDCl₃): 174.09, 171.93, 163.27, 162.82, 160.08, 157.75, 156.98, 153.10, 128.08 (2 carbons), 127.93, 114.25 (2 carbons), 103.05, 83.63, 83.38, 81.77, 79.89, 79.68, 79.13, 68.05, 47.54, 44.70, 44.15, 43.20, 42.02, 40.05, 39.95, 32.54, 31.92, 31.80, 29.69, 29.34, 29.23, 29.20, 28.24 (3 carbons), 28.23 (3 carbons), 28.05 (3 carbons), 28.03 (3 carbons), 26.01, 22.65, 14.09; HRMS (ESI m/z): calculated for [C₅₀H₈₀N₈O₁₃+H]⁺ 1001.59176, found 1001.59451.
7-(para-octyl-phenyl)-6,8-dioxatricyclo[5.2.1.0^6,8]decane-2-endo,3-exo- di-
\{N-[2-(N',N''-di-tert-butoxy carbonyl)-guanidino]-carboxamide\} (163)

**Method B: Octyl phenyl amine 142**

(81.5 mg, 142 μmol); di-boc triflylguanidine 149 (122.3 mg, 313 μmol); Et\(_3\)N (0.1 mL); R\(_f\) 0.36.8; white solid (36.8 mg, 26%); mp 79.1 – 83.2 °C; \(\nu\)\(_{\text{max}}\) (thin film/cm\(^{-1}\)) 2924 (m) (-CH\(_2\)-) 2854 (vs) (methyl C-H) 1637 (vs) (amide C=O) 1614 (vs) (aromatic C=C) 1155 (vs) (tertiary amine C-N) 1130 (vs) (acetal C-O) 859 (s) (para substitution); \(\delta\)\(_{\text{H}}\) NMR (270 MHz, CDCl\(_3\)): 11.46 (1H, br s, H27) 11.44 (1H, br s, H32) 8.64 (1H, br t, H26) 8.53 (1H, br t, H31) 8.08 (1H, br t, H23) 7.38 (2H, \(\delta\)\(_{AB}\) = 2.7 Hz, H12, H14) 7.32 (2H, \(\delta\)\(_{AB}\) = 2.7 Hz, H11, H13) 6.94 (1H, br t, H28) 5.48 (1H, s, H7) 4.21 (1H, d, \(\delta\) = 5.7 Hz, H5) 4.13 (1H, d, \(\delta\) = 5.7 Hz, H9) 3.58-3.40 (8H, m, H24-25, H29-30) 3.01-2.45 (4H, m, H1-4) 2.34 (2H, t, \(\delta\) = 6.9 Hz, H15) 1.92 (1H, d, \(\delta\) = 13.1 Hz, H10a) 1.79-1.24 (49H, m, H10b, H16-21, H33-36) 1.24 (3H, t, \(\delta\) = 3.2 Hz, H22); \(\delta\)\(_{\text{C}}\) NMR (67.5 MHz, CDCl\(_3\)): 174.13, 173.94, 172.09, 172.09, 171.83, 163.30, 162.83 (2 carbons) 157.01 (2 carbons), 153.11, 131.42, 126.58, 109.16, 83.63, 79.87, 79.85, 79.63, 79.60, 44.15, 43.37, 43.34, 40.05, 39.98, 31.93, 31.35, 29.70, 29.66, 29.36, 28.67, 28.60, 28.06 (3 carbons), 28.04 (3 carbons), 28.03 (3 carbons), 27.90 (3 carbons), 27.83, 25.36, 24.09, 22.69, 22.55, 19.44, 14.12, 14.05; HRMS (ESI m/z): calculated for [C\(_{50}\)H\(_{80}\)N\(_{8}\)O\(_{12}\)+H]\(^+\) 985.59685, found 985.59313.
Method A: Benzyloxy phenyl amine 143 (72.9 mg, 282.6 μmol); dibocchiourea 148 (156.2 mg, 565.2 μmol); Et₃N (28.8 mg, 282.6 mmol); NIS 168 (127.2 mg, 565.2 μmol); Rf 0.43; white viscous oil (26.2 mg, 21%); Method B: Benzyloxy phenyl amine 143 (94.4 mg, 166 μmol); di-boc triflylguanidine 149 (143.2 mg, 366 μmol); Et₃N (0.1 mL); Rf 0.71; white viscous oil (135.1 mg, 83%); ʋmax (thin film/cm⁻¹) 2956 (m) (methyl C-H) 1720 (m) (aromatic C=C) 1614 (s) (amide C=O) 12518 (s) (tertiary amine C-N) 1229 (vs) (benzyl ether C-O) 1131 (vs) (acetal C-O) 807 (m) (meta substitution on aromatic); δH NMR (270 MHz, CDCl3): 11.47 (1H, br s, H25) 11.45 (1H, br s, H30) 8.66 (1H, br t, H24) 8.55 (1H, br t, H29) 8.06 (1H, t, J = 2.7 Hz, H21) 7.43-6.95 (9H, m, H11-19) 6.86 (1H, br t, H26) 5.50 (1H, s, H7) 5.05 (2H, s, H20) 4.22 (1H, d, J = 3.9 Hz, H5) 4.15 (1H, d, J = 3.9 Hz, H9) 3.60-3.36 (8H, m, H22-23, H27-28) 3.01 (1H, d, J = 5.4 Hz, H2) 2.79 (1H, d, J = 5.4 Hz, H4) 2.67 (1H, s, H3) 2.52 (1H, d, J = 5.4 Hz, H1) 1.93 (1H, d, J = 7.2 Hz, H10a) 1.61-1.44 (37H, m, H10b, H31-34); δC NMR (67.5 MHz, CDCl3): 174.00, 171.89, 163.38, 162.91, 158.83, 157.80, 157.02, 153.16, 137.60, 136.93, 129.51, 128.67, 128.07, 127.60 (2 carbons), 119.44, 116.03, 112.96, 102.83, 83.68, 83.42, 82.00, 79.89, 79.65, 79.42, 70.05, 47.45, 44.84, 44.15, 43.28, 42.10, 40.03 (4 carbons), 32.59, 32.02, 29.79 (3 carbons), 28.47 (3 carbons), 28.34 (3 carbons), 28.12 (3
7-heptyl-6,8-dioxatricyclo[5.2.1.0^{6,8}]decane-2-endo,3-exo- di-{N-[2-\{N',N''-di-tert-butoxy carbonyl\}-guanidino]-carboxamide} (176)

Method A: Octyl amine 144 (78.7 mg, 162.8 μmol); dibocthiourea 148 (99.0 mg, 358.1 μmol); Et₃N (36.5 mg, 358.1 mmol); NIS 168 (80.6 mg, 358.1 μmol); Rᵣ 0.25; white viscous oil (29.4 mg, 20%); Method B: Octyl amine 144 (99.5 mg, 206 μmol); di-boc triflylguanidine 149 (177.2 mg, 453 μmol); Et₃N (0.1 mL); Rᵣ 0.64; white viscous oil (99.8 mg, 54%); ν max(thin film/cm⁻¹) 2926 (m) (methylene C-H) 1131 (vs) (acetal C-O) 1614 (s) (amide C=O) 1228 (s) (tertiary amine C-N) 2856 (m) (methyl C-H); δ H NMR (270 MHz, CDCl₃): 11.48 (1H, br s, H22) 11.45 (1H, br s, H27) 8.63 (1H, t, J = 3.5 Hz, H21) 8.51 (1H, t, J = 3.5 Hz, H26) 8.03 (1H, t, J = 2.7 Hz, H18) 6.84 (1H, t, J = 3.7 Hz, H23) 4.61 (1H, t, J = 3.2 Hz, H7) 4.03 (1H, d, J = 3.5 Hz, H5) 3.95 (1H, d, J = 3.5 Hz, H9) 3.59-3.33 (8H, m, H19-20, H24-25) 2.92 (1H, dd, J = 2.7, 5.4 Hz, H2) 2.69 (1H, dd, J = 2.7 Hz, H4) 2.56 (1H, s, H3) 2.43 (1H, d, J = 5.4 Hz, H1) 1.77 (1H, d, J = 6.9 Hz, H10a) 1.60-1.24 (37H, m, H10b, H28-31); δ C NMR (67.5 MHz, CDCl₃): 174.13, 172.03, 163.41, 162.91, 157.82, 157.01, 153.16, 104.06, 83.64, 83.36, 81.61, 79.85, 79.58, 78.94, 47.65, 44.43, 44.17, 42.98, 42.11, 40.16, 39.95, 32.92, 32.39, 31.80, 29.76, 29.60, 29.24, 24.46, 28.33 (6 carbons), 28.14 (6 carbons), 24.27, 22.69, 14.15; HRMS (ESI m/z): calculated for [C₄₉H₇₀N₈O₁₃+Na]+ 917.53184, found 917.53243.
7,7-dimethyl-6,8-dioxatricyclo[5.2.1.0^{6,8}]decane-2-endo,3-exo-di-\{N-2-(N',N''-di-\textit{tert}-butoxy carbonyl)-guanidino\}-carboxamide\} (177)

Method A: Di-methyl amine 145 (73.9 mg, 179 μmol); dibocthiourea 148 (109 mg, 393 μmol); Et$_3$N (40 mg, 393 mmol); NIS 168 (88.5 mg, 393 μmol); R$_f$ 0.25; white viscous oil (41.7 mg, 28%);

Method B: Di-methyl amine 145 (33.2 mg, 80.3 μmol); di-boc triflylguanidine 149 (69.2 mg, 176.7 μmol); Et$_3$N (0.1 mL); R$_f$ 0.41; white viscous oil (19.3 mg, 29%); $\nu_{\text{max}}$(thin film/cm$^{-1}$) 2978 (m) (methyl C-H) 1639 (s) (amide C=O) 1366 (m) (\textit{tert}-butyl C-H) 1154 (s) (tertiary amine C-N) 1130 (vs) (acetal C-O); $\delta_{\text{H}}$ NMR (270 MHz, CDCl$_3$): 11.46 (1H, br s, H17) 11.43 (1H, br s, H22) 8.63 (1H, br t, H16) 8.44 (1H, br t, H21) 8.01 (1H, br t, H13) 6.91 (1H, br s, H22) 8.63 (1H, br t, H16) 8.44 (1H, br t, H21) 8.01 (1H, br t, H13) 6.91 (1H, br t, H18) 4.11 (1H, br d, H5) 4.06 (1H, br d, H9) 3.58-3.39 (8H, m, H14-15, H 19-20) 2.92 (1H, d, $J$ = 2.7 Hz, H2) 2.65 (1H, d, $J$ = 2.7 Hz, H4) 2.51 (1H, s, H3) 2.45 (1H, d, $J$ = 2.7 Hz, H1) 1.77 (1H, d, $J$ = 10.3 Hz, H10a) 1.48 (36H, s, H23-26) 1.38 (3H, s, H11) 1.24 (3H, s, H12); $\delta_{\text{C}}$ NMR (67.5 MHz, CDCl$_3$): 173.4, 172.92, 174.1, 172.1, 163.4, 162.9, 157.8, 157.0, 153.1, 109.2, 83.6, 83.3, 81.2, 79.8, 79.5, 74.5, 47.5, 44.5, 44.0, 43.1, 42.2, 40.1, 39.9, 39.8, 32.1, 29.7, 28.3 (6 carbons), 28.1 (6 carbons), 25.4, 24.2; HRMS (ESI $m/z$): calculated for [C$_{38}$H$_{64}$N$_{8}$O$_{12}$+H]$^+$ 825.4716, found 825.47206.
**Method A:** Fluoro phenyl amine 146 (100.7 mg, 210 μmol); dibocthiourea 148 (139.3 mg, 504 μmol); Et₃N (51.4 mg, 504 mmol); NIS 168 (113.4 mg, 504 μmol); Rₐ 0.25; white viscous oil (21.3 mg, 11%); Method B: Fluoro phenyl amine 146 (68.5 mg, 143 μmol); di-boc triflylguanidine 149 (123.0 mg, 314 μmol); Et₃N (0.1 mL); Rₐ 0.51; white viscous oil (19.4 mg, 15%);

υₘₐₓ(thin film/cm⁻¹) 3330 (br m) (aromatic ring C=C) 1128 (m) (acetal C-O) 1032 (vs) (fluorine C-F) 816 (m) (1,4-substitution on aromatic C-H); δH NMR (67.5 MHz, CDCl₃): 11.48 (2H, br m, H₁₁, H₂₂) 8.56 (2H, t, J = 3.5 Hz, H₁₆, H₂₁) 7.48-7.44 (3H, m, H₁₂, H₁₃) 7.44 (2H, d, Jₐ = 5.4 Hz, H₁₁) 7.03 (2H, d, Jₐ = 5.4 Hz, H₁₂) 6.98 (1H, t, J = 3.2 Hz, H₁₈) 5.54 (1H, s, H₇) 4.14 (1H, d, J = 3,7 Hz, H₅) 4.10 (1H, d, J = 3.7 Hz, H₉) 3.61-3.42 (8H, m, H₁₄-15, H₁₉-20) 2.99 (1H, d, J = 5.4 Hz, H₂) 2.79 (1H, d, J = 5.4 Hz, H₄) 2.67 (1H, s, H₃) 2.51 (1H, d, J = 5.4 Hz, H₁) 1.90 (1H, d, J = 6.4 Hz, H₁₀ₐ) 1.67 (1H, d, J = 6.4 Hz, H₁₀₉) 1.56-1.48 (36H, m, H₂₃-2₆); δC NMR (67.5 MHz, CDCl₃): 173.4, 173.2, 172.9, 171.40, 163.5 (1C, d, J₀ = 246 Hz), 157.3 (4 carbons), 131.7 (1C, d, J₀ = 2.5 Hz) 128.6 (2C, d, J₀ = 7.5 Hz) 115.4 (2C, d, J₀ = 21 Hz) 102.54, 83.54 (2 carbons), 81.90, 79.6, 77.6 (2 carbons), 52.2, 45.5, 44.7, 42.9, 40.8 (2 carbons), 40.0 (2 carbons), 31.8, 28.3 (6 carbons), 28.0 (6 carbons); HRMS (ESI m/z): calculated for [C₄₂H₆₃FN₇O₁₂⁺2H]²⁺ 447.2402, found 447.24235.
Synthesis of guanidine salts

**General methodology:** The protected guanidines were added to a solution of HCl in Dioxane (3 M, 3 mL) and stirred for 8 hours at room temperature. The reaction solvent was removed *in vacuo*, the residue re-dissolved in EtOAc (10 mL) and transferred to a separatory flask. H$_2$O (3 × 5 mL) was used for extraction. The aqueous phases were combined and the water removed through sublimation.

Where analysis using carbon NMR spectroscopy was conducted, it was found that signals associated with carbons 1-4 of the norbornane scaffold lay under the CD$_3$OD signals as verified by HSQC NMR spectroscopy.

**7-(pentadeca)-6,8-dioxatricyclo[5.2.1.0$^6$$^8$]decane-2-endo,3-exo-dicarboxamido ethyl guanidinium chloride (30)**

Hexadeca protected guanidine 162 (30.2 mg, 29.99 μmol); white floss (9.19 mg, yield: 45%, purity: 98%); $\nu_{\text{max}}$ (thin film/cm$^{-1}$) 3062 (s) (methylene C-H) 2927 (s) (methyl C-H) 1696 (vs) (guanidine·HCl) 1638 (vs) (amide C=O) 1150 (s) (guanidine·HCl) 1074 (m) (acetal C-O); $\delta_H$ NMR (270 MHz, CD$_3$OD): 5.22 (2H, t, $J = 2.7$ Hz, H7) 4.02 (1H, br d, H9) 3.99 (1H, br d, H5) 3.46 – 3.20 (9H, H$_2$, H$_{27}$-28, H$_{33}$-34) 2.62 – 2.45 (3H, m, H$_1$, H3-4) 1.72 (1H, d, $J = 8.1$ Hz, H10a) 1.57 –
1.27 (H27, m, H10b, H12 24) 0.89 (3H, t, \( J = 8.1 \text{ Hz}, \text{H25})\); \( \delta \) C NMR (67.5 MHz, CD$_3$OD): 157.5, 152.0, 130.0, 128.6, 103.7, 84.5, 81.5, 78.6, 62.7, 54.41, 46.3, 46.2, 45.7, 43.7, 43.6, 41.1, 40.6, 38.4, 37.5, 32.5, 31.7, 31.4, 29.4, 26.9, 23.8, 22.4, 13.1, 4 carbons under solvent peak from 48.2 – 47.2; HRMS (ESI m/z): calculated for [C$_{31}$H$_{60}$N$_8$O$_4$]$^2+$ 304.23633, found 304.23596.

7-[(para-phenyl)phenyl]-6,8-dioxatricyclo[5.2.1.0$^{6,8}$]decane-2-endo,3-exo-dicarboxamido ethyl guanidinium chloride (196)

[Diagram]

Di-phenyl protected guanidine 171

(28.9 mg, 28.88 \( \mu \text{mol} \)); white floss (19.28 mg, yield: 99\%, purity: 72\%); \( \nu_{\text{max}} \) (thin film/cm$^{-1}$) 1656 (vs) (amide C=O) 1591 (vs) (guanidine·HCl) 1091 (s) (acetal C-O) 1049 (m) (aromatic C=C); Characteristic peaks in the proton NMR: 7.61 – 7.33 (H9, m, H11-19) 5.61 (1H, s, H7) 3.75 – 3.73 (2H, br m, H5, H9) 1.86 (1H, d, \( J = 8.1 \text{ Hz}, \text{H10a}) 1.49 (1H, d, \( J = 8.1 \text{ Hz}, \text{H10b}); \ MS (ESI m/z): calculated for [C$_{28}$H$_{38}$N$_8$O$_4$-H]$^+$ 549.29, found 549.29.

7-[(para-bromo)phenyl]-6,8-dioxatricyclo[5.2.1.0$^{6,8}$]decane-2-endo,3-exo-dicarboxamido ethyl guanidinium chloride (197)

[Bromo phenyl protected guanidine 172]

(30.2 mg, 31.78 \( \mu \text{mol} \)); white floss (10.93 mg, yield: 55\%, purity: 86\%); \( \nu_{\text{max}} \) (thin film/cm$^{-1}$) 3304 (br vs) (primary amine N-H) 1211 (m) (aromatic C=C) 1071 (s) (acetal C-O) 633 (m) (bromine C-Br); Characteristic peaks in proton NMR: 7.54 (2H, d, 3$^3J_{AB} = 8.1 \text{ Hz}$,
H12) 7.39 (2H, d, $^3J_{AB} = 8.1$ Hz, H11) 5.53 (1H, s, H7) 4.24 – 4.17 (2H, m, H5, H9) 3.73 – 3.30 (9H, m, H2, H14–15, H20–21) 2.72-2.56 (3H, m, H1, H3-4) 1.88 (1H, d, $J = 10.8$ Hz, H10a) 1.58 (1H, d, $J = 10.8$ Hz, H10b); HRMS (ESI m/z): calculated for [C$_{22}$H$_{33}$BrN$_8$O$_4$]$^2^+ 277.09768$, found 277.09090.

7-[(para-octynyl)-phenyl]-6,8-dioxatricyclo[5.2.1.0$^{6,8}$]decane-2-endo,3-exo-dicarboxamido ethyl guanidinium chloride (198)

Octynyl phenyl protected guanidine 173 (29.8 mg, 30.3 μmol); brown floss (18.9 mg, yield: 87%, purity: 48%);

υ$_{\text{max}}$(thin film/cm$^{-1}$) 1664 (m) (guanidine·HCl) 1648 (m) (amide C=O) 1637 (s) (aromatic C=C) 1475 (s) (methyl C-H) 1062 (m) (acetal C-O) 1062 (m) (para substitution C-H) 733 (s) (-CH$_2$-) 667 (m) (alkyne); Characteristic peaks in proton NMR: 7.47 – 7.33 (4H, m, H11-14) 5.53 (1H, s, H7) 4.24 – 4.15 (2H, m, H5, H9) 2.73 – 2.56 (3H, m, H1, H3-4) 1.91 (1H, d, $J = 10.8$ Hz, H10a); MS (ESI m/z): calculated for [C$_{30}$H$_{45}$N$_8$O$_4$]$^+$ 581.36, found 581.36; [C$_{30}$H$_{46}$N$_8$O$_4$]$^{2^+}$ 291.19, found 291.18.

7-phenyl-6,8-dioxatricyclo[5.2.1.0$^{6,8}$]decane-2-endo,3-exo-dicarboxamido ethyl guanidinium chloride (199)

Phenyl protected guanidine 158 (19.9 mg, 22.8 μmol); white floss (18.15 mg, yield: 72% purity: 40%); υ$_{\text{max}}$(thin film/cm$^{-1}$) 1591 (vs) (amide C=O) 1352 (s) (aromatic) 1098 (m) (guanidine·HCl) 1078 (m) (acetal C-
0); Characteristic peaks in proton NMR: 7.45 – 7.34 (5H, m, H11-15) 5.35 (1H, s, H7) 2.67 – 1.43 (3H, m, H1, H3-4) 1.85 (1H, d, J = 10.8 Hz, H10a); MS (ESI m/z): calculated for [C22H34N8O4-H]⁺ 473.26, found 473.26.

7-pyrenyl-6,8-dioxatricyclo[5.2.1.0⁶,8]decane-2-endo,3-exo-dicarboxamido ethyl guanidinium chloride (200)

Pyrenyl protected guanidine 174 (24.2 mg, 24.3 μmol); brown floss (16.4 mg, yield: 83%, purity: 40%); ν_max(thin film/cm⁻¹) 1970 (s) (aromatic C=C) 1596 (s) (amide C=O) 1255 (m) (aromatic C-H) 1150 (m) (guanidine·HCl) 10857 (s) (acetal C-O); Characteristic peaks in proton NMR: 7.40-6.92 (9H, m, H11-19) 6.12 (1H, s, H7) 4.20 – 4.15 (2H, m, H5, H9) 2.72 – 2.55 (3H, m, H1, H3-4); MS (ESI m/z): calculated for [C32H38N8O4]²⁺ 299.15, found 299.15.

7-[(para-octyloxy)phenyl]-6,8-dioxatricyclo[5.2.1.0⁶,8]decane-2-endo,3-exo-dicarboxamido ethyl guanidinium chloride (201)

Octyloxy phenyl protected guanidine 195 (28.9 mg, 28.9 μmol); white floss (19.3 mg, Yield 97%, purity: 79%); ν_max(thin film/cm⁻¹) 2924 (m) (methylene C-H) 1693 (s) (aromatic C=C) 1639 (s) (amide C=O) 1252 (m) (ary ether C-O) 1149 (vs) (alkyl ether C-O) 1123 (vs) (guanidine·HCl) 830 (vs) (para substitution C-H); δH NMR (270 MHz, CD3OD): 7.38 (2H, JAB = 8.1 Hz, H13-14)
6.90 (2H, $^3J_{AB} = 8.1$ Hz, H11-12) 5.50 (1H, s, H7) 4.21 (1H, d, $J = 5.4$ Hz, H9) 4.15 (1H, d, $J = 5.4$ Hz, H5) 3.77 – 3.22 (9H, m, H2, H24-25, H30-31) 2.68 – 2.56 (3H, m, H1, H3-4) 1.86 (1H, d, $J = 10.8$ Hz, H10a) 1.51 (1H, d, $J = 10.8$ Hz, H10b); $\delta_c$ NMR (62.5 MHz, CD$_3$OD): 164.0, 157.6, 157.5 (2 carbons), 154.1 (2 carbons), 128.0 (2 carbons), 113.8 (2 carbons), 100.6, 81.7, 78.9, 62.9, 46.2, 45.6, 44.6, 43.7, 49.5, 40.6, 38.3, 31.5, 29.0, 25.8, 22.3, 17.4, 4 carbons under solvent peak from 48.2 – 47.2; MS (ESI m/z): calculated for [C$_{30}$H$_{50}$N$_{8}$O$_{5}$-H]$^+$ 601.38204, found 601.38090.

7-[(para-octyl)-phenyl]-6,8-dioxatricyclo[5.2.1.0$^{6,8}$]decane-2-endo,3-exo-dicarboxamido ethyl guanidinium chloride (202)

Octyl phenyl protected guanidine 163

(29.8 mg, 30.3 μmol); brown floss

(20.5 mg, yield: 63%, purity: 36%);

$\nu_{\text{max}}$(thin film/cm$^{-1}$) 2978 (vs) (methyl C-H) 2940 (s) (methylene C-H) 1648 (vs) (amide C=O) 1206 (s) (aromatic C=C) 1151 (s) (guanidine-HCl) 1073 (m) (acetal C-O) 755 (m) (para substitution C-H); Characteristic peaks in proton NMR: 7.47 – 7.32 (4H, m, H11-14) 5.54 (1H, s, H7) 4.06 (1H, d, $J = 5.4$ Hz, H9) 4.00 (1H, d, $J = 5.4$ Hz, H5) 2.68 – 2.60 (3H, m, H1, H3-4) 1.86 (1H, d, $J = 10.8$ Hz, H10a); MS (ESI m/z): calculated for [C$_{30}$H$_{50}$N$_{8}$O$_{4}$-H]$^+$ 585.39, found 585.38.
7-[(meta-benzylloxy)-phenyl]-6,8-dioxatricyclo[5.2.1.0^6,8]decane-2-endo,3-exo-dicarboxamido ethyl guanidinium chloride (203)

Benzyloxy phenyl protected guanidine 175 (29.7 mg, 30.4 μmol); white floss (17.6 mg, yield: 89%, purity: 41%);
ν_max(thin film/cm^-1) 3138 (s) (aromatic C=C) 1693 (vs) (aromatic C=C) 1638 (s) (amide C=O) 1256 (s) (aryl ether C-O) 1147 (m) (guanidine·HCl) 1074 (s) (acetal C-O); 864 (meta substitution C-H); Characteristic peaks in proton NMR:
δ H NMR (270 MHz, CD3OD): 5.32 (1H, s, H7) 5.07 (2H, s, H20) 4.19 (1H, d, J = 5.4 Hz, H5) 3.75 (1H, d, J = 5.4 Hz, H9) 2.67 – 2.41 (3H, m, H1, H3-4) 1.72 (1H, d, J = 8.1 Hz, H10a) 1.53 (13H, m, H10b, H11-16) 0.88 (3H, t, J = 5.4 Hz, H17); δ C NMR (62.5 MHz, CD3OD): 157.48 (2 carbons), 129.09, 128.14, 103.72, 81.46, 78.56, 46.31, 45.54, 43.73, 40.63, 38.36, 32.48, 31.50, 29.21, 28.92, 23.80, 22.27, 13.00,

7-octyl-6,8-dioxatricyclo[5.2.1.0^6,8]decane-2-endo,3-exo-dicarboxamido ethyl guanidinium chloride (204)

Octyl protected guanidine 176 (33.7 mg, 37.6 μmol); white floss (15.3 mg, yield: 47%, purity: 98%);
ν_max(thin film/cm^-1) 3061 (m) (methylene C-H) 2937 (m) (methyl C-H) 1589 (s) (amide C=O) 1153 (m) (guanidine·HCl) 1124 (s) (acetal C-O); δ H NMR (270 MHz, CD3OD): 4.64 (2H, t, J = 5.4 Hz, H7) 4.03 (1H, d, J = 2.7 Hz, H5) 3.99 (1H, d, J = 2.7 Hz, H9) 3.59 – 3.21 (9H, m, H2, H19-20, H25-26) 2.61 – 2.44 (3H, m, H1, H3-4) 1.72 (1H, d, J = 8.1 Hz, H10a) 1.53 (13H, m, H10b, H11-16) 0.88 (3H, t, J = 5.4 Hz, H17); δ C NMR (62.5 MHz, CD3OD): 157.48 (2 carbons), 129.09, 128.14, 103.72, 81.46, 78.56, 46.31, 45.54, 43.73, 40.63, 38.36, 32.48, 31.50, 29.21, 28.92, 23.80, 22.27, 13.00,
4 carbons under solvent peak from 48.2 – 47.2; HRMS (ESI m/z): calculated for \([C_{23}H_{44}N_8O_4]^2+\) 248.17373, found 248.17438.

**7,7-dimethyl-6,8-dioxatricyclo[5.2.1.0^{6,8}]decane-2-endo,3-exo-dicarboxamido ethyl guanidinium chloride (205)**

Di-methyl protected guanidine 177 (41.7 mg, 50.6 μmol); white floss (24.8 mg, yield: 77%, purity: 61%); \(\nu_{\text{max}}\) (thin film/cm\(^{-1}\)) 2956 (vs) (methyl C-H) 1646 (vs) (amide C=O) 1154 (m) (guanidine·HCl) 1065 (m) (acetal C-O);

Characteristic peaks in proton NMR: 3.78 (1H, d, \(J = 5.4\) Hz, H5) 3.73 (1H, d, \(J = 5.4\) Hz, H9) 2.69 – 2.56 (3H, m, H1, H3-4) 1.88 (1H, d, \(J = 10.8\) Hz, H10a) 1.49 (1H, d, \(J = 10.8\) Hz, H10b) 1.37 (3H, s, H11) 1.25 (3H, s, H12); MS (ESI m/z): calculated for \([C_{18}H_{32}N_8O_4+K]^+\) 463.22, found 463.21.

**7-phenyl-6,8-dioxatricyclo[5.2.1.0^{6,8}]decane-2-endo,3-exo-dicarboxamido ethyl guanidinium chloride (206)**

Fluoro phenyl protected guanidine 178 (19.4 mg, 21.8 μmol); white floss (9.3 mg, yield: 76%, purity: 39%); \(\nu_{\text{max}}\) (thin film/cm\(^{-1}\)) 1639 (vs) (amide C=O) 1150 (s) (guanidine·HCl) 1075 (m) (acetal C-O) 1020 (vs) (fluorine C-F) 829 (vs) (para substitution C-H);

Characteristic peaks in proton NMR: 5.55 (1H, s, H7) 4.05 (1H, d, \(J = 5.4\) Hz, H5) 4.00 (1H, d, \(J = 5.4\) Hz, H9) 2.62 – 2.26 (3H, m, H1, H3-4) 1.86 (1H, d, \(J = 10.8\) Hz, H10a) 1.47 (1H, d, \(J = 10.8\) Hz, H10b); MS (ESI m/z): calculated for \([C_{22}H_{33}Cl_2FN_8O_4]^2+\) 281.10, found 281.10.
Chapter 4
Convergent methodology

In the current chapter, a ‘convergent’ methodology for the synthesis of guanidine functionalised norbornanes was pursued. With most reactions discussed previously in this manuscript, the chapter focuses on the synthesis of amino guanidine 208. Unfortunately, the ‘convergent’ methodology proved to be inferior and was not used.

4.1 Previous work

4.1.1 Rationale

Before the commencement of the current project, Pfeffer et. al. synthesised and tested guanidine salt 31 (Figure 77). Compound 31 displayed antibacterial activity against *P. aeruginosa* comparable to Colistin. To synthesise compound 31, Pfeffer et. al. utilised a convergent methodology to produce the precursor 162 (Figure 77).

![Figure 77 - Structure of compound 162 and the convergent methodology devised by Pfeffer et. al.](image-url)
In the current project, the ‘convergent’ methodology was explored as an efficient method of producing guanidine functionalised norbornanes. The key requirement for this approach was ethyl amino guanidine 207.

4.1.1 Synthesis of amino guanidine 207

4.1.2 Synthesis of protected guanidine 162
The initial coupling was conducted on a scale of 100 mg. To explore the robustness of the reaction, the experiment was repeated on the larger scale of 500 mg. The proton NMR spectrum of the crude oil from the larger scale reaction indicated the formation of the desired protected guanidine 162. Unfortunately even after several attempts at purification, the desired product could not be separated from the many by-products in the reaction mixture (Scheme 61).

![Scheme 61 - Failed di-amide coupling between di-carboxylic acid 76 and amino guanidine 207.](image_url)

Having obtained a yield of only 17% on a small scale di-amide coupling and experienced difficulties isolating the product from the large scale reaction, optimisation of the amide coupling step was attempted.

### 4.2 Optimisation of di-amide coupling

For the optimisation studies, di-carboxylic acids 76 and 77 were chosen as test substrates (Figure 78). As per previous trials with amide couplings, the coupling agents EDCI 92, HOBt 97 were used.

![Figure 78 - Structure of (a) di-carboxylic acid 73; (b) di-carboxylic acid 77.](image_url)
A variety of experiments were conducted exploring the following variables: solvent, temperatures, scale (100 mg vs. 500 mg) and finally the effect of conventional versus microwave irradiation. Unfortunately, in every instance, either the coupling reaction was unsuccessful or the desired guanidines could not be isolated in a sufficiently pure state.

The coupling agents and solvents were not the problem as they were purchased commercially recently. The amino guanidine 207 was being used crude from the previous guanylation reaction and as such, the substance most likely interfering with amide coupling was the crude amino guanidine 207.

4.3 Di-Boc methylisothiourea 147

In the previous section, amino guanidine 207 was used crude (Scheme 62). The guanylation of EDA 87 using di-boc methylisothiourea 147 was reconsidered as purification of amino guanidine 207 appeared difficult (amines are not normally well separated using chromatography) and there was a low efficiency associated with the guanylation reaction.
4.3.1 Purification

Work published by Nicolaou et. al. in the synthesis of integrins (act as cell surface proteins facilitating cell to cell adhesion) considered the synthesis of amino guanidine 207 using di-boc methylisothiourea 147 and EDA 87 (Scheme 63). The purification of amino guanidine 207 was reported as successfully accomplished by column chromatography with the very polar eluent of 20% MeOH in EtOAc.

In the current project, the 20% MeOH in EtOAc elution system was tested for purification for the crude amino guanidine 207. Unfortunately, even using Nicolaou’s elution system, purification by column chromatography still remained difficult with co-elution of products occurring. Several column eluent combinations were then trialled using a range of polar solvents such as CHCl₃, DCM and even isopropanol The only eluent found to give reasonable separation was isopropanol however the procedure required lengthy purification times (10 hours) even with medium pressure which were not ideal.

4.3.2 Guanylation using di-Boc methylisothiourea 147

A series of optimisation reactions were conducted to improve the efficiency of the guanylation reaction. Changes in solvent, reaction times, reaction temperatures, addition of HgCl₂ and basic work ups were explored. Despite
numerous attempts, the efficiency of the guanylation reaction between di-Boc methylisothiourea 147 and EDA 87 was still not satisfactory (Scheme 64).

Since EDA 87 is usually used in a five to seven fold equivalents in the guanylation reaction, an excess of EDA 87 was present in the crude reaction mixture post work up as well as un-reacted di-boc methylisothiourea 147. Di-guanylation was a possibility (ethyl di-guanidine 209) as well as macrocyclisation resulting in guanidine 210. With many by-products, purification was difficult.

In an effort to deal with competing by-products, a protecting group strategy was considered (Scheme 65). By capping one of the free amines on EDA 87, mono-guanylation of the compound would be ensured. Purification of the protected amine would also become easier with a greater variety of non-polar and polar solvents available.

4.3.3 Using Cbz-EDA 89

Cbz-EDA 89 was selected as the mono-protected EDA to undergo guanylation with di-boc methylisothiourea 147. The purpose of using the Cbz protecting group was the orthogonality of Cbz to the Boc groups already present on the
guanidine. Hence, hydrogenolysis required to remove Cbz from the substrate would result in high yields and not affect the Boc groups.

With Cbz-EDA 89 already in hand (Chapter 2), guanylation with di-boc methylisothiourea 147 was trialled using variety of reaction conditions. Variations in solvent, temperature and reaction time, microwave irradiation and the use of additives were explored.

![Scheme 66 - The most efficient conditions derived from the optimisation reactions conducted with di-Boc methylisothiourea 147 and Cbz-EDA 89.]

After numerous reactions, the best yield obtained was still only 36% (Scheme 66). The result was not surprising considering the poor performance of di-Boc methylisothiourea 147 previously in this project.

Triflylguanidine 149 and di-boc thiourea 148 were selected for trials in installing the guanidine moiety on EDA 87 and Cbz-EDA 89 (Table 17). The first set of guanylating agents attempted were di-boc thiourea 148 with Mukaiyama’s reagent 167 (Entries 1 and 2, Table 17). Unfortunately, these experiments did not result in the synthesis of either guanidine 207 or 211. The next guanylating agent trialled was triflylguanidine 149 (Entries 3 and 4, Table 17). The guanylation with EDA 87 was unsuccessful however using Cbz-EDA 89, the desired Cbz-amino guanidine 211 was isolated in an excellent yield of 90%. The next set of guanylating agents was di-boc thiourea 148 and NIS 168. Encouragingly, the di-boc thiourea 148 and NIS 168 combinations also resulted in the synthesis of Cbz-amino guanidine 211 albeit in a modest yield of 34%.
Table 17 – Trial guanylation using different guanylation agents and ‘activating’ agents.

<table>
<thead>
<tr>
<th>Entry No.</th>
<th>Amine</th>
<th>Solvent</th>
<th>Temp (°C)</th>
<th>Time</th>
<th>Guanylation Agents</th>
<th>Yield*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>EDA</td>
<td>DMF</td>
<td>r.t.</td>
<td>90 mins</td>
<td>Mukaiyama’s Reagent/di-Boc thiourea</td>
<td>0%</td>
</tr>
<tr>
<td>2</td>
<td>Cbz-EDA</td>
<td>DMF</td>
<td>r.t.</td>
<td>75 mins</td>
<td>Mukaiyama’s Reagent/di-Boc thiourea</td>
<td>0%</td>
</tr>
<tr>
<td>3</td>
<td>EDA</td>
<td>DCM</td>
<td>r.t.</td>
<td>16 h</td>
<td>Triflylguanidine</td>
<td>0%</td>
</tr>
<tr>
<td>4</td>
<td>Cbz-EDA</td>
<td>DCM</td>
<td>r.t.</td>
<td>16 h</td>
<td>Triflylguanidine</td>
<td>90%</td>
</tr>
<tr>
<td>5</td>
<td>Cbz-EDA</td>
<td>DCM/MeOH</td>
<td>r.t.</td>
<td>30 mins</td>
<td>NIS/di-Boc thiourea</td>
<td>34%</td>
</tr>
</tbody>
</table>

* Isolated yields

The guanylation agents di-boc thiourea 148 and NIS 168 were chosen over triflylguanidine 149 as (unlike triflylguanidine 149) di-boc thiourea 149 is easily synthesised in one step and NIS 168 is commercially available. The yield of 34% however obtained from the use of di-boc thiourea 148 and NIS 168 was quite poor and so a series of optimisation reactions conditions were conducted in an attempt to increase the yield of the reaction.

4.3.4 Cbz-EDA 89 and di-Boc thiourea 148

Following the methodology outlined in the literature, the amine, di-boc thiourea 148 and Et3N were dissolved in a solution of DCM and MeOH.247 Then NIS 168 was added and the solution stirred at room temperature.247

![Scheme 67 – Optimal reaction conditions for the synthesis of Cbz-amino guanidine 211 using di-Boc thiourea 148 and NIS 168.](image)

Variations in temperature time and comparing bench top heating and microwave irradiation were explored. The optimal reaction conditions involved
stirring a solution of Cbz-EDA 89, di-boc thiourea 148, Et₃N and NIS 168 in a solution of DCM/MeOH for 16 hours at room temperature and a yield of 78% was obtained.

### 4.5 Deprotection

#### 4.5.1 Deprotection

The next step in the synthesis of the hydrophilic component was the removal of the Cbz protecting group from Cbz amino guanidine 211. Cleaving Cbz generally proceeds by catalytic hydrogenolysis which in theory is an easily achieved process. Many attempts were made to remove the Cbz protecting group (Table 18).

<table>
<thead>
<tr>
<th>Entry No.</th>
<th>Solvent</th>
<th>Temp</th>
<th>Time</th>
<th>Pd Species</th>
<th>Additives</th>
<th>Deprotect</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MeOH</td>
<td>r.t.</td>
<td>16 h</td>
<td>Pd/C</td>
<td>-</td>
<td>No</td>
<td>197</td>
</tr>
<tr>
<td>2</td>
<td>MeOH</td>
<td>r.t.</td>
<td>16 h</td>
<td>Pd(OH)₂/C</td>
<td>-</td>
<td>No</td>
<td>188</td>
</tr>
<tr>
<td>3</td>
<td>MeOH</td>
<td>r.t.</td>
<td>16 h</td>
<td>Pd(OH)₂/C</td>
<td>CH₃CO₂H</td>
<td>No</td>
<td>269</td>
</tr>
<tr>
<td>4</td>
<td>MeOH</td>
<td>r.t.</td>
<td>16 h</td>
<td>Pd(OH)₂/C</td>
<td>HCl</td>
<td>No</td>
<td>270</td>
</tr>
<tr>
<td>5</td>
<td>EtOH</td>
<td>r.t.</td>
<td>16 h</td>
<td>Pd/C</td>
<td>C₆H₆</td>
<td>No</td>
<td>271</td>
</tr>
<tr>
<td>6</td>
<td>i-PrOH</td>
<td>80 °C MW</td>
<td>5 mins</td>
<td>Pd/C</td>
<td>HCO₂NH₄</td>
<td>No</td>
<td>272</td>
</tr>
<tr>
<td>7</td>
<td>i-PrOH</td>
<td>80 °C MW</td>
<td>15 mins</td>
<td>Pd/C</td>
<td>HCO₂NH₄</td>
<td>No</td>
<td>272</td>
</tr>
<tr>
<td>8</td>
<td>i-PrOH</td>
<td>80 °C MW</td>
<td>30 mins</td>
<td>Pd/C</td>
<td>HCO₂NH₄</td>
<td>No</td>
<td>272</td>
</tr>
<tr>
<td>9</td>
<td>MeOH</td>
<td>r.t.</td>
<td>16 h</td>
<td>Pd black</td>
<td>-</td>
<td>No</td>
<td>273</td>
</tr>
<tr>
<td>10</td>
<td>MeOH</td>
<td>r.t.</td>
<td>16 h</td>
<td>Pd/C</td>
<td>CuCl₂</td>
<td>No</td>
<td>274</td>
</tr>
</tbody>
</table>

There were many unsuccessful attempts at the deprotection of the Cbz functional group. The solvent, temperature, reaction time, heterogeneous catalyst and hydrogen source were all varied and still there was no indication of
successful deprotection. That led to a reasoning that perhaps the guanidine substrate 211 was in some way interfering with the catalytic activity of the palladium catalyst.

Catalysts may be deactivated by substances in the reaction vessels that act as ‘poisons’. The effect is particular severe when the catalyst is in a finely divided form and when the metals are deposited on active carbon for support. Traces of sulphur, phosphorus, nitrogen or mercury are known catalyst poisons. In the current project, the boc-protected guanidine moiety on compound 211 was suspected to be deactivating the catalyst.

Several groups have studied the complexes that unprotected guanidine forms with palladium. Gulko et al. sought to exploit the square planar complexes formed by sulphaguanidine and palladium as a method of separation of palladium(II) and platinum(II). The methodology proved successful achieving quantitative separation of palladium and platinum species (Scheme 68).

Another group that explored the guanidine and palladium complex was El-Sonbati et al. An interesting result from their study was that copper appeared to complex with the guanidine moiety as easily as palladium.

Turning back to the deprotection difficulties encountered in this project, perhaps protected guanidine of compound 211 could be forming a complex with the palladium catalyst. Since El-Sonbati et al. showed copper could form a
stable complex with guanidine, copper chloride was added to the hydrogenolysis reaction vessel. The reasoning was that if the guanidine moiety would complex with the copper species first, then this would prevent complexation with the palladium catalyst, thereby not affecting its catalytic activity. So, a solution of compound 211 and copper chloride in MeOH was stirred for an hour. Then the palladium catalyst was added and the reaction mixture was allowed to stir for 16 hours at room temperature under a hydrogen atmosphere. Unfortunately, once again, deprotection was unsuccessful.

4.5.2 The guanidine-palladium complex hypothesis

The hypothesis that a guanidine-palladium complex could be interfering with the catalytic activity of the palladium catalyst was tested. An interesting study conducted by Hirota et al. involved treating the heterogeneous catalyst with EDA 87 for up to 48 hours and then adding protected substrates. Hirota et al. showed that O-benzyl and N-Cbz groups were retained whilst reducing olefin, acetylene, nitro, benzyl ester and azido residues. Their hypothesis was that EDA 87 Pd/C formed a rigid five-member chelate (Figure 80) that decreased the hydrogenolytic ability of Pd/C.

![Figure 80 - Five-member chelate between EDA 89 and Pd/C proposed by Hirota et al.]

Looking at compound 211, there is the two carbon linker as is present in EDA 87. It could therefore be possible that compound 211 ‘poisons’ the palladium catalyst. To test the hypothesis, norbornene 215 (Figure 81) was selected because it contained a number of functionalities that are labile when stirred.
with palladium under a hydrogen atmosphere: the alkene and the Cbz functional groups.

![Figure 81 – Structure of norbornene di-carboxamido ethyl amine Cbz 215.](image)

First, the alkene and Cbz functional groups were proved to be labile under normal hydrogenation reaction conditions. Hence, a suspension of Pd/C in a solution of compound 215 in MeOH was stirred for 16 hours at room temperature. The substrate 216 was formed in a yield of 64%.

![Scheme 69 – Parallel hydrogenation and hydrogenolysis of compound 215.](image)

Following the reasoning by Hiruto et. al., if compound 211 ‘poisons’ in a similar manner to EDA 87, it should deactivate any hydrogenolytic activity while still allowing hydrogenation.

![Scheme 70 – Unsuccessful hydrogenation/hydrogenolysis](image)

A solution of Cbz amino guanidine 211 was stirred with a suspension of Pd/C in MeOH for 2 hours. Then test compound 215 was added and the reaction mixture was stirred 16 hours at room temperature under a hydrogen atmosphere. The Pd/C was removed by filtration. The filtrate was collected and
the solvent removed *in vacuo*. The crude oil obtained was analysed using proton NMR spectroscopy.

![Figure 82 - Proton NMR spectrum at 270 MHz in CHCl₃ of (a) di-amine 216; (b) protected amino guanidine 211; (c) Cbz-protected norbornene 215. (d) Proton NMR spectrum at 500 MHz in CHCl₃ of crude oil obtained from the hydrogenation/hydrogenolysis test reaction using compounds 211 and 215.](image)

The proton spectrum indicated that hydrogenolysis of the test compound was not successful (Scheme 70). Figure 82 lists a series of spectra for comparison. The Cbz protecting groups are still present (strong signals at δ7.34 and δ5.09) while it appeared that the alkene signals were gone.

Furthermore, the signals associated with the amide protons are clearly present in the spectrum of the crude oil (δ6.79 and δ6.60). Unfortunately, the rest of the peaks are not either the carbamate protons or alkene protons present in test compound **215**. For more information, analysis using mass spectrometry was conducted.
The mass spectrum of the product indicated the presence of norbornane 217, where hydrogenation was successfully and hydrogenolysis not. (Figure 84). The experiments indicated that it is possible the Cbz-protected amino guanidine 211 in some way interferes with the normally efficient hydrogenolytic and hydrogenating activity of Pd/C.

Furthermore to achieve successful Cbz deprotection, related examples in literature either had longer carbon spacers or used high pressures and acid additives which were considered impractical in the current project (Scheme 71). ²⁷⁷, ²⁷⁸
4.6 EDA 89 and di-Boc thiourea 147

The preceding two sections led to the conclusion that to synthesise the desired hydrophilic substrate 207, that perhaps using an orthogonal protecting group may not be the answer. With NIS 168 and di-boc thiourea 148 having a better success rate at guanylation then di-boc methylisothiourea 147, these were used to synthesise the desired substrate 207 (Scheme 72).

Figure 85 – Section of Proton NMR spectrum of crude sample of compound 207. Impurities highlighted in blue.

After altering reaction times several times, two hours was deemed optimal to produce substrate 207. Figure 85 shows the proton NMR spectrum of a crude
sample of compound 207. By using literature characterisation [δ3.34-3.33 (m, -CH₂), δ2.76 (t, -CH₂)], the ethyl peaks were identified and used to determine the purity of the sample. Generally, purity was approximately 70% (Figure 85).

4.7 Final di-amide coupling

To attach compound 207 to main scaffold, an amide coupling was conducted. The amide coupling still resulted in low yields and as before, column chromatography was again difficult with impurities constantly co-eluting.

![Scheme 73 - Di-amide coupling.](image)

Having thoroughly explored all the weak steps in the convergent methodology, no ideal solution could be reached and thus the convergent approach was abandoned in favour of the methodology devised in Chapter 3.
4.8 Conclusion

Chapter 4 concludes the sections concerned with the synthesis of guanidine- and amine functionalised norbornanes. Two methods were explored: (i) linear synthesis; (ii) Late stage variation.

Scheme 74 – Linear method for the synthesis of amine functionalised norbornanes.

The linear method for the synthesis of amine functionalised norbornanes suffered several disadvantages (Scheme 74). For instance, in the six step synthesis, purification by column chromatography was required three times. Since the hydrophobic residues were introduced early in the synthesis, there were a multitude of compounds that needed handling through the different stages of the synthesis. Finally, the di-amide coupling gave only in poor to modest yields resulting in the loss of large amounts of starting material.

Scheme 75 – Late variation of norbornanes.
Re-evaluation of the synthetic methodology led to the decision to introduce the hydrophobic residues at late a stage as possible. The new synthetic strategy had many desirable characteristics. Each step had yields of higher than 85% even when conducted on a multi-gram scale. In the six step synthesis, purification by column chromatography was only required once. Unfortunately, problems were encountered in the deprotection and salt formation step. The acetal functional group was not always stable to the conditions used to cleave Boc groups. A protocol involving treatment with an acetyl chloride in MeOH solution and a subsequent aqueous wash resulted in amine salts that had purities of 56% - 100% (8 > 85%).

Scheme 76 - Synthesis of guanidine functionalised norbornanes using amine functionalised norbornanes.

The synthesis of the guanidine functionalised norbornanes proceeded first with the guanylation of the amine functionalised norbornanes (Scheme 76). Three guanylation agents were evaluated however only di-Boc triflylguanidine 149 was successful in producing the entire library of desired guanidines. Once again, there were problems with the deprotection of Boc groups. Due to the lengthy times required for the removal of four protecting groups, purity of some ‘products’ were as low as 40%.
Finally attempts were made to optimise the ‘convergent’ methodology developed by Pfeffer et. al. (Figure 86). The incorporation of protecting groups in the synthesis of amino guanidine 207 appeared to interfere with the catalytic efficiency of Pd/C. Pursuit of the ‘convergent’ methodology ceased in light of the superior methods developed in Chapter 3.
4.9 Experimental

**Known compounds**

The following compounds are known compounds and were prepared using literature conditions:

- Di-Boc methylisothiourea $147^{240}$
- Di-Boc thiourea $148^{246}$
- Di-Boc triflylguanidine $149^{249}$
- Benzyl 2-aminoethylcarbamate $89$ (Cbz-EDA).$^{232}$

$$\text{[(2-benzyloxycarbonylaminoethyl)carbonimidoyl]bis-, bis-(1,1-dimethyl ethyl)ester (211)}$$

**Method A:** A solution of di-boc methylisothiourea $147$ (50.6 mg, 174 μmol), Cbz-EDA $89$ (33.8 mg, 174 μmol) in THF (2 mL) was irradiated in a microwave for 15 minutes at 80 °C. The reaction mixture was diluted with saturated bicarbonate solution (10 mL), transferred to a separatory funnel and extracted with EtOAc (3 × 10 mL). The organic phases were combined and the solvent removed in vacuo. The crude solid was dissolved in hot EtOH (1 mL) and precipitated by slowly adding H$_2$O (2 mL). The precipitate was collected using vacuum filtration (27.6 mg, 36%);

**Method B:** A solution of Cbz-EDA $89$ (862 mg, 4.44 mmol), dibocthiourea $148$ (1.02 g, 3.7 mmol), Et$_3$N (52 μL) and NIS $168$ (915 mg, 4.07 mmol) was stirred for 16 hours at room temperature. 1 M sodium thiosulphate solution (3 mL) was
added and then the mixture was diluted with H₂O (10 mL), transferred to a separatory flask and extracted with EtOAc (3 × 10 mL) and the organic phases were combined and dried with MgSO₄. This was filtered and the solvent removed in vacuo. The crude brown oil was purified using column chromatography [(1:39) Acetone:DCM]. Fractions containing the desired compound (Rf 0.41) were combined and the solvent removed in vacuo resulting in clear oil. (1.25 mg, 78%);

Method C: A solution of Cbz-EDA 8 (26.2 mg, 134 μmol), di-boc triflylguanidine 149 (52.8 mg, 134 μmol) and Et₃N (18 μL) in DCM (5 mL) was stirred for 16 hours at room temperature. The reaction mixture was filtered by flash chromatography through a Celite® frit and the filter cake rinsed with an EtOAc/MeOH/Et₃N (5:3:2) solution (50 mL). The filtrate was collected and the solvent removed in vacuo resulting in light brown oil. This was purified using column chromatography [(1:39) Acetone:DCM]. Fractions containing the desired compound (Rf 0.41) were combined and the solvent removed in vacuo resulting in clear oil. (53.2 mg, 90%); νmax (thin film/cm⁻¹) 3336.44 (br m) (secondary amine N-H) 1645.10 (s) (aromatic C=C) 1456.17 (w) (aromatic C=C) 1368.88 (m) (tert-butyl C-H) 1135.99 (vs) (tertiary amine C-N); δH NMR (270 MHz, CDCl₃): 11.42 (1H, br s, H11) 8.51 (1H, br t, H10) 7.31-7.27 (5H, m, H1-5) 5.88 (1H, br t, H7) 5.06 (2H, s, H6) 3.53 (2H, dd, J = 4.70, 5.43 Hz, H8) 3.36 (2H, dd, J = 4.70, 5.45 Hz, H9) 1.49-1.42 (18H, m, H12-13); δC NMR (67.5 MHz, CDCl₃): 163.1, 157.20 156.9, 153.0, 136.5, 128.4 (2 carbons), 127.9 (2 carbons), 83.3, 79.3, 78.6, 66.6, 41.6, 40.4, 27.99 (3 carbons), 27.92 (3 carbons); HRMS (ESI m/z): calculated for [C₂₁H₃₂N₄O₆+H]+ 437.2395, found 437.23409.
Section 4.5 Deprotection difficulties

$N^2,N^3$-bis(2-aminoethyl)bicycle[2.2.1]heptanes-2-endo,3-exo-dicarboxamide (216)

To a solution of nornornene 215 (50.8 mg, 192 μmol) in MeOH (6 mL), Pd/C (6 mg) was added. This was stirred at room temperature under a hydrogen atmosphere until there was a complete loss of starting material as judged by TLC. The reaction mixture was filtered through Celite®. The filtrate was collected and the solvent removed in vacuo. This resulted in viscous clear oil. (33.2 mg, 64%); $\nu_{\text{max}}$(thin film/cm$^{-1}$) 3290.47 (br s) (amine N-H) 1635.19 (vs) (amide C=O) 537.17 (vs) (amine C-N); $\delta$H NMR (270 MHz, CD$_3$OD): 3.40-2.71 (8H, m, H9-10, H13-14) 2.56-2.26 (4H, m, H1-4) 1.74-1.05 (6H, m, H5-6, H7a-b); $\delta$C NMR (67.5 MHz, CD$_3$OD): 176.38, 174.50, 72.22, 60.93, 56.65, 49.8, 43.94, 43.02, 40.50, 40.39, 37.9, 28.98, 23.34; MS (ESI m/z): calculated for [C$_{13}$H$_{24}$N$_4$O$_2$+H]$^+$ 269.20, found 269.20.
The current chapter provides a discussion of the disk diffusion assays that were conducted to evaluate the antibacterial activity of amine functionalised and guanidine functionalised norbornane based CAMP mimics. The results from the disk diffusion experiments allowed for the selection of a few active candidates to be further evaluated in Chapter 6.

5.1 Introduction

The next aim of the project was to subject the libraries of amine functionalised and guanidine functionalised norbornane salts to antibacterial testing. Figure 87 lists the twenty-four salts sent for antibacterial testing. It should be noted that the samples sent were mixtures (see discussion in Chapter 2 and 3). Since the fully degraded product was potentially the diol 135, this compound was also sent for antibacterial testing. The purpose of testing diol 135 was to ensure that
differences of antibacterial activity between compounds could be attributed to the differences in the hydrophobic regions.

![Figure 88 – Structure of diol 135.](image)

The compounds were tested using disk diffusion assays at the Monash Institute of Pharmaceutical Sciences courtesy of Professor Jian Li. Disk diffusion was the method selected for screening of antibacterial activity as it was simple, cost-effective and allowed for the testing of a large number of compounds in one batch.

A number of bacteria strains, also nominated as ‘ESKAPE’, were selected for the antibacterial assays. A brief description of each strain follows:

- *Pseudomonas aeruginosa* ATCC 27853
- *Pseudomonas aeruginosa* 19147 n/m
- *Acinetobacter baumannii* ATCC 19606
- *Acinetobacter baumannii* 07AC-336
- *Klebsiella pneumoniae* ATCC 13883
- *Klebsiella pneumoniae* #1
- Methicillin-resistant *Staphylococcus aureus* ATCC 43300
- Vancomycin-resistant *Enterococcus faecium* ATCC 700221
5.2 Bacterial strains

5.2.1 *Pseudomonas aeruginosa* ATCC 27853 and *Pseudomonas aeruginosa* 19147 n/m

The first two strains of bacteria were varieties of *Pseudomonas aeruginosa* (*P. aeruginosa*). *Pseudomonas aeruginosa* is a Gram-negative pathogen usually found in soil and when grown in cultures, it has a characteristic blue-green colour.\textsuperscript{281, 282} The colouring of *P. aeruginosa* allowed for observations of the bacteria’s presence in the discolouration of wounds as early as 1850 and was first isolated by Carle Gessard in 1882.\textsuperscript{282} The ATCC 27853 strain is resistant to Polymyxin E however the 19147 n/m strain is classified as one of the multi-drug resistant variety.\textsuperscript{283}

*Pseudomonas aeruginosa* is an extremely hardy bacteria, being able to survive in a variety of non-native environments especially in hospitals.\textsuperscript{281} In fact, humans acquire most *P. aeruginosa* infections whilst staying in hospital.\textsuperscript{282} In hospitals in the USA, *P. aeruginosa* is the most common strain of bacteria found and is one of the leading causes of hospital-acquired pneumonia, urinary tract, surgical site and bloodstream infections. Causing more concern now however is the emergence of antibiotic resistant strains of *P. aeruginosa* which are severely limiting therapeutic options by medical practitioners.\textsuperscript{281}
5.2.2 Acinetobacter baumannii ATCC 19606/ Acinetobacter baumannii 07AC-336

The next two strains of bacteria that were tested were varieties of Acinetobacter baumannii (A. baumannii). Acinetobacter baumannii are Gram-negative bacteria covering twenty-three different species. It is found ubiquitously in nature and is now increasingly associated with severe infections acquired within hospitals.\textsuperscript{284, 285} The ATCC 19606 strain is classified as multi-drug resistant still susceptible to Colistin. The 07AC-336 strain however is deemed to be Extensive Drug Resistance as it is resistant to all antibiotics effective against Gram-negative bacteria.\textsuperscript{286, 287}

Over the last few decades, A. baumannii has been the major cause of healthcare-associated infections with mortality rates rising to 54\% for infections acquired whilst in intensive care. Acinetobacter baumannii is another strain of bacteria causing grave concerns as antibiotic resistance isolates have been detected since the 1970s and infections have become increasingly difficult to treat.

5.2.3 Klebsiella pneumoniae ATCC 13883/ Klebsiella pneumoniae #1

The next two strains of bacteria that were tested were varieties of Klebsiella pneumoniae (K. pneumoniae). Klebsiella pneumonia are Gram-negative bacteria classified under the Enterobacteriaceae family and was first identified over a hundred years ago as the cause of pneumonia infections within the wider community.\textsuperscript{288} The ATCC 13883 strain is classified as multi-drug resistant while still susceptible to Polymyxin E.
Since then, *K. pneumoniae* has found its way into hospitals becoming a common pathogen causing urinary tract, bloodstream and pneumonia infection. Also cause for concern is that since the 1990s, more and more antibiotic resistant strains of *K. pneumoniae* have been detected making infections increasingly difficult to treat.

5.2.4 Methicillin-resistant *Staphylococcus aureus* ATCC 43300

Methicillin-resistant *Staphylococcus aureus* is by far the most notorious multi-drug resistant bacteria in the last few decades. More commonly known as just MRSA, it is the leading cause of bacterial infections around the world.\(^1\), \(^2\) MRSA is a Gram-positive pathogen that is resistant to methicillin (a derivative of penicillin). MRSA concerns public health officials most and so the development of an antibiotic effective against it is of utmost importance.\(^1\)

5.2.5 Vancomycin-resistant *Enterococcus faecium* ATCC 700221

*Enterococcus faecium* is a Gram-positive bacteria that is widespread in nature and even counts for 1% of the bacteria found in the human intestinal tract.\(^2\) \(^9\) *Enterococcus faecium* has the ability to survive very harsh conditions having stability in extreme temperatures and unaffected by chemical disinfectants.\(^2\) \(^9\), \(^2\) Since *Enterococcus faecium* has the ability to reside in the human gastrointestinal tract for long periods of time, antibiotic resistance has developed rapidly.\(^2\) \(^9\)\(^2\) Vancomycin-resistant *Enterococcus faecium* (VRE) is a bacterial strain that has developed resistance against the antibiotic Vancomycin. VRE is another bacterium that has received widespread attention over the last couple of decades.\(^7\) It is also the common cause of nosocomial infections ranked
second to only MRSA. There exists a need therefore to also develop drugs capable of treating infections by VRE.

5.3 Methodology

Each of the compounds were weighed and transported to the testing facility after being subjected to freeze-drying. Each test compound was dissolved in Milli-Q water and ethanol to make up a solution to the concentration of 5 mg/mL. The solutions were store at 4 °C in a refrigerator. The disk diffusion experiments were conducted in triplicate and 50μg of sample was added to each disk. Once the experiments were completed, the inhibition zones were measured and the compounds compared.
## 5.4 Disk diffusion results

### 5.4.1 Amine functionalised norbornane based CAMP mimics

Table 19 – Disk diffusion results for amine functionalised norbornane based CAMP mimics. Zone in nm). Shading indicates antibacterial activity observed.

![Chemical structure](attachment:chemical_structure.png)

<table>
<thead>
<tr>
<th>No.</th>
<th>PA ATCC 27853</th>
<th>PA 19147m</th>
<th>Ab ATCC 19606</th>
<th>Ab 07AC-336</th>
<th>Kp ATCC 13883</th>
<th>Kp #1</th>
<th>MRSA ATCC 43300</th>
<th>VRE ATCC 700221</th>
</tr>
</thead>
<tbody>
<tr>
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<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
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<td>8 mm very faint</td>
</tr>
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<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
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<td>None</td>
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</tr>
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<td>None</td>
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<td>None</td>
<td>14 mm very faint</td>
<td>15 mm very faint</td>
</tr>
<tr>
<td>146</td>
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<td>None</td>
<td>None</td>
</tr>
<tr>
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<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>14 mm</td>
<td>16 mm faint</td>
</tr>
<tr>
<td>139</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>134</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>7 mm</td>
<td>None</td>
<td>9 mm</td>
<td>11 mm faint</td>
</tr>
<tr>
<td>142</td>
<td>None</td>
<td>11 mm</td>
<td>None</td>
<td>None</td>
<td>11 mm</td>
<td>None</td>
<td>10 mm</td>
<td>9 mm faint</td>
</tr>
<tr>
<td>141</td>
<td>None</td>
<td>10 mm</td>
<td>None</td>
<td>None</td>
<td>10 mm</td>
<td>None</td>
<td>10 mm</td>
<td>17 mm faint</td>
</tr>
<tr>
<td>135</td>
<td>Diol</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>
5.4.2 Guanidine functionalised norbornane based CAMP mimics

![Chemical structure](image)

<table>
<thead>
<tr>
<th>No.</th>
<th>Kp ATCC 13883</th>
<th>MRSA ATCC 43300</th>
<th>VRE ATCC 700221</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>205</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>203</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>196</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>197</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>206</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>200</td>
<td>None</td>
<td>7 mm</td>
<td>None</td>
</tr>
<tr>
<td>204</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>199</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>198</td>
<td>None</td>
<td>7 mm</td>
<td>None</td>
</tr>
<tr>
<td>201</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>202</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>

5.5 Discussion

In the analysis of disk diffusion data, three descriptions of antibacterial activity are commonly used. Bacteria are considered resistant to a compound if the culture grows to the edge of the paper disks soaked in test compound solution. Bacteria are classified as susceptible if there is a circular zone around the paper disk where the bacteria culture has not grown. Generally, the greater the zone
around the paper disk, the more effective a compound is against a strain of bacteria. Finally, the third classification is known as intermediate. This is where there is a faint ring surrounding the paper disk since there is a lower amount of culture growth surrounds the paper disk. Figure 89 shows an agar plate where all three classifications of growth have been identified.

![Figure 89 – Picture of plate showing the three classifications of activity.](image)

5.5.1 *Activity of amine functionalised norbornane based CAMP mimics*

![Figure 90 – Structure of amine 139 (purity: 86%)](image)

Of all the amine functionalised norbornane based CAMP mimics tested, amine 140 performed the best with five strains of bacteria showing susceptibility. Generally the zones of inhibition were between 7 – 14 mm (Figure 91). Amine
also showed intermediate activity with VRE having a ring approximately 16 mm (Figure 91).

There were a number of other amines that showed activity against at least three bacterial strains (Figure 92). The strains of bacteria that showed susceptibility to many test compounds were *Pseudomonas aeruginosa* (multi-drug resistant variant) and *Klebsiella pneumoniae* (multi-drug resistant variant) with most compounds having intermediate activity against VRE.

![Figure 91 - Disk diffusion test of (a) MRSA culture featuring the zone of inhibition associated with amine 139; (b) VRE featuring a zone of intermediate activity associated with amine 139.](image)

![Figure 92 - Structures of other amines that showed activity.](image)
5.5.2 Activity of guanidine functionalised norbornane based CAMP mimics

Unfortunately, only two of the guanidine functionalised norbornanes compounds showed activity. Guanidines 198 and 200 were active against MRSA. Even then, the susceptibility exhibited was not as strong as some of the activity exhibited by the amine counterparts. The lack of activity exhibited by the guanidines compounds is likely due to the low purity in certain cases. The results obtained from the disk diffusion tests do not then accurately reflect how potent the guanidine functionalised norbornanes may be but they certainly do not indicate strong activity for the class of compounds.

Figure 93 – Structures of guanidines 198 and 200.

Figure 94 – Disk diffusion test of MRSA culture showing susceptibility to guanidines 200 and 198.
5.6 Conclusion

There were a number of active compounds identified. Across both libraries; the compounds with the pyrenyl hydrophobic moiety exhibited the most consistent antibacterial activity. The rest of the compounds that did exhibit antibacterial activity had the common characteristic of an aromatic group as part of the hydrophobic region.

One purpose of testing these compounds was to select candidates for further identification of their physicochemical properties (Chapter 6).
The physicochemical evaluation of several amphiphiles was performed to glean insight regarding a potential mode of action. First, the critical micelle concentrations of three amine functionalised norbornanes were determined. Next, Dynamic Light Scattering was utilised to analyse interactions with ‘human’ and ‘bacterial’ liposomes. Finally, Isothermal Titration Calorimetry was conducted to determine if the compounds were interacting with lipopolysaccharides.

6.1 Introduction

Evaluating physicochemical properties is common in medicinal chemistry projects. In the current project, three techniques were selected: spectrophotometry to determine critical micelle concentration (CMC), Dynamic Light Scattering (DLS) to observe interactions with liposomes and Isothermal Titration Calorimetry to detect interactions with LPS.

Figure 95 – Structures of amines subjected to physicochemical evaluation.
Four amines were selected for testing: amines 146, 142, 134 and 141 (Figure 95). These four compounds performed the best in the disk diffusion experiments being active against at least four strains of bacteria (see Chapter 5).

### 6.2 Critical Micelle Concentration

The critical micelle concentration (CMC) refers to the concentration above which the formation of micelles is observed. Amphiphiles traditionally form micelles in water and it is commonplace to determine CMCs as a number of physical properties change above the CMC. Figure 96 shows a number of physical properties that are concentration dependent and the CMC marks the point at which change in behaviour occurs. Surface tension for example steadily decreases and past CMC, remains constant.

![Figure 96](image_url) - Schematic representation of the concentration dependence of some physical properties for solutions of a micelle-forming amphiphile.

An example of influence that CMC exerts on antibacterial activity was observed when the group of Chaveriat and Gosselin tested D-galactopyranose derivatives
that were synthetically derived (Table 20). They found that a decrease in CMCs was related to a similar decrease in the compounds' MICs against Gram-positive bacteria (compare 222 to 226, Table 20). For the D-galactopyranose derivatives, it would seem that developing compounds with low CMCs would translate to lower MICs and therefore higher potencies. Furthermore, determining a compound's CMC and MIC may give evidence to the mode of action. According to Lichtenberg, the antibacterial activity of a compound occurring near or at its CMC may indicate detergent-like mechanisms as part of its mode of action against bacteria.

**Table 20 – CMCs and MICs for D-galactopyranose derivatives**

<table>
<thead>
<tr>
<th>No.</th>
<th>R</th>
<th>CMC (μg/mL)</th>
<th>Bacillus steroothermophilus</th>
<th>Micrococcus luteus</th>
</tr>
</thead>
<tbody>
<tr>
<td>222</td>
<td>C₈H₁₇</td>
<td>3506</td>
<td>&gt;250</td>
<td>&gt;250</td>
</tr>
<tr>
<td>223</td>
<td>C₁₂H₂₅</td>
<td>Did not form micelles</td>
<td>&gt;250</td>
<td>&gt;250</td>
</tr>
<tr>
<td>224</td>
<td>(CH₂)₄OC₆H₁₇</td>
<td>109</td>
<td>63</td>
<td>63</td>
</tr>
<tr>
<td>225</td>
<td>(CH₂)₄OC₁₂H₂₅</td>
<td>10</td>
<td>31</td>
<td>16</td>
</tr>
<tr>
<td>226</td>
<td>CH₂C≡CCH₂OC₁₂H₂₅</td>
<td>13</td>
<td>8</td>
<td>4</td>
</tr>
</tbody>
</table>

In the current work, the CMCs of amines 146, 142 and 141 were determined. The CMC of a compound can be determined by measuring either the testing of surface tension, reverse-phase HPLC, x-ray and light scattering, NMR or spectrophotometry using fluorescent probes. In each of the former instances, the CMC is determined by plotting different concentrations of the property being measured by the instrument. In the current project, the use of spectrophotometry using fluorescent probes was selected as the method for the determination of the CMCs of amines 146, 142 and 141 as it is relatively simple and many examples exist where this technique has been used.
6.2.1 Methodology

Pyrene, the fluorescent probe, is highly hydrophobic and insoluble in water. Above the amphiphile’s CMC, the pyrene molecules will be preferentially encapsulated in the hydrophobic micelle core.\textsuperscript{301} Solutions of varying concentrations (0.0001 - 5 mg/mL) of amines 146, 142 and 144 were prepared using Milli-Q water.\textsuperscript{302} Then pyrene was added and each suspension was sonicated for 30 minutes at room temperature. The suspensions were then allowed to stand at room temperature for 16 hours. Immediately prior to analysis, each suspension was filtered using a 0.45 micron nylon filter. Steady-state fluorescence spectra were recorded at room temperature on a Cary Eclipse Varian spectrophotofluorimeter using 10 mm slits. Each sample solution was excited at 335 nm and the emission spectrum was recorded from 350 to 600 nm at 80 nm/min.

![Fluorescence spectra](image)

Figure 97 – Fluorescence spectra of amine 146 for (a) below the CMC; (b) above the CMC.
Visually, it is possible to determine when encapsulation has occurred (Figure 97). The spectrum recorded proceeds from a ‘sawtooth’ to a ‘smooth’ signal. However, in order to precisely determine when encapsulation of the pyrene occurs, the ratio is calculated of the first vibronic band (I1) at 386 nm to that of the third vibronic band (I3) at 393 nm.\textsuperscript{301, 302} I1 exhibits a higher fluorescence signal in a polar than in a non-polar environment. I3 however is less sensitive to changes in the environment. Hence when the I1/I3 ratio of different concentrations are plotted, there should be an inflection in the graph denoting the point at which pyrene has moved from the hydrophilic environment of the aqueous solutions and has been encapsulated in the hydrophobic core of the newly formed micelles.\textsuperscript{302}

6.2.2 CMC calculations

To illustrate how the CMC calculations were conducted, amine 142 was selected as an example. A range of concentrations were tested from 3 mg/mL to 0.0001 mg/mL and the ration of I1 to I3 in the emission was calculated (Table 21).
Table 21 – Table of I1/I3 ratios for each concentration.

<table>
<thead>
<tr>
<th>Concentration (mg/mL)</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0001</td>
<td>0.9436 ± 0.10</td>
</tr>
<tr>
<td>0.001</td>
<td>0.9552 ± 0.20</td>
</tr>
<tr>
<td>0.01</td>
<td>0.9896 ± 0.17</td>
</tr>
<tr>
<td>0.1</td>
<td>1.4168 ± 0.04</td>
</tr>
<tr>
<td>1</td>
<td>1.4958 ± 0.03</td>
</tr>
<tr>
<td>1.5</td>
<td>1.5102 ± 0.002</td>
</tr>
<tr>
<td>2</td>
<td>1.5596 ± 0.01</td>
</tr>
<tr>
<td>2.5</td>
<td>1.5246 ± 0.02</td>
</tr>
<tr>
<td>3</td>
<td>1.6489 ± 0.05</td>
</tr>
</tbody>
</table>

The data contained in Table 21 was then plotted so as to identify the point of inflection denoting when pyrene was encapsulated by the micelles.

**Concentration to I1/I3 ratio**

![Graph of concentration versus ratio of I1/I3 of pyrene emission.](https://via.placeholder.com/150)

Figure 99 – Graph of concentration versus ratio of I1/I3 of pyrene emission. Concentrations ranged from 0.0001 mg/mL – 3 mg/mL.

Data was plotted and the point of inflection identified signifying the CMC of a particular compound. The calculations were conducted and the CMCs are stated in Figure 100.
Substituents in the hydrophobic region of an amphiphile will have a substantial influence on micelle formation. For example, an increased CMC is generally observed when a polar group is incorporated onto the alkyl chain of an amphiphile. In the current project, amine 141 has a higher CMC than amine 142 and this can be attributed to presence of oxygen in the hydrophobic region. Furthermore, the incorporation of fluorine can have a striking effect on the CMC of a compound. In this project, this phenomenon was observed as CMC of amine 146 was almost fifteen times the CMCs of the other amines.

Looking back, the disk diffusion assays were conducted at a concentration of 50 μg per disk (Chapter 5) which works out to be at a concentration substantially higher than the three CMCs determined above. Preliminarily, it would therefore appear that the activity observed in the disk diffusion assays were based on the behaviour of aggregates of the amines instead of monomers. To further explore this theory, DLS experiments were conducted.
6.3 Dynamic Light Scattering

Liposomes are used extensively in the evaluation of physicochemical properties of amphiphilic compounds and may be easily formulated to mimic the outer membranes of bacteria.

Alec Bangham is credited with the discovery of liposomes having first published images collected by electron microscopy of multilamellar phospholipid vesicles (Figure 101). To physicochemists, Bangham’s discovery “… was the membrane equivalent of finding the double helix structure of DNA” (David W. Deamer). Since then, the use of liposomes has become extensive featuring in drug development, membrane mimicry and even cosmetics by Dior and Lancôme.

An example where liposomes were used to evaluate membrane interactions was published by Ramamoorthy and Bhattacharjya. Using DLS, they evaluated the diameter and polydispersity index (PDI) of solutions of LPS micelles in the absence and presence of antibacterial peptides. Ramamoorthy and Bhattacharjya correlated changes in micelle diameters and PDI to the
interaction of the antibacterial peptides with LPS. They found that in the presence of their antibacterial peptides, there was a reduction in aggregation of LPS.\textsuperscript{305}

To gain some insight into the interactions of amines 146, 142 and 161 with model membranes. Liposomes were assembled, one simulating ‘human’ membranes and one simulating ‘bacterial’ membranes. These were compared to the behaviour of the detergent Triton\textsuperscript{TM} X-100 known to rapidly disintegrate liposomes.\textsuperscript{306} The two parameters that were closely observed were the diameter of micelles and the PDI.

### 6.3.1 Sample preparation

The size of liposomes depends heavily on the method of preparation. The two most common sizes are known as Small Unilamellar Vesicles (SUVs) and Large Unilamellar Vesicles (LUVs). SUVs are less than 50 nm in diameter and are obtained through sonication.\textsuperscript{307} LUVs are between 50 to 500 nm are prepared by extruding stock liposome solution through membranes with pore sizes between 50 to 500 nm.\textsuperscript{307} Although SUVs scatter less light and are more suited to analysis by DLS, their bilayer is very strained. Hence, as a model membrane, LUVs are considered better suited for the type of analysis to be conducted.\textsuperscript{307}

Two different types of LUVs were prepared, one that mimicked human cells, herein referred to as the ‘human’ liposomes and the other that mimicked a bacterial cell membrane, herein referred to as the ‘bacterial’ liposomes.
To prepare the ‘human’ liposomes, 2-oleoyl-1-palmitoyl-sn-glycero-3-phosphocholine \(227\) (POPC) was added to sodium phosphate buffer \((10 \text{ mM, pH} = 7.4)\) to obtain a lipid concentration of 1 mM (Figure 102).\(^{308, 309}\) The stock solution of the lipid dispersion was extruded three times using 450 micron nylon filter. A series of sample solutions were then prepared where different concentrations of amines were added to the ‘human’ liposomes and the samples allowed standing for 16 hours.

To prepare the ‘bacterial’ liposomes, 2-oleoyl-1-palmitoyl-sn-glycero-3-phospho-(1’-rac-glycerol) \(228\) (POPG) and LPS isolated from \textit{Pseudomonas aeruginosa} was added to a minimum amount of chloroform to ensure dissolution. Nitrogen gas was used to evaporate most of the solvent. The resulting residue was then placed under vacuum for a further twenty-four hours to ensure all chloroform was removed. Then, Milli-Q water was added to obtain a lipid concentration of 1 mM. The ‘bacterial’ liposome solution was then extruded three times using 450 micron nylon filters. A series of sample solutions were then prepared where different concentrations of amines were added to the ‘bacterial’ liposomes and the samples allowed standing for 16 hours.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{lipid_structures.png}
\caption{(a) Structure of lipid POPC 227. (b) Structure of lipid POPG 228.}
\end{figure}
Size diameter and size dispersion of liposomes were measured by Zetasizer Nano ZS (Malvern Instruments) at a light scattering angle of 173°. Each experiment was conducted at 25 °C and each solution was measured in triplicate. The data presented in the following section is an average of the three measurements.

6.3.2 Results

6.3.2.1 Amine solutions

Before evaluating the behaviour of the amine functionalised norbornanes with respect to either 'human' or 'bacterial' liposomes, each amine was subjected to DLS below and above their respective CMCs (Table 22).

<table>
<thead>
<tr>
<th>Table 22 – Peak diameters of particles in the amine solutions.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diameter (nm)</strong></td>
</tr>
<tr>
<td><strong>Below CMC</strong></td>
</tr>
<tr>
<td><strong>Above CMC</strong></td>
</tr>
<tr>
<td>146</td>
</tr>
<tr>
<td><strong>Below CMC</strong></td>
</tr>
<tr>
<td><strong>Above CMC</strong></td>
</tr>
<tr>
<td>142</td>
</tr>
<tr>
<td><strong>Below CMC</strong></td>
</tr>
<tr>
<td><strong>Above CMC</strong></td>
</tr>
<tr>
<td>141</td>
</tr>
<tr>
<td><strong>Below CMC</strong></td>
</tr>
<tr>
<td><strong>Above CMC</strong></td>
</tr>
</tbody>
</table>

For amine 146, homogeneity increased in the solution above CMC with only one population and a decrease in PDI. For amine 142, the diameter of the micelles decreased above CMC but PDI remained fairly consistent below and above CMC. For amine 141, micelle sizes decreased substantially when above CMC with a
decrease in PDI as well. With some information of how the amines behave in water below and above CMC, the evaluation of their behaviour with respect to liposomes began.

6.3.2.2 ‘Human’ liposomes

In the evaluation of physicochemical activity using liposomes, two control solutions were tested. The first contained just the relevant liposome and the second contained liposome and the known membrane disrupting agent Triton™ X-100. Then each amine in concentrations below and above their respective CMCs was mixed with the liposomes. Table 23 details the data obtained.

<table>
<thead>
<tr>
<th>Peak diameter* (nm)</th>
<th>PDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liposomes with no additive</td>
<td>133 ± 29</td>
</tr>
<tr>
<td>Triton™ X-100</td>
<td>9.7 ± 0.9</td>
</tr>
<tr>
<td>Below CMC (0.80 mg/mL)</td>
<td>Peak 1: 108 ± 3</td>
</tr>
<tr>
<td>Above CMC (3.19 mg/mL)</td>
<td>Peak 2: 620 ± 80</td>
</tr>
<tr>
<td>Below CMC (0.015 mg/mL)</td>
<td>650 ± 63</td>
</tr>
<tr>
<td>Above CMC (0.33 mg/mL)</td>
<td></td>
</tr>
<tr>
<td>Below CMC (0.056 mg/mL)</td>
<td>Peak 1: 832 ± 60</td>
</tr>
<tr>
<td>Above CMC (1 mg/mL)</td>
<td>Peak 2: 133 ± 27</td>
</tr>
<tr>
<td>Below CMC (0.056 mg/mL)</td>
<td>778 ± 10</td>
</tr>
<tr>
<td>Above CMC (1 mg/mL)</td>
<td></td>
</tr>
<tr>
<td>Below CMC (0.056 mg/mL)</td>
<td>152 ± 9</td>
</tr>
<tr>
<td>Above CMC (1 mg/mL)</td>
<td>758 ± 84</td>
</tr>
</tbody>
</table>

* Peak diameter refers to diameter of liposomes in the measured population.
The control solutions of ‘human’ liposomes showed that particle sizes were around 133 nm. The test solution using Triton™ X-100 displayed drastically reduced particle sizes, an indication that the liposomes had been disassembled.

Selecting amine 141 as an example, Figure 103 compares the peak diameters of the control experiments and the solutions containing liposomes and amines. From the control experiment, the ‘human’ liposome size is approximately 133 nm. Triton™ X-100, a known detergent, disintegrates the liposomes and results in particles sized around 9.7 nm. Considering the solutions of just amine 141 in water, particle sizes are approximately 714 nm below CMC and 118 nm above CMC. The solution containing the amine below CMC and the ‘human’ liposome shows a particle size of approximately 152 nm. It could be argued that this would be the measurement of just the ‘human’ liposomes and not an indication of the interaction with the amine. However, in the solution with the amine above CMC and the ‘human’ liposomes, a substantial increase of particle size is observed with a population of 758 nm observed. It may be argued that the
particle size change is evidence of aggregates of amine \textit{141} interacting with the 'human' liposomes by swelling particle size. Nevertheless, no drastic reduction in size was noted, tentatively indicating little disruptive effect.

6.3.2.3 ‘Bacterial’ liposomes

In the evaluation of the amines with ‘bacterial’ liposomes, once again two control solutions were tested. The first contained just ‘bacterial’ liposomes. The second contained Triton\textsuperscript{TM} X-100 and ‘bacterial’ liposomes. Then each amine solution was tested with ‘bacterial’ liposomes above and below their respective CMCs.

\begin{table}[h]
\begin{center}
\begin{tabular}{|l|c|c|}
\hline
& Peak diameter* (nm) & PDI \\
\hline
Liposomes with no additive & 177 ± 15 & 0.31 ± 0.07 \\
\textbf{Triton\textsuperscript{TM} X-100} & 9.3 ± 0.7 & 0.58 ± 0.08 \\
Below CMC (0.80 mg/mL) & 147 ± 40 & 0.23 ± 0.03 \\
Above CMC (3.19 mg/mL) & 178 ± 27 & 0.59 ± 0.1 \\
\textbf{Below CMC (0.015 mg/mL)} & 165 ± 33 & 0.32 ± 0.05 \\
\textbf{Above CMC (0.33 mg/mL)} & 161 ± 10 & 0.35 ± 0.05 \\
\textbf{Below CMC (0.056 mg/mL)} & 181 ± 23 & 0.32 ± 0.07 \\
\textbf{Above CMC (1 mg/mL)} & Peak 1: 316 ± 10 & Peak 2: 135 ± 57 & 0.35 ± 0.06 \\
\hline
\end{tabular}
\end{center}
\caption{Peak diameters obtained from the testing of ‘bacterial’ liposomes.}
\end{table}

* Peak diameter refers to diameter of liposomes in the measured population.
Once again, amine 141 was selected as an example compound of liposome interaction. Figure 104 compares the diameters of the control solutions and the solutions with amines and liposomes. In the solution of amine below CMC and ‘bacterial’ liposomes, particle size is approximately the same as the control. However, the solution containing above the CMC of the amine and ‘bacterial’ liposomes show two populations of micelles. The changes in peak diameters possibly indicate that some interaction between the ‘bacterial’ liposomes and the amine micelles occurred.

So far the experiments using DLS indicate that the amines interact with liposomes primarily above the CMC of the respective compounds. The interactions however do not conclusively indicate the amines binding preferentially to LPS. For more information about the specific interactions with LPS, ITC experiments were conducted.
6.5 Isothermal Titration Calorimetry

Isothermal Titration Calorimetry (ITC) is an analytical method used to evaluate the thermodynamics and kinetics of the interactions between molecular entities (host-guest and enzyme substrates).\textsuperscript{310-314} By accurately measuring the heat flow that occurs as one molecule interacts with another using titration, the stoichiometry of binding sites (n), the association-binding constant (K), enthalpy (H) and entropy (S) may all be determined. Furthermore, using the experimental values obtained, the Gibb’s Free energy of the system may be calculated.

![Figure 105 - Structures of amines that were subjected to ITC analysis.](image)

For analysis by ITC, two amines were selected: amine 134 and 146. It would be interesting to observe behavioural changes between the two molecules. Amine 134 contains a hydrophobic region that has alkyl and aryl functionality. Amine 146 however only has aryl functionality. Using ITC, the beneficial effect of the larger hydrophobic regions might be confirmed.

6.5.1 Methodology

Although there are a variety of ITC instruments, there are core components common to all apparatus. An ITC instrument has two cells: a sample cell and a reference cell are both encased in cases allowing for a highly regulated
environment. In the sample cell, there is a stirring syringe (Figure 106). When a known concentration of a solution of the compound is added to the vigorously stirred solution containing the appropriate ligand, the instrument measures the heat signal with respect to the reference cell and plots the signals versus time (an isotherm).

![Diagram of ITC instrument](image)

**Figure 106 – Common core components of ITC instruments.**

Having already selected the two amines that would be evaluated using ITC, it was necessary to choose an appropriate ligand. Since the behaviour of the amine functionalised norbornane based CAMPs were evaluated using liposomes doped with LPS from *Pseudomonas aeruginosa*, it was decided that the previously used LPS would be the ligand in the ITC experiments.

Microcalorimetry measurements of the amines either binding or not binding to LPS were performed on a MicroCal VP-ITV isothermal titration calorimeter. A solution of 0.1 mM LPS in 5 mM HEPES buffer (pH 7.03) filled the microcalorimetric cell (volume: 300 μL). Titrations were conducted at 25 °C and
involved 30 × 2 μL injections at intervals of 120 seconds from a 40 μL syringe. The contents of the cell were stirred constantly at 600 RPM.

In each experiment, it was ensured that the systems equilibrate and a stable baseline recorded before titrations commenced. The heat of interaction was recorded by the ITC instrument and plotted versus time. Furthermore, the total heat signal of each injection was calculated as the area under individual peaks and plotted against amine/LPS molar concentration ratio. All ITC experiments were conducted in duplicate.

6.5.2 Results and Discussion

![Structure of amine 134.](image)

Figure 107 – Structure of amine 134.

The first compound subjected to ITC analysis was amine 134 (Figure 107). As a control, the amine solution was titrated into the 5 mM HEPES buffer (pH 7.0). The heats of dilution obtained from the experiment were subtracted from the experimental data of the heats of interaction between amine 134 and LPS. The corrected data was then subjected to nonlinear least square regression analysis (Figure 108). 316
Experiments found that at an amine concentration of 4.4 mM, the binding affinity constant for amine 134 was $8 \times 10^4$ M$^{-1}$ while Polymyxin B (9) exhibits a binding affinity of $5.77 \times 10^6$ M$^{-1}$\textsuperscript{317} The attraction between Polymyxin B and LPS is 100 times stronger than the attraction between amine 134 and LPS. Furthermore, binding stoichiometry (n) was approximately 2:1 which indicated two amine 134 molecules adhering to the LPS molecule (shown in Figure 109). In comparison, the binding stoichiometry of Polymyxin B (9) to LPS is only 1:1.\textsuperscript{318}
The experiment showed that the potential mode of action in the antibacterial activity of amine 134 involved weak interaction with the LPS molecule. The information went towards validating the overall project approach of mimicking CAMPs.

![Figure 110 - Structure of amine 146.](image)

The next amine subjected to ITC analysis was amine 146. Unfortunately ITC experiments indicated there was little or no interaction between LPS and amine 146.

![Figure 111 - Isothermal calorimetric titration of LPS from Pseudomonas aeruginosa (0.1 mM) with amine 146 (5.2 mM).](image)

Both amine 134 and 146 exhibited antibacterial activity in the disk diffusion assays. However, according to ITC analysis, the mode of action of amine 134
involves an interaction with LPS in the outer membrane of Gram-negative bacteria. Amine 146 did not exhibit interaction with LPS indicating that the mode of action responsible for its antibacterial activity must involve other interactions. Due to the structural differences of amine 134 and 146, there is a tentative observation that perhaps both alkyl and aryl constituents are necessary for the interaction with LPS.

6.6 Conclusion

The current chapter sought to evaluate the physicochemical activity of a number of amine functionalised norbornane based CAMP mimics. The CMCs of three amines (141, 142 and 146) were determined. While the CMCs of amine 142 and 141 were approximately 0.1 mg/mL, amine 146 was found to have a CMC of 1.6 mg/mL. The larger CMC value was attributed to the presence of the fluorine atom in the hydrophobic region of amine 146.

Next, DLS experiments were conducted evaluating the behaviours of amine 141, 142 and 146. Unfortunately, only amine 141 exhibited significant interaction with the ‘human’ and ‘bacterial’ liposomes. Amine 141 caused significant
swelling of ‘human’ liposomes at concentrations above CMC. Amine 141 was also responsible altering particle sizes of the ‘bacterial’ liposomes.

Finally, ITC confirmed that amine 134 interacted with LPS in a 2:1 ratio. Interestingly, amine 134 showed no binding affinity to LPS which confirms results from the DLS experiments where there were no interactions of amine 146 with liposomes. Antibacterial activity was still observed of amine 146 in the disk diffusion assays. Hence, the data received in the physicochemical tests indicate while amine 146 may act on the Gram-negative bacterial membrane through interaction with LPS, amine 146 may elicit antibacterial activity through a different mode of action.
Chapter 7
Conclusion and future work

7.1 Thesis conclusion

In conclusion, this thesis details the syntheses of novel norbornane based CAMP mimics. Preliminary attempts at the synthesis of amine functionalised norbornanes lacked robustness and reproducibility. By assembling the common sections of the targets first and then introducing hydrophobic groups, a methodology for the synthesis of amine functionalised norbornane based mimics was developed. The methodology was robust, had high yields at every step and did not require chromatography for purification. The methodology also allowed for the easy synthesis of a range of amine functionalised norbornane based CAMP mimics that varied in the hydrophobic region.

Unfortunately, in the deprotection and salt formation step, the acetal functional group proved to be sensitive to deprotecting conditions. A number of deprotection conditions were trialled and some purification was attempted. Ultimately, although mixtures were obtained, the amine salts were nonetheless determined to be of sufficient purity (8 samples > 85%) to be subjected to further analysis.

The synthesis of the guanidine functionalised norbornanes was accomplished in one step from the previously synthesised amine library. Although three guanylation agents were evaluated, only di-Boc triflylguanidine 149 allowed for the synthesis of the complete guanidine library. Once again, during deprotection and salt formation procedures, the acetal functional group was found to be
susceptible to degradation. In this instance, degradation proved to be more extensive as protected guanidines required longer treatment times. Guanidines salts had purities above 70% and all salt mixtures were sent for disk diffusion assays.

In the last section of the synthetic work for this project, a ‘convergent’ methodology was explored. Unfortunately, the ‘convergent’ methodology proved to be inferior.

A series of compounds were tested for antibacterial activity using disk diffusion assays. Encouragingly, a large number of amines and a few guanidines exhibited antibacterial activity. The disk diffusion assays proved a valuable tool for selecting ‘hits’ to be further evaluated for their physicochemical activity. The candidates were subjected to CMC evaluation, DLS and ITC testing.

Critical Micelle Concentration analysis demonstrated that the amines were capable of forming micelles in water at fairly low concentrations (0.1 – 1.5 mg per mL). Dynamic Light Scattering analysis of ‘human’ and ‘bacterial’ liposomes indicated interactions occurred between the amines and the membranes. Isothermal Titration Calorimetry showed that perhaps some compounds exhibited anti-bacterial activity through interaction with LPS and others did not. The differences in modes of action were attributed to differences in the hydrophobic region of the molecules.
7.2 Future work

There are a number of ways in which the direction of the current project may proceed in the future.

![Figure 113 - Structure of amine 139.](image)

The first would be to explore a different link between the hydrophobic region and the norbornane scaffold (Figure 113). Since the acetal proved to be sensitive to Boc deprotection reaction conditions, other potential linkages should be explored, preferably a linkage not sensitive to acidic media and would therefore not suffer during the Boc deprotecting reaction conditions (Figure 114a).

![Figure 114 - (a) An alternative linkage to the acetal functional group. (b) Greater divide between aromatic functionality and the acetal.](image)

Degradation only occurred for aryl acetals so if there was a greater separation between the aromatic functionality and the acetal, and then compounds would not suffer degradation (Figure 114b).

A future direction of this project would be to subject the hydrophilic region to greater variation. One attempt may be to functionalise the guanidine functional group (Figure 115). The group of Ōmura et. al. discovered that methylated guanidines exhibited greater antibacterial or antifungal activity than non-
methylated guanidines.\textsuperscript{319, 320} Hence it would be interesting to see the influence that functionalisation of the guanidine group may have on the overall antibacterial activity of the substrate.

![Structure of future guanidine analogues.](image)

Figure 115 - Structure of future guanidine analogues.

Finally it may be interesting to pursue more studies to irrefutably establish the mode of action. More extensive DLS and ITC testing may aid in elucidation of potential modes of action.
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