SYNTHESIS OF LIPOAMINO ACID – BASED GLYCOLIPIDS FOR DRUG DELIVERY

by

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Abstract

Many drug molecules are too hydrophilic to cross biological membranes and suffer from chemical and/or enzymatic degradation within the gastrointestinal tract. One approach to this problem is to conjugate such drugs to molecules that optimise their physicochemical properties. Conjugation of lipoamino acids to poorly absorbed peptides and drugs improves oral uptake due to enhanced membrane-like character and through increased protection from enzymatic degradation. The solubility of the resulting conjugates is often poor, however, in aqueous systems.

This approach has been extended to incorporate a hydrophilic component, i.e. a glycosyl moiety, in an effort to improve solubility in aqueous media. In addition, the potential exists with such derivatives to exploit active transport systems, such as the sodium-dependent glucose transporter.

A series of novel lipoamino acid-based glycolipids was synthesised, with functional groups suitable for both covalent and non-covalent conjugation to poorly absorbed drugs. The physicochemical properties of these compounds were varied through modifications to the sugar and lipid components and to the linkage between them (the glycosidic linkage).

A series of N-linked glycolipids was synthesised from β-glycosylamines, from glycosyl azides (using a modified Staudinger reaction) and from glycosyl isothiocyanates. A series of S-linked glycolipids, with increased enzymatic stability, was synthesised using a set of novel Mitsunobu reaction conditions, following difficulties experienced (including low reactivity and disulphide formation) using other methods. Chemically and biologically stable C-linked glycolipids were prepared using a glycosyl radical-based reaction. In addition, novel C-linked glycolipids were successfully prepared directly from their S-linked isosteres using a Ramberg-Bäcklund rearrangement.

Preliminary experiments demonstrated improved oral absorption of piperacillin, a poorly absorbed β-lactam antibacterial, when administered as a novel drug-glycolipid ionic complex. In addition, the ability to form particulate systems per se and in conjunction with conventional liposomes was demonstrated by these glycolipids, with further potential applications for drug and peptide delivery.
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Results & Discussion

experiments\textsuperscript{208}, an alternative to the 2D COSY experiments described earlier (see section 4.4).

An NMR spectrum extract for compound 110 is shown in figure 4.88.

![NMR spectrum extract for compound 110](image)

**Figure 4.88**: NMR spectrum extract for compound 110

This extract of the spectrum shows the 7 sugar protons, the amine proton and the $\alpha$-carbon (with 2 signals due to the diastereomers). We could broadly deduce that the signals at 5.16, 5.04 and 4.84 corresponded to a combination of H-2, H-3 and H-4. The signal at 4.47 was due to the amine and the multiplet signal at 4.20 corresponded to the two H-6 protons. H-1, was expected at approx 3.5 – 4.

As an example of the experiments\textsuperscript{6} carried out, decoupling the signal at 4.84 affected the signal at 5.16 and the signal at 3.53 (figure 4.89 - before decoupling, and figure 4.90 – after decoupling). It appears that the signals at 5.16 and 3.53 have essentially each changed from triplets to doublets. Since we suspected that 4.84 would be either H-2, H-3 or H-4 and we expected H-1 to appear at 3.5 – 4, we could deduce that the decoupled proton was H-2 and that the affected signal at 5.16 was H-3 and that at 3.53 was H-1.

Similar experiments enabled the complete structure to be assigned (see Experimental, section 6). In addition, the anomic configuration was established as being 1,2-trans (i.e. $\beta$-linked).

\textsuperscript{6}The NMR decoupling experiments for compound 110 were performed by Dr. Istvan Jablonkai, Chemical Research Center, Hungarian Academy of Sciences.
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<td>N-1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl</td>
</tr>
<tr>
<td>DEAD</td>
<td>diethyl azodicarboxylate</td>
</tr>
<tr>
<td>DiEA</td>
<td>N,N-diisopropylethylamine</td>
</tr>
<tr>
<td>DMAP</td>
<td>4-dimethylaminopyridine</td>
</tr>
<tr>
<td>DMF</td>
<td>dimethylformamide</td>
</tr>
<tr>
<td>DMPC</td>
<td>dimyristol lecithin</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethyl sulphoxide</td>
</tr>
<tr>
<td>DMTST</td>
<td>dimethyl(methylthio)sulphonium trifluoromethanesulphonate (triflate)</td>
</tr>
<tr>
<td>EDAC</td>
<td>1-(3-dimethylaminopropyl)-3-ethylcarbodiimide</td>
</tr>
<tr>
<td>EEDQ</td>
<td>2-ethoxy-N-(ethoxycarbonyl)-1,2-dihydroquinoline</td>
</tr>
<tr>
<td>ESI</td>
<td>electrospray ionisation</td>
</tr>
<tr>
<td>FAB</td>
<td>fast atom bombardment</td>
</tr>
<tr>
<td>Fmoc</td>
<td>9-fluorenylmethoxycarbonyl</td>
</tr>
<tr>
<td>G.I.</td>
<td>gastrointestinal</td>
</tr>
<tr>
<td>hGH</td>
<td>human growth hormone</td>
</tr>
<tr>
<td>HOBt</td>
<td>hydroxybenzotriazoly</td>
</tr>
<tr>
<td>IR</td>
<td>infra red</td>
</tr>
<tr>
<td>LAA</td>
<td>lipoamino acid (2-aminoalkanoic acid)</td>
</tr>
<tr>
<td>LHRH</td>
<td>luteinising hormone-releasing hormone</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>Log P</td>
<td>apparent partition coefficient between octanol and water</td>
</tr>
<tr>
<td>MALDI</td>
<td>matrix-assisted laser desorption ionisation</td>
</tr>
<tr>
<td>mCPBA</td>
<td>3-chloroperoxybenzoic acid</td>
</tr>
<tr>
<td>Me</td>
<td>methyl</td>
</tr>
<tr>
<td>MeOH</td>
<td>methanol</td>
</tr>
<tr>
<td>Mes</td>
<td>methanesulphonate</td>
</tr>
<tr>
<td>MIC</td>
<td>minimum inhibitory concentration</td>
</tr>
<tr>
<td>MNOBA</td>
<td>3-nitrobenzyl alcohol</td>
</tr>
<tr>
<td>MS</td>
<td>mass spectrometry</td>
</tr>
<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
</tr>
<tr>
<td>PCC</td>
<td>pyridinium chlorochromate</td>
</tr>
<tr>
<td>PepT1</td>
<td>H⁺-dependent peptide co-transporter</td>
</tr>
<tr>
<td>Pfp</td>
<td>pentafluorophenyl</td>
</tr>
<tr>
<td>P-gp</td>
<td>P-glycoprotein</td>
</tr>
<tr>
<td>Ph</td>
<td>phenyl</td>
</tr>
<tr>
<td>Phe</td>
<td>phenylalanine</td>
</tr>
<tr>
<td>Phth</td>
<td>phthalimido</td>
</tr>
<tr>
<td>Pro</td>
<td>proline</td>
</tr>
<tr>
<td>PTC</td>
<td>phase-transfer catalysis</td>
</tr>
<tr>
<td>PyBOP</td>
<td>benzotriazol-1-yloxytris(pyrrolidino)phosphonium hexafluorophosphate</td>
</tr>
<tr>
<td>R₁</td>
<td>retention time</td>
</tr>
<tr>
<td>SGLT-1</td>
<td>Na⁺-dependent glucose transporter</td>
</tr>
<tr>
<td>TBDMS</td>
<td>tert-butyldimethylsilyl</td>
</tr>
<tr>
<td>TCP</td>
<td>tetrachlorophthalimido</td>
</tr>
<tr>
<td>TEM</td>
<td>transmission electron microscopy</td>
</tr>
<tr>
<td>TFA</td>
<td>trifluoroacetic acid</td>
</tr>
<tr>
<td>THF</td>
<td>tetrahydrofuran</td>
</tr>
<tr>
<td>TMSOTf</td>
<td>trimethylsilyl trifluoromethanesulphonate (triflate)</td>
</tr>
<tr>
<td>TOF</td>
<td>time of flight</td>
</tr>
<tr>
<td>Tos</td>
<td>p-toluenesulphonyl</td>
</tr>
<tr>
<td>TRH</td>
<td>thyrotrophin-releasing hormone</td>
</tr>
<tr>
<td>troc</td>
<td>trichloroethoxycarbonyl</td>
</tr>
<tr>
<td>Val</td>
<td>valine</td>
</tr>
</tbody>
</table>
1 Introduction

The successful development of any medicinal compound relies on specific and potent pharmacological activity combined with efficient delivery of the molecule to its target site. Many potential drugs and medicinal peptides fail to reach the marketplace due to poor bioavailability and thus their potential lies in the ability of pharmaceutical scientists to design effective and stable drug delivery systems.

Oral absorption, the most important and preferred route of administration for small molecular weight drugs, presents a major challenge since the drug moiety must first traverse the epithelial cells of the gut in order to enter the circulation. The anatomy and physiology of the gastrointestinal tract are as complex as the many transport processes governing the absorption across this biological barrier, and our understanding of these processes is far from complete. Indeed, it is generally accepted that it is difficult to predict from chemical structure alone whether a compound will be sufficiently bioavailable to be clinically useful.

Most drugs are transported via transcellular diffusion (passive diffusion), but others are taken up by paracellular diffusion or carrier-mediated transport. One approach to oral drug delivery is therefore to adapt the structures of drug molecules to enhance transport via these routes. A compromise between introducing and modifying structural features of a drug moiety that optimise biopharmaceutical properties (e.g. membrane permeability and metabolism) and those that optimise pharmacological activity (e.g. enzyme or receptor binding) must be sought. An understanding of the processes and mechanisms of absorption from the G.I. tract therefore seems essential for the medicinal chemist.

The following section (section 2) will begin by briefly outlining the biological barriers to absorption via the oral route and the processes of membrane transport. Some strategies to improve the oral uptake of drugs and peptides will be considered, before introducing the chemistry of the thesis (section 3).
2 Biology

2.1 Gastrointestinal Barriers to Drug Absorption

The gastrointestinal tract (G.I. tract) is a highly efficient tissue for processing ingested material essential for life while excluding many harmful substances from entering the circulation. It is a complex system of barriers that inhibit the oral delivery of many drugs and peptides. Drugs may be subjected to hydrolysis in the acidic environment of the stomach and degradation by proteolytic enzymes throughout the G.I. tract. In addition, the physicochemical properties of the drug may lead to poor solubility in physiological conditions and a lack of penetration through the epithelial cells that line the gut. Each of these factors contributes to the failure to deliver many drugs via the oral route. It is not surprising, therefore, that many drugs, particularly peptide drugs, must be administered parenterally.

2.1.1 The Epithelial Barrier

The epithelium of the G.I. tract is a natural barrier to unwanted substances, including many drugs and peptides. Its major functions include protection from the external environment, digestion, selective absorption and the secretion of water and electrolytes. 

Figure 2.1: The gastrointestinal mucosa
The epithelium itself is a single cell layer (figure 2.1). Beneath the epithelium is the lamina propria, which in turn is supported by a layer of smooth muscle. These three layers comprise the intestinal mucosa.

The surface area of the epithelium is increased dramatically by the formation of finger-like projections known as villi. Microvilli, the cells responsible for the absorption of electrolytes and nutrients, are uniform cells expressed on the surface of the villus forming a layer known as the “brush border.” It is here that the digestive enzymes are located.

Adjacent epithelial cells are adjoined by means of a three-component structural complex, known as a tight junction, which contains a network of interconnecting strands. Within these strands are pores large enough to allow the passage of certain molecules (considered later).

The apical cell membrane of a columnar absorption cell behaves like a “lipoidal sieve” with respect to drug absorption. Thus the barrier allows the transport of lipid-soluble drugs in preference to non-lipid soluble drugs. The majority of drugs appear to cross the epithelium by passive diffusion.

2.1.2 The Enzymatic Barrier

Peptide drugs are subject to extensive degradation in the G.I. tract and it is for this reason that they are generally unsuitable for oral delivery. This is exemplified by insulin, which despite having been discovered decades ago still has to be administered by injection. Multiple enzymes mediate the degradation of peptides and proteins and the mechanisms are complex.

Proteases are ubiquitous and abundant along the G.I. tract. Destruction begins in the stomach, principally by pepsins. In the intestine, trypsin, chymotrypsin and carboxypeptidases dominate. Degradation also occurs at the brush border, mainly by aminopeptidases. Some peptides are extensively broken down inside the epithelial cells of the gut. Experiments have, for example, demonstrated that insulin is stable for 30 minutes in mucosal fluid of everted rat gut sacs (90-95% intact) but is completely destroyed in rat cell homogenates within 30 minutes.
2.2 Modes of absorption via the Oral Route

Multiple transport systems are involved in drug absorption from the G.I. tract. The structure of the intestinal mucosa restricts absorption to either the *transcellular* or *paracellular* routes (Figure 2.2).

![Figure 2.2: Routes of absorption across the G.I. mucosa; (a) paracellular and (b) transcellular](image)

The structure-transport relationships are different for molecules traversing the epithelium by different routes. It is therefore useful to establish the primary mechanisms of absorption by which a drug crosses the barrier before reaching any conclusions about structure-transport relationships and before making any decisions as to how to enhance the oral uptake of such compounds. Figure 2.3 shows the four major transcellular pathways for absorption.

![Figure 2.3: Transcellular pathways for absorption; (a) passive diffusion, (b) carrier-mediated transport, (c) endocytosis and (d) polarised efflux](image)

[D = drug molecule]
2.2.1 Passive Diffusion

2.2.1.1 Transcellular pathway

Most drugs are absorbed from the G.I. tract by passive diffusion via the transcellular route (figure 2.3). In this process, the apical membrane plays a passive role and the rate and extent of transport are dependent on the physicochemical properties of the drug, the nature of the membrane and the concentration gradient across the membrane.

Chemically, the epithelial cell membrane represents a very complex environment consisting of a lipid bilayer matrix with a variety of proteins (both integral and peripheral). These proteins are important for both structural properties as well as for transportation. The hydrophobic lipids influence the fluidity of the cell membrane and anchor the protein structure to the core of the membrane.

The ability of a drug molecule to permeate the epithelium is dependent largely on its capability to partition into the cell membrane. Two major factors seem to determine the degree of absorption via this route: molecular size and lipophilicity. In addition, conformation may be important in the case of larger peptide and protein molecules.

Translocation via the transcellular pathway involves movement of the solute across the apical membrane, through the cytoplasm of the cell and across the basolateral membrane, passively down a concentration gradient.

2.2.1.2 Paracellular pathway

The paracellular pathway is an aqueous, extracellular route across the epithelium (figure 2.2). Translocation by passive diffusion is driven by electrochemical potential gradients originating from concentration differences, electric potential and hydrostatic pressure between the two sides of the epithelium. The main barriers to passage by this route are the tight junctions between the cells.

It is generally accepted that size and charge are crucial characteristics for absorption via this pathway and since this is an aqueous, extracellular route, sufficient hydrophilicity would seem to be the most important prerequisite.
Increased lipophilicity increases the likelihood that the drug will interact with the apical cell membrane, which is the first step for transcellular absorption.

### 2.2.2 Carrier-mediated transport

It has been shown recently that there are numerous carriers in the intestinal epithelium that facilitate the absorption of important molecules. These transporters, which may be enzymes or some other membrane components, represent a significant mechanism of absorption and are a potential target for drug delivery (discussed later).

Carrier-mediated transport (figure 2.3) can be down a concentration gradient (facilitated transport) or more commonly against an electrochemical or concentration gradient (active transport) and may be energy dependent being coupled to Na\(^+\)/H\(^+\) exchange pumps.

Glucose is absorbed by hexose transporters present throughout the G.I. tract, including the Na\(^+\)-dependent glucose transporter, referred to as SGLT-1, found in the apical membranes of epithelial cells. Many nutrients are absorbed by carrier-mediated transport; carriers have also been identified for amino acids, bile acids and monocarboxylic acids.

Certain peptides are transported across the epithelium by the H\(^+\)-dependent peptide co-transporter, known as PepT1. In this system, a proton gradient and membrane potential provide the driving force for peptide uptake via transporters situated primarily on apical membranes. Experiments have revealed that peptides with two or three amino acids are preferred substrates.

Kramer and colleagues suggested an essential substrate structure for the system (figure 2.4). The importance of R\(_1\) and R\(_3\) are unclear, but it has been determined that R\(_3\) should be H, CH\(_3\) or some other electrically neutral group. $X$

$$
\begin{align*}
\text{Figure 2.4: Hypothetical substrate structure for PepT1 transporter}
\end{align*}
$$
should be a group capable of accepting electrons (e.g. NH$_2$ or SH) and $n$ should be either 0 or 1.

Many orally active peptide drugs share structural features with physiological substrates for PepT1. The system accepts these drug molecules as substrates and acts as a carrier for their effective absorption. The β-lactam antibiotics are one such example.$^{12}$

2.2.2.1 Substrates for the intestinal peptide transporter

The β-lactams are a class of pharmacologically important antibacterials that include the penicillins and cephalosporins. These compounds are tripeptide mimetics that contain a β-lactam ring, two peptide bonds and a free carboxylic acid group (figure 2.5). Many β-lactams are substrates for PepT1.$^{12}$

![General structure of (a) penicillins and (b) cephalosporins](image)

**Figure 2.5:** General structure of (a) penicillins and (b) cephalosporins

These antibiotics are hydrophilic weak acids and are generally ionised in physiological conditions. Absorption is generally much higher than would be predicted on the basis of chemical and physicochemical properties, suggesting the possibility of a carrier mechanism, now known to be PepT1.

The transporter has also been shown to mediate the uptake of angiotensin converting enzyme (ACE) inhibitors$^{13}$ and renin inhibitors$^{14}$.

The "peptide" nature of these substrates for PepT1 is revealed by a comparison of their structures to di- or tripeptides. For example, cephalexin (a cephalosporin antibiotic) resembles the structure of D-Phe-Cys-Val and enalapril (an ACE inhibitor) resembles Phe-Ala-Pro (figure 2.6)$^9$. 
2.2.3 Ion pair transport

Ion pair transport\(^{15}\) has been proposed to explain the unexpected absorption of highly hydrophilic drugs such as the tetracyclines, which are charged over the range of physiological conditions and are generally lipid insoluble. The interaction of such drugs with endogenous counter-ions in effect “buries” the charge within the ion pair, forming a neutral species, which may be able to traverse the epithelium. The introduction of organic counter-ions to modify the physicochemical properties of drugs is an approach that will be discussed later.

2.2.4 Endocytosis

Many proteins and larger peptides have been shown to enter the circulation by endocytosis\(^1\). There are essentially two types of endocytosis – pinocytosis (non-specific endocytosis) and absorptive endocytosis (specific endocytosis). Pinocytosis (fluid-phase endocytosis) involves engulfing the dissolved molecule and the surrounding extracellular fluid to form a vacuole that is internalised. Absorptive endocytosis involves the binding of the molecule to the surface of the cell membrane, possibly to a receptor, followed by internalisation. Once internalised, the molecules
can diffuse to the basolateral membrane and be released intact \textit{(transcytosis)}\textsuperscript{16} or transported to lysosomes, rich in hydrolases, where they are degraded.

\textbf{2.2.5 Efflux systems}

Polarised efflux systems are present in the G.I. tract and are able to excrete a variety of drugs and peptides against a concentration gradient into the intestinal lumen\textsuperscript{17}. Recent results gained from the study of multi-drug resistance associated with cancer chemotherapy have led to insights into this mechanism of intestinal elimination. Multi-drug resistance\textsuperscript{18,19} is thought to be due to the increased expression of a membrane glycoprotein termed \textit{P-glycoprotein} (P for permeability). P-glycoprotein (P-gp) is a product of the human MDR1 gene\textsuperscript{20} and is found not only in tumour cells but is also abundant in intestinal and colonic epithelial cells\textsuperscript{21}.

After uptake into the cell, a portion of the drug substrate is returned to the lumen by the polarised efflux pathway (figure 2.3), thereby impeding absorption into the circulation. Drugs such as digoxin\textsuperscript{22} and quinidine\textsuperscript{23} have been shown to be affected in this manner. These systems show broad substrate specificities but lipophilicity is a requirement. However, highly hydrophobic drugs that are sparingly soluble in water are not substrates, suggesting that a degree of water solubility is necessary for interaction\textsuperscript{4}.

\textbf{2.3 Drug Delivery via the Oral route}

Poor oral absorption presents a significant barrier to the clinical success of many drugs and peptides. Drug delivery strategies seek to overcome those physical and chemical properties responsible for this poor bioavailability, including molecular size, charge, hydrophilicity, hydrogen bonding potential and enzymatic lability. Indeed, there are only a few reliable examples of therapeutic levels being achieved via the oral route for peptides and proteins.

The formulation scientist has focused mainly on improving oral absorption by the use of penetration enhancers\textsuperscript{24} that alter membrane permeability non-specifically or by incorporating drug molecules into drug delivery systems such as liposomes, microparticles and microemulsion systems\textsuperscript{1} that protect the drug from the environment.
The medicinal chemist has focused on modification of the drug molecule itself, either by functional group modification or by attachment of building blocks designed to impart the desired physicochemical properties.

These strategies may be reversible or irreversible. Reversible approaches (including pro-drug formation) are designed to enhance stability, alter solubility or improve bioavailability to facilitate membrane transport. These changes are temporary, however, with the drug being presented in its parent form at the target site. Irreversible approaches give rise to new chemical entities, designed with the same aim. However, the major disadvantage of irreversible approaches is the potential for altered pharmacological activity and increased toxicity resulting from such modifications.

Traditionally, lipophilicity, expressed as the logarithm of the partition coefficient (log P) between octanol and water, has been viewed as the single most important factor in membrane transport, given that the epithelial membrane is considered a "lipophilic" barrier.

2.3.1 Lipophilicity

It is believed that the more lipophilic the molecule, the faster and more completely a drug molecule crosses the intestinal barrier. This is demonstrated by Martin, who reviewed more than 100 compounds with log P<3 and found a positive correlation between intestinal transport and log P. There is a danger, however, of making a drug too lipophilic for epithelial transport. Results suggest that there is an "optimal" lipophilicity for absorption. Highly lipophilic drugs suffer from poor aqueous solubility, also necessary for successful oral uptake.

2.3.1.1 Pro-Drugs: Esterification

Pro-drug strategies consist of a transient modification of the physicochemical properties of a given drug through chemical modification. The aim is to improve bioavailability while the inherent pharmacological activity of the parent drug remains intact. A prerequisite for success by this approach is the reliable conversion of the pro-drug to the parent drug (enzymatically or otherwise) once the barrier to delivery has been overcome. Lipophilicity can be increased by simple covalent modification.
of the functional groups of a drug molecule. Frequently, ester pro-drugs are synthesised to mask the negative charge of a carboxylic acid and to increase hydrophobicity. The β-lactam antibiotics (figure 2.5) are a good example of this approach.

Esters have been developed to improve the absorption of carbenicillin\textsuperscript{27}. Alkyl (R=CH\textsubscript{3}, R=CH\textsubscript{2}CH\textsubscript{3}, figure 2.7) and aryl esters (R=benzyl, figure 2.7) are probably hydrolysed \textit{in vivo}, furnishing the parent carbenicillin (R=H, figure 2.7). The esters themselves have also been shown to exhibit antimicrobial activity\textsuperscript{27}.

![Figure 2.7: Pro-Drugs of Carbenicillin](image)

\textbf{Figure 2.7: Pro-Drugs of Carbenicillin}

It was recognised that some simple esters were not sufficiently labile \textit{in vivo}, as was the case with penicillin esters. It was demonstrated that a double ester

![Figure 2.8: Hydrolysis of Double-Ester Pro-Drugs of Ampicillin](image)

\textbf{Figure 2.8: Hydrolysis of Double-Ester Pro-Drugs of Ampicillin}
Introduction: Biology

(acyloxyethyl ester) of benzylpenicillin was hydrolysed satisfactorily in the blood\textsuperscript{28}. Successful double-esters of ampicillin include pivampicillin\textsuperscript{28} (R=pivaloyl, figure 2.8) and bacampicillin\textsuperscript{29} (R=phthalidyl, figure 2.8). The relatively sterically unhindered double esters are susceptible to enzymatic hydrolysis of the terminal ester bond with the formation of an unstable hydroxymethyl ester. This then spontaneously dissociates to give the parent penicillin and methanal.

An alternative to simple modification of functional groups is the covalent conjugation of lipophilic building blocks, such as fatty acids, long-chain alcohols or the so-called lipoamino acids\textsuperscript{30}.

2.3.1.2 Lipoderivatisation by lipoamino acids

The lipoamino acids (LAAs) are \( \alpha \)-amino acids with long alkyl side chains\textsuperscript{30} (figure 2.9). They combine the structural properties of lipids (highly hydrophobic side chains) with those of amino acids (polar amino and carboxyl functionalities). Introduction of lipophilicity to a drug molecule by incorporation of these amphipathic molecules represents a novel approach to enhancing drug absorption\textsuperscript{31}.

\[
\begin{array}{c}
\text{H}_2\text{N} - \text{CH} - \text{COOH} \\
\left(\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\right)_n
\end{array}
\]

Figure 2.9: A Lipoamino acid

Their bi-functionality also makes LAAs suitable candidates to be chemically conjugated to drugs with a wide range of functional groups and ideal molecules to incorporate into peptide structures. The degree of lipophilicity can be tailored to a particular drug by varying the number of LAAs and the length of the alkyl side chain(s). Conjugation of one or more LAAs to a molecule will enhance its membrane-like character and may confer a degree of protection from enzymatic degradation.
LAA derivatisation has proved to be a successful strategy to enhance the oral absorption of a wide range of therapeutic agents, for example benzoquinolizines, morphine and β-lactam antibacterials.

Conjugation to anti-inflammatory benzoquinolizines increased uptake considerably in vitro (across Caco-2 cell monolayers) and in vivo, without loss of pharmacological activity. Amide conjugates of β-lactam antibiotics were found to be active in vitro but inactive in vivo, probably due to the stability of the amide bond. Conversely, acylated (double ester) derivatives proved inactive in vitro but active in vivo, most likely due to the susceptibility of the ester bond to degradation by esterase enzymes yielding the parent penicillin.

LAAs have also proved effective in enhancing the absorption of therapeutic peptides and are convenient building blocks for incorporation into standard solid phase synthetic protocols. Both the tri-peptide thyrotrophin-releasing hormone (TRH) and the decapeptide luteinising hormone-releasing hormone (LHRH) are not absorbed via the oral route. Conjugation of LAAs to these peptides increased their oral uptake markedly. A significant increase in resistance to enzymatic degradation was also demonstrated in vitro.

One disadvantage of the LAA approach is that while lipophilicity is increased, solubility in aqueous media may be reduced. The introduction of a hydrophilic moiety, such as a sugar, could serve to compensate for this deficiency. Experiments have demonstrated, for example, that conjugation of a protected glucuronic acid sugar unit to a LAA-derivatised cephalosporin antibiotic led to enhanced solubility when compared to the LAA-derivatised parent. Uptake studies confirmed that oral absorption was considerably greater than the underivatised cephalosporin.

These sugar-LAA conjugates (glycolipids) are novel compounds suitable to enhance the oral absorption of poorly absorbed drugs and peptides.

2.3.2 Other factors important for effective oral absorption

The theory that increasing the lipophilicity of a drug will increase its oral absorption is generally accepted. However this does not always prove to be the case. Haberlin and co-workers, for example, demonstrated that a glycoside of...
dexamethasone (log D$_{7.4} = -1.59$) had superior permeability in vitro to the sodium salt of dexamethasone (log D$_{7.4} = 0.58$). In addition, Rubas and Cromwell$^{43}$ similarly demonstrated with a series of platelet aggregation inhibitors that lipophilicity is not always a good predictor for intestinal transport.

These observations suggest that other factors may be important in the absorption of drug molecules from the G.I. tract, besides lipophilicity. As indicated earlier, certain drugs are substrates for active transport systems, such as PepT1. In these cases, specific structural features may be more important than overall lipophilicity.

Hydrogen bonding is also a major consideration, particularly with peptide drugs. Lipinski and co-workers$^{44}$ emphasised its importance when formulating a prediction system for drug absorption. After analysing the physicochemical characteristics of 2245 drug molecules in current clinical use, they derived a set of parameters necessary for successful drug absorption. It is suggested that absorption difficulties will be experienced if one or more of these criteria are violated. The "Rule of Five" (so-named since each parameter is a multiple of 5) is set out below:

1. Molecular weight: less than 500 g mol$^{-1}$.
2. Lipophilicity expressed as log P: less than 5.
3. Number of hydrogen bond donors: less than 5.
4. Number of hydrogen bond acceptors: less than 10.

The number of OH and NH groups present in the molecule determines the number of hydrogen bond donors. Likewise, the number of N and O atoms determines the number of hydrogen bond acceptors. Therapeutic peptides commonly violate at least three of the criteria.

Covalent lipoderivatisation increases the membrane-like character of drugs and peptides and increases their uptake across the intestinal epithelium. The potential exists, however, for the pharmacological activity of the parent molecule to be affected in some way. In addition, the toxicity profile of the new conjugate needs to be considered. An ideal situation would be to introduce lipophilicity without the need for covalent modification of the parent drug.

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$^*$ Log D$_{7.4}$ = Log of the partition coefficient between octanol and aqueous buffer (pH 7.4).
2.3.3 Non-covalent carrier mediated lipophilicity

Most oral absorption enhancement approaches are aimed at causing non-specific disruption of the cell membrane or intercellular junctions\textsuperscript{45} and therefore, when used in large quantities, can cause cell damage.

It has been demonstrated that certain carrier molecules, such as those in figure 2.10, effectively enhance the absorption of drugs traditionally administered parenterally\textsuperscript{46,47}.

![Figure 2.10: Non-covalent Absorption Enhancers](image)

Leone-Bay and co-workers\textsuperscript{49} first reported a marked increase in the oral absorption of salmon calcitonin (a single-chain polypeptide hormone of 32 amino acid residues that regulates blood calcium levels) and interferon-\(\alpha\) (a lymphokine of 165 amino acid residues with non-specific antiviral activity) and suggested that a mechanism other than general penetration enhancement or protease inhibition was responsible.

Further experiments by Milstein and co-workers\textsuperscript{48} demonstrated similar effects with human growth hormone (hGH) and suggested that the transport process was both passive and transcellular. There was an absence of an observed effect by inhibitors of active transport and a lack of evidence for any effect on tight junctions.

It is known that the partial unfolding of proteins results in the increased solvent exposure of hydrophobic side chains. Experiments have shown these unfolded conformations to move through lipid bilayers by passive diffusion\textsuperscript{49}. It was suggested that the non-covalent interactions between the carrier and drug resulted in the stabilisation of a partially unfolded conformer.

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\textsuperscript{45} Reference to specific research findings or studies.

\textsuperscript{46} Reference to specific research findings or studies.

\textsuperscript{47} Reference to specific research findings or studies.

\textsuperscript{48} Reference to specific research findings or studies.

\textsuperscript{49} Reference to specific research findings or studies.
Wu and Robinson\textsuperscript{50} added support by investigating the role of the P-glycoprotein efflux system (P-gp) in Caco-2 cells. hGH cannot be transported across the intestinal epithelium alone, but in the presence of these enhancers, transport is substantial. It was determined that the hGH-enhancer complex was sufficiently lipophilic to be a substrate for P-gp. Since neither the enhancer molecules nor hGH alone are substrates for P-gp, it was deduced that a lipophilic complex was formed between the enhancer and drug.

Mlynek and co-workers\textsuperscript{51} further demonstrated the absence of any active transport mechanism, that there was no effect on tight junctions and that the intestinal membrane was not affected. It was also noted that for improved absorption, the drug and enhancer must be presented together; pre-treatment with enhancer produced no effect.

The use of enhancer molecules to modify the lipophilicity of drugs is an interesting area of research with considerable potential to deliver peptide drugs.

The presence of ionisable groups on a drug molecule provides an opportunity for ionic interactions with a lipophilic counter-ion. Ion pair transport has proved a successful means by which to deliver certain drugs.

### 2.3.4 Ion pair transport

Hydrophobic ion pairing\textsuperscript{52} represents an inexpensive and reversible means by which to modify the physicochemical properties of a drug without the need for irreversible chemical modification.

An ion pair can be defined as a neutral species formed by electrostatic attraction between oppositely charged ions in solution, which are often sufficiently lipophilic to dissolve in non-aqueous solvents\textsuperscript{53}.

The lipophilicity of hydrophilic ionised drugs can be increased by ion pair formation with lipophilic counter-ions\textsuperscript{52}. Neubert et al.\textsuperscript{52} showed that the log P of hydrophilic drugs buformine, bretylium and pholedrine was markedly increased by lipophilic ions such as hexylsalicylate. In addition, doxorubicin lipophilicity was enhanced by dioctylsulphosuccinate and dodecylsulphate.

Transport of ionised hydrophilic drugs through membranes can be enhanced by the increased lipophilic character that ion pairing provides. Irwin et al.\textsuperscript{54} were the first to test the ion pair hypothesis for lipophilisation of an ionic drug (isopropanide)
using an exogenous counter-ion (trichloroacetate) and demonstrated an increased rate of absorption. Table 2.1\textsuperscript{52} demonstrates how lipophilic counter-ions can influence the transport of drugs \textit{in vitro}. Several \textit{in vitro} membrane systems served as experimental models. The table indicates that significant increases in the rate of transport across biological membranes can be achieved.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Counter-ion</th>
<th>Increase in transport rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metoprolol tartrate</td>
<td>Oleate</td>
<td>4x</td>
</tr>
<tr>
<td>Propranolol hydrochloride</td>
<td>Taurodeoxycholate</td>
<td>5-6x</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>Dioctylsulphosuccinate</td>
<td>11x</td>
</tr>
<tr>
<td></td>
<td>Dodecylsulphate</td>
<td>17x</td>
</tr>
<tr>
<td></td>
<td>Decanesulphonate</td>
<td>11x</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>Dodecylsulphate</td>
<td>15x</td>
</tr>
</tbody>
</table>

Plakogiannis and co-workers\textsuperscript{55} determined that decylsulphate does not affect the disappearance of highly lipophilic drugs from the G.I. tracts of rats. It appears that ion pair formation only affects the partition and transport of hydrophilic drugs that are charged in the media where ion pairing takes place.

Counter-ions previously used for ion pairing include alkylsulphates, trichloroacetate and alkylcarbonates. It has been suggested that these counter-ions are too irritant to the gut at the required dosages\textsuperscript{52}. Counter-ions with the following properties are required: high lipophilicity, sufficient solubility, physiological compatibility and metabolic stability. Examples include alkanoic acids\textsuperscript{56} and alkylated salicylic acids\textsuperscript{52}.

It was first supposed that the two components of an ion pair traverse lipid membranes at an equimolar ratio. However, evidence suggests that the mechanism may be more complex. Neubert et al.\textsuperscript{52} performed experiments based on lipophilic counter-ions for cationic drug transport. It was determined that the counter-ions accumulated in the membrane and that as a result, more hydrophilic drug molecules than counter-ions were transported. Transport of the complete ion pair was also
demonstrated. Hadgraft and co-workers proposed a similar mechanism for the transport of anionic drugs.

The ability to form an ion pair and the success of improving transport by this approach depends very greatly on the physicochemical properties of both the drug and the counter-ion. Although ion pair transport is considered by many to be controversial and in some instances questionable, it should nevertheless be considered a useful method for drug delivery. It would appear that with further understanding of the processes involved, potential exists to transport more complex molecules by this approach, for example peptides and proteins.

The approaches discussed thus far are based on increasing lipophilicity for enhanced transport by passive diffusion via the transcellular pathway. An alternative strategy is to exploit the numerous active transport mechanisms present in the gastrointestinal mucosa.

2.3.5 Exploitation of active transport systems

Strategies have been designed to improve the bioavailability of poorly absorbed drugs and peptides so that they can be absorbed by specialised intestinal transporters.

2.3.5.1 Glucose Transporters

An exciting area of current research involves the elucidation of the structure and function of glucose transporters present throughout the intestinal epithelium. Conjugation of a saccharide moiety to a poorly absorbed drug will improve its solubility in aqueous media due to the poly-hydroxyl nature of sugars. In addition, sugar conjugation may allow passage of the sugar-drug conjugate across the gut via the SGLT-1 glucose transporter.

Nomoto and co-workers demonstrated the effectiveness of this approach by conjugation of a glucose derivative to a tetrapeptide not normally transported by PepT1. Interestingly, the configuration at the anomeric centre of the sugar was found to be significant to the rate of transport. A β-anomeric linkage was determined to be preferred over the α-configuration. In addition, Mizuma et al. demonstrated the β-anomeric preference of SGLT-1 for the absorption of simple naphthyl glycosides.
Subsequently, further evidence was presented for glycosides of paracetamol\textsuperscript{62}. Glucose conjugates were transported more efficiently than galactose conjugates with the $\beta$-\textit{trans}-anomeric configuration preferred in both cases. Galactose conjugates with $\alpha$-\textit{cis}-configuration were not transported at all.

Experiments have also shown that glycosylation can confer a degree of protection from enzymatic degradation, possibly due to steric effects\textsuperscript{63}. The nature of the glycosidic linkage and the stereochemistry of the anomeric centre are both important in the stability of conjugates and this will be considered later.

2.3.5.2 The P-glycoprotein efflux system

Research in the field of multi-resistant tumour cells\textsuperscript{64} has led to the synthesis of potential P-gp inhibitors to prevent efflux of anti-tumour agents from the cell. Such inhibitors have potential to enhance the oral absorption of drugs that are known substrates for the P-gp system in the G.I. tract.

It is well recognised that some pharmaceutical formulation excipients are inhibitors of P-gp in cancer cells. Surfactants such as polysorbate 80 and Cremophor EL are such inhibitors\textsuperscript{65} and have the potential to function similarly by inhibiting efflux in the G.I. tract.

Nerurkar and co-workers\textsuperscript{66} demonstrated the participation of surfactants in blocking a polarised efflux system in the intestine and in increasing the transport of a peptide that is a known substrate for the efflux pump.

2.4 Summary

Any drug delivery system is optimally designed to improve the oral absorption of a drug while not adversely affecting the pharmacological activity of the compound. It should (where relevant) improve the physicochemical properties of the drug (i.e. lipophilicity) to facilitate its passage across the intestinal membrane by passive diffusion and ideally confer some protection against enzymatic degradation. In addition, some degree of solubility in aqueous media would be an advantage.

Alternatively, the system may modify the drug in such a manner that it may exploit one of the many active transport systems throughout the intestine, e.g. the SGLT-1 and PepT1 transporters.
Ideally, the drug delivery system would allow the option to conjugate covalently (via stable or labile linkages) or non-covalently to a drug molecule to allow the possibility for ion-pair transport. As a result, appropriate functional groups for direct attachment to a drug and for forming ion pairs are a requirement.

The approach adopted with the work described in this thesis has been to synthesise a series of lipoamino acid-based glycolipids, as a potential drug delivery system. These liposaccharides consist of a hydrophilic component (a sugar) attached to a lipophilic component (lipoamino acid) via a glycosidic bond (for example, figure 2.11). They are suitable for direct attachment to poorly absorbed drugs and peptides either by covalent linkage or by ion pair formation.

![Figure 2.11: Example of an O-linked lipoamino acid-based Glycolipid](n = 5, 7, 9, etc.)

The most common linkage between sugar and aglycone is via an oxygen atom (O-glycoside). These compounds are both chemically and enzymatically labile. The synthesis of more stable glycosides linked by other atoms, for example through nitrogen, sulphur or carbon atoms (N, S and C-glycosides respectively) is a major challenge and will be addressed in this thesis.

The physicochemical properties of these glycolipids could be tailored by carrying out modifications to the following:

(a) Saccharide component
   the nature of the sugar, the number of sugar units.

(b) Lipid component
   The length of the lipid chain, the number of lipids, the nature of the lipid (e.g. presence of other functional groups such as OH, NH₂).
(c) Linkage between sugar and lipid

The glycosidic linkage would be important to the chemical and biological stability of the conjugates (the site of enzymatic degradation) and could further influence physicochemical properties.

This section has discussed the physical and biological barriers to successful oral absorption via the oral route. In addition, some strategies to overcome these challenges have been considered. Section 3 will introduce the main established methods that have been reported to synthesise simple O-, N-, S- and C-linked glycosides.
3 Chemistry

This section will introduce some of the established methods to synthesise glycoconjugates, beginning with the preparation of O-linked glycosides. Attention will then be drawn to approaches to the preparation of O-glycoside mimetics, namely N-, S- and C-linked glycoconjugates. Reference will be made throughout to the importance of the ability to produce glycosides stereoselectively.

3.1 O-linked glycosides

The recent renaissance of carbohydrate chemistry has been largely due to the discovery of the importance of glycoconjugates in biological systems, and has led to efforts to synthesise these compounds for biomedical applications. A glycoconjugate can be defined as a carbohydrate (the glycone) joined by an acetal or ketal linkage to an organic moiety (the aglycone) or a second carbohydrate. Glycoconjugate synthesis presents a major challenge to the carbohydrate chemist. Stereocontrol, the ability to synthesise one of the two anomers selectively, remains the biggest hurdle to overcome.

Most O-glycosylations involve the activation of a sugar donor to form an electron-deficient reactive intermediate, followed by nucleophilic attack of the oxygen atom of a second organic residue, the glycosyl acceptor. Deactivation by proton extraction yields the new glycoside (figure 3.1).

![Figure 3.1: Glycosylation reactions](image-url)
The stereochemistry of the glycoside depends on the stereoelectronic properties of the sugar donor, the nature of the promoter and on the reaction solvent. In addition, the reactivity of the hydroxyl acceptor will influence both reaction yield and stereochemistry. Glycosylation reactions are frequently accompanied by side reactions, commonly involving decomposition of the glycosyl donor.

3.1.1 Stereospecificity

3.1.1.1 Neighbouring-group participation

1,2-trans-glycosides are preferentially formed in glycosylation reactions where the protecting group at C-2 on the sugar (the carbon atom adjacent to the anomeric centre) is of the acyl type (figure 3.2).

![Figure 3.2: Neighbouring Group Participation](image)

The 2-O-acyl protecting group acts as an intramolecular nucleophile to give a cyclic intermediate (dioxolenium ion, figure 3.2). Since the neighbouring group blocks one face of the molecule, the acceptor can only attack from the other face, to yield the trans-glycoside. Reactive alcohols often produce a mixture of glycosides since they can undergo a reaction with the intermediate oxocarbenium ion (figure 3.1).

This type of glycosylation is widely exploited to form β-glucosides, β-galactosides and α-mannosides, commonly found in biological systems.
3.1.1.2 Manipulation of the Anomeric Effect

If the glycosyl bond is axial (and the adjacent substituent is equatorial, such as in glucose or galactose) then this is the most thermodynamically stable isomer due to the anomeric effect (section 3.1.1.2.1), i.e. α-glucosides and α-galactosides. The α-glycoside can be formed preferentially by taking advantage of the greater lability of the equatorial leaving group (figure 3.3). Lemieux\textsuperscript{71} developed a halide ion catalysed glycosylation method, in which the tendency of axial glycosyl halides to undergo rapid, partial anomérisation in the presence of tetra-alkylammonium halides is exploited. The protecting group at C-2 of the glycosyl donor must be non-participating (i.e. not of the acyl type) to avoid neighbouring group participation.

![Figure 3.3: Formation of 1,2-cis-glycosides](image)

Although less stable than axial forms, equatorial 1,2-\textit{trans}-glycosyl halides, particularly the less reactive chlorides, can be synthesised and isolated. These compounds, if sufficiently stable, then undergo nucleophilic substitution in the presence of an active catalyst to yield 1,2-cis-glycosides\textsuperscript{72}.

3.1.1.2.1 The Anomeric Effect

Studies on cyclohexane ring systems have revealed that substituents favour an equatorial orientation over an axial one. In the pyranose ring of a sugar, substituents also favour the equatorial position, with one exception. The axial orientation is favoured by electronegative substituents at the anomeric centre (for example, X=halide, O-Alkyl or O-acyl, figure 3.4). This phenomenon, first described by Lemieux\textsuperscript{73}, is thought to result from interactions between the lone pair of the ring oxygen atom and the anti-bonding $\sigma^*$-orbitals of the C-substituent bond\textsuperscript{74}. 
Newman projections along the $C_1-O$ bond (figure 3.4) reveal that in the equatorial position, the substituent (X) is gauche to both ring oxygen electron lobes, whereas it is gauche to only one lobe in the axial orientation. This effect, known as the "anomeric effect", increases with increasing electronegativity and decreases in solvents of increasing polarity.

![Figure 3.4: The anomeric effect](image)

3.1.2 $O$-glycosylation reactions

3.1.2.1 Koenigs-Knorr Glycosylation

First reported in 1901, the Koenigs-Knorr reaction (figure 3.5) involved the conversion of acetylated glycosyl chlorides or bromides into glycosides, in the presence of insoluble silver catalysts (e.g. silver carbonate).

![Figure 3.5: The Koenigs-Knorr Reaction](image)

$x = Cl, Br$
The reaction has been continuously improved to become an effective method, employing more efficient catalysts\(^7^7\) and the use of drying agents to remove water formed in the reaction\(^7^8\). Poor yields are often experienced due to the instability and sensitivity to moisture of the glycosyl halides and due to poorly reactive hydroxyl acceptors (e.g. secondary alcohols). The reactivity of the glycosyl donor can be enhanced by protecting group manipulation (particularly at C-2) and by using the more reactive glycosyl bromide in place of the chloride. Stereoselectivity can be achieved by utilising neighbouring group participation (section 3.1.1.1) to form 1,2-trans(β)-glycosides, by in-situ anomérisation (section 3.1.1.2) to form 1,2-cis(α)-glycosides and by heterogeneous catalysis to form 1,2-cis(β)-glycosides.

3.1.2.2 Thioglycosides as glycosyl donors

The use of alkyl or aryl thioglycosides, extremely stable starting materials, as glycosyl donors has proved a successful method of glycosylation\(^7^9\). The thioglycosides are activated by thiophilic reagents such as methyl trifluoromethanesulphonate (methyl triflate) and dimethyl(methylthio)sulphonium triflate (DMTST) to produce glycosides in good yield (figure 3.6)\(^8^0\).

![Figure 3.6: Thioglycosides as glycosyl donors](image-url)
Activation of thioglycosides could also be achieved in a two-step conversion by formation of the glycosyl halide \textit{in situ} in the absence of promoter, e.g. treatment of a thioglycoside with bromine gave a glycosyl bromide, which could then be activated. In addition, Kartha and Field activated thioglycosides using iodine monobromide.

3.1.2.3 Trichloroacetimidates as glycosyl donors

Glycosyl trichloroacetimidates, introduced by Schmidt, are extremely versatile donors, allowing reactions at low temperatures and short reaction times. Preparation of these compounds is relatively simple. Selective de-O-acetylation of the sugar to yield the 1-OH compound is followed by direct reaction with trichloroacetonitrile under basic conditions. The use of a weaker base, such as potassium carbonate, yields the \( \beta \)-compound. Stronger bases, such as sodium hydride, lead to formation of the thermodynamically more stable \( \alpha \)-anomer (figure 3.7).

![Figure 3.7: Trichloroacetimidate formation](image)

Glycosylation via this method proceeds smoothly, promoted by catalysts such as boron trifluoride etherate (BF\(_3\)OEt\(_2\)) and trimethylsilyl triflate (TMSOTf).
When the protecting group at C-2 is of the non-participating type (i.e. not acyl), glycosylation can be steered towards either 1,2-trans or 1,2-cis selectivity by careful choice of the donor and the promoter (figure 3.8). Boron trifluoride promoted glycosylation proceeds with inversion, forming the 1,2-cis-glycoside from the 1,2-trans-imidate. Use of TMSOTf leads to the formation of the thermodynamically more stable (α) anomer – and the 1,2-cis-glycosides were obtained from 1,2-cis-imidates under these conditions. Participating protection groups at C-2 lead to the formation of the 1,2-trans-glycosides as described earlier (section 3.1.1.1).

Figure 3.8: Glycosylation with trichloroacetimidates

3.1.2.4 1-O-Acyl sugars as glycosyl donors

The major advantage of the 1-O-acylated sugar as a glycosyl donor is the ease of its preparation. Several Lewis acids have appeared as effective promoters in the glycosylation (figure 3.9), for example SnCl₄, BF₃.OEt₂ and FeCl₃.

Figure 3.9: 1-O-Acyl Glycosyl donors
3.1.2.5 2-amino-2-deoxy sugars as glycosyl donors

2-acetamido-2-deoxy sugars are widespread in nature as components of important molecules in biological systems, such as peptidoglycans and glycoproteins. Stereocontrol in the synthesis of glucosamine derivatives is usually achieved through neighbouring-group participation of the C-2 substituent. For 2-acylamino-2-deoxy-sugars, electrophilic activation by a Lewis acid leads to the formation of a reactive electron-deficient intermediate (as in figure 3.1) which is expected to undergo nucleophilic attack from the β-face to yield exclusively 1,2-trans-glycosides (as in figure 3.2). However, with the 2-amino group acylated, e.g. N-acetyl, the intermediate can be stabilised during the reaction through abstraction of the amide proton to form a relatively stable oxazoline, which may make glycosylation difficult. Glycosylations have been reported using this relatively stable oxazoline intentionally at elevated temperatures. However, its formation and the problems associated with it have led to the search for alternative amine protecting groups. The Chloroacetyl, trichloroacetyl and allyloxycarbonyl (Aloc) protecting groups have all been explored for 2-amino protection.

The formation of this stable oxazoline can be eliminated completely by using

![Diagram of 2-acylamino-2-deoxy glycosyl formation](image)

**Figure 3.10: 2-acylamino-2-deoxy glycosyl formation**
two monovalent protecting groups\textsuperscript{102} or a bivalent protecting group, e.g. \textit{N}-tetrachlorophthaloyl\textsuperscript{103}, \textit{N}-dithiasuccinoyl\textsuperscript{104,105,106} and the recently introduced (by Schmidt and co-workers) \textit{N}-thiodiglycolyl\textsuperscript{107} (figure 3.11). In the bivalent protecting group case, electrophilic activation proceeds as with \textit{N}-acyl type protection (figure 3.10) except that the absence of the proton means that the stable oxazoline cannot be formed.

\begin{center}
\includegraphics[width=\textwidth]{figures/3.11.png}
\end{center}

\textbf{Figure 3.11: Bivalent amine protecting groups}

3.1.3 Stable Mimics of \textit{O}-glycosidic linkages

As increasing numbers of biologically active glycosides have been structurally characterised, carbohydrate chemists have sought to develop new methods for their synthesis. The main types of glycosidic linkage found in natural glycoproteins involve the oxygen in the side chain of either serine or threonine and the nitrogen in the side chain of asparagine\textsuperscript{108}.

However, like proteins, peptides and nucleic acids, carbohydrates are susceptible to biodegradation, thus limiting their therapeutic potential. \textit{O}-glycosides are susceptible to both chemical attack (acid sensitivity) and enzymatic degradation (by glycosidase enzymes). Synthetic chemists have therefore focused on the design of carbohydrate analogues that withstand these degradative forces \textit{in vivo}. 
The major effort has been to synthesise isosteric analogues in which the reactive acetal functionality of the anomeric centre is modified by substitution of the oxygen atom with a sulphur or carbon atom (figure 3.12).

![Figure 3.12: O-glycoside mimetics](image)

Figure 3.12: O-glycoside mimetics

Thioglycosides (S-glycosides) are resistant to the action of glycosidases. They are cleaved by chemical hydrolysis, but at a much slower rate than the corresponding O-glycosides. Carbon-linked glycosides (C-glycosides) are resistant to both chemical and enzymatic hydrolysis of the glycosidic bond, but are synthetically the most challenging to synthesise.

3.2 N-linked Glycosides

N-glycosides are most commonly synthesised from 1-amino sugars. Glycosylamines are commonly prepared from their corresponding reducing sugars by treatment with methanolic ammonia (figure 3.13). Kochetkov obtained β-glycosylamines from their corresponding reducing sugars using a saturated solution of ammonium bicarbonate. 1,2-trans-glycosides are formed due to the weak anomic effect of nitrogen. Unfortunately, the reaction mixtures often contain a mixture of products, which are often difficult to separate by conventional techniques.

![Figure 3.13: Synthesis of 1-amino sugars](image)
This often necessitates acetylation, crystallisation (or column chromatography) followed by de-O-acetylation, thus limiting the scope of this method. The large number of products obtained is due to epimerisations, rearrangements, degradations and intermolecular condensations\textsuperscript{112}.

Synthesis of $N$-linked glycoconjugates by this route is complicated by the high lability of unprotected 1-aminosugars and their tendency to dimerise and to deglycosylate. Other methods have utilised fully-protected sugars and the introduction of an amine functionality via a stable glycosyl azide.

The synthesis of acetylated glycosyl donors with a free anomeric amine is a crucial step in the synthesis of $N$-glycosides. Although additional steps are required, methods using fully protected glycosyl intermediates are well suited for the synthesis of glycosyl amides. Protected glycosylamines are routinely obtained by conversion of the corresponding glycosyl halide to the glycosyl azide using silver azide\textsuperscript{113}, or more commonly sodium azide. This is a high yielding reaction and produces exclusively 1,2-$\text{trans}$($\beta$)-glycopyranosyl azides. Catalytic hydrogenation of the azide using palladium on carbon or Raney nickel yields the desired glycosylamine (figure 3.14).

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure3.14.png}
\caption{Synthesis of glycosylamines}
\end{figure}

The majority of methods used to synthesise acylated glycosylamines comprise condensation of the protected glycosylamine with an acid in the presence of a reactive coupling reagent, for example $N,N'$-dicyclohexylcarbodiimide (DCC) or 2-ethoxy-$N$-(ethoxycarbonyl)-1,2-dihydroquinoline (EEDQ)\textsuperscript{114} (figure 3.15) to form a glycosyl amide (figure 3.17).
Introduction: Chemistry

Figure 3.15: Coupling reagents

Marks and Neuberger\textsuperscript{115} were the first to form an amide bond between a glycosylamine and an amino acid by this method, using a protected glucosylamine and an aspartic acid residue with DCC (\textbf{figure 3.16}). The method has subsequently been used to synthesise many glycopeptides\textsuperscript{116}. EEDQ has the advantage that glycosylated products can occasionally be crystallised without the need for purification by column chromatography, which is not the case with DCC. Yields are comparable, however.

\begin{center}
\includegraphics[width=\textwidth]{figure315.png}
\end{center}

\textbf{Figure 3.16:} Coupling of glucosylamine with aspartic acid

Other methods to form a glycosyl amide include the use of acyl chlorides in the presence of base\textsuperscript{117}, mixed anhydrides\textsuperscript{118} or via active esters (\textbf{figure 3.17})\textsuperscript{119}.

Disadvantages of the use of glycosylamines in N-glycoside synthesis include their instability, sensitivity to moisture and the possibility of acetyl migration from the $O$-acetate group at the $C$-2 position of the sugar to the anomic amine. These
shortcomings have led to the search for alternative methods to synthesise \(N\)-glycosides without the need for glycosylamines.

\[
\begin{align*}
\text{AcO} & \quad \text{O} & \quad \text{NH}_2 \\
\text{RCOOH} & & \xrightarrow{\text{DCC}} & & \text{O} & \quad \text{NH} & \quad \text{R} \\
\text{AcO} & \quad \text{O} & \quad \text{NH}_2 & & \xrightarrow{\text{Base}} & & \text{RCOX}
\end{align*}
\]

Figure 3.17: Routes to Glycosyl amides

3.3 \(S\)-linked glycosides (Thioglycosides)

Recent progress in thioglycoside synthesis has been as a result of special interest in enzyme-inhibition studies\(^{120}\). \(S\)-glycosides are much more stable to the action of glycosidases than \(O\)-glycosides and can thus inhibit enzymatic hydrolysis\(^{109}\). There is also great interest in their use as glycosyl donors\(^{79}\) (see section 3.1.2.2). Thioglycosides can be cleaved by chemical hydrolysis, but at a significantly slower rate than their oxygen-linked isosteres.

The major disadvantage in thioglycoside synthesis and in thiol chemistry in general is the unpleasant odour of many sulphides. However, with well-ventilated fume hoods and appropriate treatment of equipment and residues with oxidising agents (e.g. hypochlorite) odours can easily be eliminated from the laboratory.

A variety of methods have been described for the preparation of alkyl and aryl glycosides. These include:

(i) Acid-promoted displacement at the anomeric centre
(ii) Base-promoted displacement at the anomeric centre
(iii) Anomeric \(S\)-alkylation.
3.3.1 Preparation of thioglycosides

3.3.1.1 Acid promoted displacement at the anomeric centre

This method of preparation involves a fully acetylated glycosyl donor, which is reacted with a thiol in the presence of a Lewis acid, such as boron trifluoride. The examples shown in figure 3.18 show the formation of aryl and alkyl thioglycosides of glucose.

![Figure 3.18: Acid-catalysed S-glycosylation](image)

Eloffson and co-workers demonstrated the success of this procedure in the glycosylation of cysteine to galactose (figure 3.19).

![Figure 3.19: S-Glycoside of cysteine](image)

This method is a very general procedure to synthesise 1,2-trans-thioglycosides due to neighbouring-group participation (see section 3.1.1.1). The reactions are not entirely stereospecific, and minor amounts of 1,2-cis-product are formed. This problem can often be overcome by recrystallisation.
3.3.1.2 Base promoted displacement at the anomeric centre

This procedure involves the reaction of a fully protected glycosyl halide with a thiolate anion. It is the oldest example of thioglycoside formation, first described in 1909 by Fisher and Delbrück\(^\text{123}\). 1,2-\textit{trans}-thioglycosides are generally produced from S\(_2\)2-type substitutions. The best results with this method result from aryl thiolates since they are less basic and do not cause significant de-\(O\)-acylation of the sugar hydroxyl groups (\textbf{figure 3.20})\(^\text{123}\).

\[
\text{PhS}^-\text{Na}^+ \rightarrow \text{AcO} \quad X = \text{halogen}
\]

\textbf{Figure 3.20: Base catalysed \(S\)-glycosylation}

When alkyl thiolates are used, a re-acetylation step is often required before isolating the desired product. Kessler and co-workers\(^\text{124}\) used this procedure to glycosylate cysteine to galactose (\textbf{figure 3.21}).

\[
\text{Boc-Cys-OH} \quad \text{NaH} \quad \text{AcO} \quad \text{AcO} \quad \text{COOH}
\]

\textbf{Figure 3.21: Base promoted glycosylation of cysteine}

3.3.1.3 Anomeric \(S\)-alkylation

This procedure requires the synthesis of a 1-thiosugar, which is subsequently alkylated. An acetylated glycosyl halide is treated with thiourea, producing a pseudothiouronium salt. This salt is then hydrolysed with a weak base to give the 1-thiosugar (\textbf{figure 3.22})\(^\text{125}\).
Alternative methods to produce 1-thiosugars include the reaction of glycosyl halides with thioacetate anions followed by selective de-S-acetylation of the formed anomeric thioacetate\textsuperscript{126} and the reaction of glycosyl halides with xanthate anions\textsuperscript{127,128}, followed by treatment with cold methanolic sodium methoxide.

Once the 1-thiosugar is prepared, it is alkylated with an alkyl halide (often \textit{in situ})\textsuperscript{81}. Despite the greater number of reaction steps in this method, the intermediates are often crystalline and the yields are high throughout (figure 3.23).

3.4 C-linked glycosides

Carbon-linked glycosides are extremely stable\textsuperscript{129}. They are resistant to both chemical and enzymatic degradation, making them compounds of significant importance to the carbohydrate chemist. They are also the most difficult glycosides to synthesise and present a significant challenge.

Interest in C-glycosides was first generated in the 1970s with the discovery of C-linked nucleoside antibiotics, such as formycin and showdomycin\textsuperscript{130}. Their use as probes to understand the mechanisms of biological processes is widespread.
As with other glycosides, the two main concerns in C-glycoside synthesis are stereochemistry at the anomeric centre (i.e. \(\alpha\) or \(\beta\)) and the compatibility of reaction conditions with conventional carbohydrate protecting groups. There are several approaches to the synthesis of these compounds\textsuperscript{131,132}, including:

(i) Lewis acid mediated glycosylation  
(ii) Anomeric lactones as glycosyl donors  
(iii) Reactions of glycosyl anions with electrophiles  
(iv) Free radical mediated glycosylation.

3.4.1 Preparation of C-glycosides

3.4.1.1 Lewis acid mediated glycosylation

The most commonly used method for C-glycoside synthesis involves Lewis acid catalysed generation of an electron-deficient intermediate (figure 3.1), followed by addition of a carbon nucleophile\textsuperscript{133}. For example, Sakurai and co-workers treated a methyl glycoside with allyltrimethylsilane with a catalytic amount of TMSOTf, which led to the \(\alpha\)-linked C-glycoside (figure 3.24)\textsuperscript{134}. The axial orientation is preferred due to a combination of steric and electronic effects\textsuperscript{109}.

![Lewis acid-mediated C-glycosylation](image)

**Figure 3.24**: Lewis acid-mediated C-glycosylation

Allevi and co-workers\textsuperscript{135} used silver triflate activation to form C-glycosides from glycosyl halides and silyl enol ethers (figure 3.25), with similar configuration.
Schmidt and Hoffman\textsuperscript{136} demonstrated that glycosyl trichloroacetimidates (see section 3.1.2.3) could be utilised to form $C$-glycosides in good yields, using BF\textsubscript{3}·OEt\textsubscript{2}, ZnCl\textsubscript{2} and TMSOTf activation. Various other glycosyl donors have proved successful, including $O$-phenyl glycosides\textsuperscript{137}, glycosyl fluorides\textsuperscript{138} and glycals\textsuperscript{139}.

3.4.1.2 Anomeric lactones as glycosyl donors

A reliable method for synthesising $\beta$-$C$-glycosides involves the addition of an organometallic agent to a sugar lactone\textsuperscript{140}. Kishi and collaborators\textsuperscript{141} demonstrated the success of this approach by using allyl Grignard organometallic reagents (for example, figure 3.26).

Dondoni has used a similar reaction as access to highly versatile formyl glycosides (figure 3.27)\textsuperscript{142}.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure325}
\caption{C-glycosylation via glycosyl halides}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure326}
\caption{Anomeric lactones as glycosyl donors}
\end{figure}
Introduction: Chemistry

Th = 2-thiazole

Figure 3.27: Thiazole-based synthesis of C-glycosides

The first step involves addition of 2-lithiothiazole to the sugar lactone to give a ketol, which after acetylation is reduced to a thiazole glycoside from which the final formyl glycoside is released by hydrolytic cleavage\(^\text{143}\). This formyl glycoside can subsequently be transformed into a phosphonium iodide suitable for Wittig olefination (figure 3.28)\(^\text{144,145}\).

Figure 3.28: C-glycosides via Wittig olefination
In the example shown in figure 3.28\textsuperscript{146}, the glycosyl phosphonium salt was coupled to the aldehyde obtained from D-serine\textsuperscript{147}. Reduction of the double bond of the resulting mixture of olefins and removal of the acetonide protection transformed the glycoside into the N-Boc amino alcohol.

3.4.1.3 Reactions of Glycosyl anions with electrophiles

Several workers have reversed the natural character of the anomeric carbon centre from electrophilic to nucleophilic. Often, a stabilising substituent is utilised at C-1 such as a nitro group, which renders the remaining anomeric proton sufficiently acidic to be deprotonated by a strong base\textsuperscript{148}.

Vasella and co-workers reported the reaction of 1-nitro sugars with electrophiles such as formaldehyde (figure 3.29)\textsuperscript{149}. Subsequent reductive nitration yields the β-C-glycoside with high stereospecificity.

![Figure 3.29: Addition of an anomeric anion to an electrophile](image)

Other methods include similar procedures utilising lithiated glycosides, such as that demonstrated by Sinay and co-workers\textsuperscript{150} and by Kessler and co-workers\textsuperscript{151,152}. Organosamarium glyco-intermediates have also been used successfully to synthesise C-linked glycosides\textsuperscript{153,154}.
3.4.1.4 Free radical mediated glycosylation

The relatively mild and neutral conditions of free radical reactions make them ideally suited to carbohydrates with sensitive protecting groups. Giese and co-workers first developed the synthesis of C-glycosides by this approach, using anomic radicals with activated alkenes[^155].

An α-glucosyl bromide was used to generate an anomic radical by photolysis in the presence of tri-butyltin hydride (Bu₃SnH) and an excess of acrylonitrile to give the α-C-glycoside (figure 3.30)[^155].

![Figure 3.30: Free radical mediated C-glycosylation](image)

Mechanistic studies have revealed that the glycosyl radical adopts a configuration that leads to the preferential formation of the α-C-glycoside[^156].

In addition, radical mediated reactions have been performed using allyltributylstannane and a radical initiator, such as azobisisobutyronitrile (AIBN)[^157]. This reaction, the Keck allylation[^157] has been used successfully to synthesise C-glycosides of sialic acid (N-acetyl neuraminic acid)[^158,159] and of N-acetyl glucosamine[^160].

### 3.5 Summary

The established procedures for the preparation of glycosides have been introduced in this section. Several effective methods for the synthesis of O-linked glycosides were described. Glycosylamines provide a facile route to N-linked compounds. However, the synthesis of S- and C-linked compounds provides a greater challenge, potentially involving more complex reaction schemes.

Section 4 will describe attempts made to synthesise a series of lipoamino acid-based glycolipids, beginning with O-linked compounds, before proceeding to prepare N-, S- and C-linked derivatives.
4 Results and Discussion

The aim of the work described in this thesis was to produce a series of glycolipids designed to be suitable as conjugates to enhance the oral uptake of poorly absorbed drugs and medicinal peptides. Desirable properties previously identified were (i) the ability to enhance the lipophilicity of the drug whilst maintaining a degree of aqueous solubility, (ii) stability to chemical and enzymatic degradation in the GI tract and (iii) suitable for direct attachment to a drug or peptide either by covalent or non-covalent linkage.

The following objectives were identified as significant when planning synthetic strategies:

(i) to minimise the number of reaction steps
(ii) to obtain a reasonable overall yield
(iii) to use cheap, non-hazardous, easily obtained reagents when possible
(iv) to develop reproducible, straightforward methods that may be applicable to a larger scale reaction.

The glycoconjugates synthesised were based on the so-called lipoamino acids (see section 2.3.1.2). These LAAs were subsequently glycosylated to yield novel glycolipids, a potential drug delivery system. The nature of the sugar component, the nature of the lipid component and the linkage between sugar and lipid were all varied to produce a series of compounds with different physicochemical properties* and potential susceptibility towards the degradative action of glycosidase enzymes.

This section begins by describing the synthesis of O-linked glycolipids. Subsequently, the synthesis of N-, S- and C-linked liposaccharides is discussed.

---

* The calculated log P (CLogP) values quoted in this thesis were generated with “HyperChem Pro” release 5.1 Molecular Modelling system, using “ChemPlus QSAR properties” extension version 1.6. These values refer to fully de-protected, non-ionised compounds.
4.1 Synthesis of lipoamino acids

Lipoamino acids (LAAs), non-natural α-amino acids with long alkyl side chains, represent a class of compounds which combine the structural features of lipids with those of amino acids (figure 4.1).

![Figure 4.1: Lipoamino acid properties](image)

Lipoamino acids were synthesised according to the procedure first described by Albertson^161, with modifications. Figure 4.2 demonstrates the synthesis of 2-aminododecanoic acid.

![Figure 4.2: Synthesis of 2-aminododecanoic acid](image)

Diethyl acetamidomalonate was treated with sodium ethoxide to quantitatively yield the corresponding alkyl dicarboxylates. Subsequent hydrolysis and partial de-carboxylation leading to the long-chain α-amino acids was performed by refluxing the crude diesters in concentrated HCl. Solubility of the longer chain acids was improved by the addition of dimethylformamide (20%) to the solution^30.
Neutralisation with NH₄OH furnished the racemic lipoamino acids 1a-d. These reaction conditions were found to be suitable for synthesis on a large scale.

\[
\begin{array}{c|c}
H_2N-\text{CH-COOH} & n \\
(\text{CH}_2)n & 1a \ 9 \\
\text{CH}_3 & 1b \ 11 \\
\text{CH}_3 & 1c \ 13 \\
\text{CH}_3 & 1d \ 15 \\
\end{array}
\]

4.1.1 N-Boc-Lipoamino acids

In order for these LAAs to be useful in glycosylation reactions, the amino terminus was protected using the *tert*-butoxycarbonyl (Boc) group. Prolonged stirring of LAAs 1a-d in *tert*-butyl dicarbonate (Boc₂O) in *tert*-butyl alcohol and water mixture at pH 11 yielded N-Boc-lipoamino acids 2a-d in fair yields.

\[
\begin{array}{c|c}
\text{BocHN-CH-COOH} & n \\
(\text{CH}_2)n & 2a \ 9 \\
\text{CH}_3 & 2b \ 11 \\
\text{CH}_3 & 2c \ 13 \\
\text{CH}_3 & 2d \ 15 \\
\end{array}
\]

The Boc protecting group is stable to basic conditions (e.g. sodium methoxide and methanolic ammonia, frequently used in the de-O-protection of acyl carbohydrate protecting groups) and is stable to catalytic hydrogenation. Selective de-N-protection of the Boc group could be effected in the presence of a wide range of carbohydrate protecting groups.
4.1.2 \textit{N-Dde-Lipoamino acids}

An alternative to the Boc protection strategy is the \textit{N}-1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl (Dde) amino protecting group\textsuperscript{164}. The stability of the Dde group to both acidic and some basic conditions and its facile removal by hydrazine have led to its successful incorporation into solid phase methodologies\textsuperscript{164,165} and have made it an attractive addition to the carbohydrate chemist’s protecting group armoury.

Dde-OH 3 was synthesised by acylation of dimedone with acetic anhydride, in the presence of DMAP (figure 4.3). In a modification to the original procedure of Bycroft et al.\textsuperscript{164}, it was found that purification was easier and by-product formation less significant when triethylamine was used as base in the presence of a catalytic quantity of DMAP.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{Dde.png}
\caption{Synthesis and protection strategy for Dde protecting group}
\end{figure}

Dde-protected LAAs 4a-c were synthesised by reaction of 1a-c with Dde-OH 3 in the presence of triethylamine in refluxing ethanol (figure 4.4). In contrast to the Boc-protection reaction, Dde protection of LAAs proceeds in quantitative yield. De-\textit{N}-protection with hydrazine results in the formation of an indazole by-product (figure 4.4).

It has been suggested that the strong intermolecular hydrogen bond in the resultant Dde-protected derivative (figure 4.3) is responsible for the preference of 2-acetyldimedone (Dde-OH) to form stable derivatives only with primary amines\textsuperscript{166}.

\begin{center}
\begin{tabular}{c|c}
DdeHN\textsuperscript{--} & \textit{n} \\
\text{CH\textsubscript{2}}\textsuperscript{--}COOH & 4a 9 \\
(CH\textsubscript{2})\textit{n} & 4b 11 \\
\text{CH\textsubscript{3}} & 4c 13 \\
\end{tabular}
\end{center}
4.2 O-linked glycolipids

This section describes attempts made to synthesise O-linked lipoamino acid-based glycolipids.

4.2.1 O-linked glycolipids via 1-O-acetyl glycosyl donors

The formation of O-glycosides via anomeric acetates is possibly the simplest method available. A simple reaction between the easily synthesised per-O-acetylated sugars and an alcohol often yields the desired O-glycoside in good yield.

Glucose and galactose were acetylated with acetic anhydride using iodine as promoter (figure 4.5).

Figure 4.5: Iodine promoted acetylation of glucose
This method, reported by Kartha and Field\textsuperscript{167}, had the advantage that large volumes of pyridine could be avoided. Interestingly, the $\alpha$-anomer of both glucose and galactose was the dominant configuration ($\approx 20:1$)\textsuperscript{167}, contrary to other methods in which the ratio is closer to 1:1.

$N$-acetyl glucosamine was acetylated using acetic anhydride and pyridine\textsuperscript{168} since the reaction with iodine was extremely slow (figure 4.6).

![Acetylation of glucosamine](image)

Figure 4.6: Acetylation of glucosamine

In order to achieve the desired $O$-(ether)-linkage between sugar and lipid, an alcohol derivative of the LAAs was required, the synthesis of which is described below.

4.2.1.1 Synthesis of lipoamino alcohols

Several procedures to synthesise an alcohol derivative of the LAAs were considered (as shown in outline in figure 4.7).

![Synthesis of lipoamino alcohols](image)

Figure 4.7: Synthesis of lipoamino alcohols
The first method\(^{169}\) involved formation of a mixed anhydride using ethyl chloroformate. This was then reduced at low temperature rapidly and in good yield. This procedure was adequate but had the disadvantage of being a two-stage process.

The second method\(^{170}\) relied on the pre-formation of hydroxybenzotriazolyl (HOBt) esters of the LAA using benzotriazol-1-yloxytris(pyrrolidino)phosphonium hexafluorophosphate (PyBOP) and \(N,N\)-diisopropylethylamine (DiEA) in THF\(^{171}\). This could react quickly and cleanly with sodium borohydride in a one-pot reaction.

The third, preferred method was to reduce the LAA in borane-THF complex in a simple, clean reaction with very high yields.

\(N\)-Boc protected lipoamino alcohols 9a-c were synthesised.

\[
\text{BocHN} - \text{CH} - \text{CH}_2\text{OH} \quad \text{\text{\(n\)}} \\
\quad (\text{CH}_2)_{\text{n}} \\
\quad \text{CH}_3 \\
9a \quad 9 \\
9b \quad 11 \\
9c \quad 13
\]

However, attempts to cleanly synthesise \(N\)-Dde protected alcohols failed. Reactions often contained a mixture of products, likely to be due to the instability of the carbonyl groups of the Dde structure to the reduction conditions.

The mild conditions for reduction of \(N\)-protected amino acids recently reported by Naqvi and co-workers\(^{172}\) were also inappropriate for \(N\)-Dde protected compounds (figure 4.8).

Figure 4.8: Attempted reduction of \(N\)-Dde LAAs
The procedure involved formation of the pentafluorophenyl ester 10 of the N-Dde protected LAA using pentafluorophenol (Pfp-OH) and DCC. This was then reduced using NaBH4 in the presence of I2.

4.2.1.2 Synthesis of O-linked glycolipids via 1-O-acetyl glycosyl donors

Attempts to synthesise O-glycosides using this method failed, with extremely poor reactivity. The choice of Lewis acid did not influence the outcome of the reaction (figure 4.9).

\[ \text{O} \quad + \quad \text{SnCl}_4 \text{ or BF}_3\text{OEt}_2 \quad \rightarrow \quad \text{O} \]

Figure 4.9: Synthesis of O-glycolipids from 1-O-Acyl sugars

An alternative method was sought, using a more reactive sugar donor.

4.2.2 O-glycolipids from glycosyl trichloroacetimidates

The glycosyl trichloroacetimidate method, introduced in section 3.1.2.3, was attractive since short reaction times, mild conditions and relatively simple protocols have all been reported.

The glycosyl trichloroacetimidate of N-acetyl glucosamine was prepared from the per-O-acetylated compound 8. Selective de-O-protection of the anomeric acetate was achieved by a short reaction with hydrazinium acetate173 at 50°C in DMF to yield the 1-OH derivative 11. This compound was found to be quite stable and no acetate migration was observed (an advantage of 2-deoxy-sugars over simple glucose and similar sugars). This compound was subsequently transformed to the trichloroacetimidate 12 by reaction with trichloroacetonitrile in the presence of sodium hydride (figure 4.10). The β-configuration was the favoured orientation in this case.
The glycosyl trichloroacetimidate 12 was reacted with lipoamino alcohol 9a, in the presence of boron trifluoride dietherate (BF₃·OEt₂) as catalyst. However, the single product obtained proved to be a stable oxazoline intermediate 13 (introduced in section 3.1.2.4) as confirmed by NMR spectroscopy. It became evident that this was hindering progress of the reaction (figure 4.11).
An alternative strategy was to adopt a different N-protecting group at position 2 of the sugar. The trichloroethoxycarbonyl (troc) group\textsuperscript{174} represented an improvement. The troc group was easily introduced by dropwise addition of trichloroethoxycarbonyl chloride to an aqueous solution of glucosamine hydrochloride to yield N-troc glucosamine 14a. Following per-O-acetylation by reaction with acetic anhydride in pyridine to afford 14b, the anomeric acetate was selectively removed (as previously described) to give 1-OH compound 15. Trichloroacetimidate formation proceeded smoothly to give 16 (\textbf{figure 4.12}).

\begin{figure}
\centering
\includegraphics[width=\textwidth]{synthesis}
\caption{Synthesis of N-troc glycosyl donor}
\end{figure}

Trichloroacetimidate 16 was reacted with lipoamino alcohol 9a in the presence of BF\textsubscript{3}·OEt\textsubscript{2}. The reaction proved successful, yielding the $\beta$-O-linked glycolipid 17 (\textbf{figure 4.13}). Unfortunately, the overall yield of the reaction was poor. The troc-protected amine was readily transformed into an acetyl-protected one by treatment of 17a with zinc powder and acetic anhydride to give 17b.
Results & Discussion

\[
16 + 9a \xrightarrow{BF_3OEt_2} \begin{array}{c}
\text{AcO} \\
\text{AcO} \\
\text{TrocNH} \\
\text{O—CH—CH—NHBoc} \\
\text{(CH_2)_9} \\
\text{CH}_3
\end{array}
\]  

\hspace{1cm} \boxed{17a}

**Figure 4.13**: Synthesis of \(O\)-linked LAA-based glycolipid

**Figure 4.14** is an energy minimised representation of glycolipid \(17b\) when fully de-protected, clearly showing the amphipathic nature of the molecule.

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

**Figure 4.14**: Energy minimised structure of de-protected \(17b\); 
\(\text{CLogP}=1.59\)

Key: C(light blue); H(white); O(red); N(dark blue)

Attention was then turned to the synthesis of more chemically and biologically stable glycolipids, with glycosidic linkages other than oxygen. \(N\)-, \(S\)-, and \(C\)-linked glycolipids were considered and will be discussed in subsequent sections.
4.3 N-linked glycolipids

The objective of this part of the work was to synthesise a series of glycolipids, in which the glycosidic linkage between sugar and lipid was via a nitrogen atom rather than an oxygen atom. The most straightforward method to accomplish this appeared to be to introduce an amine functionality to the glycosyl donor, via a glycosyl azide.

4.3.1 Synthesis of β-glycosylamines

Glycosylamines have been synthesised from un-protected sugars by treatment with ammonia\textsuperscript{10} or ammonium bicarbonate\textsuperscript{111} (see section 3.2). The approach adopted, however, was to use fully protected sugars. Glycosylamines of fully protected sugars are difficult to handle. They are labile and sensitive to moisture but are not nearly as labile as their unprotected counterparts.

4.3.1.1 Synthesis of β-glycosyl azides

Introduction of the azide functionality into the sugar ring was accomplished successfully by converting the per-O-acetylated derivatives of glucose 5 and galactose 6 into their respective bromosugars 18 and 19 by treatment with 45% HBr in acetic acid\textsuperscript{175}. These compounds were then transformed into azides 22 and 23 respectively by a reaction with sodium azide in the presence of tetra-n-butyrammonium hydrogensulphate under phase-transfer conditions (PTC)\textsuperscript{176} (Path B, figure 4.15). These conditions enabled the reaction to be performed at room temperature, as opposed to the elevated temperatures required when using sodium azide in DMF\textsuperscript{113}. A more efficient route to glycosyl azides was the Lewis acid (SnCl\textsubscript{4}) promoted reaction with trimethylsilyl azide (TMSN\textsubscript{3})\textsuperscript{177}. This one-pot reaction avoided the need to synthesise the sensitive halosugar derivatives and resulted in high β-stereoselectivity due to neighbouring-group participation (see section 3.1.1.1)\textsuperscript{178}.
Synthesis of the azide 24 of per-O-acetylated N-acetyl-glucosamine 8 proved more challenging (figure 4.16). The TMSN$_3$ mediated reaction proceeded very slowly with little azide formation after 24 hours. The alternative route, via the chlorosugar 21 proved satisfactory. Compound 21 was synthesised from 8 by reaction with acetyl chloride saturated with HCl or from directly from N-acetyl glucosamine using acetyl chloride alone. The chloride reacted smoothly with sodium azide under PTC conditions$^{179}$ to give azide 24 in good yield.

However, replacement of dichloromethane with 1,2-dichloroethane and elevated temperatures allowed azide formation using the TMSN$_3$ route successfully.

Figure 4.15: Synthesis of glycosyl azides

Figure 4.16: Synthesis of the azide of N-Acetyl glucosamine
4.3.1.2 Reduction of glycosyl azides

Glycosyl azides 22 and 23 were reduced by catalytic hydrogenation\textsuperscript{178} under mild conditions to the respective glycosylamines 25 and 26 in good yield. The reaction was performed at room temperature and atmospheric pressure using 10% palladium on carbon catalyst (figure 4.17). The reduction of the azide of \(N\)-acetyl glucosamine 24 proved problematic with significant by-product formation observed. Similar problems were reported by Jeanloz and co-workers\textsuperscript{180,181} with disaccharides of glucosamine.

\[
\begin{align*}
{\text{AcO}} & \quad \text{OAc} \\
{\text{AcO}} & \quad \text{OAc} \\
{\text{AcO}} & \quad \text{N}_3 \\
22 & \quad \xrightarrow{\text{H}_2, \text{Pd} / \text{C}} \\
{\text{AcO}} & \quad \text{OAc} \\
{\text{AcO}} & \quad \text{AcO} \\
{\text{AcO}} & \quad \text{NH}_2 \\
25 &
\end{align*}
\]

\textbf{Figure 4.17: Reduction of glycosyl azides to glycosylamines}

4.3.2 Synthesis of \(N\)-(amide)-linked glycolipids via glycosylamines

Reaction of glycosylamine 25 with LAA 2a in the presence EEDQ\textsuperscript{114} (see section 2.2), proceeded smoothly to yield the amide-linked glycolipid 27a (figure 4.18). A series of \(N\)-linked glycolipids with both glucose and galactose was synthesised with varying lipid chain lengths. DCC was used in place of EEDQ in some reactions with similar success.

\[
\begin{align*}
{\text{AcO}} & \quad \text{OAc} \\
{\text{AcO}} & \quad \text{OAc} \\
{\text{AcO}} & \quad \text{OAc} \\
{\text{AcO}} & \quad \text{NH}_2 \\
25 & \quad \xrightarrow{\text{EEDQ}} \\
{\text{AcO}} & \quad \text{OAc} \\
{\text{AcO}} & \quad \text{AcO} \\
{\text{AcO}} & \quad \text{AcO} \\
{\text{AcO}} & \quad \text{NH}_{\text{Boc}} \\
{\text{CH}_{19}} & \quad \text{CH}_3 \\
27a &
\end{align*}
\]

\textbf{Figure 4.18: Synthesis of \(N\)-glycolipid via EEDQ mediated coupling}
4.3.3 Synthesis of N-linked glycolipids via a modified Staudinger reaction

The classical Staudinger reaction\textsuperscript{182} is a two-step process in which an initial electrophilic addition of a phosphine to an azide is followed by elimination of $N_2$ to give an iminophosphorane (figure 4.19)\textsuperscript{183}.

\[
P + N_3R \rightarrow PN_3R \rightarrow PN=NR + N_2
\]

Figure 4.19: Classical Staudinger reaction

Maunier and co-workers reported a modified reaction in which glycosyl amides were synthesised in one step from a reaction between glycosyl azides and acyl chlorides in the presence of triphenylphosphine\textsuperscript{184}. It was suggested that the reaction proceeded via an iminophosphorane followed by the formation of an imidoyl chloride, which was hydrolysed during work-up. (figure 4.20)\textsuperscript{184}. In addition, it was reported that no reaction occurred when carboxylic acids were used.

<table>
<thead>
<tr>
<th>R₁</th>
<th>R₂</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>27a</td>
<td>H</td>
<td>OAc</td>
</tr>
<tr>
<td>27b</td>
<td>H</td>
<td>OAc</td>
</tr>
<tr>
<td>27c</td>
<td>H</td>
<td>OAc</td>
</tr>
<tr>
<td>28a</td>
<td>OAc</td>
<td>H</td>
</tr>
<tr>
<td>28b</td>
<td>OAc</td>
<td>H</td>
</tr>
<tr>
<td>28c</td>
<td>OAc</td>
<td>H</td>
</tr>
</tbody>
</table>

Figure 4.20: Glycosyl amide formation proposed by Maunier et al.
in place of acyl chlorides.

This type of one-pot reaction was very attractive since it avoided the need for the azide reduction step. Glycosyl amides could not be obtained using this method, i.e. the reaction between glycosyl azides and N-protected LAAs in the presence of triphenylphosphine was unsuccessful. However, by using a more reactive phosphine compound, tri-n-butylphosphine (and later trimethylphosphine), glycosyl amides 29a-d were successfully synthesised in one step by reacting glycosyl azides with N-protected lipoamino acids (bearing a free carboxylic acid function). For example, glycosyl azide 24 was reacted with protected LAA 2a in the presence of tri-n-butylphosphine (Figure 4.21).

![Chemical structure](image)

**Figure 4.21**: Synthesis of glycosyl amides via a modified Staudinger reaction

<table>
<thead>
<tr>
<th>R</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>29a</td>
<td>Boc 9</td>
</tr>
<tr>
<td>29b</td>
<td>Boc 11</td>
</tr>
<tr>
<td>29c</td>
<td>Dde 9</td>
</tr>
<tr>
<td>29d</td>
<td>Dde 11</td>
</tr>
</tbody>
</table>

A search of the literature revealed only one example of a Staudinger-type reaction between glycosyl azides and carboxylic acids. Inazu and Kobayashi
reported glycosyl amide formation when glycosyl azides were reacted with an Fmoc-protected L-asparagine derivative in the presence of trialkylphosphines.

The mechanism of the reaction remains unclear. It was proposed that the reaction was not strictly a Staudinger reaction but rather an intramolecular rearrangement. It was suggested that at higher temperatures, Path A (figure 4.22) is followed, with iminophosphorane formation dominating (no reaction). At lower temperatures, Path B (figure 4.22) is followed, with an intermolecular reaction occurring. In this process, it was suggested that elimination of nitrogen, amide formation and elimination of phosphine oxide occur simultaneously. Other studies have been carried out, proposing different mechanisms. It is clear, however, that the presence of the carboxylic acid when the phosphine is added to the reaction is essential for success. When the carboxylic acid was added at a later stage, no amide formation was observed and only iminophosphoranes were identified, such as 30a. Similarly, the use of triphenylphosphine led only to iminophosphorane 30b.

Figure 4.22: A suggested mechanism for the modified Staudinger reaction
4.3.3.1 Synthesis of $N$-protected sugar building blocks

The modified Staudinger reaction previously described is an invaluable method, allowing the often troublesome and hazardous catalytic reduction step to be avoided.

An attractive goal was to be able to synthesise a protected glycosylamine that could be stored for use at a later date and that could be incorporated into solid phase synthesis methodologies\^*. In particular, a protected amine derivative of glucuronic acid would be invaluable since the sugar could be anchored to a solid support via the carboxylic acid, allowing the attachment of lipid components, followed by peptides or drugs via the (initially protected) amine.

Most reported procedures involve the direct conversion of azides into their $N$-Boc derivatives and are based on catalytic reduction followed by \textit{in situ} protection with Boc$_2$O\textsuperscript{189,190}. These procedures were not pursued since it was our intention to avoid the catalytic reduction step. In addition, $N$-Boc protected glycosylamines would not be compatible with standard Fmoc solid phase synthesis protocols.

The 9-fluorenymethoxycarbonyl (Fmoc) group\textsuperscript{191} (\textbf{figure 4.23}) is commonly used in peptide chemistry and is easily removed by treatment with piperidine\textsuperscript{192}.

\begin{figure}[h]
\centering
\includegraphics[width=0.2\textwidth]{fmc.png}
\caption{The Fmoc protecting group}
\end{figure}
The strategy adopted was to attempt to use the modified Staudinger reaction conditions to synthesise an Fmoc-protected glycosylamine from a glycosyl azide in one step. It was determined that the more reactive phosphine, trimethylphosphine, was required for a reaction to occur. However, the desired N-protected glycosylamine of glucose 31 (figure 4.24) was synthesised successfully in good yield.

![Figure 4.24: Synthesis of an Fmoc-protected glycosylamine](image)

Attempts to synthesise the N-Fmoc protected glycosylamine from the azide of methyl glucuronate proved unsuccessful (figure 4.25). Schmidt has previously observed the low reactivity of glycosyl uronates\(^{193}\).

![Figure 4.25: Synthesis of N-Fmoc protected methyl glucuronate](image)

### 4.3.4 N-linked glycolipids as a potential Drug Delivery System

The synthesised N-(amide)-linked glycolipids (27a-c, 28a-c and 29a-d) each possess either a Boc-protected or a Dde-protected amine group, which on de-N-protection is suitable for direct covalent attachment to drugs and/or peptides bearing a free carboxylic acid, for example.

Once coupling to the drug or peptide is complete, sugar O-acetate protection can be removed, yielding a glycolipid-drug conjugate with modified physicochemical properties. Alternatively, these glycolipids can be fully deprotected
for use in non-covalent conjugation to drugs and/or peptides, with the advantages that method of delivery provides (see sections 2.3.3, 2.3.4).

\(N\)-linked glycolipids 27a, 28a and 29a were de-\(O\)-acetylated by treatment with sodium methoxide (NaOMe) in methanol to give 111a, 112a and 113a respectively. De-\(N\)-protection was subsequently effected by treatment with 50% trifluoroacetic acid (TFA) in \(\text{CH}_2\text{Cl}_2\), to yield 111b, 112b and 113b respectively (figure 4.26).

\[
\begin{align*}
27a & \quad \text{H Ac Ac OAc Boc} \\
111a & \quad \text{H H H OH Boc} \\
111b & \quad \text{H H H OH H} \\
28a & \quad \text{Ac H Ac OAc Boc} \\
112a & \quad \text{H H H OH Boc} \\
112b & \quad \text{H H H OH H} \\
29a & \quad \text{H Ac Ac NHAc Boc} \\
113a & \quad \text{H H H NHAc Boc} \\
113b & \quad \text{H H H NHAc H}
\end{align*}
\]

(a) NaOMe / MeOH (b) TFA:CH\(_2\)Cl\(_2\) 1:1

**Figure 4.26:** De-protection of synthesised \(N\)-glycolipids

4.3.4.1 Synthesis of non-covalent glycolipid-drug conjugates

Piperacillin (figure 4.27) is a broad-spectrum \(\beta\)-lactam antibacterial with particular activity against *Pseudomonas aeruginosa*. Its main use is within the hospital setting, principally for the treatment of septicaemias. It has no oral activity and is administered parenterally as the sodium salt (the free acid has very poor aqueous solubility). This drug was therefore an ideal choice for glycolipid-based modification.
An ionic complex 114 between fully de-protected N-linked glycolipid 113b (bearing a free amine group) and piperacillin (bearing a free carboxyllic acid) was prepared. This was accomplished by dissolving equal molar quantities of each component in 95% acetic acid, followed by lyophilisation. The lyophilised complex was analysed by electrospray mass spectrometry, which demonstrated presence of the ionic complex itself, in addition to the positive ion usually observed in such spectra (see Experimental, section 6).

4.3.4.2 Preliminary in vivo experiments

Preliminary in vivo experiments in rats demonstrated significant uptake of piperacillin when administered orally as ionic complex 114 (table 4.1 and figure 4.28). This oral uptake was compared to that observed with oral administration of the parent piperacillin (no oral uptake, as expected) and amoxycillin, the most common clinically used orally active penicillin.

Figure 4.28 clearly demonstrates significant oral absorption of piperacillin, with a relatively rapid onset, when compared to amoxycillin. In addition, the area under the curve (AUC) was calculated as being 499.5 µgml⁻¹min for the normalised piperacillin-ionic complex 114 as compared to 130.4 µgml⁻¹min for amoxycillin, indicating an impressive degree of absorption. Despite the fact that these data apply to different drugs, they provide a useful indication as to the extent of absorption (excluding factors such as metabolism and excretion). These results indicate the potential for glycolipids to enhance the absorption of poorly absorbed drugs.

---

* The in vivo experiments (and calculations) in rats were performed by Eurand, an industrial partner.
Table 4.1: Serum levels of piperacillin (µg/ml) following oral administration to rats

<table>
<thead>
<tr>
<th>Time / min</th>
<th>Piperacillin 56 mg/kg</th>
<th>Complex 114 20 mg/kg</th>
<th>Complex 114* 45 mg/kg</th>
<th>Amoxycillin 45 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>15</td>
<td>0.0</td>
<td>4.2</td>
<td>10.5</td>
<td>0.0</td>
</tr>
<tr>
<td>30</td>
<td>0.0</td>
<td>4.5</td>
<td>11.3</td>
<td>0.1</td>
</tr>
<tr>
<td>45</td>
<td>&lt;0.3</td>
<td>3.8</td>
<td>9.5</td>
<td>0.2</td>
</tr>
<tr>
<td>60</td>
<td>&lt;0.3</td>
<td>0.3</td>
<td>0.8</td>
<td>2.1</td>
</tr>
<tr>
<td>75</td>
<td>&lt;0.3</td>
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<td>0.0</td>
<td>1.8</td>
</tr>
<tr>
<td>90</td>
<td>&lt;0.3</td>
<td>0.3</td>
<td>0.8</td>
<td>1.9</td>
</tr>
<tr>
<td>105</td>
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<td>0.0</td>
<td>1.7</td>
</tr>
<tr>
<td>120</td>
<td>&lt;0.3</td>
<td>0.3</td>
<td>0.8</td>
<td>0.7</td>
</tr>
<tr>
<td>135</td>
<td>&lt;0.3</td>
<td>0.3</td>
<td>0.8</td>
<td>0.1</td>
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<tr>
<td>150</td>
<td>&lt;0.3</td>
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<tr>
<td>165</td>
<td>&lt;0.3</td>
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<td>4.0</td>
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<tr>
<td>180</td>
<td>&lt;0.3</td>
<td>0.0</td>
<td>0.0</td>
<td>&lt;0.1</td>
</tr>
</tbody>
</table>

*calculated serum concentration – mathematically corrected to 45mg/kg dose to allow comparison with amoxycillin

Figure 4.28: Serum levels of piperacillin following oral administration to rats
*calculated serum concentration – mathematically corrected to 45mg/kg dose to allow comparison with amoxycillin
4.3.4.3 Microbiological assay

Tests were performed to determine whether complex formation had any adverse effect on the microbial activity of the drug piperacillin. Piperacillin (as the free acid) and piperacillin-complex 114 were subjected to a minimum inhibitory concentration (MIC) assay. The MIC (i.e. the lowest concentration that will inhibit visible growth in vitro) assay provides a quantitative estimate as to the susceptibility of a microorganism to a given antibacterial. Five species of bacteria were used, namely Pseudomonas aeruginosa (the main organism for which piperacillin is clinically used), Bacillus subtilis, Escherichia coli, Klebsiella aerogenes and Staphylococcus aureus.

A series of dilutions of piperacillin and piperacillin-glycolipid complex 114 were prepared in agar medium. Once the plates had set, each was inoculated with a 1μl sample of each microorganism. In addition, a sample of a 10^2- and 10^4-fold dilution of each organism (made in the broth in which they had grown) was applied. After overnight incubation, the MIC was recorded as the highest dilution in which no growth was visible (table 4.2 and table 4.3). For example, the MIC for piperacillin against B. subtilis was 1μg/ml since that was the lowest concentration at which no bacterial growth was visible (table 4.2).

Dilution testing by this agar method is said to be a very reliable susceptibility testing technique, allowing simultaneous testing of several agents. In addition, contamination is readily detected.

Literature sources suggest that the MIC standard for piperacillin is <64μg/ml for Pseudomonas aeruginosa and <16μg/ml for other gram-negative bacilli. Organisms are said to be resistant if concentrations of antibacterial agent at or above 128μg/ml are required.

The results indicate that the antimicrobial activity of the complex was marginally greater than that of the parent piperacillin. This may have been due to better solubility of the complex in the agar medium. A more thorough investigation with a wider range of compounds is planned for the future. As a broad indicator, however, these simple experiments show that piperacillin-glycolipid complex 114 fell well within the specified limits for microbial activity, with activity similar to that of the parent piperacillin.
It should also be noted that the MIC is a quantity based on mass and not molecular weight. Since piperacillin (MW = 517 g/mol) is only one of the two components comprising complex 114 (MW = 934 g/mol), a higher concentration (approx. double) is required to achieve the equivalent quantity of piperacillin found in the parent.

Table 4.2: MIC assay results for piperacillin

<table>
<thead>
<tr>
<th>Conc* (µg/ml)</th>
<th>B. subtilis</th>
<th>E. coli</th>
<th>K. aerogenes</th>
<th>P. aeruginosa</th>
<th>S. aureus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10^4</td>
<td>10^2</td>
<td>N</td>
<td>10^4</td>
<td>10^2</td>
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<tr>
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<td>+</td>
<td>+</td>
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<td>+</td>
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<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

"+" indicates microbial growth observed; "-" indicates no microbial growth observed.
N=neat organism, 10^2=100-fold dilution of organism, 10^4=10,000-fold dilution of organism
* concentration of piperacillin

Table 4.3: MIC assay results for piperacillin-glycolipid complex 114

<table>
<thead>
<tr>
<th>Conc* (µg/ml)</th>
<th>B. subtilis</th>
<th>E. coli</th>
<th>K. aerogenes</th>
<th>P. aeruginosa</th>
<th>S. aureus</th>
</tr>
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<td>N</td>
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<tr>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

"+" indicates microbial growth observed; "-" indicates no microbial growth observed.
N=neat organism, 10^2=100-fold dilution of organism, 10^4=10,000-fold dilution of organism
* concentration of glycolipid-piperacillin complex 114 (1 µg complex contains 0.55 µg piperacillin)

The ionic complex formation clearly did not drastically affect antimicrobial activity. These results demonstrate the potential of non-covalent conjugation as a means by which to deliver drugs without affecting pharmacological activity.
4.3.5 Glycosyl isothiocyanates as precursors for $N$-glycoside synthesis

An alternative approach to the synthesis of $N$-linked glycolipids is via glycosyl isothiocyanates$^{195,196}$.

Glycosyl isothiocyanates are highly versatile synthetic intermediates, which have been used as precursors for heterocyclic sugar derivatives$^{197}$ such as nucleoside analogues$^{195}$. They undergo many reactions. Isothiocyanates can react with carboxylic acids, but also form interesting products with amines, alcohols and thiols.

These compounds presented a facile route for the synthesis of glycosyl amides but also provided the opportunity to synthesise compounds with unusual and interesting linkages, which may influence physicochemical properties such as solubility and lipophilicity. In addition, it provided an opportunity to link sugars to compounds (through an $N$-glycosidic linkage) that possess functional groups other than carboxylic acids.

4.3.5.1 Synthesis of glycosyl isocyanates

Attempts to synthesise glycosyl isocyanates using trimethylsilyl isocyanate (TMSNCO) either from per-$O$-acylated sugars or via halosugars proved unsuccessful (figure 4.29). Despite product being detected in reaction mixtures by mass spectrometry, the compounds proved impossible to isolate. The isocyanates were highly reactive, extremely labile and sensitive to moisture$^{198}$, which led to significant polymerisation. Efforts were therefore focused on the synthesis of the less reactive (only by comparison) glycosyl isothiocyanates.

![Figure 4.29: Synthesis of glycosyl isocyanates](image-url)
3.3.5.2 Synthesis of glycosyl isothiocyanates

The classical method to synthesise glycosyl isothiocyanates was developed by Fischer in 1914. It involved treatment of an acylated glycosyl bromide with silver thiocyanate in an apolar solvent, e.g. xylene (figure 4.30).

$$\text{AcO} \rightarrow \text{AgSCN} \rightarrow \text{AcO} \quad \text{N} = \text{C} = \text{S}$$

**Figure 4.30:** Fischer synthesis of glycosyl isothiocyanates

Depending on the reactivity of the particular glycosyl halide and the reaction conditions, either a thiocyanate or an isothiocyanate was formed directly. Sugar thiocyanates rearranged fairly readily into the corresponding isothiocyanate. The two products were, however, difficult to separate, which has led to a search for improved methods. These include reaction of glycosylamines with thiophosgene and solvent-free preparation using thiocyanate salts.

Camarasa and co-workers introduced a modified procedure whereby sugar isothiocyanates could be synthesised by reaction with potassium thiocyanate in the presence of a tetraalkylammonium salt as catalyst.

$$\text{AcO} \quad \text{OAc} \quad \text{NCS} \quad 33$$

$$\text{AcO} \quad \text{OAc} \quad \text{NCS} \quad 34$$

$$\text{AcO} \quad \text{AcO} \quad \text{OAc} \quad \text{NCS} \quad 35$$

$$\text{AcO} \quad \text{AcO} \quad \text{AcNH} \quad \text{NCS} \quad 36$$
Results & Discussion

Using this method, glycosyl isothiocyanates of glucose 33, galactose 34, mannose 35 and N-acetyl-glucosamine 36 were synthesised from their respective halosugars.

In addition, the isothiocyanate of N-acetyl neuraminic acid (sialic acid) was synthesised by this method. Sialic acid is a highly sensitive sugar residue involved in many biological processes.

The carboxylic function of sialic acid was methyl-protected by stirring the compound in methanol with an H⁺ exchange resin. The resulting compound 37 was acetylated using acetic anhydride and pyridine to give 38. This per-O-acetylated derivative was dissolved in acetyl chloride through which HCl gas had been bubbled and allowed to react for 24 hours. This chlorosugar 39 was then treated with potassium thiocyanate to yield the isothiocyanate 40 (figure 4.31).

Figure 4.31: Synthesis of the isothiocyanate of sialic acid
4.3.5.3 Synthesis of glycosyl amides from isothiocyanates

The synthesis of glycosyl amides from isothiocyanates was first described by Khorlin and co-workers\textsuperscript{198} (1980), following the known reaction of alkyl and aryl isocyanates and isothiocyanates with aliphatic and aromatic carboxylic acids.

At first glance, the reaction between glycosyl isothiocyanates and carboxylic acids appears to be straightforward. However, it has been shown\textsuperscript{198} that the mechanism is a little more complex. It has been suggested that the formation of a mixed anhydride follows the reaction between sugar and carboxylic acid (figure 4.32). The formation of disubstituted ureas in reactions with isocyanates is usually explained by the presence of moisture\textsuperscript{198}. However, not only thioureas can arise from isothiocyanate reactions but also ureas derived from the relatively stable thiocarbamic acid derivative (figure 4.32). Khorlin suggested that the proportion of urea derivatives could not be explained by moisture alone but may depend on further rearrangements of the aforementioned mixed anhydride.

![Figure 4.32: Glycosyl amide formation via glycosyl isothiocyanates](image)
Glycosyl amides were synthesised smoothly by reaction of glycosyl isothiocyanates and protected lipoamino acids in the presence of a catalytic quantity of triethylamine (0.2 equivalents) in refluxing toluene (figure 4.33).

![Figure 4.33: N-linked glycolipids from isothiocyanates](image)

The amount of triethylamine (TEA) used in the reaction, together with the number of molar equivalents of carboxylic acid used, were thought to be influential in limiting by-product formation.

### 4.3.5.4 Glycosyl thiocarbamates

Besides glycosyl amides, glycosyl isothiocyanates are able to react with nucleophiles such as alcohols, sulphides (section 4.3.5.5) and amines (section 4.3.5.6). In these cases, the electronegative atom bonds to the carbon atom of the isothiocyanate as shown in figure 4.34.

![Figure 4.34: Reaction of alcohols with isothiocyanates](image)

Glycosyl thiocarbamates were synthesised cleanly from glycosyl isothiocyanates and N-Boc protected lipoamino alcohols 9 (figure 4.35).

It was determined that the conditions required for their preparation were identical to those for glycosyl amide formation, i.e. a catalytic quantity of TEA in refluxing toluene.
4.3.5.5 Glycosyl dithiocarbonates

The reaction of glycosyl dithiocarbonates is an extremely fast (often total conversion within five minutes) and efficient reaction. Reaction of glycosyl isothiocyanate 33 with dodecanethiol produced glycosyl dithiocarbonate 42 in excellent yield and purity (figure 4.36).

4.3.5.5.1 Synthesis of lipoamino thiols

In order to synthesise dithiocarbonates from LAAs, a thiol derivative was required. The first approach to this challenge was via the conversion of a halide derivative to a thiol derivative using potassium thioacetate (figure 4.37).
Figure 4.37: Synthesis of lipoamino thiols (1)

The $p$-toluenesulphonyl (tosyl) 43 and methanesulphonyl (mesyl) 44 derivatives of the $N$-Boc protected lipoamino alcohols 9 were synthesised by reaction with tosyl chloride in pyridine and mesyl chloride in triethylamine respectively. The tosyl reaction in particular is a low yielding reaction requiring purification by column chromatography to remove pyridinium by-products.

The iodo derivatives 45 were synthesised by halogen exchange with sodium iodide in acetone. The recently reported procedures for direct conversion of alcohols to alkyl halides via tetrahydropyranyl ethers using either dimethylphosgeniminium chloride$^{205}$ or 2,4,4,6-tetrabromo-2,5-cyclohexadienone and triphenylphosphine$^{206}$ proved unsuccessful for these compounds.
These iodo derivatives could then be reacted with potassium thioacetate in DMF at raised temperature to yield the thioacetate compounds 46. Thioacetate derivatives could also be synthesised directly from the sulphonyl compounds. De-S-protection occurs smoothly using methanolic ammonia. However, confirmation of the success of this reaction proved extremely difficult.

It is well known that in basic conditions, thiols can form disulphide by-products\(^{207}\). The mass spectrum for compound 47a indicated that the product was poorly ionisable and indicated the presence of disulphide in a sample that had been purified by column chromatography (figure 4.38).

![Figure 4.38: FAB Mass spectrum extract for compound 47a](image)

The spectrum shows the presence of the product (340=[M+Na]\(^{+}\)), but also indicates disulphide presence (633=[M\(^{1+}\)+H]\(^{+}\), 656=[M\(^{1+}\)+Na]\(^{+}\), 533=[M\(^{1-Boc+}\)+H]\(^{+}\)). The NMR spectrum was similarly ambiguous. An alternative method of analysis was therefore required.

An infrared spectrum is obtained when a sample absorbs radiation in the infrared region of the electromagnetic spectrum. Scattering of incident radiation by a sample produces a Raman spectrum. The characteristic Raman frequencies of thiols and disulphides are shown below\(^{208}\):

<table>
<thead>
<tr>
<th>Compound</th>
<th>Functional Group</th>
<th>Frequency Range (cm(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disulphide</td>
<td>C-S-S-C</td>
<td>550 - 430 cm(^{-1})</td>
</tr>
<tr>
<td>Sulphide</td>
<td>C-S</td>
<td>705 - 570 cm(^{-1})</td>
</tr>
<tr>
<td>Thiol</td>
<td>R-SH</td>
<td>2590 - 2560 cm(^{-1})</td>
</tr>
<tr>
<td></td>
<td></td>
<td>700 - 550 cm(^{-1})</td>
</tr>
<tr>
<td></td>
<td></td>
<td>340 - 320 cm(^{-1})</td>
</tr>
</tbody>
</table>
Clearly, the thiol has a unique S-H stretching frequency at 2590-2560 cm$^{-1}$. Examination of the Raman spectrum$^\text{6}$ for compound 47a (figure 4.39) reveals a peak at 2582 cm$^{-1}$, indicating the presence of a thiol.

![Figure 4.39: Raman spectrum of compound 47a](image)

The second approach to the challenge of synthesising lipoamino thiols was via a Mitsunobu reaction$^\text{209}$. The lipoamino alcohols 9a-c could be transformed directly to the thioacetate derivatives 46a-c by a reaction with diethyl azodicarboxylate (DEAD), triphenylphosphine (PPh$_3$) and thiolacetic acid (figure 4.40). This reaction had the advantage of being a one-step process and was a high yielding reaction. However, problems were encountered and these, together with the Mitsunobu reaction itself will be discussed in section 4.4.4.

$^6$ The Raman spectrum for compound 47a (ref. R338, figure 4.39) was recorded by Dr. Istvan Jablonkai, Chemical Research Center, Hungarian Academy of Sciences.
4.3.5.5.2 Synthesis of glycosyl dithiocarbonates

Glycosyl dithiocarbonates were synthesised rapidly and cleanly from glycosyl isothiocyanates and lipoamino thiols (figure 4.41).
Results & Discussion

These reactions were carried out at room temperature, with a catalytic quantity of triethylamine. Complete conversion was observed within 10 minutes.

\[
\text{AcO} \quad \text{OAc} \quad \text{OAc} \quad \text{S} \quad \text{NHCSCH}_2\text{CH} \quad \text{OAc}
\]

\[
\text{(CH}_2\text{n} \quad \text{CH}_3
\]

<table>
<thead>
<tr>
<th>n</th>
<th>48a</th>
<th>48b</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>9</td>
<td>11</td>
</tr>
</tbody>
</table>

4.3.5.6 Glycosyl thioureas

In addition to alcohols and thiols, isothiocyanates also react with amines to produce thiourea derivatives.

4.3.5.6.1 Synthesis of di-amine LAA derivatives

An amine derivative of the LAAs was synthesised by two different routes. The first involved reaction of the tosyl 43 or mesyl 44 derivatives with sodium azide in DMF at raised temperatures, in a reaction similar to that with potassium thioacetate described in section 4.3.5.5.1. The azide derivatives 49 could then be reduced by hydrogenation over 10% Pd/C to give the mono-protected di-amines 50 (figure 4.42).

\[
\text{BocHN} \quad \text{CH} \quad \text{CH}_2\text{OMes} \quad \text{NaN}_3 \quad \text{DMF} \quad \text{BocHN} \quad \text{CH} \quad \text{CH}_2\text{N}_3
\]

\[
\text{44a} \quad \text{49a}
\]

\[
\text{H}_2 \quad \text{Pd/C}
\]

\[
\text{BocHN} \quad \text{CH} \quad \text{CH}_2\text{NH}_2
\]

\[
\text{49a} \quad \text{50a}
\]

Figure 4.42: Synthesis of di-amine LAA derivatives (1)
A simpler method to introduce a second amine functionality was by a Mitsunobu reaction. An N-Phthaloyl protected derivative was synthesised by reaction of the lipoamino alcohols 9 with phthalimide in the presence of DEAD and PPh₃. Despite a relatively long reaction time, the product was obtained in one step and in good yield.

De-N-protection of the phthalimido protection proved difficult. Substitution of phthalimide (Phth) by tetrachlorophthalimide (TCP), however, meant that de-N-protection could be effected easily using hydrazine or ethylenediamine at room temperature (figure 4.43). Alternatively, the N-Boc deprotection was removed by treatment with trifluoroacetic acid (TFA) to yield 53.

Figure 4.43: Synthesis of di-amine LAA derivatives (2)
4.3.5.6.2 Synthesis of glycosyl thioureas

Glycosyl thioureas were synthesised from glycosyl isothiocyanates and di­amine LAA derivatives (figure 4.44).

\[
\begin{array}{ccc}
\text{BocHN-CH-CH}_2\text{NH}_2 & \text{AcO} & \text{AcO} \\
\text{33} & \text{AcO} & \text{AcO} \\
\text{CH}_3 & \text{AcO} & \text{AcO} \\
\text{50a} & \text{AcO} & \text{AcO} \\
\text{CH}_3 & \text{AcO} & \text{AcO} \\
\end{array}
\]

Figure 4.44: Synthesis of glycosyl thioureas bearing a protected amine

Methyl ester derivatives of LAAs 1b and 1d were prepared by treatment with thionyl chloride in methanol. Hydrochloride salt 55a was subsequently treated with triethylamine and reacted with glycosyl isothiocyanate 33 to yield a thiourea-linked derivative (figure 4.45).
Results & Discussion

\[
\begin{array}{c}
\text{H}_2\text{N} - \text{CH} - \text{COOMe} \\
\text{CH}_3 \\
\end{array}
\begin{array}{c}
\text{H}_3\text{N} - \text{CH} - \text{COOMe} \\
\text{(CH}_2\text{n)} \\
\text{CH}_3 \\
\end{array}
\]

\[55a \quad 55b \]

\[55a \quad 55b \]

\[n \]

\[55a \quad 55b \]

\[55a \quad 55b \]

\[n \]

\[55a \quad 55b \]

\[56a \quad 56b \]

\[n \]

\[56a \quad 56b \]

Figure 4.45: Synthesis of glycosyl thioureas bearing a protected carboxylic acid

\[
\text{H}_2\text{N} - \text{CH} - \text{COOMe} + (\text{CH}_2\text{)}_{11} \xrightarrow{\text{TEA}} \text{AcO} \text{AcO} \text{OAc} \text{TEA} \text{AcO} \text{OAc} \text{NHCNHCH} - \text{COOMe}
\]

\[56a \quad 56b \]

These reactions were carried out at room temperature, with a catalytic quantity of triethylamine. Complete conversion was observed within 2 hours.

A Novel thiourea derivative of sialic acid 57 was synthesised from the respective isothiocyanate 40 (figure 4.46).

\[
\text{AcO} \text{NHCNHCH} - \text{COOMe} \xrightarrow{\text{55a TEA}} \text{NCS} \text{COOCH}_3
\]

\[40 \]

\[55a \]

\[57 \]

Figure 4.46: Thiourea derivative of sialic acid
4.3.5.7 Influence of N-linkage on physicochemical properties

The compounds described in this section, namely amide-linked, thiocarbamate-linked, dithiocarbonate-linked and thiourea-linked, demonstrate the versatility of glycosyl isothiocyanates.

The linkage between sugar and lipid influences the lipophilicity of the conjugate, as demonstrated in Table 4.4, which shows the calculated log P (CLogP) values of a selection of synthesised compounds, when fully de-protected.

![Chemical structure](image)

**Table 4.4: Selected CLogP values of synthesised N-glycolipids**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Linkage (X)*</th>
<th>n</th>
<th>ClogP**</th>
</tr>
</thead>
<tbody>
<tr>
<td>27a</td>
<td>Amide</td>
<td>9</td>
<td>1.33</td>
</tr>
<tr>
<td>27b</td>
<td>Amide</td>
<td>11</td>
<td>2.12</td>
</tr>
<tr>
<td>27c</td>
<td>Amide</td>
<td>15</td>
<td>3.70</td>
</tr>
<tr>
<td>41a</td>
<td>thiocarbamate</td>
<td>9</td>
<td>2.40</td>
</tr>
<tr>
<td>41b</td>
<td>thiocarbamate</td>
<td>11</td>
<td>3.19</td>
</tr>
<tr>
<td>48a</td>
<td>dithiocarbonate</td>
<td>9</td>
<td>2.74</td>
</tr>
<tr>
<td>48b</td>
<td>dithiocarbonate</td>
<td>11</td>
<td>3.54</td>
</tr>
<tr>
<td>54a</td>
<td>thiourea</td>
<td>9</td>
<td>2.08</td>
</tr>
<tr>
<td>54b</td>
<td>thiourea</td>
<td>11</td>
<td>2.88</td>
</tr>
</tbody>
</table>

* for exact structure, refer to main text; ** refers to fully de-protected compound

These figures demonstrate that lipophilicity was increased not only by increasing the length of the lipid chain, but also by varying the nature of the glycosidic linkage. Other factors may be influenced by the nature of the linkage, such as hydrogen-bonding. Thioureas, for example are known to possess considerable hydrogen-bonding potential\textsuperscript{210}.
4.3.6 N-Glycosides of an N-Dde-protected glucosamine

Once acetylated, the amine of N-acetyl glucosamine is effectively non-accessible since the conditions required for de-protection would be extremely harsh. By protecting the amine with the Dde group\textsuperscript{164}, however, the potential existed to perform reactions at the anomeric centre and to deprotect the amine at C-2 of the sugar when required.

\(N\)-Dde glucosamine was synthesised by refluxing \(\alpha\)-d-glucosamine hydrochloride in methanol with Dde-OH (3) in the presence of base. The resulting compound 58 was then fully acetylated by reaction with acetic anhydride in pyridine to give 59. The bromosugar 60 was synthesised as previously described. However, compound 60 could be isolated as a white solid which could be stored. The azide 61 was subsequently synthesised by reaction with Na\(\text{N}_3\) under PTC conditions as

![Synthesis of N-Dde protected glucosamine derivatives](image)

\textbf{Figure 4.47:} Synthesis of \(N\)-Dde protected glucosamine derivatives
previously described. Catalytic reduction over Pd/C yielded amine 62 (figure 4.47). The Dde group is evidently stable to these conditions.

Attempts to synthesise the azide 61 from the per-acetylated compound 59 using trimethylsilyl azide proved unsuccessful. In addition, we were not able to synthesise the isothiocyanate derivative of this compound cleanly.

Glycolipid 63 was synthesised by a DCC-mediated coupling with lauric (dodecanoic) acid.

\[
\begin{align*}
\text{AcO} & \quad \text{AcO} \\
\text{DdeNH} & \quad \text{NH} \left(\text{CH}_2\right)_{10} \text{CH}_3 \\
\end{align*}
\]

4.3.7 Glycolipids via non-anomeric linkages

There are relatively few examples of glycoconjugates where the linkage between sugar and lipid or amino acid is other than via the anomeric centre. Besides the structural differences these conjugates would have with their anomerically-linked counterparts, they would represent a group of novel compounds with the potential to be further derivatised through the anomeric centre.

The first compound was synthesised by the simple EEDQ-mediated coupling of the methyl ester-protected LAA 55b to per-O-acetylated glucuronic acid 64 to give glycolipid 65 bearing a protected carboxylic acid function for attachment to drugs and peptides (figure 4.48).
The second method was by a similar DCC coupling of the \( N \)-tetrachlorophthaloyl protected di-amine LAA derivative 53 to glucuronic acid 64 (figure 4.49).

![Chemical Structure]

\[
\begin{align*}
64 + 53 & \rightarrow 66a, 66b \\
\end{align*}
\]

**Figure 4.49:** Non-anomerically linked glycolipid bearing a protected amine

Interestingly, this reaction gave a mixture of two products that were separable by column chromatography, 66a and 66b. Mass spectral analysis revealed that the products were identical. NMR spectroscopy revealed that both compounds (as would be expected) were \( \beta \)-anomers. Extracts of the \( ^1H \) NMR spectra (500MHz, CDCl\(_3\)) for 66a and 66b are shown in figure 4.50 and figure 4.51 respectively.

The NMR spectra clearly show similar chemical shifts for the following protons:

<table>
<thead>
<tr>
<th>( \delta ) 66a</th>
<th>( \delta ) 66b</th>
<th>Proton</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.44</td>
<td>6.51</td>
<td>NH</td>
<td></td>
</tr>
<tr>
<td>5.73</td>
<td>5.73</td>
<td>H-1</td>
<td>( J_{1,2} ) (66a) = 8.0 Hz = ( \beta ), ( J_{1,2} ) (66b) = 7.8Hz = ( \beta )</td>
</tr>
<tr>
<td>4.17</td>
<td>4.14</td>
<td>( \alpha )CH</td>
<td></td>
</tr>
<tr>
<td>3.92</td>
<td>3.97</td>
<td>H-5</td>
<td></td>
</tr>
</tbody>
</table>

However, the other sugar protons (H-2, H-3 and H-4) show considerable differences between compounds, induced by the effect of the stereochemistry of the lipid over the sugar ring. There are also differences in the splitting patterns for the CH\(_2\) protons adjacent to the tetrachlorophthaloyl-protected amine.
Results & Discussion

Figure 4.50: NMR spectrum extract for compound 66a

![Diagram of compound 66a](Image)

Figure 4.51: NMR spectrum extract for compound 66b

![Diagram of compound 66b](Image)
Interpretation of such spectra is obviously very difficult. One solution to this problem is two dimensional correlation spectroscopy (2D-COSY)\textsuperscript{208}, which provides additional information in relation to the coupling of protons in close proximity. The spectrum shows the presence of coupled protons by dark shaded regions. The 2D COSY spectra of compounds 66a and 66b are shown in figure 4.52 and figure 4.53 respectively.

The assignments made from the traditional spectrum above allow interpretation to be drawn from the 2D COSY spectrum. For instance, since H-1 was assigned at 5.73 ppm for compound 66a, the position of H-2 could be determined (figure 4.52). H-1, labelled signal (a), was coupled to signal (c). Similarly, since H-5 (e) was assigned at 3.92 ppm, the position of H-4 could be determined as being (c) also. H-3 was assigned as being at signal (b) since it showed coupling only with signal (c).

The missing data is therefore:

\begin{align*}
\delta 66a & \\
5.25 & H-3 \\
5.14 - 5.10 & H-4, H-2 \\
3.73 & CH_2N
\end{align*}

\begin{align*}
\delta 66b & \\
5.23 - 5.16 & H-3, H-2 \\
4.73 & H-4 \\
3.82, 3.67 & CH_2N
\end{align*}

These data show that it is H-4 that is most influenced by the change of conformation. H-2 is influenced but not by as great a margin. These two protons are axial (above the plane of the sugar ring). H-3, along with H-5 and H-1 (the equatorially orientated protons, is not influenced to any extent.
Results & Discussion

Figure 4.52: 2D COSY spectrum for compound 66a

Figure 4.53: 2D COSY spectrum for compound 66b
4.3.8 Oligosaccharide based N-linked glycolipids

The physicochemical properties of the synthesised glycolipids have thus far been influenced by modifying the nature of the lipid component (length of alkyl chain) and by varying the nature of the glycosidic linkage. An alternative approach was to modify the polar component, by introducing one or more sugar units in the form of di- or trisaccharides.

A LAA-based glycolipid was synthesised from maltose. The sugar was per-O-acetylated to give 67. Trimethylsilyl azide was used to introduce an azide group, yielding 68. Catalytic hydrogenation gave amine 69 (figure 4.54).

![Diagram of maltose azide synthesis](image)

<table>
<thead>
<tr>
<th>R</th>
<th>α/β</th>
</tr>
</thead>
<tbody>
<tr>
<td>OAc</td>
<td>α/β</td>
</tr>
<tr>
<td>N₃</td>
<td>β</td>
</tr>
<tr>
<td>NH₂</td>
<td>β</td>
</tr>
</tbody>
</table>

Figure 4.54: Synthesis of maltose azide

Amine 69 was coupled smoothly to N-Boc protected LAA 2d in the presence of EEDQ as previously described to yield glycolipid 70 (figure 4.55).

![Diagram of maltose based LAA conjugate synthesis](image)

Figure 4.55: Synthesis of maltose based LAA conjugate
Similarly, LAA conjugates were synthesised from the trisaccharide maltotriose. Per-O-acetylation gave 71, introduction of an azide group gave 72, and catalytic hydrogenation gave amine 73 (figure 4.56).

![Diagram of maltotriose azide synthesis]

Amine 73 was coupled to N-Boc protected LAA 2d in the presence of EEDQ as previously described to yield glycolipid 74 (figure 4.57).

![Diagram of maltotriose-based LAA conjugate synthesis]

Figure 4.56: Synthesis of maltotriose azide

Figure 4.57: Synthesis of maltotriose-based LAA conjugate
4.3.8.1 Influence of saccharide component on physicochemical properties

The introduction of one or more additional sugar units (i.e. di- and trisaccharides) influenced lipophilicity and as a result solubility in aqueous systems, as demonstrated by the synthesised compounds (with identical lipid component) listed in table 4.5. The physicochemical properties can in theory therefore be tailored to requirements.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Nature of sugar</th>
<th>ClogP*</th>
</tr>
</thead>
<tbody>
<tr>
<td>27c</td>
<td>monosaccharide</td>
<td>3.70</td>
</tr>
<tr>
<td>70</td>
<td>disaccharide</td>
<td>2.49</td>
</tr>
<tr>
<td>74</td>
<td>trisaccharide</td>
<td>1.27</td>
</tr>
</tbody>
</table>

* refers to fully de-protected compound

4.3.9 Particle formation properties of N-linked glycolipids

The synthesised liposaccharide conjugates, with the long hydrocarbon chains and a polar head, are ampholytic surfactants with the possibility that they may form particulate systems. A set of experiments was performed to investigate the particle forming properties of a representative sample of the synthesised glycolipids.

N-linked glycolipids 27c, 70 and 74 were de-O-acetylated as previously described (see section 4.3.4) to yield 115a, 117a and 118a respectively, subsequent de-N-protection yielded fully de-protected glycolipids 115b, 117b and 118b respectively. Glycolipid 65 was fully de-protected in one step by treatment with a 1:8:1 mixture of triethylamine:methanol:water at 40°C to give 116.
The particle forming properties of these fully de-protected glycolipids were subsequently investigated.
The ability of the liposaccharides to aggregate was examined using transmission electron microscopy (TEM). Microparticulate systems were formed by the glycolipids; spherical particles observed with monosaccharide glycolipid 115b, trisaccharide glycolipid 118b and glucuronic acid-based glycolipid 116. The general size range was determined to be 10-70 nm. More complex disperse systems were observed with disaccharide 117b.

The inclusion of varying concentrations of the phospholipid dimyristoyl lecithin (DMPC) with the respective glycolipids resulted in the formation of vesicles with bilayer structure, rather than the microparticulate systems formed when using glycolipids alone. DMPC alone under these conditions formed highly polydisperse systems with a size of ca. 1μm. The incorporation of a glycolipid, however, resulted in the formation of monodisperse vesicles of smaller size than using DMPC alone. This was demonstrated by incorporation of glucuronic acid-based glycolipid 116 with DMPC in a molar ratio of 1:3 (figure 4.58) – the concentric layers being typical of bilayer formation.

![TEM micrograph of 116; DMPC (1:3 molar ratio) liposomes (bar = 40 nm)](image)

Similarly, the incorporation of glycolipid 118b with DMPC in a molar ratio of 1:3 led to the formation of vesicles with multiple lamellae (figure 4.59).

\(^3\) These experiments were performed and interpreted by Dr. Anya Hillery, University of Brighton.
The incorporation of the glycolipids into the vesicles formed was confirmed by gel exclusion chromatography and $^1$H NMR. The suspensions containing the vesicles were passed through the column. The presence of vesicles in an eluted fraction was determined by TEM. These fractions containing vesicles were then dissolved in DMSO-$d_6$. Since DMPC is insoluble in that solvent, only the glycolipids would be detectable by NMR. Peaks characteristic of the glycolipids were observed in the NMR spectra, confirming glycolipid incorporation into the vesicles.

These experiments have demonstrated the formation of particulate systems by the synthesised glycolipids per se and their successful incorporation into conventional liposomes. The structure of the liposaccharide and the molar ratio of DMPC influenced the type and size of aggregates produced.

These interesting properties give glycolipids further potential for drug delivery, and are worthy of further investigation in the future.
4.4 S-linked Glycolipids

The objective of this part of the work was to synthesise a series of glycolipids, in which the glycosidic linkage between sugar and lipid was via a sulphur atom. Thioglycosides are less chemically labile than their O-linked isosteres and are much more stable to the action of glycosidase enzymes\(^\text{109}\).

4.4.1 S-linked glycolipids via 1-O-acetyl glycosyl donors

Like the analogous reaction for O-glycoside formation, this method was potentially very useful. A simple reaction between a per-O-acetylated sugar and a thiol often yields thioglycosides in good yields.

Efforts to synthesise S-glycosides via this method, however, proved difficult. Reaction of lipoamino thiol derivative 47a (see section 4.3.5.5.1) with per-O-acetylated glucose 5, galactose 6, N-acetyl glucosamine 8 and N-Dde glucosamine 59 all failed, with little or no conversion detected. Several catalysts were used, including BF\(_3\)OEt\(_2\), SnCl\(_4\) and FeCl\(_3\) (figure 4.60).

\[ \text{OAc} + 47a \xrightarrow{\text{Lewis acid}} \text{S-CH}_2\text{CH-NHBoc} \]

\((\text{CH}_2)_9\text{CH}_3\)

Figure 4.60: Synthesis of S-glycolipids from 1-O-Acyl sugars

Galema and co-workers\(^\text{122}\) and van Doren et al.\(^\text{211}\) both reported the facile manner in which n-alkyl thioglycosides were synthesised using this method. Average yields of 70% were reported for a series of alkyl glycosides, using BF\(_3\)OEt\(_2\) as promoter. In addition, the reactions were quenched after 15 minutes at room temperature. This avoided \(\alpha\)-anomer formation, which occurred with longer reaction times. Wunberg, Kunz and colleagues\(^\text{212}\) also reported thioglycoside formation using a similar method from a per-O-acetylated \(\beta\)-glucose derivative with yields of 80\%. 
Attempts to repeat these results by reacting dodecanethiol with per-O-acetylated glucose, galactose and N-acetyl glucosamine resulted in poor conversion, even after lengthy reaction times. Reaction of dodecanethiol with per-O-acetylated mannose 7, however, did result in complete conversion to the thioglycoside 75, with an anomeric mixture of products (figure 4.61).

\[
7 + \text{CH}_3(\text{CH}_2)_{11}\text{SH} \xrightarrow{\text{BF}_3\text{OEt}_2} \quad 75
\]

**Figure 4.61: Synthesis of mannose-lipid conjugate**

Reaction of lipoamino thiol 47a with per-O-acetylated mannose 7 resulted in the novel compound 76, again as a mixture of anomers, but in very low yield (figure 4.62).

\[
7 + 47a \xrightarrow{\text{BF}_3\text{OEt}_2} \quad 76
\]

**Figure 4.62: Synthesis of LAA-based mannose conjugate**

Possible explanations for the lack of success of this approach could be due to the reactivity of the lipoamino thiol itself. In addition, others have commented on the low reactivity of some N-Boc protected amino acid derivatives, preferring N-Fmoc protection for some reactions\(^{213}\). Another consideration could be the anomeric configuration of the starting per-O-acetates. Garegg noted that reactions were generally much faster when using 1,2-trans-acetates than with the corresponding 1,2-cis ones\(^{79}\). Our preferred method of sugar acetylation was the iodine-catalysed procedure of Kartha and Field\(^{167}\), which led almost exclusively to the \(\alpha\)-anomers of...
glucose and galactose, but more of a mixture of anomers (predominantly the β-anomer) in the case of mannose. These factors could each be used to explain the results obtained.

4.4.2 Trichloroacetimidate approaches to S-glycolipid formation

Attempts were made to synthesise S-linked glycolipids from glycosyl trichloroacetimidates (see section 3.1.2.3), successfully used to synthesise O-linked compounds (see section 4.2.2).

Lipoamino thiol derivative 47a was reacted with trichloroacetimidates of galactose, N-acetyl glucosamine 12 and N-troc glucosamine 16 under both BF₃·OEt₂ and TMSOTf catalysis (figure 4.63).

Unfortunately, reactions did not take place, again possibly due to the reactivity of the thiol acceptor.

A trichloroacetimidate derivative 77 was synthesised from lipoamino alcohol 9a in good yield (figure 4.64), using the procedure previously described. This compound was reacted with 1-thiogalactose 79 (see section 4.4.3). No product formation was observed.
4.4.3 \( S \)-linked glycolipids via anomeric \( S \)-alkylation

1-thiosugars were synthesised by reaction of their respective halosugars with thiourea, followed by basic hydrolysis as previously described (see section 3.3.1.3).

\[
\begin{array}{cccc}
\text{R}_1 & \text{R}_2 & \text{R}_3 \\
78 & \text{H} & \text{OAc} & \text{OAc} \\
79 & \text{OAc} & \text{H} & \text{OAc} \\
80 & \text{H} & \text{OAc} & \text{NHAc} \\
81b & \text{H} & \text{OAc} & \text{NHDde}
\end{array}
\]

An alternative route to the synthesis of Dde-protected glucosamine derivative 81b was via the anomeric \( S \)-acetate derivative 81a. This was synthesised readily from the bromosugar 60 by a PTC reaction with potassium thioacetate in the presence of tetra-\( n \)-butylammonium hydrogensulphate to yield 81a. Anomeric \( S \)-deprotection using hydrazine acetate, in a reaction analogous to that described for anomeric de-\( O \)-protection (see section 4.2.2) yielded 1-thiosugar 81b (figure 4.65).

\[
\begin{array}{cccc}
\text{AcO} & \text{OAc} & \text{DdeNH} \\
\text{AcO} & \text{OAc} & \text{Br} & \text{KSAc} & \text{TBAHS} & \text{AcO} & \text{OAc} & \text{DdeNH} & \text{SAc} \\
60 & \text{H}_2\text{NNH}_2 & \text{H}_2\text{NNH}_2 & \text{AcO} & \text{OAc} & \text{DdeNH} & \text{SH}
\end{array}
\]

**Figure 4.65:** Synthesis of 1-thiosugars via 1-thioacetates

Reaction of 1-thiosugar 78 with tosyl derivative 43a in the presence of sodium hydride in DMF proved successful, yielding glycolipid 82a (figure 4.66). However, these conditions required low temperatures and had the disadvantage that
acetate groups were lost during the reaction, requiring an additional re-acetylation step.

\[
\begin{array}{c}
\text{SH} \\
\text{\textbf{78}}
\end{array}
\xrightarrow{\text{NaH}}
\begin{array}{c}
\text{O} \\
\text{\textbf{43a}} \\
\text{S-CH₂-CH-NHBoc}
\end{array}
\]

\[
\begin{array}{c}
\text{\textbf{82a}} \\
\text{CH₃}
\end{array}
\]

\text{Figure 4.66: Synthesis of glycolipid by S-alkylation with NaH}

The use of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), as described by Ono and co-workers\textsuperscript{215} for simple aromatic thiols, led to considerable disulphide formation. Milder reaction conditions, such as those utilised by Witczak and Boryczewski\textsuperscript{216}, involved the use of a catalytic quantity of triethylamine in CH₂Cl₂. This method proved satisfactory, yielding glycolipids \textbf{82a} and \textbf{83a} from respectively 1-thioglucose \textbf{78} and 1-thiogalactose \textbf{79} in good yield. However, the syntheses of the tosyl- and iodo-derivatives of the LAAs were poor yielding, as previously described (see section 4.3.5.5.1).

4.4.4 S-linked glycolipids via a Mitsunobu reaction

The methods described thus far to synthesise S-linked glycolipids were satisfactory. The poor yielding synthesis of tosyl- and iodo- derivatives of the LAAs, and the side-reaction of disulphide formation led to the search for an alternative approach.

The Mitsunobu reaction\textsuperscript{217}, pioneered by Mitsunobu and co-workers, has proven useful in a wide range of synthetic applications involving alcohols\textsuperscript{209}. The original reaction, utilising a diethyl azodicarboxylate (DEAD)-triphenylphosphine (PPh₃) system, is a versatile procedure for the conversion of alcohols to other functionalities, such as esters. It is generally accepted that the reaction takes place in three steps as outlined in figure 4.67\textsuperscript{209}.

The first step (figure 4.67) involves the reaction of DEAD and PPh₃ in either CH₂Cl₂ or THF. This proceeds rapidly and smoothly and can be followed visually since a coloured solution becomes colourless.
Results & Discussion

Step One: Adduct formation

\[
\text{EtO—C—N=N—C—OEt} + \text{Ph3P} \rightarrow \text{EtO—C—N=N—C—OEt} \oplus \text{Ph3PO}
\]

Step Two: Alcohol activation

\[
\text{OH} \quad \text{EtO—C—N=N—C—OEt} \oplus \text{Ph3PO} \quad \text{HX} \rightarrow \text{EtO—C—NH—NH—C—OEt}
\]

Step Three: S_N2 Reaction

\[
\text{OPPh}_3 \oplus \text{X} \oplus \text{R}_1 \quad \text{R}_2 \quad \rightarrow \quad \text{X} \oplus \text{R}_1 \quad \text{R}_2 \quad \text{O=PPh}_3
\]

**Figure 4.67**: Proposed mechanism for the Mitsunobu reaction

The second step involves activation of the alcohol. The structure of the activated species is subject to debate. Mitsunobu originally proposed the oxyphosphonium salt structure\(^{209}\) (figure 4.67). However, more recent studies\(^{228}\) suggest that a pentavalent phosphorane may be present in equilibrium with the oxyphosphonium salt. Nevertheless, it has been suggested that the oxyphosphonium salt is most likely the key intermediate involved in the displacement of triphenylphosphine oxide leading to the product\(^{209}\).

The final step involves a simple S\(_{N2}\) reaction whereby the oxyphosphonium species undergoes nucleophilic attack.
The major use of the Mitsunobu reaction has been to invert optically pure secondary alcohols via formation of inverted esters. Variants of the reaction have emerged that extend its utility by replacing the original acidic component with alternative nucleophiles, to form alkyl halides, amines and carbon-carbon bonds. In addition, acylthiols have been used to generate carbon-sulphur bonds.

Lipoamino thiols 47a and 47b were synthesised by reaction of lipoamino alcohols 9a and 9b with thiolacetic acid under Mitsunobu conditions (see section 4.3.5.5.1). Problems were experienced, however, with the purification of these compounds. The triphenylphosphine oxide by-product could not be separated from the reaction mixture and frequently co-eluted with the desired product on purification by column chromatography. This has been noted by others, and it has been suggested that this is due to hydrogen bonding and hydrophobic effects\(^{218}\).

Various methods have been devised to combat this problem, including the use of modified phosphines (e.g. 1,2-bis[diphenylphosphino]ethane)\(^{319}\) as a substitute for triphenylphosphine, which has been used in approximately 90% of reported Mitsunobu reactions\(^{209}\). An alternative system, using 1,1′-(azodicarbonyl)dipiperidine (ADDP)\(^{220}\) in place of DEAD was considered.

It was our intention to use Mitsunobu conditions to synthesise \(S\)-linked glycolipids by reaction of 1-thiosugars with lipoamino alcohols. This would avoid the need for synthesis of halide derivatives from the LAAs and would avoid the need for the use of base in the reaction (minimising disulphide formation).

The use of ADDP and trimethylphosphine (PMe\(_3\)) represented a significant improvement, since the trimethylphosphine oxide by-product could be removed from the reaction mixture on aqueous work-up. \(S\)-linked glycolipids were synthesised under these revised Mitsunobu conditions\(^{5}\) (figure 4.68).

PMe\(_3\) was added to a solution of ADDP in THF. A yellow solution turned colourless after 30 minutes, indicating the completion of the first stage (figure 4.68). The nucleophile, in this case the thiosugar, was not added at the outset since thiols readily alkylate ADDP (and DEAD). After addition of the alcohol, a precipitate began to form, which was later identified as being the reacted ADDP. The 1-thiosugar was subsequently added, with complete conversion generally observed.

within 2 hours. Washing of the reaction mixture with water led to removal of trimethylphosphine oxide, allowing for simple product purification.

**Step One:** Adduct formation

```
\[ \text{N\text{-}C\text{-}N\text{-}N\text{-}C\text{-}N} + \text{Me}_3\text{P} \rightarrow \text{N\text{-}C\text{-}N\text{-}N\text{-}C\text{-}N} \]
```

**Step Two:** Alcohol activation

```
\text{Boc}\text{HN} - \text{CH} - \text{CH}_2\text{OH} \quad \text{(CH}_2\text{)_9} \quad \text{CH}_3 \quad 9\text{a}
```

\[ \rightarrow \text{Boc}\text{HN} - \text{CH} - \text{CH}_2\text{OPMe}_3 \quad \text{(CH}_2\text{)_9} \quad \text{CH}_3 \]

**Step Three:** S\text{\textsubscript{N}2} Reaction

```
\text{AcO} - \text{OAc} - \text{OAc} - \text{OAc} - \text{SH} \quad \text{Boc}\text{HN} - \text{CH} - \text{CH}_2\text{OPMe}_3 \quad \text{(CH}_2\text{)_9} \quad \text{CH}_3
```

\[ \rightarrow \text{AcO} - \text{OAc} - \text{OAc} - \text{SH} - \text{CH}_2\text{NHBOc} + \text{O\text{-}PMe}_3 \quad \text{(water soluble)} \]

*Figure 4.68:* Synthesis of $S$-linked glycolipids via revised Mitsunobu conditions

$S$-linked glycolipids of glucose, galactose and $N$-acetyl glucosamine were synthesised in excellent yields via this procedure.
In addition, novel S-linked glycolipids were synthesised from the 1-thiosugar of N-Dde glucosamine 81b. Simple straight chain alcohols, such as ethanol and octanol afforded glycolipids 86 and 87 respectively in excellent yields. In addition, a triethylene glycol derivative 88 was synthesised by reaction with triethylene glycol monomethyl ether. Each of these compounds has a protected amine function at C-2 of the sugar.

\[
\begin{array}{|c|c|c|c|}
\hline
R_1 & R_2 & R_3 & n \\
\hline
82a & H & OAc & OAc & 9 \\
82b & H & OAc & OAc & 11 \\
83a & OAc & H & OAc & 9 \\
83b & OAc & H & OAc & 11 \\
84a & H & OAc & NHAc & 9 \\
84b & H & OAc & NHAc & 11 \\
\hline
\end{array}
\]

The Dde protected amine at position C-2 of these conjugates could be de-N-protected to yield a free amine suitable for conjugation to drugs and peptides. Alternatively, the protection could be removed to allow attachment of a LAA derivative.

This approach was used to synthesise 90 from methyl thioglycoside 85. Interestingly, removal of the Dde group from the two anomers of methyl thioglycoside 85 proceeded at different rates (figure 4.69).
The amine of the α anomer was de-protected readily, with complete de-protection observed within 1 hour, using 2% hydrazine in CH₂Cl₂ to give 89. However, the amine of the β anomer was stable to these conditions for several hours and required higher concentrations (10%) and longer reaction times, risking loss of O-acetate protecting groups. It is possible that an intramolecular interaction between the sulphur atom and a carbonyl group of the Dde structure was the reason for this difficulty.

\( N\)-Boc protected LAA \( 2b \) was coupled to 89 via a DCC-mediated coupling to yield glycolipid 90 in fair yield.

4.4.4.2 Synthesis of disulphides under Mitsunobu conditions

The method described previously for the synthesis of S-linked glycolipids was adapted for the synthesis of the novel disulphide-linked derivative 91.
LAA thiol derivative 47a (see section 4.3.5.5.1) was reacted with the ADDP-PMe₃ adduct as previously described. Thiosugar 80 was subsequently added to the solution, leading to the successful formation of disulphide 91 (figure 4.70).

Hummel and Hindsgaul later described a reaction in which 1-thiosugars were "protected" using ethanethiol under similar conditions. Ethanethiol alkylated DEAD (in the absence of a phosphine), followed by subsequent reaction with the 1-thiosugar.

It is therefore possible in this case that LAA derivative 47a alkylated ADDP, followed by nucleophilic attack by the 1-thiosugar, rather than formation of a phosphonium ion as previously described.

4.4.5 Influence of S-linkage on physicochemical properties

The S-linked glycolipids were generally more lipophilic than their O- and N-linked isosteres, as indicated by the synthesised compounds (with identical lipid component) listed in table 4.6.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Linkage*</th>
<th>ClogP**</th>
</tr>
</thead>
<tbody>
<tr>
<td>17b</td>
<td>O</td>
<td>1.59</td>
</tr>
<tr>
<td>29a</td>
<td>Amide</td>
<td>1.14</td>
</tr>
<tr>
<td>84a</td>
<td>S</td>
<td>2.03</td>
</tr>
<tr>
<td>91</td>
<td>S-S</td>
<td>2.97</td>
</tr>
</tbody>
</table>

*for exact structure, refer to main text; ** refers to fully de-protected compound
Compound 88, derived from the Dde-protected glucosamine, has a ClogP value of $-1.88$ when fully de-protected, compared to a liposaccharide with a straight-chain lipid, 87, having a ClogP value of 1.41.

The ethylene glycol-based glycolipids are thus relatively hydrophilic, while sharing structural properties similar to the respective straight-chain compounds (figure 4.71).

Figure 4.71: Energy minimised structures of 87 (upper) and 88 (lower), when fully de-protected

Key: C(light blue); H(white); O(red); N(dark blue); S(yellow)

The final section of work describes attempts made to synthesise C-linked glycolipids.
4.5 C-linked Glycolipids

C-linked glycosides are the most stable glycosides with resistance to both chemical and enzymatic degradation. The synthesis of C-linked glycolipids based on the LAAs presented a major but exciting challenge. Established methods to synthesise C-glycosides were generally applicable only to simple molecules (see section 3.4.1).

4.5.1 Free radical-based synthesis of C-linked glycolipids

Our first approach to the synthesis of these compounds was via a spacer molecule, which was carbon-linked to the sugar. Once de-protected, attachment of a LAA was possible. The spacer molecule was an unsaturated derivative, synthesised from the simple amino acid serine. This dehydroalanine unit could then be attached to a sugar, via a carbon linkage, using a free-radical based approach.

A convenient method for the synthesis of dehydroalanine was via the β-elimination of serine. Srinivasan and co-workers\textsuperscript{222} described a reaction whereby serine was treated with phosphorous pentachloride to yield the β-chloroalanine unit, which was subsequently converted to the unsaturated compound by elimination using a tertiary amine as a base. This method produced the desired compound in good yield, but was a two-stage process with difficult purification steps to remove reagent side-products\textsuperscript{223}.

![Figure 4.72: Synthesis of dehydro amino acids](attachment:image.png)
Miller described a simple isourea-mediated procedure, whereby a protected serine derivative was converted into an O-alkylisourea by the CuCl-catalysed reaction with a carbodiimide (figure 4.72). Attempts to isolate the desired isoureas from the reactions, however, gave only the elimination products.

The carboxylic acid group of N-Boc serine was protected by both benzyl and methyl esters. The methyl ester was introduced by reaction of serine hydrochloride with HCl in methanol, followed by N-Boc protection to yield 92. The benzyl ester was introduced from N-Boc serine 93 via formation of a caesium salt, which was reacted with benzyl bromide to give 94 (figure 4.73).

![Figure 4.73: Synthesis of protected serine derivatives](image)

The dehydroalanine derivatives 95 and 96 were subsequently obtained from 92 and 94 respectively, using the method previously described.
C-glycosides were synthesised by reaction of the bromosugar derivative of galactose 19 with a protected dehydroalanine derivative, in the presence of a hydride source, tri-butyltin hydride (Bu\textsubscript{3}SnH) and a radical initiator, AIBN\textsuperscript{225} (figure 4.74).

![Figure 4.74: Radical-based synthesis of C-glycosides](image)

The first step is generation of a free radical species by the initiator (figure 4.75). AIBN gives rise to two carbon-based radicals with evolution of nitrogen at raised temperatures. These radicals then react with the hydride source, Bu\textsubscript{3}SnH, to give a tin-based radical, which attacks the bromosugar\textsuperscript{157,226}.

![Figure 4.75: Mechanism of radical-based C-glycosylation](image)
The desired sugar radical has two fates. It can either react with dehydroalanine to produce a C-glycoamino acid radical, or it can react with another Bu$_3$SnH molecule to give a reduced sugar. The C-glycoamino acid radical is subsequently reduced by Bu$_3$SnH to give the desired product.

C-glycoamino acids were synthesised successfully using this approach. Diastereomeric mixtures were obtained, which made determination of the anomeric configuration impossible by conventional means. However, experiments have demonstrated that the most favourable conformation of the glycosyl radical is the twist boat (figure 4.76), leading to the axial orientation in the chair conformation$^{226}$.

![Figure 4.76: Twist boat conformation of the glycosyl radical](image)

Polymerisation was observed when the concentration of dehydroalanine 95 was high (figure 4.77). Analysis by mass spectrometry revealed that up to hexamers ($n=5$, figure 4.77) were formed.

![Figure 4.77: Polymers of C-glycoside 97](image)
The mass spectrum extract (figure 4.78) reveals the following peaks:

- 573 \([M_1+K]^+\) \(n=0\) monomer
- 756 \([M_2+Na]^+\) \(n=1\) dimer
- 958 \([M_3+Na]^+\) \(n=2\) trimer
- 1159 \([M_4+Na]^+\) \(n=3\) tetramer
- 1359 \([M_5+Na]^+\) \(n=4\) pentamer
- 1560 \([M_6+Na]^+\) \(n=5\) hexamer

Figure 4.78: MALDI TOF mass spectrum extract for polymers of compound 97

Compounds 97 and 98 were de-N-protected using trifluoroacetic acid, yielding 99a and 100a respectively. These compounds were each subsequently coupled via a DCC mediated reaction to LAA 2b (as previously described) to give C-linked glycolipids 99b and 100b.

```
  AcO  OAc
 / \ /  \
\ AcO AcO
    |    |
CH₂—CH—NH—HBOc CH—NHBOc
    |     |
    COOR  (CH₂)₁₁
    CH₃
```

<table>
<thead>
<tr>
<th></th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>99b</td>
<td>Me</td>
</tr>
<tr>
<td>100b</td>
<td>Bn</td>
</tr>
</tbody>
</table>
4.5.2 Synthesis of C-linked glycolipids from glyconolactones

Despite having successfully synthesised a C-linked glycolipid, we attempted to synthesise a true isostere of the O-, N-, and S-linked compounds previously described (i.e. a sugar-LAA conjugate linked without a spacer).

Our approach was to attempt to synthesise C-glycolipids via a Wittig olefination, such as that described by Xie and co-workers, in which a glyconolactone was converted to a glycosylidene by reaction with a triphenylphosphine Wittig reagent.

Attempts were made to prepare the glyconolactones of glucose and glucosamine. Acetate protection was unsuitable for these reactions and was replaced by benzyl protection. The glyconolactone of glucose was prepared by oxidation of the commercially available 2,3,4,6-tetra-O-benzyl-D-glucopyranose (i.e. the 1-OH benzylated sugar) with pyridinium chlorochromate (PCC) in excellent yield (figure 4.79).

\[
\text{Figure 4.79: Synthesis of glucono-1,5-lactone}
\]

The synthesis of the glyconolactone of N-acetyl glucosamine, however, was more problematic. The basis for the synthesis was 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-D-glucopyranose. The first step was to introduce a protecting group at the anomeric hydroxyl that would be stable to the conditions of de-O-acetylation. The acetate at that position was selectively cleaved using hydrazine acetate as previously described to give 11 (see section 4.2.2). tert-Butyldimethylsilyl (TBDMS) protection was introduced in fair yield by reaction with tert-butyldimethylchlorosilane (TBDMS-Cl) in the presence of imidazole to give 102. This was de-O-acetylated smoothly to give 103 (figure 4.80). Attempts to benzylate this compound, however, led only to mixtures of partially benzylated products.
Results & Discussion

Problems have been described elsewhere regarding the benzylation of derivatives of N-acetyl glucosamine\(^\text{228}\).

\[
\begin{array}{c}
\text{AcO} & \text{AcO} & \text{AcNH} \\
\text{O} & \text{OH} & \text{O} \\
\text{OAc} & \text{AcNH} & \text{OTBDMS} \\
\end{array}
\rightarrow
\begin{array}{c}
\text{TBDMS-Cl} \\
\text{NaOMe} \\
\text{MeOH} \\
\end{array}
\begin{array}{c}
\text{AcO} & \text{OAc} & \text{AcNH} \\
\text{O} & \text{OH} & \text{O} \\
\text{OAc} & \text{AcNH} & \text{OTBDMS} \\
\end{array}
\]

\text{Figure 4.80: Attempted synthesis of glyconolactone of N-acetyl glucosamine}

A Wittig reagent (triphenylphosphorane) was prepared from the iodo-derivative 45a of LAA 2a\(^\text{145}\).

Previously, lipoamino alcohol 9a was transformed into the tosyl derivative 43a, followed by halogen exchange (see section 4.3.5.5.1) to yield iodo compound 45a. This procedure was relatively low yielding and required additional purification steps.

The modified Mitsunobu conditions utilised to synthesise S-linked glycolipids were applied to the synthesis of alkyl halides. It was found that lipoamino alcohol 9a could be transformed directly to the alkyl iodide 45a using methyl iodide under Mitsunobu conditions (figure 4.81).

\[
\begin{array}{c}
\text{BocHN—CH—CH}_2\text{OH} \\
\text{(CH}_2\text{)}_9 \\
\text{CH}_3 \\
9a \\
\end{array}
\rightarrow
\begin{array}{c}
\text{BocHN—CH—CH}_2\text{I} \\
\text{(CH}_2\text{)}_9 \\
\text{CH}_3 \\
45a \\
\end{array}
\]

\text{Figure 4.81: Synthesis of alkyl halides via a Mitsunobu reaction}
The triphenylphosphorane 104 was prepared by heating iodo derivative 45a in triphenylphosphine for 12 hours at 120°C (figure 4.82)\textsuperscript{145}.

![Figure 4.82: Synthesis of Triphenylphosphorane from alkyl iodide](image)

However, attempts to form a C-linked glycosylidene by reaction of 101 and 104 under Wittig conditions proved unsuccessful.

### 4.5.3 Synthesis of C-linked glycolipids from their S-linked isosteres

Our final approach to the synthesis of C-linked glycolipids utilised the previously described S-linked compounds, which were synthesised with ease and in excellent yield via modified Mitsunobu conditions (section 4.4.4). These S-linked compounds were transformed into their C-linked isosteres using a Ramberg-Bäcklund rearrangement reaction\textsuperscript{229}.

The Ramberg-Bäcklund rearrangement, the base-mediated conversion of α-halogenated sulphones into alkenes (figure 4.83), has been the subject of considerable research since it was first described in 1940\textsuperscript{230,231,232}.

![Figure 4.83: The Ramberg-Bäcklund Rearrangement](image)

Meyer and co-workers later described conditions in which the sulphone underwent \textit{in situ} halogenation\textsuperscript{233} (figure 4.84). High control of stereochemistry (formation of \textit{cis}- or \textit{trans}- isomers) has been described as not being a feature of the reaction.
Results & Discussion

Figure 4.84: Meyer's modification of the Ramberg-Bäcklund rearrangement

Difficulties in by-product formation were largely overcome by the use of conditions described by Chan and co-workers. Alumina-supported KOH, together with CBr₂F₂ instead of CCl₄ were found to lead to the formation of simple alkyl and aryl substituted alkenes in excellent yield.

Taylor and co-workers later described the synthesis of exo-glycals from simple methyl and benzyl 1-thioglycosides, via a Ramberg-Bäcklund rearrangement (figure 4.85). Transformation of these compounds into simple C-glycosides was described, via procedures such as radical addition and hydroboration.

Continuing this work, Campbell and co-workers described the synthesis of a C-linked glycoamino acid from an exo-glycal. Although successful, this synthesis involved 11 separate steps to produce the final unsaturated C-glycoside (excluding synthesis of the amino acid derivative). This included protection of the sugar, formation of the methyl 1-thioglycoside (2 steps), oxidation to the sulphone, Ramberg-Bäcklund rearrangement to form the exo-glycal, hydroboration followed by
oxidation (2 steps) to yield an alcohol, transformation into the alkyl iodide, coupling to the thiol derivative of the amino acid, oxidation of the newly formed sulphide to the sulphone and finally a second Ramberg-Bäcklund rearrangement to form the unsaturated C-glycoamino acid.

We were able to apply Ramberg-Bäcklund chemistry to the synthesis of LAA based C-linked glycolipids, in considerably fewer reaction steps than previously reported.

S-linked glycolipid 82a was de-O-acetylated to yield 105. The glycolipid was subsequently benzyl protected smoothly using benzyl bromide and NaH to give 106 (acetate protection was incompatible with the basic conditions of the Ramberg-Bäcklund rearrangement). The sulphone 107 was obtained by reaction of 106 with 3-chloroperoxybenzoic acid (mCPBA) in good yield (figure 4.86).

Figure 4.86: Synthesis of a Benzyl-protected glycosyl sulphone
A Ramberg-Bäcklund rearrangement reaction was performed on sulphone 107. CBr₂F₂ was added to the sulphone and alumina-supported KOH at 5°C. Conversion to the alkene 108 was observed within 3 hours. The product was reduced by catalytic hydrogenation over Pd/C (with simultaneous removal of the benzyl protecting groups) to give 109. The target compound was subsequently re-acetylated (for analytical purposes) to yield 110 (figure 4.87).

Figure 4.87: Synthesis of a LAA-based C-linked glycolipid

The exact configuration of compound 110 was determined by NMR spectroscopy. The individual protons were assigned by spin-spin decoupling.
results, an alternative to the 2D COSY experiments described earlier (see section 4.4).

An NMR spectrum extract for compound 110 is shown in Figure 4.88.

![Figure 4.88: NMR spectrum extract for compound 110](image)

This extract of the spectrum shows the 7 sugar protons, the amine proton and the α-carbon (with 2 signals due to the diastereomers). We could broadly deduce that the signals at 5.16, 5.04 and 4.84 corresponded to a combination of H-2, H-3 and H-4. The signal at 4.47 was due to the amine and the multiplet signal at 4.20 corresponded to the two H-6 protons. H-1, was expected at approx 3.5 – 4.

As an example of the experiments carried out, decoupling the signal at 4.84 affected the signal at 5.16 and the signal at 3.53 (Figure 4.89 – before decoupling, and Figure 4.90 – after decoupling). It appears that the signals at 5.16 and 3.53 have essentially each changed from triplets to doublets. Since we suspected that 4.84 would be either H-2, H-3 or H-4 and we expected H-1 to appear at 3.5 – 4, we could deduce that the decoupled proton was H-2 and that the affected signal at 5.16 was H-3 and that at 3.53 was H-1.

Similar experiments enabled the complete structure to be assigned (see Experimental, section 6). In addition, the anomeric configuration was established as being 1,2-trans (i.e. β-linked).

---

* The NMR decoupling experiments for compound 110 were performed by Dr. Istvan Jablonkai, Chemical Research Center, Hungarian Academy of Sciences.
The Ramberg-Backlund rearrangement proved to be an invaluable means by which to prepare C-glycosides in highly facile and effective manner.
5 Conclusion

A series of lipoamino acid-based glycolipids was successfully synthesised, incorporating oxygen-, nitrogen-, sulphur- and carbon-linked glycosides. The physicochemical properties of these amphipathic molecules were altered by varying the nature of the saccharide component (the nature of the sugar and the number of sugar units), by varying the lipid component (the nature of the lipid and the length of alkyl chain) and by varying the nature of the glycosidic linkage. The compounds each possessed either an amine group or a carboxylic acid (or both) that could be used for attachment to poorly absorbed drugs and peptides.

N-linked glycolipids were synthesised from glycosylamines, via carbodiimide-mediated couplings to lipoamino acids, incorporating disaccharides and trisaccharides in addition to monosaccharides. The relative difficulty of purification of these compounds, together with need for the hazardous glycosyl azide reduction step and the extreme instability of glycosylamines led to the search for alternative procedures. Amide linked glycolipids were subsequently synthesised directly from glycosyl azides using a modified Staudinger reaction. These reaction conditions enabled the facile synthesis of N-glycolipids in one step, without the need for hydrogenation.

Conditions were found to synthesise glycosyl isothiocyanates in excellent yield. These versatile compounds were the source of a series of conjugates with unusual linkages, further influencing physicochemical properties and perhaps stability. Novel N-linked glycolipids were prepared with thiocarbamate, dithiocarbonate and thiourea linkages.

Glycolipids of glucosamine were also synthesised, with the potential for a free amine group at position C-2 of the sugar, normally acetate protected in N-acetyl glucosamine. This was achieved by application of the hydrazine-labile Dde group to sugar-amine protection. Dde was also found to be highly useful in protection of the lipoamino acid series. Glucuronic acid was used to prepare glycolipids linked through the carboxylic acid (at position C-6) of the sugar, as opposed to the anomeric hydroxyl.

The preparation of S-linked glycolipids, which would possess a greater degree of chemical and biological stability, proved problematic. Established
procedures were generally unsuitable for the synthesis of these compounds, with low yields frequently experienced and disulphide formation common.

The novel Mitsunobu conditions developed here enabled the desired glycolipids to be synthesised in a facile manner with excellent yields. The advantages included the mild, neutral reaction conditions (minimising the potential for disulphide formation) and the fact that a lipoamino alcohol could be used directly in $S$-glycoside formation, without the need to generate an alkyl halide. These conditions were also found to be useful in the synthesis of a novel glycolipid with an unusual disulphide linkage. There are very few instances of such compounds in the literature.

The synthesis of $C$-linked glycolipids provided, as expected, the greatest challenge. Most published methods for the synthesis of these compounds were either for the conjugation of very simple molecules or specific to molecules of a certain type. The procedures were found to be generally unsuitable for the synthesis of the desired glycolipids.

$C$-linked glycolipids via a spacer were synthesised by reaction of glycosyl halides with dehydroalanine, in a free radical-mediated reaction. These reactions often required difficult purification steps since polymer formation was impossible to eliminate. Attachment of a lipoamino acid to the $C$-linked glycosylserine derivative furnished $C$-linked glycolipids. However, a true isostere of the previously synthesised $O$-, $N$- and $S$-linked compounds was desired.

The Ramberg-Bäcklund rearrangement allowed transformation of the synthesised $S$-glycolipids into $C$-linked derivatives. These reaction conditions provided a simple but highly effective method for novel $C$-glycoside formation. The resulting glycosylidene was reduced by catalytic hydrogenation, furnishing the desired product in excellent yield.

Experiments have demonstrated the ability of these amphipathic liposaccharides to form particulate systems *per se* and also their ability to be incorporated into liposomal systems. These compounds thus have potential to form novel colloidal systems in which flexibility is possible regarding size and the type of system formed, with further applications for drug and peptide delivery.

Preliminary *in vivo* oral absorption experiments demonstrated the potential application of these compounds to drug delivery. An ionic complex between
piperacillin (a β-lactam antibacterial with zero oral bioavailability) and an N-linked glycolipid was successfully prepared. When administered to rats in vivo, significant oral absorption of piperacillin was observed. A microbiological assay confirmed that complex formation did not adversely affect antimicrobial activity.

Summary and Future Work

The synthesised glycolipids represent a series of novel compounds with a range of physicochemical properties dependent upon the nature of the sugar, the lipid and the linkage between them. The methodology developed in this thesis allows for the simple and efficient synthesis of N-, S- and C-linked glycolipids in high yields.

The use of a modified Staudinger reaction to prepare amide-linked conjugates and later the use of isothiocyanates provide facile access to N-glycosides. The Mitsunobu conditions utilised in the synthesis of S-glycolipids proved generally applicable with a wide range of glycosyl acceptors. This type of reaction provides a new route to thioglycosides, with mild, neutral conditions. The use of the Ramberg-Bäcklund rearrangement in the synthesis of C-linked conjugates transformed an impossible task into perhaps the one of the most straightforward. The reaction itself is fast, clean and easily purified with the catalyst on an alumina support. This approach to C-glycoamino acid synthesis is an exciting one, and worthy of future research.

The application of these methods to the modification of medicinal peptides in solid-phase chemistry is a potential area of future research. The application of the modified Staudinger reaction to the synthesis of C-terminal modified N-linked glycopeptides has already proved successful (see publications arising from this work). The novel synthesis of S- and C-linked glycolipid-modified peptides on a solid support would be an extremely challenging and exciting project.

The chemical and enzymatic stability of the synthesised glycolipids would reflect the nature of the glycosidic linkage, ranging from the least stable O-linked compounds to the most stable C-linked conjugates. Investigations as to the stability of these compounds in vivo would be a logical next step, and such experiments are planned for the future to gain information such as the influence of the type of linkage, and also the effect of anomic configuration on susceptibility to
degradation by glycosidase enzymes. These properties could have applications for a pro-drug approach if the glycolipid were covalently conjugated to the drug or peptide.

The use of these conjugates for the delivery of drugs by ionic complex formation is an exciting area of research. The preliminary in vivo data presented in this thesis demonstrate the potential usefulness of this approach. Evidence to confirm that the drug is indeed transported as an ion-pair is required however, and further study is planned for the future. This method of delivery is to be investigated in experiments using Caco-2 cell monolayers, where the mechanism of transport can be examined. The influence of active transport systems and the possible involvement of the P-glycoprotein efflux system will be considered.

The use of lipoamino acid-based glycolipids as a novel drug delivery system has considerable potential for improving the bioavailability of poorly absorbed drugs and peptides. Covalent and non-covalent conjugation to a wider range of drugs and peptides, and further in vivo studies are planned for the future.
6 Experimental

6.1 General Methods

All moisture sensitive reactions were carried out under a nitrogen atmosphere using oven-dried glassware. Solvents were dried over standard drying agents and freshly distilled prior to use. Reactions were carried out at room temperature unless otherwise specified. Purification was achieved by column chromatography through Sorbsil C60-H40/60, using the mobile phases indicated for thin layer chromatography unless otherwise specified.

Reaction progress was monitored by thin layer chromatography on Kieselgel 60 F$_{254}$ using the mobile phases indicated. Visualisation was achieved by UV light and by charring with sulphuric acid. The ion exchange resin used was Amberlite IR-120(H$^+$).

NMR spectra were recorded at room temperature for CDCl$_3$ solutions (unless otherwise indicated). $^1$H NMR spectra were recorded using a Bruker AM 500 instrument operating at a field of 500 MHz. Where available, $^{13}$C spectra were recorded using a Bruker AM 250 instrument with dual proton and carbon-13 probe, using an Aspect 3000 computer, operating at a field of 62.9MHz. Chemical shifts are reported in ppm downfield from internal TMS.

Mass spectra were obtained using a VG Analytical ZAB-SE instrument using Fast Atom Bombardment (FAB) techniques – 20kV Cs$^+$ ion bombardment, with 2µl of appropriate matrix, either 3-nitrobenzyl alcohol (MNOBA) or thioglycerol with NaI (MeOH) solution added where necessary to produce natriated species when no protonated molecular ions were observed. Alternatively, spectra were obtained using a Finnigan MassLab Navigator quadropole mass spectrometer, using electrospray ionisation. (N$_2$ flow, 300 L/h; temperature, 180°C; cone voltage, 49V).

Analytical RP-HPLC was carried out on a Vydac C$_4$ Protein column (25.0 cm x 4.6 mm). Separation was achieved using a linear gradient at a flow-rate of 1.2 ml/min effected by a Waters 600S controller and 616 pump running Solvent A: 0.1% TFA; Solvent B: 0.1% TFA in 90% MeCN; 0% B to 70% B over 20 min, then 70% B to 0% B over 5 min. HPLC grade MeCN and water were filtered through a 23 micrometre membrane filter and degassed with helium flow prior to use. Separation
was monitored with a Waters 486 absorbance detector at 214 nm. Retention time ($R_t$) and purity were determined using Waters Millennium Chromatography Manager software.

### 6.2 Particle Formation

These experiments were performed by Dr. Anya Hillery, University of Brighton. Glycolipids 115b, 116, 117b and 118b were studied alone or in varying molar ratios (1:1, 1:2, 1:3 or 1:6) with dimyristoyl lecithin (DMPC: Lipid Products). The compounds were dissolved in either CHCl$_3$ or methanol:CHCl$_3$ 1:1 (v/v), followed by evaporation under vacuum at 50°C. To ensure complete removal of solvent, films were flushed with oxygen-free nitrogen (3 min). Dry films were re-hydrated at 50°C using either PBS or distilled water. Suspensions were either centrifuged (1300 g for 15 min) and the pellet washed three times with PBS or were passed down a gel exclusion column (Sephadex G50; Pharmacia) using PBS as eluent. Transmission electron microscopy (TEM) was carried out on a Phillips XL 20 scanner using negative staining with 1% phosphotungstic acid. To confirm the presence of the glycolipids in the vesicles, vesicular fractions obtained by passing the various suspensions down the gel exclusion columns were lyophilised and re-suspended in DMSO-$d_6$ or CDCl$_3$ and $^1$H NMR spectra were obtained.

### 6.3 Microbiological Assay

The minimum inhibitory concentrations (MICs) of piperacillin and piperacillin-glycolipid complex 114 were determined. Five species of bacteria were used in these experiments, namely *Bacillus subtilis*, *Escherichia coli*, *Klebsiella aerogenes*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. 1µl samples of culture, and 1µl samples from $10^2$- and $10^4$-fold dilutions (in the broth in which they had been grown) were inoculated onto a series of plates containing solid nutrient media with decreasing concentrations of the test compounds. Inoculation was performed using a Denley Multipoint Inoculator (Denley Products, Billinghamurst, UK). The plates were

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subsequently incubated at 37°C for 24 hours under aerobic conditions. The MICs were determined as the lowest concentration of antibacterial that inhibited visible growth of an organism.

6.4 Synthesised compounds

2-(R/s)\-[(\textit{t}ert\-Butyloxycarbonyl)amino\]dodecanoic acid (2a)

Diethyl acetamidomalonate (81.3 g, 0.375 mol) was added to a stirred solution of sodium (8.40 g, 0.365 mol) in abs. ethanol (300 ml). 1-bromodecane (110 g, 0.498 mol, 105 ml) was then added to the solution. The reaction mixture was refluxed for 24 hours. After evaporation of the solvent, the oily residue was taken up in ethyl acetate (500 ml) and washed with water (1 x 500 ml) and brine (1 x 500 ml). The solution was then dried over MgSO₄, filtered and evaporated. The resulting oil was dissolved in concentrated hydrochloric acid (600 ml) and DMF (70 ml) and refluxed for 48 hours. On completion, the reaction mixture was poured onto ethanol:water 3:1 (750 ml). A solid product was precipitated from ammonia, filtered off and washed with ether (2 x 100 ml). The solid lipoamino acid [2-(R/s)-aminododecanoic acid] \( \text{1a} \) was then suspended in \textit{t}ert\-butanol:water 2:3 (900 ml) and the pH corrected to 11. Di-\textit{t}ert\-butyl dicarbonate (101 g, 0.463 mol) was then added to the solution, which was subsequently stirred for 48 hours. The solution was diluted with water (360 ml) and made pH 3 by addition of potassium hydrogensulphate. The product was extracted into ethyl acetate (500 ml) and was washed with brine (1 x 500 ml). The solution was then dried over MgSO₄, filtered and evaporated. Re-crystallisation from acetonitrile gave \( \text{2a} \) (96.2 g, 82%).

\[ R_f = 0.41 \text{ hexane:ethyl acetate 4:1 (v/v); } \]

\( ^1\text{H NMR} \delta 4.99 \text{ (s, 1H, NH), 4.30 \text{ (m, 1H, } \alpha\text{CH), 1.42 \text{ (s, 9H, 3 x Boc CH}_3\text{), 1.20 - 1.29 \text{ (m, 18H, 9CH}_2\text{), 0.86 \text{ (t, 3H, CH}_3\text{);}} \]

FAB MS \( \text{C}_{17}\text{H}_{33}\text{O}_4\text{N} \) (315.45) m/z (%) 316 [M+H]⁺ (27), 338 [M+Na]⁺ (95), 216 [M-Boc+H]⁺ (68).

Lipoamino acids \( \text{2b-2d} \) were synthesised from the respective bromoalkanes, using the procedure described for \( \text{2a} \):
Experimental

2-(R/S)-[(tert-Butoxycarbonyl)amino]tetradecanoic acid (2b)

R_f = 0.26 hexane:ethyl acetate 4:1 (v/v); yield 68%; 1H NMR δ 5.00 (s, 1H, NH), 4.28 (m, 1H, αCH), 1.40 (s, 9H, 3 x Boc CH3), 1.24 (m, 22H, 11CH2), 0.87 (t, 3H, CH3); FAB MS C19H37O4N (343.50) m/z (%) 344 [M+H]^+ (20), 366 [M+Na]^+ (80), 243 [M-Boc+H]^+ (75).

2-(R/S)-[(tert-Butoxycarbonyl)amino]hexadecanoic acid (2c)

R_f = 0.41 hexane:ethyl acetate 4:1 (v/v); 1H NMR δ 4.32 (m, 1H, αCH), 1.43 (s, 9H, 3 x Boc CH3), 1.22 (m, 26H, 13CH2), 0.86 (t, 3H, CH3); FAB MS C21H41O4N (371.55) m/z (%) 372 [M+H]^+ (27), 394 [M+Na]^+ (70), 272 [M-Boc+H]^+ (40).

2-(R/S)-[(tert-Butoxycarbonyl)amino]octadecanoic acid (2d)

R_f = 0.39 hexane:ethyl acetate 4:1 (v/v); 1H NMR δ 5.01 (m, 1H, NH), 4.28 (m, 1H, αCH), 1.42 (s, 9H, 3 x Boc CH3), 1.23 (m, 28H, 15CH2), 0.87 (t, 3H, CH3); FAB MS C23H45O4N (399.61) m/z (%) 400 [M+H]^+ (37), 422 [M+Na]^+ (20), 300 [M-Boc+H]^+ (80).

2-(1-hydroxyethylidene)-5,5-dimethylcyclohexane-1,3-dione [Dde-OH] (3)

Method A (established procedure)

4-Dimethylaminopyridine [DMAP] (19.2 g, 0.157 mol) was dissolved in abs. CH2Cl2 (300 ml). Acetic anhydride (16.0 g, 0.157 mol) was added to the solution, which was stirred for 15 minutes. Dimehdone (20.0 g, 0.143 mol) was added and the solution stirred for 12 hours. The reaction mixture was washed with 5% HCl(aq) (2 x 250 ml). The product was then extracted into IM NaOH (2 x 200 ml). Ethyl acetate was added to the separating funnel and concentrated HCl was added to precipitate the product from the aqueous phase, which was subsequently shaken to transfer it to the organic phase. The organic phase was then washed with brine (2 x 200 ml). The solution was then dried over MgSO4, filtered and evaporated. The product solidified on cooling to give 3 (18.2 g, 70%).

Method B (modified procedure)

Acetic anhydride (52.5 g, 0.514 mol) and triethylamine (43.3 g, 0.429 mol) were added to a solution of DMAP (5.23 g, 42.9 mmol) in abs. CH2Cl2 (500 ml), which
was stirred for 15 minutes at 0°C. Dimedone (60.0 g, 0.429 mol) was added and the solution stirred at room temperature for 12 hours. The reaction mixture was diluted with CH₂Cl₂ (250 ml) and washed with 5% HCl(aq) (3 x 500 ml). The solution was then dried over MgSO₄, filtered and evaporated. The product was precipitated from hexane to give 3 (55.3 g, 71%).

Rₛ = 0.84 hexane:ethyl acetate 2:1 (v/v); ¹H NMR δ 2.59 (s, 3H, NH C=C(NH)CH₃), 2.52, 2.35 (2s, 4H, 2CH₂), 1.06 (s, 6H, 2CH₃); FAB MS C₁₀H₁₄O₃ (182.22) m/z (%) 183 [M+H]⁺ (100).

N-Dde Lipoamino acids 4a-4c were synthesised from the respective un-protected lipoamino acids, using the procedure described for 4b:

2-(R/s)-[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl]amino)dodecanoic acid (4a)

Rₛ = 0.54 chloroform:methanol 10:0.7 (v/v); ¹H NMR δ 13.65 (d, 1H, NH), 9.55 (s, 1H, COOH), 4.40 (m, 1H, αCH), 2.53 (s, 3H, C=C(NH)CH₃), 2.39 (2s, 4H, 2CH₂), 1.90 (m, 2H, βCH₂), 1.23 (m, 16H, 8CH₂), 1.03 (s, 6H, 2CH₃); FAB MS C₂₂H₃₇NO₄ (379.53) m/z (%) 380 [M+H]⁺ (100), 402 [M+Na]⁺ (20), 334 [M-COOH]⁺ (25).

2-(R/s)-[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl]amino]tetradecanoic acid (4b)

2-(R/s)-aminotetradecanoic acid 1b (9.60 g, 39.5 mmol) was suspended in abs. ethanol (300 ml). Dde-OH 3 (7.55 g, 41.5 mmol) and triethylamine (3.99 g, 39.5 mmol) were added to the suspension, which was subsequently refluxed for 12 hours. The clear solution was evaporated to dryness and the oily residue taken up in CH₂Cl₂ (200 ml) and washed with 5% HCl(aq) (2 x 200 ml). The solution was then dried over MgSO₄, filtered and evaporated. The product was precipitated from hexane to give 4b (13.5 g, 84%).

Rₛ = 0.74 chloroform:methanol 10:1 (v/v); ¹H NMR δ 13.75 (d, 1H, NH), 4.40 (m, 1H, αCH), 2.54 (s, 3H, C=C(NH)CH₃), 2.42 (2s, 4H, 2CH₂), 1.89 (m, 2H, βCH₂), 1.24 (m, 20H, 10CH₂), 1.04 (s, 6H, 2CH₃); FAB MS C₂₄H₄₁NO₄ (407.59) m/z (%) 408 [M+H]⁺ (100), 430 [M+Na]⁺ (45), 362 [M-COOH]⁺ (35).
2-(R/s)-{[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl]amino}hexadecanoic acid (4c)

R<sub>f</sub> = 0.52 chloroform:methanol 10:0.5 (v/v); ¹H NMR δ 13.65 (d, 1H, NH), 9.45 (s, 1H, COOH), 4.45 (m, 1H, αCH), 2.51 (s, 3H, C=CH(CH<sub>3</sub>)), 2.38 (2s, 4H, 2CH<sub>2</sub>), 1.85 (m, 2H, βCH<sub>2</sub>), 1.22 (m, 24H, CH<sub>2</sub>), 1.00 (s, 6H, CH<sub>3</sub>); FAB MS C<sub>26</sub>H<sub>45</sub>NO<sub>4</sub> (435.64) m/z (%) 436 [M+H]<sup>+</sup> (100), 458 [M+Na]<sup>+</sup> (10), 390 [M-COOH]<sup>+</sup> (15).

1,2,3,4,6-penta-O-acetyl-α-D-glucopyranose (5)

α/β-D-glucopyranose (50.0 g, 0.278 mol) was suspended in acetic anhydride (250 ml). Iodine (2.50 g [50 mg per gram sugar]) was then added to the reaction mixture which was stirred at 0°C. After 30 minutes, the reaction became exothermic and darkened. After 2 hours, the solution was evaporated and co-evaporated with toluene and benzene. The product was recrystallised from ethyl acetate:hexane 2:1 (v/v) to give 5 (96.1 g, 89%).

R<sub>f</sub> = 0.47 hexane:ethyl acetate 1:1 (v/v); ¹H NMR δ 6.33 (d, 1H, H-1, J<sub>1,2</sub>=3.6 Hz), 5.47 (t, 1H, H-2), 5.14 (t, 1H, H-3), 5.09 (dd, 1H, H-4), 4.26, 4.11 (2m, 3H, H-6, H-6', H-5), 2.18, 2.09, 2.04, 2.02, 2.01 (5s, 15H, 5Ac); FAB MS C<sub>16</sub>H<sub>22</sub>O<sub>11</sub> (390.34) m/z (%) 413 [M+Na]<sup>+</sup> (20), 523 [M+Cs]<sup>+</sup> (10), 331 [M-OAc]<sup>+</sup> (97).

1,2,3,4,6-penta-O-acetyl-β-D-galactopyranose (6)

Procedure as for 8, re-crystallisation from hexane:ethyl acetate 1:2 (v/v).

R<sub>f</sub> = 0.47 hexane:ethyl acetate 1:1 (v/v); yield 79%; ¹H NMR δ 6.13 (d, 1H, H-1, J<sub>1,2</sub>=8.8 Hz), 5.44 (d, 1H, H-4), 5.35 (dd, 1H, H-3), 5.09 (dd, 1H, H-2), 4.19 - 4.03 (m, 3H, H-6, H-6', H-5), 2.18, 2.11, 2.06, 2.05, 2.00 (5s, 15H, 5Ac); FAB MS C<sub>16</sub>H<sub>22</sub>O<sub>11</sub> (390.34) m/z (%) 413 [M+Na]<sup>+</sup> (45), 523 [M+Cs]<sup>+</sup> (40), 331 [M-OAc]<sup>+</sup> (100).

1,2,3,4,6-penta-O-acetyl-α/β-D-mannopyranose (7)

α/β-D-mannopyranose (3.00 g, 16.7 mol) was suspended in acetic anhydride (20 ml). Iodine (75.0 mg) was then added to the reaction mixture which was stirred at 0°C. After 30 minutes, the reaction became exothermic and darkened. After 1 hour, the
solution was evaporated and co-evaporated with toluene and benzene to an oily residue. The product was concentrated to give 7 as a yellow oil (5.62 g, 87%).

\[ R_f = 0.15 \text{ hexane:ethyl acetate 3:2 (v/v); } ^1\text{H NMR } \delta 6.09 (d, 1H, H-1\alpha), J_{1,2}=1.8 \text{ Hz}, \]
\[ 5.48 (d, 1H, H-1\beta), J_{1,2}=3.8 \text{ Hz}), 5.36 - 5.26 (m, 3H, H-2, H-3, H-4), 4.32 - 4.04 (m}, \]
\[ 3H, H-6, H-6', H-5), 2.21, 2.17, 2.09, 2.05, 2.01 (5s, 15H, 5Ac); \]
\[ \text{FAB MS } C_{16}H_{22}O_2 (390.34) m/z (\%) 413 [M+Na]^+ (25), 331 [M-OAc]^+ (100). \]

2-acetamido-1,3,4,6-tetra-O-acetyl-2-deoxy-\( \beta \)-d-glucopyranose (8)

\( \alpha/\beta \)-D-Glucosamine hydrochloride (40.0 g, 0.186 mmol) was suspended in pyridine (320 ml). Acetic anhydride was added slowly to the reaction mixture, which was subsequently stirred for 12 hours. The product was concentrated, taken up in CH\( \text{Cl}_2 \) (400 ml) and washed with 5% HCl(aq) (1 x 500 ml) and water (1 x 500 ml). The organic phase was dried over MgSO\( _4 \), filtered and evaporated to give a white solid foam. The product was recrystallised from ethyl acetate:hexane 2:1 (v/v) to give 8 (65.0 g, 90%).

\[ R_f = 0.30 \text{ hexane:ethyl acetate 1:3 (v/v); } ^1\text{H NMR } \delta 6.13 (d, 1H, NH), 5.74 (d, 1H, H-1, J_{1,2}=9.0 \text{ Hz}), 5.22 - 5.14 (m, 2H, H-3, H-4), 4.45 (m, 1H, H-2), 4.20, 4.02 (2m, 2H, H-6, H-6''), 3.97 (m, 1H, H-5), 2.15, 2.10, 2.04, 2.00, 1.90 (5s, 15H, 5Ac); \]
\[ \text{FAB MS } C_{16}H_{23}NO_10 (389.35) m/z (\%) 412 [M+Na]^+ (70), 552 [M+Cs]^+ (100), 330 [M-OAc]^+ (100). \]

tert-butyl N-[1-(\( \alpha /\beta \)-s)-(hydroxymethyl)undecyl]carbamate (9a)

Procedure as for 9b, Method C (using \( \alpha \text{a} \) in place of \( \alpha \text{b} \)).

\[ R_f = 0.50 \text{ hexane:ethyl acetate 4:1 (v/v); yield 87%; } ^1\text{H NMR } \delta 3.65 - 3.48 (m, 3H, \alpha \text{CH, CH}_2), 1.43 (s, 9H, 3 x Boc CH}_3), 1.24 (m, 18H, 9CH}_2), 0.86 (t, 3H, CH}_3); \]
\[ \text{FAB MS } C_{17}H_{35}NO_3 (301.46) m/z (\%) 302 [M+H]^+ (15), 324 [M+Na]^+ (5), 434 [M+Cs]^+ (10), 202 [M-Boc+H]^+ (95). \]

tert-butyl N-[1-(\( \alpha /\beta \)-s)-(hydroxymethyl)tridecy]carbamate (9b)

Method A

4-methylmorpholine (470 mg, 4.65 mmol) and ethyl chloroformate (505 mg, 4.65 mmol) were added to a stirred solution of 2-(\( \alpha /\beta \)-s)-[(tert-
butoxycarbonyl)amino]tetradecanoic acid 2b (1.60 g, 4.65 mmol) in abs. THF (20 ml) at -15°C. After 15 minutes, sodium borohydride (528 mg, 14.0 mmol) was added in one portion. Methanol (60 ml) was added dropwise to the solution at 0°C over 15 minutes. After additional stirring for 20 minutes, the reaction was quenched with 5% HCl(aq). The organic phase was evaporated, taken up in CH₂Cl₂ (40 ml) and washed with brine (2 x 40 ml). The solution was then dried over MgSO₄, filtered and evaporated. Purification by column chromatography gave 9b (998 mg, 65%).

Method B

Benzotriazol-1-yloxytris(pyrrolidino)phosphonium hexafluorophosphate [pyBOP] (166 mg, 0.319 mmol) and N,N-diisopropylethylamine (45.1 mg, 0.350 mmol) were added to a solution of 2-(R/s)-[(tert-butoxycarbonyl)amino]tetradecanoic acid 2b (100 mg, 0.292 mmol) in absolute THF (5 ml). After 10 minutes, sodium borohydride (11.0 mg, 0.290 mmol) was added. After 1 hour, the reaction was quenched with 5% HCl(aq). The organic phase was evaporated, taken up in CH₂Cl₂ (20 ml) and washed with brine (2 x 20 ml). The solution was then dried over MgSO₄, filtered and evaporated. Purification by column chromatography gave 9b (52.0 mg, 54%).

Method C

2-(R/s)-[(tert-butoxycarbonyl)amino]tetradecanoic acid 2b (1.00 g, 2.92 mmol) in abs. THF (3 ml) was added slowly dropwise to BH₃-THF complex (1.0M, 5.8 ml, 5.80 mmol) at 0°C. After stirring for 2 hours, the reaction mixture was quenched with 10% acetic acid in methanol (v/v) and evaporated. The residue was taken up in CH₂Cl₂ (10 ml) and washed with 1M KHSO₄(aq) (1 x 20 ml) and brine (2 x 20 ml). The solution was then dried over MgSO₄, filtered and evaporated. Purification by column chromatography gave 9b (821 mg, 86%).

Rₛ = 0.82 chloroform:methanol 10:1 (v/v); ¹H NMR δ 3.72 – 3.48 (m, 3H, αCH, CH₂), 1.40 (s, 9H, 3 x Boc CH₃), 1.25 (m, 22H, 11CH₂), 0.86 (t, 3H, CH₃); FAB MS C₁₉H₃₉NO₃ (329.52) m/z (%) 330 [M+H]+ (6), 352 [M+Na]+ (10), 462 [M+Cs]+ (8), 230 [M-Boc+H]+ (100).

tert-butyl N-[1-(R/s)-(hydroxymethyl)pentadecyl]carbamate (9c)

Procedure as for 9b, Method C (using 2c in place of 2b).
Experimental

R$_f$ = 0.72 chloroform:methanol 10:0.7 (v/v); yield 82%; $^1$H NMR $\delta$ 3.69 - 3.45 (m, 2H, $\alpha$CH, CH$_2$)$_2$), 2.97 (m, 1H, CH$_2$), 1.41 (s, 9H, 3 x Boc CH$_3$), 1.25 (m, 18H, 13CH$_2$), 0.88 (t, 3H, CH$_3$); FAB MS C$_{21}$H$_{43}$NO$_3$ (357.32) m/z (%) 380 [M+Na]$^+$ (15), 258 [M-Boc+H]$^+$ (100).

Pentafluorophenyl 2-(R/S)-[[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl]-amino]dodecanoate (10)

2-(R/S)-[[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl]amino]dodecanoic acid 4a (5.00 g, 13.2 mmol) and dicyclohexylcarbodiimide [DCC] (3.25 g, 15.8 mmol) were dissolved in absolute CH$_2$Cl$_2$ (25 ml) and stirred at 0°C for 15 minutes. Pentafluorophenol (2.91 g, 15.8 mmol) was added to the solution, which was stirred for 12 hours. The solvent was evaporated and the product used without purification. R$_f$ = 0.47 hexane:ethyl acetate 2:1 (v/v); $^1$H NMR $\delta$ 4.70 (m, 1H, $\alpha$CH), 2.59 (s, 3H, C=C(NH)C$_3$), 2.44, 2.38 (2s, 4H, 2CH$_2$), 1.25 (m, 18H, 9CH$_2$), 1.04 (s, 6H, 2CH$_3$), 0.87 (t, 3H, CH$_3$); FAB MS C$_{28}$H$_{36}$F$_5$NO$_4$ (545.58) m/z (%) 546 [M+H]$^+$ (97).

2-acetamido-3,4,6-tri-0-acetyl-2-deoxy-$\alpha$/p-D-glucopyranose (11)

2-acetamido-1,3,4,6-tetra-0-acetyl-2-deoxy-$\beta$-D-glucopyranose 8 (389 mg, 1.00 mmol) was dissolved in DMF (15 ml). Hydrazine acetate (110 mg, 1.20 mmol) was added to the solution, which was stirred at 50°C for 15 minutes. The solvent was evaporated and the residue was taken up in CH$_2$Cl$_2$ (20 ml). The solvent was washed with brine (2 x 20 ml). The solution was then dried over MgSO$_4$, filtered and evaporated to give 11 (320 mg, 92%). R$_f$ = 0.14 hexane:ethyl acetate 1:9 (v/v); $^1$H NMR $\delta$ 5.58 (d, 1H, H-1, $J_{1,2}$=8.0 Hz), 4.54 - 4.32 (m, 3H, H-3, H-4, NH), 3.51 - 3.44 (m, 3H, H-2, H-6, H-6'), 3.33 (m, 1H, H-5), 4.20, 4.02 (2m, 2H, H-6, H-6'), 3.97 (m, 1H, H-5), 2.15, 2.04, 2.02, 2.00, (4s, 12H, 4Ac); FAB MS C$_{14}$H$_{21}$NO$_9$ (347.32) m/z (%) 348 [M+H]$^+$ (90), 370 [M+Na]$^+$ (40), 480 [M+Cs]$^+$ (20), 330 [M-OAc]$^+$ (100).
Experimental

\[O-(2\text{-acetamido-3,4,6-tri-O-acetyl-2-deoxy-\(\alpha/\beta\)-D-glucopyranosyl)}\]

\text{trichloroacetiminate (12)}

Sodium hydride (51.0 mg, 1.29 mmol) was added to 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-\(\alpha/\beta\)-D-glucopyranose 11 (320 mg, 0.920 mmol), trichloroacetonitrile (530 mg, 3.70 mmol) and molecular sieves (100 mg) at 0°C. The solution was stirred for 2 hours. The reaction mixture was subsequently passed through a celite pad and evaporated. The residue was purified by column chromatography to give 12 (340 mg, 76%).

\[R_f = 0.45 \text{ hexane:ethyl acetate 5:95 (v/v)}; \quad \text{\textsuperscript{1}H NMR } \delta 8.78 (d, 1H, C=NH), 6.35 (d, 1H, NH), 5.65 (d, 1H, H-1, \text{J}_{1,2}=8.5 \text{ Hz}), 5.32 - 5.22 (m, 2H, H-3, H-4), 4.52 (m, 1H, H-2), 4.23 (m, 1H, H-6), 4.10 (m, 2H, H-5, H-6'), 2.15, 2.07, 2.05, 1.98, (4s, 12H, 4Ac); \quad \text{FAB MS } C_{16}H_{21}Cl_3N_2O_9 (491.70) m/z (\%) 516 [M+Na]^+ (5), 623, 625 [M+Cs]^+ (48, 50).\]

\[2\text{-methyl-(3,4,6-tri-O-acetyl-1,2-dideoxy-\(\alpha\)-D-glucopyranosyl)-[2',1':4,5]-2-oxazoline (13)}\]

Boron trifluoride etherate (430 mg, 3.00 mmol) was added to a stirred solution of 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-\(\alpha/\beta\)-D-glucopyranosyl trichloroacetiminate 12 (730 mg, 1.50 mmol), tert-butyl \text{N-[1-(R/S)-(hydroxymethyl)undecyl]carbamate 9a}} (300 mg, 1.00 mmol) and molecular sieves (500 mg) in \(\text{CH}_2\text{Cl}_2\) (30 ml) at 0°C slowly over 20 minutes. After addition was completed, the mixture was stirred at room temperature for 1 hour. The reaction mixture was then filtered through a celite pad, and washed with NaHCO\(_3\) (sat, aq) (1 x 30 ml) and water (1 x 30 ml). The solution was then dried over \(\text{MgSO}_4\), filtered and evaporated. The residue was purified by column chromatography in chloroform:methanol 95:5 (v/v) to give only 13 as a by-product.

\[R_f = 0.11 \text{ ether:ethyl acetate 7:3 (v/v)}; \quad \text{\textsuperscript{1}H NMR } \delta 5.95 (d, 1H, H-1), 5.25 (m, 1H, H-4), 4.91 (m, 1H, H-3), 4.17 - 4.11 (m, 3H, H-2, H-6, H-6'), 3.60 (m, 1H, H-5), 2.15 (s, 3H, N=C(CH\(_3\))), 2.09, 2.07, 2.06, (3s, 9H, 3Ac); \quad \text{FAB MS } C_{14}H_{19}NO_8 (329.30) m/z (\%) 330 [M+H]^+ (98), 352 [M+Na]^+ (20), 462 [M+Cs]^+ (10).\]
1,3,4,6-tetra-\(O\)-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-\(\alpha/\beta\)-D-glucopyranose (14b)

2,2,2-Trichloroethoxycarbonyl chloride (Troc-Cl) (12.7 g, 59.9 mmol) was added dropwise at room temperature to a vigorously stirred solution of \(\alpha\)-D-glucosamine hydrochloride and NaHCO\(_3\) (12.6 g, 150 mmol) in water (150 ml). The solution was stirred for 1 hour. The reaction mixture was then neutralised with 1M HCl (50 ml) and evaporated. The residue (white powder, 14a) was dissolved in pyridine (50 ml) and acetic anhydride (25 ml) and was stirred for 12 hours. Following evaporation, the residue dissolved in CH\(_2\)Cl\(_2\) (200 ml) and was washed with 1M HCl\(_{aq}\) (1 x 200 ml), water (1 x 200 ml) and sat. NaHCO\(_3\) (1 x 200 ml). The organic phase was dried over MgSO\(_4\), filtered and evaporated to give 14b (22.6 g, 72%) as white foamy crystalline material.

\[ R_f = 0.31 \text{ hexane:ethyl acetate 1:1 (v/v); } \]  \( ^1 \text{H NMR } \delta \text{ 6.22 (d, 1H, NH), 5.27 - 5.16 (m, 3H, H-1, H-3, H-4), 4.80, 4.60 (2d, 2H, Cl}_{3}\text{CCCH}_2, 4.27 - 4.10 (m, 2H, H-2, H-6), 4.06 - 3.90 (m, 2H, H-5, H-6'), 2.19, 2.10, 2.03, 2.02 (4s, 12H, 4Ac); FAB MS } \text{C}_{17}\text{H}_{22}\text{Cl}_{3}\text{NO}_{11} (522.71) \	ext{m/z (%) 546 [M+Na]^+ (18), 462 [M-OAc]^+ (43).} \]

3,4,6-tri-\(O\)-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-\(\alpha/\beta\)-D-glucopyranose (15)

1,3,4,6-tetra-\(O\)-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-\(\alpha/\beta\)-D-glucopyranose 14b (3.10 g, 5.99 mmol) and hydrazine acetate (660 mg, 7.17 mmol) were stirred in abs. DMF (30 ml) at room temperature for 40 minutes. Following evaporation, the residue dissolved in CH\(_2\)Cl\(_2\) (80 ml) and was washed with brine (1 x 50 ml) and water (1 x 30 ml). The solution was dried over MgSO\(_4\), filtered and evaporated to give 15 (2.80 g, crude), which was used in the next reaction without further purification.

\[ R_f = 0.25 \text{ hexane:ethyl acetate 1:1 (v/v); } \]  \( ^1 \text{H NMR } \delta \text{ 5.35 - 5.31 (m, 2H, H-1, H-4), 5.12 (t, 1H, H-3), 4.80, 4.63 (2d, 2H, Cl}_{3}\text{CCCH}_2, 4.23 - 4.19 (m, 2H, H-2, H-6), 4.15 - 4.00 (m, 2H, H-5, H-6'), 2.09, 2.03, 2.00 (3s, 9H, 3Ac); FAB MS } \text{C}_{15}\text{H}_{20}\text{Cl}_{2}\text{NO}_{10} (480.68) \	ext{m/z (%) 502 [M+Na]^+ (17), 464 [M-OH]^+ (48), 302 [M-troc+H]^+ (93).} \]
Experimental

$O$-[3,4,6-tri-$O$-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-$\alpha$/-$\beta$-$D$-glucopyranosyl] trichloroacetimidate (16)

Sodium hydride (0.32 g, 8.10 mmol) was added to a mixture of 3,4,6-tri-$O$-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-$\alpha$/-$\beta$-$D$-glucopyranose 15 (2.80 g, 5.83 mmol), trichloroacetonitrile (5.05 g, 34.9 mmol) and molecular sieves (500 mg) at 0°C. The reaction was then stirred for 2 hours at room temperature. The solution was subsequently filtered through a celite pad, evaporated and the residue was purified by column chromatography in hexane:ethyl acetate 6:4 (v/v) to give 16 (1.70 g, 47%).

$R_f = 0.46$ hexane:ethyl acetate 1:1 (v/v); $^1$H NMR $\delta$ 6.42 (m, 1H, H-1, $J_1$,$_2$=3.2 Hz), 5.35 - 5.20 (m, 3H, H-3, H-4, NH), 4.70 (d, 2H, Cl$_3$CCH$_2$), 4.29 - 4.25 (m, 2H, H-2, H-6), 4.15 - 4.10 (m, 2H, H-5, H-6'), 2.09, 2.05, 2.03 (3s, 9H, 3Ac); FAB MS C$_{17}$H$_{20}$Cl$_3$N$_2$O$_{10}$ (625.06) m/z (%) 648 [M+Na]$^+$ (8), 461 [M-OC(NH)CCl$_3$]$^+$ (44), 301 [M-troc+H]$^+$ (100).

tert-Butyl 1-[[3,4,6-tri-$O$-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-$\beta$-$D$-glucopyranosyloxy]methyl]-(R/s)-undecylcarbamate (17a)

$O$-[3,4,6-tri-$O$-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-$\alpha$-$D$-glucopyranosyl] trichloroacetimidate 16 (125 mg, 0.20 mmol), tert-butyl $N$-[1-(R/S)-(hydroxymethyl)undecyl]carbamate 9a (45.0 mg, 0.150 mmol) and molecular sieves (200 mg) were stirred in abs. CH$_2$Cl$_2$ (5 ml) for 15 minutes. Boron trifluoride etherate (64.0 mg, 0.451 mmol) in abs. CH$_2$Cl$_2$ (3 ml) was added dropwise at 0°C over 20 minutes. The mixture was stirred for 2 hours at room temperature. The reaction mixture was then diluted with CH$_2$Cl$_2$ (10 ml) and filtered through a Celite pad. The solution was washed with NaHCO$_3$ (sat,aq) (1 x 10 ml) and water (1 x 10 ml). The organic layer was dried over MgSO$_4$, filtered and evaporated. The residue was purified by column chromatography using hexane:ethyl acetate 6:4 (v/v) to give 17 (40.0 mg, 35%).

$R_f = 0.35$ hexane:ethyl acetate 1:1 (v/v); $^1$H NMR $\delta$ 5.28 - 5.21 (m, 2H, H-3, H-4), 4.79, 4.63 (2m, 2H, Cl$_3$CCH$_2$), 4.56 (d, 1H, H-1, $J_1$,$_2$=8.2 Hz), 4.25, 4.14 (2m, 2H, H-6, H-6'), 3.82 (m, 1H, H-2), 3.70 - 3.55 (m, 4H, H-5, $\alpha$CH, CH$_2$), 2.16, 2.08, 2.02 (3s, 9H, 3Ac), 1.44 (s, 9H, 3 x Boc CH$_3$), 1.28 - 1.23 (m, 18H, 9CH$_2$), 0.87 (t, 3H,
Experimental

CH₃); FAB MS C₃₂H₅₅Cl₃N₂O₁₂ (764.13) m/z (%) 787 [M+Na]+ (100), 462 [M-
lipid]+ (75), 663 [M-Boc+H]+ (70).

tert-Butyl 1-[(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyloxy)-
methyl]-(r/s)-undecylcarbamate (17b)

tert-Butyl 1-[(3,4,6-tri-O-acetyl-2-deoxy-2(2,2,2-trichloroethoxycarbonyl-amino)-
β-D-glucopyranosyloxy)methyl]-(r/s)-undecylcarbamate 17a (27.0 mg, 0.0353
mmol) was dissolved in acetic anhydride (1 ml) into which activated zinc powder
(4.6 mg, 0.0706 mmol) had been added. The reaction was stirred for 6 hours, after
which it was filtered and evaporated (and co-evaporated with benzene and toluene).
The residue was purified by column chromatography to give 17b (11 mg, 49%).
Rf = 0.17 hexane:ethyl acetate 1:1 (v/v); ¹H NMR δ 5.24 – 5.16 (m, 2H, H-3, H-4),
4.51 (d, 1H, H-1, J₁₂=8.5 Hz), 4.27, 4.11 (2m, 2H, H-6, H-6'), 3.72 (m, 1H, H-2),
3.71 – 3.57 (m, 4H, H-5, aCH, CH₂), 2.16, 2.08, 2.02, 1.96 (4s, 12H, 4Ac), 1.43 (s,
9H, 3 x Boc CH₃), 1.29 – 1.24 (m, 18H, 9 % ), 0.87 (t, 3H, CH₃); FAB MS
C₃₁H₅₄N₂O₁₁ (630.77) m/z (%) 653 [M+Na]+ (60), 531 [M-Boc+H]+ (90).

2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide (18)

Procedure as for 19, Method B.
Rf = 0.61 hexane:ethyl acetate 1:1 (v/v); yield 93%; ¹H NMR δ 6.52 (d, 1H, H-1,
J₁₂=3.6 Hz), 5.46 (d, 1H, H-3), 5.38 (dd, 1H, H-4), 4.94 (dd, 1H, H-2), 4.44 (t, 1H,
H-6'), 4.12 (m, 2H, H-6, H-5), 2.15, 2.10, 2.05, 1.97 (4s, 12H, 4Ac); FAB MS
C₁₄H₁₉BrO₉ (411.20) m/z (%) 433, 435 [M+Na]+ (34, 31), 543, 545 [M+Cs]+ (71,
69), 331 [M-Br]+ (80).

2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl bromide (19)

Method A
Hydrogen bromide in acetic acid (45%, 2 ml) was added to a stirred suspension of
α/β-D-galactopyranose (2.00 g, 11.1 mmol) suspended in acetic anhydride (10 ml) at
room temperature. After 8 hours, a further portion of HBr in acetic acid (45%, 10
ml) was added to the solution, which was subsequently stirred for a further 8 hours.
The clear solution was evaporated and co-evaporated with toluene and benzene. The
Experimental

oil was then taken up in CH$_2$Cl$_2$ (cold, -15°C, 50 ml), washed with water (3 x 50 ml) and NaHCO$_3$(sat, aq) (1 x 50 ml). The organic phase was dried over MgSO$_4$, filtered and evaporated. Purification by column chromatography gave 19 (2.89 g, 63%).

Method B

Acetic anhydride (1 ml) was added to HBr in acetic acid (45%, 12 ml) and allowed to stir for 30 minutes. 1,2,3,4,6-penta-O-acetyl-α/β-D-galactopyranose 6 (6.00 g, 15.4 mmol) was then dissolved in a minimal quantity of absolute CH$_2$Cl$_2$, added to the solution and stirred for 2 hours. The reaction mixture was then diluted with CH$_2$Cl$_2$ (cold, -15°C, 100 ml), washed with water (3 x 300 ml) and NaHCO$_3$(sat, aq) (1 x 300 ml). The organic phase was dried over MgSO$_4$, filtered and evaporated. Purification by column chromatography gave 19 (6.05 g, 96%).

Method C

Trimethylsilyl bromide (785 mg, 5.13 mmol) and bismuth(in) bromide (29.0 mg, 0.0646 mmol) were added to a solution of 1,2,3,4,6-penta-O-acetyl-α/β-D-galactopyranose 6 (500 mg, 1.28 mmol) in CH$_2$Cl$_2$ (5 ml) and stirred for 1 hour. The reaction mixture was diluted with CH$_2$Cl$_2$ (cold, -15°C, 10 ml), washed with water (3 x 20 ml) and NaHCO$_3$(sat, aq) (1 x 20 ml). The organic phase was dried over MgSO$_4$, filtered and evaporated. Purification by column chromatography gave 19 (402 mg, 76%).

R$_f$ = 0.52 hexane:ethyl acetate 1:2 (v/v); $^1$H NMR δ 6.71 (d, 1H, H-1, J$_{1,2}$=3.5 Hz), 5.52 (d, 1H, H-4), 5.42 (dd, 1H, H-3), 5.03 (dd, 1H, H-2), 4.50 (t, 1H, H-6'), 4.16 (m, 2H, H-6, H-5); FAB MS C$_{14}$H$_{18}$BrO$_9$ (411.20) m/z (%) 433, 435 [M+Na]$^+$ (17, 16), 543, 545 [M+Cs]$^+$ (67, 65), 331 [M-Br$^+$] (100).

2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl bromide (20)

Procedure as for 19, Method B.

R$_f$ = 0.35 hexane:ethyl acetate 1:1 (v/v); yield 91%; $^1$H NMR δ 6.27 (d, 1H, H-1, J$_{1,2}$=1.4 Hz), 5.66 (dd, 1H, H-3), 5.35 (dd, 1H, H-2), 5.27 (m, 1H, H-4), 4.25 (m, 1H, H-6'), 4.12, 4.07 (2m, 2H, H-6, H-5), 2.17, 2.11, 2.06, 2.01 (4s, 12H, 4Ac); FAB MS C$_{14}$H$_{18}$BrO$_9$ (411.20) m/z (%) 411, 412 [M+H]$^+$ (30, 30), 433, 435 [M+Na]$^+$ (29, 29), 331 [M-Br$^+$] (100).

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Experimental

2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-glucopyranosyl chloride (21)

Method A

HCl gas was bubbled through acetyl chloride (150 ml) for 15 minutes at -15°C to form a saturated solution. 2-acetamido-2-deoxy-1,3,4,6-tetra-O-acetyl-α-D-glucopyranose 8 (3.00 g, 7.71 mmol) was then added to the reaction flask, which was sealed, allowed to equilibrate to room temperature and stored for 72 hours. The acetyl chloride was then removed by evaporation and co-evaporation with toluene and benzene to give 21, a solid white foam (1.77 g, 63%).

Method B

2-acetamido-2-deoxy-α-D-glucopyranose (15.0 g, 67.8 mmol) was suspended in acetyl chloride (65 ml) and stirred at 45°C for 12 hours. The acetyl chloride was then removed by evaporation and co-evaporation with toluene and benzene. The product was purified by column chromatography using chloroform:ethyl acetate 10:4 (v/v) to give 21 (15.9 g, 64%).

R_f = 0.65 hexane:ethyl acetate 4:1 (v/v); \[^1\]H NMR δ 6.17 (d, 1H, H-1, J_{1,2}=3.6 Hz), 5.88 (d, 1H, NH), 5.29 (t, 1H, H-3), 5.20 (m, 1H, H-4), 4.50 (m, 1H, H-2), 4.25, 4.10 (2m, 3H, H-6, H-6'), 2.09, 2.03, 2.02, 1.97 (4s, 12H, 4Ac); FAB MS C_{14}H_{20}ClNO_8 (365.76) m/z (%) 366 [M+H]^+ (100), 388 [M+Na]^+ (75), 331 [M-Cl]^+ (18).

2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl azide (22)

Procedure as for 23, Method B, except re-crystallisation from ethyl acetate:hexane 2:1 (v/v) gave 22 (7.87 g, 82%).

R_f = 0.55 hexane:ethyl acetate 1:1 (v/v); \[^1\]H NMR δ 5.21, 5.09 (2t, 2H, H-3, H-4), 4.94 (t, 1H, H-2), 4.65 (d, 1H, H-1, J_{1,2}=8.8Hz), 4.27, 4.15 (2m, 2H, H-6, H-6'), 3.81 (m, 1H, H-5), 2.09, 2.07, 2.02, 1.99 (4s, 12H, 4Ac); FAB MS C_{14}H_{19}N_3O_9 (373.32) m/z (%) 396 [M+Na]^+ (20), 331 [M-N_2]^+ (100).

2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl azide (23)

Method A

2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl bromide 19 (3.99 g, 9.71 mmol) was dissolved in CH_2Cl_2 (40 ml). Sodium azide (2.52 g, 38.8 mmol) and tetra-n-
butylammonium hydrogen sulphate (3.29 g, 9.71 mmol) were dissolved in NaHCO$_3$(sat, aq) (40 ml). The two phases were stirred thoroughly for 2 hours. The organic phase was then diluted with CH$_2$Cl$_2$ (200 ml), separated and washed with NaHCO$_3$(sat, aq) (2 x 200 ml), water (1 x 200 ml) and brine (1 x 200 ml). The organic phase was dried over MgSO$_4$, filtered and evaporated. Recrystallisation from ethyl acetate:hexane 1:1 (v/v) gave 23 (2.95 g, 82%).

Method B

1,2,3,4,6-penta-O-acetyl-α/β-D-galactopyranose 6 (10.0 g, 25.6 mmol) was dissolved in abs. CH$_2$Cl$_2$ (100 ml). Trimethylsilyl azide (7.38 g, 64.1 mmol) and tin(iv) chloride (3.34 g, 12.8 mmol) were added to the solution, which was then stirred overnight. The reaction mixture was then diluted with CH$_2$Cl$_2$ (250 ml), washed with 1M KF(aq) (1 x 250 ml), brine (1 x 250 ml) and NaHCO$_3$(sat, aq) (1 x 250 ml). The organic phase was dried over MgSO$_4$, filtered and evaporated. Recrystallisation from ethyl acetate:hexane 1:1 (v/v) gave 23 (8.62 g, 90%).

R$_f$ = 0.60 hexane:ethyl acetate 1:1 (v/v); $^1$H NMR δ 5.41 (d, 1H, H-4), 5.17 (m, 1H, H-2), 5.04 (m, 1H, H-3), 4.60 (d, 1H, H-1, J$_{1,2}$=8.7 Hz), 4.19 (m, 2H, H-6, H-6'), 4.00 (m, 1H, H-5), 2.15, 2.08, 2.05, 1.98 (4s, 12H, 4Ac); FAB MS C$_{14}$H$_{19}$N$_3$O$_9$ (373.32) m/z (%) 396 [M+Na]$^+$ (100).

2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl azide (24)

Procedure as for 23, Method B.

R$_f$ = 0.50 ethyl acetate; yield 87%; $^1$H NMR δ 5.70 (d, 1H, NH), 5.24 (t, 1H, H-3), 5.09 (t, 1H, H-4), 4.76 (d, 1H, H-1, J$_{1,2}$=9.1 Hz), 4.25, 4.16 (2m, 2H, H-6, H-6'), 3.90 (m, 1H, H-2), 3.79 (m, 1H, H-5), 2.09, 2.03, 2.02, 1.97 (4s, 12H, 4Ac); FAB MS C$_{14}$H$_{20}$N$_4$O$_8$ (372.33) m/z (%) 373 [M+H]$^+$ (100), 395 [M+Na]$^+$ (30), 330 [M-N$_3$]$^+$ (97).

2,3,4,6-tetra-O-acetyl-β-D-glucopyranosylamine (25)

Procedure as for 26.

R$_f$ = 0.35 hexane:ethyl acetate 1:1 (v/v); yield 83%; $^1$H NMR δ 5.26 (d, 1H, H-3), 5.16 - 5.03 (m, 2H, H-2, H-3), 4.12 (d, 1H, H-1, J$_{1,2}$=8.5 Hz), 4.12 (m, 2H, H-6, H-6'), 3.86 (m, 1H, H-5), 2.11, 2.06, 2.04, 2.01 (4s, 12H, 4Ac); FAB MS C$_{14}$H$_{21}$NO$_9$ (347.32) m/z (%) 370 [M+Na]$^+$ (80).
2,3,4,6-tetra-O-acetyl-β-D-galactopyranosylamine (26)
Palladium catalyst (10% on carbon, 20.0 mg) was added in one portion to a solution
of 2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl azide (500 mg, 1.34 mmol) in abs.
methanol (5 ml) under a hydrogen atmosphere (a small amount of abs. THF was
added to dissolve the sugar). The solution was allowed to stir for 12 hours. The
catalyst was subsequently filtered off, and the solvent evaporated. Purification by
column chromatography gave 26 (400 mg, 86%).

Rf = 0.30 hexane:ethyl acetate 8:7 (v/v); 1H NMR δ 5.40 (d, 1H, H-4), 5.04 (m, 2H,
H-2, H-3), 4.16 (d, 1H, H-1, J1,2 = 8.0 Hz), 4.10 (m, 2H, H-6, H-6′), 3.99 (m, 1H, H-
5), 2.14, 2.07, 2.06, 1.97 (4s, 12H, 4Ac); FAB MS C14H21NO9 (347.32) m/z (%) 370
[M+Na]+ (100).

2,3,4,6-tetra-O-acetyl-N-{1-(R/S)-[(tert-butoxycarbonyl)amino]dodecyl}-β-D-
glucopyranosylamide (27a)
Procedure as for 27c, (using 2a in place of 2d)

Rf = 0.87 chloroform:methanol 10:2.5 (v/v); yield 68%; 1H NMR δ 5.31 - 5.22 (m,
2H, H-1, H-3), 5.06 (m, 1H, H-4), 4.93 (m, 1H, H-2), 4.79 (br s, 1H, NH), 4.28 (m,
1H, H-6), 4.13 - 4.05 (m, 2H, H-6', αCH), 3.80 (m, 1H, H-5), 2.06, 2.03, 2.01, 2.00
(4s, 12H, 4Ac), 1.44 (s, 9H, 3 x Boc CH3), 1.28 - 1.23 (m, 18H, 9 CH2), 0.87 (t, 3H,
CH3); FAB MS C31H52N2O12 (644.75) m/z (%) 667 [M+Na]+ (10), 777 [M+Cs]+
(100), 545 [M-Boc+H]+ (15).

2,3,4,6-tetra-O-acetyl-N-{1-(R/S)-[(tert-butoxycarbonyl)amino]tetradecyl}-β-D-
glucopyranosylamide (27b)
Procedure as for 27c, (using 2b in place of 2d).

Rf = 0.42 hexane:ethyl acetate 1:1 (v/v); yield 64%; 1H NMR δ 5.28 (m, 2H, H-1,
H-3), 5.06 (m, 1H, H-4), 4.97 (m, 2H, H-2, NH), 4.26, 4.11 (2m, 2H, H-6, H-6′),
3.83 (m, 1H, H-5), 2.08, 2.04, 2.02, 1.99 (4s, 12H, 4Ac), 1.42 (s, 9H, 3 x Boc CH3),
1.25 (m, 22H, 11CH2), 0.86 (t, 3H, CH3); FAB MS C33H56N2O12 (672.80) m/z (%) 695
[M+Na]+ (40), 805 [M+Cs]+ (65), 573 [M-Boc+H]+ (95).
2,3,4,6-tetra-O-acetyl-N-{1-(R/S)-[(tert-butoxycarbonyl)amino]octadecyl}-β-D-glucopyranosylamide (27c)

2-(R/S)-[(tert-Butoxycarbonyl)amino]octadecanoic acid 2d (575 mg, 1.44 mmol) and EEDQ (428 mg, 1.72 mmol) were added to a stirred solution of 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosylamine 25 (500 mg, 1.44 mmol) in abs. THF (10 ml). The reaction was stirred at 40°C for 6 hours. After evaporation, the residue was purified by column chromatography to give 27c (733 mg, 70%).

R_f = 0.34 hexane:ethyl acetate 2:1 (v/v); 1H NMR δ 6.75 (d, 1H, NH), 5.25 (m, 2H, H-1, H-3), 5.07 (dd, 1H, H-4), 4.94 (dd, 1H, H-2), 4.78 (s, 1H, NH-C=O), 4.22, 4.06 (m, 2H, H-6, H-6'), 3.98 (m, 1H, αCH), 3.80 (m, 1H, H-5), 2.07, 2.04, 2.02, 2.00 (s, 12H, 4Ac), 1.44 (s, 9H, 3 x Boc CH_3), 1.24 (m, 30H, 1 5 % ), 0.88 (t, 3H, CH_3); FAB MS C_{37}H_{64}N_2O_{12} (728.91) m/z (%) 751 [M+Na]^+ (33), 861 [M+Cs]^+ (27), 629 [M-Boc+H]^+ (75).

2,3,4,6-tetra-O-acetyl-N-{1-(R/S)-[(tert-butoxycarbonyl)amino]dodecyl}-β-D-galactopyranosylamide (28a)

Procedure as for 28c, alternatively:

Method B

Triethylamine (66.0 mg, 0.653 mmol) was added to a stirred solution of 2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl isothiocyanate 34 (1.28 g, 3.29 mmol) and 2-(R/S)-[(tert-Butoxycarbonyl)amino]dodecanoic acid 2a (1.24 g, 3.95 mmol) in abs. toluene (15 ml). The reaction was refluxed for 12 hours. After evaporation, the residue was taken up in CH_2Cl_2 (20 ml) and washed with NaHCO_3 (sat, aq) (2 x 20 ml). The organic phase was dried over MgSO_4, filtered and evaporated. The product was purified by column chromatography in chloroform:ethyl acetate 10:3 (v/v) to give 28a (1.40 g, 66%).

R_f = 0.54 chloroform:methanol 10:0.2 (v/v); 1H NMR δ 5.52 (d, 1H, H-4), 5.16 (m, 3H, H-1, H-2, H-3), 4.75 (br, 1H, NH), 4.21, 4.09 (m, 4H, αCH, H-5, H-6, H-6'), 2.19, 2.06, 2.03, 1.99 (s, 12H, 4Ac), 1.45 (s, 9H, 3 x Boc CH_3), 1.26 (m, 18H, 9CH_2), 0.88 (t, 3H, CH_3); FAB MS C_{31}H_{52}N_2O_{12} (644.75) m/z (%) 667 [M+Na]^+ (65), 544 [M-Boc+H]^+ (55), 331 (40).
Experimental

2,3,4,6-tetra-O-acetyl-N-[1-(R/s)-(tert-butoxycarbonyl)amino]tetradecyl]-β-D-galactopyranosylamide (28b)

Procedure as for 28c (using 2b in place of 2c).

Rf = 0.38 hexane:ethyl acetate 1:1 (v/v); yield 69%; 1H NMR δ 5.53 (m, 1H, H-4), 5.25 - 5.13 (m, 3H, H-1, H-2, H-3), 4.20 - 4.11 (m, 4H, αCH, H-5, H-6, H-6''), 2.17, 2.04, 2.03, 2.00 (4s, 12H, 4Ac), 1.43 (s, 9H, 3 x Boc CH3), 1.26 (m, 22H, IICH2), 0.87 (t, 3H, CH3); FAB MS C33H56N2O12 (672.80) m/z (%) 695 [M+Na]+ (25), 573 [M-Boc+H]+ (100).

2,3,4,6-tetra-O-acetyl-N-[1-(R/s)-(tert-butoxycarbonyl)amino]hexadecyl]-β-D-galactopyranosylamide (28c)

2-(R/s)-(tert-Butoxycarbonyl)amino]hexadecanoic acid 2c (52.0 mg, 0.144 mmol) and DCC (43.0 mg, 0.209 mmol) were added to a stirred solution of 2,3,4,6-tetra-O-acetyl-β-D-galactopyranosylamine 26 (50.0 mg, 0.144 mmol) in abs. CH2Cl2 (5 ml). The reaction was stirred at 0°C for 2 hours. The reaction mixture was diluted with CH2Cl2 (20 ml) and washed with NaHCO3 (sat, aq) (2 x 20 ml). The organic phase was dried over MgSO4, filtered and evaporated. The residue was purified by column chromatography in hexane:ethyl acetate 1:1 (v/v) to give 28c (67.0 mg, 66%).

Rf = 0.40 ethyl acetate; 1H NMR δ 5.43 (d, 1H, H-4), 5.22 (m, 1H, H-3), 5.12 (m, 2H, H-1, H-2), 4.80 (br s, 1H, NH), 4.09 (m, 3H, αCH, H-6, H-6''), 4.02 (m, 1H, H-5), 2.17, 2.03, 1.99 (3s, 12H, 4Ac), 1.46, 1.44 (2s, 9H, 3 x Boc CH3), 1.35 - 1.22 (m, 26H, IICH2), 0.88 (t, 3H, CH3); MALDI TOF MS C35H60N2O12 (700.86) m/z (%) 724 [M+Na]+ (100), 602 [M-Boc+H]+ (51).

2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-N-[1-(R/s)-(tert-butoxycarbonyl)amino]dodecyl]-β-D-glucopyranosylamide (29a)

Procedure as for 29b.

Rf = 0.64 chloroform:methanol 10:0.7 (v/v); yield 76%; 1H NMR δ 5.09 - 4.98 (m, 2H, H-3, H-4), 4.41 (d, 1H, H-1, J1,2=9.6 Hz), 4.20 - 4.08 (m, 3H, αCH, H-6, H-6''), 3.68 (m, 2H, H-2, H-5), 2.07, 1.99, 1.96 (3s, 12H, 4Ac), 1.44 (s, 9H, 3 x Boc CH3), 1.26 (m, 18H, 9CH2), 0.87 (t, 3H, CH3); FAB MS C31H53N3O11 (643.77) m/z (%) 644 [M+H]+ (40), 544 [M-Boc+H]+ (100).
2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-N-[1-(R,S)-[(tert-butoxycarbonyl)amino]tetradecyl]-β-D-glucopyranosylamide (29b)

Tributyl-n-phosphine (4.88 g, 24.2 mmol) was dissolved in abs. CH₂Cl₂ (50 ml) and added dropwise to a stirred solution of 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl azide 24 (6.00 g, 16.1 mmol) and 2-(R,S)-[(tert-butoxycarbonyl)amino]tetradecanoic acid 2b (10.2 g, 32.3 mmol) in abs. CH₂Cl₂ (100 ml) over 20 minutes. After stirring for 2 hours at room temperature, the reaction mixture was diluted with CH₂Cl₂ (100 ml) and washed with NaHCO₃ (sat, aq) (2 x 100 ml). The organic phase was dried over MgSO₄, filtered and evaporated. The product was purified by column chromatography in chloroform:methanol 10:0.2 (v/v) to give 29b (8.50 g, 82%).

Alternative procedure:

As above except the lipoamino acid 2b was activated with EDAC (1.2 molar equivalents) to give 29b (84%).

Alternative procedure:

As above except the lipoamino acid 2b was activated with EDAC (1.2 molar equivalents) to give 29b (84%).

Rᵣ = 0.64 hexane:ethyl acetate 1:3 (v/v); ¹H NMR δ 5.11, 5.01 (2m, 2H, H-3, H-4), 4.45 (d, 1H, H-1, J₁=9.5 Hz), 4.21, 4.10 (2m, 3H, αCH, H-2, H-6'), 3.81 – 3.65 (m, 2H, H-2, H-5), 2.06, 2.05, 2.00, 1.97 (3s, 12H, 4Ac), 1.43 (s, 9H, 3 x Boc CH₃), 1.25 (m, 22H, 11CH₂), 0.86 (t, 3H, CH₃); FAB MS C₃₃H₅₇N₃O₁₁ (671.82) m/z (%) 694 [M+Na]⁺ (45), 572 [M-Boc+H]⁺ (100).

2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-N-[[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl]amino]-(R,S)-dodecyl]-β-D-glucopyranosylamide (29c)

Procedure as for 29d.

Rᵣ = 0.62 chloroform:methanol 10:0.5 (v/v); yield 79%; ¹H NMR δ 5.12 – 5.07 (m, 3H, H-1, H-3, H-4), 4.28 (m, 1H, H-6), 4.12 (m, 3H, αCH, H-2, H-6'), 3.79 (m, 1H, H-5), 2.46 (s, 3H, C=CH₂), 2.40 (s, 4H, 2CH₂), 2.07, 2.04, 2.02, 1.99 (4s, 12H, 4Ac), 1.25 (m, 18H, 9CH₂), 1.02 (s, 6H, 2CH₃), 0.87 (t, 3H, CH₃); ¹³C NMR δ 199.2, 173.2, 172.3, 170.7, 169.1, 80.6, 73.6, 72.7, 67.8, 61.7, 57.9, 53.5, 52.9, 32.9 – 18.3, 14.0; FAB MS C₃₆H₆₇N₃O₁₁ (707.85) m/z (%) 730 [M+Na]⁺ (25), 840 [M+Cs]⁺ (45).
Experimental

2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-N-[[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl]amino]-(R,s)-tetradecyl-β-D-glucopyranosylamide (29d)

Tributyl-µ-phosphine (4.07 g, 20.2 mmol) was dissolved in abs. CH₂Cl₂ (10 ml) and added dropwise to a stirred solution of 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl azide 24 (5.00 g, 13.4 mmol) and 2-(R/S)-[[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl]amino]tetradecanoic acid 4b (8.21 g, 20.2 mmol) in abs. CH₂Cl₂ (50 ml) over 20 minutes. After stirring for 3 hours at room temperature, the reaction mixture was diluted with CH₂Cl₂ (100 ml) and washed with 5% HCl(aq) (3 x 100 ml). The organic phase was dried over MgSO₄, filtered and evaporated. The product was precipitated from hexane and then re-crystallised from acetonitrile to give 29d (8.00 g, 81%).

Rf = 0.66 chloroform:methanol 10:0.7 (v/v); ¹H NMR δ 7.46 (d, 1H, NH), 5.10 - 5.07 (m, 3H, H-1, H-3, H-4), 4.29 (dd, 1H, H-6), 4.15 - 4.04 (m, 3H, αCH, H-2, H-6'), 3.76 (m, 1H, H-5), 2.47 (s, 3H, C=C(NH)CH₃), 2.39 (s, 4H, 2CH₂), 2.07, 2.04, 2.02, 1.91 (4s, 12H, 4Ac), 1.24 (m, 22H, IICH₂), 1.03 (s, 6H, 2CH₃), 0.87 (t, 3H, CH₃); FAB MS C₃₈H₆₁N₃O₁ (735.90) m/z (%) 736 [M+H]^⁺ (35), 759 [M+Na]^⁺ (5), 868 [M+Cs]^⁺ (4).

2.3.4.6-tetra-O-acetyl-β-(tributylphosphoranylidene)galactopyranosylamine (30a)

This crude product was identified from a failed reaction only.

Rf = 0.50 chloroform:methanol 10:0.7 (v/v); FAB MS C₂₆H₄₆NO₉P (547.62) m/z (%) 548 [M+H]^⁺ (100).

2.3.4.6-tetra-O-acetyl-β-(triphenylphosphoranylidene)galactopyranosylamine (30b)

Rf = 0.15 acetonitrile:ethyl acetate 1:1 (v/v); ¹H NMR δ 7.68 - 7.61, 7.53 - 7.49, 7.45 - 7.42, 7.32 - 7.29 (4m, 15H, arom.H), 5.50 (dd, 1H, H-4), 5.16 - 4.85 (m, 3H, H-1, H-2, H-3), 4.00, 3.81 (2m, 2H, H-6, H-6'), 3.72 (m, 1H, H-5), 2.12, 2.03, 1.99, 1.97 (4s, 12H, 4Ac); FAB MS C₃₂H₄₆NO₉P (607.59) m/z (%) 608 [M+H]^⁺ (90).
2,3,4,6-tetra-O-acetyl-N-[(9H-fluoren-9-ylmethoxy)carbonyl]-β-D-glucopyranosylamine (31)

2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl azide 22 (1.00 g, 2.68 mmol) was dissolved in abs. THF (10 ml). Trimethylphosphine (1.0M in THF, 2.82 mmol) was added in one portion. The reaction was then stirred at room temperature for 2 hours. 9-Fluorenylmethyl chloroformate (763 mg, 2.95 mmol) was then dissolved in abs. THF (5 ml) and added to the solution with further stirring for 2 hours. The solvents were evaporated. The residue was taken up in CH$_2$Cl$_2$ (20 ml) and washed with 5% HCl(aq) (1 × 20 ml). The organic phase was dried over MgSO$_4$, filtered and evaporated. The product was purified by column chromatography to give 31 (899 mg, 59%).

R$_f$ = 0.29 chloroform:ethyl acetate 10:1 (v/v); $^1$H NMR δ 7.75, 7.54, 7.40, 7.31 (4m, 8H, Fmoc arom.H), 5.30 (d, 1H, NH), 5.08 (t, 1H, H-3), 5.08 (m, 2H, H-1, H-2), 4.95 (t, 1H, H-4), 4.40 – 4.30 (m, 3H, H-6, H-6', αCH), 4.22, 4.10 (2m, 2H, CH$_2$), 3.80 (m, H, H-5), 2.08, 2.05, 2.02 (3s, 12H, 4Ac); $^{13}$C NMR δ 170.9, 170.5, 169.6, 169.4, 155.2, 143.6, 141.3, 127.8, 127.1, 124.9, 120.0, 81.0, 73.5, 72.8, 70.3, 68.6, 68.2, 67.6, 61.7, 47.0, 29.9, 28.2, 20.5, 1.9; FAB MS C$_{29}$H$_{31}$NO$_3$ (569.56) m/z (%) 570 [M+H]$^+$ (10), 592 [M+Na]$^+$ (35), 702 [M+Cs]$^+$ (100), 331 [M-NH$_2$Fmoc]$^+$ (50).

2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate (33)

Potassium thiocyanate (2.81 g, 28.7 mmol), tetrabutylammonium hydrogen sulphate (1.22 g, 3.59 mmol) and molecular sieves (6.00 g) were stirred in absolute acetonitrile (500 ml) for 30 minutes. 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide 18 (5.90 g, 14.4 mmol) was then dissolved in acetonitrile, added to the reaction flask and refluxed for 90 minutes. The solution was then allowed to cool, filtered through a celite pad and concentrated. Purification by column chromatography in hexane:ethyl acetate 2:1 (v/v) to give 33 (4.26 g, 76%).

R$_f$ = 0.29 hexane:ethyl acetate 3:2 (v/v); $^1$H NMR δ 5.20 (t, 1H, H-2), 5.09 (m, 2H, H-3, H-4), 5.02 (d, 1H, H-1, J$_{1,2}$=8.7 Hz), 4.24, 4.14 (2m, 2H, H-6, H-6'), 3.74 (m, 1H, H-5), 2.09, 2.01, 2.00 (3s, 12H, 4Ac); $^{13}$C NMR δ 170.6, 170.1, 169.1, 168.9, 144.3, 83.5, 74.1, 72.5, 71.9, 61.8, 61.5, 20.6, 20.5, 20.5, 20.4; FAB MS
Experimental

C_{15}H_{19}NO_{9}S (389.38) m/z (%) 412 [M+Na]^+ (8), 522 [M+Cs]^+ (25), 331 [M-NCS]^+ (100).

2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl isothiocyanate (34)

Procedure as for 33.

Rf = 0.38 hexane:ethyl acetate 3:2 (v/v); yield 79%; \(^1\)H NMR δ 5.39 (d, 1H, H-4), 5.28 (m, 1H, H-2), 4.99 (dd, 1H, H-3), 4.96 (m, 1H, H-1, J\(_{1,2}\)=8.9 Hz), 4.12 (m, 2H, H-6, H-6'), 3.95 (m, 1H, H-5), 2.16, 2.10, 2.04, 1.98 (4s, 12H, 4Ac); FAB MS C_{15}H_{19}NO_{9}S (389.38) m/z (%) 412 [M+Na]^+ (5), 522 [M+Cs]^+ (50), 331 [M-NCS]^+ (100).

2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl isothiocyanate (35)

Procedure as for 33.

Rf = 0.40 hexane:ethyl acetate 1:1 (v/v); yield 84%; \(^1\)H NMR δ 5.55 (d, 1H, H-1, J\(_{1,2}\)=2.0 Hz), 5.32 (m, 1H, H-2), 5.27 (m, 2H, H-3, H-4), 4.27, 4.14 (2m, 2H, H-6, H-6'), 4.08 (m, 1H, H-5), 2.17, 2.10, 2.06, 2.01 (4s, 12H, 4Ac); \(^13\)C NMR δ 170.7, 170.4, 169.9, 169.8, 144.1, 82.8, 71.6, 69.7, 68.3, 65.4, 61.6, 20.7, 20.6, 20.5, 14.2; FAB MS C_{15}H_{19}NO_{9}S (389.38) m/z (%) 412 [M+Na]^+ (5), 522 [M+Cs]^+ (70), 331 [M-NCS]^+ (100).

2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl isothiocyanate (36)

Potassium thiocyanate (1.56 g, 15.9 mmol), tetrabutylammonium hydrogen sulphate (673 mg, 1.98 mmol) and molecular sieves (3.00 g) were stirred in absolute acetonitrile (300 ml) for 30 minutes. 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-glucopyranosyl chloride 21 (2.90 g, 7.93 mmol) was then dissolved in acetonitrile, added to the reaction flask and refluxed for 90 minutes. The solution was then allowed to cool, filtered through a celite pad and concentrated. Purification by column chromatography in hexane:ethyl acetate 3:2 (v/v) to give 36 (1.09 g, 74%).

Rf = 0.38 hexane:ethyl acetate 3:1 (v/v); \(^1\)H NMR δ 5.94 (d, 1H, NH), 5.24 (t, 1H, H-3), 5.24 (d, 1H, H-1, J\(_{1,2}\)=9.6 Hz), 5.06 (t, 1H, H-4), 4.21, 4.11 (2m, 2H, H-6, H-6'), 3.99 (m, 1H, H-2), 3.75 (m, 1H, H-5), 2.09 (s, 3H, NAc), 2.04, 2.02, 2.00 (3s, 9H, 3OAc); \(^13\)C NMR δ 170.7, 170.6, 169.5, 169.2, 143.2, 83.9, 73.9, 71.8, 68.0,
61.7, 56.0, 23.2, 20.7, 20.6, 20.5; FAB MS C₁₅H₂₀N₂O₈S (388.39) m/z (%) 411 [M+Na]+ (20), 521 [M+Cs]+ (65), 330 [M-NCS]+ (100).

**Methyl 5-acetamido-3,5-dideoxy-β-D-glycero-D-galacto-2-nonulo-pyranosonate (37)**

5-Acetamido-3,5-dideoxy-α/β-D-glycero-D-galacto-2-nonulopyranosonic acid (2.00 g, 6.46 mmol) was suspended in absolute methanol (60 ml) with ion exchange resin and stirred for 72 hours. The resin was subsequently filtered off and washed with methanol. The solution was concentrated and purified by column chromatography to give 37 (1.94 g, 93%).

Rf = 0.60 chloroform:methanol:water 5:6:2 (v/v/v); ¹H NMR δ 4.00 - 3.94 (m, 2H, H-4, H-6), 3.83 (t, 1H, H-5), 3.76 (s, 3H, OCH₃), 3.74 (dd, 1H, H-9'), 3.63 (dd, 1H, H-8), 3.53 (dd, 1H, H-9), 3.46 (d, 1H, H-7), 2.22 (dd, 1H, H-3eq), 1.82 (dd, 1H, H-3ax); FAB MS C₁₂H₁₉NO₉ (323.29) m/z (%) 324 [M+H]+ (5), 346 [M+Na]+ (100).

**Methyl 5-acetamido-2,4,7,8,9-penta-β-acetyl-3,5-dideoxy-α/β-D-glycero-D-galacto-2-nonulo-pyranosonate (38)**

Methyl 5-acetamido-3,5-dideoxy-β-D-glycero-D-galacto-2-nonulopyranosonate 37 (1.94 g, 6.01 mmol) was dissolved in pyridine (22.6 ml) and acetic anhydride (25.6 ml) and stirred overnight. The pyridine was evaporated and the residue co-evaporated with toluene and benzene. Purification by column chromatography gave 38α (570 mg, 18%) and 38β (1.58 g, 49%).

38α : Rf = 0.40 ethyl acetate:methanol 10:0.5 (v/v/v); ¹H NMR δ 5.36 (dd, 2H, NH, H-7), 5.19 (dd, 1H, H-8), 5.04 - 4.99 (m, 1H, H-4), 4.68 (dd, 1H, H-6), 4.36 (dd, 1H, H-9'), 4.16 (m, 1H, H-5), 4.06 (dd, 1H, H-9), 3.76 (s, 3H, OCH₃), 2.56 (dd, 1H, H-3eq), 2.07 (dd, 1H, H-3ax), 2.12, 2.09, 2.02, 1.89 (4s, 18H, 6Ac); FAB MS C₂₂H₃₁NO₁₄ (533.48) m/z (%) 534 [M+H]+ (5), 556 [M+Na]+ (37), 414 (100).

38β : Rf = 0.30 ethyl acetate:methanol 10:0.5 (v/v/v); ¹H NMR δ 5.37 (dd, 1H, H-7), 5.31 - 5.22 (m, 2H, H-4, NH), 5.06 (dd, 1H, H-8), 4.49 (dd, 1H, H-9'), 4.15 - 4.07 (m, 3H, H-5, H-6, H-9), 3.76 (s, 3H, OCH₃), 2.55 (dd, 1H, H-3eq), 2.14 (dd, 1H, H-3ax), 2.16, 2.08, 2.04, 1.89 (4s, 18H, 6Ac); FAB MS C₂₂H₃₁NO₁₄ (533.48) m/z (%) 534 [M+H]+ (2), 556 [M+Na]+ (38), 414 (100).
Experimental

Methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-2-chloro-3,5-dideoxy-β-D-glycero-D-galacto-2-nonulo-pyranosonate (39)

HCl gas was bubbled through acetyl chloride (150 ml) for 15 minutes at -15°C to form a saturated solution. Methyl 5-acetamido-2,4,7,8,9-penta-O-acetyl-3,5-dideoxy-α/β-D-glycero-D-galacto-2-nonulo-pyranosonate 38 (700 mg, 1.31 mmol) was added to the solution, which was stirred for 24 hours. The acetyl chloride was evaporated and the residue co-evaporated with toluene and benzene. Purification by column chromatography using ethyl acetate gave 39 (582 mg, 87%).

\[ R_f = 0.5 \text{ ethyl acetate:methanol 10:0.5 (v/v)}; \]

\[ ^1H \text{ NMR} \delta 5.51 (d, 1H, NH), 5.47 (dd, 1H, H-7), 5.38 (m, 1H, H-4), 5.16 (m, 1H, H-8), 4.43 (dd, 1H, H-9'), 4.36 (dd, 1H, H-6), 4.21 (m, 1H, H-5), 4.08 (m, 1H, H-9), 3.87 (s, 3H, OCH\_3), 2.76 (dd, 1H, H-3\text{eq}), 2.27 (dd, 1H, H-3\text{ax}), 2.12, 2.09, 2.05, 1.90 (4s, 15H, 5Ac); \]

FAB MS C\_2\text{0}H\_2\text{8}C\_1\text{NO}\_2 (509.89) m/z (%) 532 [M+Na]^+ (47), 496 (100).

Methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-2-isothiocyanato-3,5-dideoxy-α-D-glycero-D-galacto-2-nonulo-pyranosonate (40)

Potassium thiocyanate (1.10 g, 11.3 mmol), tetrabutylammonium hydrogen sulphate (478 mg, 1.41 mmol) and molecular sieves (3.00 g) were stirred in absolute acetonitrile (300 ml) for 30 minutes. Methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-2-chloro-3,5-dideoxy-β-D-glycero-D-galacto-2-nonulo-pyranosonate 39 (2.86 g, 5.63 mmol) was then dissolved in acetonitrile, added to the reaction flask and refluxed for 1 hour. The solution was then allowed to cool, filtered through a celite pad and concentrated. Purification by column chromatography gave 40 (2.01 g, 67%).

\[ R_f = 0.21 \text{ chloroform:methanol 10:1 (v/v)}; \]

\[ ^1H \text{ NMR} \delta 5.45 (d, 1H, NH), 5.42 (dd, 1H, H-7), 5.22 (m, 1H), 5.17 (m, 1H), 4.37 (dd, 1H), 4.16 (m, 2H), 4.05 (m, 1H), 3.89 (s, 3H, COOCH\_3), 2.48 (dd, 1H, H-3\text{eq}), 2.23 (dd, 1H, H-3\text{ax}), 2.10, 2.06, 2.03, 1.89 (4s, 15H, 5Ac); \]

\[ ^13C \text{ NMR} \delta 170.8, 170.5, 170.3, 170.0, 169.9, 169.7, 145.4, 107.9, 89.5, 76.8, 76.5, 73.5, 70.6, 69.7, 68.8, 68.5, 67.9, 67.9, 67.5, 67.0, 62.1, 61.9, 59.1, 53.9, 49.2, 48.9, 46.8, 38.9, 38.3, 24.2, 23.1, 20.9, 20.7, 19.6, 13.9; \]

\[ \text{FAB MS } C\_2\text{1}H\_2\text{8}N\_2O\_2S (532.52) m/z (%) 533 [M+H]^+ (20), 555 [M+Na]^+ (60), 571 [M+K]^+ (100), 665 [M+Cs]^+ (70). \]
2,3,4,6-tetra-O-acetyl-N-[(2-(R/S)-[(tert-butoxycarbonyl)amino]dodecyl]oxy]-carbonothioyl]-β-D-glucopyranosylamine (41a)

2,3,4,6-tetra-O-acetyl-N-[(2-(R/S)-[(tert-butoxycarbonyl)amino]dodecyl]oxy]-carbonothioyl]-β-D-glucopyranosylamine (41a) was prepared by dissolving 2,3.4.6-tetra-O-cetyl-β-D-glucopyranosyl isothiocyanate (1.00 g, 2.57 mmol), tert-butyl A^-l-(l-(hydroxymethyl)undecyl)carbamate (967 mg, 3.21 mmol) and triethylamine (130 mg, 1.29 mmol) in abs. toluene (10 ml) and stirring under reflux for 12 hours. Following evaporation, the residue was purified by column chromatography in hexane:ethyl acetate 2:1 to give 41a (1.36 g, 77%).

R_f = 0.69 chloroform:methanol 10:2 (v/v); 1H NMR δ 7.02 (d, 1H, NH), 5.54, 5.32, 5.05, 4.96 (4m, 4H, H-1, H-2, H-3, H-4), 4.37 (m, 1H, αCH), 4.28 (m, 1H, H-6), 4.09 (m, 3H, CH_2, H-6'), 3.81 (d, 1H, H-5), 2.05, 2.01, 2.00, 1.99 (4m, 4H, 4Ac), 1.41 (s, 9H, 3 x Boc CH_3), 1.28 - 1.21 (m, 18H, 9 %), 0.85 (t, 3H, CH_3); 13C NMR δ 170.6, 170.4, 169.9, 169.4, 155.3, 83.2, 81.9, 73.7, 72.7, 70.5, 69.8, 68.3, 67.6, 65.8, 61.6, 61.2, 60.2, 52.9, 49.6, 31.8 - 13.9 ; FAB MS C_{34}H_{54}N_2O_12S (690.84) m/z (%) 713 [M+Na]^+ (25), 823 [M+Cs]^+ (100), 591 [M-Boc+H]^+ (40).

2.3.4,6-tetra-O-acetyl-N-[(2-(R/S)-[(tert-butoxycarbonyl)amino]tetradecyl]oxy]-carbonothioyl]-β-D-glucopyranosylamine (41b)

Procedure as for 41a.

R_f = 0.29 chloroform:methanol 10:0.2 (v/v); yield 72%; 1H NMR δ 7.05 (d, 1H, NH), 5.53 - 4.99 (2m, 4H, H-1, H-2, H-3, H-4), 4.34 (m, 1H, αCH), 4.28 - 4.06 (m, 4H, H-6, H-6', CH_2), 3.79 (d, 1H, H-5), 2.07, 2.03, 2.02, 1.99 (4s, 12H, 4Ac), 1.43 (s, 9H, 3 x Boc CH_3), 1.25 (m, 22H, 11CH_2), 0.87 (t, 3H, CH_3); FAB MS C_{36}H_{58}N_2O_12S (718.90) m/z (%) 719 [M+H]^+ (25), 851 [M+Cs]^+ (50), 619 [M-Boc+H]^+ (70).

2,3,4,6-tetra-O-acetyl-N-[(tetradecyl]thio)carbonothioyl]-β-D-glucopyranosylamine (42)

2,3.4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate (100 mg, 0.257 mmol), dodecanethiol (78 mg, 0.386 mmol) and triethylamine (7.00 mg, 0.0642 mmol) were dissolved in abs. CH_2Cl_2 (10 ml) and stirred at room temperature for 20 minutes. Following evaporation, the residue was purified by column chromatography to give 42 (132 mg, 87%).
Experimental

\[ R_f = 0.46 \text{ hexane:ethyl acetate 2:1 (v/v)}; \quad ^1\text{H NMR } \delta \text{ 5.49 - 4.97 (m, 4H, H-1, H-2, H-3, H-4), 4.28, 4.11 (2m, 2H, H-6, H-6'), 3.77 (d, 1H, H-5), 2.58 (m, 2H, SCH}_2, 2.07, 2.04, 2.03, 2.00 (4s, 12H, 4Ac), 1.58 (m, 2H, \beta CH}_2), 1.31 - 1.24 (m, 18H, 9CH}_2), 0.87 (t, 3H, CH}_3); \quad \text{FAB MS C}_{27}\text{H}_{45}\text{NO}_9\text{S}_2 (591.78) m/z (%) 592 [M+H]^+ (100), 614 [M+Cs]^+ (15), 619 [M-NHC(S)S(CH}_2)_2\text{CH}_3]^+ (35). \]

**2-(R/S)-[(tert-butoxycarbonyl)amino]dodecyl 4-methylbenzenesulphonate (43a)**

Procedure as for 43b (using 9a in place of 9b).

\[ R_f = 0.46 \text{ hexane:ethyl acetate 4:1 (v/v)}; \quad \text{yield 54%}; \quad ^1\text{H NMR } \delta \text{ 7.75, 7.34 (2d, 4H, arom.H), 4.55 (d, 1H, NH), 4.04 - 3.96 (m, 2H, CH}_2), 3.69 (m, 1H, \alpha CH), 2.43 (s, 3H, tos.CH}_3), 1.41 (s, 9H, 3 x Boc CH}_3), 1.28 - 1.22 (m, 18H, 9CH}_2), 0.87 (t, 3H, CH}_3); \quad \text{FAB MS C}_{24}\text{H}_{41}\text{NO}_5\text{S (455.65) m/z (%) 456 [M+H]^+ (20), 478 [M+Na]^+ (15), 588 [M+Cs]^+ (50), 356 [M-Boc+H]^+ (100).} \]

**2-(R/S)-[(tert-butoxycarbonyl)amino]tetradecyl 4-methylbenzenesulphonate (43b)**

4-Toluenesulphonyl chloride (1.11 g, 5.83 mmol) and DMAP (10 mg, catalytic) were added to a solution of tert-butyl \( N-[1-(R/S)-(hydroxymethyl)tridecyl]carbamate \) 9b (960 mg, 2.92 mmol) in pyridine (5 ml) at 0°C. The reaction was stirred for 2 hours. NaHCO\(_3\) (sat, aq) (50 ml) was poured onto the solution, and the product extracted into CH\(_2\)Cl\(_2\) (100 ml). The organic phase was washed with NaHCO\(_3\) (sat, aq) (3 x 50 ml), dried over MgSO\(_4\), filtered and evaporated. Purification by column chromatography gave 43b (810 mg, 57%).

\[ R_f = 0.39 \text{ hexane:ethyl acetate 4:1 (v/v)}; \quad ^1\text{H NMR } \delta \text{ 7.77, 7.33 (2d, 4H, arom.H), 4.55 (d, 1H, NH), 4.05, 3.96 (2m, 2H, CH}_2), 3.71 (m, 1H, \alpha CH), 2.44 (s, 3H, tos.CH}_3), 1.39 (s, 9H, 3 x Boc CH}_3), 1.27 - 1.22 (m, 22H, 11CH}_2), 0.86 (t, 3H, CH}_3); \quad \text{FAB MS C}_{26}\text{H}_{45}\text{NO}_5\text{S (483.71) m/z (%) 484 [M+H]^+ (10), 506 [M+Na]^+ (3), 616 [M+Cs]^+ (2), 384 [M-Boc+H]^+ (100).} \]

**2-(R/S)-[(tert-butoxycarbonyl)amino]dodecyl methanesulphonate (44a)**

Methanesulphonyl chloride (285 mg, 2.49 mmol) and triethylamine (252 mg, 2.49 mmol) were added to a solution of tert-butyl \( N-[1-(R/S)-\)
hydroxymethyl)undecyl]carbamate 9a (500 mg, 1.66 mmol) in absolute CH\textsubscript{2}Cl\textsubscript{2} (10 ml) at 0°C. After stirring for 30 minutes, the reaction mixture was allowed to reach room temperature, and was stirred for an additional 1 hour. The organic phase was diluted with dichloromethane (20 ml) and washed with NaHCO\textsubscript{3}(saturated,aq) (1 x 30 ml), 1M KHSO\textsubscript{4}(aq) (1 x 30 ml) and brine (2 x 30 ml). The organic phase was dried over MgSO\textsubscript{4}, filtered and evaporated. Purification by column chromatography gave 44a (1.46 g, 64%).

\[ R_f = 0.46 \text{ hexane:ethyl acetate } 2:1 (v/v); \quad ^1\text{H NMR} \delta 4.55 (d, 1H, NH), 4.25, 4.20 (2m, 2H, CH\textsubscript{2}), 3.80 (m, 1H, \alpha CH), 3.01 (s, 3H, mes.CH\textsubscript{3}), 1.43 (s, 9H, 3 x Boc CH\textsubscript{3}), 1.28 – 1.24 (m, 18H, 9 % ), 0.87 (t, 3H, CH\textsubscript{3}); \quad \text{FAB MS } C_{18}H_{37}NO_{5}S (379.56) m/z (%) 380 [M+H]\textsuperscript{+} (7), 402 [M+Na]\textsuperscript{+} (25), 280 [M-Boc+H]\textsuperscript{+} (100).\]

2-(R/s)-(tert-butoxycarbonylamino)tetradecyl methanesulphonate (44b)

Procedure as for 44a (using 9b in place of 9a).

\[ R_f = 0.48 \text{ hexane:ethyl acetate } 2:1 (v/v); \quad \text{yield 59%}; \quad ^1\text{H NMR} \delta 4.65 (d, 1H, NH), 4.21 (m, 2H, CH\textsubscript{2}), 3.84 (m, 1H, \alpha CH), 3.03 (s, 3H, mes.CH\textsubscript{3}), 1.44 (s, 9H, 3 x Boc CH\textsubscript{3}), 1.24 (m, 22H, 11CH\textsubscript{2}), 0.86 (t, 3H, CH\textsubscript{3}); \quad \text{FAB MS } C_{20}H_{41}NO_{5}S (407.61) m/z (\%) 430 [M+Na]\textsuperscript{+} (40), 308 [M-Boc+H]\textsuperscript{+} (100).\]

tert-butyl N-[1-(R/s)-(iodomethyl)undecyl]carbamate (45a)

Trimethylphosphine (1.0M, 1.33 mmol) was added dropwise to a stirred solution of (azodicarbonyl)dipiperidine [ADDP] (336 mg, 1.33 mmol) in abs. THF (25 ml) at 0°C. After 30 minutes, iodomethane (189 mg, 1.33 mmol) and tert-butyl N-[1-(R/s)-(hydroxymethyl)undecyl]carbamate 9a (200 mg, 0.664 mmol) were added to the solution, which was subsequently stirred for 4 hours at room temperature. The precipitate was then filtered off and the solution evaporated to dryness. The residue was dissolved in ethyl acetate and the remaining hydrazide was precipitated from hexane and removed by filtration. Following evaporation, the residue was taken up in CH\textsubscript{2}Cl\textsubscript{2} (50 ml), washed with water (2 x 25 ml) and with NaHCO\textsubscript{3}(saturated,aq) (1 x 25 ml), dried with MgSO\textsubscript{4}, filtered and evaporated. The residue was purified by column chromatography in hexane:ethyl acetate 4:1 (v/v) to give 45a (176 mg, 64%).

\[ R_f = 0.79 \text{ hexane:ethyl acetate } 1:1 (v/v); \quad ^1\text{H NMR} \delta 4.47 (d, 1H, NH), 3.24 (m, 1H, \alpha CH), 2.15, 1.84 (2d, 2H, CH\textsubscript{2}I), 1.40 (s, 9H, 3 x Boc CH\textsubscript{3}), 1.23 (m, 18H, 9CH\textsubscript{2}), \]
0.83 (t, 3H, CH$_3$); $^{13}$C NMR δ 155.1, 80.8, 49.6, 38.2 – 22.6, 15.1, 14.0; FAB MS C$_{17}$H$_{34}$INO$_2$ (411.36) m/z (%) 410 [M-H]$^+$ (100), 434 [M+Na]$^+$ (30), 544 [M+Cs]$^+$ (85), 340 [M-Boc+H]$^+$ (100).

tert-butyl N-[1-(r/s)-(iodomethyl)tridecyl]carbamate (45b)

2-(r/s)-[[(tert-butoxycarbonyl)amino]tetradecyl 4-methylbenzenesulphonate 43b (500 mg, 1.04 mmol) and sodium iodide (186 mg, 1.24 mmol) were dissolved in absolute acetone (10 ml) and refluxed gently for 12 hours. The solid precipitate was filtered off, and the solution evaporated to dryness. Purification by column chromatography gave 45b (392 mg, 86%).

R$_f$ = 0.46 hexane:ethyl acetate 1:2 (v/v); $^1$H NMR δ 4.47 (d, 1H, NH), 3.22 (m, 1H, αCH), 2.16, 1.82 (2d, 2H, CH$_2$I), 1.39 (s, 9H, 3 x Boc CH$_3$), 1.28 – 1.19 (m, 22H, IICH$_2$), 0.81 (t, 3H, CH$_3$); FAB MS C$_{19}$H$_{38}$INO$_2$ (439.42) m/z (%) 572 [M+Cs]$^+$ (25), 340 [M-Boc+H]$^+$ (100).

S-(2-(r/s)-[[(tert-butoxycarbonyl)amino]dodecyl]ethanethioate (46a)

Method A

2-(r/s)-[[(tert-butoxycarbonyl)amino]dodecyl 4-methylbenzenesulphonate 43a (860 mg, 1.89 mmol) and potassium thioacetate (862 mg, 7.56 mmol) were dissolved in abs. DMF (25 ml) and stirred at 80°C for 4 hours. The solvent was then evaporated (and co-evaporated with xylene) and the residue taken up in CH$_2$Cl$_2$ (50 ml) which was then washed with NaHCO$_3$(sat, aq) (2 x 50 ml), 1M KF(aq) (1 x 50 ml), brine (1 x 50 ml) and again with NaHCO$_3$(sat, aq) (1 x 50 ml). The organic phase was dried over MgSO$_4$, filtered and evaporated. The residue was purified by column chromatography in hexane:ethyl acetate 4:1 (v/v) to give 46a (480 mg, 71%).

Method B

Diethyl azidodicarboxylate [DEAD] (579 mg, 3.32 mmol) dissolved in abs. THF (1 ml) was added to a stirred solution of triphenylphosphine (870 mg, 3.32 mmol) in abs. THF (10 ml) at 0°C. After stirring for 30 minutes, tert-butyl N-[1-(r/s)-(hydroxymethyl)undecyl]carbamate 9a (500 mg, 1.66 mmol) and thiolacetic acid (252 mg, 3.32 mmol) in abs. THF (3 ml) were added to the solution. The reaction mixture was then stirred at room temperature for 2 hours. The solvent was then evaporated. The product was dissolved in hexane, and the insoluble organic
Experimental

precipitate filtered off. The solvent was evaporated and the residue was purified by column chromatography in hexane:ethyl acetate 5:1 (v/v) to give 46a (430 mg, 72%).

Method C

Trimethylphosphine (1.0M, 0.664 mmol) was added dropwise to a stirred solution of 1,1'-azodicarbonyldipiperidine [ADD] (168 mg, 0.664 mmol) and imidazole (46.0 mg, 0.664 mmol) in abs. THF (10 ml) at 0°C. After stirring for 30 minutes, tert-butyl N-[1-(R/S)-(hydroxymethyl)undecyl]carbamate 9a (100 mg, 0.332 mmol) and thiolacetic acid (51.0 mg, 0.664 mmol) in abs. THF (2 ml) were added to the solution. The reaction was stirred at room temperature overnight. The solvent was then evaporated. The product was dissolved in hexane, and the insoluble organic precipitate filtered off. Following evaporation of the solvent, the residue was taken up in CH<sub>2</sub>Cl<sub>2</sub> (50 ml) and was washed with NaHCO<sub>3</sub>(sat, aq) (2 x 50 ml). The organic phase was dried over MgSO<sub>4</sub>, filtered and evaporated. The residue was purified by column chromatography in hexane:ethyl acetate 5:1 (v/v) to give 46a (91.0 mg, 76%).

R<sub>f</sub> = 0.47 hexane:ethyl acetate 4:1 (v/v);<sup>1</sup>H NMR δ 4.46 (d, 1H, NH), 3.74 (m, 1H, αCH), 3.12, 2.99 (2m, 2H, CH<sub>2</sub>), 2.36 (s, 3H, SAc), 1.43 (s, 9H, 3 x Boc CH<sub>3</sub>), 1.27 (m, 18H, 9CH<sub>2</sub>), 0.87 (t, 3H, CH<sub>3</sub>); <sup>13</sup>C NMR δ 195.5, 155.5, 79.3, 50.5, 34.5 – 26.4, 25.9, 22.7, 14.1; FAB MS C<sub>19</sub>H<sub>37</sub>NO<sub>3</sub>S (359.57) m/z (%) 360 [M+H]<sup>+</sup> (7), 382 [M+Na]<sup>+</sup> (20), 260 [M-Boc+H]<sup>+</sup> (100).

S-{2-(R/S)-[(tert-butoxycarbonyl)amino]tetradecyl}ethanethioate (46b)

Procedure as for 46a (using 9b in place of 9a).

R<sub>f</sub> = 0.44 hexane:ethyl acetate 4:1 (v/v); yield 72%;<sup>1</sup>H NMR δ 4.47 (d, 1H, NH), 3.71 (m, 1H, αCH), 3.09, 2.98 (2m, 2H, CH<sub>2</sub>), 2.35 (s, 3H, SAc), 1.41 (s, 9H, 3 x Boc CH<sub>3</sub>), 1.25 (m, 22H, 11CH<sub>2</sub>), 0.87 (t, 3H, CH<sub>3</sub>); FAB MS C<sub>21</sub>H<sub>41</sub>NO<sub>3</sub>S (387.62) m/z (%) 388 [M+H]<sup>+</sup> (10), 288 [M-Boc+H]<sup>+</sup> (100).

S-{2-(R/S)-[(tert-butoxycarbonyl)amino]dodecyl}ethanethiol (47a)

Method A

S-{2-(R/S)-[(tert-butoxycarbonyl)amino]dodecyl}ethanethioate 46a (1.50 g, 4.18 mmol) was dissolved in methanolic ammonia and stirred at room temperature for 1 hour. Following evaporation, the residue was taken up in CH<sub>2</sub>Cl<sub>2</sub> (50 ml) and was
washed with brine (1 x 50 ml). The organic phase was dried over MgSO₄, filtered and evaporated to give a white solid 47a (1.17 g, 88%).

Method B

Lithium aluminium hydride (1.0M, 17.3 mmol) was added dropwise to a stirred solution of S-{2-(R/s)-[(tert-butoxycarbonyl)amino]dodecyl}ethanethioate 46a (1.55 g, 4.32 mmol) in abs. THF (15 ml) and stirred at room temperature for 1 hour. The reaction was quenched with 1M HCl(aq), diluted with CH₂Cl₂ (50 ml) and was washed with brine (1 x 50 ml) and NaHCO₃(sat, aq) (1 x 50 ml). The organic phase was dried over MgSO₄, filtered and evaporated. The residue was purified by column chromatography in hexane:ethyl acetate 4:1 (v/v) to give 47a (857 mg, 63%).

Rᵣ = 0.53 hexane:ethyl acetate 4:1 (v/v); ¹H NMR δ 4.59 (d, 1H, NH), 3.71 (m, 1H, αCH), 2.66 (m, 2H, CH₂), 1.44 (s, 9H, 3 x Boc CH₃), 1.25 (m, 18H, 9CH₂), 0.88 (t, 3H, CH₃); ¹³C NMR δ 155.4, 79.9, 51.4, 32.9, 31.9, 29.5, 29.3, 28.4, 25.9, 22.6, 14.0; FAB MS C₁₇H₃₅NO₂S (317.53) m/z (%) 340 [M+Na]⁺ (15), 450 [M+Cs]⁺ (3), 533 [2M-2H-Boc+H]⁺ (10), 633 [2M-2H+H]⁺ (2), 656 [2M-2H+Na]⁺ (10); IR Raman shift: 2582 cm⁻¹ (0.26) S-H stretch; see section 4.3.5.5.1 for spectrum and for further details.

S-{2-(R/s)-[(tert-butoxycarbonyl)amino]tetradecyl}ethanethiol (47b)

Procedure as for 47a, Method A.

Rᵣ = 0.51 hexane:ethyl acetate 4:1 (v/v); yield 81%; ¹H NMR δ 4.59 (d, 1H, NH), 3.69 (m, 1H, αCH), 2.64 (m, 2H, CH₂), 1.41 (s, 9H, 3 x Boc CH₃), 1.26 (m, 22H, 11CH₂), 0.87 (t, 3H, CH₃); FAB MS C₁₉H₃₉NO₂S (345.58) m/z (%) 478 [M+Cs]⁺ (10), 245 [M-Boc+H]⁺ (15), 661 [2M-2H+H]⁺ (5).

2,3,4,6-tetra-O-acetyl-N-[(2-(R/s)-[(tert-butoxycarbonyl)amino]dodecyl]thio)carbonothioyl]-β-D-glucopyranosylamine (48a)

2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate 33 (100 mg, 0.257 mmol), S-{2-(R/s)-[(tert-butoxycarbonyl)amino]dodecyl}ethanethiol 47a (98.0 mg, 0.308 mmol) and triethylamine (5.20 mg, 0.0514 mmol) were dissolved in abs. CH₂Cl₂ and stirred at room temperature for 20 minutes. Following evaporation, the residue was purified by column chromatography in hexane:ethyl acetate 3:1 to give 48a (128 mg, 71%).
Experimental

R\text{f} = 0.61 \text{Hex:EtOAc 1:1 (v/v)}; ^1\text{H NMR} \delta 5.34, 5.16, 5.08 (3\text{H}, 4\text{H}, \text{H-1, H-2, H-3, H-4}), 4.29, 4.09 (2\text{H}, 2\text{H}, \text{H-6, H-6'}), 3.84 (d, 1\text{H}, \text{H-5}), 3.71, 3.44 (2\text{H}, 3\text{H}, \alpha\text{CH}, \text{CH}_2), 2.06, 2.04, 2.01, 2.00 (4\text{H}, 12\text{H}, 4\text{Ac}), 1.46 (s, 9\text{H}, 3 \times \text{Boc CH}_3), 1.30 - 1.25 (m, 18\text{H}, 9\text{CH}_2); \text{FAB MS} C_{32}H_{54}N_2O_{11}S_2 (706.91) m/z (%) 707 [M+H]^+ (5), 729 [M+Na]^+ (10), 839 [M+Cs]^+ (15), 607 [M-Boc+H]^+ (80), 331 (25).

2,3,4,6-tetra-O-acetyl-N-[(2-(R/s)-[\text{tert-butoxycarbonyl}amino]tetradecyl]thio)carbonothioyl]-\beta-D-glucopyranosylamine (48\text{b})

Procedure as for 48\text{a} (using 47\text{b} in place of 47\text{a}).

R\text{f} = 0.57 \text{Hex:EtOAc 1:1 (v/v)}; \text{yield} 73%; ^1\text{H NMR} \delta 5.39 - 5.01 (4\text{H}, \text{H-1, H-2, H-3, H-4}), 4.28, 4.11 (2\text{H}, 2\text{H}, \text{H-6, H-6'}), 3.84 (d, 1\text{H}, \text{H-5}), 3.67 - 3.39 (3\text{H}, \alpha\text{CH}, \text{CH}_2), 2.07, 2.02, 2.01, 1.99 (4\text{H}, 12\text{H}, 4\text{Ac}), 1.43 (s, 9\text{H}, 3 \times \text{Boc CH}_3), 1.30 - 1.24 (m, 22\text{H}, 11\text{CH}_2), 0.86 (t, 3\text{H}, \text{CH}_3); \text{FAB MS} C_{34}H_{56}N_2O_{11}S_2 (734.96) m/z (%) 735 [M+H]^+ (15), 757 [M+Na]^+ (3), 635 [M-Boc+H]^+ (60).

\text{tert-butyl N-[1-(R/S)-(azidom ethyl)undecyl]carbamate (49a)}

\text{tert-butyl N-[1-(R/S)-(iodomethyl)undecyl]carbamate (45a) (250 mg, 0.608 mmol) was dissolved in abs. DMF (10 ml). Sodium azide (79.0 mg, 1.22 mmol) was added to the solution, which was subsequently stirred at 110°C for 12 hours. Following evaporation, the residue was taken up in CH}_2\text{Cl}_2 (50 ml) and was washed with NaHCO}_3(\text{sat, aq}) (1 \times 50 ml). The organic phase was dried over MgSO}_4, filtered and evaporated. The residue was purified by column chromatography in hexane:ether 10:1 (v/v) to give 49\text{a} (100 mg, 54%).}

R\text{f} = 0.46 \text{Hex:EtOAc 5:1 (v/v)}; ^1\text{H NMR} \delta 3.61 (m, 1\text{H}, \alpha\text{CH}), 3.46 - 3.39 (m, 2\text{H}, \text{CH}_2), 1.43 (s, 9\text{H}, 3 \times \text{Boc CH}_3), 1.25 (m, 18\text{H}, 9\text{CH}_2), 0.87 (t, 3\text{H}, \text{CH}_3); \text{ESI MS} C_{17}H_{34}N_4O_2 (326.48) m/z (%) 327 [M+H]^+ (100), 349 [M+Na]^+ (15), 227 [M-Boc+H]^+ (20).

\text{tert-butyl N-[1-(R/S)-(azidom ethyl)tridecyl]carbamate (49b)}

Procedure as for 49\text{a}.

R\text{f} = 0.44 \text{Hex:EtOAc 4:1 (v/v)}; \text{yield} 59%; ^1\text{H NMR} \delta 3.63 (m, 1\text{H}, \alpha\text{CH}), 3.40 (m, 2\text{H}, \text{CH}_2), 1.44 (s, 9\text{H}, 3 \times \text{Boc CH}_3), 1.29 - 1.25 (m, 22\text{H}, 11\text{CH}_2), 0.86 (t,
Experimental

3H, CH₃); FAB MS C₁₉H₃₂N₄O₂ (354.53) m/z (%) 355 [M+H]^⁺ (15), 377 [M+Na]^⁺ (20), 255 [M-Boc+H]^⁺ (50).

tert-butyl N-[1-((R/s)-(aminomethyl)undecyl]carbamate (50a)

Method A
Palladium catalyst (10% on carbon, 10.0 mg) was added in one portion to a solution of tert-butyl N-[1-(azidomethyl)undecyl]carbamate 49a (100 mg, 0.282 mmol) in abs. methanol (5 ml) under a hydrogen atmosphere. The solution was allowed to stir for 12 hours. The catalyst was subsequently filtered off, and the solvent evaporated to give 50a (78 mg, 84%).

Method B

tert-butyl N-{1-(R/S)-[tetrachlorophthalimidomethyl]undecyl]carbamate 52 (50.0 mg, 88.0 mmol) was dissolved in CH₂Cl₂ (1 ml). Ethylenediamine (2.65 mg, 44.0 mmol) was added to the solution, which was stirred for 1 hour, after which the reaction was complete. The residue was evaporated to give crude 50a, which was used without further purification.

R_f = 0.59 hexane:ethyl acetate 1:1 (v/v); ¹H NMR δ 4.92 (d, 1H, NH), 3.74 (m, 1H, αCH), 3.05 (m, 2H, CH₂), 1.45 (s, 9H, 3 x Boc CH₃), 1.25 (m, 18H, CH₂), 0.88 (t, 3H, CH₃); FAB MS C₁₇H₃₂N₂O₂ (300.48) m/z (%) 301 [M+H]^⁺ (55), 323 [M+Na]^⁺ (20), 201 [M-Boc+H]^⁺ (85).

tert-butyl N-[1-(R/s)-(aminomethyl)tridecyl]carbamate (50b)

Procedure as for 50a, Method A.

R_f = 0.55 hexane:ethyl acetate 2:1 (v/v); yield 80%; ¹H NMR δ 4.90 (d, 1H, NH), 3.80 (m, 1H, αCH), 3.12 (m, 2H, CH₂), 1.43 (s, 9H, 3 x Boc CH₃), 1.29 - 1.26 (m, 22H, CH₂), 0.86 (t, 3H, CH₃); FAB MS C₁₉H₄₀N₂O₂ (328.53) m/z (%) 329 [M+H]^⁺ (10), 229 [M-Boc+H]^⁺ (65).

tert-butyl N-{1-(R/S)-(phthalimidomethyl)undecyl]carbamate (51a)

Diethyl azidodicarboxylate [DEAD] (188 mg, 1.08 mmol) dissolved in abs. THF (2 ml) was added to a stirred solution of triphenylphosphine (283 mg, 1.08 mmol), phthalimide (171 mg, 1.16 mmol) and tert-butyl N-[1-(R/S)-(hydroxymethyl)-undecyl]carbamate 9a (250 mg, 0.831 mmol) in abs. THF (7 ml). The reaction
mixture was then stirred at room temperature for 72 hours. The solvent was then evaporated and the residue was purified by column chromatography in hexane:ethyl acetate 7:3 (v/v) to give 51a (240 mg, 67%).

R_f = 0.23 hexane:ethyl acetate 1:1 (v/v); ^1H NMR δ 7.84, 7.69 (2m, 4H, arom.H), 4.52 (d, 1H, NH), 3.97 (m, 1H, αCH), 3.67 (m, 2H, CH_2), 1.44 – 1.23 (m, 27H, 3 x Boc CH_3, 9CH_2), 0.88 (t, 3H, CH_3); FAB MS C_{25}H_{38}N_2O_4 (430.58 m/z (%) 453 [M+Na]^+ (5), 563 [M+Cs]^+ (30), 331 [M-Boc+H]^+ (100).

**tert-butyl N-{1-(R/S)-[phthalimidomethyl]tridecyl}carbamate (51b)**

Procedure as for 51a (using 9b in place of 9a).

R_f = 0.21 hexane:ethyl acetate 1:1 (v/v); yield 61%; ^1H NMR δ 7.80 - 7.70 (m, 4H, arom.H), 3.91 (m, 1H, αCH), 3.64 (m, 2H, CH_2), 1.43 (s, 9H, 3 x Boc CH_3), 1.26 – 1.21 (m, 22H, 11CH_2), 0.87 (t, 3H, CH_3); FAB MS C_{27}H_{42}N_2O_4 (458.63 m/z (%) 459 [M+H]^+ (25), 481 [M+Na]^+ (40), 359 [M-Boc+H]^+ (80).

**tert-butyl N-{1-(R/S)-[4,5,6,7-tetrachlorophthalimidomethyl]undecyl}carbamate (52)**

Diisopropyl azidodicarboxylate (873 mg, 4.32 mmol) dissolved in abs. THF (1 ml) was added to a stirred solution of triphenylphosphine (1.13 g, 4.32 mmol), tetrachlorophthalimide (1.33 g, 4.65 mmol) and tert-butyl N-{1-(R/S)-(hydroxymethyl)undecyl}carbamate 9a (1.00 g, 3.32 mmol) in abs. THF (150 ml). The reaction mixture was then stirred at room temperature for 72 hours. The solvent was then evaporated and the residue was purified by column chromatography in hexane:ethyl acetate 5:1 (v/v) to give 52 (1.41 g, 75%).

R_f = 0.23 hexane:ethyl acetate 1:1 (v/v); ^1H NMR δ 4.44 (d, 1H, NH), 3.94 (m, 1H, αCH), 3.65 (m, 2H, CH_2), 1.44 – 1.25 (m, 27H, 3 x Boc CH_3, 9CH_2), 0.88 (t, 3H, CH_3); FAB MS C_{25}H_{34}Cl_4N_2O_4 (568.36 m/z (%) 591 [M+Na]^+ (5), 470 [M-Boc+H]^+ (100).

**2-(R/S)-(2-aminododecyl)-1-(4,5,6,7-tetrachlorophthalimide) (53)**

tert-butyl N-{1-(R/S)-[4,5,6,7-tetrachlorophthalimidomethyl]undecyl}carbamate 52 (342 mg, 0.602 mmol) was dissolved in CH_2Cl_2:TFA 1:1 (4 ml) and stirred at room temperature for 10 minutes. After evaporation, the residue was taken up in CH_2Cl_2
Experimental

(20 ml) and washed with NaHCO₃ (sat. aq) (2 x 20 ml). The organic phase was dried over MgSO₄, filtered and evaporated to give 53 (248 mg, 88%).

Rₜ = 0.41 chloroform:methanol 10:2 (v/v); ¹H NMR (CDCl₃:CD₂OD 1:1) δ 3.80 - 3.46 (m, 3H, αCH, CH₂), 1.25 (m, 18H, 9CH₂), 0.86 (t, 3H, CH₃); FAB MS C₂₀H₂₆Cl₄N₂O₂ (468.24) m/z (%) 469 [M+H]⁺ (50).

2,3,4,6-tetra-O-acetyl-N-[(2-(R/S)-[tert-butoxycarbonyl]amino)dodecyl]amino)carbonothioyl]-β-D-glucopyranosylamine (54a)

2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate 33 (25.0 mg, 0.0617 mmol), tert-butyl N-[1-(R/S)-(aminomethyl)undecyl]carbamate 50a (27.8 mg, 0.0927 mmol) and triethylamine (12.5 mg, 0.0124 mmol) were dissolved in abs. CH₂Cl₂ (5 ml) and stirred at room temperature for 1 hour. Following evaporation, the residue was purified by column chromatography to give 54a (42.0 mg, 94%)

Rₜ = 0.39 chloroform:methanol 10:0.2 (v/v); ¹H NMR δ 5.11 - 4.99 (m, 3H, H-1, H-3, H-4), 4.23, 4.10 (2m, 2H, H-6, H-6'), 3.87 - 3.61 (m, 3H, H-2, H-5, αCH), 2.09, 2.01, 2.00, 1.96 (4s, 12H, 4Ac), 1.43 (s, 9H, 3 x Boc CH₃), 1.24 (m, 3H, CH₃); FAB MS C₃₂H₅₅N₃O₁₁S (689.86) m/z (%) 690 [M+H]⁺ (10), 712 [M+Na]⁺ (30), 590 [M-Boc+H]⁺ (100).

2,3,4,6-tetra-O-acetyl-N-[(2-(R/S)-[tert-butoxycarbonyl]amino)tetradecyl]amino)carbonothioyl]-β-D-glucopyranosylamine (54b)

Proceedure as for 54a (using 50b in place of 50a).

Rₜ = 0.41 chloroform:methanol 10:0.2 (v/v); yield 85%; ¹H NMR δ 5.16 - 4.96 (m, 3H), 4.28, 4.11 (2m, 2H, H-6, H-6'), 3.84 - 3.58 (m, 3H, H-2, H-5, αCH), 2.11, 2.06, 2.04, 2.00 (4s, 12H, 4Ac), 1.44 (s, 9H, 3 x Boc CH₃), 1.25 (m, 22H, 11CH₂), 0.84 (t, 3H, CH₃); FAB MS C₃₄H₆₅N₃O₁₁S (717.91) m/z (%) 718 [M+H]⁺ (40), 618 [M-Boc+H]⁺ (85).

Methyl 2-(R/S)-aminotetradecanoate hydrochloride (55a)

2-(R/S)-aminotetradecanoic acid 1a (4.00 g, 14.3 mmol) and thionyl chloride (1.79 g, 15.0 mmol) were dissolved in methanol (100 ml) and heated under reflux for 24
Experimental

hours. On cooling, the precipitated product was removed and re-crystallised from methanol to give 55a (3.15 g, 74%)

\[ R_f = 0.26 \text{ chloroform:methanol 10:0.2 (v/v); } \]

\[ ^1H \text{ NMR } \delta 3.90 (m, 1\text{H}, \alpha\text{CH}), 3.80 (s, 3\text{H}, \text{COOCH}_3), 1.35 (m, 2\text{H}, \beta\text{CH}_2), 1.24 (m, 20\text{H}, 10\text{CH}_2), 0.87 (t, 3\text{H}, \text{CH}_3); \]

FAB MS \( C_{13}H_{31}NO_2 \) (257.41) m/z (%) 258 [M+H]^+ (100).

**Methyl 2-(R/s)-aminooctadecanoate hydrochloride (55b)**

Procedure as for 55a (using 1d in place of 1a).

\[ R_f = 0.29 \text{ chloroform:methanol 10:0.1 (v/v); } \]

yield 69%; \[ ^1H \text{ NMR } \delta 3.88 (m, 1\text{H}, \alpha\text{CH}), 3.81 (s, 3\text{H}, \text{COOCH}_3), 1.32 (m, 2\text{H}, \beta\text{CH}_2), 1.28 - 1.24 (m, 20\text{H}, 14\text{CH}_2), 0.86 (t, 3\text{H}, \text{CH}_3); \]

FAB MS \( C_{19}H_{39}NO_2 \) (313.52) m/z (%) 314 [M+H]^+ (100).

**2,3,4,6-tetra-O-acetyl-\( β \)-D-glucopyranosylamine (56a)**

2,3,4,6-tetra-O-acetyl-\( β \)-D-glucopyranosyl isothiocyanate 33 (50.0 mg, 0.129 mmol) and methyl 2-(R/s)-aminotetradecanoate hydrochloride 55a (50.0 mg, 0.193 mmol) were dissolved in \( CH_2Cl_2 \) (5 ml). Triethylamine (2.60 mg, 0.0257 mmol) was added to the solution, which was subsequently stirred for 1 hour. Following evaporation, the residue was purified by column chromatography to give 56a (65.0 mg, 78%)

\[ R_f = 0.39 \text{ chloroform:methanol 10:0.2 (v/v); } \]

\[ ^1H \text{ NMR } \delta 5.36 - 5.03 (m, 4\text{H}, H-1, H-2, H-3, H-4), 4.26, 4.13 (2m, 2\text{H}, H-6, H-6'), 3.84 - 3.71 (m, 5\text{H}, H-5, \alpha\text{CH}, \text{COOCH}_3), 2.07, 2.03, 2.01, 1.99 (4s, 12\text{H}, 4\text{Ac}), 1.44 (s, 9\text{H}, 3 \times \text{Boc CH}_3), 1.29 - 1.24 (m, 22\text{H}, 11\text{CH}_2), 0.86 (t, 3\text{H}, \text{CH}_3); \]

FAB MS \( C_{30}H_{56}N_2O_{11}S \) (646.79) m/z (%) 647 [M+H]^+ (30).

**2,3,4,6-tetra-O-acetyl-\( β \)-D-glucopyranosylamine (56b)**

Procedure as for 56a (using 55b in place of 55a).

\[ R_f = 0.35 \text{ chloroform:methanol 10:0.1 (v/v); } \]

yield 74%; \[ ^1H \text{ NMR } \delta 5.38 - 5.00 (m, 4\text{H}, H-1, H-2, H-3, H-4), 4.24, 4.11 (2m, 2\text{H}, H-6, H-6'), 3.84 - 3.70 (m, 5\text{H}, H-5, \alpha\text{CH}, \text{COOCH}_3), 2.06, 2.03, 2.01, 2.00 (4s, 12\text{H}, 4\text{Ac}), 1.45 (s, 9\text{H}, 3 \times \text{Boc CH}_3), \]
1.25 (m, 30H, 15%); FAB MS C_{34}H_{58}N_{2}O_{11}S (702.90) m/z (%) 703 [M+H]^+ (45), 603 [M-Boc+H]^+ (100).

Methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-2-\{N-[[\{2-(R/s)-(tert-butoxycarbonyl)amino\}tetradecyl]amino]carbonothioyl\}-3,5-dideoxy-\alpha-L-galacto-2-nonulopyranosonate (57a)

Methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-2-isothiocyanato-3,5-dIDEOXY-\alpha-D-glycero-\beta-D-galacto-2-nonulopyranosonate 40 (100 mg, 0.188 mmol), ethyl 2-aminotetradecanoate 55a (58.0 mg, 0.226 mmol) and triethylamine (3.80 mg, 0.0376 mmol) were dissolved in CH₂Cl₂ (10 ml) and stirred at room temperature for 1 hour. Unfortunately, this compound proved to be unstable.

Rf = 0.52 chloroform:methanol 10:0.5 (v/v); FAB MS C_{36}H_{59}N_{3}O_{14}S (789.93) m/z (%) 790 [M+H]^+ (6), 812 [M+Na]^+ (10), 922 [M+Cs]^+ (30).

1,3,4,6-tetra-O-acetyl-2-deoxy-2-\{1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl\}amino-\alpha-D-glucopyranose (59)

2-acetyldimedone 3 (21.9 g, 121 mmol) was added to a stirred solution of sodium (2.13 g, 92.7 mmol) in absolute methanol (100 ml). 2-amino-2-deoxy-\alpha-D-glucopyranose (20.0 g, 92.7 mmol) was added to the solution, which was then refluxed for 4 hours. The solvent was then evaporated to give an off-white solid 58. The residue was suspended in pyridine (160 ml) and acetic anhydride (105 ml) and stirred for 12 hours. The reaction was concentrated, taken up in CH₂Cl₂ (250 ml) and washed with 5% HCl (aq) (2 x 250 ml). The organic phase was dried over MgSO₄, filtered and evaporated. Recrystallisation from methanol gave 59 (38.8 g, 82%).

Rf = 0.47 hexane:ethyl acetate 1:2 (v/v); \(^1\)H NMR δ 6.22 (d, 1H, H-1, J_{1,2}=3.6 Hz), 5.42 (t, 1H, H-3), 5.16 (t, 1H, H-4), 4.36, 4.24 (2m, 2H, H-6, H-6'), 4.14 (m, 1H, H-5), 4.08 (m, 1H, H-2), 2.59 (s, 3H, C=CH(NH)CH₃), 2.36, 2.33 (2s, 4H, 2CH₂), 2.31, 2.09, 2.03, 1.97 (4s, 12H, 4Ac), 1.00 (s, 6H, 2CH₃); FAB MS C_{24}H_{33}NO_{11} (511.52) m/z (%) 512 [M+H]^+ (65), 534 [M+Na]^+ (20), 452 [M-OAc]^+ (30).
Experimental

3.4.6-tri-O-acetyl-2-deoxy-2-\{[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)-ethyl]amino\}-α-D-glucopyranosyl bromide (60)

Acetic anhydride (2.0 ml) was added to HBr in acetic acid (45%, 31 ml) and allowed to stir for 30 minutes. 1,3,4,6-tetra-O-acetyl-2-deoxy-2-\{[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl]amino\}-α-D-glucopyranose 59 (16.0 g, 31.3 mmol) was then dissolved in a minimal quantity of absolute CH₂Cl₂ and added to the solution and stirred for 4 hours. The reaction mixture was then diluted with cold (-15°C) CH₂Cl₂ (500 ml), washed with water (3 x 500 ml) and NaHCO₃ (s a t, a q) (1 x 500 ml). The organic phase was dried over MgSO₄, filtered and evaporated to give a white solid which could be washed with ether (2 x 150 ml) and filtered to give 60 (12.5 g, 75%).

Rₚ = 0.47 hexane:ethyl acetate 1:2 (v/v); ¹H NMR δ 6.41 (d, 1H, H-1, J₁₂=3.2 Hz), 5.52 (t, 1H, H-3), 5.20 (t, 1H, H-4), 4.38 (m, 2H, H-6, H-6'), 4.25 (m, 1H, H-5), 4.14 (m, 1H, H-2), 2.63 (s, 3H, C=C(NH)CH₃), 2.40 (s, 4H, 2CH₂), 2.11, 2.05, 1.96 (3s, 9H, 3Ac), 1.02 (s, 6H, 2CH₃); FAB MS C₂₂H₃₀BrNO₉ (532.28) m/z (%) 532, 534 [M+H]^+ (100, 98), 554, 556 [M+Na]^+ (5, 4), 452 [M-Br]^+ (25).

Rₚ = 0.40 hexane:ethyl acetate 1:3 (v/v); ¹H NMR δ 5.18 (t, 1H, H-3), 5.09 (t, 1H, H-4), 4.85 (d, 1H, H-1, J₁₂=8.9 Hz), 4.34, 4.15 (m, 2H, H-6, H-6'), 3.85 (m, 2H, 2CH₂), 2.09, 2.02, 1.95 (3s, 9H, 3Ac), 1.02 (s, 6H, 2CH₃); FAB MS C₂₂H₃₀N₄O₉ (494.50) m/z (%) 495 [M+H]^+ (20).
Experimental

3,4,6-tri-O-acetyl-2-deoxy-2-{[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)-ethyl]amino}-β-D-glucopyranosylamine (62)

Palladium catalyst (10% on carbon, 50.0 mg) was added in one portion to a solution of 3,4,6-tri-O-acetyl-2-deoxy-2-{[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl]amino}-β-D-glucopyranosyl azide 61 (520 mg, 1.05 mmol) in abs. methanol (5 ml) under a hydrogen atmosphere. The solution was allowed to stir for 36 hours. The catalyst was subsequently filtered off, and the solvent evaporated to give 62 (420 mg, 85%).

R_f = 0.13 hexane:ethyl acetate 1:3 (v/v); ¹H NMR δ 5.16 (t, 1H, H-3), 5.09 (t, 1H, H-4), 4.39 (d, 1H, H-1, J_1,2=9.0 Hz), 4.26 (m, 2H, H-6, H-6'), 3.89 - 3.71 (m, 2H, H-2, H-5), 2.56 (s, 3H, C=C(NH)CH₃), 2.37 (s, 4H, 2CH₂), 2.07, 2.03, 1.99 (3s, 9H, 3Ac), 1.01 (s, 6H, 2CH₃); FAB MS C₂₂H₃₂N₂O₉ (468.50) m/z (%) 469 [M+H]^⁺ (60), 491 [M+Na]^⁺ (10), 452 [M-NH₂+H]^⁺ (5).

3,4,6-tri-O-acetyl-2-deoxy-2-{[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl]amino}-N-dodecyl-β-D-glucopyranosylamide (63)

Dodecanoic acid (198 mg, 0.986 mmol) and DCC (204 mg, 0.986 mmol) were added to a stirred solution of 3,4,6-tri-O-acetyl-2-deoxy-2-{[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl]amino}-β-D-glucopyranosylamine 62 (420 mg, 1.44 mmol) in abs. CH₂Cl₂ (10 ml). The reaction was stirred for 12 hours. After evaporation, the residue was purified by column chromatography to give 63 (376 mg, 64%).

R_f = 0.67 hexane:ethyl acetate 1:3 (v/v); ¹H NMR δ 5.13 (t, 1H, H-3), 5.11 (t, 1H, H-4), 4.44 (d, 1H, H-1, J_1,2=8.5 Hz), 4.31 - 4.11 (m, 2H, H-6, H-6'), 3.85 (m, 1H, H-5), 3.76 (m, 1H, H-2), 2.56 (s, 3H, C=C(NH)CH₃), 2.39 (s, 4H, 2CH₂), 2.08, 2.04, 2.00 (3s, 9H, 3Ac), 1.29 - 1.21 (m, 18H, 9CH₃), 0.88 (t, 3H, CH₃); FAB MS C₃₄H₅₄N₂O₁₀ (650.80) m/z (%) 651 [M+H]^⁺ (20), 673 [M+Na]^⁺ (45), 452 (65).

1,2,3,4-tetra-O-acetyl-β-D-glucopyranuronic acid (64)

D-Glucuronic acid (6.00 g, 31.0 mmol) was suspended in acetic anhydride (85 ml). Iodine (425 mg, 2.00 mmol) was added slowly to the solution at 0°C. The resulting solution was stirred 2 hours and then at room temperature for a further 1 hour. The
solution was re-cooled to 0°C. Methanol (30 ml) was added to the solution to react with the excess acetic anhydride. After standing overnight, the solvents were evaporated and co-evaporated with toluene and benzene. The solid residue was recrystallised from ethyl acetate:hexane 1:1 to afford 64 as a white solid (10.3 g, 92%).

\[ R_f = 0.10 \text{ hexane:ethyl acetate 1:1 (v/v); } ^1\text{H NMR } \delta 5.79 (\text{d, 1H, H-1, } J_{1,2}=7.3 \text{ Hz), 5.29 (m, 2H, H-3, H-4), 5.13 (m, 1H, H-2), 4.24 (m, 1H, H-5), 2.11, 2.04, 2.03, 2.02 (4s, 12H, 4Ac); FAB MS } C_{14}H_{18}O_{11} (362.29) \text{ m/z(%) 385 [M+Na]}^+ (86), 303 [M-OAc]^+ (34), 407 [M+2Na-H]^+ (100). \]

1.2.3.4-tetra-O-acetyl-\(\beta\)-L-glucopyranuronamide (65)

1,2,3,4-tetra-O-acetyl-\(\beta\)-D-glucopyranuronic acid 64 (360 mg, 0.994 mmol), methyl 2-(R/S)-aminooctadecanoate hydrochloride 55b (310 mg, 0.990 mmol) and EEDQ (366 mg, 1.481 mmol) were dissolved in abs. THF (5 ml) and stirred at 40°C for 12 hours. Following evaporation, the residue was purified by column chromatography to give 65 (235 mg, 36%).

\[ R_f = 0.24 \text{ hexane:ethyl acetate 1:3 (v/v); } ^1\text{H NMR } \delta 5.76 (\text{d, 1H, H-1}), 5.30 - 5.05 (\text{m, 3H, H-2, H-3}), 4.49 (\text{m, 1H, } \alpha\text{CH}), 4.09 (\text{m, 1H, H-5}), 3.72, 3.71 (2\text{s, 3H, COOCH}_3), 2.06 - 2.00 (4\text{s, 12H, 4Ac}), 1.29 - 1.23 (\text{m, 30H, 15CH}_2), 0.87 (\text{t, 3H, CH}_3); \text{ Anal. Calcd. for } C_{33}H_{55}NO_{12} (657.79): C, 60.26; H, 8.43; N, 2.13. \text{ Found C, 60.53; H, 8.39; N, 2.11; FAB MS } (657.79) \text{ m/z(%) 658 [M+H]}^+ (19), 680 [M+Na]^+ (100). \]

1,2,3,4-tetra-O-acetyl-N-[1-(R/S)-(methoxycarbonyl)heptadecyl]-\(\beta\)-D-glucopyranuronamide (66)

DCC (68.0 mg, 0.329 mmol) was added to a stirred solution of 1,2,3,4-tetra-O-acetyl-\(\beta\)-D-glucuronic acid 64 (119 mg, 0.329 mmol) in THF (5 ml) at 0°C. After 10 minutes, 2-(R/S)-(2-aminododecyl)-1-(4,5,6,7-tetrachlorophthalimido)-undecyl-\(\beta\)-D-glucopyranuronamide (66)

DCC (68.0 mg, 0.329 mmol) was added to a stirred solution of 1,2,3,4-tetra-O-acetyl-\(\beta\)-D-glucuronic acid 64 (119 mg, 0.329 mmol) in THF (5 ml) at 0°C. After 10 minutes, 2-(R/S)-(2-aminododecyl)-1-(4,5,6,7-tetrachlorophthalimido) 53 (123 mg, 0.263 mmol) was dissolved in CH2Cl2 (2 ml). The reaction was stirred at room temperature for 12 hours. The reaction mixture was then filtered and evaporated. The residue was taken up in CH2Cl2 (20 ml) and washed with NaHCO3(sat, aq) (2 x 20 ml). The organic phase was dried over MgSO4, filtered and evaporated. The residue
was purified by column chromatography in hexane:ethyl acetate 2:1 to give 66 (152 mg, 71%) as two diastereomers (66a and 66b) in a ratio of 4:3 (see Section 4.3.7).

66a: \( R_f = 0.43 \) hexane:ethyl acetate 1:1 (v/v); \(^1H\) NMR \( \delta \) 6.44 (d, 1H, NH), 5.72 (d, 1H, H-1, \( J_{1,2} = 8.0 \) Hz), 5.25 (t, 1H, H-3), 5.14 – 5.10 (m, 2H, H-4, H-2), 4.16 (m, 1H, \( \alpha CH \)), 3.91 (d, 1H, H-5), 3.73 (m, 2H, CH\(_2\)), 2.18, 2.04, 2.00, 1.97 (4s, 12H, 4Ac), 1.45 - 1.20 (m, 18H, 9CH\(_2\)), 0.88 (t, 3H, CH\(_3\)); ESI MS C\(_{34}\)H\(_{42}\)Cl\(_4\)N\(_2\)O\(_{12}\) (812.51) m/z (% 835 [M+Na]\(^+\) (100).

66b: \( R_f = 0.48 \) hexane:ethyl acetate 1:1 (v/v); \(^1H\) NMR \( \delta \) 6.50 (d, 1H, NH), 5.72 (d, 1H, H-1, \( J_{1,2} = 7.8 \) Hz), 5.21 – 5.17 (m, 2H, H-2, H-3), 4.73 (t, 1H, H-4), 4.14 (m, 1H, \( \alpha CH \)), 3.96 (d, 1H, H-5), 3.81, 3.66 (2m, 2H, CH\(_2\)), 2.19, 2.04, 1.98, 1.79 (4s, 12H, 4Ac), 1.25 (m, 18H, 9CH\(_2\)), 0.88 (t, 3H, CH\(_3\)).

\( O-[2',3',4',6'-tetra-O-acetyl-\alpha-D-glucopyranosyl(1'\rightarrow4)]-1,2,3,6-tetra-O-acetyl-\beta-D-glucopyranose (67) \)

Procedure as for 8.

\( R_f = 0.25 \) hexane:ethyl acetate 8:7 (v/v); yield 89%; \(^1H\) NMR \( \delta \) 5.74 (d, 1H, H-1, \( J_{1,2} = 8.1 \) Hz), 5.40 (d, 1H, H-1', \( J_{1,2} = 3.9 \) Hz), 5.29 (t, 1H, H-2'), 5.35 (t, 1H, H-2), 5.05 (t, 1H, H-3'), 4.97 (t, 1H, H-3), 4.86 (dd, 1H, H-4'), 4.44 (dd, 1H, H-4), 4.24 (m, 1H, H-6'), 4.22 (m, 1H, H-6), 4.06 (m, 1H, H-6a), 4.03 (m, 1H, H-6a'), 3.94 (m, 1H, H-5), 3.84 (m, 1H, H-5), 2.13, 2.10, 2.05, 2.02, 2.01, 2.02 (6s, 24H, 8Ac); FAB MS C\(_{28}\)H\(_{38}\)O\(_{19}\) (678.59) m/z (% 701 [M+Na]\(^+\) (65).

\( O-[2',3',4',6'-tetra-O-acetyl-\alpha-D-glucopyranosyl(1'\rightarrow4)]-2,3,6-tri-O-acetyl-\beta-D-glucopyranosyl azide (68) \)

Procedure as for 22.

\( R_f = 0.30 \) hexane:ethyl acetate 8:7 (v/v); yield 84%; \(^1H\) NMR \( \delta \) 5.41 (d, 1H, H-1', \( J_{1,2} = 4.0 \) Hz), 4.85 (dd, 1H, H-4'), 4.78 (t, 1H, H-2), 4.70 (d, 1H, H-1, \( J_{1,2} = 8.7 \) Hz), 2.15 – 1.99 (7s, 21H, 7Ac); FAB MS C\(_{28}\)H\(_{35}\)N\(_3\)O\(_{17}\) (661.57) m/z (% 684 [M+Na]\(^+\) (100), 360 (25).
Experimental

\[O-[2',3',4',6'-\text{tetra-}O-\text{acetyl}-\alpha-D-\text{glucopyranosyl}(1'\rightarrow4)]-2,3,6-\text{tri-}O-\text{acetyl}-\beta-D-\text{glucopyranosylamine (69)}\]

Procedure as for 26.

\[R_f = 0.50 \text{ chloroform:ethyl acetate 1:2 (v/v); yield 72%}; \quad ^1H \text{ NMR } \delta 5.43 (d, 1H, H-1'), \ 4.13 (d, 1H, H-1), \ 2.14 - 2.00 (7s, 21H, 7Ac); \quad \text{MALDI TOF MS } C_{28}H_{37}NO_{17} (635.57) \text{ m/z } (\%) 659 [M+Na]^+ (100), 1278 (43).\]

\[O-[2',3',4',6'-\text{tetra-}O-\text{acetyl}-\alpha-D-\text{glucopyranosyl}(1'\rightarrow4)]-2,3,6-\text{tri-}O-\text{acetyl}-N-\{1-(R/S)-[(\text{tert-butoxycarbonyl})amino]octadecyl\}-\beta-D-\text{glucopyranosylamido (70)}\]

Procedure as for 27c (using 69 in place of 25).

\[R_f = 0.56 \text{ chloroform:methanol 10:0.3 (v/v); yield 64%}; \quad ^1H \text{ NMR } \delta 5.40 - 5.22 (m, 4H), \ 4.86 (m, 1H), \ 4.77 (m, 1H), \ 4.39 (m, 2H), \ 4.02 (m, 2H), \ 3.94 (m, 2H), \ 3.78 (m, 1H), \ 2.12 - 1.99 (7s, 21H, 7Ac), \ 1.70 (m, 2H, \beta CH_2), \ 1.44, 1.43 (2s, 9H, 3 x Boc CH_3), \ 1.25 (m, 28H, 14CH_2), \ 0.87 (t, 3H, CH_3); \quad \text{Anal. Calcd. for } C_{49}H_{80}N_2O_{20} (1017.16): C, 57.87; H, 7.87; N, 2.75. \quad \text{Found: } C, 57.72; H, 7.91; N, 2.81; \quad \text{FAB MS (1017.16) m/z } (\%) 1039 [M+Na]^+ (97), 918 (100).\]

\[O-[O-[2'',3'',4'',6''-\text{tetra-}O-\text{acetyl}-\alpha-D-\text{glucopyranosyl}(1''\rightarrow4'')]\cdot2',3',6'-\text{tetra-}O-\text{acetyl}-\alpha-D-\text{glucopyranosyl}(1'\rightarrow4)]-1,2,3,6-\text{tetra-}O-\text{acetyl}-\beta-D-\text{glucopyranose (71)}\]

Procedure as for 8.

\[R_f = 0.50 \text{ hexane:ethyl acetate 4:10 (v/v); yield 86%}; \quad \text{MALDI TOF MS } C_{40}H_{54}O_{27} (966.84) \text{ m/z } (\%) 989 [M+Na]^+ (100).\]

\[O-[O-[2'',3'',4'',6''-\text{tetra-}O-\text{acetyl}-\alpha-D-\text{glucopyranosyl}(1''\rightarrow4'')]\cdot2',3',6'-\text{tetra-}O-\text{acetyl}-\alpha-D-\text{glucopyranosyl}(1'\rightarrow4)]-1,2,3,6-\text{tetra-}O-\text{acetyl}-\beta-D-\text{glucopyranosyl azide (72)}\]

Procedure as for 22.

\[R_f = 0.70 \text{ hexane:ethyl acetate 4:10 (v/v); yield 79%}; \quad \text{FAB MS } C_{38}H_{51}N_3O_{25} (949.82) \text{ m/z } (\%) 973 [M+Na]^+ (100), 945 (38).\]
Experimental

\[O\{O-[2''',3'''',4'''',6'''-tetra-O-acetyl-\alpha-D-glucopyranosyl(1''\rightarrow4')]\}-2',3',6'-tetra-O-acetyl-\alpha-D-glucopyranosyl(1'\rightarrow4)\}-1,2,3,6-tetra-O-acetyl-\beta-D-glucopyranosyl-amine (73)

Procedure as for 26.

\[R_f = 0.30 \text{ hexane:ethyl acetate 6:10 (v/v); yield 66\%; FAB MS } C_{38}H_{53}NO_{25} (923.82) m/z(\%) \text{ 925 [M+H]^+ (100), 229 (48).}\]

\[O\{O-[2''',3'''',4'''',6'''-tetra-O-acetyl-\alpha-D-glucopyranosyl(1''\rightarrow4')]\}-2',3',6'-tetra-O-acetyl-\alpha-D-glucopyranosyl(1'\rightarrow4)\}-1,2,3,6-tetra-O-acetyl-N-\{1-(\tau/s)-[\text{(tert-butoxycarbonyl)amino}]octadecyl\}-\beta-D-glucopyranosylamide (74)

Procedure as for 27c.

\[R_f = 0.11 \text{ chloroform:methanol 10:0.2 (v/v); yield, 53\%; } ^{1}H \text{ NMR } \delta 5.40 - 5.33 (m, 4H), 5.25 (m, 2H), 5.06 (dd, 1H), 4.90 (dd, 1H), 4.76 (m, 2H), 4.43 (m, 1H), 4.23 (m, 2H), 4.16 (d, 1H), 4.06 (dd, 1H), 3.94 (m, 5H), 3.82 (m, 1H), 2.15 - 1.99 (10s, 30H, 10Ac), 1.70 (m, 2H, } \beta \text{CH}_2\), 1.44, 1.43 (2s, 9H, 3 x } \text{Boc } \text{CH}_3\), 1.25 (m, 28H, 14CH_2), 0.88 (t, 3H, } \text{CH}_3\); Anal. Calcd. for } C_{49}H_{80}N_{20}O_{20} (1305.41): C, 56.13; H, 7.36; N, 2.15. Found C, 56.02; H, 7.42; N, 2.19; FAB MS (1305.41) m/z(\%) 1328 [M+Na]^+ (28), 1438 [M+Cs]^+ (18), 439 (10).

Dodecyl 2,3,4,6-tetra-O-acetyl-1-thio-\alpha/\beta-D-mannopyranoside (75)

1,2,3,4,6-penta-O-acetyl-\alpha/\beta-D-mannopyranose 7 (300 mg, 0.769 mmol), dodecanethiol (186 mg, 0.923 mmol) and BF_3 OEt_2 (546 mg, 3.85 mmol) were dissolved in abs. CH_2Cl_2 (2 ml) and stirred at room temperature for 12 hours. A colourless solution turned red. The solution was evaporated, taken up in CHCl_3 (10 ml) and washed with NaHCO_3(sat, aq) (3 x 10 ml). The organic phase was dried over MgSO_4, filtered and evaporated. The residue was purified by column chromatography in hexane:ethyl acetate 3:1 (v/v) to give 75 (319 mg, 78%) as a mixture of anomers.

\[R_f = 0.74 \text{ hexane:ethyl acetate 1:1 (v/v); } ^{1}H \text{ NMR } \delta 75a: 5.31 - 5.25 (m, 4H, H-1, H-2, H-3, H-4), 4.41 (m, 1H, H-5), 4.34, 4.11 (2m, 2H, H-6, H-6'), 2.69 - 2.53 (m, 2H, SCH_2), 2.16, 2.10, 2.05, 1.99 (4s, 12H, 4Ac), 1.61 (m, 2H, } \beta \text{CH}_2\), 1.38 (m, 2H, } \gamma \text{CH}_2\), 1.26 (m, 16H, 8CH_2), 0.88 (t, 3H, } \text{CH}_3\); \delta 75b: 5.50 (d, 1H, H-4), 5.25 (t, 1H,
Experimental

H-3), 5.06 (dd, 1H, H-2), 4.74 (d, 1H, H-1β), 4.26, 4.14 (2m, 2H, H-6, H-6’), 3.69
(m, 1H, H-5), 2.69 (t, 2H, SCH2), 2.18, 2.07, 2.04, 1.98 (4s, 12H, 4Ac), 1.63 (m, 2H,
βCH2), 1.36 (m, 2H, γCH2), 1.26 (m, 16H, 8CH2), 0.89 (t, 3H, CH3); FAB MS
C26H44O9S (532.69) m/z (%) 555 [M+Na]+ (5), 665 [M+Cs]+ (35), 331 [M-
S(CH2)11CH3]+ (100).

tert-butyl 1-[(2,3,4,6-tetra-O-acetyl-β-D-mannopyranosyl)thio]methyl]-(R/S)-
undecylcarbamate (76)
1,2,3,4,6-penta-O-acetyl-α/β-D-mannopyranose 7 (123 mg, 0.315 mmol), S-{2-(R/S)-
[(tert-butoxycarbonyl)amino]dodecyl}ethanethiol 47a (120 mg, 0.379 mmol) and
BF3OEt2 (224 mg, 1.58 mmol) were dissolved in abs. CH2Cl2 (1 ml) and stirred at
room temperature for 12 hours. The solution was evaporated, taken up in CH2Cl2
(10 ml) and washed with NaHCO3 (sat, aq) (2 x 10 ml). The organic phase was dried
over MgSO4, filtered and evaporated. The residue was purified by column
chromatography to give 76 (78.0 mg, 38%).

Rf = 0.74 hexane:ethyl acetate 1:1 (v/v); 1H NMR δ 5.35 – 5.27 (m, 4H, H-1, H-2,
H-3, H-4), 4.37 - 4.34, 4.13 (2m, 4H, H-5, H-6, H-6’, αCH), 2.72 (m, 2H, SCH2),
2.14, 2.10, 2.05, 1.99 (4s, 12H, 4Ac), 1.43 (s, 9H, 3 x Boc CH3), 1.30 - 1.24 (m, 18H,
9CH2), 0.87 (t, 3H, CH3); FAB MS C31H53NO11S (647.82) m/z (%) 648 [M+H]+ (5),

2-(R/S)-[(tert-butoxycarbonyl)amino]dodecyl 2,2,2-trichloroethanimidoate (77)
tert-butyl N-[1-(R/S)-(hydroxymethyl)undecyl]carbamate 9a (500 mg, 1.66 mmol)
was dissolved in absolute CH2Cl2 (30 ml) and cooled to 0°C. Molecular sieves (100
mg) and trichloroacetonitrile (959 mg, 6.64 mmol) were subsequently added.
Sodium hydride (55.8 mg, 2.33 mmol) was added in one portion and stirred at 0°C for
2 hours. The solution was then stirred for 90 minutes at room temperature. The
reaction mixture was filtered through a celite pad and evaporated. The residue was
purified by column chromatography in hexane:ethyl acetate 5:1 (v/v) to give 77 (450
mg, 61%).

Rf = 0.79 hexane:ethyl acetate 3:1 (v/v); 1H NMR δ 8.32 (s, 1H, NH=CCCl3), 4.65
(d, 1H, NHC=O), 4.27 (m, 2H, αCH, CH2α), 3.95 (m, 1H, CH2β), 1.44 (s, 9H, 3 x
Experimental

Boc CH₃, 1.25 (m, 18H, 9CH₂), 0.87 (t, 3H, CH₃); FAB MS C₁₉H₃₅Cl₃N₂O₃ (445.85) m/z (%) 445, 447 [M+H]⁺ (85, 75), 467, 469 [M+Na]⁺ (43, 42).

2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucopyranose (78)

2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide 18 (1.00 g, 2.43 mmol) and thiourea (185 mg, 2.43 mmol) were dissolved in absolute acetone (20 ml) and refluxed for 12 hours. The solvent was evaporated and the solid residue suspended in 1,2-dichloroethane (8 ml). Sodium metabisulphite (485 mg, 2.55 mmol) was dissolved in water (5 ml), which was added to the organic phase and stirred vigorously for 15 minutes. The organic phase was diluted with CH₂Cl₂ (25 ml) and washed with brine (2 x 30 ml) and NaHCO₃ (s a t, a q) (1 x 30 ml). The organic phase was dried over MgSO₄, filtered and evaporated. Purification by column chromatography gave 78 (658 mg, 74%).

Rₛ = 0.48 hexane:ethyl acetate 1:1 (v/v); ¹H NMR δ 5.17 (t, 1H, H-3), 5.08 (t, 1H, H-2), 4.95 (t, 1H, H-4), 4.53 (t, 1H, H-1, J₁₂=9.8 Hz), 4.22, 4.11 (m, 2H, H-6, H-6'), 3.71 (m, 1H, H-5), 2.29 (d, 1H, SH), 2.08, 2.07, 2.00, 1.98 (4s, 12H, 4Ac); FAB MS C₁₄H₂₀O₉S (364.37) m/z (%) 387 [M+Na]⁺ (15), 497 [M+Cs]⁺ (100), 331 [M-SH]⁺ (35).

2,3,4,6-tetra-O-acetyl-1-thio-β-D-galactopyranose (79)

Procedure as for 78.

Rₛ = 0.47 hexane:ethyl acetate 1:1 (v/v); yield 75%; ¹H NMR δ 5.41 (d, 1H, H-4), 5.16 (t, 1H, H-2), 5.00 (dd, 1H, H-3), 4.52 (t, 1H, H-1), 4.10 (m, 2H, H-6, H-6'), 3.93 (m, 1H, H-5), 2.35 (d, 1H, SH), 2.14, 2.07, 2.03, 1.96 (4s, 12H, 4Ac); FAB MS C₁₄H₂₀O₉S (364.37) m/z (%) 365 [M+H]⁺ (27), 387 [M+Na]⁺ (14), 331 [M-SH]⁺ (100).

2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-1-thio-β-D-glucopyranose (80)

2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-glucopyranosyl chloride 21 (3.00 g, 8.21 mmol) and thiourea (2.50 g, 32.8 mmol) were dissolved in absolute acetone (50 ml) and refluxed for 12 hours. The solvent was evaporated and the solid residue suspended in 1,2-dichloroethane (50 ml). Sodium metabisulphite (1.72 g, 9.03 mmol) was dissolved in water (30 ml), which was added to the organic phase and
stirred vigorously for 15 minutes. The organic phase was diluted with CH$_2$Cl$_2$ (50 ml) and washed with brine (2 x 100 ml) and NaHCO$_3$(sat, aq) (1 x 100 ml). The organic phase was dried over MgSO$_4$, filtered and evaporated. Purification by column chromatography gave 80 (2.06 g, 69%).

R$_s$ = 0.16 hexane:ethyl acetate 1:4 (v/v); $^1$H NMR $\delta$ 5.69 (d, 1H, H-1), 5.23 (t, 1H, H-3), 5.07 (t, 1H, H-4), 4.62 (t, 1H, H-1, $J_{1,2}$=9.0 Hz), 4.54 (m, 1H, H-2), 4.16, 4.05 (m, 2H, H-6, H-6'), 3.69 (m, 1H, H-5), 2.04, 2.03, 2.00, 1.98 (4s, 12H, 4Ac); FAB MS C$_{14}$H$_{21}$NO$_2$S (363.38) m/z (%) 364 [M+H]$^+$ (85), 386 [M+Na]$^+$ (80), 330 [M-SH]$^+$ (100).

3,4,6-tri-O-acetyl-2-deoxy-2-[[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)-ethyl]amino]-α/β-D-glucopyranosyl thiolate (81a)

3,4,6-tri-O-acetyl-2-deoxy-2-[[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)-ethyl]amino]-α-D-glucopyranosyl bromide 60 (14.8 g, 27.8 mmol) was dissolved in ethyl acetate (150 ml). Potassium thioacetate (3.80 g, 33.8 mmol) and tetra-butyl-n- ammonium hydrogensulphate (944 mg, 2.78 mmol) were added. NaHCO$_3$(sat, aq) (75 ml) was added to the solution and the reaction was stirred for 90 minutes. The reaction was diluted with ethyl acetate (75 ml) and the layers separated. The organic phase was washed with water (1 x 200 ml) and brine (1 x 200 ml). The solution was then dried over MgSO$_4$, filtered and evaporated. The residue was recrystallised from isopropanol to give 81a (12.5 g, 85%).

R$_s$ = 0.55 hexane:ethyl acetate 1:2 (v/v); $^1$H NMR $\delta$ 13.92 (d, 1H, NH), 5.31 (d, 1H, H-1, $J_{1,2}$=10 Hz), 5.23, 5.10 (2t, 2H, H-3, H-4), 4.30 (dd, 1H, H-2), 4.14 (m, 2H, H-6, H-6'), 3.89 (m, 1H, H-5), 2.56 (s, 3H, C=C(NH)CH$_3$), 2.34 (m, 7H, 3Ac, 2CH$_2$), 2.07, 2.03, 1.95 (3s, 9H, 3Ac), 0.99 (s, 6H, 2CH$_3$); FAB MS C$_{24}$H$_{33}$NO$_3$S (527.59) m/z (%) 528 [M+H]$^+$ (100), 550 [M+Na]$^+$ (5), 484 (34).

3,4,6-tri-O-acetyl-2-deoxy-2-[[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)-ethyl]amino]-1-thio-α/β-D-glucopyranose (81b)

Method A

3,4,6-tri-O-acetyl-2-deoxy-2-[[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl]amino]-α-D-glucopyranosyl bromide 60 (2.50 g, 4.70 mmol) and thiourea (1.43 g, 18.8 mmol) were dissolved in absolute acetone (40 ml) and refluxed for 12 hours.
The solvent was evaporated and the solid residue suspended in 1,2-dichloroethane (30 ml). Sodium metabisulphite (983 mg, 5.17 mmol) was dissolved in water (20 ml), which was added to the organic phase and stirred vigorously for 15 minutes. The organic phase was diluted with dichloromethane (20 ml) and washed with brine (2 x 50 ml) and NaHCO₃(sat, aq) (1 x 50 ml). The organic phase was dried over MgSO₄, filtered and evaporated. Purification by column chromatography gave 81b (1.46 g, 64%).

Method B

3,4,6-tri-O-acetyl-2-deoxy-2-\{1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl\}-amino-\(\alpha/\beta\)-D-glucopyranosyl thiolate 81a (200 mg, 0.380 mmol) was dissolved in abs. DMF (5 ml). Hydrazine acetate (41.9 mg, 0.455 mmol) was added and the solution was stirred for 5 minutes at 50°C. The reaction was diluted with dichloromethane (20 ml) and washed with water (1 x 25 ml) to give 81b (145 mg, 79%)

\(R_f = 0.38\) hexane:ethyl acetate 1:3 (v/v); \(^1\)H NMR \(\delta 5.63\) (t, 1H, H-1\(\alpha\)), 5.17 - 5.05 (t, 2H, H-3, H-4), 4.74 (t, 1H, H-1\(\beta\)), 4.35 - 4.28, 4.11 (2m, 2H, H-6, H-6'), 3.96 (m, 1H, H-2), 3.78 (m, 1H, H-5), 2.59 (s, 3H, C=\(\text{C(NH)}\)CH₃), 2.35 (s, 4H, 2CH₂), 2.09, 2.02, 1.95 (3s, 9H, 3Ac), 1.02 (s, 6H, 2CH₃); FAB MS C₂₂H₃₁NO₉S (485.55) m/z (%): 486 [M+H]⁺ (100), 508 [M+Na]⁺ (10), 452 [M-SH]⁺ (15).

\textit{tert}-butyl 1-\{[(2,3,4,6-tetra-O-acetyl-\(\beta\)-D-glucopyranosyl)thio]methyl\}-(\(R/s\))-undecylcarbamate (82a)

Procedure as for 83a, Method B (using 78 in place of 79).

\(R_f = 0.63\) hexane:ethyl acetate 1:1 (v/v); yield 81%; \(^1\)H NMR \(\delta 5.20\) (t, 1H, H-2), 5.11 - 4.97 (m, 2H, H-3, H-4), 4.49 (d, 1H, H-1, J₁₂=10.1 Hz), 4.25 - 4.09 (m, 2H, H-6, H-6'), 3.72 (m, 2H, \(\alpha\)CH, H-5), 2.76, 2.73 (2m, 2H, CH₂), 2.08, 2.05, 2.03, 1.99 (4s, 12H, 4Ac), 1.43 (s, 9H, 3 x Boc CH₃), 1.39 - 1.24 (m, 18H, 9CH₂), 0.86 (t, 3H, CH₃); \(^{13}\)C NMR \(\delta 170.5, 170.1, 169.8, 169.5, 155.3, 85.4, 84.1, 76.5, 76.4, 74.7, 74.4, 71.6, 67.5, 67.3, 67.1, 66.1, 61.4, 61.2, 53.0, 49.9, 36.6 - 20.5, 14.0; FAB MS C₃₁H₅₃NO₁₁S (647.82) m/z (%): 648 [M+H]⁺ (40), 670 [M+Na]⁺ (20), 548 [M-Boc+H]⁺ (80).
**Experimental**

**tert-butyl 1-{{(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)thio}methyl}-(R/S)-tridecylcarbamate (82b)**

Procedure as for 83a, Method B (using 78 in place of 79, and 9b in place of 9a).

R <sub>f</sub> = 0.46 hexane:ethyl acetate 2:1 (v/v); yield 84%; ¹H NMR δ 5.17 (t, 1H, H-2), 5.10, 4.94 (2m, 2H, H-3, H-4), 4.46 (d, 1H, H-1, J<sub>1,2</sub>=9.6 Hz), 4.23, 4.12 (2m, 2H, H-6, H-6'), 3.69 (m, 2H, αCH, H-5), 2.75 (m, 2H, CH₂), 2.07, 2.04, 2.03, 1.99 (4s, 12H, 4Ac), 1.43 (s, 9H, 3 x Boc CH₃), 1.25 (m, 22H, 11CH₂), 0.87 (t, 3H, CH₃); FAB MS C<sub>33</sub>H<sub>57</sub>NO<sub>11</sub>S (675.87) m/z (%) 676 [M+H]<sup>+</sup> (65), 808 [M+Cs]<sup>+</sup> (40), 576 [M-Boc+H]<sup>+</sup> (95).

**tert-butyl 1-{{(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)thio}methyl}undecyl-carbamate (83a)**

**Method A₁**

2,3,4,6-tetra-O-acetyl-1-thio-β-D-galactopyranose 79 (115 mg, 0.316 mmol) was dissolved in abs. DMF (2ml) and cooled to −30°C. Sodium hydride (7 mg, 0.290 mmol) was added portionwise to the solution, which was stirred for 5 minutes. 2-(R/S)-[{(tert-butoxycarbonyl)amino}dodecyl methanesulphonate 44a (100 mg, 0.264 mg) was dissolved in abs. DMF and added to the reaction mixture. The solution was stirred for a further 2 hours, then for 1 hour at room temperature. The reaction mixture was diluted with CH₂Cl₂ (20 ml) and was washed with 1M KHSO₄(aq) (1 x 25 ml), NaHCO₃(sat, aq) (1 x 25 ml) and brine (1 x 25 ml). The organic phase was dried over MgSO₄, filtered and evaporated. The residue was purified by column chromatography in hexane:ethyl acetate 2:1 (v/v) to give 83a (116 mg, 57%).

**Method A₂**

2-(R/S)-[{(tert-butoxycarbonyl)amino}dodecyl 4-methylbenzenesulphonate 43a was used as a substitute for 2-(R/S)-[{(tert-butoxycarbonyl)amino}dodecyl methanesulphonate 44a with similar results. Product yield: 59%

**Method B**

Trimethylphosphine (1.0M, 1.731 mmol) was added dropwise to a stirred solution of ADDP (437 mg, 1.73 mmol) in abs. THF (20 ml) at 0°C. After stirring for 30 minutes, tert-butyl N-[1-(R/S)-(hydroxymethyl)undecyl]carbamate 9a (261 mg, 0.865 mmol) and 2,3,4,6-tetra-O-acetyl-1-thio-β-D-galactopyranose 79 (630 mg, 1.73 mmol) in abs. THF (5 ml) were added to the solution. The reaction was stirred at
room temperature overnight. The solvent was then evaporated. The product was dissolved in hexane, and the insoluble organic precipitate filtered off. Following evaporation of the solvent, the residue was taken up in CH₂Cl₂ (50 ml) and was washed with NaHCO₃ sat. (2 x 50 ml). The organic phase was dried over MgSO₄, filtered and evaporated. The residue was purified by column chromatography in hexane:ethyl acetate 2:1 (v/v) to give 83a (460 mg, 82%).

\[ R_f = 0.68 \text{ hexane:ethyl acetate 1:1 (v/v)}; \text{ } ^1H \text{ NMR } \delta 5.43 (d, 1H, H-4), 5.19 (m, 1H, H-2), 5.03 (m, 1H, H-3), 4.48 (d, 1H, H-1, J₁₂=9.8 Hz), 4.17, 4.12 (m, 3H, } \alpha \text{CH, H-6, H-6}^-; 3.94 (m, 1H, H-5), 2.88, 2.79 (2m, 2H, CH₂), 2.13, 2.08, 2.03, 1.97 (4s, 12H, 4Ac), 1.43 (s, 9H, 3 x Boc CH₃), 1.24 (m, 18H, 9% ), 0.88 (t, 3H, CH₃); FAB MS C₃₃H₅₃NO₇S (647.82) m/z (%) 670 [M+Na]^⁺(10), 780 [M+Cs]^⁺ (100), 548 [M-Boc+H]^⁺ (20).

**tert-butyl** 1-[(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)thio]methyl)-(R/s)-tridecylcarbamate (83b)

Procedure as for 83a, Method A₁ (using 44b in place of 44a).

\[ R_f = 0.49 \text{ hexane:ethyl acetate 2:1 (v/v); yield 55%; } ^1H \text{ NMR } \delta 5.44 (dd, 1H, H-4), 5.20 (m, 1H, H-2), 5.03 (m, 1H, H-3), 4.41 (d, 1H, H-1, J₁₂=9.8 Hz), 4.16 (m, 3H, } \alpha \text{CH, H-6, H-6}^-; 3.92 (m, 1H, H-5), 2.88, 2.78 (2m, 2H, CH₂), 2.13, 2.07, 2.04, 1.99 (4s, 12H, 4Ac), 1.43 (s, 9H, 3 x Boc CH₃), 1.29 – 1.24 (m, 22H, 11CH₂), 0.87 (t, 3H, CH₃); FAB MS C₃₃H₅₇NO₁₇S (675.87) m/z (%) 698 [M+Na]^⁺ (20), 808 [M+Cs]^⁺ (80), 576 [M-Boc+H]^⁺ (100).

**tert-butyl** 1-[(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)thio]methyl)-(R/s)-undecylcarbamate (84a)

Procedure as for 83a, Method B (using 80 in place of 79, and 9b in place of 9a).

\[ R_f = 0.31 \text{ hexane:ethyl acetate 4:1 (v/v); yield 77%; } ^1H \text{ NMR } \delta 5.04 (m, 2H, H-3, H-4), 4.56 (d, 1H, H-1, J₁₂=10.1 Hz), 4.16, 4.09 (2m, 3H, } \alpha \text{CH, H-6, H-6}^-; 3.65 (m, 2H, H-2, H-5), 2.77, 2.71 (2m, 2H, CH₂), 2.02, 1.96, 1.89 (3s, 12H, 4Ac), 1.39 (s, 9H, 3 x Boc CH₃), 1.19 (m, 18H, 9CH₂), 0.81 (t, 3H, CH₃); ^13C NMR δ 168.5, 167.9, 167.5, 166.8, 153.4, 82.6, 81.8, 76.9, 73.6, 73.4, 71.1, 65.9, 65.8, 59.8, 59.5,
Experimental

51.2, 50.4, 48.4, 47.1, 33.8 – 17.9, 11.4; FAB MS C_{31}H_{54}N_{2}O_{10}S (646.83) m/z (%) 669 [M+Na]^+ (40), 547 [M-Boc+H]^+ (80).

1-[(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)thio]methyl)-(R/S)-tridecylcarbamate (84b)

Procedure as for 83a, Method B (using 80 in place of 79).

R_f = 0.37 hexane:ethyl acetate 3:1 (v/v); yield 77%; \(^1^H\) NMR \(\delta\) 5.09 – 5.02 (m, 2H, H-3, H-4), 4.53 (d, 1H, H-1, J_{1,2} = 10.1 Hz), 4.18 – 4.11 (2m, 3H, αCH, H-6, H-6'), 3.67 – 3.52 (m, 2H, CH_2), 2.77, 2.73 (2m, 2H, CH_2), 2.04, 2.01, 1.99 (3s, 12H, 4Ac), 1.43 (s, 9H, 3 x Boc CH_3), 1.24 (m, 22H, 1ICH_2), 0.87 (t, 3H, CH_3); FAB MS C_{33}H_{58}N_{2}O_{10}S (674.89) m/z (%) 675 [M+H]^+ (20), 576 [M-Boc+H]^+ (90).

Methyl 3,4,6-tri-O-acetyl-2-deoxy-2-[[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl]amino]-1-thio-α/β-D-glucopyranose (85)

Procedure as for 86 (using methanol in place of ethanol).

R_f = 0.49 hexane:ethyl acetate 1:4 (v/v); yield 85%; \(^1^H\) NMR \(\delta\) 5.24 (d, 1H, H-1α, J_{1,2} = 4.8 Hz), 4.98 (m, 2H, H-3, H-4), 4.39 (m, 1H, H-2), 4.32 – 4.06 (3m, 3H, H-5, H-6, H-6'), 2.67 (s, 3H, C=CH(NH)CH_3), 2.52, 2.34 (2s, 4H, 2CH_2), 2.14 (s, 3H, SCH_3), 2.07, 2.06, 2.02 (3s, 9H, 3Ac), 1.09 (s, 6H, 2CH_3); FAB MS C_{33}H_{33}NO_{9}S (499.58) m/z (%) 499 [M+H]^+ (100), 521 [M+Na]^+ (20).

Ethyl 3,4,6-tri-O-acetyl-2-deoxy-2-[[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl]amino]-1-thio-α/β-D-glucopyranose (86)

Trimethylphosphine (1.0M, 0.344 mmol) was added dropwise to a stirred solution of ADDP (87.0 mg, 0.344 mmol) in abs. THF (10 ml) at 0°C. After stirring for 30 minutes, abs. ethanol (8.00 mg, 0.172 mmol) and 3,4,6-tri-O-acetyl-2-deoxy-2-[[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl]amino]-1-thio-α/β-D-glucopyranose 81b (100 mg, 0.206 mmol) in abs. THF (5 ml) were added to the solution. The reaction was stirred at room temperature overnight. The solvent was then evaporated. The product was dissolved in hexane, and the insoluble organic precipitate filtered off. Following evaporation of the solvent, the residue was taken up in CH_2Cl_2 (50 ml) and was washed with NaHCO_3(aq) (2 x 50 ml). The organic phase was dried over MgSO_4, filtered and evaporated. The residue was purified by
column chromatography in hexane:ethyl acetate 1:1 (v/v) to give 86 (460 mg, 77%, α:β = 3:2).

**86α:** \( R_f = 0.49 \) hexane:ethyl acetate 1:4 (v/v); \(^1\)H NMR δ 5.27 (d, 1H, H-1α, J\(_{1,2}\)=5.0 Hz), 5.27 (t, 1H, H-3), 5.07 (t, 1H, H-4), 4.53 (m, 1H, H-2), 4.35, 4.06 (2m, 3H, H-5, H-6, H-6′), 2.61 (s, 3H, C=C(NH)CH\(_3\)), 2.38 (s, 4H, 2CH\(_2\)), 2.10, 2.03, 1.93 (3s, 9H, 3Ac), 1.25 (m, 2H, SCH\(_2\)), 1.01 (s, 6H, 2CH\(_3\)), 0.87 (t, 3H, CH\(_3\)).

**86β:** \( R_f = 0.57 \) hexane:ethyl acetate 1:4 (v/v); \(^1\)H NMR δ 5.17 (t, 1H, H-3), 5.08 (t, 1H, H-4), 4.60 (d, 1H, H-1β, \( J_{1,2}=9.9 \) Hz), 4.28, 4.11 (2dd, 2H, H-6, H-6′), 3.97 (m, 1H, H-2), 3.75 (m, 1H, H-5), 2.56 (s, 3H, C=C(NH)CH\(_3\)), 2.37 (s, 4H, 2CH\(_2\)), 2.08, 2.03, 1.95 (3s, 9H, 3Ac), 1.26 (m, 2H, SCH\(_2\)), 1.01 (s, 6H, 2CH\(_3\)), 0.86 (t, 3H, CH\(_3\)); FAB MS C\(_{24}\)H\(_{35}\)NO\(_9\)S (513.60) m/z (%) 514 [M+H]\(^{+}\) (85), 647 [M+Cs]\(^{+}\) (75), 452 (40).

**Octyl 3,4,6-tri-O-acetyl-2-deoxy-2-[[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl]amino]-1-thio-α/β-D-glucopyranose (87)**

Procedure as for 86 (using octanol in place of ethanol).

\( R_f = 0.56 \) hexane:ethyl acetate 1:4 (v/v); yield 66%, α:β = 5:2; \(^1\)H NMR δ 5.33 (d, 1H, H-1α, \( J_{1,2}=4.98 \) Hz), 5.27 (t, 1H, H-3), 5.06 (t, 1H, H-4), 4.35 (m, 1H, H-2), 4.11 (m, 2H, H-6, H-6′), 3.71 (m, 1H, H-5), 2.62 (s, 3H, C=C(NH)CH\(_3\)), 2.39 (s, 4H, 2CH\(_2\)), 2.11, 2.04, 1.94 (3s, 9H, 3Ac), 1.24 (m, 14H, 7%, 3CH\(_2\)CH\(_2\)), 1.02 (s, 6H, 2CH\(_3\)), 0.88 (t, 3H, CH\(_3\)); FAB MS C\(_{30}\)H\(_{47}\)NO\(_9\)S (597.76) m/z (%) 598 [M+H]\(^{+}\) (95), 730 [M+Cs]\(^{+}\) (100), 452 (25), 392 (95).

**2-[2-(2-methoxyethoxy)ethoxy]ethyl 3,4,6-tri-O-acetyl-2-deoxy-2-[[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl]amino]-1-thio-α/β-D-glucopyranose (88)**

Procedure as for 86 (using 2-[2-(2-methoxyethoxy)ethoxy]ethyl alcohol in place of ethanol).

\( R_f = 0.18 \) hexane:ethyl acetate 1:5 (v/v); yield 67%, α:β = 1:1; \(^1\)H NMR δ 5.54 (d, 1H, H-1α, \( J_{1,2}=4.91 \) Hz), 5.26 (t, 1H, H-3), 5.08 (t, 1H, H-4), 4.49 (d, 1H, H-1β, \( J_{1,2}=8.2 \) Hz), 4.36 (m, 2H, H-6, H-6′), 4.07 (dd, 1H, H-2), 3.76 – 3.44 (m, 13H, H-5, 3CH\(_2\)CH\(_2\)), 3.37 (s, 3H, OCH\(_3\)), 2.62 (s, 3H, C=C(NH)CH\(_3\)), 2.39 (s, 4H, 2CH\(_2\)).
Experimental

2.10, 2.03, 1.94 (3s, 9H, 3Ac), 1.01 (s, 6H, 2CH₃); FAB MS C₂₉H₄₅NO₁₂S (631.73) m/z (%) 632 [M+H]⁺ (25), 655 [M+Na]⁺ (100).

**Methyl 2-[(R/s)-((tert-butoxycarbonyl)amino)tetradecanamido]-3,4,6-tri-O-acetyl-2-deoxy-1-thio-β-D-glucopyranose (90)**

Methyl 3,4,6-tri-O-acetyl-2-deoxy-2-[[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl]amino]-1-thio-α/β-D-glucopyranose 85 (4.40 g, 8.82 mmol) was dissolved in a 2% solution of hydrazine monohydrate in CH₂Cl₂ (30 ml). After 2 hours, the solution was diluted with CH₂Cl₂ (20 ml) and washed with water (2 x 50 ml). The organic phase was dried over MgSO₄, filtered and evaporated. The residue was purified by column chromatography (to remove by-products which included the unreacted β-anomer, see section 4.4.4) in hexane:ethyl acetate 1:3 (v/v) to give 89α. 89α (1.84 g, 5.49 mmol) was dissolved in abs. CH₂Cl₂ (20 ml). l-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDAC, 1.58 g, 8.24 mmol) and 2-(R/S)-[(tert-butoxycarbonyl)amino]tetradecanoic acid 2b (1.88 g, 5.49 mmol) were added to the solution which was stirred at room temperature for 12 hours. The solution was diluted with CH₂Cl₂ (30 ml) and washed with NaHCO₃(sat, aq) (2 x 50 ml). The organic phase was dried over MgSO₄, filtered and evaporated. The residue was purified by column chromatography in hexane:ethyl acetate 1:3 (v/v) to give 90 (2.07 g, 57%).

Rₛ = 0.73 hexane:ethyl acetate 1:4 (v/v); ¹H NMR δ 5.30 (d, 1H, H-1), 5.05 - 4.95 (m, 2H, H-3, H-4), 4.30 - 4.15 (2m, 3H, H-5, H-6, H-6'), 4.05 (m, 1H, H-2), 3.80 (m, 1H, αCH), 2.15 (s, 3H, SCH₃), 2.05, 2.04, 2.00 (3s, 9H, 3Ac), 1.44 (s, 9H, 3 x Boc CH₃), 1.31 - 1.25 (m, 22H, IICH₂), 0.88 (t, 3H, CH₃); FAB MS C₃₂H₅₆N₂O₁₀S (660.86) m/z (%) 683 [M+H]⁺ (5), 793 [M+Cs]⁺ (10), 561 [M-Boc+H]⁺ (5), 499 (100).

2-acetamido-3,4,6-tri-O-acetyl-1-S-[(2-(R/S)-((tert-butoxycarbonyl)amino)decyl)sulphanyl]-2-deoxy-1-thio-β-D-glucopyranose (91)

Trimethylphosphine (1.0M, 0.918 mmol) was added dropwise to a stirred solution of ADDP (232 mg, 0.918 mmol) in abs. THF (15 ml) at 0°C. After stirring for 30 minutes, S-[(2-(R/S)-[(tert-butoxycarbonyl)amino]decyl]ethanethiol 47a (146 mg,
0.459 mmol) and 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-1-thio-α/β-D-glucopyranose 80 (250 mg, 0.689 mmol) in abs. THF (10 ml) were added to the solution. The reaction was stirred at room temperature overnight. The solvent was then evaporated. The product was dissolved in hexane, and the insoluble organic precipitate filtered off. Following evaporation of the solvent, the residue was taken up in CH\textsubscript{2}Cl\textsubscript{2} (50 ml) and was washed with NaHCO\textsubscript{3} (sat. aq) (2 x 50 ml). The organic phase was dried over MgSO\textsubscript{4}, filtered and evaporated. The residue was purified by column chromatography in hexane:ethyl acetate 3:1 (v/v) to give 91 (194 mg, 62%).

R\textsubscript{f} = 0.35 hexane:ethyl acetate 3:1 (v/v); \textsuperscript{1}H NMR \( \delta \) 5.30 (d, 1H, H-1), 5.19, 5.08 (2t, 2H, H-3, H-4), 4.66 (d, 1H, H-1, \( \text{J}_{2} \)=10.7 Hz), 4.29 - 4.11 (m, 3H, \( \alpha \text{CH. H-6}, \text{H-6}^{'} \)), 3.79 - 3.44 (3m, 4H, H-2, H-5, CH\textsubscript{2}), 2.08, 2.02, 2.00, 1.96 (4s, 12H, 4Ac), 1.48, 1.45 (2s, 9H, 3 x Boc CH\textsubscript{3}), 1.25 (m, 18H, 9CH\textsubscript{2}), 0.88 (t, 3H, CH\textsubscript{3}); FAB MS C\textsubscript{31}H\textsubscript{55}NO\textsubscript{11}S (679.88) m/z (%) 702 [M+Na\textsuperscript{+} (20), 579 [M-Boc+H]\textsuperscript{+} (100), 330 (75).

**Methyl N-(
\textit{tert}-butoxycarbonyl)serinate (92)**

HCl gas was introduced to a suspension of L-serine (20.0 g, 0.190 mmol) in methanol (200 ml) at 0°C. This was stirred for 20 minutes, at which point the solid had dissolved. The reaction vessel was sealed and allowed to stand for 12 hours. The solvent was subsequently evaporated and co-evaporated with toluene to give the methyl ester (30g). A portion of the residue (15.0 g, 0.126 mmol) was dissolved in a \textit{tert}-butyl alcohol and water mixture (1:2). Boc\textsubscript{2}O (35.7 g, 0.164 mmol) and NaOH (6.56 g, 0.164 mmol) were added to the solution, which was subsequently stirred for 12 hours. The solution was adjusted to pH 3 using KHSO\textsubscript{4}. The product was extracted into ethyl acetate (500 ml) and washed with 1M KHSO\textsubscript{4} (aq) (1 x 500 ml). The organic phase was dried over MgSO\textsubscript{4}, filtered and evaporated to give 92 (23.5 g, 85%).

R\textsubscript{f} = 0.27 hexane:ethyl acetate 1:1 (v/v); \textsuperscript{1}H NMR \( \delta \) 5.95 (br s, 1H, NH), 4.40 (m, 1H, \( \alpha \text{CH} \)), 3.93 - 3.85 (m, 2H, CH\textsubscript{2}), 3.69 (s, 3H, COOCH\textsubscript{3}), 1.41 (s, 9H, 3 x Boc CH\textsubscript{3}); FAB MS C\textsubscript{9}H\textsubscript{17}NO\textsubscript{5} (219.24) m/z (%) 220 [M+H]\textsuperscript{+} (95).

**N-(
\textit{tert}-butoxycarbonyl)serine (93)**

L-Serine (15.0 g, 0.143 mmol) was dissolved in a \textit{tert}-butyl alcohol and water mixture (1:2). Boc\textsubscript{2}O (40.5 g, 0.186 mmol) and NaOH (7.40 g, 0.186 mmol) were
Experimental

added to the solution, which was subsequently stirred for 12 hours. The solution was
adjusted to pH 3 using KHSO₄. The product was extracted into ethyl acetate (800
ml) and washed with 1M KHSO₄(aq) (1 x 500 ml). The organic phase was dried over
MgSO₄, filtered and evaporated to give 93 (21.1 g, 72%).
Rₛ = 0.45 chloroform:methanol 10:4 (v/v); ¹H NMR δ 5.85 (br, 1H, NH), 4.38 (m,
1H, αCH), 3.90 (m, 2H, CH₂), 1.40 (s, 9H, 3 x Boc CH₃); FAB MS C₈H₁₅NO₅
(205.21) m/z (%) 228 [M+Na]⁺ (100).

Benzyl N-(tert-butoxycarbonyl)serinate (94)
Caesium carbonate (3.97 g, 12.2 mmol) was dissolved in methanol (10 ml). Water (3
drops) was added to the solution to fully dissolve the solid. This solution was then
added dropwise to a solution of N-(tert-butoxycarbonyl)serine 93 (5.00 g, 24.4
mmol) in methanol (25 ml). The solution was then stirred for 30 minutes. The
solvent was subsequently evaporated, yielding the caesium salt. The residue was
dissolved in abs. DMF (20 ml). Benzyl bromide (6.26 g, 36.6 mmol) was added to
the solution, which was stirred for 4 hours. The reaction mixture was filtered and
then evaporated. The residue was taken up in CH₂Cl₂ (50 ml) and washed with brine
(2 x 50 ml). The organic phase was dried over MgSO₄, filtered and evaporated. The
residue was purified by column chromatography in hexane:ethyl acetate 2:1 (v/v) to
give 94 (5.33 g, 74%).
Rₛ = 0.58 chloroform:methanol 10:1 (v/v); ¹H NMR δ 7.24 (m, 5H, arom.H), 5.40
(br s, 1H, NH), 5.11 (s, 2H, PhCH₂), 4.32 (m, 1H, αCH), 3.87, 3.81 (2m, 2H, CH₂),
1.34 (s, 9H, 3 x Boc CH₃); FAB MS C₁₅H₂₁NO₅ (295.33) m/z (%) 296 [M+H]⁺ (15),
318 [M+Na]⁺ (27), 196 [M-Boc+H]⁺ (85), 240 [M-tBu+H]⁺ (100).

Methyl 2-[(tert-butoxycarbonyl)amino]acrylate (95)
Methyl N-(tert-butoxycarbonyl)serinate 92 (2.00 g, 9.12 mmol) was dissolved in abs.
acetonitrile (5 ml). Copper(I) chloride (271 mg, 2.74 mmol) and diisopropyl
 carbodiimide (1.29 g, 10.0 mmol) were subsequently added. After stirring for 12
hours, the solvent was evaporated. The residue was dissolved in CH₂Cl₂ (20 ml) and
the solid urea by-product filtered off. The solution was passed through a celite pad.
The solution was washed with NaHCO₃(aq) (1 x 30 ml) and with brine (1 x 30 ml).
The organic phase was dried over MgSO₄, filtered and evaporated to give 95 (1.29 g, 70%).

Rₛ = 0.53 hexane:ethyl acetate 1:1 (v/v); ¹H NMR δ 7.26 (br s, 1H, NH), 6.15 (s, 1H, CH₃), 5.72 (s, 1H, CH₂), 3.82 (s, 3H, COOCH₃), 1.48 (s, 9H, 3 x Boc CH₃); FAB MS C₉H₁₅NO₄ (201.22) m/z (%) 202 [M+H]⁺ (100), 224 [M+Na]⁺ (15), 192 (90).

**Benzyl 2-[(tert-butoxycarbonyl)amino]acrylate (96)**

Procedure as for 95 from 94.

Rₛ = 0.46 hexane:ethyl acetate 4:1 (v/v); yield 73%; ¹H NMR δ 7.36, 7.01 (2m, 5H, arom.H), 6.15, 5.77 (2s, 2H, CH₂, CH₃), 5.24 (s, 2H, PhCH₂), 1.46 (s, 9H, 3 x Boc CH₃); ESI MS C₁₅H₁₉NO₄ (277.32) m/z (%) 278 [M+H]⁺ (100), 178 [M-Boc+H]⁺ (65).

**3,7-anhydro-4,5,6,8-tetra-O-acetyl-2-[(tert-butoxycarbonyl)amino]-2-deoxy-D-galacto-oct-1-methoxycarbonyl-enitol (97)**

**Method A**

2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl bromide 19 (2.87 g, 6.98 mmol) and methyl 2-[(tert-butoxycarbonyl)amino]acrylate 95 (1.17 g, 5.80 mmol) were dissolved in benzene (7 ml) and stirred at 80°C. Tri-butyltin hydride (2.03 g, 6.98 mmol) and AIBN (70 mg) were added to the solution over 20 minutes. The solvent was evaporated and the residue taken up in acetonitrile (10 ml). This was washed with hexane (5 x 20 ml), followed by evaporation of the acetonitrile. The residue was purified by column chromatography to give 97 (1.73 g, 56%).

Rₛ = 0.60 chloroform:methanol 10:3 (v/v); ¹H NMR δ 5.41 (m, 1H, H-7), 5.30 – 5.15 (m, 3H, H-5, H-6, NH), 4.40 (m, 1H, H-2), 4.35 – 4.05 (m, 4H, H-4, H-8, H-9, H-9'), 3.75 (s, 3H, COOCH₃), 2.45 (m, 1H, CH₂a), 2.11, 2.09, 2.08 (3s, 9H, 3Ac), 2.06 – 2.03 (m, 4H, CH₂b, Ac), 1.43 (s, 9H, 3 x Boc CH₃); FAB MS C₂₃H₃₅NO₁₃ (533.52) m/z (%) 555 [M+Na]⁺ (25).

**Method B** (polymer formation).

As Method A, except 95 was added in a 5 molar excess with respect to 19.

MALDI TOF MS (533.52) m/z (%) 573 (70), 756 (91), 958 (100), 1159 (91), 1359 (73), 1560 (68); see section 4.5.1 for further details.
Experimental

3.7-anhydro-4,5,6,8-tetra-O-acetyl-2-[(tert-butoxycarbonyl)amino]-2-deoxy-D-galacto-oct-1-benzyloxy carbonyl-enitol (98)


Rf = 0.45 chloroform:methanol 10:1 (v/v); yield 53%; ^1H NMR δ 7.43 − 4.31 (m, 5H, arom.H), 5.43 (m, 1H, H-7), 5.35 − 5.12 (m, 5H, PhCH2, H-5, H-6, NH), 4.37 (m, 1H, H-2), 4.32 − 4.09 (m, 4H, H-4, H-8, H-9, H-9'), 2.28 − 2.10 (m, 1H, CH2), 2.09, 2.05, 2.03, 2.00 (4s, 12H, 3Ac), 1.42 (s, 9H, 3 x Boc CH3); FAB MS C29H39NO13 (609.62) m/z (%) 632 [M+Na]^+ (60).

3.7-anhydro-4,5,6,8-tetra-O-acetyl-2-{2-(R/s)-[(tert-butoxycarbonyl)amino]-dodecyl}-2-deoxy-D-galacto-oct-1-methoxycarbonyl-enitol (99b)

3.7-anhydro-4,5,6,8-tetra-0-acetyl-2-[(tert-butoxycarbonyl)amino]-2-deoxy-D-galacto-oct-1-methoxycarbonyl-enitol 97 (35.0 mg, 0.0656 mmol) was dissolved in a 1:1 solution of trifluoroacetic acid and CH2Cl2 and allowed to stir for 30 minutes. The solution was subsequently evaporated and taken up in CH2Cl2 (1 ml). Triethylamine (5 drops) was added and the solution was again evaporated. Residue 99a was dissolved in CH2Cl2 (0.5 ml). 2-(R/s)-[(tert-Butoxycarbonyl)amino]-tetradecanoic acid 2b (19.0 mg, 0.0554 mmol) was added to solution, which was cooled to 0°C. DCC (13.0 mg, 0.0631 mmol) was added and the reaction mixture was stirred at room temperature for 2 hours. The solution was then diluted with CH2Cl2 (2 ml) and washed with 1M KHSO4(aq) (2 x 2 ml). The organic phase was dried over MgSO4, filtered and evaporated. The residue was purified by column chromatography to give 99b (33 mg, 67%).

Rf = 0.51 chloroform:methanol 10:0.1 (v/v); ^1H NMR δ 5.40 (m, 1H, H-7), 5.28, 5.19 (2m, 2H, H-5, H-6), 4.46 (m, 1H, H-2), 4.37 − 4.01 (m, 5H), 3.71 (s, 3H, COOCH3), 2.41 − 2.29 (m, 2H, 2CH2), 2.10, 2.07, 2.06, 2.02 (4s, 12H, 4Ac), 1.44 (s, 9H, 3 x Boc CH3), 1.26 (m, 22H, 11CH2), 0.88 (t, 3H, CH3); FAB MS C37H62N2O14 (758.89) m/z (%) 759 [M+H]^+ (6), 781 [M+Na]^+ (47), 659 [M-Boc+H]^+ (92).

3.7-anhydro-4,5,6,8-tetra-O-acetyl-2-{2-(R/s)-[(tert-butoxycarbonyl)amino]-dodecyl}-2-deoxy-D-galacto-oct-1-benzyloxy carbonyl-enitol (100b)

Procedure as for 99b, using C-glycoside 98 in place of 97.
Experimental

R_f = 0.44 chloroform:methanol 10:0.1 (v/v); yield 61%: _1^H NMR δ 7.45 – 7.27 (m, 5H, arom.H), 5.41 (m, 1H, H-7), 5.37 - 5.10 (m, 4H, PhCH2, H-5, H-6), 4.41 (m, 1H, H-2), 4.39 - 4.03 (2m, 5H), 2.31 – 2.17 (m, 2H, 2CH2), 2.10, 2.05, 2.03, 2.01 (4s, 12H, 4Ac), 1.45 (s, 9H, 3 x Boc CH3), 1.32 - 1.25 (m, 22H, 11CH2), 0.87 (t, 3H, CH3); FAB MS C_{34}H_{68}N_{2}O_{14} (834.99) m/z (%) 835 [M+H]^+ (15), 857 [M+Na]^+ (30), 757 [M-Boc+H]^+ (100).

2,3,4,6-tetra-O-benzyl-D-glucono-1,5-lactone (101)^{239}

Method A

2,3,4,6-tetra-O-benzyl-α/β-D-glucopyranose (500 mg, 0.925 mmol) was dissolved in abs. CH_{2}Cl_{2} (10 ml). 4-methylmorpholine-N-oxide (217 mg, 1.85 mmol) and molecular sieves (500 mg) were added. After stirring at room temperature for 10 minutes, tetra-n-propylammonium perruthenate(vii) (33.0 mg, 0.0939 mmol) was added. The reaction mixture was stirred for 12 hours. The solution was filtered through a celite-silica gel pad (1:1) and evaporated. The residue was taken up in CH_{2}Cl_{2} (5 ml) and washed with NaHCO_{3}(sat, aq) (1 x 5 ml), with 5% Na_{2}S_{2}O_{3}(aq) (1 x 10 ml), with brine (1 x 10 ml) and with CuSO_{4}(sat, aq) (1 x 10 ml). The organic phase was dried over MgSO_{4}, filtered and evaporated. The residue was purified by column chromatography to give 101 (442 mg, 89%).

Method B

2,3,4,6-tetra-O-benzyl-α/β-D-glucopyranose (4.00 g, 7.40 mmol) was dissolved in abs. CH_{2}Cl_{2} (100 ml). Molecular sieves (7 g) were added. The reaction mixture was stirred for 15 minutes. Pyridinium chlorochromate (7.20 g, 33.3 mmol) was added in one portion. The solution was stirred for 1 hour. Cyclohexane (100 ml) and ether (200 ml) were added to the flask. The mixture was filtered through silica gel (60 g) to give a colourless solution. Evaporation gave 101 (3.87 g, 97%).

R_f = 0.31 hexane:ethyl acetate 2:1 (v/v); for NMR data see Lit. ref. 239. FAB MS C_{34}H_{34}O_{6} (538.63) m/z (%) 539 [M+H]^+ (65), 561 [M+Na]^+ (100).

tert-Butyldimethylsilyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranoside (102)

2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α/β-D-glucopyranose 11 (1.86 g, 5.35 mmol), tert-butylidemethylchlorosilane (967 mg, 6.42 mmol) and imidazole (727 mg,
10.7 mmol) were dissolved in abs. CH$_2$Cl$_2$ (20 ml) and stirred for 12 hours. The reaction was diluted with CH$_2$Cl$_2$ (50 ml) and washed with H$_2$O (50 ml). The organic phase was dried over MgSO$_4$, filtered and evaporated. The residue was purified by column chromatography to give 102 (1.53 g, 62%).

R$_f$ = 0.37 chloroform:ethyl acetate 1:2 (v/v); $^1$H NMR $\delta$ 5.54 (d, 1H, NH), 5.22 (t, 1H, H-3), 5.02 (t, 1H, H-4), 4.83 (d, 1H, J$_{1,2}$=8.0 Hz), 4.19 – 4.11 (m, 2H, H-6, H-6'), 3.86 (m, 1H, H-5), 3.68 (m, 1H, H-5), 2.06, 2.02, 2.01, 1.91 (4s, 12H, 4Ac), 0.87 (s, 9H, C(CH$_3$)$_3$), 0.11, 0.08 (2s, 6H, Si(CH$_2$)$_2$); FAB MS C$_{29}$H$_{39}$NO$_9$Si (461.58) m/z (%) 462 [M+H]$^+$ (45), 484 [M+Na]$^+$ (25), 594 [M+Cs]$^+$ (55), 330 [M-OTBDMS]$^+$ (40), 404 [M-tBu]$^+$ (100).

tert-Butyldimethylsilyl 2-acetamido-2-deoxy-$\beta$-D-glucopyranoside (103)

 tert-Butyldimethylsilyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-$\beta$-D-glucopyranoside 102 (960 mg, 2.08 mmol) was stirred in methanol (10 ml). Sodium methoxide (0.5M, 2.08 mmol) was added to the solution, which was then stirred for a further 90 minutes. The reaction was neutralised with Amberlite H$^+$ ion exchange resin. The solution was then filtered and the resin washed with methanol. Evaporation gave 103 (662 mg, 95%).

R$_f$ = 0.55 chloroform:methanol 10:3 (v/v); $^1$H NMR $\delta$ 4.69 (d, 1H, J$_{1,2}$=6.5 Hz), 3.82 – 3.34 (m, 7H), 2.03 (s, 3H, Ac), 0.91, 0.88 (2s, 9H, C(CH$_3$)$_3$), 0.09 (s, 6H, Si(CH$_2$)$_2$); FAB MS C$_{14}$H$_{29}$NO$_6$Si (335.47) m/z (%) 358 [M+Na]$^+$ (100), 468 [M+Cs]$^+$ (10).

{2-(R/S)-[(tert-butoxycarbonyl)amino]dodecyl}(triphenyl)phosphonium iodide (104)

tert-butyl N-[1-(R/S)-(iodomethyl)undecyl]carbamate 45a (314 mg, 0.764 mmol) was placed in a round-bottomed flask with triphenylphosphine (1.00 g, 3.82 mmol) and heated to 120°C. The molten mixture was stirred at 120°C for 12 hours to give crude 418, which was used without further purification.

FAB MS C$_{32}$H$_{49}$INO$_2$P (673.65) m/z (%) 546 [M-I]$^+$ (100).
**Experimental**

**tert-butyl 1-\{[(β-D-glucopyranosyl)thio]methyl\}-(R/S)-undecylcarbamate (105)**

**tert-butyl**

1-\{[(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)thio]methyl\}-(R/S)-undecylcarbamate 82a (4.00 g, 6.182 mmol) was dissolved in abs. methanol (40 ml). Sodium methoxide was added (0.5M, 0.618 mmol) and the reaction was stirred for 3 hours. The reaction was neutralised with Amberlite H⁺ ion exchange resin. The solution was then filtered and the resin washed with methanol. Evaporation gave 105 (2.70 g, 91%).

R⁰ = 0.43 chloroform:methanol 10:2 (v/v); ¹H NMR δ 4.31 (t, 1H, H-1), 3.80 (m, 1H, H-6), 3.60 (m, 2H, CH₂), 1.42 (s, 9H, 3 × Boc CH₃), 1.28 (m, 18H, 9 % ), 0.87 (t, 3H, CH₃); FAB MS C₂₃H₄₅NO₇S (479.67) m/z (%) 502 [M+Na]⁺ (100), 380 [M-Boc+H]^⁺ (15).

**tert-butyl 1-\{[(2,3,4,6-tetra-benzyl-β-D-glucopyranosyl)thio]methyl\}-(R/S)-undecylcarbamate (106)**

**tert-butyl**

1-\{[(β-D-glucopyranosyl)thio]methyl\}-(R/S)-undecylcarbamate 105 (2.70 g, 5.64 mmol) was dissolved in abs. DMF (20 ml) and added slowly dropwise to a stirred suspension of NaH (1.08 g, 45.1 mmol) in abs. DMF (40 ml) at 0°C. After stirring the mixture for 2 hours, benzyl bromide (7.71 g, 45.1 mmol) was added dropwise to the reaction mixture at 0°C. The solution was stirred for a further 90 minutes. Methanol was cautiously added to quench the reaction. The solution was evaporated, taken up in ether (100 ml) and washed with water (3 x 100 ml). The organic phase was dried over MgSO₄, filtered and evaporated. The residue was purified by column chromatography in hexane:ethyl acetate 9:1 (v/v) to give 106 (3.05 g, 64%).

R⁰ = 0.56 hexane:ethyl acetate 2:1 (v/v); ¹H NMR δ 7.37 – 7.17 (m, 20H, arom.H), 4.92 – 4.52 (m, 8H, 4CH₂Ar), 4.41 (d, 1H, H-1), 3.73 – 3.65 (m, 5H, αCH, H-2, H-3, H-4, H-6), 3.48 (m, 2H, H-5, H-6'), 2.90, 2.75 (2m, 2H, CH₂), 1.45, 1.42 (2s, 9H, 3 × Boc CH₃), 1.28 – 1.20 (m, 18H, 9CH₂), 0.89 (t, 3H, CH₃); ¹³C NMR δ 157.5, 140.1, 130.3, 130.1, 129.9, 129.8, 129.7, 129.6, 129.5, 88.9, 88.3 – 71.0, 52.3, 39.0 – 24.6, 15.9; FAB MS C₅₁H₆₉NO₇S (840.16) m/z (%) 841 [M+H]^⁺ (5), 863 [M+Na]^⁺ (7), 972 [M+Cs]^⁺ (100).
2,3,4,6-tetra-O-benzyl-1-{2-(R/s)-[(tert-butoxycarbonyl)amino]dodecyl}sulphonyl)-1,5-anhydro-d-glucohexitol (107)

tert-butyl 1-\{[(2,3,4,6-tetra-O-benzyl-\beta-D-glucopyranosyl)thio]methyl\}-(R/s)-undecylcarbamate 106 (2.03 g, 2.42 mmol) was dissolved in CHCl₃ (50 ml). 4-chloroperoxybenzoic acid (728 mg, 3.63 mmol) was added. The reaction was stirred at room temperature for 3 hours. The solution was then diluted with CHCl₃ (25 ml) and washed with 1M K₂HPO₄ₚₐₗ₉ (2 x 25 ml). The organic phase was dried over MgSO₄, filtered and evaporated. The residue was purified by column chromatography in hexane:ethyl acetate 4:1 (v/v) to give 107 (1.60 g, 76%).

Rₛ = 0.51 hexane:ethyl acetate 2:1 (v/v); ¹H NMR δ 7.38 - 7.25 (m, 20H, arom.H), 4.97 - 4.52 (m, 8H, 4CH₂Ar), 4.27 (d, 1H, H-1), 4.13 - 3.66 (m, 7H), 3.47, 3.30 (2m, 2H, CH₂), 1.43 (s, 9H, 3 x Boc CH₃), 1.25 (m, 18H, 9 x CH₃), 0.88 (t, 3H, CH₃); ¹³C NMR δ 155.7, 155.3, 153.3, 137.8, 137.7, 137.4, 128.8, 128.5, 128.3, 127.9, 127.8, 127.7, 90.8, 89.8, 86.4, 86.1, 79.6 - 68.8, 55.8, 54.9, 46.9, 46.8, 35.7 - 22.7, 14.1; FAB MS C₅₁H₆₉NO₉S (872.16) m/z (%) 894 [M+Na]⁺ (60), 1004 [M+Cs]⁺ (100), 772 [M-Boc+H]⁺ (15).

2,3,4,6-tetra-O-acetyl-1-{2-[(tert-butoxycarbonyl)amino]dodecylidene}-1,5-anhydro-d-glucohexitol (108)

CBr₂F₂ (1.27 g, 6.06 mmol) dissolved in CH₂Cl₂ (2 ml) was added to a stirred mixture of 2,3,4,6-tetra-O-benzyl-1-{2-(R/s)-[(tert-butoxycarbonyl)amino]dodecyl}sulphonyl)-1,5-anhydro-d-glucohexitol 107 (440 mg, 0.505 mmol) and KOH/Al₂O₃ catalyst in CH₂Cl₂:tert-butanol 1:2 (v/v) (10.5 ml) at 4°C. The mixture was stirred for 15 minutes then for 3 hours at room temperature. The reaction was diluted with CH₂Cl₂ (50 ml) and passed through a celite pad. The solvents were evaporated and the residue purified by column chromatography in hexane:ethyl acetate 9:1 to give 108 (590 mg, 67%).

Rₛ = 0.51 hexane:ethyl acetate 2:1 (v/v); ¹H NMR δ 7.36 - 7.15 (m, 20H, arom.H), 4.79 - 4.53 (m, 10H), 3.92 - 3.54 (m, 6H), 1.43, 1.41 (2s, 9H, 3 x Boc CH₃), 1.25

Preparation of KOH catalyst: Potassium hydroxide pellets (10g) were dissolved in methanol and shaken with neutral alumina (70-230 mesh) for 4 hours. The solvent was then evaporated at 60°C until the powder was free-flowing.
(m, 18H, 9CH₂), 0.88 (t, 3H, CH₃); FAB MS C₅₁H₆₇NO₇ (806.08) m/z (%) 829 [M+Na]+ (70), 845 [M+K]+ (25), 705 [M-Boc+H]+ (40).

1-{2-(R/S)-[[(tert-butoxycarbonyl)amino]dodecyl]-1,5-anhydro-D-gluco-hexitol (109)

2,3,4,6-tetra-O-acetyl-1-{2-[(tert-butoxycarbonyl)amino]dodecylidene}-1,5-anhydro-D-gluco-hexitol 108 (270 mg, 0.335 mmol) was dissolved in methanol (7 ml). THF (2 ml) was added to fully dissolve the material. Palladium catalyst (10% on carbon, 50.0 mg) was added in one portion to the solution, which was stirred for 12 hours under a hydrogen atmosphere. The catalyst was subsequently filtered off, and the solvent evaporated to give 109 (120 mg, 80%), which was used for the next reaction step unpurified. Rᵢ = 0.09 ethyl acetate; ESI MS C₂₃H₄₅NO₇ (447.61) m/z (%) 448 [M+H]+ (100), 470 [M+Na]+ (10).

2,3,4,6-tetra-O-acetyl-1-{2-(R/S)-[(tert-butoxycarbonyl)amino]dodecyl}-1,5-anhydro-D-gluco-hexitol (110)

1-{2-(R/S)-[(tert-butoxycarbonyl)amino]dodecyl}-1,5-anhydro-D-gluco-hexitol 109 (120 mg, 26.9 mmol) was dissolved in acetic anhydride (2 ml) and pyridine (3 ml). The reaction mixture was stirred for 12 hours. The product was concentrated, taken up in CH₂Cl₂ (10 ml) and washed with 1M KHSO₄ (aq) (1 x 10 ml) and water (1 x 10 ml). The organic phase was dried over MgSO₄, filtered and evaporated. The product was purified by column chromatography in hexane:ethyl acetate 1:2 to give 110 (130 mg, 79%) as a mixture of diastereomers 110a and 110b in a ratio of 4:3. Rᵢ = 0.66 ethyl acetate; 110a : ¹H NMR δ 5.16 (t, 1H, H-3), 5.04 (m, 1H, H-4), 4.84 (t, 1H, H-4), 4.47 (d, 1H, NH), 4.22 (dd, 1H, H-6), 4.10 (dd, 1H, H-6'), 3.81 (m, 1H, αCH), 3.60 (m, 1H, H-5), 3.53 (t, 1H, H-1β), 2.13, 2.04, 2.03, 1.99 (4s, 12H, 4Ac), 1.60 (m, 2H, CH₂), 1.45 (s, 9H, 3 x Boc CH₃), 1.26 (m, 18H, 9CH₂), 0.88 (t, 3H, CH₃); ¹H NMR δ 5.15 (t, 1H, H-3), 5.02 (m, 1H, H-4), 4.85 (t, 1H, H-4), 4.47 (d, 1H, NH), 4.21 (dd, 1H, H-6), 4.13 (dd, 1H, H-6'), 3.70 (m, 1H, αCH), 3.62 (m, 1H, H-5), 3.53 (t, 1H, H-1β), 2.13, 2.04, 2.03, 1.99 (4s, 12H, 4Ac), 1.60 (m, 2H, CH₂), 1.45 (s, 9H, 3 x Boc CH₃), 1.26 (m, 18H, 9CH₂), 0.88 (t, 3H, CH₃); ¹³C NMR δ 170.5, 170.3, 169.7, 169.6, 155.5, 79.0, 76.1, 75.8, 75.7, 75.2, 74.5, 72.1, 68.9,
Experimental

68.8, 62.4, 48.9, 47.5, 36.8 - 20.5, 14.0; FAB MS C₃₁H₅₃NO₁₁ (615.75) m/z (%) 638 [M+Na]^+ (8), 654 [M+K]^+ (15), 516 [M-Boc+H]^+ (100).

\[\text{N-}\{1-\text{R/s)-[\text{tert-butoxycarbonyl}amino]dodecyl}\}-\beta-\text{D-glucopyranosylamide (111a)}\]

De-O-protection procedure as for 105.

Rf = 0.51 chloroform:methanol 10:2.5 (v/v); yield 87%; \(^1\)H NMR δ 4.86 (d, 1H, H-1, J₁₂ = 9.3 Hz), 3.98 (m, 1H, αCH), 3.79, 3.62 (2m, 2H, H-6, H-6'), 3.37 - 3.21 (m, 4H), 1.41 (s, 9H, 3 x Boc CH₃), 1.26 (m, 18H, 9 CH₂), 0.87 (t, 3H, CH₃); FAB MS C₂₃H₄₄N₂O₁₁ (476.60) m/z (%) 477 [M+H]^+ (3), 499 [M+Na]^+ (80), 377 [M-Boc+H]^+ (10).

\[\text{N-(2-amino-(R/s)-dodecyl)-\beta-\text{D-glucopyranosylamine (111b)}\]

Residue 111a (1.34 g, 2.82 mmol) was dissolved in CH₂Cl₂:TFA 1:1 (v/v) (6 ml) and stirred at room temperature for 15 minutes. The solvent was evaporated and co-evaporated with benzene and toluene to give 111b (860 mg, 81%).

Rf = 0.05 chloroform:methanol 10:2 (v/v); \(^1\)H NMR δ 4.88 - 3.30 (m, 8H), 1.28 - 1.16 (m, 18H, 9 CH₂), 0.78 (t, 3H, CH₃); FAB MS C₁₈H₃₆N₂O₆ (376.49) m/z (%) 377 [M+H]^+ (10), 399 [M+Na]^+ (30).

\[\text{N-}\{1-\text{R/s)-[\text{tert-butoxycarbonyl}amino]dodecyl}\}-\beta-\text{D-galactopyranosylamide (112a)}\]

De-O-protection procedure as for 105.

Rf = 0.28 chloroform:methanol 10:2 (v/v); yield 85%; \(^1\)H NMR δ 5.49 (d, 1H, NH), 4.87 (m, 1H, H-1), 4.10 - 3.95 (m, 1H, αCH), 3.89 (d, 1H, H-4), 3.70 - 3.51 (3m, 5H, H-2, H-3, H-5, H-6, H-6'), 1.45 (s, 9H, 3 x Boc CH₃), 1.29 (m, 18H, 9 CH₂), 0.90 (t, 3H, CH₃); FAB MS C₂₃H₄₄N₂O₈ (476.60) m/z (%) 499 [M+Na]^+ (35), 399 [M-Boc+H]^+ (90).

\[\text{N-(1-amino-(R/s)-dodecyl)-\beta-\text{D-galactopyranosylamine (112b)}\]

De-N-protection procedure as for 111b.
Experimental

R_f = 0.05 chloroform:methanol 10:2 (v/v); yield 97%; 'H NMR δ 4.20 – 3.24 (m, 8H), 1.38 – 1.16 (m, 18H, 9CH_2), 0.78 (t, 3H, CH_3); FAB MS C_{18}H_{36}N_2O_6 (376.49) m/z (%) 399 [M+Na]^+ (60).

2-acetamido-2-deoxy-N-{1-(R/s)-[(tert-butoxycarbonyl)amino]dodecyl}-β-D-glucopyranosylamide (113a)
De-O-protection procedure as for 105.
R_f = 0.39 chloroform:methanol 10:2 (v/v); yield 87%; 'H NMR δ 3.86 – 3.38 (m, 8H), 1.41 (s, 9H, 3 × Boc CH_3), 1.28 – 1.21 (m, 18H, 9CH_2), 0.86 (t, 3H, CH_3); FAB MS C_{23}H_{47}N_3O_8 (517.66) m/z (%) 518 [M+H]^+ (40), 540 [M+Na]^+ (50).

2-acetamido-2-deoxy-N-(1-amino-(R/s)-dodecyl)-β-D-glucopyranosylamine (113b)
De-N-protection procedure as for 111b.
R_f = 0.05 chloroform:methanol 10:2 (v/v); yield 95%; 'H NMR δ 7.35 (m, 1H, NH), 4.91 (m, 1H, H-1), 3.94 – 3.31 (m, 8H), 1.28 – 1.20 (m, 18H, 9CH_2), 0.82 (t, 3H, CH_3); FAB MS C_{20}H_{39}N_3O_6 (417.54) m/z (%) 418 [M+H]^+ (3), 440 [M+Na]^+ (5).

Piperacillin / 2-acetamido-2-deoxy-N-(1-amino-(R/s)-dodecyl)-β-D-glucopyranosylamine Ionic Complex (114)

Piperacillin (2.00 g, 3.87 mmol) and 2-acetamido-2-deoxy-N-(1-amino-(R/s)-dodecyl)-β-D-glucopyranosylamine 113b (1.61 g, 3.87 mmol) were dissolved in 95% acetic acid. Once fully dissolved, the solution was filtered and lyophilised to give 114 as a white solid (3.50 g, 97%).
RP-HPLC: R_t=12.46 min. ESI MS [M (complex 114) = 934; M^+ (glycolipid 113b) = 417] m/z (%) 935 [M+H]^+ (100), 418 [M^++H]^+ (45).

N-(1-amino-(R/s)-octadecoyl)-β-D-galactopyranosylamine (115b)
De-O-protection of 27c was effected using the procedure described for 105 to give 115a, which was subsequently de-N-protected, using the procedure described for 111b to give 115b.
Experimental

R_f = 0.26 chloroform:methanol 4:1 (v/v); yield 88%; 1H NMR (DMSO-d_6) δ 4.90 – 3.50 (m, 8H), 1.45 (m, 2H, βCH_2), 1.10 (m, 30H, 15CH_2), 0.75 (t, 3H, CH_3); Anal. Calcd. for C_{24}H_{49}N_2O_6 (460.63): C, 62.61; H, 10.43; N, 6.16. Found C, 62.75; H, 10.32; N, 6.08; FAB MS (460.63) m/z (%) 461 [M+H]^+ (32), 483 [M+Na]^+ (15); HRMS Calcd. for C_{24}H_{49}N_2O_6: 461.3591. Found: 461.3585.

N-[1-(R/S)-carboxyheptadecyl]-β-D-glucopyranuronamide (116)

1,2,3,4-tetra-0-acetyl-N-[1-(R/S)-(methoxycarbonyl)heptadecyl]-β-D-glucopyranuronamide 65 (33 mg, 0.0502 mmol) was dissolved in a solution of triethylamine:methanol:water 1:8:1 (v/v/v) (1 ml), and stirred at 40°C for 2 hours. The reaction mixture was then evaporated to give 116 (22 mg, 92%).

R_f = 0.36 chloroform:methanol 10:1 (v/v); 1H NMR (DMSO-d_6) δ 4.35 – 3.50 (m), 1.62 (m, 2H, βCH_2), 1.21 (m, 28H, 14CH_2), 0.84 (t, 3H, CH_3); Anal. Calcd. for C_{24}H_{45}NO (460.63): C, 60.61; H, 9.54; N, 2.94. Found C, 60.73; H, 9.42; N, 2.88; FAB MS (475.62) m/z (%) 498 [M+Na]^+ (80), 521 [M+2Na]^+ (65); HRMS Calcd. for C_{24}H_{43}NO: 498.3043. Found 498.3050.

O-[α-D-glucopyranosyl(1’→4)]-N-{1-amino-(R/S)-octadecoyl}-β-D-glucopyranosylamine (117b)

De-O-protection of 70 was effected using the procedure described for 105 to give 117a, which was subsequently de-β-protected, using the procedure described for 111b to give 117b.

R_f = 0.31 chloroform:methanol 1:1 (v/v); yield 81%; 1H NMR (CD_3OD) δ 5.18 – 4.96 (m, 2H), 3.88 – 3.42 (m, 12H), 1.28 (m, 30H, 15CH_2), 0.88 (t, 3H, CH_3); Anal. Calcd. for C_{30}H_{58}N_2O_{11} (622.40): C, 57.87; H, 9.32; N, 4.50. Found C, 57.82; H, 9.37; N, 4.44; FAB MS (622.40) m/z (%) 623 [M+H]^+ (3), 645 [M+Na]^+ (6), 307 (100); HRMS Calcd. for C_{30}H_{58}N_2O_{11}: 623.4119. Found 623.4110.
Experimental

\[ O-\{O-[\alpha-D-glucopyranosyl(1'\rightarrow4')]-\alpha-D-glucopyranosyl(1'\rightarrow4')\}-N-\{1-amino-(R/S)-octadecoyl\}-\beta-D-glucopyranosylamine (118b) \]

De-\(O\)-protection of 74 was effected using the procedure described for 105 to give 118a, which was subsequently de-\(N\)-protected, using the procedure described for 111b to give 118b.

\[ R_f = 0.39 \text{ chloroform: methanol 3:2 (v/v)}; \text{ yield 75\%}; \]  
\[ ^1\text{H NMR (CD}_3\text{OD)} \delta 5.08 (m, 3H), 3.90 - 3.37 (m, 18H), 1.29 (m, 30H, 15CH}_2, 0.89 (t, 3H, CH}_3); \]  
\[ \text{Anal. Calcd. for C}_{36}\text{H}_{68}\text{N}_2\text{O}_{16}: C, 55.10; H, 8.67; N, 3.57. \]  
\[ \text{Found C, 55.33; H, 8.44; N, 3.63; FAB MS (784.46) m/z (\%)} 785 [M+H]^+ (50), 807 [M+Na]^+ (100); \]  
\[ \text{HRMS Calcd. for C}_{36}\text{H}_{68}\text{N}_2\text{O}_{16}\text{Na: 807.4467. Found 807.4460.} \]
7 References


References


40 Toth, I.; Flinn, N.; Hillery, A.; Gibbons, W.A.; Artursson, P. Lipidic conjugates of luteinising hormone releasing hormone (LHRH) and thyrotrophin releasing hormone (TRH) that release and protect native hormones in homogenates of human intestinal epithelial (Caco-2) cells. *Int. J. Pharm.*, 1994, 105, 241-247.


References


56 Green, P.G.; Hadgraft, J. Facilitated transfer of cationic drugs across a lipoidal membrane by oleic acid and lauric acid. *Int. J. Pharm.*, 1987, 37, 251-255.

References


210
References


82 Jain, R.K.; Matta, K.L. Methyl 3,4-O-isopropylidene-2-O-(4-methoxybenzyl)-1-thio-β-L-fucopyranoside – A Novel, Efficient Glycosylating Reagent for the


References


References


References


164 Bycroft, B.W.; Chan, W.C.; Chhabra, S.R.; Hone, N.D. A Novel Lysine-
protecting Procedure for Continuous Flow Solid Phase Synthesis of Branched
165 Kellam, B.; Bycroft, B.W.; Chhabra, S.R. Solid Phase Applications of Dde and
1997, 38, 4849-4852.
166 Kellam, B.; Bycroft, B.W.; Chan, W.C.; Chhabra, S.R. Solid phase strategies:
Applications of 2-acetyl-4-nitroindane-1,3-dione as a selective protecting group
167 Kartha, K.P.R.; Field, R.A. Iodine: A Versatile Reagent in Carbohydrate
Chemistry IV. Per-O-Acetylation, Regioselective Acylation and Acetolysis.
169 Kokotos, G. A convenient one-pot conversion of N-protected amino acids and
170 McGeary, R.P. Facile and chemoselective reduction of carboxylic acids to
29, 3319-3322.
171 Castro, B.; Dormoy, J.-R.; Dourtoglou, B.; Evin, G.; Selve, C.; Ziegler, J.-C.
Peptide Coupling Reagents VI. A Novel, Cheaper Preparation of
Benzotriazolyloxy-tris(dimethylamino)phosphonium hexafluorophosphate.
172 Naqvi, T.; Bhattacharya, M.; Haq, W. An Efficient Method for the Reduction
of N-Protected Amino Acids and Peptides to the Corresponding Alcohols. J.
173 Excoffier, G.; Gagnaire, D.; Utille, J.-P. Coupure selective par l'hydrazine des
groupements acetyl ansomeres de residus glycosyles acetyles. Carbohydr.
174 Dullenkopf, W.; Castro-Palomino, J.C.; Manzoni, L.; Schmidt, R.R. N-
Trichloroethoxycarbonyl-glucosamine derivatives as glycosyl donors.
References


References


References


