Synthesis of S-glycolipids and peptidomimetics

By

Ciarán O’Reilly

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For the degree of
Doctor of Philosophy

Based on the research carried out in the
Department of Chemistry,
National University of Ireland,
Galway

Under the supervision and direction of
Prof. Paul V. Murphy
National University of Ireland,
Galway.
For my mother and father
Declaration

This thesis has not been submitted before, in whole or in part, to this or any other university for any degree, and is, except where otherwise stated, the original work of the author.

_____________________

Ciarán O’Reilly
Abstract

Chapters one and two of this thesis describe the synthesis of two novel glycosphingolipid derivatives. Glycosphingolipids are amphiphilic molecules consisting of a carbohydrate head group glycosidically linked to a sphingoid lipid chain. Our interest is in the synthesis of α-glycosphingolipids of bacterial origin, particularly those isolated from the cell walls of Sphingomonas bacteria. These glycolipid derivatives bear a striking resemblance to the known immunostimulant KRN7000 and have shown activity in both human and mouse models. The Murphy group has previously reported the synthesis of a bacterial glycosphingolipid termed PBS-59 and its glucuronic acid derivative; therefore, this thesis focuses on our efforts to synthesise thio-linked analogues of these lipid derivatives in the hope of improving their in vivo stability and immunostimulatory activity. The synthetic route includes a stereoselective anomerisation reaction to form α-glycosyl thiol precursors as well as a new route to sphinganine chains starting from the Myers pseudoephedrine chiral auxiliary.

Chapters three and four investigate the use of carbohydrates as scaffolds for the development of novel biologically active peptidomimetics. Of particular interest is the synthesis of a novel macrocyclic structure with embedded carbohydrates capable of mimicking the α-helical domain. The hydroxyl groups of the carbohydrate act as a functional handle onto which pharmacophoric groups can be grafted. Two key protein-protein interactions (Bcl-2 family of proteins and the p53-MDM2 interaction) were identified as targets for peptidomimetic development. The synthesis of these macrocyclic structures was developed, a route which includes a rare example of the use of a reductive amination/ring closing macrocyclisation sequence, and a number of novel α-helical peptidomimetics were obtained. Some of these mimetics have shown activity in triggering apoptosis in ML-1 cells.

Presented in chapter five is the synthesis of a novel bicyclic iminocyclitol derivative. This compound was synthesised via a novel one pot nucleophilic substitution, Huisgen cycloaddition, triazoline decomposition, aziridine formation and aziridine ring opening by an azide anion. The conversion of this compound into a tricyclic derivative is also described showing its potential for use in peptidomimetic development or as a building block in the synthesis of more complex molecules.
Acknowledgements

Firstly, I would like to say a heartfelt thank you to my supervisor and mentor, Professor Paul V. Murphy, to whom I owe a huge debt of gratitude. It has been a real privilege and an honour to have been part of his group for the last four years. His dedication and knowledge of chemistry is truly inspirational and his guidance, advice and support to me personally throughout my time in his group was second to none and is something I will never forget.

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Finally I would like to say a special thank you to the most amazing parents and brother in the world. To my parents, Carmel and Martin O’Reilly and my brother Aaron, I’d like to dedicate this thesis to you all. Your unwavering support, belief, kindness and love has made me the person I am today. I could never have achieved any of this without you. I love you all and I hope I have made you proud.
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<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \alpha )</td>
<td>Alpha</td>
</tr>
<tr>
<td>ADDP</td>
<td>1,1’-(azidodicarbonyl)dipiperidine</td>
</tr>
<tr>
<td>Ar</td>
<td>Aromatic</td>
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<tr>
<td>BAIB</td>
<td>Bisacetoxyiodobenzene</td>
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<tr>
<td>Bcl</td>
<td>B-Cell lymphoma</td>
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<td>BH</td>
<td>Bcl-2 homology</td>
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<td>Butyllithium</td>
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<tr>
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<td>Ceramide</td>
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<td>COSY</td>
<td>Correlation Spectroscopy</td>
</tr>
<tr>
<td>( \delta )</td>
<td>Chemical shift in ppm downfield from TMS</td>
</tr>
<tr>
<td>d</td>
<td>Doublet (spectral)</td>
</tr>
<tr>
<td>dd</td>
<td>Doublet of doublets (spectral)</td>
</tr>
<tr>
<td>ddd</td>
<td>Doublet of doublets of doublets (spectral)</td>
</tr>
<tr>
<td>DEPT</td>
<td>Distortionless Enhancement by Polarisation Transfer</td>
</tr>
<tr>
<td>DIPEA</td>
<td>Diisopropylethylamine</td>
</tr>
<tr>
<td>DMJ</td>
<td>Deoxymannojirimycin</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>DNJ</td>
<td>Deoxynojirimycin</td>
</tr>
<tr>
<td>dt</td>
<td>Doublet of triplets (spectral)</td>
</tr>
<tr>
<td>dr</td>
<td>Diastereomeric ratio</td>
</tr>
<tr>
<td>EDC</td>
<td>1-ethyl-3-(3-dimethylaminopropyl) carbodiimide</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>eq.</td>
<td>Equivalents</td>
</tr>
<tr>
<td>er</td>
<td>Enantiomeric ratio</td>
</tr>
<tr>
<td>Et</td>
<td>Ethyl</td>
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<tr>
<td>ES-HRMS</td>
<td>High-Resolution Mass Spectrometry - Electrospray Ionization</td>
</tr>
<tr>
<td>FTIR</td>
<td>Fourier transform infrared (spectroscopy)</td>
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</tr>
<tr>
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</tr>
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<td>GSL</td>
<td>Glycosphingolipid</td>
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<tr>
<td>HMBC</td>
<td>Heteronuclear multiple bond correlation</td>
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<td>Hexamethyldisilylazide</td>
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<td>High performance liquid chromatography</td>
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<td>Heteronuclear single quantum correlation</td>
</tr>
<tr>
<td>Hz</td>
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</tr>
<tr>
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<td>Interferon-γ</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>IR</td>
<td>Infrared (spectroscopy)</td>
</tr>
<tr>
<td>J</td>
<td>Coupling constant (nmr), in Hz</td>
</tr>
<tr>
<td>kDa</td>
<td>Kilo Dalton</td>
</tr>
<tr>
<td>lit.</td>
<td>Literature reference</td>
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<tr>
<td>LHMDS</td>
<td>Lithium hexamethyldisilazide</td>
</tr>
<tr>
<td>N</td>
<td>Normal</td>
</tr>
<tr>
<td>m</td>
<td>Multiplet</td>
</tr>
<tr>
<td>M</td>
<td>Molar</td>
</tr>
<tr>
<td>Symbol</td>
<td>Definition</td>
</tr>
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<td>--------</td>
<td>------------</td>
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<tr>
<td>$M^+$</td>
<td>Mass of the molecular ion (mass spectrometry)</td>
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<td>MDM2</td>
<td>Murine double-minute 2</td>
</tr>
<tr>
<td>Mcl</td>
<td>Myeloid cell leukaemia</td>
</tr>
<tr>
<td>Me</td>
<td>Methyl</td>
</tr>
<tr>
<td>Ms</td>
<td>Mesyl</td>
</tr>
<tr>
<td>MHz</td>
<td>Megahertz</td>
</tr>
<tr>
<td>mL, µL</td>
<td>Milliliter, microliter</td>
</tr>
<tr>
<td>mol, mmol</td>
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</tr>
<tr>
<td>mM, µM</td>
<td>Millimolar, micromolar</td>
</tr>
<tr>
<td>Mp</td>
<td>Melting point</td>
</tr>
<tr>
<td>NK</td>
<td>Natural Killer</td>
</tr>
<tr>
<td>NMM</td>
<td>N-methylmorpholine</td>
</tr>
<tr>
<td>NMO</td>
<td>N-Methylmorpholine-(\text{N-oxide})</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear Magnetic Resonance</td>
</tr>
<tr>
<td>NOE</td>
<td>Nuclear Overhauser Effect</td>
</tr>
<tr>
<td>NOESY</td>
<td>Nuclear Overhauser Effect Spectroscopy</td>
</tr>
<tr>
<td>PTSA</td>
<td>para-toluenesulfonic acid</td>
</tr>
<tr>
<td>ppm</td>
<td>Parts per million (NMR)</td>
</tr>
<tr>
<td>Prop</td>
<td>Propyl</td>
</tr>
<tr>
<td>q</td>
<td>Quartet (spectral)</td>
</tr>
<tr>
<td>$R_f$</td>
<td>Retention factor</td>
</tr>
<tr>
<td>rt</td>
<td>Room temperature</td>
</tr>
<tr>
<td>$[\alpha]_D$</td>
<td>Specific rotation</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
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<tr>
<td>--------------</td>
<td>-----------------------------------------</td>
</tr>
<tr>
<td>s</td>
<td>Singlet (spectral)</td>
</tr>
<tr>
<td>$S_N^2$</td>
<td>Bimolecular nucleophilic substitution</td>
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<td>t</td>
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<td>TEMPO</td>
<td>(2,2,6,6-Tetramethylpiperidin-1-yl)oxyyl</td>
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<tr>
<td>Ts</td>
<td>Tosyl</td>
</tr>
<tr>
<td>td</td>
<td>Triplet of doublets (spectral)</td>
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<tr>
<td>t-Bu</td>
<td>tert-butyl</td>
</tr>
<tr>
<td>TCR</td>
<td>T cell receptor</td>
</tr>
<tr>
<td>$T_b$</td>
<td>T helper</td>
</tr>
<tr>
<td>TLC</td>
<td>Thin Layer Chromatography</td>
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</table>
## Chapter 1: Introduction to glycosphingolipids

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1.1 Biology of Natural Killer T-Cells

CD1d restricted Natural Killer T cells (NKT cells) constitute an exclusive subset of natural killer (NK) lymphocytes. Possessing a number of unique characteristics, these lymphocytes play a key role in the regulation of immune responses\(^1\)\(^-\)\(^2\). NKT cells present in the liver and spleen are capable of co-expressing an invariant T cell receptor (TCR). It has been shown that in both mouse and human models TCR’s can recognize glycosphingolipid antigens presented by the monomorphic antigen presenting glycoprotein known as CD1d\(^3\). This glycoprotein is expressed on the cell surface of an antigen presenting cell or APC. Upon activation, NKT cells stimulate the production of numerous signalling peptides (cytokines) such as interferon-\(\gamma\) (IFN-\(\gamma\)) and members of the interleukin family including IL-4 and IL-12. These cytokines are capable of exerting rapid and substantial immune responses. The response triggered can be of two distinct types; T helper 1 (T\(_h\)1) responses and T helper 2 (T\(_h\)2) responses. The effects on the immune system that these responses promote are summarised below\(^4\):

1. T\(_h\)1 response: this constitutes a pro-inflammatory response which assists in the control of various bacterial, parasitic and viral infections and can have an effect against certain tumours. These responses are triggered by cytokines such as IFN-\(\gamma\). The major caveat of this response however, is that a number of autoimmune diseases such as diabetes, multiple sclerosis, lupus and rheumatoid arthritis are also T\(_h\)1 mediated.

2. T\(_h\)2 response: this constitutes an immunomodulatory response brought about by cytokines such as IL-4. This response is antagonistic and hinders the T\(_h\)1 response meaning T\(_h\)2 responses could help alleviate the effects of autoimmune diseases.

Stimulation of NKT cells by glycosphingolipid antigens can lead to production of both T\(_h\)1 and T\(_h\)2 cytokines simultaneously. Should this happen, the antagonistic nature of these cytokines means that any immunogenic response will be effectively abrogated\(^5\). This means that any potential medicinal use of NKT cell stimulation by glycosphingolipid antigens must be under strict control in order to elicit a biased response towards the production of either T\(_h\)1 or T\(_h\)2 cytokines.
Presented in Figure 1 is a simplified overview of the interaction between glycosphingolipids and NKT cells. In step 1 an antigen presenting cell (APC) of the innate immune system expressing the CD1d protein on its cell surface binds the hydrophobic chain of the glycolipid and displays the sugar head group on the cell surface. The sugar groups are recognised by TCR’s on the surface of NKT cells (step 2). Recognition leads to the rapid expression of cytokines (IFN-γ and IL-4 by NKT cells and IL-12 from the CD1d bearing APC) which in turn elicit an immune response (step 3).

1.2 Structure of glycosphingolipids

Glycosphingolipids are a diverse subset of glycolipids, many of which have varied and complex biological roles. These compounds have been shown to be involved in many cellular interactions including cell signalling, host-pathogen interactions, migration and apoptosis. Typically made up of a sphingoid base glycosidically linked to a single sugar residue or oligosaccharide moiety examples of glycosphingolipids include:
1. Cerebrosides Containing one sugar residue
2. Sulfatides Containing one sulfated sugar residue
3. Neutral glycosphingolipids Containing more than one sugar residue
4. Gangliosides Containing neuraminic acid residues

Much of the diversity found in glycosphingolipid structure comes as a result of the sphingoid portion of the molecule. There are three main classes of sphingoid base present in nature. The most abundant of which are sphingosines, which contain an amino-diol and are unsaturated at C4-C5. Phytosphingosines are 2-amino-1,2,4-triols and sphinganines (dihydrosphingosines) are amino-diols without the double bond at C4-C5. Being the most abundant, the synthesis of sphingosines and phytosphingosines has been extensively reviewed. This thesis will focus on synthetic efforts towards sphinganines and their use in the synthesis of bacterial glycosphingolipid mimics.

**Figure 2** The three most common sphingoid bases present in nature

### 1.3 Discovery of glycolipid ligands for NKT cell stimulation

In 1993, workers at the Kirin brewery in Japan isolated a number of unusual glycolipids from the Okinawan marine sponge *Agelas mauritianus*. These compounds were called agelasphins (Figure 3) and a biological evaluation showed that they possessed potent antitumour properties due to their ability to stimulate NKT cells. These agelasphins were seen as unusual because they contained an α-glycosidic bond between the sugar and ceramide moieties (glycolipids in higher organisms are typically β-linked with respect to their glycosidic bonds).
Extensive structure activity relationships were carried out on both the sugar and ceramide portions of the molecules in order to elucidate the reasons for the high biological potency of these compounds. Modifications of the sugar portion showed that α-galactosyl ceramide 5 was more active than α-glucosylceramide 6 (see figure 4)\textsuperscript{12}. It also showed that both α-mannosylceramide 7 and β-galactosylceramide 8 were inactive. This suggests that both the nature of the glycone and the presence of an α-glycosidic linkage are important factors in the ability of glycolipids to stimulate NKT cells.

![Figure 4 Structures of monoglycosylceramides](image)

Investigations into the ceramide portion showed that the C2’ hydroxyl group does not significantly alter the activity of the bound glycolipid. The C4 hydroxyl group only plays a minor role, however, the C3 hydroxyl group is vital to maximise the biological activity of the lipid derivatives\textsuperscript{13} (Figure 5).
From these studies α-GalCer 9 or KRN7000 was identified as a lead drug candidate and has been extensively studied in vivo for its immunostimulatory properties. Phase 1 clinical trials showed KRN7000 to be safe and active as an immunostimulant, however, the results of further trials were less than optimistic. As previously stated, stimulation of NKT cells by glycolipids can trigger both Th1 and Th2 responses simultaneously. The antagonistic nature of these immune responses could be a reason for the impaired efficacy of KRN7000.

1.4 The CD1d/KRN7000 interaction

In recent years structure activity relationships have been aided by crystal structures of lipid bound mouse\textsuperscript{14} and human\textsuperscript{15} CD1d and even more recently by the ternary structure of the TCR/CD1d/KRN7000 complex\textsuperscript{16}. The crystal structure of the CD1d/KRN7000 interaction is shown in Figure 6. The lipid-binding pocket of CD1d is particularly well adapted to bind both self and microbial glycosphingolipids. As shown in Figure 6 C the acyl chain occupies A’ hydrophobic pocket while the sphingosine chain occupies the F’ hydrophobic pocket. For KRN7000 and the closely related α-glycuronylceramides, the α1 helix Arg79 and Asp80 establish hydrogen bonds with the hydroxyl groups of the sphingosine. The α2 helix Asp153 stabilises the galactose through hydrogen bonds with the 2”’ and 3”’ hydroxyl groups, this anchors the sugar in a position parallel to the plain of the α helices (Figure 6 B) and therefore allows it to sit atop the binding groove. This is in sharp contrast to β-GalCer (Figure 6 B), in which the galactose moiety is orientated away from the binding groove indicating why an α-linkage is so important for NKT cell stimulation. The α2 helix also shows a hydrogen bond between the anomeric oxygen and Thr156, this is interesting as this hydrogen bond is absent from the ternary structure published recently (Figure 6 E)\textsuperscript{16}.
Figure 6 A.) Crystal structure of the KRN7000/CD1d complex showing hydrogen bonding to sphingosine chain. B.) KRN7000 (yellow) and β-GalCer (green) bound to CD1d. C.) sphingosine and acyl chains occupying F and A’ hydrophobic pockets respectively. E.) Ternary structure TCR/CD1d/KRN7000. (Reprinted by permission from Macmillan Publishers Ltd: Nature, Ref. 16, copyright 2007)
1.5 Polarising the immune response

Recently, research has focused on the design and synthesis of glycolipid derivatives which are capable of eliciting biased immune responses. These compounds offer an enormous challenge to synthetic chemists, not only are there inherent challenges in the synthesis of the sphingoid portion of the molecule, controlling the stereochemistry of the anomeric linkage poses a great challenge in itself. Therefore the development of novel strategies towards α-glycolipids has been of great interest in recent years. Several synthetic analogues have been developed and a number of these including OCH \textsuperscript{10} and α-C-GalCer \textsuperscript{11} (Figure 7) have been successful in polarizing the type of response triggered.

![Figure 7 Structures of important glycolipid analogues](image)

OCH \textsuperscript{10} is a glycolipid derivative which is truncated in the sphingosine chain of KRN7000. This compound showed a bias in the production of IL-4 cytokines from NKT cells. It is believed the reason for this biased response is that OCH is less stable in the binding site of the CD1d protein and therefore exerts a short lived NKT cell stimulation. On the other hand, α-C-GalCer \textsuperscript{11} induces an IFN-γ biased immune response presumably due to a more prolonged NKT-cell stimulation time (IFN-γ production requires longer TCR stimulation than that of IL-4). α-C-GalCer is an analogue of KRN7000 in which the anomeric oxygen is replaced with a methylene group. Interest in compound \textsuperscript{11} has been immense in recent years due, in part, to its superiority to KRN7000 in cure ratios for malaria (1000/1 α-C-GalCer/KRN7000) and melanoma (100/1 α-C-GalCer/KRN7000). The reason for this dramatic increase in NKT cell stimulation is poorly understood at present. Crystal structures show that the KRN7000/CD1d complex has a key hydrogen bond to the anomeric oxygen (however, as pointed out previously, this hydrogen bond is absent in the ternary structure).
α-C-GalCer lacks this oxygen it would be anticipated that the binding interaction would not be as strong and that a decrease in cytokine production would be observed, however, this is not the case. This implies that, although crystal structures and considerable SAR information is available for some of these compounds, it is still difficult to predict how modified glycolipids will bind. The success of α-C-GalCer as an NKT cell agonist suggests that anomeric modifications/replacements could be important in both biasing responses and also as mechanistic tools to further elucidate the mechanism by which NKT cell stimulation occurs.

1.6 Bacterial glycolipids as natural NKT cell antigens

Detection and response to microbial infection is one of the main aspects of innate immunity, therefore it is reasonable to suggest that NKT cells play a key role in the hunt for pathogenic bacteria or parasites in vivo. In 2004 Schaible and co-workers showed that glycolipids isolated from mycobacterium such as PIM$_4$ (Figure 8) could stimulate NKT cells causing the modest release of cytokines compared to KRN7000. This was the first report of a glycolipid of bacterial origin stimulating NKT cells.

![Figure 8](image)

**Figure 8** Structure of PIM$_4$, a glycolipid from mycobacterium that stimulates NKT cells

In recent years it has been shown that glycolipids isolated from certain gram-negative bacteria can also trigger an immune response. Gram-negative bacteria are ubiquitous in nature. In general, the majority of these bacteria have cell walls comprised of lipopolysaccharide (LPS). The innate immune system is capable of recognizing these pathogens and NKT cells play an indirect role in this process. However numerous gram-negative bacteria have cell walls which do not contain LPS (Figure 9), so the question arises: how does the innate immune system address this problem?
Investigations into the structure of the membranes of non-LPS-producing gram-negative bacteria of the *Sphingomonadaceae*\(^{23-24}\) family turned up some very interesting results. Zahringer\(^{25}\), along with contributions from Kawasaki\(^{26}\) and co-workers managed to elucidate the structures of a number of glycosphingolipids from the cell walls of these bacteria which they termed GSL-1 \(^{13}\), GSL-3 \(^{15}\) and GSL-4. A GSL-1 (GSL-1’ \(^{14}\)) derivative containing a galacturonic acid residue in place of the glucuronic acid residue (Figure 10)\(^{27}\) has also been isolated. Similar glycolipids have also been isolated from members of the *Ehrlichia* family which belong to the same class of α-proteobacteria\(^{28}\).  

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**Figure 9** Graphical representation of the *Sphingomonas* cell wall
It can be clearly seen that some of these compounds bear a striking resemblance to KRN7000 differing only in the presence of uronic acid residues in the sugar moiety. These similarities led a number of groups to test bacterial glycolipids for their ability to activate NKT cells. As expected it has been shown that these compounds and some synthetic analogues such as PBS-30 \(16\) and PBS-59 \(17\) (Figure 10) strongly activated NKT cells in a CD1d dependant fashion \(^{28-29}\). For example, injection of *Sphingomonas* into mice triggered septic shock followed by bacterial clearance, however, in NKT cell-deficient mice; a marked decrease in bacterial clearance was noted. Although a number of syntheses of GSL-1 derivatives have been reported, there is still a lack of SAR evaluation on these glycolipids.
1.7 α-S-glycolipids

With the impressive results obtained from the biological evaluations of α-C-GalCer\(^6\) it comes as no surprise that many groups have become interested in modifying the anomeric linkage. S-glycosides have long been used as O-glycoside isosteres owing to their similar properties and enhanced stability against the action of glyco-processing enzymes in vivo. Although a C-S bond is longer than a C-O bond, the C-S-C bond angle is a lot smaller than the corresponding C-O-C bond angle. This means that there is a relatively small difference in their positions within a glycosidic bond. These properties have led a number of groups, including our own, to pursue S-linked glycolipids as novel lipid derivatives. It is believed that these compounds could provide an invaluable mechanistic insight into NKT cell activation by glycolipids as well as an extra degree of stability in vivo over their O-linked counterparts.

1.7.1 Previous synthesis of S-linked analogues of KRN7000

At present, only two reports have appeared containing the synthesis of thioglycoside analogues of KRN7000, the first of which was reported by Zhu and Dere\(^{30}\). Their synthetic approach includes a novel synthesis of α-glycosyl thiols via the treatment of 1,6-anhydrogalactose 20 with hexamethyldisilathiane and TMSOTf at reflux to provide the corresponding α-glycosyl thiol 21 exclusively in 88% yield (Scheme 1). This reaction is believed to proceed via an S\(_{\text{N}2}\) displacement on the 1,6 anhydrosugar\(^{31}\).

![Scheme 1 Novel synthesis of α-glycosyl thiols](image-url)
With the α-glycosyl thiols in hand, attention was focused on the synthesis of the phytosphingosine chain (Scheme 2). Following the procedure outlined by Schmidt, compound 23 was synthesised from D-galactose via intermediate 22 in 6 steps. Protection of 23 as an isopropylidene followed by mesylation and Finkelstein type reaction gave the iodide derivative 24 in 95% yield.

**Scheme 2** Zhu’s approach to the synthesis of phytosphingosines

Coupling of glycosyl thiol 21 and the phytosphingosine derivative 24 under phase transfer conditions gave the glycolipid derivative 25 in a 73% yield. Staudinger reduction of the azide followed by amide coupling and subsequent deprotection under Birch conditions gave α-S-GalCer 27 in a 63% yield.

**Scheme 3** Zhu’s synthesis of α-s-GalCer

Around the same time as the paper from Zhu’s group appeared in the literature, a second total synthesis of the thioglycoside analogue of KRN7000 appeared. Howells group synthesised the bromide derivative 29 in 4 steps from ribo-phytosphingosine in excellent yield (Scheme
4). The glycosyl thiol 30 was synthesised in 3 steps using the procedure described by Yamamoto as a 2:1 α:β mixture of anomers. Coupling of the fragments 29 and 30 under phase transfer conditions gave glycolipid derivative 31 in modest yield. Subsequent deprotection followed by amide formation gave α-S-GalCer 27.

Scheme 4 Howells synthesis of α-S-GalCer

Despite the obvious similarities between KRN7000 and its thioglycoside analogue, the Howell group detected no immune response or cytokine production for α-S-GalCer in both in vivo and in vitro tests. In order to decipher a possible reason for this lack of NKT stimulation, they carried out a docking study between α-S-GalCer and the ternary crystal structure of the CD1d/TCR complex (Figure 11). Although several key hydrogen bonds were retained between the two complexes, three key hydrogen bonds were either absent or greatly weakened in the α-S-GalCer model. KRN7000 forms two hydrogen bonds to Asp 151, one to the carbonyl of Gly 96 and another to the hydroxyl group of Thr 154. In α-S-GalCer, only one bond exists with Asp 151 although the other could be possible if there was a slight shift of the sugar residue. The hydrogen bond to the Gly 96 carbonyl is completely absent and the bond to the Thr 154 hydroxyl group is very weak. However a new hydrogen bond does exist between α-S-GalCer and Asp 80. This evidence demonstrates that binding is indeed possible between α-S-GalCer and CD1d, however, the reason for the lack of NKT cell stimulation is still unclear and further investigation is needed.
Figure 11 Howells model of α-S-GalCer (purple) and KRN7000 (cyan) overlapping and docked into the ternary structure of CD1d-NKT-TCR (reprinted from Ref. 32 with permission from Elsevier, copyright 2008)

1.7.2 Bacterial S-linked glycosphingolipid mimics

Since 2005 a number of total syntheses of GSL-1’ and PBS-59 have appeared in the literature. Although these compounds are not as active as the highly potent α-GalCer, there exists the opportunity for modification of their structures in an effort to improve their immunostimulatory properties. Amide bond formation for instance, (to maintain a hydrogen bonding group in the structure) has yet to be explored and to the best of our knowledge, there is only one report which attempts to modify the anomeric linkage.

Within the Murphy group, there has been a long standing interest in the stereoselective synthesis of glycuronic acid derivatives. With this in mind, PBS-59 and its analogues presented ideal synthetic targets. Vital to this work was the development of a novel strategy to gain stereoselective control in the synthesis of α-linked glycuronic acid derivatives. It was known within the group that treatment of glycuronic acid derivatives with Lewis acids such as TiCl₄ and SnCl₄ highly favoured the formation of α-anomers by way of a chelation induced anomerisation reaction. This reaction will be discussed further in the next chapter. Recently, Pilgrim and Murphy have successfully applied this reaction to the synthesis of
PBS-59 and a sulfone analogue\textsuperscript{36} (Scheme 5). Glycosidation of the benzyolated galacturonic acid trichloroacetimidate donor \textbf{32} and the sphinganine acceptor \textbf{33}, synthesised in 14 steps from D-galactal, gave the $\beta$-glycolipid derivative \textbf{34} in a 96% yield. Chelation induced anomerisation was facilitated through treatment with a catalytic amount of TiCl$_4$ to generate the $\alpha$-sphingolipid derivative \textbf{35} in high $\alpha:\beta$ ratio and 96% yield. Staudinger reduction of the azide followed by amide formation gave the ceramide derivative \textbf{36} in modest yield over 2 steps. Unfortunately, deprotection of these compounds proved challenging. It is well known that under basic conditions, glycuronic acid derivatives (particularly galacturonic acid derivatives) undergo elimination reactions across C4 and C5 to give unsaturated derivatives such as \textbf{38} via an E1cB type elimination process. This elimination reaction poses a challenge to any synthetic route involving the use of uronic acid derivatives. All attempts to remove the benzoate protecting groups from \textbf{36} under basic conditions led to this elimination process. After the evaluation of a number of deprotection conditions, successful deprotection of \textbf{36} was facilitated via treatment with hydrogen peroxide and sodium propoxide. The PBS-59 derivative \textbf{37} was isolated in 36% yield.

\begin{center}
\textbf{Scheme 5} Pilgrim and Murphy’ synthesis of PBS-59 via chelation induced anomerisation reaction
\end{center}
Also in this report, Murphy and Pilgrim also made an initial attempt to modify the anomeric linkage to give access to an S-linked analogue of a bacterial glycosphingolipid (Scheme 6). Chelation induced anomerisation reactions of S-glycosides is rare in the literature and this paper provides a rare glimpse at the potential such reactions could have in the stereoselective synthesis of S-linked glycosides. The β-thioglycolipid 41 was synthesised via the coupling of bromide 40 with the β-glycosyl thiol derivative 39 in a 55% yield. With compound 41 in hand, the chelation induced anomerisation reaction was attempted using 2 equivalents of TiCl₄. Gratifyingly the anomerisation reaction proceeded to give the α-glycolipid derivative 42 in 55% yield with a 4:1 α:β selectivity. Staudinger reduction and amide coupling gave ceramide derivative 43 in 40% yield over 2 steps. However, deprotection of 43 under the conditions set out for compound 36 led to oxidation of the sulfur atom to give the sulfone derivative 44 in 56% yield and all efforts to preclude this oxidation reaction proved unsuccessful.

Scheme 6 First attempt to synthesise S-linked bacterial glycosphingolipids via chelation induced anomerisation reaction
1.8 Target glycolipids and objectives

![Target glycosphingolipids](image)

Although the sulfone 44 is in itself an interesting and novel glycosphingolipid derivative, the thio-analogue of PBS-59 was still seen as a desirable and very achievable goal. The next chapter of this thesis will focus on attempts made to synthesise the novel S-linked PBS-59 derivative 45 and the glucuronic derivative 46. A second generation route will be provided and deprotection conditions to preclude sulfur oxidation will be explored. It is hoped these glycolipid derivatives may help to further the current understanding of NKT cell stimulation by lipid antigens.

1.9 References


(36) Pilgrim, W.; Murphy, P. V. *Organic Letters* 2009, 11, 939-942.
Chapter 2: Synthesis of S-linked bacterial glycosphingolipid derivatives

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2.1 Retrosynthetic analysis

Although the route devised by Pilgrim and Murphy was highly successful in delivering a number of glycosphingolipid derivatives, it did, however, suffer from a number of drawbacks. The route to the sphinganine chain 40 from D-galactal (Scheme 7) was a lengthy process, consisting of 14 steps. Synthesis of the benzyolated glycosyl thiol 39 also proved difficult. A 7 step synthetic sequence involved an unreliable allylation reaction which often led to low yields and poor reproducibility. It was our opinion that a shorter route to these key intermediates was possible which would allow for faster access to the desired thioglycolipid derivatives.

A re-evaluation of the retrosynthetic scheme led us to a number of conclusions (Scheme 8). As the rate of saponification for acetate protecting groups is much greater than that of benzoate protecting groups, it was believed that a change in the protecting group strategy could considerably aid the efforts in finding suitable deprotection conditions for the final compounds. It was also envisaged that the glycolipid derivative 53 could be obtained through an anomic alkylation of α-glycosyl thiol 54 and the bromo-sphinganine derivative 55. Amide formation and subsequent deprotection would then give the α-S-linked bacterial

Scheme 7 Previous syntheses of a sphinganine chain and β-glycosyl thiols from the Murphy group
glycosphingolipid derivative 45. It was proposed that α-glycosyl thiols such as 54 could be prepared via a chelation induced anomerisation reaction of the easily obtainable β-glycosyl thiol derivative 56. This would offer a key intermediate of considerable value, not only in the synthesis of glycolipids but also in the synthesis of other α-S-linked glycoconjugates.

Scheme 8 Proposed “2nd generation” retrosynthetic analysis

It was proposed that a route to the alkyl bromide 55 starting from the so called ‘Myers auxiliary’ 59 would also be examined. A number of simple organic transformations would give the sphinganine derivative 57 which could then be treated under Appel conditions to obtain the desired bromide derivative 55.

Scheme 9 Retrosynthesis of sphinganine derivative
2.2 Previous syntheses of sphinganine chains

The 1950’s saw a flurry of interest in the synthesis of sphinganines. Over a period of 5 years, several groups working independently reported the synthesis of sphinganines via similar methods. Gregory and Malkin\(^2\) were the first to show that oxaminating the \(\beta\)-ketoester 60 gave oxime derivative 61, subsequent hydrogenation of 61 gave amine derivative 62. A double reduction then gave access to sphinganine 63 (Scheme 10). Both Shapiro\(^3\) and Fischer\(^4\) used a similar reaction sequence to obtain compound 63. Each of these syntheses gave rise to mixtures of both the threo and erythro isomers and it was not until the work of Grob and Jenny that pure samples of each isomer were obtained\(^5\).

![Scheme 10 Gregory and Malkin’s synthesis of sphinganines](image)

Since those early pioneering efforts, a number of new approaches have been developed for the stereoselective synthesis of sphinganines. These approaches can be broken down into a number of categories\(^6\):

1. Serine derived syntheses
2. Carbohydrate based syntheses
3. Asymmetric synthesis
4. Chiral auxiliary based approaches

Some important examples will be discussed in this section.

2.2.1 Syntheses from L-serine

In general, serine is the most widely used starting material for the synthesis of sphinganines. Of the many routes reported with serines, the two most successful are those using the Garner aldehydes and those using serine derived Weinreb amides.
Garner’s aldehyde 64 was first reported in the 1980s and is still the most common method to synthesise 1,2 amino alcohols and 2-amino-1,3-diols. In one example D-erythro-sphinganine was synthesised through the addition of Lithium pentadecyne to Garners aldehyde to give 65 as a mixture of diastereomers (8:1). Catalytic hydrogenation followed by deprotection under acidic conditions gave sphinganine 63 in 80% yield.

Scheme 11 Synthesis of sphinganines from Garner aldehyde

In 2004 Howell et al. reported a highly efficient synthesis of sphinganines from serine derived Weinreb amide 67 in five steps. Displacement of tertiary amide using an alkyl magnesium bromide gave ketone derivative 68 which underwent the stereoselective reduction conditions set out by Hoffman to give alcohol 69. Finally treatment with TFA gave sphinganine 63 in good yield.

Scheme 12 Synthesis of sphinganines from serine derived Weinreb amides

2.2.2 Synthesis from carbohydrates

Carbohydrates offer convenient chiral templates for the synthesis of sphingoid bases. The main drawback of this approach is the requirement for the introduction of an amino group onto the molecule. This is usually achieved through the introduction of an azide functionality onto the molecule which can then be reduced at a later stage in the synthesis.
Reist and Christie\textsuperscript{10} have reported the use of a protected $\alpha$-allofuranose for the synthesis of sphinganines. Protection of the amino group of 70 followed by removal of the more labile isopropylidene group gave a diol intermediate which was oxidatively cleaved using NaIO\textsubscript{4} to give aldehyde derivative 71. Wittig reaction followed by deprotection of the remaining isopropylidene gave 72 as a mixture of isomers. Oxidative cleavage, reduction of the subsequent carbonyl, catalytic hydrogenation and deprotection gave sphinganine 63.

Scheme 13 Reist and Christie’s synthesis of sphinganines from $\alpha$-allofuranose

In the early nineties Schmidt reported the synthesis of sphinganines from commercially available perbenzylated D-galactal 47\textsuperscript{11}. Treatment of 47 with sulfuric acid gave hemiacetal derivative 73. Wittig reaction of 73 gave compound 74 with concomitant loss of the benzyl group at C4 of the galactose residue. Mesylation followed by catalytic hydrogenation, acetate protection and displacement of the azide using NaN\textsubscript{3} gave sphinganine derivative 75 in high yield. Finally, saponification of the acetate groups followed by reduction of the azide gave sphinganine 63. D-galactal was also used in the synthesis of sphinganines in the Murphy group as shown Scheme 7\textsuperscript{12}.
2.2.3 Chiral auxiliary based approaches

Chiral auxiliaries have also been employed in the synthesis of sphinganines. One such example shows the utility of the known RAMP/SAMP hydrazones to prepare sphinganines in excellent yields with a high degree of stereochemical control\(^{13}\). Alkylation of RAMP hydrazone 76 followed by cleavage of the hydrazone using oxalic acid gave ketone 77 in 96\% ee. Selective reduction gave syn alcohol 78. Introduction of the amino group by way of an azide reduction gave anti amine 79. Finally deprotection gave sphinganine 80 in high yield and high overall enantiomeric excess (ee).

Scheme 15 RAMP/SAMP chiral auxiliary approach to sphinganines

2.3 Novel approach to sphinganines from pseudoephedrine glycinamide

Myers and co-workers have demonstrated the utility of the inexpensive amino alcohol pseudoephedrine (which is readily available in both enantiomeric forms) as a chiral auxiliary in stereoselective \(\alpha\)-alkylation reactions of amino acid derivatives\(^{1}\). Of particular interest was the highly selective alkylation reactions carried out on pseudoephedrine glycinamide, usually used for the formation of unnatural amino acids. However, these pseudoephedrine auxiliaries can also be readily displaced with alkyl lithium or Grignard reagents, giving access to highly enantioenriched amino ketones\(^{14}\). With this in mind, it was decided to exploit these useful properties in the synthesis of sphinganine chains. It was believed this new approach could be
useful in obtaining new sphinganine derivatives in an efficient manner. An important aspect of this proposed route is the potential to modify the stereochemistry of the substrate at almost any step in the process to give access to numerous isomers of erythro-sphinganine for SAR studies.

Scheme 16 Synthesis of pseudoephedrine glycinamide monohydrate

To investigate this hypothesis, pseudoephedrine glycinamide 59 was prepared in a one step procedure from glycine methyl ester. Under basic conditions a direct condensation between the amino group of the pseudoephedrine moiety and the carbonyl group of glycine occurs without substantial competition from the unprotected primary amine on glycine to give access to pseudoephedrine glycinamide in 72% yield. This process is believed to occur through an initial transesterification between glycine methyl ester and the secondary hydroxyl group of pseudoephedrine, followed by a rapid N-O acyl transfer. The product is highly crystalline and pure 59 can be obtained via recrystallization from THF. Alternatively 59 can also be synthesised in a 2 step process involving the mixed anhydride of N-Boc-glycine to give compound 82. Hydrolysis and recrystallisation then give 59 in high yield (Scheme 16).

The drawback of this particular procedure is the rather substantial drying process of 59 which is vital to the attainment of high yields in subsequent alkylation reactions. The anhydrous compound is also a white solid; however, we have found it to be highly hygroscopic and can be extremely difficult to keep dry leading to incomplete alkylation reactions. Considerable care must also be taken when treating 59 with strong bases such as n-butyllithium during the course of the alkylation reactions as to prevent decomposition of the starting material. A recent paper from the Myers group has reported a way of circumventing
these problems through the use of lithium hexamethyldisilazide (LHMDS) as a base in alkylation reactions with the monohydrate of 59. Unfortunately, in our hands the alkylation reactions using this reagent were extremely slow and poor isolated yields were obtained.

It has been shown that compound 82 can also be used directly in the alkylation reactions. This compound proved less susceptible to decomposition under the reaction conditions and maintained the same high yields and good diastereomeric ratios (dr) (this is in good agreement with the results of Myers work); however, unlike compound 59, 82 does not have the advantage of being a crystalline solid.

Scheme 17 Synthesis of N-Boc pseudoephedrine allyl glycinamide

The asymmetric alkylation of compound 59 was next examined. Initial focus concentrated on choosing a suitable electrophilic partner for use in the alkylation reaction. Using paraformaldehyde as an electrophile gave a serine derivative in 26% yield which could then be protected, for example, as a silyl ether. The low yield of this reaction coupled with the extra protection step involved in the synthesis forced us to consider alternative electrophiles. Alkylation of 59 and 82 was also attempted with (chloromethoxy)triisopropylsilane. It was hoped that this would lead to the introduction of a silylated methylene group which could then undergo a late stage oxidation under Tamao-Fleming conditions to reveal the desired sphinganine chain. Unfortunately, this alkylation reaction failed to yield any of the desired products. The failure of this reaction is probably due to the lack of electrophilic character of such substrates in alkylation reactions coupled with the steric hindrance provided by the bulky isopropyl groups. The use of large excesses of potentially carcinogenic oxymethylchlorides when conducting the reaction on large scales was also considered a major drawback of this proposed route.

It was finally decided that the use of allyl bromide as an electrophile would provide a pseudoephedrine allyl glycinamide derivative such as 83 (Scheme 17), a compound which is well known in the literature. This compound was identified as an ideal starting material for the synthesis of sphinganines. The presence of the allylic alkene would allow for further
Synthesis of S-linked bacterial glycosphingolipid derivatives

Chapter 2

functionalization of the lipid chain in order to obtain natural sphinganines as well as novel sphinganine derivatives. A late stage isomerisation of the allylic bond followed by oxidative cleavage would give access to the desired sphinganine derivative. This route could also provide easier access to C-glycolipid derivatives and glycolipid derivatives which contain an extra methylene group in the sphinganine chain. Derivatives of this type could provide valuable SAR information.

Scheme 18 Proposed reactive conformation of pseudoephedrine amides

Therefore treatment of 82 with LDA following the procedures outlined in the literature\textsuperscript{16} gave diastereomERICally pure (99:1 \textit{dr}) L-allyglycinamide in 58\% yield after recrystallisation. \textit{N}-Boc protection was then achieved through treatment with Boc\textsubscript{2}O to give compound 84 in 95\% yield. Alternatively, treatment of 83 with 3.2 equivalents of LDA at -78°C followed by addition of 1.2 equivalents of allyl bromide gave \textit{N}-Boc-pseudoephedrine allyl-glycinamide 84 in 70\% yield with a 96:4 \textit{dr}. Treatment of either compound with LDA presumably gives rise to a trianion such as 85. In simple terms, the asymmetric induction is as a result of the electrophile entering on the same side as the methyl group of the auxiliary giving a 1,4 syn addition. The reason for the high diastereoselectivities observed in these reactions is not obvious and still remains open for debate. As pseudoephedrine is a linear auxiliary, it lacks a lot of the characteristics of a ‘traditional’ chiral auxiliary\textsuperscript{17}. One proposed reason for this highly diastereoselective reaction is shown in Scheme 18\textsuperscript{1}. Myers has suggested a reactive conformation such as 87. The secondary lithium alkoxide on the pseudoephedrine, potentially with the aid of some chelating THF molecules, effectively blocks the \pi-face of the enolate 87\textsuperscript{18}. This model also takes into account the allylic strain present in the molecule as the C-H
bond α to the nitrogen atom lies in plane with the enolate oxygen therefore forcing the pseudoephedrine to adopt a staggered conformation\textsuperscript{19}.

![Scheme 19](image_url)

**Scheme 19** Synthesis of enantiomerically enriched amino ketone and the iodoheptadecane intermediate

With compound 84 in hand, attention was focused on displacement of the auxiliary to give access to the ketone derivative 88. It is well known that treatment of tertiary carboxamides with organometallic nucleophiles gives rise to a stable tetrahedral intermediate\textsuperscript{20}. This intermediate breaks down upon aqueous work-up to give ketone products. This tetrahedral intermediate is key to the success of this reaction, premature breakdown of this intermediate can allow the ketone to react further giving rise to tertiary alcohol by-products. The conversion of N-Boc pseudoephedrine derivatives into ketones is well documented in the literature, however, the use of longer chain organometallic reagents is as yet untested\textsuperscript{14}. As a 17 carbon chain organolithium reagent was required to test this reaction, heptadecanol 89 was treated with mesyl chloride in CH\textsubscript{2}Cl\textsubscript{2} to give the mesylate derivative 90. Subsequent Finkelstein type reaction using sodium iodide in refluxing acetone gave 1-iodoheptadecane 91 in 80% yield over 2 steps. The organolithium reagent was then generated according to the procedure of Bailey\textsuperscript{21}, treatment of iodoheptadecane with 2.2 equivalents of t-BuLi in Et\textsubscript{2}O-pentane at -78°C gave a 0.5 M solution of lithiated heptadecane. Pseudoephedrine derivative 84 was treated with 3.1 equivalents of 0.5M lithiated heptadecane at -78°C. The reaction was warmed to room temperature for 2 hours and quenched with aqueous NH\textsubscript{4}Cl. The product ketone was obtained in 70% yield after flash chromatography. In general we have found that organolithium reagents are far superior to Grignard reagents for these reactions. Treatment of 84 with the Grignard reagent derived from 91 gave the ketone in 45% yield and required a much longer reaction time.
Hoffman has shown that 1,2 anti amino alcohols can be synthesised from 1,2 amino ketones via a highly stereoselective reduction with LiAlH(OtBu)₃ in EtOH at -78°C²². This method has been used successfully in a number of previous sphinganine syntheses. In the stereoselective reduction of any 1,2 protected amino ketone; the stereochemical outcome of the reaction is under control of two different modes (Figure 13). In the case of carbamate protected amino ketones, chelation control enforces a syn-periplanar relationship between the carbonyl and the amino group due to the presence of a Lewis acid or counterion. This chelation effect gives rise to anti-selectivity in the reduction e.g. ⁹³. Felkin-Anh control can be gained by placing steric bulk onto the nitrogen through the use of trityl or N,N benzyl protecting groups. This orientates the amino group perpendicular to the carbonyl in order to minimize repulsions in the transition state. This effect gives rise to syn-amino alcohols upon reduction e.g. ⁹⁴.

Hoffman has observed that carbamate protected amino alcohols undergo highly selective reductions under chelation control when treated with LiAlH(O-t-Bu)₃ in a protic solvent such as ethanol at low temperatures. With regards to the mechanism of such a selective reduction, it is believed that the lithium counter-ion does not play a role in the stereochemical outcome.
of the reaction and that chelation occurs between the amino ketone and the aluminium of the reducing agent through various exchange-disproportionation reactions in ethanol (Figure 14). As aluminium metals form weak complexes with neutral species and much stronger complexes with anionic species it has been postulated that small amounts of ethoxide generated during the course of the reaction deprotonate the carbamate nitrogen and facilitate chelation through ligand exchange to aluminium.

![Proposed mechanism for reduction reaction](image)

**Figure 14** Proposed mechanism for reduction reaction

As expected, this reaction proved extremely reliable giving the anti amino alcohol product **92** in 85% yield. There were some slight problems with the solubility of the ketone in EtOH at such low temperatures; however, the reaction proceeded to completion when left to stir for 24 hours.

a.)

![Scheme 20](image)

**Scheme 20** Protection of 1,2-amino alcohol

The amino alcohol derivative **92** was then protected, initially, as a benzyl ether via treatment with benzyl bromide and NaH to give the benzylated derivative **99** in 90% yield. It was
anticipated that this protecting group strategy may lead to problems at a later stage in the synthesis as the sulfur atom present in the final glycolipid derivatives would prevent the use of catalytic hydrogenation conditions for debenzylation due to poisoning of the palladium catalyst. As an alternative approach, the protection of the secondary hydroxyl group as an $N,O$-acetal by treatment of 92 with dimethoxypropane and catalytic pyridinium $p$-toluenesulfonate in refluxing toluene gave the acetal derivative 100 in 88% yield$^{24}$.

\[ \text{Scheme 21 Synthesis of bromosphinganines for use in alkylation reactions} \]

In order to synthesise the desired sphinganine derivative \( 55 \), it was first necessary to isomerise the allylic double bond of 100 to give the propenyl derivative 102. This propenyl derivative could then be subjected to oxidative cleavage followed by an Appel reaction to give the desired bromo-sphinganine derivative \( 55 \). Compound 100 could also be useful in the synthesis of glycolipid derivatives possessing an extra methylene group in the sphinganine moiety for SAR studies.

\[ \text{Scheme 22 Thermally modified Grubbs II catalysed isomerisation reaction} \]
Hanessian has recently reported an efficient method for the isomerisation of allylic double bonds to propenyl derivatives via treatment with a thermally modified Grubbs II catalyst\textsuperscript{25}. Grubbs catalysts (Scheme 22 I and II) have revolutionised strategic planning in organic synthesis due to their success in ring closing metathesis reactions (RCM). It has been reported however, that some substrates undergo unwanted isomerisation side reactions during the RCM process. Hanessian’s group saw the potential to develop this unwanted side reaction into a useful and mild method for the isomerisation of terminal double bonds. In our experience, the reaction proceeds very smoothly using 15 mol\% Grubbs II catalyst (the paper reports the reaction using 10 mol\% however we have found that in many cases the reaction does not proceed to completion) in MeOH at 60°C. The reaction is believed to proceed via a ruthenium-hydrido species (Scheme 22 III) (also reported by Grubbs et al.) however, due to the complex nature of these ruthenium species an exact mechanism or catalytic cycle has yet to be elucidated. Compound 100 was therefore treated with commercially available Grubbs II at 60°C overnight. Upon purification the propenyl derivative 102 was obtained in 71\% yield as a 1:1 E/Z mixture of isomers with no detection of dimerization products arising from potential cross coupling reactions. NMR spectra of these compounds were further complicated due to the presence of rotational isomer due to the N-Boc group. Interestingly when the isomerisation reaction was performed using freshly prepared Grubbs II catalyst (prepared according to the procedure of Scholl et. al\textsuperscript{26}), the yield of the reaction increased to 95\%. Propenyl derivative 102 could be viewed a valuable intermediate in the synthesis of C-linked-glycolipids. To the best of our knowledge this is the shortest route to obtain sphinganine derivatives of this type. This compound could therefore prove a valuable intermediate which could be used in cross metathesis reactions to give access C-glycolipids.

![Scheme 23 Oxidative cleavage and Appel reaction](image)

With propenyl derivative 102 in hand, oxidative cleavage of the double bond was attempted in order to obtain the protected sphinganine derivative 57. It was envisaged that ozonolysis followed by a reductive work-up would give access to compound 57; however, the ozonolysis
reaction of 102 was extremely unreliable and failed on a number of occasions. In the rare occasions when the reaction did proceed to completion, a complex mixture of products was obtained along with substantial decomposition of the starting material. Another common method of carrying out oxidative cleavage reactions is the use of NaIO₄ and catalytic OsO₄, the so called Lemieux-Johnson oxidation. It has been reported that in many cases these reactions are often low yielding and give rise to substantial by-products such as α-hydroxy ketones. Recently, Yu has reported a modified Lemieux-Johnson in which a stoichiometric amount of 2,6-lutidine was added to the reaction mixture. This led to a dramatic improvement in the yield of the reactions due to the suppression of by-product formation. To this end, 102 was treated under Yu’s modified conditions. The reaction went to completion after four days and the crude aldehyde was treated with NaBH₄. Purification gave compound 55 in a disappointing 30% yield. Attempts were made to modify this procedure with the hope of improving the reaction yield. The reaction time was changed and the equivalents of the reagents used were adjusted with no discernable effect on the outcome of the reaction. An attempted Upjohn dihydroxylation (NMO, OsO₄), followed by treatment with NaIO₄ also gave the product alcohol in low yield. Nicolaou has recently reported the use of hypervalent iodine compounds in oxidative cleavage reactions. BAIB is an excellent reagent for the cleavage of 1,2-diols and when combined with OsO₄ becomes a very useful tool in the oxidative cleavage of double bonds. Unlike Lemieux-Johnson type reactions, this reaction is completely homogenous and it was believed that this homogeneity could be key to increasing the yield of the oxidative cleavage of 102. Therefore compound 102 was treated with NMO, catalytic OsO₄ and 2,6-lutidine in acetone-water and stirred for 3 days. BAIB was added and the reaction mixture was stirred for a further 3 hours. Reduction of the crude product with NaBH₄ and purification gave the desired primary alcohol in 54% yield over 2 steps.

Conversion of the primary alcohol 57 into the bromide derivative 55 was then carried out under standard Appel conditions to give the bromide derivative in high yields.

2.4 Synthesis of α-glycosyl thiols

Novel methods for the stereoselective synthesis of α-glycosyl thiols have garnered considerable interest in recent years. The inherent stability of the thioglycoside bond against the action of carbohydrate processing enzymes has led to widespread interest in the synthesis of S-linked glycomimetics. Glycosyl thiols, unlike sugar hemiacetals, do not undergo the same mutarotation processes under basic conditions, meaning that their stereochemistry can
be maintained during the course of anomeric alkylation reactions. Although many methods exist to synthesise β-glycosyl thiols, there are currently very few reports on the synthesis of α-glycosyl thiols. In general, β-glycosyl thiols are synthesised through S_N2 displacement reactions of glycosyl halides with either thiourea or sodium thioacetate, hydrolysis of the former or selective deacetylation of the latter gives β-glycosyl thiols in high yields.

The method mentioned in chapter 1 by Zhu shows a rare example of direct synthesis of α-thiols. β-glycosyl chlorides have also been used in multistep procedures however it has been reported that the reproducibility of this method is low^{31}. Recently Davis reported the use of Lawesson reagent to produce anomeric mixtures of glycosyl thiols from sugar hemiacetals^{32}.

It was our belief that chelation induced anomerisation reactions could be used to control the stereoselectivity of glycosyl thiols as the thermodynamically favoured α-anomer would be formed in preference to the β-anomer. The work reported by Murphy and Pilgrim^{33} recently shows a rare example of anomerisation reactions of S-glycosides. Aside from this report, very few instances of S-glycoside anomerisation have appeared in the literature and there are no accounts of the use of anomerisation reactions in the stereoselective synthesis of glycosyl thiols. From previous studies on the rates of Lewis acid catalysed anomerisation reactions, it was well known that:

1.) Anomerisation of S-glycosides is generally faster than that of O-glycosides
2.) The rates generally follow the trend galacturonide > glucuronide > galactoside > glucoside
3.) Anomerisation reactions are faster with TiCl4

\[
\begin{align*}
104 & \xrightarrow{\text{MCl}_4} 105 \\
105 & \xrightarrow{\text{MCl}_4} 106
\end{align*}
\]

\(X = O, S; M = Sn, Ti\)

**Figure 15** Proposed mechanism for anomerisation reaction which involves an endocyclic cleavage followed by equilibration to thermodynamically favoured α-anomer.

It has been suggested that these anomerisation reactions proceed via chelation induced endocyclic cleavage between C1 and the pyranose oxygen (105) which then allows for
equilibration to the thermodynamically favoured α-anomer 106. This chelation comes as a result of an interaction between the pyranose oxygen and the substituent on C6. In general chelation is much faster for galacturonides and glucuronides due to the presence of a carbonyl group at C6. The high α:β ratios for compounds of this type is due to an increased anomeric effect.

![Scheme 24 Glycosidation of lactone 107 with hexamethyldisilathiane](image)

It was anticipated that the glycosidation of 1,6-lactone 107 with hexamethyldisilathiane in the presence of a Lewis acid would lead to the formation of β-glycosyl thiols which would be followed by anomerisation to the desired α-glycosyl thiols. The reaction of this particular lactone with silylated nucleophiles is well known from our own group and typically gives a highly stereoselective reaction favouring the α-anomer. To this end, the 1,6-lactone 107 was synthesised as previously described and treated with hexamethyldisilathiane and SnCl4. The reaction was left stirring overnight, however both TLC and mass spectrum analysis showed no product formation. With this disappointing result, a range of Lewis acids were screened with the hope of obtaining the desired glycosyl thiol 108. The results of these experiments are summarised in Table 1. Unfortunately, all efforts ultimately resulted in failure of the S-glycosidation, even the addition of small amounts of TBAF to try to generate a thiolate anion and heating the reaction at reflux proved ineffective.

<table>
<thead>
<tr>
<th>Lewis acid equivalents</th>
<th>(TMS)2S equivalents</th>
<th>Additive</th>
<th>Yield</th>
<th>Temp (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SnCl4 0.5</td>
<td>5</td>
<td>-</td>
<td>S.M</td>
<td>RT</td>
</tr>
<tr>
<td>SnCl4 3.5</td>
<td>5</td>
<td>TBAF</td>
<td>S.M</td>
<td>RT</td>
</tr>
<tr>
<td>TiCl4 0.5</td>
<td>5</td>
<td>-</td>
<td>S.M</td>
<td>RT</td>
</tr>
<tr>
<td>TiCl4 0.5</td>
<td>5</td>
<td>TBAF</td>
<td>S.M</td>
<td>RT</td>
</tr>
</tbody>
</table>
Synthesis of \( S \)-linked bacterial glycosphingolipid derivatives

<table>
<thead>
<tr>
<th>Lewis Acid</th>
<th>Temperature</th>
<th>Time (h)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BF(_3).Et(_2)O</td>
<td>RT</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>Sc(OTf)(_2)</td>
<td>Reflux</td>
<td>3.5</td>
<td>S.M</td>
</tr>
<tr>
<td>Yb(OTf)(_2)</td>
<td>RT</td>
<td>3.5</td>
<td>S.M</td>
</tr>
<tr>
<td>TMSOTf</td>
<td>RT</td>
<td>3.5</td>
<td>S.M</td>
</tr>
<tr>
<td>TMSOTf</td>
<td>Reflux</td>
<td>3.5</td>
<td>S.M</td>
</tr>
</tbody>
</table>

**Table 1** Summary of the Lewis acid catalysed glycosidation conditions which were used in the attempts to facilitate the formation of 108

Although the direct synthesis of thiols from 1,6 glucuronolactone would be a useful synthetic protocol to develop, difficulties were encountered when attempting to synthesise the corresponding 1,6-galacturonolactone. Only one synthesis of this compound has appeared in the literature through an indirect 5 step route\(^{36}\) and the conditions reported within our own group for the synthesis of the 1,6-glucuronolactone failed to yield the desired galacturonolactone derivative.

Due to the problems encountered with both the formation of the galacturonolactone and the failure of the \( S \)-glycosidation reaction, an alternative approach to glycosyl thiols was devised. It was decided to focus on the synthesis of pure \( \beta \)-glycosyl thiols which we could then attempt to anomerise, under Lewis acid catalysed conditions, to the desired \( \alpha \)-glycosyl thiol derivatives.

**Scheme 25** Synthesis of \( \beta \)-glycosyl thiols

The peracetylated glucosyl and galactosyl thiols were prepared in good yields from commercially available peracetylated glucose and galactose. Treatment of the peracetylated
glycosides with hydrogen bromide in acetic acid gave glycosyl bromides 110a and 110b. S_N2 displacement of the bromide with potassium thioacetate gave the fully protected β-thioglycoside derivatives. Selective S-deacetylation via thiolysis with sodium thiomethoxide gave the β-glycosyl thiols 111a and 111b in good yield.37

Scheme 26 Synthesis of 2,3,4-Tri-O-acetyl-β-thio-D-glucopyranosiduronic acid, methyl ester

The synthesis of the β-gluconic acid thiol derivatives started from commercially available D-gluconono-6,3-lactone 112. Opening of lactone 112 using Et_3N in methanol, followed by iodine catalysed acetylation gave 113 in 34% yield. The α-bromide of 113 was generated through treatment with hydrobromic acid in acetic acid followed by recrystallization from ethanol. Displacement of the bromine with potassium thioacetate and selective S-deacetylation as previously described gave the pure β-gluconic thiol derivative 115 as a single anomer.37
Synthesis of S-linked bacterial glycosphingolipid derivatives

Scheme 27 Synthesis of 2,3,4-Tri-O-acetyl-β-thio-D-galactopyranosiduronic acid, methyl ester

The galacturonosyl thiol 56 was synthesised in a similar sequence to the above. D-galacturonic acid monohydrate 116 was acetylated with Ac₂O and catalytic perchloric acid (other acid catalysts were tried but gave the desired galacturonic acid derivative in poor yields) to give 1,2,3,4-tetra-O-acetyl-galacturonic acid 38. Esterification with methyl iodide under phase transfer conditions gave the fully protected galacturonic acid methyl ester 117 in high yields 39. Compound 117 was subjected to an identical reaction sequence as outlined above to give the β-galacturonosyl thiol 56 in good yield after purification by flash chromatography.

Scheme 28 Lewis acid catalysed anomerisation of 115

With the four β-glycosyl thiols in hand, attention was turned to the Lewis acid catalysed anomerisation reactions. As an initial experiment, compound 115 was treated with 0.5 equivalents of TiCl₄ in anhydrous CH₂Cl₂ at room temperature for 20 hours. Gratifyingly a 1:1 mixture of anomers was obtained. Evidence had suggested that increasing the number of equivalents of TiCl₄ and lowering the reaction temperature could give a higher α:β ratio.
Therefore a number of experiments were conducted at 0°C while varying the amount of TiCl₄. The results of these experiments are given in table 2.

<table>
<thead>
<tr>
<th>eq.TiCl₄</th>
<th>Time (h)</th>
<th>Temp. (°C)</th>
<th>Ratio (α:β)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>24</td>
<td>0</td>
<td>60:40</td>
</tr>
<tr>
<td>1.5</td>
<td>24</td>
<td>0</td>
<td>80:20</td>
</tr>
<tr>
<td>2.5</td>
<td>24</td>
<td>0</td>
<td>88:11</td>
</tr>
<tr>
<td>3.5</td>
<td>24</td>
<td>0</td>
<td>83:17</td>
</tr>
<tr>
<td>4.5</td>
<td>24</td>
<td>0</td>
<td>78:22</td>
</tr>
</tbody>
</table>

Table 2 Effects of Lewis acid concentration on anomerisation

The results in the table indicate that the anomerisation reaction reached its peak ratio after about 2.5 equivalents of TiCl₄ after which the α:β ratio begins to decline. Cooling the reaction further to -20°C did not have a further influence on the ratio obtained. The results obtained here are in very good agreement with previous observations in the group ³³.

Scheme 29 Application of anomerisation conditions in the synthesis of 54

The optimised conditions were then applied to the galacturonic thiol. As expected under these conditions the galacturonic acid thiol 56 gave a higher α:β ratio than the glucuronic thiols. on 50-100 mg scale the ratio was determined to be > 95% α-anomer, however when the reaction was scaled up to 0.5-1 gram scale, the ratio was slightly lower giving 9:1 ratio α:β.
Interestingly the anomerisation reaction with both peracetylated galactose thiol 111b and peracetylated glucose thiol 111a failed to give any of the desired α-thiols indicating that the presence of the carbonyl at C6 was pivotal to the success of these anomerisation reactions, perhaps giving rise to an intermediate such as 120.

### 2.5 Coupling reactions and endgame

![Scheme 30 Anomeric alkylation reactions](image)

With the bromide 55 in hand, the direct anomeric alkylation was attempted with thiols 54 and 119 in the hope of obtaining the 2 glycolipid derivatives 53 and 121. Deprotonation of the thiols with less than 1 equivalent of NaH gave the glycolipid derivatives in 35-40% yield (The use of more than 1 equivalent in model reactions led to unsaturation across the C4-C5 bond via elimination of acetic acid to give compounds such as 123)\(^37\). Addition of additives such as tetra butyl ammonium iodide (TBAI) did not increase the yield of the reaction. In an effort to improve the yield a number of other coupling conditions were attempted. The use of \(\text{K}_2\text{CO}_3\) and \(\text{NaHCO}_3\) as a base led to lower yields of the desired glycolipid derivatives. Interestingly in one model reaction, treatment of the β-glucosyl thiol and alkyl halide in the presence of \(\text{CsCO}_3\) and TBAI\(^40\) brought about an alkylation reaction followed by an anomerisation reaction which gave the coupled product in 4:1 α:β mixture.

Toth and co-workers have recently reported a modified Mitsunobu type reaction to couple glycosyl thiols and alcohols\(^41\). The proposed mechanism for this transformation is shown in Figure 16. Initially, an ADDP-PMe3 salt (124) is formed (indicated by a colour change from yellow to clear). Reaction of alcohol 125 with the ADDP-PMe3 salt 124 gives rise to an
oxyphosphonium ion such as 126. Nucleophilic displacement with glycosyl thiol 54 then gives the final coupled product with release of trimethylphosphine as a by-product.

Therefore, the alcohol 57 and the thiols 54 and 119 were added to stirring solutions of PMe3 and 1,1'-(azidodicarbonyl)dipiperidine (ADDP) in THF. The product glycolipids 53 and 121 were obtained in modest yields after chromatography, although this reaction gave the product lipid derivatives in a similar low yield to the direct anomeric alkylation reactions, it reduced the synthetic Scheme by 1 step.

![Figure 16](image)

**Figure 16** Toth’s proposed mechanism for the modified Mitsunobu reaction

Treatment of the glycolipid derivatives with Formic acid removed the Boc and isopropylidene groups concomitantly. Acylation of the crude amine with the N-hydroxysuccinimide ester of nonadecanoic acid in the presence of DIPEA gave the fully protected glycolipid derivatives 128 and 129 in good yields.

![Scheme 31](image)

**Scheme 31** Removal of Boc and isopropylidene groups and ceramide formation

In an attempt to find suitable deprotection conditions a number of small scale reactions were carried out on compound 128 and monitored by mass spectrum analysis. As was the case with Dr. Pilgrims’s synthesis, all attempts to directly deprotect the glycolipid derivative under basic conditions led to unsaturation across the C4-C5 bond via elimination of acetic acid to
give unsaturated compounds such as 123. It was then proposed that a 2 step deprotection sequence be evaluated to deprotect compound 128. The basis for this proposal was due to a recent report by Mayato et al., in which they reported the selective deprotection of methyl esters using lithium iodide in refluxing EtOAc. It was our belief that, under the basic conditions required for the removal of acetate protecting groups, the presence of a carboxylate anion may suppress the unwanted E1Cb side reaction. Therefore, treatment of glycolipid derivative 128 with 5 equivalents of lithium iodide in refluxing EtOAc gave the free carboxylic acid derivative 131. Removal of the acetates was then achieved under mildly basic conditions (~pH 8) via treatment with a solution of guanidine-guanidinium nitrate as described by Ellervik and co-workers. Gratifyingly the fully deprotected bacterial glycolipid 45 was obtained in 55% yield over the two steps. The same deprotection sequence was then applied to compound 129 to give the fully deprotected novel bacterial glycolipid derivative 46.

Scheme 32 Two step deprotection of glycolipid derivatives
2.6 Conclusion

![Figure 17 Synthesised glycolipid derivatives](image)

In summary the synthesis of two novel bacterial glycosphingolipids has been achieved. It is our belief that this represents the first synthesis of $S$-linked bacterial glycosphingolipid mimetics based on uronic acids. The synthetic route illustrates the use of chelation induced anomerisation reactions to generate $\alpha$-glycosyl thiols of uronic acids. It is believed this reaction could find widespread use in the synthesis of novel $S$-linked glycoconjugates. In addition, these results also provide scope for further investigation into the anomerisation reactions of glycosyl thiols in the Murphy group in the hope of finding suitable conditions for the anomerisation of glycosyl thiols $111a$ and $111b$. This is currently under investigation by Michelle McKinney and Shane O’Sullivan.

We have also reported a new and versatile route to sphinganine chains from the Myers auxiliary. We believe this route has the potential to give easy access to further glycolipid derivatives via manipulation of chain length and stereochemistry which could provide useful insights into the mechanism of NKT cell stimulation via SAR studies. The glycolipids 45 and 46 are currently awaiting biological testing in order to establish their immunostimulatory activity.
2.7 References


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(3) Shapiro, D.; Segal, H.; Flowers, H. M. *Journal of the American Chemical Society* 1958, 80, 2170-2171.


### Chapter 3: Novel peptidomimetics: synthesis of Bcl-2 family inhibitors

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3.1 The role of the Bcl-2 family of proteins in cancer

Apoptosis or programmed cell death is the process by which unwanted, damaged or compromised cells are removed in mammalian systems. This process is vital during embryogenesis and in maintaining cellular homeostasis. Cells die in response to numerous stimuli in a controlled and regulated manner. These properties distinguish apoptosis from other mechanisms of cell death such as necrosis, in which the mechanism of cell death is uncontrolled and can lead to serious health problems. Apoptosis is therefore sometimes referred to as cell suicide as the cell plays an active role in its own death. Cell death through apoptosis can proceed via two distinct mechanisms (Figure 18), triggering of cell surface death receptors (extrinsic pathway) or perturbation of the mitochondria (intrinsic or Bcl-2 regulated pathway).

![Figure 18](Reprinted by permission from Macmillan Publishers Ltd: Nature Reviews Cancer, Ref. 2, copyright 2003)
These apoptotic pathways play an important role in fighting pathogenic infections and in executing the triggers from cytotoxic agents used in chemotherapy. Resistance by tumour cells to chemotherapeutic agents is a major problem in modern cancer therapy. This resistance is usually attributed to dysfunctional or impaired apoptosis\(^4\). This theory was hypothesised by the discovery that B-cell lymphoma 2 (Bcl-2) proteins are over-expressed in many tumour cells and lead to cell survival but not cell proliferation. It has been suggested that targeting the apoptotic machinery of this intrinsic pathway of apoptosis could provide novel cancer therapies which would circumvent the resistance problems\(^5\). Therefore, recent years has seen a flurry of research in developing drug like molecules capable of promoting cell death by antagonizing members of the Bcl-2 family of proteins.

The intrinsic pathway of apoptosis is governed by members of the Bcl-2 family of proteins. This family of proteins can be divided into 2 subsets; pro-apoptotic family members and anti-apoptotic (pro-survival) members. Members of the pro-apoptotic family include the proteins BAK and BAX. BAK and BAX cause the mitochondrial membrane to become permeable in response to death signals. In turn, the mitochondrion releases cytochrome c into the cytosol which causes a proteolytic cascade (caspase cascade) that induces apoptosis. Pro-survival members of the Bcl-2 family bind to these mitochondrial pro-apoptotic proteins which leads to inhibition of apoptosis\(^6\). Members of the pro-survival family include Bcl-2, Bcl-X\(_L\), Mcl-1, Bcl-w and A1. Interestingly each of the proteins mentioned share a remarkably similar sequence of homology known as the Bcl-2 homology. The Bcl-2 homology is made up of four domains, BH1, BH2, BH3 and BH4\(^7\).

**Figure 19** Crystal structures of pro-apoptotic proteins bound to pro-survival proteins (Reprinted by permission from Macmillan Publishers Ltd: Nature Reviews Drug Discovery, Ref. 1, copyright 2008)
Aside from these proteins, a number of similar pro-apoptotic proteins exist which only share the BH3 domain. These ‘BH3 only’ proteins are up-regulated in response to cellular stress signals and eight of these are known to exist in mammals, BIM, BID, PUMA, NOXA, BAD, BMF, HRK and BIK. These proteins are capable of binding selectively into a hydrophobic groove made up of the BH1, BH2 and BH4 domains on pro-survival proteins leading to BAK/BAX activation. Crystal structures of a number of these binding interactions have been elucidated (Figure 19). It has also been demonstrated that a number of these ‘BH3 only’ proteins bind to specific pro-survival proteins. For instance, BIM and PUMA can bind all pro-survival proteins tightly, BAD interacts with Bcl-2, Bcl-Xw and Bcl-w and NOXA will only bind to Mcl-1 and A1. The reasons for this selectivity are currently still unclear and are the subject of much debate. Therefore, this makes the synthesis of BH3 domain mimetics an important goal in medicinal chemistry as they can be employed as mechanistic probes to further elaborate current understanding of the interactions of Bcl-2 family proteins. In many tumour cells pro-survival members of the Bcl-2 family are over expressed leading to cell survival. It is therefore reasonable to suggest the idea that molecular mimicry of the BH3 domain should provide drug-like molecules which can bind to pro-survival proteins and in turn, trigger apoptosis.

3.2 Synthetic and naturally occurring Bcl-2 family inhibitors

Development of novel BH3 mimetics can be broken down into 3 categories:

1. Small molecule antagonists
2. Modified peptides
3. Peptidomimetics

3.2.1 Small molecule antagonists

Recently a number of small molecule compounds have been discovered which show binding to members of the Bcl-2 family (Figure 20). (-)-Gossypol, also known as AT-101 is currently being developed as a potential Bcl-2 inhibitor by Ascenta®. Studies have shown to have high affinity for Bcl-2 (Ki 230nM), Bcl-XI (Ki 480nM) and Mcl-1 (Ki 180 nM). This compound also shows good pharmacokinetics and has entered clinical trials. Extensive investigation of Gossypol derivatives has also proved successful and have allowed for the
identification of a number of analogues with submicromolar affinities for Bcl-2 and Mcl-1 including apogossypolone 135.  

**Figure 20** Structures of some important Bcl-2 family inhibitors

Abbott pharmaceuticals have recently reported two Bcl-2 inhibitors called ABT-737 and ABT-263. These compounds are the benchmark synthetic inhibitors of Bcl-2 proteins. Developed via a combination of NMR based structure activity relationships and fragment based screening, multiple iterations of medicinal chemistry led to the development of ABT-737 136. This compound showed extremely high affinity for Bcl-2 (IC\(_{50}\) 1nM) and Bcl-X\(_L\) (IC\(_{50}\) 0.5nM). Critically however, it does not have any affinity for Mcl-1. Further studies led to the discovery of ABT-263 137 which retains the activity of ABT-737 but is advantageous as it can be administered orally. ABT-263 has currently entered phase I clinical trials. The lack of interaction with Mcl-1, however, is the main drawback of the Abbott compounds as they only show single agent efficacy against malignancies with low Mcl-1 levels and would require the use of combination therapies against many cancers.

### 3.2.2 Modified peptides

\(\alpha\)-helices are the most common form of protein secondary structure and account for a high proportion of the secondary structures in proteins. Surprisingly, there has been little interest in \(\alpha\)-helical mimics for therapeutic use even though these secondary structures play an important role in many protein-protein interactions. This has recently changed however and interest in the synthesis of \(\alpha\)-helices is growing. Initially, small modified peptides were
designed and synthesised to represent the BH3 domain, however, these compounds failed to show any binding affinity, this is possibly due to their lack of helicity. In order to combat this, a number of efforts were made to reinforce their helical structures. Walensky et al. have reported the use of ring closing metathesis reaction (RCM) to effectively staple the peptide and force it to adopt a helical type structure. These “stapled” helical mimics show good cell membrane permeability and are resistant to the action of proteases. These mimics have currently entered preclinical trials. Cell based assays have shown a reduction in leukaemia progression and an extended median time of death in animal models. Recently, the same group have shown an Mcl-1 helix mimic which is an exclusive Mcl-1 inhibitor and apoptosis inducer indicating that targeting specific pro-survival proteins is a very achievable goal.

3.2.3 Peptidomimetics

Owing to the importance that the α-helix plays in protein-protein interactions it is reasonable to assume that mimicking the α-helical structure on a suitable backbone or scaffold would allow for the identification of novel biologically active structures (Figure 21). Hamilton and co workers have been successful in attaching a range of alkyl or aryl groups to a terphenyl backbone (Figure 21) in an effort to mimic the display of key amino acid epitopes on an α-helix. By examining crystal structures between Bcl-X<sub>L</sub> and Bak BH3 they were able to identify a number of ‘hot residues’ which are key to the binding interaction between the two proteins. In this case the so-called ‘hot residues’ are present at the i, i+3 and i+7 positions on an α-helix. These compounds have shown binding affinities in the nanomolar range (Kₐ 114 nM) against Bcl-X<sub>L</sub>. However the compounds are poorly soluble due to their high lipophilicity and attempts to overcome these issues have resulted in a loss of binding affinity.
3.3 Macrocycles with embedded carbohydrates as peptidomimetic scaffolds

Recently, carbohydrate structures have been introduced and validated as biologically relevant scaffolds on which pharmacophoric groups can be grafted\(^\text{31}\). The Murphy group has recently reported the synthesis of the rigid bivalent saccharide structure 141 (Scheme 33). Glycosidation of lactone 107 with the silylated nucleophile in the presence of SnCl\(_4\) gave the \(\alpha\)-carboxylic acid derivative 138 via a glycosidation-anomerisation reaction as described in previous chapter in high yield. Acid chloride formation followed by amide coupling gave dimeric compound 139 which underwent ring closing metathesis with the Grubbs 1\(^{\text{st}}\) generation catalyst in CH\(_2\)Cl\(_2\) to give the macrocyclic structure 140 in high yield as a mixture of cis and trans isomers. Deprotection then gave macrocycle 141 in 77% yield. The use of monosaccharides as scaffolds for the development of peptidomimetics such as somatostatin and \(\beta\)-turn mimetics has been well documented\(^\text{32}\). With this in mind macrocycle 141 was investigated for its potential in peptidomimetic design. Molecular modelling indicated that these structures do have a relationship to the \(\alpha\)-helix peptide backbone and could be used to display the i, i+1, i+2, i+5, i+6 and i+7 residues.

**Figure 21** Hamilton’s terphenyl peptidomimetics and the crystal structure of the BAK/Bcl-\(X_L\) interaction
Inspired by this work, we set about designing and synthesising a number of novel type III peptidomimetic derivatives which we felt could mimic the BH3 α-helical domain with the aim of triggering apoptosis. Type-III peptidomimetics are considered the ideal in peptidomimetic development. Even though they appear unrelated to the original peptide, they contain the necessary groups grafted onto a novel non-peptide scaffold which serve as geometrical mimetics (Figure 22). In this case the scaffold will be represented by a macrocycle with embedded monosaccharides. Each hydroxyl group can then act as a functional handle onto which the binding epitopes (amino acid side chains/or related motifs) can be grafted. The presence of a macrocycle will allow for the confinement of the amino acid epitopes into a strict spatial orientation which will mimic the display of an α-helix. This means that the three hot residues, i, i+4 and i+7, aligned along one side of the BH3 helix will be transferred onto one side of the novel scaffold.
The preliminary molecular modelling studies on compound 144 were carried out by Professor Murphy using Macromodel® software and the results are shown in Figure 23. The macrocyclic structure was compared to the stapled peptide Mcl-1 sensitizer synthesised recently by Walensky et al.\textsuperscript{26}. The important hydrophobic residues that facilitate binding of this peptide to Mcl-1 are Leu213, Val216, Gly217 and Val220 (i, i+3, i+4 and i+7). Placing these residues onto the macrocyclic scaffold generated compound 145. It is evident that the, i (1), i+4 (2) and i+7 (3) residues are presented along one side of the scaffold and perhaps the methylene group (4) could be considered a glycine mimic. The average distances (1-2, 2-3, 1-3) and bond angles (1-2-3) for 145 and the stapled helical mimic were calculated. Also shown in parenthesis are the range of interatomic distances and angles that were observed for the retained structures. Although not identical to the α-helix, the results are within a reasonable range to suggest that 145 could mimic the α-helix. Similar results were obtained when 145 was modelled with other anti-apoptotic members of the Bcl-2 family.

**Figure 22** Structures of the proposed macrocyclic peptidomimetics
RESULTS OF MOLECULAR MODELLING

A.) Bond angles and distances for stapled peptidomimetics by Walensky. B.) Bond angles and distances of 145 C.) 145 docked into the binding site of Mcl-1

3.4 Retrosynthetic analysis

With the molecular modeling data in hand and the previous success in the synthesis of macrocyclic carbohydrate structures, a novel approach to carbohydrate α-helical peptidomimetics was developed. In order to achieve this objective, a number of structural modifications needed to be made to the initial macrocyclic structures. The proposed retrosynthetic analysis is shown in Scheme 34. It was envisaged that replacement of the O-glycosidic linkage with a C-glycosidic linkage would provide enhanced stability of the
compounds towards the action of carbohydrate processing enzymes in the body and provide an extra degree of rigidity to the structures. Easier access to these macrocycles could be gained through the use of a copper catalyzed azide-alkyne cycloaddition reaction, this would insert a triazole into the backbone of the macrocycle, replacing the phenylenediamine backbone present in previous structures. A suitable orthogonal protecting strategy was needed in order to introduce the desired amino acid epitopes on the i-th and i+7 residues. It was suggested that the i+4 residue could be introduced through the use of an under-utilized double reductive amination-ring closing macrocyclisation sequence.

![Scheme 34 Proposed route to peptidomimetics starting from D-glucose](image)

**3.5 Synthesis of novel macrocyclic peptidomimetics with embedded carbohydrates**

**3.5.1 Synthesis of Alkyne monomers**

Working concurrently with Dr. Dilip Jarikote, the synthesis of these macrocycles starting from D-glucose was explored. Dr. Jarikote had already devised a route to the desired azide and alkyne monomers starting from commercially available methyl α-D-glucopyranoside. The route to the alkyne monomers is shown in Scheme 35. Methyl α-D-glucopyranoside was benzylated via treatment with benzyl bromide and sodium hydride to give the fully protected glucose derivative 150 in 83% yields. Allylation of 150 was achieved by treatment with allyltrimethylsilane and catalytic TMSOTf in acetonitrile gave the C-glycoside derivative 151 in 77% yield as a mixture of anomers (11:1 α:β). The use of acetonitrile as a solvent in these reactions is key to the high α ratios obtained, Schmidt and later Fraser-Reid have suggested that acetonitrile interacts with the oxocarbenium ion to form equatorial isonitrilium species which then undergo S_N2 type displacement with inversion to give the observed axial...
attack. One explanation provided for this solvent effect is that the β-isonitrilium salt is much more stable than the α-isonitrilium salt and does not revert to the axial isomer.

With the C-allylated derivatives in hand, attention then focused on selective manipulation of the C2 and C6 benzyloxy groups. Wong has shown that the 6-O-benzyl group of a glycoside can be regioselectively converted into an acetate group via treatment with TMSOTf and Ac₂O at low temperatures. A proposed mechanism for this reaction is shown in Scheme 36. It is believed that 151 reacts with Ac₂O in the presence of TMSOTf giving the cationic intermediate 155, AcOTMS and the triflate anion. 155 then reacts with AcOTMS to give the acetylated product 156 along with a second cationic intermediate. Reaction of this cationic intermediate with the triflate anion then regenerates the catalyst. A Zemplén deacetylation gave the primary alcohol derivative in high yield.
Scheme 36 Proposed mechanism for regioselective acetylation of 151

Alkylation of the primary alcohol with sodium hydride and propargyl alcohol gave propargyl derivative 152 in 90% yield. As alkylation at the C2 position was desired in order to mimic the amino acid side-chain, selective de-O-benzylolation via reductive ring opening using the conditions described by Nicotra was carried out on compound 152. Treatment of 152 with I₂ affords the iodonium ion 157 (Scheme 37). It is well known that the oxygen of a benzyl ether can react with an iodonium ion. In this case the only benzyloxy group close enough to interact with the iodonium ion is that of the C2 position. Attack of benzylic oxygen followed by loss of the subsequent benzyl cation affords cyclic iodoether 158. Zinc mediated reductive elimination restores the allylic double bond and reveals compound 153.
Alkylation of 153 with 1-bromo-3-methyl-butane afforded the benzylated monomer 154 in 79% yield.

**3.5.2 Synthesis of azide monomers**

Access to azide monomers was achieved through a similar reaction sequence (Scheme 38); regioselective deprotection of the C2 benzyloxy group was carried out on compound 151 as described for compound 153. Alkylation with iodopropane gave the benzylated derivative 160. Regioselective deprotection of the C6 benzyloxy group via treatment with Ac₂O and TMSOTf followed by Zemplén deacetylation gave a primary alcohol intermediate. Mesylation of the primary alcohol followed by S_N2 displacement with NaN₃ gave the azide derivative 161.
3.5.3 Coupling of monomers, macrocyclisation and deprotection

In 2001, K. Barry Sharpless coined the term “click chemistry” in reference to reactions which are wide in scope, high yielding, give minimal by-products, are stereospecific, easy to perform and can be carried out in volatile removable solvents. One such reaction which has become synonymous with click chemistry is the 1,3 dipolar cycloaddition reaction which occurs between an azide and a terminal alkyne. Initially introduced by Rolf Huisgen, the thermal 1,3-dipolar cycloaddition of azides and alkynes fulfilled many of the criteria for a click reaction, however, the classical reaction fell short due, in part, to the elevated temperatures required for the reaction to occur and the mixture of regioisomers obtained after purification. Development of this reaction into a fully-fledged click reaction was realised in 2002 when the groups of Mendel and Sharpless, working independently, introduced the copper catalysed variant. This reaction is facilitated via a different mechanism to the classical reaction and can even be conducted in aqueous solutions at room temperature. The copper catalysed variant also has the advantage of giving rise to one regioisomer, the 1,4 disubstituted triazole. More recently, a ruthenium based cycloaddition has also been introduced which gives the 1,5 disubstituted regioisomers. Sharpless has described this reaction as the “cream of the crop” in click chemistry and so it comes as no surprise then that there has been considerable interest in this area in recent years. The mechanism for the copper catalysed azide alkyne cycloaddition is presented in Scheme 39.
Scheme 39 Mechanism of copper catalysed azide-alkyne cycloaddition

To exploit the useful properties of this reaction, alkyne 154 and azide 161 were treated with CuI in MeCN-H₂O at reflux for 6 hours. After work-up and purification the triazole derivative 162 was obtained in 80% yield.

Scheme 40 Synthesis of triazole 162

With compound 162 in hand, attention turned into macrocyclisation and the introduction of the final amino acid epitope. It was suggested that the final amino acid epitope could be introduced by a double reductive amination reaction. Oxidative cleavage of dialkene 162 would give a bisaldehyde intermediate which could then undergo a double reductive amination/macrocyclisation when treated with an amine to deliver the desired macrocyclic peptidomimetics. At the conception of this route, a double reductive amination, ring closing
macrocyclisation reaction between a bisaldehyde and an amine was unprecedented in the literature, however, recently Madsen et al. have reported the use of such reactions in the synthesis of some novel macrocyclic compounds.

Scheme 41 Oxidative cleavage and reductive amination/macrocyclisation sequence

Compound 162 was treated with a catalytic amount of OsO₄, NaIO₄ and 2,6-lutidine in dioxane-H₂O giving the bisaldehyde intermediate 163. Reaction of crude 163 with NaCNBH₃ and isopentylamine in THF for 24 hours gave compound 164 according to mass spectrum analysis. The solvents were removed and purification via flash chromatography was attempted. Unfortunately the yield of the macrocyclic structures using this method was poor (~10-15%) and it proved very difficult to purify the compounds to a high degree. In addition to the poor yield, the NMR analysis of the compound was also complicated due to poorly resolved spectra. As a result, it became very difficult to characterise the macrocyclic structures. At this point, an extensive investigation into this reaction conditions was undertaken. NMR spectra of the crude bisaldehyde 163 showed well resolved clean spectra which were relatively free from impurities. Evidence pointed to a problem during the reductive amination reaction. In an effort to improve the reaction, alternative reducing agents were first examined. NaCNBH₃ has a number of undesirable properties including its toxicity and the potential for toxic by products such as NaCN and HCN to be generated during work-up, meaning it is not ideal for large scale processes. It can also, in some cases, contaminate products with cyanide. A cleaner and milder method for reductive amination has been reported by Abdel-Magid and co-workers, in which sodium triacetoxyborohydride was used as the reducing agent. Following their procedure, the bisaldehyde 163 was treated with isopentylamine and NaBH(OAc)₃ in dichloroethane (dichloromethane can also be used in this reaction, making removal of solvent easier). Interestingly, upon work-up with aqueous sodium bicarbonate, compound 164 was isolated in 75% yield. The NMR spectra obtained after purification were much cleaner and had a higher degree of resolution than those.
obtained when using NaCNBH$_3$. When this reaction was carried out without work up with sodium bicarbonate, a poor yield was observed and the spectra were also poorly resolved. This suggests that, perhaps, there is a chelation between the nitrogen of the macrocycle and the boron of the reducing agent which leads to complicated NMR spectra. Upon work-up, however, this interaction is broken down and 164 was revealed.

The mechanism for reductive amination is shown in Scheme 42. Reaction of an aldehyde (I) under acid catalysed conditions with an amine (II) generates an iminium ion (III). Loss of a proton then generates the imine (IV) which undergoes a reduction to give the secondary amine (V). In the case above this secondary amine then undergoes a second intramolecular reductive amination to close the ring.

**Scheme 42** Mechanism for reductive amination

With the macrocyclic structure in hand, attention was then focused on deprotection to yield the final polyhydroxylated macrocyclic structures. The most common method to remove benzyl ethers is through catalytic hydrogenation. Unfortunately, with this substrate, all attempts to remove the benzyl ethers under catalytic hydrogenation conditions failed to deliver the desired deprotected macrocycles. The hydrogenation was attempted with numerous palladium catalysts and solvents, these efforts are summarised in table 3. In each case, only unreacted starting material was recovered. Initially, it was thought that the nitrogen atoms present on the scaffolds could potentially poison the palladium catalyst. In an effort to overcome this potential problem, a number of reactions were performed in the presence of organic acids such as TFA and formic acid, however, these attempts also failed to deliver the desired product.
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**Table 3** Summary of catalytic hydrogenation conditions attempted for the deprotection of 164

While working on similar macrocyclic structures, Dr. Jarikote was able to successfully achieve a global debenzylation using the Birch reduction conditions$^{50}$. In this case the macrocycles were obtained in low yields. The low yield of these reactions coupled with the harsh conditions under which the Birch reduction is carried out led us to explore alternative ways to deprotect the macrocyclic derivatives.

![Scheme 43](image)

**Scheme 43** Lewis acid mediated deprotection of 164

Lewis acids have been reported in the literature for the deprotection of benzyl ethers. Recently Brar *et al.* reported the use of a reagent system consisting of BF$_3$.Et$_2$O, NaI and Ac$_2$O to facilitate a deprotective acetylation reaction$^{51}$. When 164 was subjected to these conditions, the presence of the fully acetylated derivative 165 was detected by both mass spectrum and TLC analysis after 24 hours. Gratifyingly, purification gave the macrocyclic...
derivative 165 in 57% yield. Although this reaction worked well on small scale, larger scale reactions were less successful, complex mixtures were obtained. Prolonged reaction times and the use of excesses of reagents failed to push the reaction to completion. The minor success of this reaction suggested that Lewis acids are particularly useful in the deprotection of these macrocyclic substrates. BF$_3$.Et$_2$O used in conjunction with ethane thiol has shown to be an excellent reagent system for the debenzylation of sugar derivatives. Therefore, treatment of 164 with this reagent system gave fully deprotected derivative in 85% crude yield. Acetylation of the crude product gave the macrocycle derivative 165 in 90% yield. It was believed that the acetylated macrocyclic derivatives would be more suitable for biological evaluation owing to the greater lipophilicity of the compounds being advantageous when crossing cell membranes.

Scheme 44 Approach to macrocyclic peptidomimetics from D-galactose

3.6 A second generation approach to macrocyclic peptidomimetics

3.6.1 Synthesis of 1-C-Allyl-1-deoxy-3,4-O-isopropylidene-α-D-galactopyranoside

Although the route from D-glucose did yield some positive results, it was decided that a more concise and flexible route should be developed. It was proposed that selective protecting group manipulations would be easier on macrocycles derived from D-galactose. A revised retrosynthesis is shown in Scheme 44. Selective introduction of an isopropylidene group onto compound 170 would give the diol 173 which could be further manipulated into the desired azide and alkyne derivatives.
The synthesis of 173 started from commercially available peracetylated D-galactose. Allylation of 171 was achieved through treatment with allyltrimethylsilane and 5 equivalents of BF$_3$.Et$_2$O at 80°C for 6 hours to give 172 as a 4:1 mixture of anomers$^{53}$. Zemplén deacetylation of 172 gave the compound 170 in 93% yield on a multi-gram scale. Selective introduction of the isopropylidene was then achieved under thermodynamic conditions using conditions set out by Ben and co-workers. Treatment of 170 with dimethoxypropane and p-toluenesulfonic acid in acetone gave 173 in 73% yield$^{53}$. With this key intermediate in hand, attention was then focused on conversion of this material into the desired alkyne and azide monomers.

**Scheme 45** Synthesis of key intermediate 173

**Scheme 46** Synthetic route to azide 175

### 3.6.2 New approach to azide monomers

Initially, attempts were made to introduce a mesylate selectively onto the primary alcohol of 173 in order to facilitate an S$_{N}$2 displacement reaction with NaN$_3$. 173 was treated with MsCl and Et$_3$N in CH$_2$Cl$_2$, this however, led to a mixture of the di-mesylate, mono-mesylate and unreacted starting material. As an alternative, the less reactive tosylate group was selectively introduced onto the primary alcohol to give 174 in excellent yield. Displacement of the tosylate was achieved through treatment with NaN$_3$ in DMF to give the azide derivative 175.
in good yield. This new route to azide monomers represents a significant improvement over the previous route in both the number of steps required and also overall yield of the sequence. The presence of the free secondary hydroxyl group at C2 also offers the possibility to develop a library of azide monomers for use in macrocycle synthesis.

**Scheme 47** Synthesis of azide monomers 176 and 177

In order to obtain a small library of potential Bcl-2 inhibitors for biological screening, alkylation of azide 175 was carried out with iodoethane and 1-bromo-3-methyl-butane to give the alkylated derivatives 176 and 177. O-alkylation was also attempted with 1-bromo-2-methyl-propane however it is believed that a competing elimination reaction prevents the alkylation from occurring on this particular substrate.

**Scheme 48** Synthesis of alkyne monomer 180
3.6.3 Revised approach to alkyne monomers

With the azide monomers now in hand, attention was turned to the synthesis of the alkyne monomer (Scheme 48). It was initially believed that a propargyl group could be introduced selectively on the free primary alcohol of 173. Treatment of compound 173 with propargyl bromide gave a mixture of both the di-alkylated and mono-alkylated products as well as a considerable amount of unreacted starting material. As an alternative approach, the primary alcohol was selectively protected as a silyl ether via treatment with TBSCI and imidazole to give 178 in 76% yield. Alkylation of the free secondary hydroxyl group at C2 was then achieved through treatment with 1-bromo-3-methyl-butane in DMF to give 179 in good yield. Removal of the silyl ether with TBAF followed by alkylation with propargyl bromide gave the alkyne derivative 180 in 59% yield over 2 steps. Again, this represents a marked improvement over the previous approach in terms of both the yield and the number of steps required to obtain the desired alkyne monomer.

![Scheme 49 Click reaction of 176 and 180](image)

3.6.4 Coupling reaction, macrocyclisation and final manipulations

Coupling of the azide 176 with alkyne 180 under copper catalysed conditions gave compounds 181 in good yield. An identical reaction sequence was conducted with azide 177 to generate the dialkene derivative 182. Gratifyingly, treatment of dialkene derivative 181 with the conditions outlined previously for the double reductive amination macrocyclisation reaction gave macrocycle 183 in 55% yield. Replacement of isopentylamine with 2-(2-Naphthyl)ethylamine hydrochloride and benzyl amine in the reductive amination reaction generated the macrocyclic derivatives 184 and 185 respectively. Treatment of compound 182
with isopentylamine and benzylamine generated two further macrocyclic derivatives 186 and 187.

Scheme 50 Macrocyclisation reaction and protected peptidomimetic compounds

Deprotection of macrocyclic derivatives 183-187 was facilitated via treatment with TFA-H<sub>2</sub>O 4:1 to give the fully deprotected macrocycles 188-192 in excellent yields. In order to improve
the bioavailability of the compounds, acetylation of 188-192 was achieved through treatment with pyridine-acetic anhydride to give acetylated macrocycles 193-197 in high yields.

Scheme 51 Summary of macrocyclic peptidomimetics synthesised

3.7 Preliminary biological results

Some preliminary cell viability testing has been carried out on a number of the proposed BH3 mimics by the cell stress and apoptosis research group of Professor Afshin Samali at the National University of Ireland, Galway. A series comprised of the isopropylidene protected compound 183, its polyhydroxylated analogue 188 and the acetylated derivative 193 were tested (alongside the two acetylated derivatives 196 and 197) and the results were compared to the known Bcl-2 inhibitor ABT 737. The candidate compounds were incubated with ML-1 acute myeloid leukaemia cells for 24 hours and the loss of cell viability was measured by an MTT assay. The results are shown in Figure 24.
Figure 24 Candidate compounds are active in ML-1 acute myeloid leukaemia cells. ML-1 cells were treated with the indicated concentrations of the candidate BH3-mimetic compounds or ABT737 for 24 h after which loss of cell viability was assessed with MTT assay.

The results show that, although not as potent as the highly optimised compound ABT737, there is activity in acute myeloid leukaemia cells. As expected the protected derivatives 183 and 193 were much more active than the related polyhydroxylated compound 188. It is believed that the increased hydrophobicity of these compounds allows them to cross the cell membrane easier. Interestingly the isopropylidene protected compound 183 showed a much higher degree of activity than the acetylated derivative indicating the choice of protecting group may be pivotal to obtaining good biological activity (although each compound only showed good activity at the 100 µM range). Compounds 196 and 197 showed little activity, perhaps indicating the ethyl side chain is not long enough to elicit suitable binding in the active site of the protein. Although these preliminary results show throw up some promising results, it must be noted that some solubility issues were identified with these compounds during these experiments and this must be addressed in the future.

3.8 Conclusion

The synthesis of six novel macrocyclic α-helical peptidomimetics has been successfully achieved. These compounds provide the first example of the use of bivalent carbohydrate macrocycles as rigid scaffolds for the synthesis of α-helical peptidomimetics. These scaffolds could find use in drug design and as mechanistic probes to further understand the basis for protein-protein interactions. The route provided is quick and flexible and allows for the generation of numerous macrocyclic derivatives in high yields for biological evaluation. With the promising biological data obtained for compounds such as 183, the future will allow for
the use of rational design and molecular modelling to identify potential lead compounds and targets for drug development. Improvements to the solubility of the compounds must be addressed and could lead to improvements in the biological results, this work is on going in the Murphy group and is being carried out by Dr. Jian Zhou and Mark Farrell.

3.9 References


Novel peptidomimetics: Synthesis of Bcl-2 family inhibitors

Chapter 3


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Chapter 4: Novel peptidomimetics: disrupting the p53/MDM2 interaction

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4.1 The p53/MDM2 interaction as a target for cancer therapy

p53 (“p” for protein and 53 for its molecular weight of 53 kDa) is a well known transcription factor and tumour suppressor protein\(^1\). In times of cellular stress (e.g. DNA damage, hypoxia), an increase in the expression of wild type p53 induces cell cycle arrest\(^2\) or apoptosis\(^3\) (Figure 25).

![Figure 25 The p53 mediated response](image)

In normal cells p53 is present in low levels and is kept under strict control by the murine double minute 2 protein (MDM2) (the human form of this protein is called HDM2, however for the purposes of this thesis the MDM2 terminology will be used). The regulation of p53 by MDM2 is represented in Figure 26. Binding of p53 to MDM2 blocks the DNA binding affinity of p53 and helps export it away from the nucleus where it can be targeted by proteases and broken down via the ubiquitin-dependent proteasome pathway\(^4,5\). Under times of cellular stress, p53 is phosphorylated at or near to its MDM2 binding site thereby decreasing its affinity for MDM2 and activating it as a transcription factor\(^1\). In many tumours and especially soft-tissue cancers, osteosarcomas and oesophageal carcinomas\(^6,7\), MDM2 expression is found to be highly amplified. This overexpression inhibits the ability of p53 to cause cell cycle arrest and/or apoptosis and leads to uncontrolled cell-proliferation\(^8\). Overexpression of MDM2 causes many modern cancer treatments to show a decrease in potency as the tumour cells are much less susceptible to the signals which trigger apoptosis through the p53 pathway. This in turn, leads to poor patient prognosis. Therefore, molecules
which disrupt the interaction of p53 and MDM2 have attracted interest from many research groups and have become a major goal in anticancer drug development⁹.

Figure 26 Regulation of p53 by MDM2

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4.2 Characteristics of the p53/MDM2 interaction

Interest in disruption of the p53/MDM2 interaction was sparked in 1996 when the co-crystal structure was published showing the exact protein-protein interface¹⁰. This structure showed that binding occurs in a hydrophobic cleft of MDM2 consisting of amino acid residues 18-102 and an α-helical portion of p53 consisting of residues 16-28. Three key ‘hot residues’ corresponding to Phe 19, Trp 23, and Leu 26 projected on one side of the p53 α-helical domain are ultimately responsible for this strong binding interaction¹ (Figure 27).
4.3 Synthesis of macrocyclic peptidomimetics as potential MDM2 inhibitors

Since the elucidation of the p53/MDM2 crystal structure, numerous synthetic and natural inhibitors which show a disruption of this interaction have appeared in the literature.
Successful disruption of the p53/MDM2 interaction has been achieved with small molecule inhibitors such as nutlins (198)\textsuperscript{11}, benzodiazepinediones (199)\textsuperscript{12} and spiro-oxindoles (200)\textsuperscript{13} (Figure 28) as well as oligomeric α-helical peptidomimetics\textsuperscript{14-16}. It is the latter type of inhibitor which is of interest to us. With the success of using carbohydrate based macrocycles in the synthesis of Bcl-2 family peptidomimetics as described in the previous chapter, it was decided to show the versatility of the novel macrocyclic scaffold in the synthesis of other α-helical peptidomimetics. The three ‘hot residues’, i, i+4 and i+7, that facilitate the p53/MDM2 interaction presented an ideal target for peptidomimetic development. The alkyne 180 was chosen to mimic the Leu 26 residue on the α-helix and was readily available through our previous work on Bcl-2 family inhibitors. With this monomer already in hand, attention was focused on synthesis of suitable azide monomers possessing aromatic groups on the C2 hydroxyl group. Following a similar reaction sequence to those described in section 3.6.2 the azide monomers 201 and 202 were synthesised via treatment of azide 175 with benzyl bromide and 2-(bromomethyl)naphthalene to give the aromatic derivatives in 77% and 68% yield respectively. The synthesis of a third aromatic derivative was also attempted via treatment of azide 175 with 2-(bromoethyl)benzene. This reaction failed to give any of the desired product, this is possibly due to a β-elimination process being more favoured under the basic reaction conditions.

**Scheme 52** Synthesis of aromatic monomers

Azides 201 and 202 were coupled with alkyne 180 under copper catalysed azide-alkyne cycloaddition conditions to give the dialkene derivatives 203 and 204 in high yields.
Scheme 53 Click reactions to give triazole derivatives 200 and 201

Oxidative cleavage of dialkenes 203 and 204 using conditions described previously followed by the reductive amination macrocyclisation sequence gave access to the potential MDM2 inhibitors 205-210 in good yields (Scheme 54).
Scheme 54 Reductive amination/macrocyclisation sequence in the synthesis of potential p53/MDM2 inhibitors and a summary of the macrocycles synthesised

Compounds 205-210 were deprotected using TFA-H₂O 4:1 to give the polyhydroxylated macrocycles 211-216 in high yields (Figure 29).
Figure 29 Polyhydroxylated p53/MDM2 inhibitors

In an effort to increase their cell permeability the compounds were acetylated with acetic anhydride in pyridine to give protected derivatives 217-221 in excellent yield (Figure 30).

Figure 30 Acetylated p53/MDM2 inhibitors
4.4 Synthesis of a polyhydroxylated macrocycle

The hydrophobicity of such macrocycles means that there is a potential for them to participate in non-specific interactions in biological systems. In order to discount this possibility, a macrocycle lacking the amino acid epitopes at i, i+4 and i+7 was synthesised. The route to this compound is presented in Scheme 55.

Scheme 55 Synthesis of polyhydroxylated macrocycle 224

Azide 201 and alkyne 152 were coupled under copper catalysed conditions to give the dialkene derivative 222 in 85% yield. As there was no desire to mimic the i+4 residue in this compound, macrocyclisation was achieved via the venerable ring closing metathesis reaction using the Hoveyda-Grubbs II catalyst in toluene at 90°C to give the protected macrocyclic derivative 223. The compound was isolated as the trans isomer exclusively, indicated by \( J \) values of 15 Hz between the two alkenyl protons. Chauvin’s mechanism for the ring closing metathesis reaction is shown in Figure 31. [2+2] cycloaddition between an alkene and a transition metal alkylidene forms a metallocyclobutane intermediate. This metallocyclobutane then undergoes cycloreversion to give the initial alkylidene or a new alkylidene species. A second metallocyclobutane intermediate is then formed between this new alkylidene complex and a second alkene derivative. Cycloreversion of this metallocyclobutane releases the desired coupled product and gives a new alkylidene complex to restart the cycle.
Finally, debenzylation with concomitant removal of the isopropylidene was facilitated via treatment with BF$_3$.Et$_2$O and ethane thiol 1:4\textsuperscript{18} to give the fully deprotected macrocycle derivative \textit{224} in 89% yield.

### 4.5 Conclusion

In an effort to show the utility of the novel macrocyclic scaffold designed and synthesised in the previous chapter, six novel p53 mimics have been developed in the hope of disrupting the p53/MDM2 interaction. These compounds are currently awaiting biological evaluation. From the results obtained, it can be seen how easily the macrocyclic scaffold we have developed can be adapted to target other important protein-protein interactions. It is hoped that these initial compounds can provide a valuable insight into the p53/MDM2 interaction which in turn will lead to optimisation of the epitopes on the scaffold to provide $\alpha$-helical mimetics with high potency. The synthesis of a macrocycle free of any amino acid epitopes, \textit{224}, has also been achieved. This compound will be used as a negative control in the biological studies which will be performed in order to discount the possibility of non-specific interactions taking place.
4.5 References


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5.1 Introduction to iminosugars

Iminosugars or iminocyclitols, make up a diverse family of alkaloids. These alkaloids are sugar analogues in which the endocyclic oxygen is replaced by a nitrogen atom. Recent years has seen a huge interest in both synthetic and naturally occurring iminosugars as biological tools and as potential therapeutic agents. This interest is due in part to the ability of iminosugars to interfere with carbohydrate processing enzymes. It is thought that these sugar analogues are capable of mimicking the transition state in the active site of pyranosidic or furanosidic glycosidase inhibitors thereby endowing them with potent biological activity. The inhibition of such glycosidase enzymes can cause wide and varied responses in vivo and can have considerable effects against numerous disease states including diabetes, bacterial infections, cancer and some sphingolipid storage diseases. A number of both natural and non-natural iminosugars have subsequently found their way into clinical use\(^1\)\(^2\). The three main families of naturally occurring iminosugars are: polyhydroxylated piperidines e.g. 225 & 226, indolizidines (fused piperidine and pyrrolidine) e.g. 227 & 228, and nortropanes e.g. 229 & 230 (Figure 32).

![Figure 32](image)

**Figure 32** Naturally occurring iminosugars: Piperidines (DMJ & DNJ), indolizidines (Castanospermine & 6-epi-Castanospermine) and nortropanes (Calystegines B\(_2\) & A\(_3\))
5.2 Use of iminosugars as scaffolds for peptidomimetic development

As stated in chapters 3 and 4, recent years has seen widespread interest in the use of sugars as scaffolds for peptidomimetic development. The pioneering work of Nicolaou and Hirschmann showed that β-D-glucopyranoside can be used as a suitable scaffold onto which pharmacophoric amino acid side chains can be grafted. This strategy was used to great effect in the synthesis of somatostatin mimics such as 232. Monosaccharides have a number of advantages which make them ideal for peptidomimetic development, they are chiral, have rigid conformations and contain multiple sites on which to graft pharmacophoric groups. Iminosugars however, are under-utilised as scaffolds, perhaps due to the difficulties often encountered during their synthesis. These sugar analogues would offer a number of advantages over other monosaccharide scaffolds as the presence of the nitrogen atom in the ring would allow for the introduction of pharmacophoric groups at a very useful site on the molecule and also for the possibility of incorporating a charged nitrogen atom which could led to increased hydrogen bonding interactions between ligand and receptor. Indeed, some success has already been achieved within the Murphy group through the synthesis of novel somatostatin mimics using both DNJ (233) and DMJ (234) as scaffolds (Figure 33).

![Figure 33](image-url) Somatostatin 230, β-D-glucopyranoside mimic 232 and representative Murphy group iminosugar derivatives 233, 234 and 235
5.3 Novel access to iminosugar derivatives

There is, however, still a great desire to develop new and more efficient approaches to the synthesis of both natural and novel iminosugar derivatives. This would allow for their widespread use in medicinal and bioorganic chemistry. Recently, intense efforts within in the Murphy group have focused on the development of novel methodology to access iminosugar derivatives.

The intramolecular Huisgen 1,3-dipolar cycloaddition reaction\(^8\) between an azide and an alkene has recently been utilised by Dr. Ying Zhou as part of a sequence of reactions in the synthesis of DNJ derivatives\(^9\).

As an extension of this methodology, it was envisaged that a novel polyhydroxylated iminosugar \(\text{236}\) (Figure 34) could be derived from the intramolecular Huisgen cycloaddition of the azide derivative \(\text{236}\). It was our belief that a thermally promoted 1,3-dipolar cycloaddition would give a triazoline derivative such as \(\text{237}\). As with the DNJ synthesis it was anticipated that minimization of allylic strain would lead to the formation of a single triazoline stereoisomer \(\text{237}\). Extrusion of molecular nitrogen was then expected to give aziridine derivative \(\text{238}\)\(^{10}\). It was then anticipated that \(\text{238}\) would then undergo nucleophilic ring opening to give novel polyhydroxylated iminosugar derivatives. It was proposed that the synthesis of \(\text{236}\) could be achieved from commercially available L-sorbose.
5.4 Synthesis of a novel bicyclic iminosugar derivative

Treatment of L-sorbose 241 (Scheme 56) with I$_2$ in acetone gave di-isopropylidene-L-sorbose 242 in high yield and multi-gram quantities$^{11}$. Oxidation of the remaining free primary alcohol under Swern conditions followed by Wittig olefination gave alkene derivative 243$^{12}$. Regioselective ring opening of the more labile isopropylidene group was achieved through treatment with 60% acetic acid at 60°C to give the diol 244 in good yield. In order to facilitate the introduction of the azide, the diol 244 was converted into a cyclic sulfite via treatment with thionyl chloride and pyridine. The crude cyclic sulfite was treated with 5 equivalents of sodium azide at 120°C in the hopes of obtaining 236. However upon isolation, compound 245 was obtained as a white crystalline solid and no other by-products were detected. The formation of 245 can be explained by a cascade sequence which can occur under the reaction conditions. The proposed mechanism for this transformation is shown in Scheme 57. The structure of 245 was confirmed through x-ray analysis (Figure 35)$^{13}$. 

Scheme 56 Synthesis of novel iminosugar derivative from L-sorbose
The reaction was repeated using differing numbers of equivalents of sodium azide in the hope of trapping the aziridine intermediate however these attempts all failed to deliver the desired compound 238. Removal of the remaining isopropylidene from 245 was facilitated with HCl in methanol to give the bicyclic iminocyclitol derivative 246 in 60% yield.

**Scheme 57** Proposed mechanism for the formation of 245

### 5.5 Synthesis of a novel tricyclic framework

In an effort to show the potential utility of compound 245 as a starting material for the synthesis of more complex molecules, alkylation of the secondary amino group with ethyl bromoacetate was carried out in the presence of catalytic tetrabutylammonium iodide at reflux to give the protected sugar amino acid derivative in 61% yield (Scheme 58). Compound 248 could be considered a protected sugar amino acid$^{14}$. Catalytic hydrogenation of 248 led to spontaneous lactam formation to give the novel tricyclic derivative 249 in high...
Synthesis of a novel bicyclic iminocyclitol

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yield. Deprotection with methanolic HCl as before gave polyhydroxylated tricycle 250 in 70% yield.

Scheme 58 conversion of 245 in a novel tricyclic framework

5.6 Conclusion

A novel iminocyclitol containing an ether bridge has been synthesised in a stereoselective and efficient manner starting from L-sorbose. The route involves a one pot nucleophilic substitution, Huisgen cycloaddition, triazoline decomposition, aziridine formation and aziridine ring opening by an azide anion. This provides a molecule which could be of great interest to medicinal chemists. 8-oxa-3-azabicyclo[3.2.1]-octanes such as 246 are of medicinal interest\textsuperscript{15,16}. Cope has reported the achiral 8-oxa-3-azabicyclo[3.2.1]-octane as an analgesic and anti-inflammatory agent which shows efficacy \textit{in vivo}\textsuperscript{17}. The protected derivative 245 can be considered a conformationally constrained morpholine type structure\textsuperscript{18} and possess a similar structural motif to nortropane type iminosugars which have shown to be potent glycosidase inhibitors\textsuperscript{19}. The constrained structure of this amine means that this molecule could also be of use as an organocatalyst although this has not been examined. The protected derivative also gives the opportunity for further development as a scaffold for peptidomimetic research, both the secondary alcohol and the secondary amine can be selectively alkylated, the presence of the azido group means this compound could be used in ‘click reactions’ or alternatively reduction to the primary amino group and alkylation would give access to further derivatives. Also shown is the utility of this compound as a scaffold for the synthesis of the tricyclic lactam 250.
5.7 References


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# Chapter 6: Experimental Data

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6.1 General Experimental Conditions

Optical rotations were determined at the sodium D line at 20°C. NMR spectra were recorded with 600, 500 and 400 MHz Varian spectrometers. Chemical shifts are reported relative to internal Me$_4$Si in CDCl$_3$ (δ 0.0), HOD for D$_2$O (δ 4.84) or CD$_2$HOD (δ 3.31) for $^1$H and Me$_4$Si in CDCl$_3$ (δ 0.0) or CDCl$_3$ (δ 77.0) or CD$_3$OD (δ 49.05) for $^{13}$C. $^1$H NMR signals were assigned with the aid of COSY. $^{13}$C NMR signals were assigned with the aid of DEPT, gHSQCAD and/or gHMBCAD. Coupling constants are reported in hertz. The IR spectra were recorded using thin film on a NaCl plate or with ATR attachment. Low and high resolution mass spectra were in positive and/or negative mode as indicated in each case. Thin layer chromatography (TLC) was performed on aluminium sheets precoated with silica gel and spots visualized by UV and charring with H$_2$SO$_4$-EtOH (1:20), or cerium molybdate. Flash chromatography was carried out with silica gel 60 (0.040-0.630 mm) and using a stepwise solvent polarity gradient correlated with TLC mobility. CH$_2$Cl$_2$, MeOH, and THF reaction solvents were used as obtained from a Pure Solv™ Solvent Purification System. Anhydrous DMF, pyridine, and toluene were used as purchased from Sigma-Aldrich. Chromatography solvents, petroleum ether, cyclohexane and ethyl acetate were used as obtained from suppliers (Sigma-Aldrich).
6.2 Chapter 2-Experimental

2-Amino-N-((1R,2R)-1-hydroxy-1-phenylpropan-2-yl)-N-methylacetamide (Pseudoephedrine glycinamide)\(^1\) (59). A flame dried round bottom flask stored under argon was charged with anhydrous LiCl (5.13 g, 121 mmol), \((R,R)-(-)-\text{pseudoephedrine}\) (10 g, 61 mmol) and THF (90 mL). The resulting slurry was stirred at 0°C for 15 min. Solid lithium methoxide (1.1 g, 30 mmol) was then added to the slurry and the reaction mixture stirred for a further 10 mins. A solution of glycine methyl ester (6.7 g, 76 mmol) in THF (20 mL) was then added dropwise over 1 h and the reaction mixture was stirred at 0°C for 8 h. The reaction was terminated via the addition of H\(_2\)O (100 mL) and the bulk of the THF was removed under reduced pressure. The aqueous solution was then diluted with H\(_2\)O and extracted into CH\(_2\)Cl\(_2\). The combined organic extracts were then dried with K\(_2\)CO\(_3\) and the solvents were removed under reduced pressure. The crude product was then recrystallized from THF-H\(_2\)O to give \((R,R)-(-)-\text{pseudoephedrine glycinamide monohydrate}\) as a white crystalline solid (10.3 g, 72%). The monohydrate was then dissolved in CH\(_2\)Cl\(_2\) and stirred for 1 h. To this was added anhydrous K\(_2\)CO\(_3\) and the reaction stirred until translucent. The mixture was then filtered and concentrated under reduced pressure. The oily residue was recrystallised from hot toluene to give anhydrous 59 as a white solid. Analytical data were in good agreement with those reported in the literature; [\(\alpha\)]\(_D\) \(-103.1^\circ\) (c 1.1, MeOH); IR (film) cm\(^{-1}\): 3360, 2989, 1630, 1486, 1454, 1312, 1126, 1040, 926; \(^1\)H NMR (500 MHz, 1:1 mixture of rotamers, CDCl\(_3\)) \(\delta\) 7.37-7.26 (5H, m, Ar-H), 4.64-4.55 (1.5H, m), 3.78 (0.5H, m), 3.67 (0.5H, d, \(J\) 15.4), 3.30 (1.5H, m), 2.91 (1.5H, s), 2.76 (1.5H, s), 0.99 (1.5H, d, \(J\) 6.6), 0.93 (1.5H, d, 6.6); \(^{13}\)C NMR (125 MHz, CDCl\(_3\)) \(\delta\) 174.0, 173.5, 142.1, 141.8, 128.5, 128.4, 128.1, 126.7, 126.6, 75.6, 74.9, 57.4, 57.2, 43.5, 43.2, 26.9, 15.3, 14.1; ESI-HRMS calcd for C\(_{12}\)H\(_{19}\)N\(_2\)O\(_2\) 223.1447, found m/z 223.1440 [M+H]\(^+\).
(S)-2-Amino-N-((1R,2R)-1-hydroxy-1-phenylpropan-2-yl)-N-methylpent-4-enamide\(^1\) (83). A flame dried round bottom flask stored under argon was charged with THF (30 mL) and diisopropylamine (5 mL, 36 mmol). The solution was cooled to 0°C and deoxygenated under high vacuum. To this was added n-BuLi (12 mL of 2.5 M in hexanes) dropwise over 20 min. After a further 20 min the resulting LDA solution was transferred via cannula to a separate flask containing a solution of anhydrous lithium chloride (4.6 g, 107 mmol) and 59 (4 g, 18 mmol) in THF (40 mL) at 0°C. The rate of addition was carefully monitored so as the internal temperature of the mixture did not rise above 5°C. The yellow enolate solution was stirred for a further 30 min and allyl bromide (1.7 mL, 20 mmol) was added dropwise while keeping the temperature below 5°C. After stirring for 1 h the reaction was terminated via the addition of H\(_2\)O. The biphasic mixture was then extracted into EtOAc. Phases were separated and the organic phase was extracted into 3M HCl solution. The aqueous layers were combined and cooled to 0°C. The solution was basified via the addition of cold 50% NaOH solution. The basic solution was then extracted into CH\(_2\)Cl\(_2\). The combined organic layers were then dried over K\(_2\)CO\(_3\) and the solvents were concentrated under reduced pressure. The crude residue was recrystallized from hot toluene to give (R,R)-(-)-Pseudoephedrine-L-allylglycinamide (2.7 g, 58%) as a white solid. NMR data \(^1H\) and \(^{13}C\) was in agreement with reported literature data; \([\alpha]_D\) -79.0° (c 1.0 MeOH); IR (film) cm\(^{-1}\): 3356, 3071, 2978, 1631, 1451, 1453, 1109, 1049, 703; \(^1H\) NMR (500 MHz, 3:1 rotamer ratio, CDCl\(_3\)) major rotamer δ 7.38-7.23 (5H, m), 5.85-5.64 (1H, m, CH\(_2\)CH=CH\(_2\)), 5.14-5.07 (2H, m, CH\(_2\)CH=CH\(_2\)), 4.59-4.45 (2H, m), 3.65 (1H, dd, J 7.5, 5.3), 2.87 (3H, s, NCH\(_3\)), 2.23 (1H, m, CHHCH=CH\(_2\)), 2.13 (1H, m, CHHCH=CH\(_2\)), 1.03 (3H, d, J 6.4, CH\(_3\)); \(^{13}C\) NMR (125 MHz, CDCl\(_3\)) δ 176.1 (C=O), 142.1 (CH\(_2\)CH=CH\(_2\)), 133.7, 128.2, 127.6, 126.5, 118.1 (CH\(_2\)CH=CH\(_2\)), 75.5 (CHOH), 57.6 (CHN), 51.2 (CH), 39.6 (CH\(_2\)CH=CH\(_2\)), 31.4 (CH\(_3\)N), 14.4 (CH\(_3\)); ESI-HRMS calcd for C\(_{15}\)H\(_{23}\)N\(_2\)O\(_2\) 263.1759, found m/z 263.1749 [M+H]\(^+\)
Trimethyl acetyl chloride (10.5 mL, 85.7 mmol) was added dropwise to a stirring solution of \(N\)-Boc-glycine (15 g, 85.7 mmol) and triethylamine (13 mL, 94 mmol) in \(\text{CH}_2\text{Cl}_2\) (300 mL) at 0°C. After 40 min a second portion of triethylamine (13 mL, 94 mmol) was added, followed by the rapid addition of solid \((R,R)-(\cdot)-\text{pseudoephedrine}\) (14 g, 85.7 mmol). The reaction mixture was stirred for 1 h, diluted with 1 M HCl solution, brine and EtOAc. Phases were separated and the aqueous layer was extracted into EtOAc. The combined organic phases were washed with satd \(\text{K}_2\text{CO}_3\), dried over MgSO\(_4\) and the solvents were concentrated under reduced pressure. The crude residue was purified via flash chromatography (EtOAc-petroleum ether 1:1) to give 82 (23.8 g, 86%) as a viscous oil; \(^1\text{H}\) NMR (500 MHz, 1:1 rotamer ratio, CDCl\(_3\)) \(\delta\) 7.39 – 7.29 (5H, m, Ar-H), 5.62 (1H, m, RCHOH), 4.66 – 4.57 (1H, m), 4.16 (1H, d, J 16.4), 4.00 (1H, dd, J 16.5, 4.6), 3.88 (1H, t, J 11.3, CH\(\text{HNH}_2\)), 3.86 – 3.78 (1H, m, CH\(\text{HNH}_2\)), 2.92 (1H, d, J 11.1), 2.81 (2H, s), 1.45 (9H, s, Boc), 1.02 (2H, d, J 6.6), 0.95 (1H, d, J 6.6); \(^{13}\text{C}\) NMR (125 MHz, CDCl\(_3\)) \(\delta\) 170.1, 169.6*, 156.0, 155.9*, 141.7, 141.5*, 128.7, 128.4*, 128.4, 127.9, 126.8, 126.5*, 79.6, 79.5*, 75.8, 75.1, 57.5, 57.2*, 42.8, 42.5*, 28.3, 28.3*, 27.12, 15.1, 14.2*; ESI-HRMS calced for \(\text{C}_{17}\text{H}_{27}\text{N}_2\text{O}_4\) 323.1971, found \(m/z\) 323.1966 [M+H]\(^+\)

\(N\)-Boc-(S)-2-amino-\(N\)-((1\(R,2R\))-1-hydroxy-1-phenylpropan-2-yl)-\(N\)-methylpent-4-enamide (84). n-BuLi (28.8 mL of 2.5 M in hexanes) was added dropwise to a stirred suspension of anhydrous lithium chloride (5.9 g, 139.6 mmol) and diisopropylamine (10.5 mL, 74.4 mmol) in THF (140 mL) at -78 °C. After stirring for 10 min a solution of 82 (7.5 g, 23.3 mmol) in THF (130 mL) was added via cannula. The resulting yellow solution was stirred for 20 min at -78 °C and then warmed to 0 °C. After 20 min at 0 °C, allyl bromide was added dropwise. After 2 h the reaction was terminated by the addition of 1 M aqueous HCl.
The phases were separated and the aqueous phase was extracted with EtOAc. The combined organic phases were then washed with satd NaHCO₃, dried over MgSO₄ and the solvent was removed under reduced pressure. Flash chromatography of the residue (petroleum ether-EtOAc, 1:1) gave 84 (5.9 g, 70%) as a yellow oil; [α]D -26.1° (c 0.79, CHCl₃); ¹H NMR (500 MHz, 2:1 ratio of rotamers, the asterisk denotes signals of the minor rotamer, CDCl₃); δ 7.35-7.24 (5H, m, Ar-H), 5.76* (1H, ddt, J CH=CH₂), 5.68 (1H, ddt, J , CH=CH₂), 5.55 (1H, d, J 8.1.), 5.45 (1H, d, J 9.1), 5.08 (2H, m), 4.68* (1H, m), 4.57 (3H, m), 4.17* (1H, m), 2.89 (3H, s), 2.54* (1H, m), 2.37* (1H, m), 2.25 (1H, m), 1.40 (9H, s, t-Bu), 0.98 (3H, d, J 6.5), 0.90* (3H, d, J 6.6); ¹³C NMR (125 MHz, CDCl₃) δ 173.3, 172.4*, 155.3, 155.1*, 141.7, 141.4*, 133.7*, 132.6, 128.5, 128.1*, 127.5, 126.5*, 126.5, 118.3, 117.6*, 79.5, 79.2*, 75.3, 75.1*, 57.8, 50.4, 49.7*, 37.1* 36.9, 28.1, 26.7, 15.3*, 14.1; IR (film) cm⁻¹: 3411(br), 2978, 2933, 1697, 1625, 1493, 1453, 1366, 1249, 1165; ESI-HRMS calcd for C₂₀H₃₁N₂O₄ 363.2283, found m/z 363.2285 [M+H]+

1-Iodoheptadecane (91). Iodoheptadecanol (25 g, 97 mmol) and triethylamine (40.7 mL, 292 mmol) were dissolved in CH₂Cl₂ (200 mL) and cooled to 0°C. To this was added methanesulfonyl chloride (9 mL, 116 mmol) dropwise. The solution was allowed warm to room temperature and stirred for 4 h. The reaction was diluted with H₂O and washed with 1M HCl solution. The organic phase was washed with brine, dried over MgSO₄ and the solvents were concentrated under reduced pressure. The crude mesylate was dissolved in acetone (250 mL) and NaI (72 g, 485 mmol) was added and the reaction was heated at reflux overnight. H₂O and EtOAc were added and the phases were separated. The organic phase was washed with water, brine dried over MgSO₄ and concentrated under reduced pressure. The crude product was purified via flash chromatography (petroleum ether-EtOAc 19:1) to give iodide 91 (28.4 g, 80%) as a white solid; ¹H NMR (500 MHz, CDCl₃) δ 3.17 (2H, t, J 7.5, CH₂), 1.82-1.77 (2H, m, CH₂), 1.39 (2H, dd, J 14.9, 7.0, CH₂), 1.24 (26H, s, each alkyl CH₂), 0.88 (3 H, t, J 6.9, CH₃); ¹³C NMR (125 MHz, CDCl₃) δ 31.9, 30.9, 29.6, 29.6, 29.6, 29.5, 29.3, 29.0, 28.7, 25.5, 24.5, 22.6 (each CH₂), 14.1 (CH₃)
**Experimental data**

Chapter 6

**(S)-t-Butyl 5-oxodocos-1-en-4-ylcarbamate (88).** To a stirred solution of 91 (5.4 g, 14.86 mmol) in pentane-Et₂O (50 mL, 3:2) at -78 °C was added t-BuLi (18.2 mL, 1.6M in hexanes) dropwise. The solution was stirred for 10 min at -78°C, warmed to room temperature and stirred for 1 h. The resultant suspension was then added via cannula to a stirred solution of 84 (4.4 g, 11.4 mmol) in THF (25 mL) at -78 °C. After a further 10 min the reaction flask was transferred to an ice-bath and stirred for 2 h. The reaction mixture was then poured slowly onto a mixture of crushed ice and satd NH₄Cl and extracted with EtOAc. The combined organic layers were dried (MgSO₄) and the solvent was removed under reduced pressure. Flash chromatography of the residue (petroleum ether-EtOAc 15:1) gave 88 (3.7 g, 75%) as a white solid. R_f 0.75 (petroleum ether-EtOAc, 9:1); [α]D +31.5° (c 1.4, CHCl₃); IR (film) cm⁻¹: 2925, 2854, 1706, 1493, 1367, 1264, 1167; ¹H NMR (500 MHz, CDCl₃) δ 5.66 (1H, ddt, J 17.1, 9.9, 7.1, H-2), 5.22 (1H, d, J 6.7, NH), 5.11 (2H, d, J 13.5, H-1), 4.36 (1H, dd, J 12.1, 5.9, H-4), 2.64 – 2.54 (1H, m, H-3a), 2.48 (2H, dd, J 9.2, 5.6, H-6), 2.43 – 2.33 (1H, m, H-3b), 1.63 – 1.53 (2H, m, H-7), 1.44 (9H, s, t-Bu), 1.26 (28H, s, each CH₂), 0.88 (3H, t, J 6.8, CH₃). NMR (125 MHz, CDCl₃) δ 208.8 (C=O), 155.3 (C=O), 132.4 (C-2), 118.9 (C-1), 79.6 (tBu), 58.6 (C-4), 40.0 (C-6), 36.0 (C-3), 31.9, 29.7 (4s) 29.6, 29.5 (each CH₂), 29.4 (t-Bu), 29.2, 28.3, 23.4, 22.7 (each CH₂), 14.1 (CH₃); ESI-HRMS calcd for C₂₇H₄₁NO₃Na 460.3767, found m/z 460.3759 [M+Na]⁺

**t-Butyl (4S,5R)-5-hydroxydocos-1-en-4-ylcarbamate (92).** The ketone 88 (1.94 g, 4.4 mmol) was taken up in dry EtOH (35 mL) and cooled to -78 °C. LiAl(O-t-Bu)₃H (2.88 g, 26.6 mmol) was added portion-wise over 1 h. After stirring at -78 °C overnight the reaction was then diluted with CH₂Cl₂ and treated with a 10% citric acid (60 mL). The mixture was stirred at room temperature for 2 h and extracted into CH₂Cl₂. The combined organic layers were washed with H₂O, brine, dried over MgSO₄ and the solvent removed under reduced pressure. Flash chromatography of the residue (petroleum ether-EtOAc 6:1) gave the alcohol
92 (1.65 g, 85%) as a white solid; Rf 0.25 (petroleum ether-EtOAc 9:1) [α]D +7.6° (c 1.2, CHCl3); IR (film) cm⁻¹: 3345, 2917, 2849, 1684, 1527, 1262, 1172, 1014; ¹H NMR (500MHz, CDCl3) δ 5.82 (1H, ddt, J 17.1, 9.9, 7.1, H-2), 5.14 – 5.06 (2H, m, H-1), 4.68 (1H, br s, OH), 3.65 (2 H, overlapping signals, H-4 & H-5), 2.32 (1H, m, H-3a), 2.23 – 2.13 (1H, m, H-3b), 1.51 (1H, s), 1.44 (12H, overlapping signals, CH₂ & t-Bu), 1.26 (30H, s, each alkyl CH₂), 0.88 (3H, t, J 6.8, CH₃); NMR (125MHz, CDCl3): δ 156.2 (C=O), 134.9 (CH=CH₂), 117.5 (CH=CH₂), 79.4, 74.1 (-CH-), 54.6 (-CH-), 34.1, (CH₂-CH=CH₂), 33.3, 32.0, 29.7 (3s), 29.6 (2s), 29.4 (each CH₂), 28.4 (t-Bu), 26.1, 22.7 (each CH₂), 14.1 (CH₃); ESI-HRMS calced for C₂₇H₅₃NO₃Na 462.3923, found m/z 462.3924 [M+Na]⁺

(4S,5R)-t-Butyl 4-allyl-5-heptadecyl-2,2-dimethylxazolidine-3-carboxylate (100). The alcohol 92 (1.16 g, 2.64 mmol) was taken up in toluene (4 mL) and 2,2-dimethoxypropane (0.81 mL, 6.6 mmol) was added along with pyridinium p-toluenesulphonate (7 mg). The resulting suspension was stirred at 85 °C for 6 h. Upon cooling the reaction was neutralised with solid NaHCO₃ (10 mg) and the solvent was removed under reduced pressure. Flash chromatography of the residue (petroleum ether-EtOAc 9:1) gave 100 (1.18 g, 88%) as a colourless oil; [α]D +1.8° (c 0.7, CHCl₃); IR (film) cm⁻¹: 2923, 2853, 1698, 1384, 1364, 1256, 1178, 1075; ¹H NMR (500MHz, CDCl3) δ 5.78 (1H, ddt, J 16.9, 10.1, 6.8, H-2), 4.99 (2H, m, H-1), 3.97 – 3.91 (1H, m), 3.77 (1H, dd, J 11.1, 5.7), 2.40 – 2.28 (1H, m, H-3a), 2.17 (1H, ddd, J 20.2, 13.9, 6.5, H-3b), 1.53 (2H, s), 1.48 (2H, s), 1.47 (2H, s), 1.43 (2H, d, J 3.9), 1.42 (9H, s, t-Bu), 1.38 (2H, s), 1.23 (30H, s, each CH₂), 0.83 (3H, t, J 6.9). ; ¹³C NMR (125MHz, CDCl3) δ 152.2*, 151.7 (C=O), 135.9 (CH=CH₂), 116.7 (CH=CH₂), 116.4*, 92.6, 92.1*, 79.7*, 79.3 (t-Bu), 77.1 (CHOOCR₂), 76.8*, 59.0 (CHN), 58.8*, 34.9, 34.4* (CH₂CH=CH₂), 32.0, 29.8, 29.7 (2s), 29.6, 29.5, 29.4, 29.1 (2s) (each CH₂), 28.5, 28.4 (2 s), 27.8, 27.0, 26.5, 26.4, 24.9, 23.6 (each CH₃), 22.8 (CH₂), 14.2 (CH₃); ESI-HRMS calcd for C₃₀H₅₇NO₃Na 502.4236, found m/z 502.4231 [M+Na]⁺
(4S,5R)-\textit{t}-Butyl-5-heptadecyl-2,2-dimethyl-4-(prop-1-enyl)oxazolidine-3-carboxylate (102). Oxazolidine 100 (0.49 g, 1.0 mmol) was taken up in methanol (14 mL) and the Grubbs-II catalyst, (9 mg, 0.1 mmol)) was added. The reaction mixture was placed in a pre-heated oil bath at 60 °C for 12 h. After cooling the solvent was removed under reduced pressure and the residue purified by flash chromatography (petroleum ether-EtOAc 9:1) to give compound 102 (467 mg, 95 %) as a colourless oil; IR (film) cm$^{-1}$: 2922, 2853, 1696, 1384, 1375, 1364, 1251, 1176, 1075; $^1$H NMR (500MHz, CDCl$_3$ 1:1 E,Z mixture) δ 5.56 (1H, ddd, J 17.5, 12.0, 6.9), 5.46 (1H, m), 5.24 (2H, dd, J 15.1, 8.8), 4.15 (1H, d, J 5.9), 3.98 (1H, dd, J 8.4, 5.3), 3.88 (2H, dd, J 11.5, 5.8), 1.63 (3H, d, J 6.5), 1.54 (2H, s), 1.49 (2H, s), 1.43 (5H, s), 1.40 (7H, s), 1.18 (51H, s), 0.80 (6H, t, J 6.8); $^{13}$C NMR (125MHz, CDCl$_3$): δ 151.8, 129.0*, 128.7, 126.8, 126.34*, 92.7, 92.2*, 79.7*, 78.9, 76.9, 76.7*, 62.6, 62.3*, 56.6, 32.0, 29.7 (3s), 29.6, 29.5, 29.4, 28.45, 27.3, 25.7, 23.8, 22.7, 17.8*, 17.6, 14.1.; ESI-HRMS calcd for C$_{30}$H$_{57}$NO$_3$Na 502.4236, found m/z 502.4234[M+Na]$^+$

(4S,5R)-\textit{t}-Butyl 5-heptadecyl-4-(hydroxymethyl)-2,2-dimethyloxazolidine-3-carboxylate (57). To a stirred solution of alkene 102 (0.3 g, 0.63 mmol) in 10:1 acetone-water (6 mL) were added 2,6-lutidine (0.15 mL, 1.25 mmol), 4-methylmorpholine-N-oxide (0.11 g, 0.9 mmol) and a catalytic amount of osmium tetroxide (0.16 mL, 2 mol %,). The solution was stirred vigorously for 2 days at room temperature. Then PhI(OAc)$_2$ was added and the reaction mixture was stirred for a further 3 h. The reaction was quenched with aqueous sodium thiosulfate and extracted with EtOAc. The combined organic extracts were washed with water, brine, dried over MgSO$_4$ and the solvent was removed under reduced pressure. The residue was taken up in THF (8 mL) and cooled to 0 °C. Sodium borohydride was added and the reaction mixture allowed to attain room temperature over 1 h. Brine was then added and the mixture was extracted with EtOAc. The combined organic phases were dried over
MgSO₄ and the solvent was removed under reduced pressure. Flash chromatography of the residue (petroleum ether-EtOAc 9:1, Rf 0.20) gave the title compound (189 mg, 64%) as a colourless oil; [α]D +3.6° (c 0.2, CHCl₃); IR (film) cm⁻¹: 3514 (br), 2922, 2853, 1698, 1466, 1392, 1366, 1256, 1176, 1049; ¹H NMR (500MHz, CDCl₃) δ 4.03 (2H, overlapping signals, H-2 & H-3), 3.80 (1H, m, H-1a), 3.63 (1H, m, H-1b), 1.56 (4H, s, CH₃ & CHH), 1.50 (4H, m, CH₃ CHH), 1.48 (9H, s, t-Bu), 1.25 (30H, s, each CH₂), 0.87 (3H, t, J 6.9); ¹³C NMR (125MHz, CDCl₃) δ 154.8 (C=O), 92.8, 81.3, 75.8 (CH), 63.5 (CH₂), 61.35 (CH), 32.1, 29.8 (3 s), 29.7, 29.6, 29.5, 29.1, 28.5 (each CH₂), 28.0, 26.6, 24.7, 22.8, 14.3 (each CH₃); ESI-HRMS calcd for C₂₈H₅₆N₂O₄ 470.4209, found m/z 470.4220 [M+H]+.

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\text{(4R,5R)-} \text{tert-butyl-4-(bromomethyl)-5-heptadecyl-2,2-dimethyloxazolidine-3-carboxylate (55).} \]

(4S,5R)-t-Butyl 5-heptadecyl-4-(hydroxymethyl)-2,2-dimethyloxazolidine-3-carboxylate (529 mg, 1.1 mmol) was dissolved in CH₂Cl₂ (40 mL). To this was added carbon tetrabromide (710 mg, 2.15 mmol), triphenylphosphine (4.30 mmol), and triethylamine (300µl, 2.15 mL). The solution was stirred for 5 h. Silica gel was added and solvents were removed under reduced pressure. Flash chromatography of the residue (petroleum ether-EtOAc 9:1) gave bromide 55 (511mg, 85%) as a colourless oil. [α]D +16.8° (c 1.1, CHCl₃); ¹H NMR (500MHz, CDCl₃ ; the asterisk denotes the minor rotamer.) δ 4.15* (1H, s), 4.05 (2H, d, J 8.0, H-2 & H-3 overlapping), 3.49* (1H, m), 3.42 (1H, dd, J 16.0, 8.8, H-1a), 3.31 (1H, d, J 10.2, H-1b), 1.76 (1H, m), 1.64 (1H, m) (CH₂), 1.56 (3H, s, CH₃), 1.49 (12H, overlapping signals, t-Bu & CH₃), 1.25 (30H, s), 0.87 (3H, t, J 6.7, CH₃); ¹³C NMR (125MHz, CDCl₃): δ 152.21*, 151.40 (C=O), 92.7, 92.35*, 80.8*, 80.4, 76.6, 76.3* (CH), 60.5, 60.2* (CH), 32.1, 29.8 (2s), 29.7 (2s), 29.65, 29.5, 28.6 (each CH₂), 28.5, 27.9, 27.3, 27.2, 26.9, 24.6 23.35, 22.8, 14.3 (CH₃)
**1,2,3,4-Tetra-\(\beta\)-D-glucopyranosiduronic acid, methyl ester\(^2\)** (113).

Glucuronolactone (10 g, 56.8 mmol) was suspended in dry MeOH (160 mL). Dimethylethylamine (0.1 mL) was added and the reaction was stirred for 16 h until all the glucuronolactone had dissolved. The solvent was evaporated and the foam was used without purification. Acetic anhydride (50 mL) and sodium acetate (5 g, 61.0 mmol) were added and the suspension was stirred for 8 days. The reaction was poured onto ice water (300 mL) and stirred overnight. The \(\beta\)-acetate was separated by filtration, washed with water and recrystallised from Et\(_2\)O-petroleum ether to give 113 as a white solid (8.5 g, 39%). \(R_f\) 0.57 (1:1 EtOAc-cyclohexane); NMR data \(\left(\text{\textsuperscript{1}H and \textsuperscript{13}C}\right)\) was in agreement with reported literature data; \([\alpha]_D\) +9.3\(^\circ\) (c 1.16, CHCl\(_3\)); IR (film) cm\(^{-1}\): 2958, 1757, 1439, 1370, 1215, 1039; \(\text{\textsuperscript{1}H NMR}\) (CDCl\(_3\), 600 MHz) \(\delta\) 5.77 (1H, d, \(J\) 7.7 Hz, H-1), 5.31 (1H, t, \(J\) 9.3 Hz, H-3), 5.25 (1H, t, \(J\) 9.3 Hz, H-4), 5.14 (1H, dd, \(J\) 9.3 Hz, \(J\) 7.7 Hz, H-2), 4.18 (1H, d, 9.3 Hz, H-5), 3.76 (3H, s, OMe), 2.13 (3H, s), 2.05 (6H, s), 2.04 (3H, s) (each OAc); \(\text{\textsuperscript{13}C NMR}\) (CDCl\(_3\), 150 MHz) \(\delta\) 169.9, 169.4, 169.2, 168.8, 166.8 (each C=O), 91.4 (C-1), 73.0 (C-5), 71.8 (C-3), 70.1 (C-2), 68.9 (C-4), 53.0 (OMe), 20.8, 20.6, 20.5, 20.5 (each OAc); ESI-HRMS calcd for C\(_{15}\)H\(_{20}\)O\(_{11}\)Na 399.0903, found \(m/z\) 399.0885 [M+Na]\(^+\).

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**1-Bromo-1-deoxy-2,3,4-tri-\(\alpha\)-D-glucopyranosiduronic acid, methyl ester\(^3\).**

Methyl ester 113 (4.0 g, 10.5 mmol) was dissolved in CH\(_2\)Cl\(_2\) (8 mL) and cooled to 0 °C. To this HBr (33% in AcOH, 16 mL) was added and the reaction allowed to warm to room temperature over 4 h. The solvent was removed under reduced pressure and the residue was dissolved in chloroform. The organic layer was washed with satd NaHCO\(_3\), water, brine, dried over MgSO\(_4\), filtered and the solvents were removed under reduced pressure. Recrystallisation of the residue from absolute EtOH gave the bromide precursor (2.66 g, 63%) as a white solid; \(R_f\) 0.69 (1:1 EtOAc-cyclohexane); IR (film) cm\(^{-1}\): 2975, 1752, 1370, 1213, 1115, 1044; \(\text{\textsuperscript{1}H NMR}\) (CDCl\(_3\), 500 MHz) \(\delta\) 6.64 (1H, d, \(J\) 4.1 Hz, H-1), 5.61 (1H, t, \(J\) 10.0 Hz, H-3), 5.24 (1H, t, \(J\) 10.0 Hz, H-4), 4.86 (1H, dd, 10.0, 4.1 Hz, H-2), 4.58 (1H, d,
10.0 Hz, H-5), 3.76 (3H, s, OMe), 2.10 (3H, s), 2.06 (3H, s), 2.05 (3H, s) (each OAc); $^{13}$C NMR (CDCl$_3$, 125 MHz) $\delta$ 169.6, 169.5, 169.3, 166.6 (each C=O), 85.3 (C-1), 72.0, 70.3, 69.3, 68.5, 53.0 (OMe), 20.6 (2s), 20.4 (each OAc)

**1-S-Acetyl-2,3,4-tri-O-acetyl-1-$\beta$-thio-$\delta$-glucopyranuronic acid, methyl ester**\(^4\) (114).

Bromide precursor (2.66 g, 6.62 mmol) was dissolved in DMF (20 mL) and KSAc (0.92 g, 8.07 mmol) was added. The reaction stirred at room temperature for 3 h, diluted with EtOAc and the solvent was removed under reduced pressure. The residue filtered through silica (EtOAc-cyclohexane 1:1), solvents were removed and the title compound was recovered by recrystallising from absolute EtOH to give **114** (1.34 g, 51%) as an off white solid; $R_f$ 0.59 (EtOAc-cyclohexane 3:1); [α]$_D$ +17.2° (c 2.16, CHCl$_3$); Mp 163.6-164.0 °C; IR (film) cm$^{-1}$: 2956, 1654, 1711, 1375, 1217, 1077, 1036; $^1$H NMR (CDCl$_3$, 600 MHz) $\delta$ 5.33 (1H, t, $J$ 9.7 Hz, H-3), 5.30 (1H, d, $J$ 10.4 Hz, H-1), 5.20 (1H, t, $J$ 9.7 Hz, H-4), 5.14 (1H, dd, $J$ 10.4 Hz, $J$ 9.7 Hz, H-2), 4.16 (1H, d, $J$ 9.7 Hz, H-5), 3.73 (3H, s, OMe), 2.38 (3H, s, SAc), 2.03 (3H, s, OAc), 2.02 (6H, s, OAc); $^{13}$C NMR (CDCl$_3$, 150 MHz) $\delta$ 191.7, 169.8, 169.3, 169.2, 166.7 (each C=O), 80.2 (C-1), 76.5 (C-5), 73.1 (C-3), 69.3 (C-4), 68.7 (C-2), 52.9 (OMe), 30.8 (SAc), 20.5 (2s), 20.4 (each OAc); ESI-HRMS calced for C$_{15}$H$_{20}$O$_{10}$Na 415.0675, found m/z 415.0656 [M+Na]$^+$

2,3,4-Tri-O-acetyl-1-$\beta$-thio-$\delta$-glucopyranosiduronic acid, methyl ester\(^4\) (115). Thioacetate 114 (400 mg, 1.01 mmol) was dissolved in CHCl$_3$-MeOH 1:1 (8 mL) and cooled to 0 °C. Nitrogen was bubbled through the solution for 5 min, followed by the addition of NaSMe (70 mg, 1.01 mmol). The reaction was stirred for 5 min at 0 °C and then poured onto 1% aqueous HCl and extracted with CH$_2$Cl$_2$. The combined organic layers were washed with brine, dried over MgSO$_4$, filtered, and concentrated under reduced pressure. Recrystallisation from absolute EtOH gave **115** as a yellow solid (217 mg, 61%); $R_f$ 0.52 (EtOAc-cyclohexane 3:1); [α]$_D$ 2.7° (c 0.94, CHCl$_3$); Mp 122.6-122.9 °C; IR (film) cm$^{-1}$: 2955, 2559, 1752, 1375, 1218, 1072, 1036; $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$ 5.24 (2H, m, H-3, H-4), 5.00 (1H, m, H-2), 4.28 (1H, d, $J$ 9.4 Hz, H-5), 3.73 (3H, s, OMe), 2.38 (3H, s, SAc), 2.03 (3H, s, OAc), 2.02 (6H, s, OAc); $^{13}$C NMR (CDCl$_3$, 150 MHz) $\delta$ 191.7, 169.8, 169.3, 169.2, 166.7 (each C=O), 80.2 (C-1), 76.5 (C-5), 73.1 (C-3), 69.3 (C-4), 68.7 (C-2), 52.9 (OMe), 30.8 (SAc), 20.5 (2s), 20.4 (each OAc); ESI-HRMS calced for C$_{15}$H$_{20}$O$_{10}$Na 415.0675, found m/z 415.0656 [M+Na]$^+$
4.58 (1H, t, J 9.9 Hz, H-1), 4.05 (1H, d, J 9.6 Hz, H-5), 3.76 (3H, s, OMe), 2.38 (1H, d, J 9.9 Hz, SH), 2.08 (3H, s), 2.03 (3H, s), 2.02 (3H, s) (each OAc); $^{13}$C NMR (CDCl$_3$, 100 MHz) δ 169.9, 169.5, 169.3, 166.7 (each C=O), 79.0 (C-1), 76.6 (C-5), 73.2 (C-2), 72.8, 69.3, 53.0 (OMe), 20.7, 20.6, 20.5 (each OAc).

**1,2,3,4-Tetra-O-acetyl-α-D-galactopyranosiduronic acid**. To a stirred solution of HClO$_4$ (500 µL) in Ac$_2$O (130 mL) at 0°C was added d-galacturonic acid monohydrate (25 g, 118 mmol). The reaction was warmed to room temperature and stirred for 3 h. The reaction was then re-cooled to 0°C and MeOH (15 mL) was added cautiously. After stirring for 30 mins the reaction was partitioned between EtOAc and H$_2$O. The aqueous layer was extracted into EtOAc and the combined organic layers were washed with water, brine, dried over MgSO$_4$, filtered and the solvents were concentrated under reduced pressure. The product was recrystallised from EtOAc-pentane to give the acid derivative (38g, 89%) as a white solid; R$_f$ 0.27 (EtOAc-cyclohexane 1:1); IR (film) cm$^{-1}$: 3507, 2945, 1756, 1370, 1217, 1039; $^1$H NMR (CDCl$_3$, 500 MHz) δ 9.31 (1H, bs, OH), 6.37 (1H, d, J 3.4 Hz, H-1), 5.75 (1H, m, H-4), 5.30 (1H, dd, J 10.8 Hz, J 3.1 Hz, H-3), 5.23 (1H, dd, J 10.8 Hz, J 3.4 Hz, H-2), 4.71 (1H, d, J 0.7 Hz, H-5), 2.07 (3H, s), 2.02 (3H, s), 1.92 (3H, s), 1.90 (3H, s) (each OAc); $^{13}$C NMR (CDCl$_3$, 125 MHz) δ 170.4, 170.2, 170.1, 169.1, 168.6 (each C=O), 89.5 (C-1), 70.5 (C-5), 67.3 (C-3), 66.2 (C-2), 20.8, 20.7, 20.6 (2s) (each OAc); ESI-HRMS calcd for C$_{14}$H$_{18}$O$_{51}$Na 385.0747, found m/z 385.0755 [M+Na]$^+$

**1,2,3,4-Tetra-O-acetyl-α-D-galactopyranosiduronic acid, methyl ester** (117). To a solution of 1,2,3,4-tetra-O-α-D-galactopyranuronic acid (18 g, 50 mmol) in H$_2$O (35 mL) was added solid NaHCO$_3$ (4.6 g, 55 mmol). After the cessation of gas evolution, CH$_2$Cl$_2$ (90 mL) was added followed by tetra-n-butyl ammonium bromide (17.5 g, 55 mmol) and methyl iodide (4.1 ml, 65 mmol). The resulting suspension was stirred overnight at room temperature. The phases were separated and the aqueous phase was extracted into CH$_2$Cl$_2$. 

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The combined organic phases were washed with water, dried over MgSO$_4$, filtered and the solvents were concentrated under reduced pressure. The residue was dissolved in warm EtOAc and cooled to -5°C where the ammonium salts precipitated and were removed by filtration. The filtrate and washings were then concentrated under reduced pressure. Recrystallization from Et$_2$O gave 117 (13.4 g, 71%). NMR data ($^1$H and $^{13}$C) was in agreement with reported literature data; R$_f$ 0.27 (EtOAc-cyclohexane 1:1); IR (film) cm$^{-1}$: 2958, 1755, 1438, 1372, 1224, 1078, 940, 730; $^1$H NMR (CDCl$_3$, 500 MHz) $\delta$ 6.37 (1H, d, $J$ 2.8 Hz, H-1), 5.68 (1H, m, H-4), 5.25 (2H, overlapping signals, H-2, H-3), 4.67 (1H, d, $J$ 1.2 Hz, H-5), 3.63 (3H, s, OMe), 2.03 (3H, s), 1.99 (3H, s), 1.90 (3H, s), 1.89 (3H, s) (each OAc); $^{13}$C NMR (CDCl$_3$, 125 MHz) $\delta$ 169.6, 169.4, 169.3, 168.2, 166.3 (each C=O), 89.2 (C-1), 70.4 (C-5), 68.3 (C-4), 66.7, 65.7, 52.4 (OMe), 20.4, 20.2, 20.1 (2s) (each OAc); ESI-HRMS calcd for C$_{15}$H$_{20}$O$_{10}$Na 399.0903, found m/z 399.9012 [M+Na]$^+$

1-Bromo-1-deoxy-2,3,4-tri-$O$-acetyl-$\alpha$-D-galactopyranosiduronic acid, methyl ester$^6$ (117) (10 g, 27 mmol) was dissolved in CH$_2$Cl$_2$ (30 mL). To this was added Ac$_2$O (3.3 mL), AcOH (13 mL) and AcBr (18 mL). The reaction mixture was brought to 0°C and a solution of H$_2$O (4.2 mL) in AcOH (14 mL) was added dropwise. After 10 min the reaction was warmed to room temperature and stirred for 3 h. The mixture was poured onto ice, the layers were separated and the aqueous layer was washed into CH$_2$Cl$_2$. The combined organic layers were washed with ice water, cold satd NaHCO$_3$, water, brine, dried over MgSO$_4$, filtered and the solvents were concentrated under reduced pressure. The crude material (80%) was used without further purification; NMR data ($^1$H and $^{13}$C) was in good agreement with the reported literature data; R$_f$ 0.58 (EtOAc-cyclohexane 1:1); IR (film) cm$^{-1}$: 2992, 2957, 1755, 1372, 1220, 1093, 1013; $^1$H NMR (CDCl$_3$, 500 MHz) $\delta$ 6.72 (1H, d, $J$ 3.9 Hz, H-1), 5.77 (1H, dd, $J$ 3.2 Hz, $J$ 1.2 Hz, H-4), 5.40 (1H, dd, $J$ 10.6 Hz, $J$ 3.2 Hz, H-3), 5.05 (1H, dd, $J$ 10.6 Hz, $J$ 3.9 Hz, H-2), 4.84 (1H, d, $J$ 1.2 Hz, H-5), 3.73 (3H, s, OMe), 2.06 (3H, s), 1.97 (6H, s) (each OAc); $^{13}$C NMR (CDCl$_3$, 125 MHz) $\delta$ 169.7, 169.5, 169.3, 165.7 (each C=O), 87.2 (C-1), 72.3 (C-5), 67.8 (C-4), 67.5 (C-3), 67.1 (C-2), 52.8 (OMe), 20.5, 20.4, 20.3 (each OAc)
1-S-Acetyl-2,3,4-tri-O-acetyl-β-thio-D-galactopyranosiduronic acid, methyl ester (118).

The bromide precursor 117 (5 g, 12.6 mmol) was dissolved in DMF (40 mL) and KSAc (1.72 g, 15.1 mmol) was added. The reaction was stirred at room temperature for 3 h, diluted with EtOAc and washed with water, brine, dried over MgSO₄ and the solvent was removed under reduced pressure. Flash chromatography (petroleum ether-EtOAc, 1:1) gave the thioacetate 118 (2.87 g, 56%) as an off white solid; R_f 0.57 (EtOAc-cyclohexane 3:1); [α]_D +53.5° (c 2.2, CHCl₃); IR (film) cm⁻¹: 3021, 1753, 1721, 1372, 1214, 1086, 1061; ¹H NMR (CDCl₃, 500 MHz) δ 5.77 (1H, d, J 3.0, H-4), 5.41 – 5.32 (2H, overlapping signals, H-2 and H-1), 5.27 (1H, dd, J 8.7, 3.2, H-3), 4.61 (1H, s, H-5), 3.75, 2.41, 2.11, 2.04, 1.99 (each s, each 3H, each CH₃); ¹³C NMR (126 MHz, CDCl₃) δ 192.1, 169.7 (2s), 169.4, 166.0 (each C=O), 80.4 (C-1), 76.1 (C-5), 71.5 (C-4), 68.5 (C-3), 65.9 (C-2), 52.8 (OMe), 30.7, 20.6, 20.5 (2s, each CH₃); ESI-HRMS calcd for C₁₅H₂₀O₁₀SNa 415.0675, found m/z 415.0656 [M+Na]^+.

2,3,4-Tri-O-acetyl-β-thio-D-galactopyranosiduronic acid, methyl ester (56). Thioacetate 118 (1.5 g, 3.7 mmol) was dissolved in CHCl₃-MeOH 1:1 (30 mL) and cooled to 0 °C. Nitrogen was bubbled through the solution for 5 min, followed by the addition of NaSMe (257 mg, 3.67 mmol). The reaction was stirred for 5 min at 0 °C and then poured onto 1M HCl (20 mL), and extracted with CH₂Cl₂ (2 x 30 mL). The combined organic layers were washed with brine, dried over MgSO₄, filtered, and the solvent was removed under reduced pressure. Flash chromatography (EtOAc-petroleum ether 3:1) gave 56 as an off-white solid (1.01 g, 79%); R_f 0.52 (EtOAc-cyclohexane 3:1); [α]_D +39.7° (c 1.85, CHCl₃); IR (film) cm⁻¹: 1750, 1372, 1220, 1059; ¹H NMR (500 MHz, CDCl₃) δ 5.76 (1H, dd, J 3.4, J 1.3, H-4), 5.22 (1H, t, J 9.9, H-2), 5.09 (1H, dd, J 10.1, 3.4, H-3), 4.56 (1H, t, J 9.8, H-1), 4.35 (1H, d, J 1.2, H-5), 3.77 (3H, s, OCH₃), 2.50 (1H, d, J 9.9, SH), 2.13 (3H, s), 2.09 (3H, s), 2.00 (3H, s) (each OAc); ¹³C NMR (CDCl₃, 125 MHz) δ 169.8, 169.7, 166.1 (each C=O), 79.3, 76.2, 71.2, 70.4, 68.4, 52.9 (OMe) 20.6, 20.5 (each 3H, each s, each CH₃). ESI-HRMS calcd for C₁₃H₁₇O₉S 349.0593, found m/z 349.0588 [M-H]^−.
2,3,4-Tri-O-acetyl-α-thio-β-glucopyranosiduronic acid, methyl ester (119). To a stirred solution of 115 (100 mg, 0.28 mmol) in CH$_2$Cl$_2$ (3 mL) was added TiCl$_4$ (76 µL, 0.7 mmol) dropwise. The reaction mixture was stirred for 10 min at room temperature and then cooled to 0°C overnight. The reaction was diluted with CH$_2$Cl$_2$ and quenched with satd NH$_4$Cl. Phases were separated and the aqueous phase was extracted with CH$_2$Cl$_2$. The combined organic layers were washed with water, brine, dried over MgSO$_4$ and concentrated under reduced pressure to give 119 (65 mg, 65%) as a white foam; IR (film) cm$^{-1}$: 2955, 2572, 1747, 1438, 1370, 1214, 1077, 1042; $^1$H NMR (CDCl$_3$, 500 MHz) δ 5.98 (1H, t, J 5.7, H-1), 5.39 (1H, t, J 9.0, H-3), 5.17 (1H, t, J 8.9, H-4), 5.02 (1H, dd, J 9.3, 5.2, H-2), 4.76 (1H, d, J 9.2, H-5), 3.76 (3H, s, OCH$_3$), 2.09 (3H, s), 2.06 (3H, s), 2.05 (3H, s) (each OAc), 2.02 (1H, s, SH); $^{13}$C NMR (126 MHz, CDCl$_3$) δ 169.5, 169.4, 167.7 (each C=O), 76.4 (C-1), 69.8 (C-2), 69.4 (C-3), 68.9 (C-4) 68.6 (C-3), 52.8 (OMe), 20.6, 20.5, 20.5 (each OAc); ESI-HRMS calcd for C$_{13}$H$_{17}$O$_7$S 349.0593, found m/z 349.0584 [M-H].

2,3,4-Tri-O-acetyl-α-thio-β-galactopyranosiduronic acid, methyl ester (54). To a stirred solution of 56 (100 mg, 0.28 mmol) in CH$_2$Cl$_2$ (3 mL) was added TiCl$_4$ (76 µL, 0.7 mmol) dropwise. The reaction mixture was stirred for 10 min at room temperature and was then cooled and left at 0 °C overnight. The reaction was then diluted with CH$_2$Cl$_2$ and quenched with satd NH$_4$Cl. The phases were separated and the aqueous phase was extracted into CH$_2$Cl$_2$. The combined organic layers were washed with water, brine, dried over MgSO$_4$ and the solvent was removed under reduced pressure to give 54 (61 mg, 61%) as a white foam; $[\alpha]_D$ +186.4° (c 3.3, CHCl$_3$); IR (film) cm$^{-1}$: 3059, 1748, 1439, 1370, 1266, 1217, 1125, 1069; $^1$H NMR (500 MHz, CDCl$_3$) δ 6.08 (1H, t, J 5.1, H-1 ), 5.72 (1H, s, H-4), 5.25 (1H, dd, J 10.8, 5.2, H-2), 5.21 (1H, dd, J 10.8, 3.0, H-3), 5.02 (1H, s, H-5), 3.71 (3H, s, OCH$_3$), 2.05 (3H, s), 2.03 (3H, s), 1.95 (3H, s) (each OAc), 1.89 (1H, d, J 5.1, SH). $^{13}$C NMR (125 MHz,
CDCl₃ δ 169.7, 169.6, 167.0 (each C=O), 77.7 (C-1), 69.0 (C-5), 68.6 (C-4), 67.1 (C-3), 66.9 (C-2), 52.7 (OMe), 20.6, 20.5, 20.4 (each CH₃); ESI-HRMS calcd for C₁₃H₁₇O₉S 349.0593, found m/z 349.0588 [M-H]⁻

1-S-(((4R,5R)-3-(tert-Butyloxycarbonyl)-5-heptadecyl-2,2-dimethyloxazolidin-4-yl)methyl)-2,3,4-Tri-O-acetyl-α-thio-β-glucopyranosiduronic acid, methyl ester (121).

Compound 119 (155 mg, 0.44 mmol) was dissolved in dry DMF (3 mL) and cooled to 0°C. NaH (60% in mineral oil, 16 mg, 0.4 mmol) was added slowly and the reaction stirred for 30 min. A solution of bromide 55 (179 mg, 0.15 mmol) in DMF was then added dropwise and the reaction mixture allowed warm to room temperature overnight. EtOAc and water were added and the aqueous layer was washed with EtOAc. The combined organic layers were washed with water, brine, dried over MgSO₄ and concentrated under reduced pressure. Purification via flash chromatography (petroleum ether-EtOAc 4:1) gave 121 (42 mg, 39%) as a colourless oil. [α]D +59.5° (c, CHCl₃); IR (film) cm⁻¹: 2923, 2853, 1752, 1697, 1457, 1375, 1177, 1040; ¹H NMR (500MHz, CDCl₃) δ 5.71 (1H, m, H₁), 5.41 – 5.31 (1H, m, H₃), 5.24 – 5.14 (1H, m, H-4), 5.03 (1H, dd, J 9.8, 5.5, H-2), 4.72 (1H, m, H-5), 3.96 (2H, overlapping signals, H-2’ & H-3’), 3.74 (3H, s, OMe), 2.08 – 2.00 (9H, overlapping signals, OAc), 1.54 (6H, m), 1.48 (9H, s, t-Bu), 1.45 (3H, s), 1.25 (30H, s, each CH₂), 0.88 (3H, t, J 6.7, CH₃); ¹³C NMR (125MHz, CDCl₃) δ 169.8, 169.5, 169.4, 167.9 (C=O), 92.5, 83.3 (C-1), 80.1, 76.6 (C-3’), 70.0 (C-2), 69.5 (C-3), 69.1 (C-4), 68.9 (C-5), 58.6 (C-2’), 52.9 (OMe), 32.1, 29.9, 29.7, 29.5, 28.6 (each CH₂), 22.8, 20.8, 20.7 20.7, 14.3 (each CH₃); ESI-HRMS calcd for C₄₁H₇₁NO₁₂SNa 824.4595, found m/z 824.4586 [M+Na]⁺
Experimental data

Chapter 6

1-S-(((4R,5R)-3-(tert-Butoxycarbonyl)-5-heptadecyl-2,2-dimethyloxazolidin-4-yl)methyl)-2,3,4-Tri-O-acetyl-α-thio-β-galactopyranosiduronic acid, methyl ester (53). Trimethylphosphine (330 µL, 1.0M in THF) and ADDP (84 mg, 0.33 mmol) were stirred for 30 min at 0 °C in THF. Compound 54 (58 mg, 0.165 mmol) and alcohol 57 (60 mg, 0.127 mmol) were added and the resulting mixture was stirred at room temperature for 2 days. The mixture was filtered and diluted with EtOAc. Remaining hydrazide was precipitated from hexane and removed via filtration. The filtrate was washed with H₂O, brine, satd NaHCO₃, dried over MgSO₄ and the solvents were concentrated under reduced pressure. Flash chromatography of the residue (petroleum ether-EtOAc, 4:1) gave 53 (36 mg, 36%) as a colourless oil; [α]D +58.4° (c, CHCl₃); IR (film) cm⁻¹: 2923, 2853, 1752, 1697, 1457, 1375, 1177, 1040; ¹H NMR (500MHz, CDCl₃): δ 5.87 (1H, d, J 5.4 H-1), 5.76 (1H brs, H-4), 5.22 (1H, m, H-2), 5.05 (1H, m, H-3), 4.96 (1H, m, H-5), 4.01 (1H, m, H-3’), 3.81 (1H, m, H-2’), 3.74 (3H, s, OMe), 2.84 (1H, dd, J 12.6, 7.4), 2.78 (1H, dd, J 13.1, 8.9 ), 2.70 (1H, dd, J 12.7, 3.7), 2.60 (1H, dd, J 12.7, 1.1), 2.08 – 2.00 (9H, m, OAc), 1.56 (9H, brs, t-Bu), 1.50 (6H, s), 1.47 (4H, s), 1.25 (27H, s, each CH₂), 0.88 (3H, t, J 6.7, CH₃); ¹³C NMR (125MHz, CDCl₃): δ 169.7, 167.35, 167.1, 151.3 (each C=O), 92.7, 92.3*, 83.6*, 82.6 (C-1), 80.4 , 76.1 (C-3’), 68.9 (C-4), 68.8 (C-3), 68.6 (C-5), 67.7 (C-2), 59.2 (C-2’), 52.6 (OMe), 31.9, 29.7 (2s), 29.6 (2s), 29.4, 28.4, 28.3, 27.1, 26.7, 24.6, 23.4, 22.7, 20.7, 20.6, 20.55, 14.1; ESI-HRMS calcd for C₄₁H₇₁N₉O₁₉S Na 824.4595, found m/z 824.4586 [M+Na]⁺

1-N-succinimidyl-nonadecanoate. N-hydroxysuccinimide (0.76 g, 6.56 mmol) and EDC (1.25 g, 6.56 mmol) were added to a solution of nonadecanoic acid (1.87 g, 6.56 mmol) in CH₂Cl₂ (50 mL) and the mixture was stirred overnight at room temperature. The solvent was concentrated under reduced pressure and the resulting residue was dissolved in CH₂Cl₂. The
solution was washed with water, dried over MgSO₄ and concentrated under reduced pressure to yield the title compound as a white solid (2.45 g, 98%). The compound was used without further purification; ¹H NMR (500 MHz, CDCl₃) δ 2.83 (4H, s, O=CH₂CH₂C=O), 2.60 (2H, t, J 7.5, CH₂C=O), 1.79 – 1.69 (2H, m, CH₂), 1.39 (2H, dd, J 14.9, 7.0, CH₂), 1.27 (29H, s, each alkyl CH₂), 0.88 (3H, t, J 6.9, CH₃); ¹³C NMR (125 MHz, CDCl₃) δ 169.1, 168.6 (each C=O), 31.9, 30.9, 29.6, 29.6, 29.6, 29.5, 29.3, 29.0, 28.8, 25.6, 24.6, 22.7 (each CH₂), 14.1 (CH₃)

1-S-((2R,3R)-3-Hydroxy-2-nonadecanamidoicosyl)-2,3,4-Tri-O-acetyl-α-thio-D-galactopyranosiduronic acid, methyl ester (128). Compound 53 (30 mg, 0.037 mmol) was taken up in formic acid (2 mL) and the reaction mixture was stirred vigorously for 30 min. Toluene (5 mL) was added and the volatile components were then removed under reduced pressure. The resulting residue was dissolved in water and basified with solid NaHCO₃. The mixture was then extracted into CH₂Cl₂, dried over MgSO₄ and the solvent was removed under reduced pressure. The crude product was then taken up in CH₂Cl₂ and DIPEA (31 µL, 0.18 mmol) was added. To this was added a solution of 1-N-succinimidyl nonadecanoate (50 mg, 0.13 mmol) in CH₂Cl₂ and the mixture was stirred at room temperature for 16 h. The reaction mixture was partitioned between EtOAc and satd NaHCO₃. Phases were separated and the aqueous phase extracted into EtOAc. The combined organic phases were washed with brine, dried over MgSO₄ and the solvent was removed under reduced pressure. Flash chromatography of the residue (petroleum ether-EtOAc 3:1) gave 128 (19 mg, 54% over 2 steps) as a white solid; [α]D +51.5 (c, CHCl₃); IR (film) cm⁻¹: 3293 br, 2917, 2850, 1751, 1648, 1467, 1371, 1223, 1080; ¹H NMR (500MHz, CDCl₃) δ 6.04, (1H, d, J 8.9, -NH-), 5.84 (1H, d, J 5.6, H-1), 5.79 (1H, br s, H-4), 5.31 – 5.25 (1H, m, H-2), 5.18 (1H, dd, J 10.9, 3.3, H-3), 5.05 (1H, brs, H-5), 4.07 (1H, brs, H-2'), 3.76 (3H, s, OMe), 3.62 (1H, m, H-3’), 2.94 (1H, dd, J 14.2, 8.4, -CHHS-), 2.82 (1H, m,-CHHS- ), 2.24 – 2.18 (2H, m, CH₂), 2.10, 2.08, 2.00 (each 3H, each s, acetate CH₃) , 1.63 (4H, m, each CH₂), 1.46 (2H, m, CH₂), 1.28 (58 H, s each CH₂), 0.88 (6H, t, J 6.9); ¹³C NMR (125MHz, CDCl₃): δ 173.5, 170.0, 169.7, 169.6, 167.25 (each C=O), 84.7 (C-1), 73.7 (C-3’), 68.8 (C-5), 68.5 (C-4), 67.7 (C-3), 67.5 (C-2),

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53.6 (C-2'), 52.8 (OMe), 36.75, 34.0, 32.4, 31.9, 29.7, 29.7, 29.6, 29.4, 25.9, 25.8 (each CH₂), 22.7, 20.8, 20.6, 20.5, 14.1 (each CH₃); ESI-HRMS calcd. for C₅₂H₉₄N O₁₁S 940.6548, found \textit{m/z} 940.6558 [M-H]⁻

![Chemical Structure Image]

1-S-((2R,3R)-3-Hydroxy-2-nonadecanamidoicosyl)-2,3,4-Tri-O-acetyl-α-thio-D-glucopyranosiduronic acid, methyl ester (129). Compound 121 (30 mg, 0.037 mmol) was taken up in formic acid (3 mL) and stirred vigorously for 30 min. Toluene (5 mL) was added and the solvents were removed under reduced pressure. The resulting residue was taken up in water and basified with solid NaHCO₃. The mixture was then extracted into CH₂Cl₂, dried over MgSO₄ and concentrated under reduced pressure. The resulting residue was taken up in CH₂Cl₂ (2 mL) and DIPEA (22 µL, 0.131 mmol) was added. To this was added a solution of 1-N-succinimidyl nonadecanoate (37 mg, 0.094 mmol) in CH₂Cl₂ (1.5 mL) and the mixture was stirred at room temperature for 16 h. The reaction mixture was partitioned between EtOAc and satd NaHCO₃. Phases were separated and the aqueous phase extracted into EtOAc. The combined organic phases were washed with brine, dried over MgSO₄ and concentrated under reduced pressure. Purification via flash chromatography (petroleum ether-EtOAc 3:1) gave 129 (19 mg, 57% over two steps) as a white solid; [α]₀⁺47.8° (c 1.0 in CHCl₃); IR (film) cm⁻¹: 3294 br, 2917, 2850, 1751, 1650, 1467, 1219, 1043; ¹H NMR (500MHz, CDCl₃) δ 5.69 (1H, d, J 5.3, H-1), 5.33 – 5.27 (1H, m, H-3), 5.21 (1H, t, J 8.8, H-4), 4.99 (1H, dd, J 9.3, 5.2, H-2), 4.74 (1H, dd, J 9.1, H-5), 4.01 (1H, s, H-3'), 3.79 – 3.73 (3H, m, OMe), 3.68 (1H, brs, H-2'), 3.03 (1H, dd, J 14.0, 8.0, CH₂CHOH), 2.84 (1H, dd, J 14.0, 3.5, CH₂CHOH), 2.19 (2H, d, J 4.3, CH₂), 2.08 (3H, s), 2.04 (3H, s), 2.03 (3H, s) (each CH₃), 1.62 (4H, s, 2 x CH₂), 1.45 (2H, m, CH₂), 1.32 – 1.22 (60H, m, each CH₂), 0.87 (6H, t, J 6.9, each CH₃); ¹³C NMR (125MHz, CDCl₃) δ 173.68, 169.76, 169.49, 169.42, 167.94 (each C=O), 83.52 (C-1), 73.55 (C-3'), 70.02 (C-2), 69.23 (C-3), 69.18 (C-5), 68.77 (C-4), 54.04 (C-2'), 52.95 (OMe), 36.74, 34.02, 31.93 (each CH₂), 29.72, 29.68, 29.67, 29.56, 29.41, 29.37, 25.96, 25.72, 22.70 (each CH₂), 20.71, 20.62, 20.60, 14.13 (each CH₃); ESI-HRMS calcd. for C₅₂H₉₄NO₁₁S 940.6548, found \textit{m/z} 940.6578 [M-H]⁻
1-S-((2R,3R)-3-Hydroxy-2-nonadecamidoicosyl)-α-thio-D-galactopyranosiduronic acid (45). Protected lipid derivative 128 (9 mg, 0.92 µmol) was dissolved in anhydrous EtOAc (20 µL) and LiI (13 mg, 0.09 mmol) was added. The reaction mixture was heated at reflux for 6 h. Upon cooling the reaction mixture was washed with H₂O, brine, dried over MgSO₄ and concentrated under reduced pressure. The resulting residue was then taken up in a guanidine-guanidinium nitrate solution (2 mL, 1M in CH₂Cl₂-MeOH 1:9) the reaction was stirred at room temperature for 1h. The reaction was neutralised by the addition of Amberlite® IR-20, filtered and concentrated under reduced pressure. The crude product was purified via lipophilic Sephadex® LH-20 to give the title compound (4.2 mg, 55%) as a white powder; ¹H NMR (500 MHz, CDCl₃-MeOD 2:1); δ ¹H NMR (500 MHz, CDCl₃-MeOD 2:1) δ 5.43 (1H, d, J 5.5, H-1), 4.73 (1H, m, H-5), 4.26 (1H, m, H-4) 4.08 (1H, m, H-2), 3.95 (1H, dd, J 11.2, 6.3, CHNHR), 3.61 (1H, dd, J 10.2, 3.3, H-3), 3.51 (1H, m, CHOH), 2.85 (1H, m, CHHS), 2.60 (1H, m, CHHS) 2.32 – 2.23 (1H, m), 2.18 (2H, t, J 7.5), 1.58 (3H, m, CH₂), 1.47 (3H, m, CH₂), 1.25 (56H, s, each CH₂), 0.85 (6H, t, J 6.9, each CH₃); ¹³C NMR (125 MHz, CDCl₃-MeOD 2:1) δ 175.2, 171.8 (each C=O), 87.9 (C1), 73.1 (CHOH), 71.4 (C5), 70.7 (C3), 70.1 (C4), 68.5 (C2), 54.8 (CHN), 36.6, 32.3 (CH₂S), 34.1, 29.6, 26.1, 26.0, 22.9, 22.8, 19.4 (each CH₂), 14.1 (CH₃); ESI-HRMS calcd. for C₄₅H₈₆NO₈S 800.6074, found m/z 800.6072 [M-H]⁻

1-S-((2R,3R)-3-Hydroxy-2-nonadecamidoicosyl)-α-thio-D-glucopyranosiduronic acid (46). Protected lipid derivative 129 (3 mg, 0.3 µmol) was dissolved in anhydrous EtOAc (200 µL) and LiI (15 mg, 0.11 mmol) was added. The reaction mixture was heated at reflux for 6 h. Upon cooling the reaction mixture was washed with H₂O, brine, dried over MgSO₄ and concentrated under reduced pressure. The resulting residue was then taken up in a guanidine-guanidinium nitrate solution (2 mL, 1M in CH₂Cl₂-MeOH 1:9) the reaction was stirred at
room temperature for 1 h. The reaction was neutralised by the addition of Amberlite® resin IR-20, filtered and the solvents was removed under reduced pressure. The crude product was purified via lipophilic Sephadex® LH-20 to give the title compound (1.4 mg, 58% over 2 steps) as a white powder; $^1$H NMR (500 MHz, CDCl$_3$-MeOD 2:1) $\delta$ 5.17 (1H, d, J 4.7), 4.31 (1H, s), 3.78 (1H, s), 3.58 (2H, s), 3.45 (1H, s), 3.38 (1H, s), 2.80 – 2.72 (1H, m), 2.66 (1H, s), 2.10 (1H, s), 1.97 (2H, s), 1.83 (1H, s), 1.46 (1H, s), 1.39 (2H, s), 1.28 (2H, s), 1.04 (40H, s), 0.65 (7H, d, J 6.6); $^{13}$C NMR (125 MHz, CDCl$_3$-MeOD 2:1) $\delta$ 175.5 (C=O), 87.6 (C1), 73.5 (CHOH), 71.4 (C2), 70.4 (C-3), 69.0 (C-5), 67.2 (C-4), 53.6 (CHN), 35.3, 31.3 (CH$_2$S), 34.0, 29.4, 26.1, 26.0, 22.9, 22.7, 19.3 (each CH$_2$), 14.1 (CH$_3$); ESI-HRMS calcd for C$_{45}$H$_86$NO$_8$S 800.6074, found m/z 800.6077 [M-H]$^-$.}

**6.3 Chapter 3-Experimental**

Methyl 2,3,4,6-tetra-O-benzyl-$\alpha$-D-glucopyranoside (150). Methyl $\alpha$-D-glucopyranoside (10 g, 51.5 mmol) was dissolved in DMF (250 mL) and cooled to 0 °C. To this was added sodium hydride (60% in mineral oil dispersion, 10.3 g, 257 mmol) portion-wise over 1 h. Benzyl bromide (31 mL, 257 mmol) was then added dropwise and the reaction was allowed to attain room temperature over 24 h. The reaction was quenched via the slow addition of MeOH and diluted with EtOAc. The organic layer was washed with H$_2$O, brine, dried over MgSO$_4$ and the solvents were concentrated under reduced pressure. Flash chromatography of the residue (EtOAc-cyclohexane 1:8) gave 150 (23.7 g, 83%) as a yellow oil; IR (film) cm$^{-1}$: 3032, 1605, 1495, 1161, 1048, 736; $^1$H NMR (500MHz, CDCl$_3$) $\delta$ 7.12–7.38 (20H, m, Ar-H), 4.99 (1H, d, J 10.9, PhCH$_2$O), 4.84 (1H, d, J 10.7, PhCH$_2$O), 4.83 (1H, d, J 10.9, PhCH$_2$O), 4.81 (1H, d, J 12.1, PhCH$_2$O), 4.68 (1H, d, J 12.1, PhCH$_2$O), 4.64 (1H, d, J 3.6, H-1), 4.62 (1H, d, J 12.4 Hz, PhCH$_2$O), 4.49 (1H, d, J 12.4, PhCH$_2$O), 4.48 (1H, d, J 10.7, PhCH$_2$O), 3.99 (1H, t, J 9.2 Hz, H-3), 3.71–3.77 (2H, overlapping signals, H-5 & H-6a), 3.62–3.68 (2H, overlapping signals, H-4 & H-6b), 3.57 (1H, dd, J 5.6, 9.6, H-2), 3.39 (3H, s, OCH$_3$); $^{13}$C NMR (125MHz, CDCl$_3$) $\delta$ 138.7, 138.2, 138.1, 138.0 (each Ar-C), 128.7 (2s), 128.6, 128.5, 128.2, 128.2, 128.1 (2s), 128.0, 127.96, 127.8 (2s) (each Ar-CH), 98.2 (C-1), 92.8 (C-1), 86.5 (C-1), 80.8 (C-1).
Experimental data

82.1 (C-3), 79.9 (C-2), 77.7 (C-4), 75.7, 75.0, 73.4, 73.3 (each OCH\_2Ph), 70.1 (C-5), 68.6 (C-6), 55.1 (OCH\_3); ESI-HRMS calcd for C\textsubscript{35}H\textsubscript{39}O\textsubscript{5} 555.2746, found m/z 555.2740 [M+H]^+

1-C-allyl-1-deoxy-2,3,4,6-tetra-O-benzyl-\alpha-D-glucopyranoside\textsuperscript{7} (151). Compound 150 (15 g, 27 mmol) was placed under high vacuum and heated to 60 °C for 2 h. The resulting syrup was kept under an atmosphere of argon. Acetonitrile (300 mL) was added and the solution was cooled to 0 °C. Allyltrimethylsilane (12.8 mL, 81 mmol) was added and the mixture stirred for five min. Trimethylsilyl triflate (2.44 mL, 13.5 mmol) was added dropwise and the reaction was left to stir overnight at room temperature. Satd. NaHCO\textsubscript{3} was added and the aqueous layer was extracted into EtOAc. The organic layers were combined and washed with H\textsubscript{2}O, brine and dried over MgSO\textsubscript{4} and filtered. The solvent was concentrated under reduced pressure and the crude residue was purified via flash chromatography (EtOAc-Cyclohexane 1:7) to give 151 (11.7 g, 77%) as a white solid; \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) \(\delta\) 7.40-7.00 (20H, m, Ar-H), 5.86-5.76 (1 H, ddt, J 17.1, 10.1, 7.0, CH\_2CH=CH\_2), 5.15-5.05 (2H, m, CH\_2CH=CH\_2), 4.92 (1H, d, J 10.9 Hz, OCH\_2Ph), 4.80 (2H, dd, J 2.9, 7.7, OCH\_2Ph), 4.69 (1H, d, J 11.6 Hz, OCH\_2Ph), 4.63-4.60 (2H, dd, OCH\_2Ph), 4.48-4.45 (2H, dd, OCH\_2Ph), 4.15-4.10 (1H, m, H-1), 3.82-3.73 (2H, overlapping signals, H-3 & H-2), 3.71-3.68 (1H, ddd, J 9.8, 4.3, 2.5, H-5), 3.65-3.60 (3H, overlapping signals, H-4 & H-6), 2.55-2.42 (2H, m, CH\_2CH=CH\_2); \textsuperscript{13}C NMR (100 MHz, CDCl\textsubscript{3}) \(\delta\) 138.9, 138.4, 138.4, 138.3 (each Ar-C), 134.9 (CH\_2CH=CH\_2), 128.7, 128.6 (2s), 128.5, 128.2, 128.1 (2s), 128.0 (2s),127.9, 127.8 (2s) (Ar-C), 117.1 (CH\_2CH=CH\_2) 82.6, 80.3, 78.4, 75.8, 75.5 (OCH\_2Ph), 73.9 (CH), 73.7, 73.3 (OCH\_2Ph), 71.4 (CH), 69.2 (C-6), 30.1 (CH\_2CH=CH\_2); ESI-HRMS calcd for C\textsubscript{37}H\textsubscript{41}O\textsubscript{5} 565.2954, found m/z 565.2958 [M+H]^+
**1-C-allyl-1-deoxy-2,3,4-tri-o-benzyl-α-D-glucopyranoside**. Compound 151 (5 g, 8.8 mmol) was dissolved in Ac₂O-CH₂Cl₂ (80 mL, 1:1) and cooled to -78 °C. To this was added Trimethylsilyl triflate (477 µL, 2.6 mmol) dropwise. After 3 h, the reaction was brought to 0°C and quenched with satd. NaHCO₃. Phases were separated and the aqueous phase was extracted into CH₂Cl₂. The combined organic layers were washed with brine, dried over MgSO₄, filtered and the solvents were concentrated under reduced pressure to give the 6-O-acetylated intermediate 156 as a brown oil. The crude product was dried for 3 h under high vacuum and dissolved in MeOH (40 mL). To this was added freshly prepared solution of NaOMe-MeOH (10 mL of a 1M solution). The reaction mixture was stirred at room temperature overnight. Solvents were removed and the crude residue was taken up in CH₂Cl₂ and washed with water, brine, dried over MgSO₄, filtered and the solvents were concentrated under reduced pressure. The crude residue was purified via flash chromatography (petroleum ether-EtOAc 9:1) to give the title compound as a white solid (3.3 g, 79% over two steps); H NMR (500 MHz, CDCl₃) δ 7.35-7.27 (15 H, m, Ar-H), 5.80-5.72 (1 H, dd, J 17.1, 10.1, 7.0, CH₂CH=CH₂), 5.12-5.07 (2H, m, CH₂CH=CH₂), 4.93 (1H, d, J 10.9, OCH₂Ph), 4.86 (1H, d, J 10.9, OCH₂Ph), 4.81 (1H, d, J 10.9, OCH₂Ph), 4.71-4.69 (1H, d, J 10.9, OCH₂Ph), 4.63-4.61 (2H, each d, OCH₂Ph), 4.06-4.03 (1H, m, H-1), 3.80 (1H, apt t, J 8.8, H-3), 3.75 (1H, ddd, J 11.6, 4.4, 2.3, H-6a), 3.69 (1H, dd, J 9.3, 5.8, H-2), 3.65-3.59 (1H, m, H-6b), 3.54 (1H, ddd, J 9.6, 4.2, 2.5, H-5), 3.49 (1H, t, J 8.5, H-4), 2.52-2.42 (2H, m, CH₂CH=CH₂); C NMR (125 MHz, CDCl₃) δ 138.7, 138.1 (2s) (each Ar-C), 134.5 (CH₂CH=CH₂), 128.5, 128.4 (2s), 128.0, 127.9, 127.8 (2s), 127.79, 127.61 (each Ar-CH), 117.16 (CH₂CH=CH₂) 82.2 (C-3), 80.1 (C-2), 78.1 (C-4), 75.4, 75.1 (each OCH₂Ph), 73.6 (C-1), 73.1 (CH), 71.6 (C-6), 29.9 (CH₂CH=CH₂); ESI-HRMS calcd for C₃₀H₃₅O₅ 475.2484, found m/z 475.2480 [M+H]⁺
1-C- Allyl-1-deoxy-2,3,4-tri-O-benzyl-6-O-propargyl-α-D-glucopyranoside (152). Primary alcohol (1 g, 2.1 mmol) was dissolved in DMF (20 mL) and cooled to 0 °C. Sodium hydride (60% dispersion in mineral oil, 127 mg, 3.2 mmol) was added to the reaction mixture portion-wise and stirring was continued for 10 min. Propargyl bromide (341 µL, 3.2 mmol, 80% solution in toluene) was added dropwise and the reaction mixture was allowed warm to room temperature overnight. The reaction was quenched via the slow addition of MeOH at 0 °C. EtOAc was added and the reaction mixture was washed with H₂O, brine, dried over MgSO₄, filtered and the solvents were concentrated under reduced pressure. The crude residue was purified via flash chromatography (petroleum ether-EtOAc 6:1) to give 152 (973 mg, 90%) as a yellow oil; ¹H-NMR (500 MHz, CDCl₃) δ 7.36-7.26 (15H, m, Ar-H), 5.84-5.78 (1H, ddt, J 17.1, 10.1, 7.0, CH₂CH=CH₂), 5.13-5.07 (2H, m, CH₂CH=CH₂), 4.90 (1H, d, J 11.0 OCH₂Ph), 4.86 (1H, d, J 10.8, OCH₂Ph), 4.82 (1H, d, J 11.0 Hz, OCH₂Ph), 4.70 (2H, dd, J 4.2, 10.8, OCH₂Ph), 4.62 (1H, d, J 11.6, OCH₂Ph), 4.24 (1H, dd, J 4.2, 15.9 alkyne CH₂), 4.15 (1H, d, J 4.2 alkyne CH₂), 4.11 (1H, ddd, J 8.0, 1.8, 8.4, H-1), 3.84 (1H, dd, J 7.7, 2.5, H-3) 3.81-3.78 (1H, m, H-6a), 3.74 (1H, dd, J 9.4, 5.8, H-2), 3.64 (3H, overlapping signals, H-6b, H-5 & H-4), 2.49-2.48 (2H, m, CH₂CH=CH₂), 2.36 (1H, t, J 2.36, alkyne C-H); ¹³C NMR (150 MHz, CDCl₃) δ 138.8, 138.4, 138.2 (each Ar-C), 134.6 (CH₂CH=CH₂), 128.4 (2s), 128.3, 128.0, 127.8 (2s), 127.7, 127.5 (each Ar-CH), 116.9 (CH₂CH=CH₂) 82.3 (C-3), 80.0 (C-2), 79.6 (C-4), 77.9 (alkyne C), 77.2, 77.0, 76.0 (OCH₂Ph), 75.4, 75.1 (CH), 74.7 (C-1), 73.8 (CH₂), 70.9 (C-5), 68.5 (alkyne CH₂), 58.5 (C-6), 29.8 (alkyne CH), 29.7 (CH₂CH=CH₂); ESI-HRMS calcd for C₃₃H₃₆O₅Na 535.2460, found m/z 535.2465 [M+Na]⁺

1-C- Allyl-1-deoxy-3,4-di-O-benzyl-6-O-propargyl-α-D-glucopyranoside (153). Compound 152 (800 mg, 1.56 mmol) was taken up in THF (10 mL) and cooled to 0 °C. I₂
(1.98 g, 7.8 mmol) was added and the reaction mixture was allowed to stir for 2 h. The reaction mixture was diluted with EtOAc and 1M Na$_2$S$_2$O$_3$ solution was added and stirring was continued for 10 min. Phases were separated and aqueous layer was extracted into EtOAc. The combined organic layers were washed with brine, dried over MgSO$_4$ and concentrated under reduced pressure. The crude product was purified via flash chromatography (petroleum ether-EtOAc 9:1) to give a colourless syrup. The product was dissolved in MeOH-Et$_2$O 1:1 (12 mL). To this was added Zn dust (1.0 g, 15.3 mmol) and glacial acetic acid (100 µL). The reaction mixture was stirred at room temperature overnight, filtered through Celite® and the solvents were concentrated under reduced pressure. The crude residue was taken up in CH$_2$Cl$_2$, washed with 1M HCl, satd NaHCO$_3$, brine, dried over MgSO$_4$, filtered and the solvents were concentrated under reduced pressure. Flash chromatography of the residue (petroleum ether-EtOAc 9:1) gave 153 (395 mg, 60%) as a white solid; $^1$H-NMR (500 MHz, CDCl$_3$) $\delta$ 7.37-7.3 (10H, m, Ph-H), 5.88-5.80 (1H, ddt, $J$ 17.1, 10.1, 7.0, CH$_2$CH=CH$_2$), 5.16-5.06 (2H, m, CH$_2$CH=CH$_2$), 4.70 (1H, d, $J$ 11.7 OCH$_2$Ph), 4.68 (2H, d, $J$ 11.4 OCH$_2$Ph), 4.62 (1H, d, $J$ 11.7 OCH$_2$Ph), 4.23 (1H, dd, $J$ 15.9, 2.3, alkyne CH$_2$), 4.20 (1H, dd, $J$ 15.9, 2.3, alkyne CH$_2$), 4.01-3.98 (2H, overlapping signals, H-1 & H-3), 3.90 (1H, dd, $J$ 10.2, 5.6, H-2), 3.76 (1H, t, $J$ 5.8, H-6a), 3.72 (1H, dd, $J$ 10.8, 4.6, H-6b), 3.70-3.68 (1H, m, H-5), 3.63 (1H, t, $J$ 5.2, H-4), 2.47-2.40 (2H, m, CH$_2$CH=CH$_2$), 2.38 (1H, t, $J$ 2.4 alkyne C-H); $^{13}$C-NMR (125 MHz, CDCl$_3$) $\delta$ 138.0, 137.5 (each Ar-C), 134.6 (CH$_2$CH=CH$_2$), 128.5, 128.4, 127.9 (2s), 127. 6, 127.4 (each Ar-C), 116.9 (CH$_2$CH=CH$_2$) 79.6, 77.9, 75.2 (CH), 75.2, 73.1, 73.0 (each OCH$_2$Ph), 71.4 (C-1), 73.1 (CH), 69.3 (C-5), 67.6 (alkyne C-H), 58.4 (CH), 32.8 (CH$_2$CH=CH$_2$); ESI-HRMS calcd for C$_{26}$H$_{31}$O$_5$ 423.2171, found m/z 423.2168 [M+H]$^+$

1-C-allyl-1-deoxy-2-O-isopentyl-3,4-di-O-benzyl-6-O-propargyl-α-D-glucopyranoside (154). Sodium hydride (60% dispersion in mineral oil, 37 mg, 0.92 mmol) was added to a stirring solution of 153 (300 mg, 0.71 mmol) in DMF (7 mL) at 0 °C. After 10 min, 1-bromo-
3-methyl-butane (212 µL, 1.8 mmol) was added dropwise. The reaction mixture was allowed warm to room temperature overnight and quenched via the slow addition of MeOH at 0 °C. The reaction mixture was diluted with EtOAc, washed with H₂O, brine, dried over MgSO₄, filtered and the solvents were concentrated under reduced pressure. The crude residue was purified via flash chromatography (petroleum ether-EtOAc 6:1) to give 154 (276 mg, 79 %) as a white solid; [α]₀ +32.4° (c 0.01 in CHCl₃); ¹H-NMR (500 MHz, CDCl₃): 7.38-7.28 (10 H, m Ar-H), 5.88-5.80 (1 H, ddt, J 17.1, 10.1, 7.0, CH₂CH=CH₂), 5.14-5.07 (2H, m, CH₂CH=CH₂), 4.93 (1H, ddt, J 17.1, 10.1, 7.0, CH₂CH=CH₂), 4.86 (1H, d, J 10.8, OCH₂Ph), 4.80 (1H, d, J 11.06, OCH₂Ph), 4.68 (1H, d, J 11.8, OCH₂Ph), 4.24 (1H, d, J 11.8, OCH₂Ph), 3.84 (1H, d, J 10.4, OCH₂Ph), 3.72 (1H, t, J 8.8, H-2), 3.66-3.57 (4H, overlapping signals, H-5, H-6 & H-4 overlapping), 3.57 (2H, t, J 6.5, OCH₂CH₂CH(CH₃)₂), 2.50-2.36 (2H, m, CH₂CH=CH₂), 2.36 (1H, t, J 2.3, alkyne C-H), 1.75-1.67 (1H, m, OCH₂CH₂CH(CH₃)₂), 1.48 (1H, dd, J 6.7, 1.6, OCH₂CH₂CH(CH₃)₂), 1.48 (1H, dd, J 6.8, 1.8, OCH₂CH₂CH(CH₃)₂), 0.88 (6H, dd, J 6.6, 1.5, OCH₂CH₂CH(CH₃)₂); ¹³C NMR (125 MHz, CDCl₃) δ 138.9, 138.4 (each Ar-C), 134.8 (CH₂CH=CH₂), 128.3 (2s), 128.0, 127.8, 127.6, 127.4 (each Ar-CH), 116.7 (CH₂CH=CH₂), 82.2, 80.6, 79.6 (CH), 77.8 (OCH₂CH₂CH(CH₃)₂), 75.2, 75.0, (each OCH₂Ph), 73.8 (C-1), 70.9 (alkyne CH₂), 69.3 (CH), 68.5, 58.5 (alkyne C-H), 39.0 (OCH₂CH₂CH(CH₃)₂), 29.7 (CH₂CH=CH₂), 24.8 (OCH₂CH₂CH(CH₃)₂), 22.6, 22.5 (OCH₂CH₂CH(CH₃)₂); ESI-HRMS calcd for C₃₁H₄₀O₅Na 515.2773, found m/z 515.2770 [M+Na]+

1-C- Allyl-1-deoxy-3,4,6-tri-O-benzyl-α-D-glucopyranoside⁸ (159). Compound 151 (1.0 g, 1.9 mmol) was taken up in THF (12 mL) under and cooled to 0 °C. I₂ (1.98 g, 7.8 mmol) was added and the reaction mixture was allowed to stir for 2 h. The reaction mixture was diluted with EtOAc and 1M Na₂S₂O₃ solution was added. Phases were separated and aqueous layer was extracted into EtOAc. The combined organic layers were washed with brine, dried over MgSO₄, filtered and the solvents were concentrated under reduced pressure. The crude product was dissolved in MeOH-Et₂O (14 mL, 1:1). To this was added Zn dust (1.0 g, 15.3 mmol) and glacial acetic acid (0.110 mL). The reaction mixture stirred at room temperature overnight. The reaction mixture was then filtered through Celite® and the solvents were
Experimental data

Chapter 6

concentrated under reduced pressure. The crude residue was taken up in CH₂Cl₂, washed with 1M HCl, satd NaHCO₃, brine, dried over MgSO₄, filtered and the solvents were concentrated under reduced pressure. Flash chromatography of the residue (petroleum ether-EtOAc 9:1) gave 159 (622 mg, 69%) as a white solid; ¹H NMR (500 MHz, CDCl₃) δ 7.34 – 7.27 (10H, m, Ar-H), 7.25 – 7.22 (5H, m, Ar-H), 5.83 (1H, ddt, J 17.1, 10.2, 6.9, CH₂CH=CH₂), 5.16 – 5.03 (2H, m, CH₂CH=CH₂), 4.64 (1H, d, J 11.7, OCHHPh), 4.61 (1H, d, J 11.5, OCHHPh), 4.56 (3H, dd, J 11.9, 2.1, each OCHHPh), 4.52 – 4.48 (1H, m, OCHHPh), 4.05 (1H, dd, J 10.1, 5.2, CH), 3.93 (1H, ddd, J 8.7, 5.5, 3.0, H-1), 3.81 (1H, dd, J 10.2, 5.8, H-6a), 3.75 (1H, t, J 5.3, CH), 3.69 (1H, dd, J 10.2, 5.2, H-6b), 3.67 – 3.62 (2H, m, CH), 2.47 – 2.34 (2H, m, CH₂CH=CH₂); ¹³C NMR (125 MHz, CDCl₃) δ 138.1, 137.9, 137.4 (each Ar-C), 134.7 (CH₂CH=CH₂), 128.5, 128.4, 128.3, 127.9 (3s), 127.7, 127.6, 127.5 (each Ar-CH), 116.9 (CH₂CH=CH₂), 77.4 (CH), 74.7 (CH), 73.5 (CH), 73.2 (OCH₂Ph), 72.7 (OCH₂Ph), 71.0 (C-1), 69.0 (CH), 68.0 (C-6), 33.2 (CH₂CH=CH₂); ESI-HRMS calcd for C₃₀H₃₅O₅ 475.2484, found m/z 475.2480 [M+H]⁺

1-C-Allyl-1-deoxy-2-O-propyl-3,4,6-tri-O-benzyl-α-D-glucopyranoside (160). Sodium hydride (60% in mineral oil dispersion, 66 mg, 1.64 mmol) was added portionwise to a stirring solution of 160 (600 mg, 1.26 mmol) in DMF (13 mL) at 0 °C. After 10 min, 1-iodopropane (393 µL, 4.0 mmol) was added dropwise. The reaction mixture was allowed warm to room temperature overnight and quenched via the slow addition of MeOH at 0 °C. The reaction mixture was diluted with EtOAc, washed with H₂O, brine, dried over MgSO₄, filtered and the solvents were concentrated under reduced pressure. Flash chromatography of the residue (petroleum ether-EtOAc 6:1) gave 160 (553 mg, 71%) as a white solid; ¹H-NMR (500 MHz, CDCl₃) δ 7.38 – 7.26 (14H, m, Ar-H), 7.14 (1H, d, J 5.8, Ar-H), 5.89-5.81 (1H, ddt, J 17.1, 10.1, 7.0, CH₂CH=CH₂), 5.14-5.07 (2H, m, CH₂CH=CH₂), 4.93 (1H, d, J 10.8, OCH₂Ph), 4.79 (2H, dd, J 17.8, 10.8, OCH₂Ph), 4.62 (1H, d, J 12.0, OCH₂Ph), 4.48 (2H, d, J 12.3, OCH₂Ph), 4.20 (1H, m, H-1), 3.7 (2H, overlapping signals, each CH), 3.64-3.59 (6H, overlapping signals, CH, CH₂ & OCH₂CH₂CH₃), 2.45 (2H, m, CH₂CH=CH₂), 1.65 – 1.53 (2H, m, OCH₂CH₂CH₃), 0.94 (3H, t, J 7.4, OCH₂CH₂CH₃); ¹³C NMR (125 MHz, CDCl₃) δ
138.9, 138.2, 138.1 (Ar-C), 134.8 (CH₂CH=CH₂), 128.3 (2s), 127.9, 128.8, 127.8, 127.6, 127.5 (2s) (each Ar-CH), 116.7 (CH₂CH=CH₂), 82.3, 80.6, 78.0 (CH), 75.0, 75.4 (each OCH₂Ph), 73.7 (C-1), 75.4 (OCH₂CH₂(CH₃)₂), 72.7 (OCH₂CH₂CH₃), 71.4 (CH), 62.4 (C-6), 29.9 (CH₂CH=CH₂), 23.4 (OCH₂C₂H₂CH₃), 10.7 (OCH₂CH₂C₃H₃); ESI-HRMS calcd for C₃₃H₄₁O₅ 517.2954, found m/z 517.2951 [M+H]⁺

1-C-allyl-1-deoxy-2-O-propyl-3,4-di-O-benzyl-a-D-glucopyranoside. Compound 160 (550 mg, 1.1 mmol) was dissolved in Ac₂O-CH₂Cl₂ (10 mL, 1:1) and cooled to -78 °C. To this was added trimethylsilyltriflate (60 µL, 0.33 mmol) dropwise. After 3 h, the reaction was brought to 0 °C and quenched with satd NaHCO₃. Phases were separated and the aqueous phase was extracted into CH₂Cl₂. The combined organic layers were washed with brine, dried over MgSO₄, filtered and the solvents were concentrated under reduced pressure to give the 6-O-acetylated intermediate as a brown oil. The crude product was dried for 3 h under high vacuum and dissolved in MeOH (10 mL). To this was added freshly prepared solution of NaOMe-MeOH (2mL of a 1M solution) and the reaction mixture was stirred at room temperature overnight. Solvents were concentrated under reduced pressure and the crude residue was taken up in CH₂Cl₂ and washed with H₂O, brine, dried over MgSO₄, filtered and the solvents were concentrated under reduced pressure. The crude residue was purified via flash chromatography (petroleum ether-EtOAc 9:1) to give the title compound (351 mg, 75%) as a white solid; [α]D +18.1 (c 1.0 in CHCl₃); ¹H-NMR (500 MHz, CDCl₃) δ 7.32 (10H, ddd, J 20.2, 14.0, 7.0, Ar-H), 5.85 – 5.74 (1H, dtt, J 17.1, 10.1, 7.0, CH₂CH=CH₂), 5.16 – 5.06 (2H, m, CH₂CH=CH₂), 4.95 (1H, d, J 11.0, OCHHPh), 4.86 (1H, d, J 10.9, OCHHPh), 4.79 (1H, d, J 11.0, OCHHPh), 4.62 (1H, d, J 10.9, OCHHPh), 4.16 – 4.10 (1H, m, H-1), 3.80 – 3.72 (2H, overlapping signals, H-6a & CH), 3.67 – 3.60 (1H, m, H-6b), 3.58 – 3.50 (4H, overlapping signals, OCH₂CH₂CH₃, CH & CH), 3.51 – 3.43 (1H, m, CH), 2.45 (2H, m, CH₂CH=CH₂), 1.65 – 1.53 (2H, m, OCH₂CH₂CH₃), 0.94 (3H, t, J 7.4, OCH₂CH₂CH₃); ¹³C NMR (125 MHz, CDCl₃) δ 138.7 (CH₂CH=CH₂), 138.0, 134.6 (each Ar-C), 128.4, 128.3, 128.0, 127.8, 127.5 (each Ar-CH), 117.0 (CH₂CH=CH₂), 82.0 (CH), 80.6 (CH), 77.9 (CH), 75.3 (OCH₂Ph), 75.1 (OCH₂Ph), 73.5 (CH), 72.7 (OCH₂CH₂CH₃), 71.4
1-C-Allyl-1,6-dideoxy-2-O-propyl-3,4-di-O-benzyl-6-azido-α-D-glucopyranoside (161).

Primary alcohol (350 mg, 0.82 mmol) was dissolved in CH$_2$Cl$_2$ (10 mL) and cooled to 0 °C. To this was added triethylamine (400 µL, 2.87 mmol) followed by the dropwise addition of MsCl (95 µL, 1.2 mmol). The reaction was stirred for 2 h, diluted with H$_2$O and extracted into EtOAc. The organic phase was washed with brine, dried over MgSO$_4$, filtered and the solvents were concentrated under reduced pressure. The crude residue was dissolved in DMF (10 mL) and NaN$_3$ (267, 4.1 mmol) was added. The reaction was heated to 110 °C for 26 h. Upon cooling the reaction was diluted with Et$_2$O and washed with H$_2$O, brine, dried over MgSO$_4$, filtered and the solvents were concentrated under reduced pressure. The crude residue was purified via flash chromatography (petroleum ether-EtOAc 6:1) to give 161 (241 mg, 65%) as a white solid; [α]$_D$ +39.1 (c 1.0 in CHCl$_3$); $^1$H-NMR (500 MHz, CDCl$_3$) δ 7.32 (10H, ddd, $J$ 20.2, 14.0, 7.0, Ar-H), 5.85 – 5.74 (1H, ddt, $J$ 17.1, 10.1, 7.0, CH$_2$CH=CH$_2$), 5.16 – 5.06 (2H, m, CH$_2$CH=CH$_2$), 4.95 (1H, d, $J$ 11.0, OCH$_2$HPh), 4.86 (1H, d, $J$ 10.9, OCH$_2$HPh), 4.79 (1H, d, $J$ 11.0, OCH$_2$HPh), 4.62 (1H, d, $J$ 10.9, OCH$_2$HPh), 4.16 – 4.10 (1H, m, H-1), 3.80 – 3.72 (2H, overlapping signals, H-6a & CH), 3.67 – 3.60 (1H, m, H-6b), 3.58 – 3.50 (4H, overlapping signals, OCH$_2$CH$_2$CH$_3$, CH & CH), 3.51 – 3.43 (1H, m, CH), 2.45 (2H, m, CH$_2$CH=CH$_2$), 1.65 – 1.53 (2H, m, OCH$_2$CH$_2$CH$_3$), 0.94 (3H, t, $J$ 7.4, OCH$_2$CH$_2$CH$_3$); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 138.7, 137.9 (Ar-C), 134.4 (CH$_2$CH=CH$_2$), 128.4, 128.3, 128.0, 127.9, 127.8, 127.6 (each Ar-CH), 116.9 (CH$_2$C=CH$_2$), 81.9, 80.5, 78.6 (CH & OCH$_2$CH$_2$CH$_3$ overlapping), 75.2, 75.1, (each OCH$_2$Ph), 73.6 (C-1), 70.9, 69.4 (each CH), 51.7 (C-6), 29.9 (CH$_2$CH=CH$_2$), 23.4 (OCH$_2$CH$_2$CH$_3$), 10.7 (OCH$_2$CH$_2$CH$_3$); ESI-HRMS calcd for C$_{26}$H$_{34}$N$_3$O$_4$ 452.2549, found m/z 452.2444 [M+H]$^+$.
1-C-allyl-1,6-dideoxy-6-(((1-C-allyl-1,6-dideoxy-2-O-isopentyl-3,4-di-O-benzyl-α-D-glucopyranos-6-yl)oxymethyl)-1H-1,2,3-triazol-1-yl)-2-O-propyl-3,4-di-O-benzyl-α-D-glucopyranoside (162). Alkyne 154 (200 mg, 0.41 mmol) and azide 161 (183 mg, 0.41 mmol) were dissolved in a mixture of Acetonitrile-H₂O 1:1 (4 mL). To this was added CuI (41 mg, 0.22 mmol) and the reaction was heated at reflux for 24 h. Upon cooling the reaction was diluted with EtOAc, washed with H₂O, satd NH₄Cl, brine, dried over MgSO₄, filtered and the solvents were concentrated under reduced pressure. The crude residue was purified via flash chromatography (EtOAc-petroleum ether 1:1) to give compound 162 (329 mg 85%) as a colourless oil; ¹H NMR (500 MHz, CDCl₃) δ 7.59 (1H, s, triazole H), 7.39 – 7.18 (20H, m, Ar-H), 5.82 (1H, ddt, J 16.9, 10.1, 6.8, CH₂CH=CH₂), 5.59 (1H, ddt, J 16.9, 10.1, 6.8, CH₂CH=CH₂), 5.14 – 5.03 (2H, m, CH₂CH=CH₂), 4.99 – 4.89 (4H, overlapping signals, CH₂CH=CH₂ & OCHHPh), 4.85 (1H, dd, J 10.7, 1.9, OCHHPh), 4.81 (1H, d, J 11.0, OCHHPh), 4.79 – 4.74 (1H, d, J 11.1 OCHHPh), 4.72 (3H, overlapping signals, OCHHPh & OCHHC=C), 4.63 (1H, d, J 12.5, OCHHC=C), 4.50 (1H, d, J 10.8, OCHHPh), 4.46 (1H, dd, J 14.2, 5.6, H-6’a), 4.37 (1H, dd, J 14.2, 2.6, H-6’b), 4.20 – 4.13 (1H, m, CH), 4.12 – 4.02 (1H, m, CH), 3.79 – 3.65 (5H, overlapping signals), 3.64 – 3.54 (5H, overlapping signals), 3.49 (2H, td, J 6.5, 3.0, CH₂), 3.43 (1H, dd, J 9.2, 5.8, CH), 3.08 (1H, dd, J 9.6, 8.8, CH), 2.51 – 2.33 (4H, overlapping signals, each CH₂CH=CH₂), 1.70 (1H, dt, J 13.4, 6.7, OCH₂CH₂CH(CH₃)₃), 1.57 (2H, dd, J 14.1, 6.9, OCH₂CH₂CH(CH₃)₃), 1.45 (2H, dt, J 19.6, 9.8, OCH₂CH₂CH₃), 0.91 (3H, t, J 7.4, OCH₂CH₂CH₃), 0.88 (6H, d, J 6.7, OCH₂CH₂CH(CH₃)₂); ¹³C NMR (125 MHz, CDCl₃) δ 144.7, 138.8, 138.4, 138.2, 137.9 (each Ar-C), 134.7 (CH₂CH=CH₂), 134.1 (CH₂CH=CH₂), 128.5, 128.4, 128.3 (3s), 128.0, 127.9, 127.8 (3s), 127.7, 127.6, 127.5 (2s), (each Ar-CH), 124.3 (triazole CH=C), 117.1 (CH₂CH=CH₂), 82.2, 81.8, 80.5, 80.2, 78.0, 77.9 (each CH), 75.3, 75.2, 75.0 (2s), 74.7 (each OCH₂Ph), 73.7 (CH), 73.7 (CH), 72.6 (CH₂), 71.0 (CH), 70.8 (CH), 70.0 (CH₂), 69.3 (CH₂),
68.4, 65.0 (OCH₂C=C), 50.8 (OCH₂CH₂CH₃), 29.8 (CH₂CH=CH₂), 29.7 (CH₂CH=CH₂), 24.8 (OCH₂CH₂CH(CH₃)₂), 23.4 (OCH₂CH₂CH(CH₃)₂), 22.6, 22.5 (each OCH₂CH₂CH(CH₃)₂), 10.6 (OCH₂CH₂CH₃); ESI-HRMS calcd for C₅₇H₇₄N₃O₉ 944.5425, found m/z 944.5423 [M+H]+

Macrocycle 164. To a stirred solution of 162 (200 mg, 0.212 mmol) in dioxane-H₂O (3:1, 2 mL) was added 2,6-lutidine (86 µL, 0.742 mmol), NaIO₄ (204 mg, 0.95 mmol) and a catalytic amount of OsO₄ (2 drops, 2.5% solution in t-BuOH). The reaction mixture was stirred at room temperature for 2.5 h. H₂O and CH₂Cl₂ were added, layers were separated and the aqueous layer was extracted into CH₂Cl₂. The combined organic phases were washed with brine, dried over MgSO₄, filtered and the solvents were concentrated under reduced pressure. The resulting residue was taken up in 1,2-dichloroethane (5 mL) and isopentylamine (25 µL, 0.212 mmol) was added dropwise. The solution was stirred at room temperature for 20 min. Sodium triacetoxyborohydride (157 mg, 0.742 mmol) was then added and the reaction mixture stirred for 3 h. The reaction was quenched by the addition of satd NaHCO₃ and the product was extracted into EtOAc, dried with MgSO₄, filtered and the solvents were concentrated under reduced pressure. The crude residue was purified via flash chromatography (CH₂Cl₂-EtOAc 1:9) to give compound 164 (160 mg, 75%) as an off white solid; ¹H NMR (500 MHz, CDCl₃) δ 7.73 (1H, s, triazole H), 7.40 – 7.26 (20H, m, Ar-H), 4.89 (4H, overlapping signals, each OCH₂Ph), 4.76 (4H, overlapping signals, OCH₂Ph, H-6’a and OCHHC=C), 4.65 (1H, d, J 11.2, OCHHPh), 4.60 – 4.52 (2H, overlapping signals, OCHHPh & OCHHC=C), 4.06 (3H, overlapping signals, 2 x CH & H-6’b), 3.77 (3H, overlapping signals, 2 x CH & H-6a), 3.67 (2H, dd, J 18.1, 9.1, each CH), 3.55 (7H, overlapping signals), 3.32 – 3.22 (2H, overlapping signals, each CH), 2.46 (3H, m), 2.38 (2H,
m), 2.18 (1H, m) (each CH₂N), 1.73 (5H, overlapping signals), 1.60 (2H, m, OCH₂CH₂CH(CH₃)₂), 1.52 (1H, NCH₂CH₂CH(CH₃)₂) 1.45 (2H, m OCH₂CH₂CH₃), 1.25 (2H, m, NCH₂CH₂CH(CH₃)₂) 0.93 (3H, t, J 7.4, OCH₂CH₂CH₃), 0.88 (12H, d, J 6.2, CH₂CH₂CH(CH₃)₂); ¹³C NMR (125 MHz, CDCl₃) δ 146.3 (triazole CH=C), 138.0, 137.6 (each Ar-C), 128.5, 128.4, 128.3, 128.0, 127.9, 127.8, 127.7, 127.5 (each Ar-CH), 122.9 (triazole CH=C), 82.6, 81.8, 80.7, 80.4, 79.2, 78.7 (each CH), 75.3 (CH₂Ph), 75.1 (CH₂Ph), 74.8 (CH₂Ph), 72.7 (CH₂), 72.3, 72.0, 71.9, 70.7, 70.2, 69.3 (CH₂), 65.2 (OCH₂C=C), 51.8, 51.7, 51.3, 49.0 (each CH₂N), 39.1 (CH₂), 26.6 (CH), 24.9 (CH), 23.4 (CH₂), 22.6, 22.5 (each CH₂CH₂CH(CH₃)₂), 21.7 (CH₂), 10.7 (OCH₂CH₂CH₃); ESI-HRMS calcd for C₆₀H₈₃N₄O₉ 1003.6160, found m/z 1003.6158 [M+H]+

Macrocycle 165. Compound 164 (50 mg, 0.049 mmol) was dissolved in EtSH-BF₃·Et₂O (2 mL, 4:1). The reaction was stirred at room temperature for 24 h. The solvents were removed under reduced pressure and the crude residue was dissolved in pyridine-Ac₂O (1:1, 1.5 mL). The reaction mixture was stirred at room temperature for 5 h. Solvents were removed under reduced pressure and the residue was taken up in EtOAc and washed with H₂O, brine, dried over MgSO₄, filtered and the solvents were concentrated under reduced pressure. Flash chromatography of the residue (MeOH-CH₂Cl₂, 1:9) gave 165 (30 mg, 76%) as a white solid; ¹H NMR (500 MHz, CDCl₃) δ 7.75 (1H, s, triazole H), 5.20 (2H, overlapping signals, H-3 & H-3’), 4.88 – 4.82 (1H, m, H-4’), 4.74 (3H, overlapping signals, H-4 & OCH₂C=C), 4.62 (2H, m, H-6’), 4.27 – 4.22 (1H, m, H-1’), 4.17 (1H, dd, J 14.1, 9.9, H-5’), 4.11 – 4.04 (1H, m, H-1), 3.93 – 3.86 (1H, m, H-5), 3.84 – 3.79 (1H, m, H-2), 3.61 – 3.50 (7H, overlapping signals, each CH₂, H-2 & H-2’), 2.52 – 2.41 (3H, m), 2.41 – 2.33 (3H, m) (each CH₂N), 2.11 (3H, s), 2.07 (3 H, s), 2.05 (6H, each s) (each acetate CH₃), 1.72 – 1.57 (4H, m), 1.54 (4H, m), 1.45 – 1.33 (2H, m, OCH₂CH₂CH(CH₃)₂), 1.23 (2H, m, NCH₂CH₂CH(CH₃)₂), 0.88 (15H,
dd, \( J \) 17.4, 7.0, \( \text{CH}_2\text{CH}_2\text{CH}((\text{CH}_3)_2 \& \text{CH}_2\text{CH}_2\text{H}_2 \text{ overlapping)\}; \)

\(^{13}\text{C} \text{NMR (125 MHz, CDCl}_3) \delta 170.1, 170.1, 169.9, 169.7 \text{ (each C=O)}, 146.3 \text{ (C=CH)}, 123.2 \text{ (C=CH)}, 77.4, 77.3 \text{ (each CH)}, 73.1 \text{ (CH}_2), 72.4, 72.3, 72.2, 72.1, 71.4, 71.1, 70.9, 69.9, 69.9 \text{ (each CH)}, 69.5 \text{ (CH}_2), 69.4 \text{ (3s)} 68.2, 65.4, 58.6, 51.6, 51.5, 51.1, 38.7 \text{ (each CH}_2), 26.2, 25.9, 24.6 \text{ (CH)}, 23.1 \text{ (CH}_2), 22.5, 22.4, 22.3, 21.4, 20.8 \text{ (2s)}, 20.7 \text{ (2s)}, 10.36 \text{ (each CH}_3); \) ESI-HRMS calcd for \( \text{C}_{40}\text{H}_{67}\text{N}_4\text{O}_{13} \) 811.4705, found \( m/\text{z} \) 811.4703 \([\text{M+H}]^+\)

**1,2,3,4,6-Penta-O-acetyl-\( \alpha/\beta\)-D-galactopyranose (171)**. To a suspension of \( \text{D-galactose (100 g, 554 mmol)} \text{ in Ac}_2\text{O (500 mL)} \) was added \( \text{I}_2 \text{ (7 g, 55 mmol)} \). The reaction was cooled to 0°C in an ice bath and stirred for 3 h. The reaction was quenched with sodium thiosulphate and extracted into \( \text{CH}_2\text{Cl}_2 \text{. The combined organic layers were washed with H}_2\text{O, brine, dried over MgSO}_4\text{, filtered and the solvents were concentrated under reduced pressure. The resulting yellow oil was recrystallised from ethanol to give 171 (205 g, 95%) (4:1 \( \alpha/\beta \) ratio) as a white solid. NMR data (\(^1\text{H and }^{13}\text{C} \text{) was in agreement with reported literature data.**

\( \alpha \)-anomer: \(^1\text{H NMR (500 MHz, CDCl}_3) \delta 6.36 \text{ (1H, d, } J 1.7, \text{ H-1)}, 5.48 \text{ (1H, s, H-4)}, 5.32 \text{ (2H, overlapping signals, H-2 & H-3)}, 4.32 \text{ (1H, t, } J 6.6, \text{ H-5)}, 4.12 – 4.06 \text{ (2H, m, H-6)}, 2.14 \text{ (6H, s, CH}_3), 2.02 \text{ (3H, s, CH}_3), 2.00 \text{ (3H, s, CH}_3), 1.98 \text{ (3H, s, CH}_3); \) \(^{13}\text{C NMR (126 MHz, CDCl}_3) \delta 170.4, 170.2 \text{ (2s)}, 169.9, 169.0 \text{ (each C=O)}, 89.8 \text{ (C-1)}, 68.8 \text{ (C-5)}, 67.5 \text{ (C-4)}, 67.4, 66.5, 61.3 \text{ (C-6)}, 20.9, 20.7 \text{ (3s)}, 20.6 \text{ (each CH}_3)\)

\( \beta \)-anomer: \(^1\text{H NMR (500 MHz, CDCl}_3) \delta 5.72 \text{ (1H, d, } J 8.3, \text{ H-1)}, 5.4 \text{ (1H, d, } J 3.3, \text{ H-4)}, 5.3 \text{ (1H, dd, } J 8.8, 9.8, \text{ H-2)}, 5.1 \text{ (1H, dd, } J 10.3, 3.5, \text{ H-3)}, 4.35 \text{ (1H, m, H-5)}, 4.13 \text{ (2H, m, H-6)}, 2.20 \text{ (3H, s, CH}_3), 2.15 \text{ (3H, s, CH}_3), 2.09 \text{ (3H, s, CH}_3), 1.99 \text{ (3H, s, CH}_3), 1.95 \text{ (3H, s, CH}_3); \) \(^{13}\text{C NMR (125 MHz, CDCl}_3): \delta 170.2, 169.6, 169.4, 169.2, 167.7 \text{ (each C=O)}, 92.3 \text{ (C-1)}, 70.2, 68.4, 68.0, 65.1, 61.7, 20.5, 20.4 \text{ (2s)}, 20.3, 20.2 \text{ (each CH}_3)\)
1-C-Allyl-1-deoxy-2,3,4,6-tetra-O-acetyl-α-D-galactopyranoside (172). To a stirred suspension of 171 (30 g, 76 mmol) and allyltrimethylsilane (36 mL, 230 mmol) in Acetonitrile (150 mL) was added BF₃.Et₂O (47 mL, 380 mmol). The reaction was heated at reflux overnight, diluted with EtOAc and quenched with satd NaHCO₃. Phases were separated and the aqueous layer was washed with EtOAc. The combined organic phases were washed with H₂O, brine, dried over MgSO₄, filtered and the solvents were concentrated under reduced pressure. Purification of the crude residue via flash chromatography gave 172 (21 g, 75%) as a viscous oil. NMR data (¹H and ¹³C) was in agreement with reported literature data. Rₚ 0.6 (petroleum ether-EtOAc 2:1); [α]D +84° (c 0.01 in CHCl₃); ¹H NMR (500 MHz, CDCl₃) δ 5.80–5.69 (1H, ddt, J 17.1, 10.1, 7.0, CH₂C=CH₂), 5.42–5.40 (1H, m, H-4), 5.28–5.25 (1H, dd, J 4.8, 9.3 Hz, H-2), 5.22–5.21 (1H, dd, J 3.2, 9.3 Hz, H 3), 5.15–5.08 (2H, m, CH₂CH=CH₂), 4.31–4.28 (1H, q, J 4.8, 10.0 Hz, H-1), 4.22–4.17 (1H, dd, J 8.8, 12.8 Hz, H-6), 4.12–4.06 (2H, overlapping signals, H-5 & H-6), 2.52–2.41 (1H, m, CHHCH=CH₂), 2.32–2.22 (1H, m, CHHCH=CH₂), 2.12 (3H, s, CH₃), 2.07 (3H, s, CH₃), 2.04 (3H, s, CH₃), 2.03 (3H, s, CH₃); ¹³C NMR (125 MHz, CDCl₃) δ 168.1, 167.6, 167.5, 167.3 (each C=O) 130.8 (CH₂CH=CH₂), 115.3 (CH₂CH=CH₂), 69.1 (CH), 65.9 (CH), 65.6 (CH), 65.3 (CH), 59.2 (C-6), 28.7 (CH₂CH=CH₂), 18.7, 18.6, 18.5 (each CH₃); ESI-HRMS calcd for C₁₇H₂₅O₉ 373.1498, found m/z 373.1492 [M+H]+.

1-C-Allyl-1-deoxy-α-D-galactopyranoside⁹ (170). Compound 172 (25 g, 67 mmol) was dissolved in MeOH (250 mL) and cooled to 0 °C. To this was added a solution of NaOMe-MeOH (13.4 mL of a 1M solution) and the resulting mixture was stirred at room temperature for 4 h. The reaction was acidified with Amberlite® resin, filtered and the solvents were concentrated under reduced pressure to give 170 (12.7, 93%) as a white solid. NMR data (¹H and ¹³C) was in agreement with reported literature data; ¹H NMR (500 MHz, CD₃OD) δ 5.90.
(1H, ddt, J 17.1, 10.1, 6.9, CH$_2$CH=CH$_2$), 5.10 (2H, m, CH$_2$CH=CH$_2$), 4.04 – 3.99 (1H, m), 3.97 (1H, s), 3.91 (1H, dd, J 8.6, 5.2), 3.77 (2H, dt, J 11.8, 8.1), 3.71 (1H, dd, J 8.6, 5.2), 3.70 (2H, t, J 11.8, 8.1), 3.65 (1H, dd, J 7.8, 4.6), 3.35 – 3.31 (1H, m), 2.47 (1H, m, CH$_2$CH=CH$_2$), 2.40 (1H, m, CH$_2$CH=CH$_2$); $^{13}$C NMR (125 MHz, CD$_3$OD) δ 135.3 (CH$_2$CH=CH$_2$), 115.4 (CH$_2$CH=CH$_2$), 74.2, 72.7, 70.5, 68.7, 68.5, 60.5, 29.7 (CH$_2$CH=CH$_2$); ESI-HRMS calcd for C$_9$H$_{17}$O$_5$ 205.1076, found m/z 205.1071 [M+H]$^+$

1-C- Allyl-1-deoxy-3,4-O-isopropylidene-$\alpha$-D-galactopyranoside$^9$ (173). To a solution of 170 (10 g, 49 mmol) and dimethoxypropane (24 mL, 195 mmol) in acetonitrile (80 mL), was added p-TsOH monohydrate (186 mg, 1 mmol) and the reaction mixture was stirred for 2 h. H$_2$O (15 mL) was then added and after 30 min the reaction was neutralized with triethylamine and the solvents were removed under reduced pressure. Flash chromatography of the crude residue (CH$_2$Cl$_2$-EtOAc 1:9) afforded 173 (8.4 g, 70%) as white crystalline solid. NMR data ($^1$H and $^{13}$C) was in agreement with reported literature data. $^1$H NMR (500 MHz, CDCl$_3$) δ 5.86 (1H, ddt, J 17.1, 10.1, 7.0, CH$_2$CH=CH$_2$), 5.15-5.09 (2H, m, CH$_2$CH=CH$_2$) 4.33-4.25 (2H, overlapping signals), 4.08-4.02 (2H, overlapping signals), 3.87-3.77 (2H, overlapping signals), 2.45-2.30 (2H, overlapping signals), 2.20 (1H, m, CH=CH=CH$_2$), 2.00 (1H, m, CHHCH=CH$_2$), 1.47 (3H, s, isopropylidene CH$_3$), 1.32 (3H, s, isopropylidene CH$_3$); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 134.3 (CH$_2$CH=CH$_2$), 117.7 (CH$_2$CH=CH$_2$), 109.7 (isopropylidene C), 74.8 (C-2), 73.1 (C-4), 70.6 (C-3), 69.6 (C-1), 69.1 (C-5), 63.6 (C-6), 34.9 (CH$_2$CH=CH$_2$), 26.8 (CH$_3$), 24.6 (CH$_3$); ESI-HRMS: calcd for C$_{12}$H$_{21}$O$_5$Na 267.1162, found 267.1208 [M+Na]$^+$
**Experimental data**

**Chapter 6**

**1-C- Allyl-1- deoxy-3,4- O- isopropylidene-6- O-p- toluenesulfonyl-α-D- galactopyranoside** *(174).* A solution of 173 (1.00 g, 4.27 mmol) in pyridine-acetone (1:1, 25 mL) was treated at 0 °C with p-toluenesulfonyl chloride (896 mg, 4.70 mmol). The solution was allowed to warm to room temperature and stirred for 6 h. The reaction mixture was repeatedly azeotroped with toluene and the crude residue was taken up in CH₂Cl₂ and H₂O. The aqueous phase was extracted with CH₂Cl₂ and the organic extracts were combined, dried with MgSO₄, filtered and the solvents were concentrated under reduced pressure. Flash chromatography of the crude residue (petroleum ether-EtOAc 2:1) gave the tosylate 174 (1.07 g, 64%) as a white solid. Rf 0.51 (EtOAc). [α]D +27.1° (c 0.01 in CHCl₃); ¹H NMR (500 MHz, CDCl₃) δ 7.78 (2H, d, J 8.3, Ar-H), 7.32 (2H, d, J 8.0, Ar-H), 5.77 (1H, ddt, J 17.1, 10.1, 7.0, CH₂CH=CH₂), 5.11 (1H, dd, J 17.2, 1.5, CH₂CH=CHH), 5.05 (1H, d, J 10.2, CH₂CH=CHH), 4.23 (2H, overlapping signals, H-4 & H-3), 4.21 (1H, apt t, J 6.5, H-5), 4.16 – 4.05 (2H, m, H-6), 3.92 (1H, td, J 7.1, 2.2, H-1), 3.74 (1H, brs, H-2), 2.42 (3H, s, Ar-CH₃), 2.35 – 2.22 (2H, m, CH₂CH=CH₂), 1.38 (3H, s, isopropylidene CH₃), 1.25 (3H, s, isopropylidene CH₃); ¹³C NMR (125 MHz, CDCl₃) δ 144.8 (Ar-C), 134.2 (CH₂CH=CH₂), 132.8, 129.8, 128.0 (each Ar-CH), 117.5 (CH₂CH=CH₂), 109.6 (isopropylidene C), 74.1 (C-4), 71.6 (C-3), 70.5 (C-1), 69.4 (C-6), 68.4 (C-2), 67.5 (C-5), 35.2 (CH₂CH=CH₂), 26.5 (isopropylidene CH₃), 24.4 (isopropylidene CH₃), 21.6 (Ar-CH₃); ESI-HRMS calcd for C₁₉H₂₇O₇S 399.1478, found m/z 399.1474 [M+H]+

**1-C- Allyl-1,6-dideoxy-3,4-O-isopropylidene-6-azido-α-D-galactopyranoside** *(175).* The tosylate 174 (5.0 g, 12.5 mmol) was dissolved in DMF-H₂O (10:1). To this was added NaN₃ (4.07 g, 62.7 mmol) and the reaction heated to 120 °C for 24 h. Upon cooling to room temperature, the reaction mixture was partitioned between EtOAc and H₂O. The organic
phase was washed with H₂O, brine, dried over MgSO₄, filtered and the solvents were concentrated under reduced pressure. The resulting residue was purified via flash chromatography (petroleum ether-EtOAc 1:1) to give the title compound 175 (2 g, 60%) as a colourless oil. [α]₀ +32.3° (c 0.03 in CHCl₃); IR (film) cm⁻¹: 3446, 2917, 2101 1642, 1376, 1211, 1062; ¹H NMR (500 MHz, CDCl₃) δ 5.86 (1H, ddt, J 17.1, 10.1, 7.0, CH₂CH=CH₂), 5.16 (1H, ddd, J 17.0, 3.2, 1.4, CH₂CH=CHH), 5.10 (1H, ddt, J 10.3, 2.0, 1.0, CH₂CH=CHH), 4.28 (1H, dd, J 7.4, 5.2, 2.0, CH₃), 4.03 (1H, td, J 7.2, 2.5, H-1), 3.80 (1H, brs, H-2), 3.51 (1H, dd, J 12.6, 7.8, H-6a), 3.26 (1H, dd, J 12.6, 5.2, H-6b), 2.45 – 2.32 (2H, m, CH₂CH=CH₂), 1.48 (3H, s, isopropylidene CH₃), 1.32 (3H, s, isopropylidene CH₃); ¹³C NMR (125 MHz, CDCl₃) δ 133.2 (CH₂CH=CH₂), 116.6 (CH₂CH=CH₂), 108.7 (isopropylidene C), 73.5 (C-2), 71.5 (C-4), 69.8 (C-3), 68.0 (C-1), 67.7 (C-5), 51.0 (C-6), 34.0 (CH₂CH=CH₂), 25.7 (isopropylidene CH₃), 23.5 (isopropylidene CH₃); ESI-HRMS calcd for C₁₂H₂₀N₃O₄ 270.1376, found m/z 270.1374 [M+H]⁺.

1-C-Allyl-1,6-dideoxy-2-O-isopentyl-3,4-O-isopropylidene-6-azido-α-D-galactopyranoside (176). To a stirred suspension of 175 (1.76 g, 6.5 mmol) in DMF (25 mL) at 0 °C was added sodium hydride (60% in mineral oil dispersion, 3.9 g, 9.7 mmol) slowly with vigorous stirring. After 15 min, 1-bromo-3-methylbutane (2.5 mL, 20.8 mmol) was added and the reaction was warmed to room temperature and stirred for 14 h. The reaction was quenched by the slow addition of MeOH (5 mL) and partitioned between EtOAc and H₂O. Phases were separated and the organic phase was washed with H₂O, brine, dried over MgSO₄, filtered and the solvents were concentrated under reduced pressure. The crude residue was purified via flash chromatography (petroleum ether-EtOAc 4:1) to give compound 176 (1.58g, 72%) as a colourless oil. [α]₀ +9.3° (c 0.05 in CHCl₃); IR (film) cm⁻¹: 2925, 2908 1642, 1372, 1210, 1061; ¹H NMR (500 MHz, CDCl₃) δ 5.81 (1H, ddt, J 17.1, 10.1, 7.0, CH₂CH=CH₂), 5.12 (1H, ddd, J 17.5, 3.1, 1.6, CH₂CH=CHH), 5.07 (1H, ddt, J
10.4, 2.1, 1.1, CH₂CH=CHH), 4.37 (1H, dd, J 7.5, 3.3, H-3), 4.20 (1H, dd, J 7.5, 1.7, H-4), 4.07 – 4.00 (2H, overlapping signals, H-1 & H-5), 3.66 (1H, dt, J 9.2, 6.7, OCH/H₂CH(CH₃)₂), 3.52 – 3.43 (2H, overlapping signals, OCH/HCH₂CH(CH₃)₂ & H-6a), 3.37 (1H, t, J 3.1, H-2), 3.26 (1H, dd, J 12.5, 5.5, H-6b), 2.45 – 2.38 (1H, m, CH₂CH=CH₂), 2.37 – 2.30 (1H, m, CHHCH=CH₂), 1.71 (1H, dq, J 20.1, 6.7, OCH₂CH₂CH(CH₃)₂), 1.49 (3H, s, isopropylidene CH₃), 1.46 (2H, m, OCH₂CH₂CH(CH₃)₂), 1.34 (3H, s, isopropylidene CH₃), 0.90 (6H, dd, J 6.7, 0.9, OCH₂CH₂CH₂CH(CH₃)₂), 

13C NMR (125 MHz, CDCl₃) δ 134.7 (CH₂C=CH₂), 117.2 (CH₂CH=CH₂), 109.9 (isopropylidene C), 75.8 (C-2), 73.1 (C-4), 71.7 (C-3), 71.4 (C-5), 69.6 (C-1), 69.0 (OCH₂CH₂CH(CH₃)₂), 52.1 (C-6), 39.0, 35.2, 26.8, 25.0, 24.7, 22.7; ESI-HRMS calcd for C₁₇H₂₉N₃O₄ 339.2258, found m/z 339.2258 [M+Na]⁺

1-C- Allyl-1,6-dideoxy-2-O-ethyl-3,4-O-isopropylidene-6-azido-α-D-galactopyranoside (177). To a stirred suspension of 175 (870 mg, 3.23 mmol) in DMF (7 mL) at 0°C was added sodium hydride (60% dispersion in mineral oil 193 mg, 4.85 mmol) slowly with vigorous stirring. After 15 min, iodoethane (904 µL, 11.3 mmol) was added and the reaction was warmed to room temperature and stirred for 14 h. EtOAc and H₂O were added and the phases were separated. The organic phase was washed with H₂O, brine, dried over MgSO₄, filtered and the solvents were concentrated under reduced pressure. The crude residue was purified via flash chromatography (petroleum ether-EtOAc 4:1) to give 177 (749 mg, 78%) as a colourless oil; [α]D +14.8° (c 0.06 in CHCl₃); IR (film) cm⁻¹: 2986, 2101 1641, 1375, 1059 905; ¹H NMR (500 MHz, CDCl₃) δ 5.79 (1H, ddt, J 17.1, 10.1, 7.0, CH₂C=CH₂), 5.07 (2H, ddd, J 13.7, 11.0, 1.2, CH₂CH=CH₂), 4.34 (1H, dd, J 7.4, 3.3, H-3), 4.19 (1H, dd, J 7.4, 1.8, H-4), 4.06 – 3.98 (2H, overlapping signals, H-1 & H-5), 3.68 (1H, dq, J 9.2, 7.0, OCH/HCH₂), 3.51 – 3.43 (2H, overlapping signals, H-6a & OCH/HCH₂), 3.36 (1H, t, J 3.1, H-2), 3.24 (1H, dd, J 12.5, 5.5, H-6b), 2.39 (1H, ddd, J 14.1, 7.7, 6.3, CH₂CH=CH₂), 2.32 (1H, dt, J 14.3, 7.3, CH₂CH=CH₂), 1.47 (3H, s, isopropylidene CH₃), 1.32 (3H, s, isopropylidene CH₃), 1.18 (H, t, J 7.0, OCH₂CH₂), ¹³C NMR (126 MHz, CDCl₃) δ 134.4 (CH₂C=CH₂), 117.2 (CH₂CH=CH₂), 109.9 (isopropylidene C), 75.8 (C-2), 73.1 (C-4), 71.7 (C-3), 71.4 (C-5), 69.6 (C-1), 69.0 (OCH₂CH₂CH(CH₃)₂), 52.1 (C-6), 39.0, 35.2, 26.8, 25.0, 24.7, 22.7; ESI-HRMS calcd for C₁₇H₂₉N₃O₄ 339.2258, found m/z 339.2258 [M+Na]⁺
24.5 (isopropylidene CH$_3$), 15.5 (OCH$_2$CH$_3$); ESI-HRMS calcd for C$_{14}$H$_{23}$N$_3$O$_4$Na 320.1689 found m/z 320.1683 [M+Na]$^+$

1-C-Allyl-1-deoxy-3,4-O-isopropylidene-6-O-dimethyltertbutylsilyl-α-D-galactopyranoside$^9$ (178). Compound 173 (8.8 g, 36 mmol) was dissolved in DMF (70 mL) and cooled to 0 °C. Imidazole (8.56 g, 126 mmol) was added followed by the addition of TBDMSCl (7.05 g, 46.8 mmol). The reaction mixture was stirred for 2 h at room temperature, diluted with EtOAc and washed with H$_2$O, brine, dried over MgSO$_4$, filtered and the solvents were concentrated under reduced pressure. The crude residue was purified via flash chromatography to give 178 (9.8 g, 76%) as a colourless oil. NMR data ($^1$H and $^{13}$C) was in agreement with reported literature data; $^1$H NMR (500 MHz, CDCl$_3$) δ 5.82 (1H, ddt, $J_{17.1, 10.2, 7.0}$, CH$_2$C=CH$_2$), 5.09 (1H, ddd, $J_{17.2, 3.3, 1.5}$, CHHCH=CH$_2$), 5.02 (1H, ddt, $J_{10.1, 2.0, 1.1}$, CHHCH=CH$_2$) 4.37 (1H, dd, $J_{7.1, 1.9}$, H-3), 4.20 (1H, dd, $J_{7.1, 3.4}$, H-4), 4.02 – 3.96 (2H, overlapping signals, H-5 & H-1), 3.79 – 3.67 (3H, overlapping signals, H-2 & H-6), 2.34 (2H, ddd, $J_{7.2, 2.4, 1.2}$, CH$_2$CH=CH$_2$), 1.46 (3H, s, isopropylidene CH$_3$), 1.31 (3H, s, isopropylidene CH$_3$), 0.91 – 0.85 (9H, m), 0.04 (6H, d, $J_{1.5}$); $^{13}$C NMR (126 MHz, CDCl$_3$) δ 134.5 (C2), 117.5 (C-1), 109.1 (C), 74.9 (C-7), 71.9 (C-6), 70.8, 69.9, 69.6 (C-5), 62.8 (C-9), 34.8 (C-3), 27.2, 25.9, 25.7, 24.7, 18.4, -5.2, -5.3 (each SiCH$_3$)

To a stirred suspension of 178 (4.2 g, 11.7 mmol) in DMF (15 mL) at 0°C was added sodium hydride (60% dispersion in mineral oil, 609 mg, 15.2 mmol) slowly with vigorous stirring. After 15 min, 1-bromo-3-methylbutane (4 mL, 35.1 mmol) was added and the reaction was warmed to room temperature and stirred for 14 h. MeOH (5 mL) was
added to quench the reaction and EtOAc and H₂O were added. The phases were separated and the organic phase was washed with H₂O, brine, dried over MgSO₄, filtered and the solvents were concentrated under reduced pressure. The crude residue was purified via flash chromatography (petroleum ether-EtOAc 6:1) to give compound 179 (3.76 g, 75%) as a colourless oil; [α]D +19.4° (c 0.03 in CHCl₃); IR (film) cm⁻¹: 2954, 2905, 2869, 1477, 1361, 1198, 882; ¹H NMR (500 MHz, CDCl₃) δ 5.79 (1H, ddt, J 17.1, 10.1, 7.1, CH₂CH=CH₂), 5.10 (1H, ddd, J 16.8, 3.1, 1.4, CH₂CH=CHH), 5.05 (1H, ddt, J 10.4, 2.1, 1.0, CH₂CH=CHH), 4.37 (1H, dd, J 7.3, 1.6, H-4), 4.29 (1H, dd, J 7.3, 3.2, H-3), 3.99 (1H, td, J 7.3, 2.8, H-1), 3.96 – 3.90 (1H, m, H-5), 3.73 (2H, dt, J 9.6, 6.7, H-6), 3.66 (1H, dt, J 9.2, 7.0, OCHHCH₂CH(CH₃)₂), 3.44 (1H, dt, J 9.2, 6.7, OCHHCH₂CH(CH₃)₂), 3.31 (1H, t, J 3.0, H-5), 2.42 – 2.30 (2H, m, CH₂CH=CH₂), 1.72 (1H, tt, J 13.4, 6.7, OCH₂CH₂CH(CH₃)₂), 1.49 (3H, s, isopropylidene CH₃), 1.45 (2H, dd, J 13.8, 7.1, OCH₂CH₂CH(CH₃)₂), 1.33 (3H, s, isopropylidene CH₃), 0.89 (15H, overlapping signals, OCH₂CH₂CH(CH₃)₂ & t-Bu-H), 0.07 (6H, dd, J 5.1, 2.8, tBuSi(CH₃)₂); ¹³C NMR (125 MHz, CDCl₃) δ 134.9 (CH₂CH=CH₂), 117.1 (CH₂CH=CH₂), 109.1 (isopropylidene C), 76.6 (C-2), 72.1 (C-4), 71.7 (C-3), 71.0 (C-1), 69.8 (C-5), 69.2 (OCH₂CH₂CH(CH₃)₂), 62.6 (C-6), 39.0 (OCH₂CH₂CH(CH₃)₂), 35.0 (CH₂CH=CH₂), 27.1, 26.0, 25.0 (each CH₃), 24.7 (OCH₂CH₂CH(CH₃)₂), 22.7 (2s), 18.4, 14.2, -5.1, -5.2 (each CH₃). ESI-HRMS calc'd for C₂₃H₄₅O₅Si 429.3035 found m/z 429.3036 [M+H]⁺

1-C-Allyl-1-deoxy-2-O-isopentyl-3,4-O-isopropylidene-α-D-galactopyranoside.

Compound 179 (1.4 g, 3.3 mmol) was dissolved in THF (25 mL) and TBAF (10 mL of a 1.0 M solution in THF) was added dropwise. The resulting solution was stirred for 3 h at room temperature. EtOAc was added and the organic layer was washed with 1M HCl, H₂O, brine, dried over MgSO₄, filtered and the solvents were concentrated under reduced pressure. The resulting residue was used without further purification (955 mg, 92% crude yield). IR (film) cm⁻¹: 3500 br, 2926, 1381, 1210, 1060; ¹H NMR (500 MHz, CDCl₃) δ 5.78 (1H, ddt, J 17.1, 10.1, 7.1, CH₂CH=CH₂), 5.08 (2H, dd, J 20.7, 13.8, CH₂CH=CH₂), 4.34 (1H, dd, J 7.4, 3.4,
Experimental data

1-C-Allyl-1-deoxy-2-O-isopentyl-3,4-O-isopropylidene-6-O-propargyl-α-D-galactopyranoside (180). To a stirred suspension of alcohol (955 mg, 3 mmol) in DMF (6 mL) at 0°C was added sodium hydride (60% dispersion in mineral oil, 145 mg, 3.6 mmol) slowly and with vigorous stirring. After 15 min, propargyl bromide (80% solution in toluene, 620 µL, 9 mmol) was added and the reaction was warmed to room temperature and stirred for 14 h. EtOAc and H₂O were added, phases were separated and the organic phase was washed with H₂O, brine, dried over MgSO₄, filtered and the solvents were concentrated under reduced pressure. The crude residue was purified via flash chromatography to give compound 180 (676 mg 64%) as a colourless oil; [α]D +21.7° (c 0.03 in CHCl₃); IR (film) cm⁻¹: 3529 sharp, 2954, 2905, 2869, 1477, 1361, 1198, 882; ¹H NMR (500 MHz, CDCl₃) δ 5.84 – 5.75 (1H, ddt, J 17.1, 10.1, 7.0, CH₂CH=CH₂), 5.08 (2H, ddd, J 13.7, 11.0, 1.3, CH₂CH=CH₂), 4.33 (1H, dd, J 7.4, 3.2, H-3), 4.29 (1H, dd, J 7.4, 1.5, H-4), 4.21 (2H, qd, J 15.7, 2.4, alkyne CH₂), 4.11 (1H, td, J 6.4, 1.6), 4.01 (1H, td, J 7.3, 2.9, H-1), 3.71 (1H, dd, J 9.8, 6.0, H-6a), 3.69 – 3.62 (2H, overlapping signals, OCHHCH₂CH(CH₃)₂ & H-6b), 3.44 (1H, dt, J 9.1, 6.6, OCHHCH₂CH(CH₃)₂), 3.35 (1H, t, J 3.0, H-2), 2.43 (1H, t, J 2.3, alkyne C-H), 2.37 (2H, td, J 14.0, 7.0, CH₂CH=CH₂), 1.76 – 1.67 (1H, m, OCH₂CH₂CH(CH₃)₂), 1.49 (3H, s, isopropylidene CH₃), 1.44 (2H, dd, J 13.5, 6.8, OCH₂CH₂CH(CH₃)₂), 1.33 (3H, s, isopropylidene CH₃), 0.89 (6H, s, CH₃); ¹³C NMR (125 MHz, CDCl₃) δ 134.7 (CH₂CH=CH₂), 117.2 (CH₂CH=CH₂), 109.7 (isopropylidene C), 76.0 (C-2), 73.5 (C-4), 71.9 (C-3), 71.1 (C-1), 69.6 (C-5), 69.4 (OCH₂CH₂CH(CH₃)₂), 63.5 (C-6), 38.9 (OCH₂CH₂CH(CH₃)₂), 34.9 (CH₂CH=CH₂), 26.8 (CH₃), 25.7 (OCH₂CH₂CH(CH₃)₂), 24.9, 24.7, 22.6 (each CH₃); ESI-HRMS calcd for C₁₇H₃₄NO₅ 332.2437, found m/z 332.2433 [M+NH₄]⁺
isopropylidene CH$_3$), 1.45 (2H, q, $J$ 6.7, OCH$_2$CH$_2$CH(CH$_3$)$_2$), 1.34 (3H, s, isopropylidene CH$_3$), 0.90 (6H, d, $J$ 6.7, OCH$_2$CH$_2$CH(CH$_3$)$_2$); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 134.8 (CH$_2$CH=CH$_2$), 117.2 (CH$_2$CH=CH$_2$), 109.5 (isopropylidene C), 79.9, 76.0 (C-2), 74.5, 73.0 (C-4), 71.6 (C-3), 71.1 (C-1), 70.1 (OCH$_2$CH$_2$CH(CH$_3$)$_2$), 69.3 (C-6), 68.5 (C-2), 58.7 (C-10), 39.0 (CH$_2$CH=CH$_2$), 35.1 (OCH$_2$CH$_2$CH(CH$_3$)$_2$), 26.9 (CH$_3$), 24.9 (OCH$_2$CH$_2$CH(CH$_3$)$_2$), 24.8 (CH$_3$), 22.7 (CH$_3$); ESI-HRMS calcd for C$_{20}$H$_{32}$O$_5$Na 375.2147, found m/z 375.2143 [M+Na]$^+$

1-C- Allyl-1,6-dideoxy-6-((1-C-allyl-1,6-dideoxy-2-O-isopentyl-3,4-$O$-isopropylidene-$\alpha$-D-galactopyranos-6-yl)oxymethyl)-1H-1,2,3-triazol-1-yl)-2-$O$-isopentyl-3,4-$O$- isopropylidene-$\alpha$-D-galactopyranoside (181). Alkyne 180 (110 mg, 0.31 mmol) and azide 176 (106 mg, 0.31 mmol) were dissolved in acetonitrile-H$_2$O (6 mL, 1:1). To this was added Cul (41 mg, 0.22 mmol) and the reaction was heated at reflux for 24 h. Upon cooling the reaction was diluted with EtOAc, washed with H$_2$O, satd NH$_4$Cl, brine, dried over MgSO$_4$, filtered and the solvents were concentrated under reduced pressure. The crude residue was purified via flash chromatography (EtOAc-petroleum ether 1:1) to give compound 181 (118 mg 55%) as a colourless oil. [$\alpha$]$_D$ +24.2$^o$ (c 0.002 in CHCl$_3$); IR (film) cm$^{-1}$: 3572, 2955, 2871, 1466, 1361, 1200, 882; $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.71 (1H, s, triazole H), 5.84 – 5.74 (1H, ddt, $J$ 17.1, 10.1, 7.0, CH$_2$CH=CH$_2$), 5.71 (1H, ddd, $J$ 17.3, 8.7, 5.3, CH$_2$CH=CH$_2$), 5.13 – 4.99 (4H, m, each CH$_2$CH=CH$_2$), 4.70 (2H, q, $J$ 12.4,-C=C-CH$_2$-O), 4.60 (1H, dd, $J$ 13.9, 3.4, H-6’a), 4.39 (2H, overlapping signals, H6’b & H-4’), 4.34 – 4.28 (2H, overlapping signals, H-3’ & H-4), 4.21 (1H, dd, $J$ 9.7, 2.3, H-3), 4.18 (1H, brd, H-5), 4.11 (1H, t, $J$ 6.2, H-5’), 4.09 – 4.04 (1H, m, H-1’), 4.01 (1H, td, $J$ 7.3, 2.7, H-1), 3.73 – 3.59 (4H, overlapping
Experim

tnental data

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signals, OCH₂CH₂CH(CH₃)₂ & H-6), 3.42 (3H, overlapping signals, OCH₂CH₂CH(CH₃)₂ & H-2), 3.33 (1H, apt t, J 2.7, H-2’), 2.43 – 2.34 (2H, m, CH₂CH=CH₂), 2.29 (1H, dd, J 14.6, 7.4, CHHCH=CH₂), 2.22 (1H, dd, J 13.9, 6.9, CHHCH=CH₂), 1.74 – 1.61 (2H, m, each OCH₂CH₂CH(CH₃)₂), 1.52 (3H, s, isopropylidene CH₃), 1.48 (3H, s, isopropylidene CH₃), 1.43 (4H, dq, J 13.6, 6.8, each OCH₂CH₂CH(CH₃)₂), 1.36 (3H, s, isopropylidene CH₃), 1.34 (3H, s, isopropylidene CH₃), 0.88 (12H, dd, J 7.8, 6.8, each OCH₂CH₂CH(CH₃)₂); ¹³C NMR (125 MHz, CDCl₃) δ 134.4 (CH₂C=CH₂), 124.1 (triazole C-H), 117.2 (CH₂CH=CH₂), 110.0 (C), 109.3 (C), 76.1 (CH), 75.5 (CH), 73.1 (CH), 72.9 (CH), 71.6 (CH), 71.6 (CH), 71.5 (CH), 71.0 (CH), 70.5 (CH), 69.8 (CH), 69.3 (CH₂), 68.9 (CH), 68.5 (CH), 65.0 (CH₂), 51.9 (CH₂), 39.0 (OCH₂CH₂CH(CH₃)₂), 35.2 (CH₂CH=CH₂), 27.0 (isopropylidene CH₃), 26.7 (isopropylidene CH₃), 24.9 (OCH₂CH₂CH(CH₃)₂), 24.9 (isopropylidene CH₃), 24.8 (isopropylidene CH₃), 22.7 (2s), 22.6 (2s), (each OCH₂CH₂CH(CH₃)₂); ESI-HRMS calcd for C₃₇H₆₂N₃O₉ 692.4486, found m/z 692.4472 [M+H]^+.

1-C-allyl-1,6-dideoxy-6-((1-C-allyl-1,6-dideoxy-2-O-ethyl-3,4-O-isopropylidene-α-D-galactopyranos-6-yl)oxymethyl)-1H-1,2,3-triazol-1-yl)-2-O-isopentyl-3,4-O-isopropylidene-α-D-galactopyranoside (182). Alkyne 180 (189 mg, 0.54 mmol) and azide 177 (159 mg, 0.54 mmol) were dissolved in a mixture of acetonitrile-H₂O (6 mL, 1:1). To this was added CuI (51 mg, 0.27 mmol) and the reaction was heated at reflux for 6 h. Upon cooling the reaction was diluted with EtOAc, washed with 1M HCl, H₂O, brine, dried over MgSO₄, filtered and the solvents were concentrated under reduced pressure. The crude residue was purified via flash chromatography (EtOAc-petroleum ether, 1:1) to give the title compound (288 mg, 82%) as a colourless oil; [α]D +35.5° (c 0.004 in CHCl₃); IR (film) cm⁻¹:
2923, 1559, 1372, 1098, 935; $^1$H NMR (500 MHz, CDCl$_3$) δ 7.72 (1H, s, triazole H), 5.85 – 5.65 (2H, m, each CH$_2$CH=CH$_2$), 5.14 – 4.99 (4H, m, each CH$_2$CH=CH$_2$), 4.71 (2H, q, $J$ 12.4, O-CH$_2$C=C), 4.62 (1H, dd, $J$ 14.0, 3.8, H-6’a), 4.42 – 4.36 (2H, overlapping signals, H-6’b & H-3’), 4.34 – 4.27 (2H, overlapping signals, H-3 & H-4’), 4.23 (1H, dd, $J$ 8.2, 3.3, H-5), 4.20 (1H, d, $J$ 7.6, H-4), 4.11 (1H, apt t, $J$ 6.2, H-5’), 4.08 – 4.03 (1H, m, H-1’), 4.01 (1H, apt t, $J$ 7.2, H-1), 3.73 – 3.61 (4H, overlapping signals, H-6, OCHHCH$_2$CH(CH$_3$)$_2$ & OCHHCH$_3$), 3.44 (2H, overlapping signals, OCHHCH$_2$CH(CH$_3$)$_2$ & OCHHCH$_3$), 3.40 (1H, t, $J$ 2.7, H-2’), 3.33 (1H, apt s, H-2), 2.36 (2H, m, CH$_2$CH=CH$_2$), 2.30 (1H, dd, $J$ 14.3, 7.0, CHHCH=CH$_2$), 2.23 (1H, dd, $J$ 13.9, 6.9, CHHCH=CH$_2$), 1.70 (1H, dt, $J$ 13.4, 6.7, OCH$_2$CH$_2$CH(CH$_3$)$_2$), 1.52 (3H, s, isopropylidene CH$_3$), 1.48 (3H, s, isopropylidene CH$_3$), 1.44 (2H, dd, $J$ 13.4, 6.7, OCH$_2$CH$_2$CH(CH$_3$)$_2$), 1.35 (3H, s, isopropylidene CH$_3$), 1.33 (3H, s, isopropylidene CH$_3$), 1.16 (3H, t, $J$ 6.9, OCH$_2$CH$_3$), 0.89 (6H, d, $J$ 6.6, OCH$_2$CH$_2$CH(CH$_3$)$_2$); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 144.89 (triazole CH=C), 134.38 (CH$_2$CH=CH$_2$), 124.23 (triazole CH=C), 117.28 (CH$_2$CH=CH$_2$), 110.03 (isopropylidene C), 109.33 (isopropylidene C), 76.01 (C-2), 75.21 (C-2), 72.91 (C-4), 72.81 (C-4’), 71.57 (C-3’), 71.48 (C-3), 71.35 (C-1’), 70.84 (C-1), 70.44 (C-6), 69.20 (OCH$_2$), 68.76 (C-5), 68.37 (C-5’), 66.57 (OCH$_2$), 64.88 (OCH$_2$C=C), 51.88 (C-6’), 38.86 (OCH$_2$CH$_2$CH(CH$_3$)$_2$), 35.07 (CH$_2$CH=CH$_2$), 26.85 (isopropylidene CH$_3$), 26.62 (isopropylidene CH$_3$), 24.82 (isopropylidene CH$_3$), 24.65 (OCH$_2$CH$_2$CH(CH$_3$)$_2$), 24.62 (isopropylidene CH$_3$), 22.58 (OCH$_2$CH$_2$CH(CH$_3$)$_2$), 15.50 (OCH$_2$CH$_3$); ESI-HRMS calcd for C$_{34}$H$_{55}$N$_3$O$_9$Na 672.3836, found m/z 672.3861 [M+Na]$^+$

**Macrocycle 183.** To a stirred solution of 181 (118 mg, 0.171 mmol) in dioxane-H$_2$O (3:1, 2 mL) was added 2,6-lutidine (69 µL, 0.599 mmol), NaIO$_4$ (165 mg, 0.77 mmol) and a catalytic
amount of OsO₄ (2 drops, 2.5% solution in t-BuOH). The reaction mixture was stirred at room temperature for 2.5 h. H₂O and CH₂Cl₂ were added, layers were separated and the aqueous layer was extracted into CH₂Cl₂. The combined organic phases were washed with brine, dried over MgSO₄, filtered and the solvents were concentrated under reduced pressure. The resulting residue was taken up in 1,2-dichloroethane (5 mL) and isopentylamine (20 µL, 0.171 mmol) was added dropwise. The solution was stirred at room temperature for 20 min. Sodium triacetoxyborohydride (127 mg, 0.599 mmol) was then added and the reaction mixture stirred for 3 h. The reaction was quenched by the addition of satd NaHCO₃ and the product was extracted into EtOAc, dried with MgSO₄, filtered and the solvents were concentrated under reduced pressure. The crude residue was purified via flash chromatography (CH₂Cl₂-EtOAc 1:9) to give compound 183 (69 mg, 54%) as an off white solid; [α]D +50.7° (c 0.002 in CHCl₃); IR (film) cm⁻¹: 2957, 1466, 1382, 1212, 1099, 905; ¹H NMR (500 MHz, CDCl₃) δ 7.81 (1H, s, triazole H), 4.77 (1H, d, J 11.7, OCH=C), 4.70 (1H, d, J 11.7, OCHHC=C), 4.65 (1H, d, J 13.2, H-6’a), 4.40 (1H, dd, J 7.6, 3.2, H-3), 4.36 (1H, dd, J 14.3, 10.0, H-6’b), 4.27 (1H, dd, J 7.7, 1.4, H-3’), 4.24 (1H, apt d, J 6.1, H-4’), 4.14 (1H, dd, J 6.4, 1.9, H-4), 4.09 (2H, overlapping signals, H-5 & H-5’), 4.06 – 4.00 (1H, m, H-1’), 3.98 (1H, dt, J 9.6, 3.2, H-1), 3.80 – 3.70 (2H, m, H-6’), 3.64 (2H, overlapping signals, OCH₂CH₂CH(CH₃)₂ & H-2’), 3.51 (2H, overlapping signals, OCHHCH₂CH(CH₃)₂ & H-2’), 3.36 – 3.40 (1H, m, OCH/HCH₂CH(CH₃)₂), 3.36 – 3.32 (1H, m, H-2), 2.72 (3H, d, J 11.3), 2.52 (2H, dd, J 19.5, 11.0), 2.44 (1H, dd, J 17.4, 10.4), 1.87 – 1.74 (2H, m) (each CH₂CH₃N), 1.67 (5H, overlapping signals, 3 x CH and CH₂), 1.53 (3H, s, isopropylidene CH₃), 1.52 (3H, s, isopropylidene CH₃), 1.50 – 1.40 (4H, overlapping signals, OCH₂CH₂CH(CH₃)₂), 1.38 (3H, s, isopropylidene CH₃), 1.33 (5H, overlapping signals, CH₂ & isopropylidene CH₃), 0.94 – 0.82 (18H, m, OCH₂CH₂CH(CH₂)₂); ¹³C NMR (125 MHz, CDCl₃) δ 146.0 (triazole C=C), 123.2 (triazole CH=C), 110.2 (isopropylidene C), 109.7 (isopropylidene C), 76.5, 74.6 (each C-2), 73.9, 73.6 (each C-4), 71.6 (C-3), 71.3 (C-1), 71.1 (C-6), 70.4 (C-1), 69.9 (OCH₂CH₂CH(CH₃)₂), 69.5 (OCH₂CH₂CH(CH₃)₂), 69.1 (C-5), 68.7 (C-5), 65.9 (OCH₂C=C) 52.7 (C-6), 51.6 (CH₂CH₂N), 51.1 (CH₂CH₂N), 49.6 (CH₂CH₂N), 38.9 (CH₂), 38.7 (CH₂), 27.6, 26.7, 26.6, 25.8, 25.0, 24.8, 24.6, 22.9, 22.7 (3s), 22.6, 22.5; ESI-HRMS calcd for C₄₀H₇₁N₄O₉ 751.5221, found m/z 751.5220 [M+H]+
Macrocycle 185. To a stirred solution of 181 (110 mg, 0.159 mmol) in dioxane-H₂O (3:1, 2 mL) was added 2,6-lutidine (65 µL, 0.565 mmol), NaIO₄ (152 mg, 0.715 mmol) and a catalytic amount of OsO₄ (2 drops, 2.5% solution in t-BuOH). The reaction mixture was stirred at room temperature for 2.5 h. H₂O and CH₂Cl₂ were added, the layers were separated and the aqueous layer was extracted into CH₂Cl₂. The combined organic phases were washed with brine, dried over MgSO₄, filtered and the solvents were concentrated under reduced pressure. The resulting residue was taken up in 1,2-dichloroethane (5 mL) and benzyl amine (17 µL, 0.159 mmol) was added dropwise. The solution was stirred at room temperature for 20 min. Sodium triacetoxyborohydride (127 mg, 0.599 mmol) was then added and the reaction mixture stirred for 3 h. The reaction was quenched by the addition of satd NaHCO₃ and the product was extracted into EtOAc, dried with MgSO₄, filtered and the solvents were concentrated under reduced pressure. The crude residue was purified via flash chromatography (CH₂Cl₂-EtOAc 1:9) to give compound 185 (92 mg, 75%) as an off white solid; [α]D +50.7° (c 0.002 in CHCl₃); IR (film) cm⁻¹: 2957, 1466, 1382, 1212, 1099, 905; ¹H NMR (500 MHz, CDCl₃) δ 7.82 (1H, s, triazole H), 7.37 – 7.19 (5H, m, Ar-H), 4.75 (2H, q, J 12.0, OCH₂C=C), 4.64 (1H, dd, J 14.3, 1.3, H-6’a), 4.37 (1H, dd, J 7.6, 3.3, H-3’), 4.32 (1H, dd, J 14.4, 9.9, H-6’b), 4.27 – 4.22 (2H, overlapping signals, H3 & H-4’), 4.17 (1H, dd, J 6.6, 1.9, H-4), 4.12 – 4.08 (1H, m, H-1), 4.0 (2H, overlapping signals, H-5 and H-5’), 3.95 – 3.90 (1H, m, H-1’), 3.77 – 3.72 (1H, m, H-6a), 3.70 (1H, dd, J 10.3, 3.0), 3.62 (1H, dt, J 9.4, 6.7, OCHHCH₂CH(CH₃)₂), 3.59 – 3.53 (2H, m, PhCH₂N), 3.54 – 3.47 (1H, m, OCHHCH₂CH(CH₃)₂), 3.45 (1H, dd, J 6.7, 2.7, OCHHCH₂CH(CH₃)₂), 3.42 (1H, dd, J 8.8, 3.8, OCHHCH₂CH(CH₃)₂), 3.27 (1H, m, H-2’), 3.23 (1H, apt t, J 3.2, H-2), 2.75 – 2.67 (1H, m), 2.67 – 2.54 (2H, m), 2.53 – 2.45 (1H, m) (each CH₂N), 1.87 – 1.75 (2H, m), 1.69 (2H, dt,
J 18.8, 6.1, OCH₂CH₂CH(CH₃)₂, 1.64 – 1.58 (1H, m, CH₂CH₂CH(CH₃)₂), 1.51 (3H, s, isopropylidene CH₃), 1.49 (3H, s, isopropylidene CH₃), 1.43 (4H, dt, J 9.6, 5.9, each OCH₂CH₂CH(CH₃)₂), 1.35 (3H, s, isopropylidene CH₃), 1.34 (3H, s, isopropylidene CH₃), 0.87 (12H, ddd, J 14.1, 6.6, 2.1, OCH₂CH₂CH(CH₃)₂); ¹³C NMR (125 MHz, CDCl₃) δ 145.7 (triazole CH=), 128.6, 128.3, 128.1, 128.1, 126.9, 126.7 (each Ar-C), 123.3 (triazole CH=C), 110.1 (isopropylidene C), 109.4 (isopropylidene C), 77.4 (C-2), 76.0 (C-2), 74.0 (C-4), 73.7 (C-4), 73.5 (C-3), 71.5 (C-3’), 71.1, 70.3 (C-1), 70.3 (C-1’), 69.7 (C-5), 69.3 (C-5’), 69.0 (OCH₂CH₂CH(CH₃)₂), 68.7 (OCH₂CH₂CH(CH₃)₂), 65.9 (OCH₂C=C), 56.8 (PhCH₂N), 53.1 (C-6), 51.5 (CH₂N), 50.8 (CH₂N), 38.8 (CH₂), 38.6 (CH₂), 29.6, 27.3 (isopropylidene CH₃), 26.5 (isopropylidene CH₃), 25.5 (isopropylidene CH₃), 24.8 (isopropylidene CH₃), 24.7, 24.5, 22.6, 22.5, 22.3; ESI-HRMS calcd for C₄₂H₆₆N₄O₉ 771.4908, found m/z 771.4901 [M+H]⁺

**Macrocycle 184.** To a stirred solution of 181 (120 mg, 0.173 mmol) in dioxane-H₂O (3:1, 2 mL) was added 2,6-lutidine (71 µL, 0.607 mmol), NaIO₄ (165 mg, 0.77 mmol) and a catalytic amount of OsO₄ (2 drops, 2.5% solution in t-BuOH). The reaction mixture was stirred at room temperature for 2.5 h. H₂O and CH₂Cl₂ were added, the layers were separated and the aqueous layer was extracted into CH₂Cl₂. The combined organic phases were washed with brine, dried over MgSO₄, filtered and the solvents were concentrated under reduced pressure. The resulting residue was taken up in 1,2-dichloroethane (5 mL) and 2-(2-Naphthyl)ethylamine hydrochloride (36 mg, 0.173 mmol) was added dropwise. The solution was stirred at room temperature for 20 min. Sodium triacetoxyborohydride (127 mg, 0.599 mmol) was then added and the reaction mixture stirred for 3 h. The reaction was quenched by
the addition of satd NaHCO₃ and the product was extracted into EtOAc, dried with MgSO₄, filtered and the solvents were concentrated under reduced pressure. The crude residue was purified via flash chromatography (CH₂Cl₂-EtOAc 1:9) to give compound 184 (88 mg, 61%) as an off white solid; [α]D +40.7° (c 0.010 in CHCl₃); ¹H NMR (500 MHz, CDCl₃) δ 7.80 (1H, s, Ar-H), 7.80 – 7.73 (3H, m, Ar-H), 7.61 (1H, s, Ar-H), 7.42 (2H, tt, J 13.4, 6.7, Ar-H), 7.31 (1H, dd, J 8.4, 1.2, Ar-H), 4.77 (1H, d J 11.7, OCHH=C), 4.70 (1H, d J 11.7, OCHH/C=C), 4.64 (1H, d, J 13.6, H-6′a), 4.35 (2H, overlapping signals, H-3 & H-6′b), 4.22 – 4.18 (1H, m, H-4), 4.15 (1H, apt t, J 6.1, H-4′), 4.03 (5H, overlapping signals, H-5, H-5′, H-1 & H-1′), 3.77 – 3.71 (1H, m, H-6α), 3.68 (1H, dd, J 10.3, 2.2, H-6β), 3.65 – 3.56 (2H, m, OCH₂CH₂CH(CH₃)₂), 3.48 (2H, overlapping signals, H-2′ & OCHHCH₂CH(CH₃)₂), 3.42 – 3.36 (1H, m, OCHHCH₂CH(CH₃)₂), 3.30 (1H, t, J 3.0, H-2), 2.92 – 2.85 (2H, m), 2.84 – 2.71 (5H, m), 2.61 (1H, m) (each CH₂N), 1.79 (1H, dd, J 16.9, 6.9), 1.69 (4H, dt, J 13.3, 6.7), 1.61 (1H, dt, J 20.0, 6.7), 1.52 (3H, s, isopropylidene CH₃), 1.50 (3H, s, isopropylidene CH₃), 1.48 – 1.37 (4H, m, each CH₂), 1.35 (3H, s, isopropylidene CH₃), 1.32 (3H, s, isopropylidene CH₃), 0.85 (12H, ddd, J 9.0, 6.6, 2.8, OCH₂CH₂CH(CH₃)₂); ¹³C NMR (125 MHz, CDCl₃) δ 146.0 (triazole CH=C), 138.1, 133.6, 132.0, 127.9, 127.6, 127.5, 127.4, 126.8, 126.0, 125.3 (each Ar-C), 123.1 (triazole CH=C), 110.1 (isopropylidene CH₃), 109.6 (isopropylidene CH₃), 76.6 (C-2), 74.6 (C-2), 73.8 (C-4), 73.5 (C-4), 71.7 (C-3), 71.3 (C-3), 71.1 (C-6), 70.4 (C-1), 69.8 (C-1), 69.4 (C-5), 69.0 (C-5′), 68.6 (OCH₂CH₂CH(CH₃)₂), 66.1 (OCH₂=C=C), 53.3 (CH₃N), 52.7 (C-6), 52.0 (CH₂N), 50.9 (CH₂N), 38.8 (CH₂), 38.7 (CH₂), 27.6 (isopropylidene CH₃), 26.7 (isopropylidene CH₃), 25.8 (isopropylidene CH₃), 24.9 (isopropylidene CH₃), 24.8, 24.6, 22.7, 22.6 (2s), 22.4; ESI-HRMS calcd for C₄₇H₇¹N₄O₉
835.5221, found m/z 835.5218 [M+H]⁺
Macrocycle 186. To a stirred solution of 182 (112 mg, 0.172 mmol) in dioxane-\(\text{H}_2\text{O}\) (3:1, 2 mL) was added 2,6-Lutidine (69 µL, 0.599 mmol), \(\text{NaIO}_4\) (165 mg, 0.77 mmol) and a catalytic amount of \(\text{OsO}_4\) (2 drops, 2.5% solution in t-BuOH). The reaction mixture was stirred at room temperature for 2.5 h. \(\text{H}_2\text{O}\) and \(\text{CH}_2\text{Cl}_2\) were added, the layers were separated and the aqueous layer was extracted into \(\text{CH}_2\text{Cl}_2\). The combined organic phases were washed with brine, dried over \(\text{MgSO}_4\), filtered and the solvents were concentrated under reduced pressure. The resulting residue was taken up in 1,2-dichloroethane (5 mL) and isopentylamine (20 µl, 0.171 mmol) was added dropwise. The solution was stirred at room temperature for 20 min. Sodium triacetoxyborohydride (127 mg, 0.599 mmol) was then added and the reaction mixture stirred for 3 h. The reaction was quenched by the addition of satd \(\text{NaHCO}_3\) and the product was extracted into \(\text{EtOAc}\), dried with \(\text{MgSO}_4\), filtered and the solvents were concentrated under reduced pressure. The crude residue was purified via flash chromatography (\(\text{CH}_2\text{Cl}_2\)-\(\text{EtOAc}\) 1:9) to give compound 186 (86 mg, 71%) as an off white solid; \([\alpha]_D^{20}+43.3^\circ\) (c 0.003 in \(\text{CHCl}_3\)); \(^1\text{H}\) NMR (500 MHz, \(\text{CDCl}_3\)) \(\delta\) 7.78 (1H, s, triazole H), 4.75 (1H, d, \(J_{11.8}\), O\(\text{CH}_2\text{C}')) 4.68 (1H, d, \(J_{11.8}\), O\(\text{CH}_2\text{C}')) 4.64 (1H, apt d, \(J_{13.9}\), H-6’a), 4.37 (1H, dd, \(J_{7.6, 3.2}\), H-3), 4.32 (1H, dd, \(J_{14.4, 10.0}\), H-6’b), 4.26 (1H, dd, \(J_{7.6, 1.2}\), H-3), 4.21 (1H, t, \(J_{6.1}\), H-4), 4.11 (1H, dd, \(J_{6.4, 1.8}\), H-4), \(4.10 – 4.03\) (2H, overlapping signals, H-5 & H-5’), \(4.03 – 3.98\) (1H, m, H-1), \(3.97 – 3.92\) (1H, m, H-1), \(3.76 – 3.67\) (2H, m, H-6), \(3.63\) (2H, overlapping signals O\(\text{CH}_2\text{H}_3\) & O\(\text{CH}_2\text{H}_2\text{C}(\text{CH}_3)_2\)), \(3.47\) (3H, overlapping signals, H-2’, O\(\text{CHHCH}_3\) & O\(\text{CHHCH}_2\text{CH(CH}_3)_2\)), \(3.31\) (1H, t, \(J_{2.5}\), H-2), \(2.67\) (2H, dd, \(J_{24.4, 12.4}\), \(\text{CH}_2\text{N}\)), \(2.49\) (2H, m, \(\text{CH}_2\text{N}\)), \(2.45 – 2.33\) (2H, m, \(\text{CH}_2\text{N}\)), \(1.82 – 1.60\) (5H, overlapping signals, \(\text{CH}_2\text{N}\) & O\(\text{CH}_2\text{CH}(\text{CH}_3)_2\)), \(1.54\) (1H, m, CH) \(1.51\) (3H, s, isopropylidene \(\text{CH}_3\)) \(1.49\) (3H, s, isopropylidene \(\text{CH}_3\)), \(1.47 – 1.38\) (4H, m,
OCH₂CH₂CH(CH₃)₂), 1.35 (3H, s, isopropylidene CH₃), 1.31 (3H, s, isopropylidene CH₃), 1.13 (3H, t, J 7.0, OCH₂CH₂), 0.91 – 0.82 (12H, m, OCH₂CH₂CH(CH₃)₂); ¹³C NMR (125 MHz, CDCl₃) δ 144.9 (triazole C=CH), 122.0 (triazole C=CH), 109.0 (isopropylidene C), 108.5 (isopropylidene C), 76.4 (C-2), 75.3 (C-2), 73.6 (C-4), 72.8 (C-4), 72.5 (C-3’), 70.6 (C-3), 70.2 (C-6), 70.0 (C-1’), 69.2 (C-1), 68.3 (C-5’), 68.0 (OCH₂CH₂CH(CH₃)₂), 67.5 (C-5), 65.7 (OCH₂CH₃), 65.0 (OCH₂C=C), 51.7 (C-6’), 50.7, 50.2, 48.6 (each CH₂N), 37.8 (OCH₂CH₂CH(CH₃)₂), 26.5, 25.6, 25.5, 24.7, 23.9, 23.4, 21.8, 21.6 (2s), 21.5 (OCH₂CH₂CH(CH₃)₂), 14.5 (OCH₂CH₃); ESI-HRMS calcd for C₃₇H₆₅N₄O₉ 709.4751, found m/z 709.4745 [M+H]⁺

**Macrocycle 187.** To a stirred solution of 182 (100 mg, 0.154 mmol) in dioxane-H₂O (3:1, 2 mL) was added 2,6-lutidine (63 µL, 0.539 mmol), NaIO₄ (148 mg, 0.69 mmol) and a catalytic amount of OsO₄ (2 drops, 2.5% solution in t-BuOH). The reaction mixture was stirred at room temperature for 2.5 h. H₂O and CH₂Cl₂ were added, layers were separated and the aqueous layer was extracted into CH₂Cl₂. The combined organic phases were washed with brine, dried over MgSO₄, filtered and the solvents were concentrated under reduced pressure. The resulting residue was taken up in 1,2-dichloroethane (5 mL) and benzyl amine (17 µL, 0.154 mmol) was added dropwise. The solution was stirred at room temperature for 20 min. Sodium triacetoxyborohydride (127 mg, 0.599 mmol) was then added and the reaction mixture stirred for 3 h. The reaction was quenched by the addition of satd NaHCO₃ and the product was extracted into EtOAc, dried with MgSO₄, filtered and the solvents were concentrated under reduced pressure. The crude residue was purified via flash
chromatography (CH$_2$Cl$_2$-EtOAc 1:9) to give compound 187 (73 mg, 65%) as an off white solid; [α]$_D$ +33.4° (c 0.004 in CHCl$_3$); $^1$H NMR (500 MHz, CDCl$_3$) δ 7.75 (1H, s, triazole H), 7.30 – 7.12 (5H, m, Ar-H), 4.68 (2H, q, J 12.1, O-CH$_2$C=C), 4.56 (1H, d, J 14.0, H-6’a), 4.31 – 4.27 (1H, m, H-3), 4.27 – 4.21 (1H, m, H-6’b), 4.20 – 4.15 (2H, overlapping signals, H-3’ & H-4), 4.10 (1H, dd, J 6.6, 1.2, H-4), 4.02 (3H, overlapping signals, H-1’, H-5 & H-5’), 3.87 – 3.80 (1H, m, H-1), 3.64 (2H, dt, J 10.2, 8.8, H-6), 3.57 – 3.50 (2H, m, OCH$_2$CH$_2$CH(CH$_3$)$_2$), 3.50 – 3.42 (2H, overlapping signals, NC=HPh & OCH$_2$CH$_3$), 3.42 – 3.34 (1H, m, NCH$_2$Ph), 3.33 (1H, t, J 4.8, H-2’), 3.24 – 3.14 (2H, overlapping signals, H-2 & OCH$_2$CH$_3$), 2.60 (2H, d, J 6.7, CHHN), 2.48 (1H, dd, J 15.6, 8.3, CHH-N), 2.45 – 2.36 (1H, m, CHHN), 1.74 (2H, m, CH$_2$CH$_2$N) 1.67 – 1.54 (2H, overlapping signals, CHHCH$_2$N & OCH$_2$CH$_2$CH(CH$_3$)$_2$), 1.47 (1H, m, CHHCH$_2$N), 1.43 (3H, s, isopropylidene CH$_3$), 1.42 (3H, s, isopropylidene CH$_3$), 1.36 (2H, dd, J 13.4, 6.6, OCH$_2$CH$_2$CH(CH$_3$)$_2$), 1.27 (3H, s, isopropylidene CH$_3$), 1.26 (3H, s, isopropylidene CH$_3$), 1.00 (3H, t, J 7.0, OCH$_2$CH$_3$), 0.81 (6H, dt, J 17.1, 8.6, OCH$_2$CH$_2$CH(CH$_3$)$_2$); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 145.8 (Ar-C), 140.3 (C=CH), 128.7, 128.4, 128.2, 128.2, 127.0 (each Ar-C), 123.4 (C=CH), 110.2 (isopropylidene C), 109.5 (isopropylidene C), 77.5 (C-2), 75.8 (C-2’), 74.0 (C-4), 73.8 (C-4), 73.6 (C-3’), 71.7 (C-3’), 71.2 (C-6), 70.3, (C-1’) 70.2 (C-1), 69.4 (C-5), 69.1 (C-5’), 68.7 (OCH$_2$CH$_2$CH(CH$_3$)$_3$, 66.7 (OCH$_2$CH$_3$), 65.9 (OCH$_2$C=C), 57.0 (PhCH$_2$N), 53.2 (C-6’), 51.4 (CH$_2$CH$_3$N), 50.9 (CH$_2$CH$_3$N), 38.9 (OCH$_2$CH$_2$CH(CH$_3$)$_3$, 27.4 (isopropylidene CH$_3$), 26.6 (isopropylidene CH$_3$), 25.5 (isopropylidene CH$_3$), 24.9 (isopropylidene CH$_3$), 24.6 (OCH$_2$CH$_2$CH(CH$_3$)$_3$, 22.7 (OCH$_2$CH$_2$CH(CH$_3$)$_3$, 15.5 (OCH$_2$CH$_3$)); ESI-HRMS calcd for C$_{39}$H$_{61}$N$_4$O$_9$ 729.4438, found m/z 729.4431 [M+H]$^+$
Macrocycle 188. Compound 183 (50 mg, 0.066 mmol) was dissolved in TFA-H$_2$O (4:1, 1.5 mL) and stirred at room temperature for 2 h. Solvents were removed under reduced pressure and the residue was azeotroped with toluene to remove excess TFA. The resulting residue was taken up in MeOH and basified to pH 8 using Dowex® M-43 ion exchange resin, filtered and concentrated under reduced pressure to give 183 (34 mg, 75%) as a white powder. $^1$H NMR (500 MHz, CD$_3$OD) δ 7.84 (1H, s, triazole-H), 4.62 (1H, dd, J 14.3, 10.3, CHHN), 4.54 (2H, q, J 11.9, OCH$_2$C=CH), 4.51 – 4.43 (1H, m, CHHN), 4.04 – 3.98 (1H, m, CH), 3.91 (3H, overlapping signals, each CH), 3.73 (1H, br s, CH), 3.68 (3H, overlapping signals, CH), 3.63 – 3.51 (6H, overlapping signals, each CH$_2$), 3.47 (2H, dd, J 15.4, 7.0, CH$_2$), 2.49 – 2.24 (6H, m, each CH$_2$N), 1.72 – 1.53 (7H, overlapping signals, 2 x CH$_2$ and 3 x OCH$_2$CH$_2$CH(CH$_3$)$_2$), 1.45 – 1.32 (6H, m, OCH$_2$CH$_2$CH(CH$_3$)$_2$), 0.81 (18H, brs, each OCH$_2$CH$_2$CH(CH$_3$)$_2$); $^{13}$C NMR (125 MHz, CD$_3$OD) δ 145.7 (C=CH), 124.0 (C=CH), 77.0 (CH), 76.9 (CH), 72.0 (CH$_2$C=CH), 71.5, 71.3, 70.6, 70.0, 69.9, 69.3, 69.2 (each CH), 69.1 (CH$_2$), 69.0 (CH), 64.1, 51.5, 51.4, 50.9, 48.4, 38.7 (each CH$_2$), 33.9, 26.6, 24.7 (CH), 22.1, 21.8, 21.8 (each CH$_2$), 21.7, 21.6, 21.5, 21.4 (each CH$_3$); ESI-HRMS calcd for C$_{34}$H$_{63}$N$_4$O$_9$ 671.4595, found m/z 671.4592 [M+H]$^+$
Macrocycle 189. Compound 184 (70 mg, 0.084 mmol) was dissolved in TFA-H_2O (4:1, 1.5 mL) and stirred at room temperature for 2 h. Solvents were removed under reduced pressure and the residue was azeotroped with toluene to remove excess TFA. The resulting residue was taken up in MeOH and basified to pH 8 using Dowex® M-43 ion exchange resin, filtered and the solvents were concentrated under reduced pressure to give 189 (48 mg, 76%) as a white powder. \(^1\)H NMR (500 MHz, CD_3OD) δ 7.84 (1H, s, triazole H), 7.68 (3H, dd, J 18.1, 8.0, ArH), 7.51 (1H, s, ArH), 7.32 (2H, td, J 13.2, 6.3, ArH), 7.20 (1H, d, J 8.4, ArH), 4.61 (1H, dd, J 14.3, 10.2, H-6’a), 4.56 (2H, m, CH_2), 4.51 – 4.41 (1H, m, H-6’b), 4.03 (2H, overlapping signals, each CH), 3.90 – 3.84 (2H, m), 3.69 (4H, dd, J 18.3, 9.0), 3.63 – 3.50 (5H, overlapping signals), 3.50 – 3.41 (3H, overlapping signals), 2.73 (4H, overlapping signals, CH_2N), 2.53 (2H, d, J 6.6, CH_2N), 2.33 (1H, m, CHH), 2.16 (1H, m, CHH), 1.66 (6H, overlapping signals, 2 x CH & 2 x CH_2), 1.32 (4H, dt, J 21.6, 6.9), 0.80 – 0.72 (6H, m), 0.68 (6H, dd, J 13.9, 8.6); \(^1^3\)C NMR (125 MHz, CD_3OD) δ 145.6 (C=CH), 136.0, 133.3, 133.1 (each Ar-C), 127.7, 127.5, 127.2, 126.4, 125.7, 125.6 (each Ar-CH), 124.1 (C=CH), 76.9, 76.7, 72.9, 71.3, 71.2, 70.0, 69.9, 69.5, 69.2 (each CH), 69.0, 64.0, 51.4, 51.2, 50.9, 48.3, 38.8 (Each CH_2), 33.8, 26.4 (CH), 24.7, 21.8, 21.7 (each CH_2), 21.5, 21.4 (each CH_3); ESI-HRMS calcd for C_{41}H_{63}N_{6}O_{9} 755.4595, found m/z 755.4592 [M+H]^+.
**Macrocycle 190.** Compound 185 (50 mg, 0.065 mmol) was dissolved in TFA-H$_2$O (4:1, 1.5 mL) and stirred at room temperature for 2 h. Solvents were removed under reduced pressure and the residue was azeotroped with toluene to remove excess TFA. The resulting residue was taken up in MeOH and basified to pH 9 using Dowex® M-43 ion exchange resin, filtered and the solvents were concentrated under reduced pressure to give 190 (32 mg, 71%) as a white powder. $^1$H NMR (500 MHz, CD$_3$OD) $\delta$ 7.94 (1H, s, triazole-H), 7.34 (3H, d, J 3.9, ArH), 7.29 (2H, d, J 6.3, ArH), 4.74 (1H, d, J 10.6, H-6’a), 4.68 (1H, d, J 12.2, H-6’b), 4.63 – 4.55 (2H, brs, OCH$_2$C=C), 4.53 (1H, m, CH), 4.09 (1H, brs, CH), 4.01 (2H, overlapping signals, each CH), 3.81 (4H, overlapping signals, each CH), 3.70 (2H, overlapping signals, each CH), 3.67 – 3.47 (6H, overlapping signals, each CH$_2$), 3.47 – 3.41 (2H, m, CH$_2$), 2.51 (2H, m), 2.47 – 2.39 (1H, m), 2.20 – 2.11 (1H, m) (each CH$_2$N), 1.74 (2H, m, CH$_2$), 1.65 (4H, overlapping signals, each CH$_2$), 1.47 – 1.34 (4H, overlapping signals, each CH$_2$), 0.86 (12H, dd, J 10.0, 6.6, OCH$_2$CH$_2$CH(CH$_3$)$_2$); $^{13}$C NMR (125 MHz, CD$_3$OD) $\delta$ 145.6 (C=CH), 138.5 (Ar-C), 127.9, 127.6, 127.3 (each Ar-CH), 124.1 (C=CH), 76.9, 76.5, 72.8, 71.5, 71.3, 70.5, 69.8, 69.4, 69.2 (each CH), 69.0 (CH$_2$), 64.0 (CH), 59.2, 51.4, 51.3, 50.9, 38.8 (each CH$_2$), 33.6, 26.4, 24.7 (each CH$_2$), 21.8, 21.7, 21.5, 21.4 (each CH$_3$); ESI-HRMS calcd for C$_{36}$H$_{59}$N$_4$O$_9$ 791.4282, found m/z 791.4275 [M+H]$^+$
Macrocycle 191. Compound 186 (50 mg, 0.070 mmol) was dissolved in TFA-H₂O (4:1, 1.5 mL) and stirred at room temperature for 2 h. Solvents were removed under reduced pressure and the residue was azeotroped with toluene to remove excess TFA. The resulting residue was taken up in MeOH and basified to pH 8 using Dowex® M-43 ion exchange resin, filtered and the solvents were concentrated under reduced pressure to give 191 (30 mg, 69%) as a white powder. ¹H NMR (500 MHz, CD₃OD) δ 7.88 (1H, s, triazole-H), 4.70 – 4.62 (1H, m, H-6’a), 4.58 – 4.48 (3H, overlapping signals, OCH₂C=C & H-6’b), 4.02 (2H, overlapping signals, each CH), 3.93 – 3.86 (2H, overlapping signals, each CH), 3.77 (2H, overlapping signals, each CH), 3.69 (2H, overlapping signals, each CH), 3.64 – 3.45 (7H, overlapping signals, 3 x CH & 2 x CH₂), 2.56 (4H, m), 2.06 (1H, m), 1.84 (1H, m) (each CH₂N), 1.63 (3H, td, J 13.5, 6.8), 1.47 (1H, dd, J 13.2, 6.6), 1.42 – 1.32 (2H, m, each OCH₂CH₂CH(CH₃)₂), 1.32 – 1.22 (4H, overlapping signals, each OCH₂CH₂CH(CH₃)₂), 1.09 (3H, t, J 7.0, OCH₂CH₃), 0.83 (12H, ddd, J 8.9, 6.5, 3.5, each OCH₂CH₂CH(CH₃)₂); ¹³C NMR (125 MHz, CD₃OD) δ 145.7 (C=CH), 124.0 (C=CH), 78.4, 78.1, 73.3, 73.1, 73.0, 72.4, 71.8, 71.1, 70.6, 70.5 (each CH), 67.5, 65.4, 53.4, 52.6, 52.2, 40.2 (each CH₂), 27.7, 26.1 (CH), 23.2 (CH), 23.1, 22.9, 22.8 (each CH₃), 16.0 (CH₃); ESI-HRMS calcd for C₃₁H₅₆N₄O₉ 628.4047, found m/z 628.4040 [M+H]⁺
Macrocycle 192. Compound 187 (62 mg, 0.085 mmol) was dissolved in TFA-H$_2$O (4:1, 1.5 mL) and stirred at room temperature for 2 h. Solvents were removed under reduced pressure and the residue was azeotroped with toluene to remove excess TFA. The resulting residue was taken up in MeOH and basified to pH 8 using Dowex® M-43 ion exchange resin, filtered and the solvents were concentrated under reduced pressure to give 192 (42 mg, 76%) as a white powder. $^1$H NMR (500 MHz, CD$_3$OD)  $\delta$ 7.85 (1H, s, triazole H), 7.34 – 7.03 (5H, m, Ar-H), 4.65 (1H, dd, $J$ 14.4, 10.5, H-6’a), 4.59 (1H, d, $J$ 12.1, OCHHC=C), 4.52 (1H, d, $J$ 12.4, OCHHC=C), 4.45 (1H, d, $J$ 15.2, H-6’b), 4.01 – 3.96 (1H, m, CH), 3.96 – 3.87 (3H, overlapping signals, each CH), 3.74 (1H, s, CH), 3.72 – 3.59 (4H, overlapping signals), 3.56 (1H, dd, $J$ 9.3, 3.2), 3.53 – 3.43 (3H, m), 3.42 (2H, d, $J$ 6.1), 3.36 (3H, ddd, $J$ 16.3, 12.2, 6.2), 2.40 (2H, m), 2.30 (1H, m), 2.05 (1H, m) (each CH$_2$N), 1.75 – 1.60 (2H, m, CH$_2$), 1.60 – 1.49 (3H, overlapping signals, CH$_2$ & OCH$_2$CH$_2$CH(CH$_3$)$_2$), 1.31 (2H, tt, $J$ 13.6, 6.7, OCH$_2$CH$_2$CH(CH$_3$)$_2$), 1.01 (3H, t, $J$ 7.0, OCH$_2$CH$_3$), 0.84 – 0.75 (6H, m, OCH$_2$CH$_2$CH(CH$_3$)$_2$); $^{13}$C NMR (125 MHz, CD$_3$OD)  $\delta$ 145.7 (C=CH), 128.6, 128.2, 128.0, 127.9, 126.6, 124.1 (C=CH), 76.9, 76.7, 71.6, 71.4, 71.1, 70.3, 69.8, 69.7, 69.1 (each CH), 68.9, 65.8, 63.9, 59.2, 56.2, 52.1, 51.9, 51.7, 50.7, 38.7 (each CH$_2$), 24.6 (CH), 21.7 (CH$_3$), 21.4 (CH$_3$), 16.0 (CH$_3$); ESI-HRMS calcd for C$_{33}$H$_{53}$N$_4$O$_9$ 649.3812, found m/z 641.3811 [M+H]$^+$
Macrocycle 193. Compound 188 (20 mg, 0.029 mmol) was dissolved in pyridine-Ac₂O (1:1, 3 mL) and stirred at room temperature for 5 h. Solvents were removed under reduced pressure and the residue was taken up in EtOAc and washed with H₂O, brine, dried over MgSO₄, filtered and the solvents were concentrated under reduced pressure to give 193 (20mg, 80%) as a white solid; IR (film) cm⁻¹: 2956, 2253, 1745, 1370, 1239, 1107, 903; ¹H NMR (500 MHz, CDCl₃) δ 7.70 (1H, s, triazole H), 5.51 – 5.47 (1H, m, H-4’), 5.37 (1H, dd, J 2.7, 1.2, H-4), 5.15 (1H, dd, J 9.5, 3.4, H-3’), 5.05 (1H, dd, J 9.7, 3.3, H-3), 4.68 (2H, q, J 12.5, CH₂), 4.55 (1H, dd, J 14.1, 0.9, H-6’a), 4.37 (1H, dd, J 14.1, 10.2, H-6’b), 4.27 – 4.20 (1H, m, H-1’), 4.19 – 4.08 (2H, overlapping signals, H-5’ & H-1), 3.87 (1H, d, J 6.9, H-5), 3.79 – 3.72 (2H, overlapping signals, H-2 & H-2’), 3.66 – 3.58 (2H, m, OCH₂CH₂CH(CH₃)₂), 3.59 – 3.50 (4H, overlapping signals, OCH₂CH₂CH(CH₃)₂ & C-6), 2.47-2.25 (6H, m, each CH₂N), 2.20, 2.14, 2.06, 2.03 (each 3H, each s, each acetate CH₃), 1.84 – 1.59 (6H, overlapping signals, 2 x CH₂ & CH), 1.41 (4H, qd, J 13.7, 6.9, OCH₂CH₂CH(CH₃)₂), 1.23 (2H, m, -NCH₂CH₂CH(CH₃)₂), 0.88 (18H, t, J 6.6, OCH₂CH₂CH(CH₃)₂); ¹³C NMR (125 MHz, CDCl₃) δ 170.4, 170.4, 170.1, 170.0 (each C=O), 146.3 (triazole CH=C), 123.5 (triazole CH=C), 74.4 (C-2), 74.3 (C-2’), 72.4 (C-1), 72.0 (C-1’), 70.3 (C-3), 70.2 (C-5), 70.0 (CH₂), 69.7 (CH₂), 69.5 (C-4), 69.4 (C-3’), 69.3 (C-5’), 69.0 (C-4’), 65.2 (CH₂), 51.7 (CH₂N), 51.6 (CH₂N), 50.4 (C-6’), 49.5 (CH₂N), 38.9 (CH₂), 30.4 (CH), 29.7, 29.5, 26.7 (CH), 24.9 (CH), 23.2, 22.9, 22.8 (each CH₂), 22.7, 22.6, 21.0, 20.9 (2s), 20.8 (each CH₃); ESI-HRMS calcd for C₄₂H₇₁N₄O₁₃ 839.5017, found m/z 839.5015 [M+H]⁺
Macrocycle 194. Compound 189 (30 mg, 0.039 mmol) was dissolved in pyridine-Ac$_2$O (1:1, 3 mL) and stirred at room temperature for 5 h. Solvents were removed under reduced pressure and the residue was taken up in EtOAc and washed with H$_2$O, brine, dried over MgSO$_4$, filtered and the solvents were concentrated under reduced pressure to give 194 (33 mg, 91%) as a white solid; $[\alpha]_D$ +60° (c 0.006 in CHCl$_3$); IR (film) cm$^{-1}$: 2956, 2253, 1745, 1370, 1107, 903; $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.82 – 7.73 (4H, m, ArH), 7.59 (1H, s, triazole H), 7.43 (2H, td, $J$ 13.1, 6.2, ArH), 7.30 – 7.27 (1H, m, ArH), 5.45 – 5.41 (1H, m, H-4'), 5.35 (1H, dd, $J$ 3.0, 1.2, H-4), 5.14 (1H, dd, $J$ 9.2, 3.4, H-3'), 5.06 (1H, dd, $J$ 9.7, 3.4, H-3), 4.72 – 4.65 (2H, q, $J$ 12.5, OCH$_2$C=C), 4.49 (1H, dd, $J$ 14.1, 1.4, H-6'a), 4.39 (1H, dd, $J$ 13.8, 10.6, H-6'b), 4.29 – 4.22 (1H, m, H-1'), 4.18 – 4.09 (2H, m, H-1 & H-5'), 3.87 (1H, d, $J$ 7.0, H-5), 3.80 – 3.71 (2H, overlapping signals, H-2 & H-2'), 3.69 – 3.60 (1H, m), 3.59 – 3.51 (5H, overlapping signals) (each CH$_2$), 2.90 – 2.78 (4H, m), 2.69 – 2.61 (2H, m), 2.54 – 2.46 (1H, m), 2.40 – 2.32 (1H, m), 2.19, 2.15, 2.06, 2.04 (each 3H, each s, each acetate CH$_3$), 1.93 – 1.81 (3H, m), 1.66 (2H, ddt, $J$ 27.0, 13.4, 6.7, OCH$_2$CH$_2$CH(CH$_3$)$_2$), 1.41 (4H, dtd, $J$ 20.8, 13.7, 6.9, OCH$_2$CH$_2$CH(CH$_3$)$_2$), 0.89 – 0.81 (12H, m, OCH$_2$CH$_2$CH(CH$_3$)$_2$); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 170.4, 170.3, 170.1, 170.0 (each C=O), 146.1 (triazole CH=C), 133.6, 132.1 (each Ar-C), 128.2, 127.7, 127.5, 127.2, 126.9, 126.1, 125.4 (each Ar-CH), 123.7 (triazole CH=C), 74.4 (C-2), 74.3 (C-2), 72.3 (C-1), 71.8 (C-1), 70.3, 70.2 (C-5), 70.0 (C-3), 69.8 (CH$_2$), 69.6 (CH), 69.3 (CH), 68.8 (CH), 65.2 (OCH$_2$C=C), 52.3, 51.4, 51.1, 50.3 (each CH$_2$N), 38.9, 38.9 (each CH$_2$), 24.9 (CH), 24.9 (CH), 22.7, 22.6, 22.5 (2s) (each CH$_3$), 21.0, 20.9 (2s), 20.8 (each acetate CH$_3$); ESI-HRMS calcd for C$_{49}$H$_{71}$N$_4$O$_{13}$ 923.5017, found m/z 923.5016 [M+H]$^+$
Macrocyclic **195**: Compound **190** (25 mg, 0.036 mmol) was dissolved in pyridine-Ac₂O (1:1, 2.5 mL) and stirred at room temperature for 5 h. Solvents were removed under reduced pressure and the residue was taken up in EtOAc and washed with H₂O, brine, dried over MgSO₄, filtered and the solvents were concentrated under reduced pressure to give **195** (27 mg, 89%) as a white solid; [α]_D^25 +51.2° (c 0.009 in CHCl₃) IR (film) cm⁻¹: 2956, 2253, 1745, 1370, 1239, 1107, 903;¹H NMR (500 MHz, CDCl₃) δ 7.72 (1 H, s, triazole H), 7.41 – 7.30 (2H, m, ArH), 7.19 (3H, m, ArH), 5.49 – 5.46 (1H, m, H-4’), 5.38 – 5.35 (1H, m, H-4), 5.13 (1H, dd, J 8.9, 3.3, H-3’), 5.04 (1H, dd, J 9.4, 3.4, H-3), 4.71 (1H, d, J 12.6, OCH₃HC=C), 4.64 (1H, d, J 12.6, OCH₃HC=C), 4.54 (1H, d, J 13.0, H-6’a), 4.41 (1H, m, H-6’b), 4.24 – 4.16 (2H, overlapping signals, H-1 & H-5), 4.07 – 4.01 (1H, m, H-1), 3.88 (1H, d, J 7.6, H-5), 3.70 – 3.63 (3H, overlapping signals, H-2, H-2’ & H-6a), 3.58 – 3.53 (3H, overlapping signals, H-6b & NCH₂Ph), 3.47 (2H, ddd, J 16.1, 11.4, 4.6, OCH₂CH₂CH(CH₃)₂), 3.41 – 3.36 (2H, m, OCH₂CH₂CH(CH₃)₂), 2.55 – 2.39 (2H, m, CH₂N), 2.32 (2H, dd, J 9.6, 7.3, CH₂N), 2.19, 2.13, 2.07, 2.04 (each 3H, each s, each acetate CH₃), 1.79 (2H, m, CH₂), 1.70 (2H, m, CH₂) 1.60 (2H, td, J 13.5, 6.8, OCH₂CH₂CH(CH₃)₂), 1.34 (4H, dtd, J 20.6, 13.7, 6.8, OCH₂CH₂CH(CH₃)₂), 0.87 – 0.82 (12H, m, OCH₂CH₂CH(CH₃)₂);¹³C NMR (125 MHz, CDCl₃) δ 170.2, 170.2, 169.9, 169.8 (each C=O), 146.1 (triazole CH=C), 128.9 (Ar-C), 128.5, 128.2, 128.2, 126.3 (each Ar-CH), 123.4 (triazole CH=C), 74.2, 74.2 (each C-2), 71.8, 71.6 (each C-1), 70.0 (C-5), 69.9 (C-3), 69.7 (CH₂), 69.5 (CH₂), 69.4 (C-5), 69.4 (C-3), 69.1 (C-6), 68.9 (C-4), 68.6 (C-4’), 65.1 (OCH₂C=C), 56.5 (NCH₂Ph) 51.8, 51.6, 50.7 (each -CH₂N-), 38.7, 38.6 (each CH₂), 24.7 (CH), 22.5, 22.5 (CH₃), 22.4 (CH₂), 22.4, 21.6, 20.8, 20.8, 20.7, 20.7 (each acetate CH₃). ESI-HRMS calcd for C₄₄H₆₇N₄O₁₃ 859.4704, found m/z 859.4710 [M+H]^+
Macrocycle 196. Compound 191 (20 mg, 0.032 mmol) was dissolved in pyridine-Ac₂O (1:1, 3 mL) and stirred at room temperature for 6 h. Solvents were removed under reduced pressure and the residue was taken up in EtOAc and washed with H₂O, brine, dried over MgSO₄, filtered and the solvents were concentrated under reduced pressure to give 196 (24 mg, 93%) as a white solid; [α]D +83.4° (c 0.003 in CHCl₃) IR (film) cm⁻¹: 2956, 2253, 1745, 1370, 1239, 1107, 903; ¹H NMR (500 MHz, CDCl₃) δ 7.70 (1H, s, triazole H), 5.53 – 5.48 (1H, m, H-4'), 5.37 (1H, dd, J 3.1, 1.5, H-4), 5.37 (1H, dd, J 9.2, 3.1, H-3'), 5.15 (1H, dd, J 9.7, 3.3, H-3), 4.68 (2H, q, J 12.5, OCH₂C=C), 4.55 (1H, dd, J 14.0, 1.0, H-6’a), 4.37 (1H, dd, J 14.1, 10.4, H-6’b), 4.28 – 4.21 (1H, m, H-1’), 4.17 (1H, d, J 10.4, H-5’), 4.15 – 4.10 (1H, m, H-1), 3.90 – 3.84 (1H, m, H-5), 3.82 – 3.72 (2H, overlapping signals, H-2 & H-2’), 3.70 – 3.59 (3H, overlapping signals, H-6 & OCHHCH₃), 3.58 – 3.50 (3H, overlapping signals, OCHHCH₃ & OCH₂CH₂CH(CH₃)₂), 2.45 (3H, dt, J 16.1, 8.3), 2.36 (2H, ddd, J 20.2, 11.0, 7.0), 2.26 (1H, m), 2.19, 2.14, 2.07, 2.03 (each 3H, each s, each acetate CH₃), 1.70 – 1.60 (3H, m), 1.55 – 1.47 (2H, m), 1.44 – 1.37 (3H, m), 1.23 (2H, m, -NCH₂CH₂CH(CH₃)₂), 1.18 (3H, t, J 7.0, OCH₂CH₂), 0.88 (12H, d, J 6.7, each OCH₂CH₂CH(CH₃)₂); ¹³C NMR (125 MHz, CDCl₃) δ 170.2, 170.2, 170.0, 169.9 (each C=O), 146.2 (triazole CH=C), 123.4 (triazole CH=C), 74.2, 74.1 (C-2 & C-2’), 72.3, 71.8 (C-1 & C-1’), 70.2 (C-5), 70.1 (C-3), 69.6 (CH₂), 69.4 (CH₂), 69.2 (C-5’), 69.2 (C-3’), 68.8 (C-4), 66.9 (C-4’), 65.1 (CH₂), 51.6, 51.5 (each CH₂N), 50.2 (C-6), 49.5 (CH₂N), 38.8 (CH₂), 35.0 (CH), 26.5 (CH), 24.8 (CH), 23.2, 22.8 (CH₂), 22.7, 22.5 (OCH₂CH₂CH(CH₃)₂), 21.2 (CH₂), 20.8, 20.8 (2s), 20.7 (each CH₃), 15.5 (OCH₂CH₃); ESI-HRMS calcd for C₃₉H₆₅N₄O₁₃ 797.4548, found m/z 797.4541 [M+H]+
**Experimental data**

Chapter 6

**Macrocycle 197.** Compound 192 (25 mg, 0.038 mmol) was dissolved in pyridine-Ac₂O (1:1, 1.5 mL) and stirred at room temperature for 5 h. Solvents were removed under reduced pressure and the residue was taken up in EtOAc and washed with H₂O, brine, dried over MgSO₄, filtered and the solvents were concentrated under reduced pressure to give 197 (29 mg, 92%) as a white solid; [α]D +44.0° (c 0.007 in CHCl₃) IR (film) cm⁻¹: 2956, 2253, 1745, 1370, 1239, 1107, 903; ¹H NMR (500 MHz, CDCl₃) δ 7.63 (1H, s, triazole H), 7.35 – 7.21 (3H, m, Ar-H), 7.12 (2H, m, Ar-H), 5.42 (1H, apt t, J 3.0, H-4’), 5.30 (1H, dd, J 3.0, 2.3, H-4), 5.05 (1H, dd, J 8.8, 3.3, H-3’), 4.97 (1H, dd, J 9.4, 3.4, H-3), 4.65 (1H, d, J 12.7, OCHHC=C), 4.57 (1H, d, J 12.6, OCHHC=C), 4.48 (1H, dd, J 14.3, 1.3, H-6a’), 4.33 (1H, dd, J 13.9, 10.6, H-6b’), 4.16 – 4.08 (2H, overlapping signals, H-1’ & H-5’), 4.01 – 3.96 (1H, m, H-1), 3.83 – 3.79 (1H, m, H-5), 3.63 – 3.56 (3H, overlapping signals, H-2, H-2’ & H-6a), 3.46 (4H, overlapping signals, H-6b, NCH₂Ph & OCHHCH₃), 3.39 (1H, m, OCHHCH₂CH(CH₃)₂), 3.36–3.29 (2H, overlapping signals, OCHHCH₃ & OCHH/CH₂CH(CH₃)₂), 2.49–2.32 (3H, m), 2.28–2.20 (1H, m) (each CH₂N), 2.11, 2.06, 2.00, 1.97 (each 3H, each s, each acetate CH₃), 1.64 (4H, overlapping signals, each CH₂) 1.53 (1H, td, J 13.4, 6.7, OCH₂CH₂CH(CH₃)₂), 1.27 (2H, dt, J 21.3, 6.9, OCH₂CH₂CH(CH₃)₂), 1.00 (3H, t, J 7.0, OCH₂CH₃), 0.77 (6H, t, J 6.4, OCH₂CH₂CH(CH₃)₂); ¹³C NMR (125 MHz, CDCl₃) δ 170.3, 170.0 (2s) (each C=O), 146.2 (triazole CH=C), 129.1 (Ar-C), 128.7, 128.4, 128.3, 126.5 (each Ar-CH), 123.5 (triazole CH=C), 74.3, 74.1 (C-2 & C-2’), 71.9, 71.6 (C-1 & C-1’), 70.2 (C-5), 70.0 (C-3), 69.6 (C-3’), 69.3 (2s) (CH₂ & C-5’), 69.2 (C-4), 69.0 (C-6), 68.8 (C-4’), 66.9 (CH₂), 65.2 (OCH₂=C=C), 56.9, 52.0, 51.8, 50.8, 50.1 (each CH₂N), 38.8 (CH₂), 24.9 (CH), 22.7, 22.5, 21.0 (2s), 20.9, 20.8, 15.5 (each CH₃); ESI-HRMS calcd for C₄₁H₆₁N₂O₁₃ 817.4235, found m/z 817.4227 [M+H]⁺
6.4 Chapter 4-Experimental

1-C- Allyl-1,6-dideoxy-2-O-benzyl-3,4-O-isopropylidene-6-azido-α-D-galactopyranoside (201). To a stirred suspension of 175 (1.5 g, 5.5 mmol) in DMF (15 mL) at 0 °C was added sodium hydride (60% dispersion in mineral oil, 290 mg, 7.2 mmol) slowly with vigorous stirring. After 15 min, benzyl bromide (1.65 mL, 13.9 mmol) was added and the reaction was allowed warm to room temperature and stirred for 14 h. EtOAc and H₂O were added, phases separated and the organic phase was washed with H₂O, brine, dried over MgSO₄, filtered and the solvents were concentrated under reduced pressure. The crude residue was purified via flash chromatography to give 201 (1.54 g, 77%) as a colourless oil; [α]D + 7.0° (c 0.05 in CHCl₃); IR (film) cm⁻¹: 2981, 2927, 1711, 1380, 1210, 1059; ¹H NMR (500 MHz, CDCl₃) δ 7.37 – 7.28 (5H, m, PhH), 5.81 – 5.71 (1H, ddt, J 17.1, 10.1, 7.0, CH₂CH=CH₂), 5.06 (2H, ddd, J 10.3, 8.4, 3.3 CH₃CH=CH₂), 4.70 (1H, d, J 11.8, CHHPh), 4.55 (1H, d, J 11.8, CHHPh), 4.41 (1H, dd, J 7.3, 3.5, H-3), 4.21 (1H, dd, J 7.3, 1.8, H-4), 4.09 (1H, ddd, J 7.4, 5.5, 1.8, H-5), 4.05 (1H, ddd, J 7.9, 6.7, 3.1, H-1), 3.55 (1H, t, J 3.3, H-2), 3.50 (1H, dd, J 12.5, 7.6, H-6a), 3.26 (1H, dd, J 12.5, 5.5, H-6b), 2.43 (1H, ddd, J 18.8, 7.9, 6.5, CHHCH=CH₂), 2.33 (1H, dt, J 14.3, 7.1, CHHCH=CH₂), 1.47 (3H, s, isopropylidene CH₃), 1.34 (3H, s, isopropylidene CH₃); ¹³C NMR (125 MHz, CDCl₃) δ 137.9 (Ar-C), 134.5 (CH₂CH=CH₂), 128.5, 128.0, 127.8 (each Ar-CH), 117.3 (CH₂CH=CH₂), 109.9 (isopropylidene C), 75.3 (C-2), 73.0 (C-4), 72.7 (CH₂Ph), 71.8 (C-3), 71.5 (C-1), 69.0 (C-5), 52.0 (C-6), 34.9 (CH₂CH=CH₂), 26.8 (isopropylidene CH₃), 24.8 (isopropylidene CH₃); ESI-HRMS calcd for C₁₉H₂₅N₃O₄Na382.1732 found m/z 382.1721 [M+Na]⁺
1-C-ALLYL-1,6-DIDEOXY-2-O-NAPHTHALEN-2-YLMETHYL-3,4-O-ISOPROPYLIDENE-6-AZIDO-α-D-
GALACTOPYRANOSIDE (202). To a stirred suspension of 175 (1.4 g, 5.2 mmol) in DMF (10 mL)
at 0 °C was added sodium hydride (60 % dispersion in mineral oil, 312 mg, 7.8 mmol) slowly
with vigorous stirring. After 15 min, 2-(bromomethyl)-naphthalene (4 mL, 18.2 mmol) was
added and the reaction was warmed to room temperature and stirred for 24 h. EtOAc and
H2O were added, phases were separated and the organic phase was washed with H2O, brine,
dried over MgSO4, filtered and the solvents were concentrated under reduced pressure. The
crude residue was purified via flash chromatography (Petroleum ether-EtOAc 4:1) to give
202 (1.44 g, 68%) as a colourless oil. [α]D + 7.9° (c 0.07 in CHCl3) IR (film) cm⁻¹: 2986, 2101
1641, 1375, 1059, 905; ¹H NMR (500 MHz, CDCl3) δ 7.84 – 7.79 (3H, m, ArH), 7.75 (1H, s,
ArH), 7.46 (3H, m, ArH), 5.77 (1H, ddt, J 17.1, 10.1, 7.0, CH2CH=CH2), 5.09 – 4.99 (2H, m,
CH2CH=CH2), 4.83 (1H, d, J 11.9, CH2Ar), 4.69 (1H, d, J 11.9, CH2Ar), 4.42 (1H, dt, J 15.3,
7.7, H-3), 4.20 (1H, dd, J 7.3, 1.8, H-4), 4.11 (1H, ddd, J 7.3, 5.5, 1.7, H-5), 4.06 (1H, ddd, J
8.1, 6.5, 3.1, H-1), 3.59 (1H, t, J 3.3, H-2), 3.50 (1H, dd, J 12.5, 7.6, H-6a), 3.26 (1H, dd, J
12.5, 5.5, H-6b), 2.45 (1H, ddd, J 14.4, 7.9, 6.6, CH2CH=CHH), 2.35 (1H, dt, J 14.2, 6.7,
CH2CH=CHH), 1.46 (3H, s, isopropylidene CH3), 1.32 (3H, s, isopropylidene CH3); ¹³C
NMR (125 MHz, CDCl3) δ 135.3 (Ar-C), 134.4 (CH2CH=CH2), 133.2, 133.1 (each Ar-C),
128.3, 127.9, 127.7, 126.6, 126.3, 126.1, 125.7 (each Ar-CH), 117.3 (CH2CH=CH2), 109.8
(isopropylidene C), 75.3 (C-2), 73.0 (C-4), 72.8 (CH2-Ar), 71.9 (C-3), 71.4 (C-1), 68.9 (C-5),
52.0 (C-6), 34.8 (CH2CH=CH2), 26.8 (isopropylidene CH3), 24.7 (isopropylidene CH3); ESI-
HRMS calcd for C23H27N3O4Na 432.2002 found m/z 432.2011 [M+Na]+
1-C-Allyl-1,6-dideoxy-6-(4-(((1-C-allyl-1,6-dideoxy-2-O-isopentyl-3,4-O-isopropylidene-α-D-galactopyranos-6-yl)oxy methyl)-1H-1,2,3-triazol-1-yl)-2-O-isopentyl-3,4-O-isopropylidene-α-D-galactopyranoside (203). Alkyne 180 (135 mg, 0.383 mmol) and azide 201 (135 mg, 0.383 mmol) were dissolved in a mixture of acetonitrile-H$_2$O (4 mL, 1:1). To this was added CuI (66 mg, 0.345 mmol) and the reaction was heated at reflux for 24 h. Upon cooling the reaction was diluted with EtOAc, washed with H$_2$O, brine, dried over MgSO$_4$, filtered and the solvents were concentrated under reduced pressure. The crude residue was purified via flash chromatography (EtOAc-Petroleum ether 1:1) to give compound 203 (209 mg, 77%) as a yellow oil. [α]$_D$ +19.4° (c 0.05 in CHCl$_3$); IR (film) cm$^{-1}$: 2923, 1512, 1373, 1092, 908; $^1$H NMR (500 MHz, CDCl$_3$) 7.70 (1H, s, triazole H), 7.36 – 7.31 (2H, m, Ar-H), 7.29 (3H, dd, $J_{12.5}$, 5.1, ArH), 5.79 (1H, ddt, $J_{16.9}$, 10.1, 7.0, CH$_2$C=CH$_2$), 5.64 (1H, ddt, $J_{17.2}$, 10.1, 7.0, CH$_2$C=CH$_2$), 5.13 – 4.93 (4H, overlapping signals, each CH$_2$CH=CH$_2$), 4.74 – 4.67 (2H, q, $J_{12.4}$, OCH$_2$C=C), 4.65 (1H, d, $J_{11.7}$, CHHPh), 4.61 (1H, dd, $J_{14.0}$, 4.1, H-6’a), 4.52 (1H, d, $J_{11.8}$, CHHPh), 4.44 – 4.36 (2H, overlapping signals, H-6’b, H-3’), 4.33 – 4.23 (3H, overlapping signals, H-4, H-3 & H-5’), 4.20 (1H, dd, $J_{7.4}$, 1.4, H-4’), 4.11 (1H, t, $J_{6.3}$, H-5), 4.09 – 4.03 (1H, m, H-1’), 4.01 (1H, td, $J_{7.3}$, 2.7, H-1), 3.74 – 3.61 (3H, overlapping signals, H-6 & OCHHCH$_2$CH(CH$_3$)$_2$), 3.56 (1H, t, $J_{3.4}$, H-2’), 3.46 – 3.39 (1H, m, OCHHCH$_2$CH(CH$_3$)$_2$), 3.33 (1H, t, $J_{2.7}$, H-2), 2.38 (2H, dt, $J_{16.1}$, 7.3, CH$_2$CH=CH$_2$), 2.34 – 2.27 (1H, m, CH$_2$CH=CH$_2$), 2.22 – 2.15 (1H, m, CH$_2$CH=CH$_2$), 1.70 (1H, tt, $J_{13.4}$, 6.7, OCH$_2$CH$_2$CH(CH$_3$)$_2$), 1.50 (3H, s, isopropylidene CH$_3$), 1.48 (3H, s, isopropylidene CH$_3$), 1.44 (2H, dd, $J_{13.5}$, 6.8, OCH$_2$CH$_2$CH(CH$_3$)$_2$), 1.35 (3H, s, isopropylidene CH$_3$), 1.33 (3H, s, isopropylidene CH$_3$), 0.89 (6H, d, $J_{6.7}$, OCH$_2$CH$_2$CH(CH$_3$)$_2$); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 144.9 (triazole C=C), 137.6, 134.5 (CH$_2$CH=CH$_2$), 134.3 (CH$_2$CH=CH$_2$),
Experimental data

128.5, 128.0, 127.9, 124.3 (each Ar-C), 117.5 (CH₂CH=CH₂), 116.8 (CH₂CH=CH₂), 110.2 (isopropylidene C), 109.4 (isopropylidene C), 76.1 (C-2), 74.9 (C-2’), 73.0 (CH₂C=CH₂), 71.7 (C-3), 71.6 (C-3), 71.6 (C-1), 70.9 (C-1), 70.5 (C-6), 69.3 (OCH₂CH₂CH(CH₃)₂), 68.8 (C-5’), 68.5 (C-5), 65.0 (OCH₂C=CH₂), 51.9 (C-6’), 39.0 (OCH₂CH₂CH(CH₃)₂), 35.2 (CH₂=CH₂), 34.9 (CH₂CH=CH₂), 26.9 (isopropylidene CH₃), 26.8 (isopropylidene CH₃), 24.9 (isopropylidene CH₃), 24.8 (isopropylidene CH₃), 24.7 (OCH₂CH₂CH(CH₃)₂), 22.7 (OCH₂CH₂CH(CH₃)₂); ESI-HRMS calcd for C₃₉H₆₈N₃O₉ 712.4173, found m/z 712.4171 [M+H]⁺

1-C- Allyl-1,6-dideoxy-6-(4-(((1-C-allyl-1,6-dideoxy-2-O-isopentyl-3,4-O-isopropylideneα-D-galactopyranos-6-yl)oxymethyl)-1H-1,2,3-triazol-1-yl)-2-O-naphthalen-2-ylmethyl-3,4-O-isopropylidene-α-D-galactopyranoside (204). Alkyne 180 (173 mg, 0.49 mmol) and azide 202 (201 mg, 0.49 mmol) were dissolved in acetonitrile-H₂O 1:1 (5 mL). To this was added CuI (75 mg, 0.39 mmol) and the reaction was heated at reflux for 24 h. Upon cooling the reaction was diluted with EtOAc, washed with H₂O, brine, dried over MgSO₄, filtered and the solvents were concentrated under reduced pressure. The crude residue was purified via flash chromatography (EtOAc-petroleum ether 1:1) to give compound 204 (280 mg, 75%) as a yellow oil; [α]D +24.7° (c 0.06 in CHCl₃); IR (film) cm⁻¹: 2926, 1641, 1381, 1090, 907; ¹H NMR (500 MHz, CDCl₃) δ 7.81 (3H, m, ArH), 7.70 (2H, d, J 8.1, ArH), 7.46 (2H, dd, J 8.9, 5.2), 7.40 (1H, d, J 8.4, ArH), 5.79 (1H, ddt, J 16.9, 10.1, 7.0, CH₂CH=CH₂), 5.64 (1H, ddt, J 17.2, 10.3, 7.0, CH₂CH=CH₂), 5.13 – 4.93 (4H, overlapping signals, each CH₂CH=CH₂), 4.79 (1H, d, J 12.0, CHHAr), 4.68 (3H, overlapping signals, CHHAr & OCH₂C=CH₂), 4.60 (1H, dd, J 14.0, 3.9, H-6’a), 4.43 (1H, dd, J 7.6, 3.7, H-3’), 4.41 – 4.36 (1H, m, H-6’b), 4.31
– 4.25 (3H, overlapping signals, H-4’, H-5’ & H-3), 4.19 (1H, d, J 7.4, H-4), 4.12 – 4.03 (2H, overlapping signals, H-1’ & H-5), 3.99 (1H, td, J 7.2, 2.4, H-1), 3.72 – 3.57 (4H, overlapping signals, OCH2CH2CH(CH3)2, H-2’ & H-6), 3.41 (1H, dd, J 15.7, 6.7, OCH2CH2CH(CH3)2), 3.32 (1H, t, J 2.7, H-2), 2.34 (3 H, m), 2.23 – 2.14 (1H, m) (each CH2CH=CH2), 1.68 (1H, td, J 13.3, 6.7 OCH2CH2CH(CH3)2), 1.48 (3H, s, isopropylidene CH3), 1.46 (3H, s, isopropylidene CH3), 1.43 (2H, dd, J 13.5, 6.7, OCH2CH2CH(CH3)2), 1.33 (3H, s, isopropylidene CH3), 1.31 (3H, s, isopropylidene CH3), 0.88 (6H, d, J 6.6, OCH2CH2CH(CH3)2); 13C NMR (125 MHz, CDCl3) δ 144.9 (triazole C=C), 135.0 (Ar-C), 134.7 (CH2CH=CH2), 134.3 (CH2CH=CH2), 133.2, 133.1 (each Ar-C), 128.4, 128.0, 127.8, 126.8, 126.3, 126.2, 125.9, 124.3 (each Ar-CH), 117.5 (CH2CH=CH2), 117.1 (CH2CH=CH2), 110.2 (isopropylidene C), 109.4 (isopropylidene C), 76.1 (C-2), 74.9 (C-2’), 73.1 (C-4), 73.0 (C-4’), 72.9 (CH2Ph), 71.9 (C-3’), 71.6 (C-1’), 71.5 (C-1), 71.0 (C-3), 70.5 (C-6), 69.3 (OCH2CH2CH(CH3)2), 68.8 (C-5’), 68.4 (C-5), 65.0 (O-CH2C=C), 51.8 (C-6’), 39.0 (OCH2CH2CH(CH3)2), 35.2 (CH2CH=CH2), 34.8 (CH2CH=CH2), 26.9 (isopropylidene CH3), 26.8 (isopropylidene CH3), 24.9 (isopropylidene CH3), 24.9 (OCH2CH2CH(CH3)2), 24.7 (isopropylidene CH3), 22.7 (OCH2CH2CH(CH3)2); ESI-HRMS calcd for C43H59N3O9Na 784.4149, found m/z 784.4161 [M+Na]+

**Macrocycle 205.** To a stirred solution of 203 (99 mg, 0.139 mmol) in dioxane-H2O (3:1, 2 mL) was added 2,6-lutidine (56 µL, 0.486 mmol), NaIO4 (133 mg, 0.625 mmol) and a catalytic amount of OsO4 (2 drops, 2.5% solution in t-BuOH). The reaction mixture was stirred at room temperature for 2.5 h. H2O and CH2Cl2 were added, layers were separated and the aqueous layer was extracted into CH2Cl2. The combined organic phases were washed
with brine, dried over MgSO₄, filtered and the solvents were concentrated under reduced pressure. The resulting residue was taken up in 1,2-dichloroethane (5 ml) and isopentylamine (16 µl, 0.139 mmol) was added. The solution was stirred at room temperature for 20 min. Sodium triacetoxyborohydride (103 mg, 0.486 mmol) was then added and the reaction mixture stirred for 3 h. The reaction was quenched by the addition of satd NaHCO₃ and the product was extracted into EtOAc, dried with MgSO₄, filtered and the solvents were concentrated under reduced pressure. The crude residue was purified via flash chromatography (CH₂Cl₂-EtOAc 1:9) to give compound 205 (60 mg, 54%) as an off white solid; [α]D +47.2° (c 0.01 in CHCl₃); ¹H NMR (500 MHz, CDCl₃) δ 7.80 (1H, s, triazole H), 7.36 – 7.26 (5H, m, ArH), 4.78 (1H, d, J 11.6, OCH=CH), 4.74 – 4.61 (3H, overlapping signals, OCH=CH, PhCHO & H-6’a), 4.51 (1H, d, J 12.0, PhCHO), 4.42 (1H, m, H-3), 4.40 – 4.32 (1H, m, H-6‘b), 4.29 (1H, m, H-3’), 4.23 (1H, m, H-4’), 4.15 (2H, overlapping signals, H-4 & H-5), 4.07 (2H, overlapping signals, H-5 & H-1’), 3.95 (1H, m, H-1), 3.79 – 3.70 (2H, m, H-6), 3.69 – 3.62 (1H, m, OCHHCH₂CH(CH₃)₂), 3.56 – 3.44 (3H, overlapping signals, OCHHCH₂CH(CH₃)₂, H-2 & H-2’), 2.68 (2H, m), 2.59 (1H, m), 2.48 (2H, m), 2.29 (1H, m), (each CH₂N), 1.71 (4H, dd, J 13.4, 6.7), 1.51 (6H, s, 2 x isopropylidene CH₃), 1.47 (2H, d, J 4.7), 1.42 (1H, d, J 5.8), 1.37 (3H, s, isopropylidene CH₃), 1.34 (3H, s, isopropylidene CH₃), 1.27 (3H, overlapping signals, CH₂ and CH) 0.95 – 0.81 (12H, m, each OCH₂CH₂CH(CH₃)₂); ¹³C NMR (125 MHz, CDCl₃) δ 146.0 (triazole CH=C), 137.3 (Ar-C), 128.4, 128.1, 128.0 (each Ar-Ch), 123.0 (triazole CH=C), 110.1 (isopropylidene C), 109.5 (isopropylidene C), 77.3 (C-2), 75.3 (C-2), 74.5 (C-4), 73.8 (C-4), 73.4 (C-3), 72.7 (OCH₂Ph), 71.6 (C-3), 71.2 (C-6), 71.1 (C-1), 70.2 (C-1), 69.3 (OCH₂CH₂CH(CH₃)₂), 69.0 (C-5), 68.6 (C-5), 66.0 (OCH₂C=C), 52.6 (C-6), 51.3 (-CH₂N-), 49.4 (CH₂N), 38.8 (CH₂), 31.6, 29.6, 29.6, 27.5, 26.6, 26.5, 25.7, 24.9, 24.5, 22.8, 22.6, 22.5; ESI-HRMS calcd for C₄₂H₆₇N₄O₉ 771.4908, found m/z 771.4901 [M+H]⁺
**Macrocycle 206.** To a stirred solution of 203 (111 mg, 0.155 mmol) in dioxane-H$_2$O (3:1, 2 mL) was added 2,6-lutidine (64 µL, 0.545 mmol), NaIO$_4$ (149 mg, 0.697 mmol) and a catalytic amount of OsO$_4$ (2 drops, 2.5% solution in t-BuOH). The reaction mixture was stirred at room temperature for 2.5 h. H$_2$O and CH$_2$Cl$_2$ were added, layers were separated and the aqueous layer was extracted into CH$_2$Cl$_2$. The combined organic phases were washed with brine, dried over MgSO$_4$, filtered and the solvents were concentrated under reduced pressure. The resulting residue was taken up in 1,2-dichloroethane (5 mL) and 2-phenylethylamine hydrochloride (24 mg, 0.155 mmol) was added. The solution was stirred at room temperature for 20 min. Sodium triacetoxyborohydride (115 mg, 0.542 mmol) was then added and the reaction mixture was stirred for 3 h. The reaction was quenched by the addition of satd NaHCO$_3$ and the product was extracted into EtOAc, dried with MgSO$_4$, filtered and the solvents were concentrated under reduced pressure. The crude residue was purified via flash chromatography (CH$_2$Cl$_2$-EtOAc 1:9) to give compound 206 (75 mg, 60%) as an off white solid; $^1$H NMR (500 MHz, CDCl$_3$) δ 7.80 (1H, s, triazole H), 7.36 – 7.08 (10H, m, PhH), 4.78 – 4.60 (4H, overlapping signals, OCHHPh, H-6a & OCH$_2$C=C), 4.49 (1H, d, J 11.8, OCHHPh), 4.40 (2H, overlapping signals, H-3, H-6’b), 4.32 – 4.21 (2H, overlapping signals, H-3’ & H-4’), 4.15 (2H, overlapping signals, H-5 & H-4), 4.04 (2H, overlapping signals, H-5 & H-1’), 3.94 (1H, m, H-1), 3.75 (2H, m, H-6), 3.70 – 3.60 (1H, m, OCHHHCH$_2$CH(CH$_3$)$_2$), 3.50 (3H, overlapping signals, OCHHCH$_2$CH(CH$_3$)$_2$, H-2 & H-2’), 2.71 (8H, overlapping signals, each CH$_2$N), 1.74 – 1.63 (3H, m), 1.52 (6H, m, isopropylidene CH$_3$), 1.47 (4H, dd, J 13.3, 6.5), 1.37 (3H, s, isopropylidene CH$_3$), 1.34 (3H, s, isopropylidene CH$_3$), 0.90 (6H, dd, J 6.2, 3.5, OCH$_3$CH$_2$CH(CH$_3$)$_2$); $^{13}$C NMR, (125 MHz, CDCl$_3$) δ 146.0 (triazole CH=C), 137.4 (Ar-C), 128.7, 128.5, 128.4, 128.3, 128.1 (each Ar-
CH), 123.2 (triazole CH=C), 110.2 (isopropylidene C), 77.3 (C-2), 75.3 (C-2), 74.6 (C-4), 73.9 (C-4), 73.5 (C-3), 72.7 (OCH₂Ph), 71.8 (C-3), 71.3 (C-6), 71.0 (C-1), 70.0 (C-1) 69.5 (OCH₂CH₂CH(CH₃)₂), 69.0 (C-5), 68.8 (C-5), 66.1 (OCH₂C=CH₂), 52.6 (C-6), 51.8, 51.3 (each CH₂N), 38.8 (CH₂), 27.5 (isopropylidene CH₃), 26.7 (isopropylidene CH₃), 25.8, 25.0 (isopropylidene CH₃), 24.6 (isopropylidene CH₃), 22.7 (OCH₂CH₂CH(CH₃)₂). ESI-HRMS calecd for C₄₅H₆₅N₄O₈ 805.4751, found m/z 805.4745 [M+H]⁺

**Macrocycle 207.** To a stirred solution of 203 (118 mg, 0.154 mmol) in dioxane-H₂O (3:1, 2 mL) was added 2,6-lutidine (63 µL, 0.540 mmol), NaIO₄ (148 mg, 0.69 mmol) and a catalytic amount of OsO₄ (2 drops, 2.5% solution in t-BuOH). The reaction mixture was stirred at room temperature for 2.5 h. H₂O and CH₂Cl₂ were added, layers were separated and the aqueous layer was extracted into CH₂Cl₂. The combined organic phases were washed with brine, dried over MgSO₄, filtered and the solvents were concentrated under reduced pressure. The resulting residue was taken up in 1,2-dichloroethane (5 mL) and 2-(2-Naphthyl)ethanamine hydrochloride (32 mg, 0.154 mmol) was added. The solution was stirred at room temperature for 20 min. Sodium triacetoxyborohydride (114 mg, 0.539 mmol) was then added and the reaction mixture stirred for 3 h. The reaction was quenched by the addition of satd NaHCO₃ and the product was extracted into EtOAc, dried with MgSO₄, filtered and the solvents were concentrated under reduced pressure. The crude residue was purified via flash chromatography (CH₂Cl₂-EtOAc 1:9) to give compound 207 (75 mg, 57%) as an off white solid; ¹H NMR (500 MHz, CDCl₃) δ 7.84 – 7.71 (5H, m, ArH), 7.59 (1H, s, ArH), 7.49 – 7.37 (3H, m, ArH), 7.27 (4H, dd, J 13.0, 7.7, ArH), 4.80 – 4.55 (5H, overlapping signals, OCH₂C=CH₂, OCHHPh & H-6'), 4.47 (1H, d, J 11.9, OCHHPh), 4.39 (1H,
dd, $J$ 7.2, 3.3, H-3), 4.24 (2H, overlapping signals, H-3' & H-4'), 4.06 (4H, overlapping signals, H-4, H-5, H-5' & H-1'), 3.96 (1H, m, H-1), 3.74 (2H, $J$ 19.0, 10.6, H-6), 3.65 (1H, m, OCHHCH$_2$CH(CH$_3$)$_2$), 3.54–3.47 (2H, overlapping signals, OCHHCH$_2$CH(CH$_3$)$_2$ & H-2), 3.45 (1H, m, H-2), 2.83 (6H, overlapping signals, each CH$_3$N), 1.74–1.65 (2H, m), 1.52 (3H, s, isopropylidene CH$_3$), 1.50 (3H, s, isopropylidene CH$_3$), 1.46 (5H, overlapping signals), 1.36 (3H, s, isopropylidene CH$_3$), 1.33 (3H, s, isopropylidene CH$_3$), 1.28 (2H, m), 0.88 (6H, dt, $J$ 10.9, 5.4, OCH$_2$CH$_2$CH(CH$_3$)$_2$); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 145.9 (triazole CH=C), 137.3, 133.5, 132.0 (each Ar-C), 128.4, 128.4, 128.2, 128.0, 127.6, 127.5, 127.3, 126.8, 126.0, 125.4 (each Ar-CH), 123.2 (triazole CH=C), 110.1 (isopropylidene C), 109.6 (isopropylidene C), 77.2 (C-2), 75.2 (C-2), 74.4 (C-4), 73.7 (C-4), 73.3 (C-3), 72.7 (OCH$_2$Ph), 71.7 (C-3), 71.2 (C-6), 70.9 (C-1), 70.0 (C-1), 69.5 (OCH$_2$CH$_2$CH(CH$_3$)$_2$, 68.9 (C-5), 68.8 (C-5), 65.9 (OCH$_2$C=C), 52.9 (C-6), 52.5 (CH$_2$N), 51.6 (CH$_2$N), 51.2 (CH$_2$N), 38.8 (CH$_2$), 27.4, 26.6, 25.6, 24.9, 24.8, 24.6, 22.6 (OCH$_2$CH$_2$CH(CH$_3$)$_2$; ESI-HRMS calcd for C$_{49}$H$_{67}$N$_4$O$_9$ 855.4908, found m/z 855.4901 [M+H]$^+$

**Macrocycle 208.** To a stirred solution of 203 (116 mg, 0.162 mmol) in dioxane-H$_2$O (3:1, 2 mL) was added 2,6-lutidine (66 µL, 0.570 mmol), NaIO$_4$ (156 mg, 0.730 mmol) and a catalytic amount of OsO$_4$ (2 drops, 2.5% solution in t-BuOH). The reaction mixture was stirred at room temperature for 2.5 h. H$_2$O and CH$_2$Cl$_2$ were added, layers were separated and the aqueous layer was extracted into CH$_2$Cl$_2$. The combined organic phases were washed with brine, dried over MgSO$_4$, filtered and the solvents were concentrated under reduced pressure. The resulting residue was taken up in 1,2-dichloroethane (5 mL) and tryptamine hydrochloride (32 mg, 0.162 mmol) was added. The solution was stirred at room temperature
for 20 min. Sodium triacetoxyborohydride (120 mg, 0.567 mmol) was then added and the reaction mixture stirred for 3 h. The reaction was quenched by the addition of satd NaHCO$_3$ and the product was extracted into EtOAc, dried with MgSO$_4$, filtered and the solvents were concentrated under reduced pressure. The crude residue was purified via flash chromatography (CH$_2$Cl$_2$-EtOAc 1:9) to give compound **208** (88 mg, 65%) as an off white solid; $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.81 (1H, s, triazole H), 7.34 – 7.22 (5H, m, ArH), 7.23 – 7.15 (2H, m, ArH), 7.12 (2H, d, J 7.1), 6.94 (1H, d, J 7.6), 4.73 (2H, overlapping signals, OCH$_2$C=C), 4.66 (2H, overlapping signals, OCHHPh & H-6’a), 4.49 (1H, d, J 12.0, OCHHPh), 4.42 (1H, dd, J 7.4, 3.4, H-3), 4.38 (1H, dd, J 14.3, 10.0, H-6’b), 4.31 – 4.24 (1H, overlapping signals, H-3’ & H-4’), 4.15 (2H, overlapping signals, H-4 & H-5), 4.09 – 4.05 (1H, m, H-5’), 4.03 (1H, ddd, J 11.3, 4.2, 2.8, H-1’), 3.95 – 3.91 (1H, m, H-1), 3.78 – 3.73 (1H, m, CHH), 3.68 – 3.61 (2H, m, each CHH), 3.51 (2H, overlapping signals, H-2 & CHH), 3.47 – 3.44 (1H, m, H-2), 2.73 (8H, overlapping signals, each CH$_2$), 1.70 (3H, overlapping signals), 1.52 (1H, s, isopropylidene CH$_3$), 1.51 (3H, s, isopropylidene CH$_3$) 1.50 – 1.42 (4H, overlapping signals, each CH$_2$), 1.36 (3H, s, isopropylidene CH$_3$), 1.34 (3H, s, isopropylidene CH$_3$), 1.32 (2H, m, CH$_2$) 0.92 – 0.85 (6H, m, OCH$_2$CH$_2$CH(CH$_3$)$_2$); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 139.8, 138.2, 133.4, 130.9, 129.4 (each Ar-C), 129.2, 128.8, 128.6, 125.6, 123.1, 122.3, 119.6, 119.3, 112.3 (each Ar-CH), 110.1 (isopropylidene C), 109.6 (isopropylidene C) 78.4, 78.0, 74.3 (CH$_2$), 73.3, 73.2, 72.9, 72.7, 72.0, 71.3, 71.2, 70.9, 70.6 (CH), 70.5, 65.5, 60.7, 53.2, 52.9, 52.7, 52.5, 52.3, 40.2 (each CH$_2$), 26.9, 26.8, 24.9, 24.7 (each CH$_3$), 23.2 (CH), 22.8 (CH$_2$), 22.3 (CH$_3$); ESI-HRMS calcd for C$_{47}$H$_{66}$N$_5$O$_8$ 844.4860, found m/z 844.4855 [M+H]$^+$
Macrocycle 209. To a stirred solution of 204 (105 mg, 0.137 mmol) in dioxane-H₂O (3:1, 2 mL) was added 2,6-lutidine (56 µL, 0.482 mmol), NaIO₄ (131 mg, 0.616 mmol) and a catalytic amount of OsO₄ (2 drops, 2.5% solution in t-BuOH). The reaction mixture was stirred at room temperature for 2.5 h. H₂O and CH₂Cl₂ were added, layers were separated and the aqueous layer was extracted into CH₂Cl₂. The combined organic phases were washed with brine, dried over MgSO₄, filtered and the solvents were concentrated under reduced pressure. The resulting residue was taken up in 1,2-dichloroethane (5 mL) and isopentylamine (16 µL, 0.137 mmol) was added. The solution was stirred at room temperature for 20 min. Sodium triacetoxyborohydride (127 mg, 0.599 mmol) was then added and the reaction mixture stirred for 3 h. The reaction was quenched by the addition of satd NaHCO₃ and the product was extracted into EtOAc, dried with MgSO₄, filtered and the solvents were concentrated under reduced pressure. The crude residue was purified via flash chromatography (CH₂Cl₂-EtOAc 1:9) to give compound 209 (66 mg, 59%) as an off white solid; [α]D +21.3° (c 0.02 in CHCl₃); ¹H NMR (500 MHz, CDCl₃) δ 7.84 – 7.76 (3H, m, ArH), 7.74 (1H, s, ArH), 7.50 – 7.42 (4H, m, ArH), 4.79 (2H, overlapping signals, OCH=HC=CH₂ & OCH=HArc), 4.67 (3H, overlapping signals, OCH=HC=C, OCH=HAr & H-6’a), 4.45 (1H, dd, J 7.0, 2.2, H-3), 4.37 (1H, m, H-6’b), 4.31 (1H, m, H-3’), 4.22 (1H, m, H-4’), 4.17 (1H, m H-5’), 4.12 (1H, m, H-4), 4.05 (2H, overlapping signals, H-5 & H-1’), 3.92 (1H, m, H-1), 3.79 – 3.63 (3H, overlapping signals, H-6 & OCHHCH₂CH(CH₃)₂), 3.51 (3H, overlapping signals, OCHHCH₂CH(CH₃)₂, H-2 & H-2’), 2.67 (2H, m), 2.53 (1H, m), 2.43 (1H, m), 2.30 (1H, m), 2.08 (1H, m), (each CH₂N) 1.78 – 1.60 (5H, m), 1.51 (3H, s, isopropylidene CH₃), 1.50 (5H, overlapping signals, isopropylidene CH₃ & CH₂), 1.42 (1H, d, J 6.5), 1.36 (3H, s, isopropylidene CH₃), 1.33 (3H, s, isopropylidene CH₃), 1.17 (2H, m,
CH$_2$) 0.91 (6H, d, $J$ 6.6, CH$_2$CH$_2$CH(CH$_3$)$_2$), 0.78 (6H, dd, $J$ 26.9, 6.3, CH$_2$CH$_2$CH(CH$_3$)$_2$); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 146.2 (triazole CH=), 134.9, 133.2, 133.2, 128.4, 128.0, 127.8, 126.4, 126.3, 126.2, 110.3 (isopropylidene C), 109.7 (isopropylidene C), 75.3 (C-2), 74.9 (C-2), 74.0 (C-4), 73.6 (C-4), 72.9 (C-3), 71.9 (OCH$_2$Ar), 71.4 (C-3), 71.3 (C-6), 71.2 (C-1), 70.3 (C-1), 69.4 (OCH$_2$CH$_2$CH(CH$_3$)$_2$), 69.2 (C-5), 68.7 (C-5), 66.2 (OCH$_2$C=C), 52.8 (C-6), 51.8, 51.56, 49.3 (each CH$_2$N), 38.9 (CH$_3$), 36.3 (CH$_2$), 29.8, 27.7 (isopropylidene CH$_3$), 26.7 (isopropylidene CH$_3$), 26.6 (isopropylidene CH$_3$), 25.9 (isopropylidene CH$_3$), 25.1, 24.6, 22.9, 22.8; ESI-HRMS calcd for C$_{46}$H$_{69}$N$_4$O$_9$ 821.5065, found m/z 821.5063 [M+H]$^+$

**Macrocycle 210.** To a stirred solution of 204 (115 mg, 0.151 mmol) in dioxane-H$_2$O (3:1, 2 mL) was added 2,6-lutidine (62 µL, 0.528 mmol), NaIO$_4$ (145 mg, 0.679 mmol) and a catalytic amount of OsO$_4$ (2 drops, 2.5% solution in t-BuOH). The reaction mixture was stirred at room temperature for 2.5 h. H$_2$O and CH$_2$Cl$_2$ were added, layers were separated and the aqueous layer was extracted into CH$_2$Cl$_2$. The combined organic phases were washed with brine, dried over MgSO$_4$, filtered and the solvents were concentrated under reduced pressure. The resulting residue was taken up in 1,2-dichloroethane (5 mL) and benzyamine (16 µL, 0.151 mmol) was added. The solution was stirred at room temperature for 25 min. Sodium triacetoxyborohydride (112 mg, 0.528 mmol) was then added and the reaction mixture stirred for 3 h. The reaction was quenched by the addition of satd NaHCO$_3$ and the product was extracted into EtOAc, dried with MgSO$_4$, filtered and the solvents were concentrated under reduced pressure. The crude residue was purified via flash chromatography (CH$_2$Cl$_2$-EtOAc 1:9) to give compound 210 (74 mg, 58%) as an off white
solid; $[\alpha]_D +62.4^\circ$ (c 0.03 in CHCl$_3$); $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.86 – 7.78 (3H, m, ArH), 7.68 (1H, s, ArH), 7.52 – 7.44 (2H, m, ArH), 7.41 (1H, d, $J$ 9.3, ArH), 7.37 – 7.29 (2H, m, ArH), 7.26 (1H, s, ArH), 7.24 – 7.16 (2H, m, ArH), 7.13 (1H, d, $J$ 7.0, ArH), 4.75 (2H, q, 12.0, OCH$_2$C=C), 4.67 (2H, overlapping signals, H-6’a & OCH$_2$H), 4.52 (1H, d, $J$ 12.1, OCHHAr), 4.40 (1H, dd, $J$ 7.5, 3.2, H-3), 4.33 (1H, dd, $J$ 14.3, 9.9, H-6’b), 4.28 (1H, dd, $J$ 7.5, 1.0, H-3’), 4.26 – 4.21 (2H, m, ArH), 4.15 (2H, d, $J$ 12.1, OCH$_2$HAr), 4.40 (1H, d, $J$ 7.6, H-5), 4.07 – 4.02 (1H, m, H-1’), 3.90 – 3.84 (1H, m, H-1), 3.73 (2H, dt, $J$ 7.5, 1.0, H-3’), 3.62 (1H, dt, $J$ 9.2, 6.4, OCH$_2$HCH$_2$CH(CH$_3$)$_2$), 3.51 – 3.37 (4H, overlapping signals, OCH$_2$HCH$_2$CH(CH$_3$)$_2$, PhCH$_2$N, H-2 & H-2’), 3.25 (1H, d, $J$ 13.7, PhCH$_2$N), 2.70 (1H, m), 2.55 (2H, d, $J$ 8.5), 2.40 (1H, m), (each CH$_2$N), 1.71 (5H, overlapping signals, 2 x CH$_2$ & OCH$_2$CH$_2$CH(CH$_3$)$_2$), 1.50 (3H, s, isopropylidene CH$_3$), 1.45 (5H, overlapping signals, OCH$_2$CH$_2$CH(CH$_3$)$_2$ & isopropylidene CH$_3$), 0.90 (6H, dd, $J$ 6.6, 0.9, OCH$_2$CH$_2$CH(CH$_3$)$_2$); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 146.0 (triazole CH=C), 140.4, 134.9, 133.2 (each Ar-C), 128.6, 128.5, 128.3, 128.2, 128.0, 127.8, 127.4, 127.0, 126.4, 126.3, 126.2 (each Ar-CH), 123.4 (triazole CH=C), 110.2 (isopropylidene C), 109.6 (isopropylidene C), 77.4 (C-2), 75.0 (C-2), 74.2 (C-4), 73.8 (C-4), 73.6 (C-3), 73.0 (OCH$_2$Ar), 71.9 (C-4), 71.3 (C-6), 70.7 (C-1), 70.2 (C-1), 69.5 (OCH$_2$CH$_2$CH(CH$_3$)$_2$), 69.2 (C-5), 68.8 (C-5), 66.0 (OCH$_2$C=C), 56.8 (PhCH$_2$N), 52.7 (C-6), 51.4 (CH$_2$N), 51.2 (CH$_2$N), 38.9 (CH$_2$), 29.8, 27.5 (isopropylidene CH$_3$), 26.6 (isopropylidene CH$_3$), 25.6, 25.0 (isopropylidene CH$_3$), 24.7 (isopropylidene CH$_3$), 22.7 (OCH$_2$CH$_2$CH(CH$_3$)$_2$); ESI-HRMS calcd for C$_{48}$H$_{68}$N$_4$O$_9$ 841.4752, found m/z 841.4751 [M+H]$^+$

**Macrocycle 211.** Compound 205 (40 mg, 0.052 mmol) was dissolved in TFA-H$_2$O (4:1, 1.5 mL) and stirred at room temperature for 2 h. Solvents were removed under reduced pressure
and the residue was azeotroped with toluene to remove excess TFA. The resulting residue was taken up in MeOH and basified to pH 8 using Dowex® M-43 ion exchange resin, filtered and the solvents were concentrated under reduced pressure to give 211 (27 mg, 77%) as a white powder; $^1$H NMR (500 MHz, CD$_3$OD) $\delta$ 7.84 (1H, s, triazoleH), 7.28 (1H, d, J 7.2, ArH), 7.23 (2H, t, J 7.3, ArH), 7.18 (3H, d, J 7.1, ArH), 4.65 (1H, d, J 11.8, OCHH), 4.63 – 4.55 (1H, m, CHH), 4.51 (4H, overlapping signals, each CH$_2$), 3.91 (3H, overlapping signals, each CH), 3.78 – 3.70 (3H, overlapping signals, each CH), 3.67 (1H, t, J 9.2, CH), 3.60 (1H, d, J 8.4, CH), 3.58 – 3.50 (2H, m, CH$_2$), 3.47 (1H, dd, J 16.2, 6.8, CH$_2$), 2.46 (2H, m), 2.36 (2H, m), 2.32 – 2.23 (1H, m), 1.99 (1H, s) (each CH$_3$N), 1.71 (1H, s), 1.66 – 1.56 (4H, m), 1.35 (4H, ddd, J 29.5, 14.7, 8.0, CH$_2$CH$_2$CH(CH$_3$)$_2$), 1.17 (4H, overlapping signals, each CH$_2$), 0.81 (6H, dt, J 8.8, 4.4, OCH$_2$CH$_2$CH(CH$_3$)$_2$), 0.77 (6H, t, J 6.2, OCH$_2$CH$_2$CH(CH$_3$)$_2$); $^{13}$C NMR (125 MHz, CD$_3$OD) $\delta$ 145.6 (C=CH), 138.5 (Ar-C), 127.9, 127.6, 127.3 (each Ar-CH), 124.1 (C=CH), 76.9, 76.5 (each CH), 72.8 (OCH$_2$Ph), 71.5, 71.3, 70.5, 69.8, 69.4, 69.2 (each CH), 69.0, 64.0, 59.2, 51.4, 51.3, 50.9, 38.8 (each CH$_2$), 26.4, 24.7 (each CH), 21.8, 21.7, 21.5, 21.4 (each CH$_3$); ESI-HRMS calcd for C$_{36}$H$_{59}$N$_4$O$_9$ 691.4282, found m/z 691.4276 [M+H]$^+$

**Macrocycle 212.** Compound 206 (50 mg, 0.062 mmol) was dissolved in TFA-H$_2$O (4:1, 1.5 mL) and stirred at room temperature for 2 h. Solvents were removed under reduced pressure and the residue was azeotroped with toluene to remove excess TFA. The resulting residue was taken up in MeOH and basified to pH 8 using Dowex® M-43 ion exchange resin, filtered and concentrated under reduced pressure to give 212 (33 mg, 73%) as a white powder; $^1$H NMR (500 MHz, CD$_3$OD) $\delta$ 7.85 (1H, s, triazole H), 7.25 (3H, d, J 6.4, ArH), 7.13 (6H, dd, J 12.0, 7.0, ArH), 7.10 – 7.04 (1H, m, ArH), 6.98 (1H, d, J 7.2, ArH), 4.65 (1H, d, J 11.6,
Experim\ntal data

Macrocycle 213. Compound 207 (45 mg, 0.053 mmol) was dissolved in TFA-H₂O (4:1, 1.5 mL) and stirred at room temperature for 2 h. Solvents were removed under reduced pressure and the residue was azeotroped with toluene to remove excess TFA. The resulting residue was taken up in MeOH and basified to pH 8 using Dowex® M-43 ion exchange resin, filtered and concentrated under reduced pressure to give 213 (32 mg, 78%) as a white powder. $^1$H NMR (500 MHz, CD₃OD) $\delta$ 7.82 (1H, s, triazole H), 7.70 – 7.64 (2H, m, ArH), 7.63 – 7.57 (2H, m, ArH), 7.43 (1H, s, ArH), 7.31 (2H, ddd, J 9.6, 8.2, 6.1, ArH), 7.21 (2H, d, J 6.3, ArH), 7.11 (1H, d, J 8.5, ArH), 7.09 – 7.02 (2H, m, ArH), 4.62 (2H, dd, J 18.0, 6.5, CH₂), 4.55 (2H, brs, CH₂), 4.45 (2H, m, CH₂), 4.02 – 3.95 (2H, overlapping signals, each CH), 3.88 (2H, overlapping signals, each CH), 3.75 (2H, overlapping signals, each CH), 3.70 (1H, apt d, J 9.6, CH), 3.64 (1H, apt d, J 8.3, CH), 3.58 (2H, m), 3.55 – 3.48 (2H, m, CH₂), 3.48 – 3.42 (1H, m, CHH), 2.71 – 2.57 (4H, overlapping signals, CH₂N), 2.49 (2H, m, CH₂N), 2.46 – 2.41 (1H, m, CHHN), 2.31 – 2.22 (1H, m, CHHN), 2.09 (1H, dd, J 19.9, 10.1), 1.76 – 1.55
Experimental data

(5H, overlapping signals), 1.42 – 1.28 (3H, m), 1.22 (1H, t, J 7.2), 0.81 – 0.69 (6H, dd, J 6.0, 4.2, OCH₂CH₂CH(CH₃)₂); ¹³C NMR (125 MHz, CD₃OD) δ 145.7 (C=CH), 138.4, 137.6, 133.6, 132.1 (each Ar-C), 128.0, 127.6, 127.3, 127.2, 127.0, 126.9, 126.3, 125.6 (Ar-CH), 124.9, 124.1 (C=CH), 77.0, 76.7, 72.9 (CH₂), 71.8, 71.4, 71.2, 70.6, 70.0, 69.9, 69.4, 69.2 (each CH), 69.0, 64.1 (each CH₂), 59.2, 52.1, 52.0, 51.2, 51.2, 50.9, 48.5, 38.8, 31.2 (each CH₂), 24.7 (CH), 21.9, 21.5 (CH₃); ESI-HRMS calcd for C₄₃H₅₉N₄O₇ 775.4282, found m/z 775.4277 [M+H]+

Macrocycle 214. Compound 208 (50 mg, 0.059 mmol) was dissolved in TFA-H₂O (4:1, 1.5 mL) and stirred at room temperature for 2 h. Solvents were removed under reduced pressure and the residue was azeotroped with toluene to remove excess TFA. The resulting residue was taken up in MeOH and basified to pH 8 using Dowex® M-43 ion exchange resin, filtered and concentrated under reduced pressure to give 214 (36 mg, 79%) as a white powder; ¹H NMR (500 MHz, CD₃OD) δ 7.84 (1H, s, triazole H), 7.40 (1H, d, J 7.9, ArH), 7.29 – 7.12 (4H, m, ArH), 7.08 – 7.02 (2H, m, ArH), 6.98 (1H, t, J 7.5, ArH), 6.86 (2H, dd, J 16.0, 8.9, ArH), 4.53 (6H, overlapping signals, each CH₂), 3.98 (1H, m, CH), 3.93 (1H, m, CH), 3.92 – 3.84 (2H, overlapping signals, each CH), 3.73 (2H, overlapping signals, each CH), 3.68 (1H, m, CH), 3.61 (1H, m, CH), 3.56 (1H, d, J 4.9, CHH), 3.55 – 3.45 (3H, overlapping signals, CHH & CH₂), 2.76 – 2.63 (3H, overlapping signals, CH₂ & CHH), 2.54 (1H, m, CHH), 2.30 (1H, m, CHH), 2.09 (1H, m, CHH), 1.60 (5H, overlapping signals, 2 x CH₂ & OCH₂CH₂CH(CH₃)₂), 1.42 – 1.28 (2H, m, OCH₂CH₂CH(CH₃)₂), 0.72 (6H, d, J 6.7, OCH₂CH₂CH(CH₃)₂); ¹³C NMR (125 MHz, CD₃OD) δ 139.8 (C=CH), 138.2, 133.4, 130.9, 129.4, 129.2, 128.8, 128.6, 125.6, 123.1 (C=CH), 122.3, 119.6, 119.3, 112.3 (each Ar-C), 78.4, 78.0, 74.3 (CH₂), 73.3, 73.2, 72.9, 72.7, 72.0, 71.3, 71.2, 70.9, 70.6 (CH), 70.5, 65.5,
60.7, 53.2, 52.9, 52.7, 52.5, 52.3, 40.2 (each CH₂), 26.1, 23.2 (CH), 22.8 (CH₂), 22.3 (CH₃);
ESI-HRMS calcd for C₄₁H₅₈N₅O₉ 763.4156, found m/z 763.4151 [M+H]^+

Macrocycle 215. Compound 209 (45 mg, 0.055 mmol) was dissolved in TFA-H₂O (4:1, 1.5 mL) and stirred at room temperature for 2 h. Solvents were removed under reduced pressure and the residue was azeotroped with toluene to remove excess TFA. The resulting residue was taken up in MeOH and basified to pH 8 using Dowex® M-43 ion exchange resin, filtered and concentrated under reduced pressure to give 215 (32 mg, 78%) as a white powder; ¹H NMR (500 MHz, CD₃OD) δ 7.81 (1H, s, triazole H), 7.77 – 7.70 (4H, m, ArH), 7.44 – 7.35 (3H, m, ArH), 4.84 (1H, d, J 11.9, OCHH), 4.63 (1H, d, J 11.9, OCHH), 4.57 (1H, d, J 10.2, CHH), 4.55 – 4.42 (3H, overlapping signals, CH₂ & CHH), 3.95 – 3.87 (4H, overlapping signals, each CH), 3.79 (2H, overlapping signals, each CH), 3.72 (1H, m, CH), 3.65 (1H, d, J 8.7, CHH), 3.59 (2H, overlapping signals, CHH & CH), 3.56 – 3.50 (3H, overlapping signals, CHH & 2 x CH), 3.47 (1H, t, J 8.0, CHH), 2.37 (2H, m, CH₂N), 2.21 (2H, m, CH₂N), 2.09 (1H, m, CHHN), 1.93 (1H, m, CHHN), 1.62 (3H, overlapping signals, CH₂ & CH), 1.54 – 1.46 (2H, m, CH₂), 1.44 – 1.30 (2H, m, CH₂), 1.30 – 1.21 (1H, m, CH), 1.08 (2H, m, CH₂), 0.84 – 0.79 (6H, m, CH₂CH₂CH(CH₃)₂), 0.68 (5H, t, J 6.3, CH₂CH₂CH(CH₃)₂); ¹³C NMR (125 MHz, CD₃OD) δ 145.6 (C=CH), 136.0, 133.3, 133.1 (each Ar-C), 127.7, 127.5, 127.2, 126.4, 125.7, 125.6 (each Ar-CH), 124.1 (C=CH), 76.9, 76.7 (CH), 72.9 (CH₂), 71.3, 71.2 (each CH), 70.0 (CH₂), 69.9, 69.5, 69.2 (each CH), 69.0 (CH₂), 64.0 (CH₂), 51.4, 51.2, 50.9, 48.3, 38.8 (each CH₂), 33.8, 26.4 (CH), 24.7 (CH), 21.8, 21.7, 21.5 (CH₃), 21.4 (CH₃); ESI-HRMS calcd for C₄₀H₆₁N₄O₉ 741.4438, found m/z 741.4430 [M+H]^+
Macrocycle 216. Compound 210 (50 mg, 0.059 mmol) was dissolved in TFA-H₂O (4:1, 1.5 mL) and stirred at room temperature for 2 h. Solvents were removed under reduced pressure and the residue was azeotroped with toluene to remove excess TFA. The resulting residue was taken up in MeOH and basified to pH 8 using Dowex® M-43 ion exchange resin, filtered and concentrated under reduced pressure to give 216 (36 mg, 79%) as a white powder; \(^1\)H NMR (500 MHz, CD₃OD) \(\delta\) 7.81 (1H, s, triazole H), 7.70 (3H, dd, \(J\) 15.4, 8.6, ArH), 7.39 – 7.33 (2H, m, ArH), 7.23 (1H, d, \(J\) 4.2, ArH), 7.13 – 7.07 (3H, m, ArH), 4.73 (1H, d, \(J\) 12.1, OCHH), 4.66 – 4.59 (1H, m, CHH), 4.51 (4H, overlapping signals, each CH₂), 3.96 – 3.87 (2H, overlapping signals, each CH), 3.85 (1H, brs, CH), 3.78 (1H, m, CH), 3.72 (1H, brs, CH), 3.70 – 3.55 (3H, overlapping signals, CH & CH₂), 3.53 (1H, dd, \(J\) 9.4, 3.3, CH), 3.49 – 3.41 (2H, overlapping signals, CH & CHH), 3.34 (1H, dd, \(J\) 11.4, 4.7, CHH), 3.29 (2H, t, \(J\) 10.0, CH₂), 2.34 (2H, m, CH₂N), 2.18 – 2.11 (1H, m, CHHN), 1.99 (1H, m, CHHN), 1.59 (4H, overlapping signals, CHH, CH₂ & OCH₂CH₂CH(CH₃)₂), 1.49 (1H, m, CHH), 1.37 – 1.26 (2H, m, OCH₂CH₂CH(CH₃)₂), 0.78 (6H, dd, \(J\) 12.2, 6.7, OCH₂CH₂CH(CH₃)₂); \(^{13}\)C NMR (125 MHz, CD₃OD) \(\delta\) 145.6 (C=CH), 135.9, 133.2, 133.0, 128.5, 128.2, 128.0, 127.9, 127.7, 127.5, 127.2, 126.8, 126.6, 126.4, 125.8, 125.8, 125.6 (each Ar-C), 124.1 (C=CH), 76.9, 76.7 (each CH), 72.8 (CH₂), 71.6, 71.4, 70.4 (CH₂), 69.8, 69.7, 69.2, 69.0 (each CH), 68.9, 64.0, 59.2, 55.8, 52.1, 51.9, 51.5, 50.6, 38.7 (each CH₂), 24.6 (CH), 21.8, 21.5 (CH₃); ESI-HRMS calcd for C₄₂H₅₉N₇O₉ 761.4125, found \(m/z\) 761.4122 [M+H]⁺
Macrocycle 217. Compound 211 (20 mg, 0.029 mmol) was dissolved in pyridine-Ac₂O (1:1, 3 mL) and stirred at room temperature for 5 h. Solvents were removed under reduced pressure and the residue was taken up in EtOAc and washed with H₂O, brine, dried over MgSO₄, filtered and the solvents were concentrated under reduced pressure to give 217 (20 mg, 80%) as an off-white foam; [α]₀ᵣ +64.1° (c 0.08 in CHCl₃); IR (film) cm⁻¹: 2956, 2253, 1745, 1370, 1239, 1107, 903; ¹H NMR (500 MHz, CDCl₃) δ 7.76 (1H, s, triazole H), 7.37 – 7.28 (5H, m, ArH), 5.53 – 5.51 (1H, m, H-4‘), 5.36 (1H, dd, J 3.2, 1.7, H-4), 5.24 (1H, dd, J 9.0, 3.2, H-3‘), 5.06 (1H, dd, J 9.6, 3.2, H-3), 4.68 (3H, d, J 11.7, CH₂ & CHH), 4.59 (1H, d, J 11.9, CHH), 4.53 (1H, d, J 13.8, H-6’a), 4.45 – 4.36 (1H, m, H-6’b), 4.22 (2H, d, J 6.9, each CH), 4.14 – 4.07 (1H, m, each CH), 3.91 (2H, dd, J 8.7, 5.0, each CH), 3.75 (1H, dd, J 9.2, 5.7, CH), 3.65 (2H, ddd, J 20.9, 10.5, 5.2), 3.55 (3H, dt, J 12.5, 4.5, H-6a & OCH₂CH₂CH(CH₃)₂), 2.43 (7H, m, each CH₂), 2.22 (2H, m, CH₂), 2.18, 2.14, 2.05, 2.04 (each 3H, each s, each acetate CH₃), 1.71 – 1.62 (2H, m, each OCH₂CH₂CH(CH₃)₂), 1.53 – 1.43 (2H, m, OCH₂CH₂CH(CH₃)₂), 1.44 – 1.36 (2H, m, OCH₂CH₂CH(CH₃)₂), 0.91 – 0.82 (12H, m, each OCH₂CH₂CH(CH₃)₂); ¹³C NMR (125 MHz, CDCl₃) δ 170.2, 170.2, 169.9, 169.9 (C=O), 146.1 (triazole CH=C), 137.6 (Ar-C), 128.4, 128.0, 127.7 (each Ar-CH), 123.4 (triazole CH=C), 74.2 (C-2), 73.5 (C-2), 73.2 (OCH₂Ph), 72.1 (C-1), 71.7 (CH), 70.2 (CH), 70.0 (CH), 69.7 (CH₂), 69.4 (CH), 69.3 (CH), 69.1 (CH), 65.1 (CH₂), 53.4, 51.3 (CH₂N), 38.8 (CH₂), 26.4, 24.8 (CH), 22.7, 22.6, 22.5, 22.4, 20.8, 20.8, 20.7, 20.7 (each CH₃); ESI-HRMS calcd for C₄₄H₆₇N₄O₁₃ 859.4704, found m/z 859.4712 [M+H]^⁺
Macrocycle 218. Compound 212 (25 mg, 0.0344 mmol) was dissolved in pyridine-Ac₂O (1:1, 3 mL) and stirred at room temperature for 5 h. Solvents were removed under reduced pressure and the residue was taken up in EtOAc and washed with H₂O, brine, dried over MgSO₄, filtered and the solvents were concentrated under reduced pressure to give 218 (25 mg, 82%) as a brown foam; [α]D +65.7° (c 0.07 in CHCl₃); IR (film) cm⁻¹: 2956, 2253, 1745, 1370, 1239, 1107, 903; ¹H NMR (500 MHz, CDCl₃) δ 7.73 (1H, s, triazole H), 7.33 – 7.26 (5H, m, ArH), 7.24 (3H, t, J 7.3, ArH), 7.18 (1H, t, J 7.3, ArH), 7.07 (1H, d, J 7.0, ArH), 5.54 – 5.50 (1H, m, H-4’), 5.36 (1H, dd, J 3.1, 1.4, H-4), 5.22 (1H, dd, J 9.2, 3.3, H-3’), 5.05 (1H, dd, J 9.7, 3.4, H-3), 4.67 (3H, d, J 12.0, overlapping signals, OCH₂C=O & OCH₂Ph), 4.58 (1H, d, J 11.8, OCH₂Ph), 4.53 (1H, dd, J 14.3, 1.4, H-6’a), 4.38 (1H, dd, J 14.2, 10.3, H-6’b), 4.24 – 4.19 (1H, m, H-1’), 4.19 – 4.14 (1H, m, H-5’), 4.14 – 4.10 (1H, m, H-1), 3.92 (1H, dd, J 9.2, 5.2, H-2), 3.89 – 3.85 (1H, m, H-5), 3.77 (1H, dd, J 9.7, 5.7, H-2’), 3.69 – 3.60 (2H, m, H-6), 3.59 – 3.51 (2H, m, OCH₂CH₂CH(CH₃)₂), 2.75 – 2.56 (6H, overlapping signals, CH₂N), 2.44 – 2.37 (1H, m, CH₂), 2.33 – 2.26 (1H, m, CH₂), 2.18, 2.14, 2.04, 2.02 (each 3H, each s, each acetate CH₃), 1.86 – 1.75 (2H, m), 1.69 (3H, overlapping signals), 1.42 (3H, overlapping signals), 0.88 (6H, d, J 6.6, OCH₂CH₂CH(CH₃)₂); ¹³C NMR (125 MHz, CDCl₃) δ 170.2, 170.2, 170.0, 169.9 (each C=O), 146.1 (triazole CH=C), 139.9, 137.6 (each Ar-C), 128.6, 128.4, 128.4, 127.9, 127.7, 126.0 (each Ar-CH), 123.4 (triazole CH=C), 74.2 (C-2), 73.5 (C-2), 73.2 (OCH₂Ph), 72.2 (C-1), 71.7 (C-1), 70.2 (C-3), 70.1 (C-5), 69.7 (CH₂), 69.4 (CH₂), 69.3 (C-3), 69.2 (C-5), 69.2 (C-4), 68.8 (C-4), 65.1 (CH₂), 52.5, 51.3, 51.0 (each CH₂N), 50.2 (C-6), 38.8 (CH₂), 31.9 (CH), 24.8, 22.7 (each CH₂), 22.5 (CH₂), 22.4 (CH₃), 21.3 (CH₂), 20.8, 20.8, 20.7, 20.7 (each CH₃); ESI-HRMS calcd for C₄₇H₆₅N₄O₁₃ 845.4548, found m/z 845.4542 [M+H]^+.
Macrocyle 219. Compound 213 (22 mg, 0.028 mmol) was dissolved in pyridine-Ac$_2$O (1:1, 3 mL) and stirred at room temperature for 5 h. Solvents were removed under reduced pressure and the residue was taken up in EtOAc and washed with H$_2$O, brine, dried over MgSO$_4$, filtered and the solvents were concentrated under reduced pressure to give 219 (22 mg, 83%) as a white foam; [α]$_D$ +44.5° (c 0.06 in CHCl$_3$); IR (film) cm$^{-1}$: 2956, 2253, 1745, 1370, 1239, 1107, 903; $^1$H NMR (500 MHz, CDCl$_3$) δ 7.82 – 7.78 (1H, m, ArH), 7.74 (3H, d, J 5.7, ArH), 7.54 (1H, s, triazole H), 7.48 – 7.40 (2H, m, ArH), 7.30 – 7.21 (6H, m, ArH), 5.45 (1H, m, H-4’), 5.34 (1H, dd, J 3.2, 1.5, H-4), 5.21 (1H, dd, J 9.1, 3.3, H-3’), 5.06 (1H, dd, J 9.7, 3.4, H-3), 4.67 (2H, m, OCH$_2$C=O), 4.56 (1H, d, J 11.8, CHH), 4.49 (2H, overlapping signals, CHH & H-6’a), 4.38 (2H, overlapping signals, H-6’b & CH), 4.24 – 4.18 (2H, overlapping signals, each CH), 4.16 – 4.09 (2H, overlapping signals, each CH), 3.90 (1H, dd, J 9.1, 5.1, CH), 3.87 – 3.83 (1H, m, CH), 3.77 (1H, dd, J 9.7, 5.7, H-6a), 3.63 (1H, dd, J 10.2, 8.0, H-6b), 3.57 – 3.51 (3H, overlapping signals, CH$_2$ & CH), 2.80 (4H, overlapping signals, each CH$_2$N), 2.65 (2H, t, J 7.8, CH$_2$N), 2.51 – 2.44 (1H, m), 2.33 (2H, ddd, J 17.0, 9.4, 4.1, CH$_2$N), 2.17, 2.15, 2.05, 2.04 (each 3H, each s, each acetate CH$_3$), 1.91 – 1.79 (4H, overlapping signals, each CH$_2$), 1.79 – 1.70 (2H, m), 1.71 – 1.64 (1H, m, OCH$_2$CH$_2$CH(CH$_3$)$_2$), 1.43 (2H, ddd, J 14.7, 13.7, 6.8, OCH$_2$CH$_2$CH(CH$_3$)$_2$), 0.87 (6H, d, J 6.7, OCH$_2$CH$_2$CH(CH$_3$)$_2$); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 170.0, 170.0, 169.0, 169.9 (each C=O), 160.7, 160.4, 160.1, 159.7, 144.1, 137.1, 133.5, 132.6, 132.1, 129.3, 128.7, 128.6, 128.4, 128.3, 127.8, 127.7, 127.6, 126.8, 126.4, 126.1, 125.0 (each Ar-C), 74.8, 74.5, 73.9, 73.6 (each CH), 73.3 (CH$_2$), 70.8, 70.2, 70.2, 70.1, 69.9, 68.6, 68.1, 68.1, 68.1, 68.0, 67.6, 67.3, 66.9, 66.7 (each CH), 63.3, 53.9, 52.6, 50.8, 50.7, 49.6, 38.7, 29.6 (each CH$_2$), 25.0 (CH), 22.5, 22.3, 20.8, 20.7, 20.6, 20.6 (each CH$_3$); ESI-HRMS calcd for C$_{51}$H$_{67}$N$_4$O$_{13}$ 943.4704, found m/z 943.4708 [M+H]$^+$
Macrocycle 220. Compound 215 (26 mg, 0.035 mmol) was dissolved in pyridine-Ac$_2$O (1:1, 3 mL) and stirred at room temperature for 5 h. Solvents were removed under reduced pressure and the residue was taken up in EtOAc and washed with H$_2$O, brine, dried over MgSO$_4$, filtered and the solvents were concentrated under reduced pressure to give 220 (26 mg, 82%) as a white foam; [α]$_D$ +67.4° (c 0.1 in CHCl$_3$); IR (film) cm$^{-1}$: 2956, 2253, 1745, 1370, 1239, 1107, 903 cm$^{-1}$ 1H NMR (500 MHz, CDCl$_3$) δ 7.83 (3H, d, J 7.8, ArH), 7.75 (1H, s, ArH), 7.52 – 7.47 (2H, m, ArH), 7.43 (1H, d, J 8.1, ArH), 5.54 (1H, m, H-4’), 5.36 (1H, m, H-4), 5.30 – 5.24 (1H, m, H-3’), 5.04 (1H, m, H-3), 4.82 (1H, d, J 12.2, OCH$_2$Ph), 4.76 (1H, d, J 11.7, OCH$_2$Ph), 4.67 (2H, m, OCH$_2$C=C), 4.53 (1H, d, J 13.9, H-6’a), 4.41 – 4.32 (1H, m, H-6’b), 4.20 (1H, m, H-1’), 4.09 (1H, m, H-5’), 3.97 (1H, m, H-1), 3.90 – 3.82 (1H, m, H-5), 3.75 (2H, m, H-2 & H-2’), 3.67 (1H, m, H-6a), 3.61 (1H, m, H-6b), 3.53 (2H, m, OCH$_2$CH$_2$CH(CH$_3$)$_2$), 3.46 (1H, s), 2.46 (2H, m, CH$_2$), 2.37 (2H, m, CH$_2$), 2.33 – 2.22 (2H, m, CH$_2$), 2.15 (6H, m, each acetate CH$_3$), 2.04 (6H, s, each acetate CH$_3$), 1.84 (3H, s), 1.68 (6H, s), 1.40 (5H, dd, J 21.7, 15.4), 0.84 (12H, m, CH$_2$CH$_2$CH(CH$_3$)$_2$); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 169.23, 169.22, 168.96, 168.90 (each C=O), 146.2 (triazole CH=C), 134.9, 133.2, 133.2, 128.4, 128.0, 127.8, 126.4, 126.3, 126.2 (each Ar-C), 75.3 (CH), 74.9 (CH), 74.0 (CH), 73.6 (CH), 72.9 (CH), 71.9 (CH$_3$), 71.4 (CH), 71.3 (CH$_3$), 71.2 (CH), 70.3 (CH), 69.4 (OCH$_2$CH$_2$CH(CH$_3$)$_2$), 69.2 (CH), 68.7 (CH) , 66.2 (OCH$_2$C=C), 52.8 51.8, 51.56, 49.3, 38.9, 36.3, 29.8 (each CH$_2$), 25.1, 24.6 (CH), 22.9, 22.8 (CH$_3$); ESI-HRMS calced for C$_{48}$H$_{60}$N$_4$O$_{13}$ 909.4861, found m/z 909.4855 [M+H]$^+$
Macrocycle 221. Compound 216 (22 mg, 0.029 mmol) was dissolved in pyridine-Ac₂O (1:1, 3 mL) and stirred at room temperature for 5 h. Solvents were removed under reduced pressure and the residue was taken up in EtOAc and washed with H₂O, brine, dried over MgSO₄, filtered and the solvents were concentrated under reduced pressure to give 221 (23 mg, 84%) as a white solid; [α]D +54.0° (c 0.06 in CHCl₃); IR (film) cm⁻¹: 2956, 2253, 1745, 1370, 1239, 1107, 903; ¹H NMR (500 MHz, CDCl₃) δ 7.85 – 7.75 (4H, m, ArH), 7.68 (3H, m, ArH), 7.52 – 7.45 (3H, m, ArH), 7.39 – 7.28 (4H, m, ArH), 7.26 – 7.14 (8H, m, ArH), 5.54 – 5.50 (1H, m, H-4’), 5.38 – 5.34 (1H, m, H-4), 5.24 (1H, dd, J 9.0, 3.3, H-3’), 5.03 (1H, dd, J 9.4, 3.3, H-3), 4.69 (2H, overlapping signals, each CHH), 4.64 (2H, d, J 12.5), 4.60 (3H, overlapping signals), 4.54 (1H, dd, J 14.3, 1.3), 4.42 – 4.36 (1H, m), 4.19 (3H, overlapping signals), 4.06 – 4.01 (1H, m), 3.90 – 3.83 (2H, m), 3.71 – 3.60 (2H, m, each CH), 3.55 (2H, overlapping signals, CHH & CH), 3.49 (2H, dd, J 9.8, 2.3, CH₂), 3.45 (1H, dd, J 10.9, 4.3, CH₂), 3.41 – 3.35 (1H, m, CH₂), 2.56 – 2.42 (3H, m, CH₂N), 2.41 – 2.33 (2H, m,CH₂N), 2.33 – 2.24 (2H, m, CH₂N), 2.14 (3H, s, acetate CH₃), 2.13 (3H, s, acetate CH₃), 2.04 (6H, s, each acetate CH₃), 1.65 – 1.56 (3H, m), 1.40 – 1.29 (5H, m), 0.85 (7H, t, J 6.5, OCH₂CH₂CH(CH₃)₂); ¹³C NMR (125 MHz, CDCl₃) δ 169.23, 169.22, 168.96, 168.90 (each C=O), 145.10, 133.99, 132.11, 132.02 (Ar-C), 127.95, 127.57, 127.54, 127.23, 126.83, 126.69, 125.63, 125.37, 125.26, 125.10, 124.64, 122.46 (each Ar-CH), 74.46, 73.57 (each CH), 73.24 (CH₂), 72.44, 72.01, 69.10 (CH₂), 68.94 (CH₂), 68.52, 68.45, 68.16, 67.93, 67.91, 67.71, 67.70 (each CH), 64.11 (CH₂), 50.84, 50.80, 49.75, 49.08, 37.72, 28.68 (each CH₂), 23.77 (CH), 21.56, 21.43, 19.88, 19.77, 19.68 (each CH₃); ESI-HRMS calcd for C₅₀H₆₅N₄O₁₃ 929.4548, found m/z 929.4555 [M+H]⁺
Experimental data

1-C-Allyl-1,6-dideoxy-6-((1-C-allyl-1,6-dideoxy-2,3,4-tri-O-benzyl-α-D-glucopyranos-6-yloxy)methyl)-1H-1,2,3-triazol-1-yl)-2-O-benzyl-3,4-O-isopropylidene-α-D-galactopyranoside (222). Alkyne 152 (200 mg, 0.390 mmol) and azide 201 (140 mg, 0.390 mmol) were dissolved in a mixture of acetonitrile-H2O (5 mL, 1:1). To this was added CuI (75 mg, 0.39 mmol) and the reaction was heated at reflux for 24 h. Upon cooling the reaction was diluted with EtOAc, washed with H2O, brine, dried over MgSO4, filtered and the solvents were concentrated under reduced pressure. The crude residue was purified via flash chromatography (EtOAc-petroleum ether 1:1) to give compound 222 (289 mg, 85%) as a yellow oil; IR (film) cm⁻¹: 2915, 1640, 1381, 1092, 906; ¹H NMR (500 MHz, CDCl₃) δ 7.64 (1H, s, triazole H), 7.35 – 7.26 (20H, m, ArH), 5.86 – 5.72 (1H, m, CH₂C=CH₂), 5.61 (1H, dd, J 17.1, 10.2, CH₂CH=CH₂), 5.07 (2H, dd, J 17.1, 13.8, CH₂CH=CH₂), 4.99 – 4.88 (3H, overlapping signals, CH₂CH=CH₂ & OCH₃Ph), 4.72 – 4.60 (5H, overlapping signals, OCH₃Ph & OCH₂C=C), 4.56 – 4.47 (3H, overlapping signals, H-6’a & OCH₂Ph), 4.40 – 4.33 (2H, overlapping signals, CH & H-6”b), 4.23 (1H, m, CH), 4.15 (1H, d, J 9.0, CH), 4.09 (1H, dd, J 10.3, 5.4, CH), 4.06 – 4.02 (1H, m, CH), 3.81 – 3.77 (1H, m, CH), 3.74 (2H, overlapping signals, H-6a and CH), 3.67 (1 H, d, J 10.6, H-6b), 3.60 (3 H, overlapping signals, each CH), 3.53 (1 H, t, J 3.4, CH), 2.52 – 2.44 (2 H, m, CH₂CH=CH₂), 2.31 – 2.24 (1H, m, CHHCH=CH₂), 2.18 (1H, dd, J 14.6, 6.6, CHHCH=CH₂), 1.49 (3H, s, isopropylidene CH₃), 1.33 (3H, s, isopropylidene CH₃); ¹³C NMR (125 MHz, CDCl₃) δ 144.7 (triazole CH=C), 138.9, 138.3, 138.3, 137.6 (each Ar-C), 134.8 (CH₂CH=CH₂), 134.5 (CH₂CH=CH₂), 134.3, 133.9, 133.3, 128.6, 128.5, 128.5, 128.5, 128.4, 128.1, 128.1, 127.9, 127.9, 127.9, 127.9, 127.6 (each Ar-CH), 124.3 (triazole CH=C), 117.4 (CH₂CH=CH₂), 117.0 (CH₂CH=CH₂), 110.2 (isopropylidene C), 82.4 (CH), 80.1 (CH), 78.1 (CH), 75.5 (OCH₃Ph), 75.2 (OCH₂Ph), 74.9 (CH), 73.8 (CH), 73.2 (CH), 73.0 (OCH₂Ph),
72.8 (OCH₂Ph), 71.7 (CH), 71.6 (CH), 69.2 (CH₂), 68.8 (CH), 65.0 (OCH₂C=C), 51.8 (C-6), 34.9 (CH₂CH=CH₂), 29.9 (CH₂CH=CH₂), 26.8 (isopropylidene CH₃), 24.8 (isopropylidene CH₃); ESI-HRMS calcd for C₅₂H₆₂N₃O₉ 872.4486, found m/z 872.4479 [M+H]+

**Macrocycle 223.** Compound 222 (280 mg, 0.321 mmol) was dissolved in toluene (6 mL) and degassed at -78 °C. Upon warming to room temperature, Hoveyda-Grubbs II catalyst (30 mg, 0.048 mmol) was added and the reaction heated to 90 °C for 8 h. The reaction was cooled to room temperature and the solvents were concentrated under reduced pressure. The crude residue was purified via flash chromatography (EtOAc-petroleum ether 1:5) to give 223 (251 mg, 93%) as a colourless oil; IR (film) cm⁻¹: 2913, 1641, 1381, 1090, 905; ¹H NMR (500 MHz, CDCl₃) δ 7.85 (1H, s, triazole H), 7.37 – 7.22 (20H, m, ArH), 5.71 – 5.63 (1H, dt, CH=CH, J 15.0, 6.9), 5.48 – 5.39 (1H, dt, J 15.1, 7.1, CH=C₃H), 4.92 (1H, d, J 11.0, OCH₂Ph), 4.88 (1H, d, J 11.0, OCH₃Ph), 4.81 (2H, dd, J 11.3, 8.8, overlapping signals, OCH₂Ph, OCH₃H-C), 4.70 (1H, d, J 11.6, OCH₃Ph), 4.66 – 4.54 (4H, overlapping signals, OCH₂Ph, OCH₃HPh and H-6a), 4.50 (2H, dd, J 13.8, 7.4, OCH₂Ph), 4.46 (1H, d, J 11.7, OCH₂Ph), 4.34 (1H, dd, J 14.4, 9.3, CHH), 4.27 (1H, d, J 7.8, CH), 4.10 (1H, d, J 9.2, CH), 4.03 (1H, dd, J 10.7, 3.3, CH), 3.97 (1H, d, J 11.7, CH), 3.86 – 3.80 (1H, m, CH), 3.73 (3H, overlapping signals, 2 x CH & CHH), 3.62 – 3.59 (1H, m, CHH), 3.58 (1H, t, J 3.3, CH), 3.30 (1H, dd, J 9.7, 5.0, 3.3, CH), 2.56 – 2.46 (1H, m, CHHCH=CH), 2.45 – 2.39 (1H, m, CHHCH=CH), 2.36 (1H, dd, J 15.4, 6.0, CHHCH=CH), 1.80 (1H, dd, J 15.2, 7.9, CHHCH=CH), 1.48 (3H, s, isopropylidene CH₃), 1.35 (3H, s, isopropylidene CH₃); ¹³C NMR (125 MHz, CDCl₃) δ 146.07 (triazole CH=C), 138.63, 138.21, 137.97, 137.36 (each Ar-C, 130.05 (CH=CH), 128.52 (CH=CH), 128.48, 128.46, 128.41, 128.04, 128.02, 127.94, 127.88, 127.85, 127.82, 127.80 (each Ar-CH), 123.91 (triazole CH=C), 110.61 (isopropylidene C), 82.58, 80.14, 78.85, 75.48 (CH), 75.34 (OCH₂Ph), 75.11 (OCH₂Ph), 74.04 (CH), 73.76 (CH), 186
Experimental data

Chapter 6

73.30 (OCH\textsubscript{2}Ph), 73.15 (OCH\textsubscript{2}Ph), 72.63 (CH), 72.21 (CH), 71.38, 70.44 (CH\textsubscript{2}), 69.62, 65.71 (OCH\textsubscript{2}C=C), 52.05 (C-6), 35.18 (CH\textsubscript{2}), 26.88 (CH\textsubscript{2}), 26.41 (isopropylidene CH\textsubscript{3}), 24.76 (isopropylidene CH\textsubscript{3}); ESI-HRMS calcd for C\textsubscript{50}H\textsubscript{58}N\textsubscript{3}O\textsubscript{9} 844.4173, found m/z 844.4171 [M+H]\textsuperscript{+}

Macrocycle 224. Compound 223 (250 mg, 0.296 mmol) was dissolved in EtSH-BF\textsubscript{3},Et\textsubscript{2}O (5 mL, 4:1) and stirred at room temperature for 24 h. Solvents were removed and the residue was purified via flash chromatography (CH\textsubscript{2}Cl\textsubscript{2}-MeOH 8:2) to give 224 (116 mg, 89%) as a white solid; \textsuperscript{1}HNMR (D\textsubscript{2}O, 500 MHz) \textsuperscript{δ} 7.92 (1H, s, triazole H), 5.19 (1H, dt, CH=CH, J 15.0, 6.9), 5.01 (1H, dt, CH=CH, J 15.1, 7.0), 4.69 (1H, d, J 12.9, OCHHC=C), 4.52 (2H, dd, J 18.6, 11.0, H-6'), 4.45 (1H, d, J 12.8, OCHHC=C), 4.01 (1H, m, H-5'), 3.97 (1H, m, H-3'), 3.86 (2H, overlapping signals, H-4' & H-1'), 3.74 (2H, overlapping signals, H-5 & H-6a), 3.67 (1H, ddd, J 9.0, 5.7, 2.5, H-1) 3.61 (1H, brs, H-2'), 3.59 (1H, dd, J 11.4, 7.0, H-6b), 3.51 (1H, dd, J 9.6, 5.9, H-2), 3.40 (1H, t, J 9.1, H-3), 3.08 (1H, t, J 9.1-H-4), 2.10 (4H, overlapping signals, each CH\textsubscript{2}C=C); \textsuperscript{13}C NMR (125 MHz, D\textsubscript{2}O) \textsuperscript{δ} 142.1 (C=CH), 131.9, 131.6 (each CH=CH), 127.7 (C=CH), 78.9 (C-1), 78.1 (C-1') 75.6 (C-3), 74.1 (C-5'), 73.9 (C-5), 73.5 (C-6), 73.4 (C-4), 73.1 (C-2) 72.1 (C-3'), 72.0 (C-2'), 70.5 (H-4'), 63.4 (OCH\textsubscript{2}C=C), 51.5 (C-6'), 26.2 (CH\textsubscript{2}C=C); ESI-HRMS calcd for C\textsubscript{19}H\textsubscript{30}N\textsubscript{3}O\textsubscript{9} 444.1982, found m/z 444.1978 [M+H]\textsuperscript{+}

6.5 Chapter 5-Experimental

2,3:4,6-Di-O-isopropylidene-\textalpha-\textl-sorbofuranose\textsuperscript{10} (242). Iodine (1.14 g, 4.5 mmol) was added to a solution of L-sorbose (5 g, 27.7 mmol) in dry acetone (350 mL). The solution was
stirred at room temperature for 20 h. A 0.2 M solution of sodium sulfite was then added dropwise until the red colour dissipated. The acetone was removed under reduced pressure and the remaining aqueous layer was extracted with CH$_2$Cl$_2$. The organic layers were then combined, washed with H$_2$O, dried over MgSO$_4$ and the solvents were concentrated under reduced pressure. The residue was then purified via flash chromatography (EtOAc-cyclohexane 1:1 R$_f$ 0.33) to give the desired product as a white solid (85%); IR (film) cm$^{-1}$: 3487, 2990, 2938, 1375, 1243, 1197, 1122, 1078; $^1$H NMR (500MHz, CDCl$_3$) $\delta$ 4.49 (1H, s, H-3), 4.33 (1H, s, H-4), 4.10 (1H, s, H-5), 4.07(2H, m, H-6), 3.84 (2H, m, H-1), 1.44, 1.37, 1.31, 1.30 (each 3H, each s, each CH$_3$); $^{13}$C NMR (125MHz, CDCl$_3$) $\delta$ 114.5 (C$_{-}$2), 111.9 (C(Me)$_2$), 97.5 ((C(Me)$_2$)$_{2}$), 84.8 (C-3), 73.3 ( C-5), 72.3 (C-4), 63.4 (C-1), 29.0, 27.5, 26.6, 18.8 (each CH$_3$); ESI-HRMS calcd for C$_{12}$H$_{20}$O$_6$Na 283.1158, found m/z 283.1144 [M+Na]$^+$

2,3:4,6-Di-O-isopropylidene-α-L-xylo-hexos-2-ulo-2,5-furanose$^{11}$ To a stirring solution of oxalyl chloride (2.9 mL, 34.5 mmol) in CH$_2$Cl$_2$ at -78 °C was added anhydrous DMSO (3.2 mL, 46 mmol). After 15 min a solution of 242 (3 g, 11.5 mmol) in CH$_2$Cl$_2$ was added. Stirring was continued for a further 4 h before the temperature was increased to -50°C and anhydrous triethylamine was added. After 30 min the temperature was increased to room temperature and H$_2$O was added. The layers were separated and the aqueous layer was extracted into CH$_2$Cl$_2$. The organic layers were combined and washed with brine, dried over MgSO$_4$ and the solvents were concentrated under reduced pressure. The residue was purified via flash chromatography (EtOAc-cyclohexane 1:1 R$_f$ 0.2) to give the title compound as an off-white solid (83%); $^1$H NMR (500MHz, CDCl$_3$) $\delta$ 9.62 (1H, s, CHO), 4.55 (1H, s, H-3), 4.36 (1H, m, H-4), 4.23 (1H, m, H-5), 4.12 (2H, m, H-6), 1.50, 1.40, 1.35, 1.29 (each 3H, each s, each CH$_3$); $^{13}$C NMR (125MHz, CDCl$_3$) $\delta$ 194.5 (CHO), 114.5 (C$_{-}$2), 112.1 (C-2), 97.8 ((C(Me)$_2$)$_{2}$), 90.7 (C-3), 86.7 ( C-5), 74.0 (C-4), 60.1 (C-6), 29.1, 27.3, 26.3, 18.9 (each CH$_3$)
**1,2-Dideoxy-4,5:6,8-di-O-isopropylidene-α-L-xylo-hept-1-ene-3-ulo-3,6-furanose (243).**

Ph$_3$PCH$_2$Br (4.15 g, 11.6 mmol) was taken up in THF (50 mL) and stirred at -78 °C. NaHMDS (11.6 mL, 1.0 M in THF) was then added dropwise. The reaction was stirred at -78 °C for 30 min and a solution of 2,3:4,6-Di-O-isopropylidene-α-L-xylo-hexos-2-ulo-2,5-furanose (2 g, 7.74 mmol) in THF (50 mL) was added via cannula. The reaction was stirred for a further 10 min at -78 °C and left to stir at room temperature overnight. The reaction was then quenched with satd NH$_4$Cl. The aqueous layer was extracted into EtOAc and the combined organic layers were washed with H$_2$O, brine, dried over MgSO$_4$ and the solvents were concentrated under reduced pressure. The compound was then purified via flash chromatography (EtOAc-cyclohexane 1:8 R$_f$ 0.5) to yield compound 243 as a clear oil (63%); IR (film) cm$^{-1}$: 2991, 2933, 1650, 1453, 1409, 1373, 1195, 1120, 1074, 990, 912, 873, 831; $^1$H NMR (500MHz, CDCl$_3$) δ 6.05 (1H, dd, J 10.5, 17.2, H-2), 5.70 (1H, dd, J 1.6, 10.5, H-1a), 5.29 (1H, dd, J 1.6, 17.2, H-1b), 4.29 (1H, d, J 2.1, H-6), 4.28 (1H, s, H-4), 4.09 (1H, dd, J 2.0, 3.9, H-5), 4.04 (2H, m, H-7), 1.52, 1.43, 1.37, 1.35 (each 3H, each s, each CH$_3$); $^{13}$C NMR (125MHz, CDCl$_3$) δ 136.3 (C-2), 117.4 (C-1), 113.2 (C-3), 111.7 (C(Me)$_2$), 97.5 ((C(Me)$_2$), 88.0 (C-4), 73.8 (C-6), 72.6 (C-5), 60.5 (C-7), 29.1, 27.3, 26.3, 18.9 (each CH$_3$); ESI-HRMS calcd for C$_{13}$H$_{21}$O$_5$ 257.1389, found m/z 257.1398 [M+H]$^+$

![Image of 1,2-Dideoxy-4,5:6,8-di-O-isopropylidene-α-L-xylo-hept-1-ene-3-ulo-3,6-furanose (243)](image.png)

**1,2-Dideoxy-4,5-O-isopropylidene-α-L-xylo-hept-1-ene-3-ulo-3,6-furanose (244).**

Compound 243 (1.55g, 6.05mmol) was dissolved in AcOH-H$_2$O (60% V/V) and heated to 60 °C for 2 h. It was then concentrated and columned (EtOAc-cyclohexane 1:1 R$_f$ 0.28) to yield 244 (87%) as a clear oil; [α]$_D$ +20.3° (c 1.0, CHCl$_3$); IR (film) cm$^{-1}$: 3495 (broad), 2988, 2935, 2895, 1649, 1456, 1411, 1384, 1175, 1078, 1045, 989, 912, 868, 832, 787, 737; $^1$H NMR (500MHz, CDCl$_3$) δ 6.05 (1H, dd, J 10.5, 17.2, H-2), 5.70 (1H, dd, J 1.6, 10.5, H-1a), 5.29 (1H, dd, J 1.6, 17.2, H-1b), 4.20 (3H, m, H-3, H-4 & H-5 overlapping), 4.04 (2H, m, H-7), 1.46, 1.28 (each 3H, each s, each CH$_3$); $^{13}$C NMR (125MHz, CDCl$_3$) δ 135.9 (C-2), 116.9
Experimental data

10-Azidomethyl-3,3-dimethyl-2,4,11-trioxa-9aza-tricyclo undecan-6-ol (245). To compound 244 (1.14 g, 5.3 mmol) dissolved in dry CH$_2$Cl$_2$ (70 mL) was added pyridine (1.02 mL, 12.7 mmol). The reaction was stirred at 0 °C and SOCl$_2$ (0.47 mL, 6.34 mmol) in dry CH$_2$Cl$_2$ (35 mL) was added dropwise. The reaction was then stirred for a further 2 h at 0 °C before being washed with H$_2$O, brine, dried over MgSO$_4$ and the solvents were concentrated under reduced pressure. The crude product was then dissolved in dry DMF and NaN$_3$ (1.42 g, 17.9 mmol) was added. The reaction was then stirred at 110 °C for 20 h. H$_2$O was then added and the resulting mixture was extracted into Et$_2$O. The organic layers were combined, washed with H$_2$O, dried over MgSO$_4$, filtered and the solvents were concentrated under reduced pressure. The residue was purified via flash chromatography (EtOAc-cyclohexane 1:1 R$_f$ 0.18) to yield compound 245 (40%). $[\alpha]_D^{24}$ +24.9° (c 1.0, CHCl$_3$); IR (film) cm$^{-1}$: 3504, 3319, 2853, 2099, 1453, 1375, 1288, 1234, 1199, 1134, 1109, 1051, 1014, 941, 870, 837; $^1$H NMR (500MHz, CDCl$_3$) $\delta$ 4.52 (1H, d, J 6.7, H-6), 4.40 (1H, d, J 6.7, H-5), 4.38 (1H, s, H-4), 3.76 (1H, dd, J 3.0, 11.1, H-1a), 3.32 (1H, dd, J 3.1, 9.0, H-1b), 3.24 (1H, dd, J 9.4, 11.7, H-2), 3.04 (1H, dd, J 12.1, 1.0, H-7a) 2.99 (1H, dd, J 12.0, 2.1, H-7b), 1.55, 1.36 (each 3H, each s, Each CH$_3$); $^{13}$C NMR (125MHz, CDCl$_3$) $\delta$ 116.4 (C-3), 110.0 (C(Me)2), 88.3 (C-4), 79.3 (C-6), 75.7 (C-5), 57.2 (C-2), 52.1 (C-1), 44.9 (C-7), 28.3 (CH3), 26.8 (CH3)$^1$; ESI-HRMS calcd for C$_{10}$H$_{17}$N$_4$O$_4$ 257.1250, found m/z 257.1249 [M+H]$^+$

2-Azidomethyl-6-oxa-3-aza-bicyclo[3.2.2]nonane-1,8,9-triol (246). Compound 245 was taken up in methanolic HCl (3mL) and stirred overnight at room temperature. Solvents were
removed under reduced pressure and the resulting residue was taken up in H₂O and lyophilised. The product was obtained as a brown foam (60%); [α]D +73.3° (c 1.0, H₂O); IR (film) cm⁻¹: 3398 (br), 2928, 2109, 1733, 1623, 1443; ¹H NMR (600 MHz, D₂O) δ 4.26 (1H, d, J 7.63, H-6), 4.22 (1H, d, J 9.5, H-5), 4.03 (1H, s, H-4), 3.64 (1H, dd, J 13.0, 4.0, H-1a), 3.33-3.28 (1H, dd, J 13.0, 7.86, H-2), 3.07 (1H, dd, J 7.8, 4.0, H-1b), 2.93 (1H, d, J 13.5, H-7a), 2.83 (1H, dd, J 11.0, H-7b); ¹³C NMR (150 MHz, D₂O) δ 100.0 (C-3), 78.3 (C-4), 75.4 (C-5), 75.0 (C-6), 59.4 (C-2), 51.0 (C-1), 42.8 (C-7); ESI-HRMS calcd for C₇H₁₅N₄O₄ 217.0937, found m/z 217.0933 [M+H]+

(10-Azidomethyl-6-hydroxy-3,3-dimethyl-2,4,12-trioxa-9-aza-tricyclo[5.3.2.0₁,₅]dodec-9-yl)-acetic acid ethyl ester (248). Compound 245 (235 mg, 0.9 mmol) was taken up in THF (50 mL) and triethylamine (435 uL, 3.1 mmol) was added. The solution was stirred at room temperature and ethyl bromoacetate (298 uL, 2.7 mmol) was added to the solution along with tetra-n-butylammonium iodide (299 mg, 0.8 mmol). The reaction was heated at reflux for 5 h, washed with H₂O, brine, dried over MgSO₄ and the solvents were concentrated under reduced pressure. The residue was purified via column chromatography (EtOAc-cyclohexane 1:1, Rₜ 0.38) to give the title compound as a clear oil (490 mg, 61%); [α]D -26.2° (c 1.0, CHCl₃); IR (film) cm⁻¹: 3502, 3319, 2986, 2936, 2852, 2104, 1445, 1287, 1201, 864; ¹H NMR (500MHz, CDCl₃) δ 4.47 (2H, m, H-6 & H-4 overlapping), 4.36 (1H, dd, J 7.1, 13.0, H-5), 4.23 (2H, q, J 7.0, H-10), 3.90 (1H, dd, J 2.1, 13.4, H-1a), 3.7 (1H, d, J 18.0, H-8a), 3.45 (1H, dd, J 5.2, 14.0 H-1b) 3.15 (1H, d, J 18.2, H-8b), 3.09 (1H, dd, J 2.0, 5.3, H-2), 2.93 (1H, d, J 11.0, H-7a), 2.65 (1H, dd, J 2.1, 11.4, H-7b) 1.56, 1.40 ( each 3H, each s, Each CH₃), 1.29 (t, 3H, J 7 Hz H-11); ¹³C NMR (125MHz, CDCl₃) δ 171.51 (C=O), 116.4, 109.0, 88.9, 78.2, 76.2, 62.2, 61.5, 53.3, 52.2, 49.2, 28.4, 26.7, 14.1; ESI-HRMS calcd for C₁₄H₂₃N₄O₆ 343.1618, found m/z 343.1624
Tricyclic compound (249). Compound 248 (158 mg, 0.46 mmol) was dissolved in EtOAc (8 mL) and 10% palladium on carbon was added. The reaction was stirred overnight under an atmosphere of hydrogen. The solution was filtered through Celite® and the solvent was concentrated under reduced pressure to give compound as a white solid (108 mg, 90%); mp 189-191°C; $[\alpha]_D^{+15.8^\circ}$ (c 1.0, CHCl₃); $R_f$ 0.13 EtOAc-cyclohexane 1:1; IR (film) cm⁻¹: 3331, 2943, 2247, 1668, 1213, 1048; $^1$H NMR (500MHz, CDCl₃) δ 7.34 (1H, d, NHC=O), 4.46 (1H, d, $J$ 7.0, H-6), 4.42 (1H, s, H-5), 4.34 (1H, d, $J$ 4.1) 3.48 (1H, d, $J$ 16.7), 3.42 (1H, dt, $J$ 11.1, 4.0), 3.21 (1H, t, $J$ 11.4, H-7a), 3.00-2.93 (3 H, m), 2.41 (1H, dd, $J$ 11.9, 2.0, H-7b), 1.49, 1.28 (each 3H, each s, each CH₃); $^{13}$C NMR (125 MHz, CDCl₃) δ 168.5, 116.57, 109.7, 87.6, 77.93, 75.9, 57.8, 56.6, 52.3, 41.85, 28.35, 27.1; ESI-HRMS calcd for C₁₂H₁₉N₂O₅ 271.1294, found m/z 271.1290 [M+H]$^+$

Tricyclic compound 250. Tricyclic compound 249 (97 mg, 0.36 mmol) was taken up in methanolic HCl (13 mL) and stirred for 10 hours. Solvents were concentrated under reduced pressure and the residue was taken up in H₂O and lyophilized to give the product as a white foam (73%); $[\alpha]_D^{+106.6^\circ}$ (c 1.0, H₂O); IR (film) cm⁻¹: 3300 (br), 2492, 1671, 1377, 1095; $^1$H NMR (500 MHz, D₂O) δ 4.55 (1H, d, $J$ 7.0, H-6), 4.35 (1H, d, $J$ 7.0 Hz, H-5), 4.03 (1H, s, H-4), 3.90 (1H, d, $J$ 16.6, H-8a), 3.72 (1H, d, $J$ 17.0, H-8b), 3.67 (1H, dd, $J$ 4.1, 13.1, H-1a), 3.64 (1H, dd, $J$ 4.1, 11.5, H-2), 3.52 (1H, d, $J$ 12.7, H-7a), 3.38 (1H, t, $J$ 12.21, H-1b), 3.05 (1H, d, $J$ 12.7, H-7b); $^{13}$C NMR (150 MHz, D₂O) δ 109.9 (C), 100 (C-4), 77.0 (C-5), 74.7 (C-4), 72.2 (C-6), 61.2 (C-2), 54.5 (C-8), 51.6 (C-7), 39.0 (C-1); ESI-HRMS calcd for C₉H₁₅N₂O₅ 231.0981, found m/z 231.0977 [M+H]$^+$
6.6 References


(9) Czechura, P.; Tam, R. Y.; Dimitrijevic, E.; Murphy, A. V.; Ben, R. N. *Journal of the American Chemical Society* **2008**, *130*, 2928-2929.
