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<td>Author(s)</td>
<td>McDonagh, Anthony W.</td>
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Synthesis of Novel Glycolipids and Investigation into the Anomerisation of Selenium Glycosides

By

Anthony W. McDonagh

A Thesis presented to
The National University of Ireland
For the degree of
Doctor of Philosophy

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

Under the supervision and direction of
Prof. Paul V. Murphy
National University of Ireland,
Galway.
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.

____________________

Anthony McDonagh
For Mum & Dad
Abstract

The first section of this thesis describes the synthesis of novel glycosphingolipids with an \( \alpha \)-triazole at the anomeric carbon. Glycosphingolipids are amphiphilic molecules containing a carbohydrate head glycosidically linked to a sphingoid base. The \( \alpha \)-linked derivatives KRN7000 (\( \alpha \)-GalCer) and PBS-59 are of particular interest, due to their ability to stimulate iNKT cells and produce cytokines that can assist against various infections or alleviating the effects of autoimmune diseases. The Murphy group has previously reported the synthesis of \( \alpha \)-O, \( \alpha \)-SO\(_2\) and \( \alpha \)-S PBS-59 analogues. Key to the success of each synthesis was a stereoselective anomerisation reaction in forming the \( \alpha \)-anomer. These anomerisation reactions are promoted by either TiCl\(_4\) or SnCl\(_4\) and are successful in generating \( \alpha \)-O and \( \alpha \)-S-glycosides from the corresponding \( \beta \)-anomer. A more recent development in the Murphy group has illustrated the anomerisation for glycosyl azides. Therefore, chapter two is focused on applying these \( \alpha \)-glycosyl azides as intermediates to synthesise new PBS-59 analogues with either a 1,5- or 1,4-triazole linkage.

Chapter 3 describes an investigation into the anomerisation of selenium glycosides. There are various procedures in the literature for the synthesis of \( \beta \)-Se-glycosides and a lesser number reported for \( \alpha \)-Se-glycosides. It was therefore envisioned that Lewis acid promoted anomerisation could give \( \alpha \)-Se-glycosides from the corresponding \( \beta \)-anomer. The anomerisation was successful for galacturonic acid derivatives with TiCl\(_4\) as the reaction promoter. To investigate the substrate scope, various alkyl, alkenyl, alkynyl, saccharide and steroid groups were attached to the anomeric selenium atom. The anomerisation was found only to be unsuccessful for 1→4 linked disaccharides. Yields were higher when the reactions were carried out at higher dilution. Increasing the complexity of the aglycon led to the requirement to increase the amount of Lewis acid and/or temperature to ensure reaction completion.

The selenium anomerisation was then applied to the first synthesis of selenoglycoside analogues of the potent immunostimulant \( \alpha \)-GalCer. Chapter four presents the synthesis of \( \alpha \)-Se-GalCer and analogues thereof. Synthetic challenges deemed the initial route involving an azide intermediate to be unsuccessful. However, revising the retrosynthesis to omit the azido gave a successful route to the target glycolipids.
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<td>Alpha</td>
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<tr>
<td>Å</td>
<td>Angstrom</td>
</tr>
<tr>
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<td>Acetyl</td>
</tr>
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<td>iNKT</td>
<td>Invariant natural killer T</td>
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<td>Infrared (spectroscopy)</td>
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<td>J</td>
<td>Coupling constant (nmr), in Hz</td>
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<tr>
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<td>LiI</td>
<td>Lithium iodide</td>
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<td>LPS</td>
<td>Lipopolysaccharide</td>
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<tr>
<td>m</td>
<td>Multiplet</td>
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<tr>
<td>M</td>
<td>Molar</td>
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<td>M⁺</td>
<td>Mass of the molecular ion (mass spectrometry)</td>
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MAD  Multi-wavelength anomalous dispersion
Me  Methyl
MeCN  Acetonitrile
MeOH  Methanol
Mg  Magnesium
MgBr₂·OEt₂  Magnesium bromide ethyl etherate
MHz  Megahertz
min  Minutes
mL, µL  Milliliter, microliter
mol, mmol  Mole, millimole
mM, µM  Millimolar, micromolar
mp  Melting point
Ms  Mesylate
MsCl  Methanesulfonyl chloride
MW  Microwave
η  Eta
Na₂CO₃  Sodium carbonate
NaBH₄  Sodium borohydride
NaBH₄CN  Sodium cyanoborohydride
NaH  Sodium hydride
NaHCO₃  Sodium bicarbonate
NaHSe  Sodium hydrogen selenide
NaIO₄  Sodium periodate
NaN₃  Sodium azide
NaOH  Sodium hydroxide
NaOMe  Sodium methoxide
NaOPr  Sodium propoxide
NaSMe  Sodium thiomethoxide
NaSO₄  Sodium sulfate
NCRD  N-terminal carbohydrate recognition domain
NEt₃  Triethylamine
NK  Natural Killer
NKT  Natural Killer T
NMO  4-Methylmorpholine N-oxide
NMR  Nuclear Magnetic Resonance
NOE  Nuclear Overhauser Effect
NOESY  Nuclear Overhauser Effect Spectroscopy
OTf  Trifluoromethanesulfonate/triflate
OTs  Tosylate
OsO₄  Osmium tetroxide
Ox  Oxidation
Ph  Phenyl
PhCHO  Benzaldehyde
Phe  Phenylalanine
PMA  Phorbol 12-myristate 13-acetate
PMe₃  Trimethylphosphine
PPh₂  Triphenylphosphine
ppm  Parts per million (NMR)
PPTS  Pyridinium p-toluenesulfonate
Pr, n-Pr  Normal (primary) propyl
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<tr>
<td>Py</td>
<td>Pyridine</td>
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<td>q</td>
<td>Quartet (spectral)</td>
</tr>
<tr>
<td>quant.</td>
<td>Quantitative</td>
</tr>
<tr>
<td>Rf</td>
<td>Retention factor</td>
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<td>Room temperature</td>
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<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
</tr>
<tr>
<td>[α]D</td>
<td>Specific rotation</td>
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<td>s</td>
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<td>SAD</td>
<td>Single wavelength anomalous dispersion</td>
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<td>SAR</td>
<td>Structure activity relationship</td>
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<td>Methylselenide</td>
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<td>Tin tetrachloride</td>
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<td>SnCl₃ClO₄</td>
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<td>Bimolecular nucleophilic substitution</td>
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<td>Triplet of doublets (spectral)</td>
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<tr>
<td>TBAB</td>
<td>Tetraethylammonium bromide</td>
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<td>TBAF</td>
<td>Tetraethylammonium fluoride</td>
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<td>TBAHS</td>
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<tr>
<td>TES</td>
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<td>Cis</td>
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Chapter 1: Introduction to Glycosphingolipids

1.1 Structure of Glycosphingolipids 2

1.2 Biology of Invariant Natural Killer T (iNKT) Cells 3

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1.4.3 Substitution and Variating the Lipid Chains. 10

1.4.4 Modifying the Nature and Configuration (α or β) of the Anomeric Bond. 11

1.5 Bacterial Glycosphingolipids Stimulate NKT Cells 14

1.5.1 Stereoselective Synthesis of Bacterial Glycolipids 16

1.6 Aims of this Thesis and Target Compounds 20

1.7 References 21
1.1 Structure of Glycosphingolipids

Glycosphingolipids (GSL’s) are a diverse set of glycolipid found in the lipid membranes of organisms from prokaryotic to eukaryotic cells. They have varied molecular structures and are involved in biology, through cell-cell recognition, host-pathogen interactions, cell signalling, migration and apoptosis. Each GSL has a hydrophobic ceramide and a hydrophilic carbohydrate head (single sugar or oligosaccharide). Due to the high structural variation of GSL’s they are generally classified into four categories:

- Cerebrosides Containing one sugar residue
- Sulfatides Containing one sulfated residue on the saccharide
- Neutral glycosphingolipids Containing one or more uncharged sugar residues
- Gangliosides Containing one or more neuraminic acid residues

The major types of sugars found in GSL’s are glucose, galactose, fucose, N-acetylglucosamine and N-acetylgalactosamine. The majority of diversity for GSL’s is found in the sphingoid region of the molecule. Three main categories of sphingoid are present in nature: sphingosine, sphinganine and phytosphingosine (Figure 1). N-Acylation of a sphingosine can then give a ceramide.

![Figure 1. Structure of sphingoid bases and ceramide.](image)

Sphingosines have an amino diol and an olefin at C4-C5. Sphinganines, also known as dihydrosphingosines, are the reduced form of sphingosines with an amino diol and are saturated at C4-C5, phytosphingosines are 2-amino-1,2,4-triols, while ceramides are made up of a sphingosine and a long fatty acid chain.
1.2 Biology of Invariant Natural Killer T (iNKT) Cells

Invariant natural killer T (iNKT) cells as a specialised subset of T cells that exist in a ‘poised effector’ state\(^2\) which allows them to rapidly produce signalling molecules involved in cellular communication called cytokines. Using their T cell receptor (TCR), iNKT cells recognise self and foreign lipid antigens presented by CD1d\(^3\), a protein carried by antigen presenting cells such as dendritic cells or macrophages. CD1d recognises lipid antigens to form a binary CD1d/glycolipid complex and presents them for recognition by a TCR. A ternary CD1d/glycolipid/TCR complex results from this mechanism and activates certain immune cells (NK cells, B cells, T cells, macrophages, neutrophils) to assist against antitumor, antimicrobial and antiviral infection (T\(\text{H}1\)) or to alleviate the effect of autoimmune diseases (T\(\text{H}2\))\(^4\) (Figure 2)\(^5\). The cytokines involved in a T\(\text{H}1\) response are interferon γ (IFN-γ) and for a T\(\text{H}2\) involve interleukin 4 and 12 (IL-4, IL-12).

**Figure 2.** Recognition of glycolipid antigens presented by CD1d on dendritic cells by the TCR on iNKT cells. (Reprinted with permission from Nature Reviews Immunology, Copyright 2013, Nature Publishing Group)
1.3 KRN7000 (α-GalCer): Interaction with iNKT Cells

In 1993, the Kirin Brewery Co. characterised a number of glycolipids isolated from the Japanese marine sponge *Agelas mauritianus* and were found to have both anti-tumour and immunostimulatory properties in mice and humans (Figure 3)\(^6\). A unique structural feature in these molecules is the α-configuration of the glycosidic linkage between the saccharide and the ceramide. Structure activity relationship (SAR) studies of agelasphins from the Kirin Brewery Co. in 1995 revealed an anti-cancer drug candidate KRN7000 (α-GalCer)\(^7\). In 1997, it was found that KRN7000 formed a complex with CD1d and stimulated NKT cells to produce an equally high portion of \(\text{T}_1\) and \(\text{T}_2\) cytokines\(^8\). However despite the high cytokine profile, \(\text{T}_1\) and \(\text{T}_2\) cytokines antagonise each other’s biological functions which limited the use of KRN7000 in clinical trials.

![Figure 3. Structure of agelasphin-9b and KRN7000.](image)

The X-ray crystallography analysis of the mouse\(^9\) and human\(^10\) CD1d/KRN7000 complexes revealed both the architecture of CD1d and the binding mode for the glycolipid. CD1d consists of a C-terminal moiety composed of β-sheets in two layers and a more complex N-terminal domain, consisting of two α-helices (which essentially act as a cavity for the glycolipid antigen) and a β-sheet floor. The two lipid alkyl chains are accommodated in separate hydrophobic pockets of CD1d. The fatty acyl chain is situated in the A’ pocket while the phytosphingosine chain is located in the C’ pocket (sometimes denoted F’ pocket). This interaction presents the hydrophilic sugar head group outside the pocket ready to interact with the TCR of NKT cells (Figure 4). X-ray analysis also revealed stabilising hydrogen bonding interactions with the hydrophilic sugar and CD1d. Both human and mouse NKT cells can recognise the CD1d/KRN7000 complex. A major structural difference between the mouse
and human binary complex is the positioning of the sugar head group. A 3 Å shift was observed for the human CD1d/glycolipid complex due to a steric repulsion caused by the indole group on tryptophan 153 (Trp 153), while the corresponding residue for mouse CD1d is glycine 155 (Gly 155).

Figure 4. Structure of CD1d and interaction with KRN7000. (Reprinted with permission by Organic and Biomolecular Chemistry, Copyright 2011, Royal Society of Chemistry)
In 2007, the ternary CD1d/KRN7000/human-TCR complex was published by Rossjohn and co-workers, resulting in a development for understanding the mechanistic interaction of the TCR with the binary CD1d/glycolipid complex\textsuperscript{11}. No conformational change was observed when the NKT cell TCR recognised the CD1d/glycolipid complex, indicating a lock and key style mechanism. The galactose head group was shown to be situated between Trp153 of CD1d and the hydrophobic portion of Arg95\textalpha{} of the TCR. The TCR was found to interact with the 2′, 3′ & 4′-hydroxyl groups of galactose and the 3-hydroxyl group of the phytosphingosine chain. No interaction was observed with the 6′-hydroxyl and the pyranose ring oxygen with either the TCR or CD1d. A similar hydrogen bonding network was identified in the CD1d/KRN7000/mouse-TCR crystal structure\textsuperscript{12}.

\textbf{Figure 5.} Hydrogen bonding network in the human CD1d/KRN7000 binary complex. (Reprinted with permission by Organic and Biomolecular Chemistry, Copyright 2011, Royal Society of Chemistry)
1.4 Analogues of KRN7000 and Promoting a Bias Cytokine Production

KRN7000’s limited success in clinical trials was due to the equal portions of $T_H^1$ and $T_H^2$ cytokines being produced. This caveat lead to the synthesis of various analogues of KRN7000 to promote a bias cytokine profile. Analogues have four types of modifications (Figure 7):13:

1. Modification of the sugar head group.
2. Varying the polar portion of the ceramide.
3. Substitution and variation of the lipid chains.
4. Modifying the nature and configuration ($\alpha$ or $\beta$) of the anomeric bond.

Figure 7. Illustrating the portions of KRN7000 that are modified for analogue synthesis. (Reprinted with permission by Organic and Biomolecular Chemistry, Copyright 2011, Royal Society of Chemistry)
Some of these modifications have shown promising results. It will be discussed below a short description of selected analogues related to the above categories.

### 1.4.1 Modification of the Sugar Head Group.

The saccharide of KRN7000 is α-D-galactopyranose. An SAR investigation showed the order of the favourable pyranose for cytokine stimulation is α-D-galactopyranose > α-D-glucopyranose (7) > α-D-mannopyranose (8)\(^{11,14}\). These results are consistent with CD1d being restricted to galacto- and glucopyranose respectively, while D-mannosyl compounds are restricted to CD1b. X-ray crystallography analysis implied the 2’, 3’ and 4’ hydroxyl groups are involved in hydrogen bonding with the TCR and therefore play an important role in stabilizing the ternary CD1d/glycolipid/TCR complex. Substitution at the 2’, 3’ and 4’ hydroxyls revealed any form of derivatisation at 2’ resulted in complete deactivation of the glycolipid for iNKT cell stimulation\(^{15}\). Substitution of the 3’ and 4’ hydroxyl groups showed some activity, for example the 3-O-sulfate\(^{16}\) and the 4-O-phenyl propyl\(^{17}\) compounds 9-10 gave a similar iNKT response compared to KRN7000.

![Figure 8. 2’, 3’ and 4’ derivatives of KRN7000.](image)

New C-6’ analogues have been synthesised since the crystal structure of the ternary complex indicated the 6’-hydroxy group is not involved in the hydrogen bonding network. Most do not significantly increase the ability of the glycolipid to stimulate iNKT cells and are used to investigate loading and the interaction of the glycolipid with CD1d. Analogues synthesised to date include deoxy (RCAI-58)\(^{18}\), methoxy (RCAI-61)\(^{19}\), acid\(^{24,20}\), ureido\(^{21}\) and amide\(^{27,22}\) derivatives. RCAI-61 (12), uronic acid (13), 6’-napthylurea (14) and electron withdrawing amide (15) were observed however to induce a strong Th1 bias. The last modification for the saccharide is varying both the substitution and structure of the internal pyranose ring. Cyclitol analogues RCAI-59 (16) and RCAI-92 (17) were found to stimulate a Th1 response\(^{23}\). More recently Du and co-workers replaced the oxygen of the core with sulfur along with varying the chain length of the fatty acyl chain to generate three new analogues (18-20)\(^{24}\). They found analogues 18 and 19 stimulated a higher amount of IFN-γ cytokines in vitro than that of
KRN7000 and S-analogue 19 could suppress the production of IL-4 in the presence of high amounts of IFN-γ. These results suggest 19 can be a specific therapeutic to produce T\textsubscript{H}1 cytokines. Other examples of core modifications include acyclic AraCer (21)\textsuperscript{25} and ThrCer (22)\textsuperscript{26} and were found to stimulate iNKT cells.

**Figure 9.** Illustrating 6’ and core modifications.

### 1.4.2 Varying the Polar Portion of the Ceramide.

Structural requirements for the ceramide to stimulate iNKT cells have been investigated. When both the 3 and 4 hydroxyl groups are omitted no activity is present\textsuperscript{14}. The loss in activity is not surprising as the 3-OH is involved in recognition by the TCR and both hydroxyl groups position the ligand correctly in CD1d for recognition by the TCR. For the 4-deoxy analogue 24 stimulation is still observed comparable to that of KRN7000\textsuperscript{7,14,27}. The stereochemistry of the amide and diol is also important for activity. The absolute configuration of the ceramide in KRN7000 is 2\textsubscript{S}, 3\textsubscript{S}, 4\textsubscript{R} respectively, with any deviation from this stereochemistry resulting in a loss of activity\textsuperscript{28}. Substitution of the 4 position with CF\textsubscript{2} resulted in a slight T\textsubscript{H}1 bias with loss of activity (see reference 29 for structure)\textsuperscript{29}. The loss in activity was attributed to the electron withdrawing nature of the CF\textsubscript{2} rendering the ability of the 3-OH to accept a hydrogen bond from Arg\textsubscript{95} of the NKT cell TCR. Nonetheless the presence of the CF\textsubscript{2} increases the hydrogen bonding donating ability of the 3-OH with Asp\textsubscript{80} of CD1d which may explain the T\textsubscript{H}1 bias. Amide side chain substitution has shown some interesting biological results. Isoteric substitution of the amide with a 1,4-triazole (25)
increased the bias for secreting IL-4 cytokines\textsuperscript{30}. The same IL-4 bias was observed for ester\textsuperscript{31}, sulphonamide\textsuperscript{32}, and difluoro analogues\textsuperscript{33} 26-28.

![Chemical structures](image)

**Figure 10.** Modifying the polar portion of the ceramide.

### 1.4.3 Substitution and Variating the Lipid Chains.

Lipid chain modifications include truncation and/or functionalization. Some analogues have shown very interesting results to date. OCH\textsuperscript{34} (29) a truncated C\textsubscript{18} to C\textsubscript{9} analogue of KRN7000 gave a specific Th2 response, however only a weak stimulation of human iNKT cells. Savage and co-workers investigated truncation of the phytosphingosine (30) or the acyl chain (31-32) revealing compounds with similar activity to OCH\textsuperscript{35}. Further investigation into the truncated analogues suggested that shorting the lipid chain leads to a less stable CD1d/glycolipid complex and augments IL-4 release\textsuperscript{36}. IFN-\gamma production requires longer stimulation and therefore a more stable binary complex. Wong and co-workers investigated the introduction of both short and medium chains with terminal aromatic rings (33-35)\textsuperscript{37}. On the phytosphingosine, truncating the chain from C\textsubscript{14} to either C\textsubscript{2} or C\textsubscript{4} with a terminal phenyl significantly induced a Th1 response. The presence of the phenyl ring stabilises the binary complex by enabling for \(\pi-\pi\) stacking with Phe18 and Phe77 located in the entry of the hydrophobic C’ pocket. Terminal phenyl substitution on the acyl chain gives a more noticeable response. Truncating the chain length from C\textsubscript{5} to C\textsubscript{10} (36-38) allows for an additional \(\pi-\pi\) stacking with Tyr73 and Trp40 in the CD1d hydrophobic pocket. Substitution of the terminal phenyl on the acyl chain with a \textit{para} fluoro\textsuperscript{37c} (39) or \textit{para} (4-fluorophenoxy)\textsuperscript{38} (40) gave a more positive Th1 response. These aromatic analogues listed above have shown the most relevant Th1 bias to date. Chung and co-workers adopted the same idea of aromatic substitution and replaced both the acyl and sphingosine chain with a methylene spacer and a terminal aromatic ring (41-43), surprisingly no analogues showed any
NKT cell stimulation\textsuperscript{39}. Park and co-workers recently investigated heteroaromatic pyrazoles with varying the position of a phenyl group on the sphingosine backbone. One of these analogues \textsuperscript{44} gave a greater polarization towards $T_{H2}$\textsuperscript{40}.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure11.png}
\caption{Examples of lipid chain modifications.}
\end{figure}

\subsection*{1.4.4 Modifying the Nature and Configuration ($\alpha$ or $\beta$) of the Anomeric Bond.}

The configuration of the glycosidic bond has been investigated to conclude the $\beta$ anomer is less potent than the corresponding $\alpha$ anomer of KRN7000\textsuperscript{41}. Various $\beta$ analogues have been synthesised to improve the weak activity but have unfortunately shown no activity\textsuperscript{16b, 42}. These results highlight the necessity of the $\alpha$ configuration for potency, in providing the correct special orientation of the glycolipid for recognition. Glycosidic bond modifications have been investigated for reasons which include limiting the degradation of the glycolipid by glycosidases \textit{in vivo} and with the view to investigate whether such derivatives can display bias towards the production of $T_{H1}$ or $T_{H2}$ cytokines. In 2003, $\alpha$-C-GalCer \textsuperscript{45} was developed by Frank, Tsuji and co-workers and has been one of the most interesting glycosidic analogues.
to date, showing a strong preference for a T<sub>H</sub>1 response compared to KRN7000 in mice. α-C-GalCer has a stable ether bond compared to an acetal linkage and can resist enzymatic degradation and glycosidic bond cleavage at low pH. However, Tsuji and co-workers showed that 45 had rather weak activities with human iNKT cells and gave no significant level of cytokines. The trans (E) analogue 46 had a better T<sub>H</sub>1 response than KRN7000 but was less active than α-C-GalCer while the cis (Z) 47 was completely inactive.

![Figure 12. C-glycoside analogues.](image)

With the impressive results obtained for C-glycoside analogues, S-glycoside analogues of KRN7000 (α-S-GalCer) have been investigated. To date only two reported procedures for α-S-GalCer have been presented. The first report was that by Zhu and Dere in 2008 and involved a convergent synthesis between α-glycosyl thiol 51 and phytosphingosine derivative 53. Thiol 51 was synthesised from 1,6 anhydrogalactose by a selective ring-opening with bis(trimethylsilyl)sulfide and TMSOTf at reflux for 6 h. The S<sub>N</sub>2 displacement gave exclusively the α-anomer in 88% isolated yield (Scheme 1).

![Scheme 1. Synthesis of α-glycosyl thiol 51.](image)

Attention was then focused on synthesising phytosphingosine derivative 53 (Scheme 2). Following Schmidt’s procedure, the synthesis began with compound 52 synthesised from D-galactose in 6 steps. Reaction of the triol 52 with 2,2-dimethoxypropane gave the acetonide in
80% yield. Mesylation of the primary alcohol followed by a Finkelstein type reaction with LiI in DMF gave iodide 53 in 90% yield. Coupling of thiol 51 and iodide 53 under phase transfer conditions gave glycolipid 54 in 73% yield. To complete the synthesis the corresponding azide was reduced to its amine via the Staudinger reaction. Coupling of the resulting amine with cerotic acid in the presence of EDC gave an amide and deprotection over two steps with HCl, to remove the acetonide, followed by a global benzyl deprotection via the birch reduction gave α-S-GalCer 55 in 36% yield over four steps.

Scheme 2. Zhu’s synthesis of α-S-GalCer. (Reprinted with permission from Organic Letters, Copyright 2008, American Chemical Society)

Independently in 2008, Howell and co-workers presented an alternative synthesis for 55 (Scheme 3)48. The glycosyl thiol derivative 59 was synthesised via Yamamoto’s method giving an inseparable 2:1 α:β mixture49. Coupling of the mixture with bromophytosphingosine derivative 57 under phase transfer conditions followed by separation of the α-anomer gave glycolipid 60 in 48% yield. Subsequent deprotection followed by ceramide formation gave α-S-GalCer 55.
Despite the similarity between KRN7000 and α-S-GalCer, the glycosyl thiol analogue was found to have no bioactivity in mice\textsuperscript{48}. However, recent evidence suggested that α-S-GalCer is bioactive in humans\textsuperscript{50}. Other anomeric modifications include an oxime\textsuperscript{51} 61 and an amide\textsuperscript{52} derivative 62. However, these analogues were reported without biological information.

\textbf{Figure 13}. Oxime and amide analogues of KRN7000.

\section*{1.5 Bacterial Glycosphingolipids Stimulate NKT Cells}

Bacteria are divided into two categories been either Gram-positive (one lipid bilayer and a layered peptidoglycan) and Gram-negative (possessing two lipid bilayers and a thin peptidoglycan). Many Gram-negative bacteria (e.g. \textit{Escherichia coli}) have their outer membrane consisting of lipopolysaccharides (LPS). The innate immune system is capable of recognising these LPS antigens on pathogens through NKT cells with a similar mechanism compared to KRN7000. \textit{Sphingomonadaceae}, a subset of Gram-negative bacteria do not contain LPS and instead have a layer of glycosphingolipids. An investigation into the membrane structure of these bacteria reviled glycolipids GSL-1 (63) and GSL-1’ (64) (Figure 14)\textsuperscript{53}. These glycosphingolipids have a striking resemblance to KRN7000. Compound 63 is
an α-linked glycosylceramide containing a sphinganine base ceramide and a C-6’ oxidised sugar residue and 64 is the galacturonic acid derivative. Other higher order glycosphingolipids GSL-3 and GSL-4 were also identified, the primary difference of these being the presence of additional saccharides. Previous reports for isolated GSL-4 suggested it to be a strong iNKT antigen. When the synthetic forms of GSL-3 and GSL-4 were successful, biological results indicated these higher order structures to be poor stimulants$^{54}$. This was thought to be surprising as it is known, when additional sugars are attached to KRN7000 they are enzymatically cleaved in the lysosome to give the potent antigenic cerebroside and a similar process would be observed for GSL-3 or GSL-4 to give GSL-1. Further investigation reviled that lysosome truncation does not occur for GSL’s and the iNKT stimulation for isolated GSL-4 was likely due to contamination with GSL-1$^{54}$. This result implies the importance of organic synthesis in the structure determination and biological evaluation of isolated natural products. Some synthetic analogues of bacterial glycolipids such as PBS-30 (65), PBS-59 (66), GSL-1B (67), GSL-1C (68) have been reported and shown to stimulate NKT cells$^{55,20}$. As mentioned previously galactosylceramides are more prevalent stimulants of iNKT than their corresponding gluco isomer. In order to determine if this stereochemistry is retained for C-6’ oxidised saccharides found in bacterial glycolipids, Savage and co-workers synthesised glucuronic and galacturonic acid glycolipids 67-68 (including 63 and 64) and compared their NKT cell stimulatory properties$^{20}$. Their results indicated in the context of C-6’ oxidised sugars that glycolipids with gluco stereochemistry are more active.

Figure 14. Structure of isolated and synthetic bacterial glycolipids.
1.5.1 Stereoselective Synthesis of Bacterial Glycolipids

α-Glycoside bond formation reactions can be difficult to achieve with high stereocontrol for uronic acid derivatives. Early syntheses of uronic acid based glycolipids have involved C-6’ oxidation after glycoside formation\textsuperscript{18} or glycoside bond formation reactions using uronic acid donors with a non-participating group at C-2\textsuperscript{56}, with the latter approach giving anomic mixtures in some cases. In recent years the Murphy group has developed an efficient method to generate α-linked glycuronic acid derivatives in high yields and selectivity. It was found that treatment of these acid derivatives with both TiCl\textsubscript{4} and SnCl\textsubscript{4} strongly favoured the formation of α-anomers via a chelate induced anomerisation reaction. These anomerisation reactions will be discussed further in later chapters. This reaction has been successfully applied to synthesise bacterial glycolipids within the Murphy group. In 2009, Pilgrim and Murphy applied anomerisation in the synthesis of PBS-30, PBS-59 and sulfone analogues\textsuperscript{57}. The initial synthesis involved glycosidation of a benzoylated galacturonic acid trichloroacetimidate donor 69 with sphinganine acceptor 70 giving β-glycolipid 71 in 96% yield. Subsequent anomerisation with TiCl\textsubscript{4} gave the corresponding α-anomer 72 in high yield (96%) and selectivity (α:β 97:3) with chemoselective removal of the benzyl protecting group. Staudinger reduction of the azide in 72 followed by amide bond formation gave protected glycolipid 73 in a 44% yield over two steps. Ester deprotection is not straightforward for uronic acid derivatives. Under basic conditions deprotonation of the acidic H-5’ proton results in an E1cB elimination across C-4’ and C-5’ to give an unsaturated derivative. This was the case for glycolipid 73 were upon deprotection under strong basic conditions gave 75. However mild basic conditions with hydrogen peroxide and sodium propoxide gave the PBS-30 analogue 74 in 36% isolated yield. The PBS-59 derivative 75 was achieved from an identical procedure for the corresponding galacturonic acid donor (Scheme 4).
Scheme 4. Pilgrim and Murphy’s synthesis of bacterial glycolipid analogues via chelate induced anomerisation. (Reprinted with permission from Organic Letters, Copyright 2009, American Chemical Society)

To synthesise the sulfone analogue Pilgrim and Murphy investigated anomerisation of S-glycolipids (Scheme 5). This provided a rare example of chelate induced anomerisation for S-glycosides and highlighted the scope of anomerisation in pseudoglycoside synthesis. The thiol derivative 78 was obtained in two steps from glycosyl bromide 77. The $S_N2$ displacement of bromide derivative 79 with 78 (previously deprotonated with $<$ 1 equiv. NaH) gave $\beta$-thioglycolipid 80 in a 52% yield. Anomerisation of 80 with TiCl$_4$ gratifyingly gave a 4:1 $\alpha$:$\beta$ mixture of anomers which could be separated by silica gel chromatography to give 81 in a 52% yield. Staudinger reaction, amide formation and deprotection with oxidation of the sulfur atom gave sulfone 83 in a 22% isolated yield over 3 steps. The $\alpha$-S-glycolipid would be of interest however, conditions to omit sulfur oxidation were not developed at this time.
Scheme 5. Pilgrim and Murphy’s synthesis of a sulfonate bacterial glycolipid analogue via chelate induced anomeration. (Reprinted with permission from Organic Letters, Copyright 2009, American Chemical Society)

The above glycolipid synthesis had a number of disadvantages:

1. Synthesis of sphingosine derivative 79 was a long 14 step synthesis.

2. Synthesis of the β-glycosyl thiol 78 was troublesome with low yield and reproducibility.

3. Deprotection conditions to prevent sulfur oxidation were not identified.

To overcome these caveats, O’Reilly and Murphy reinvestigated the synthesis of the S-glycolipids based on uronic acids. The galactosyl thiol was redesigned to contain acetate protecting groups as the rate of saponification is greater than benzoates, therefore deprotection conditions could possibly be identified to prevent E1cB elimination. Bromide 84 was reacted with potassium thio acetate followed by subsequent selective deacetylation to give β-glycosyl thiol 86 in a 44% isolated yield over two steps. Anomerisation of 86 to α-glycosyl thiol 87 was then successful with TiCl₄ at 0 °C (Scheme 6).
Scheme 6. Synthesis of α-glycosyl thiol 87 via anomerisation. (Reprinted with permission from Organic Letters, Copyright 2011, American Chemical Society)

To synthesise the sphingosine derivative a new route was presented (Scheme 7). Alkylation of Myers chiral auxiliary 88 with allyl bromide stereoselectively gave 89 in a 96:4 dr and a 70% yield. Treatment of 89 with heptadecyl lithium gave the corresponding α-aminoketone 90 in a 75% yield. Reduction of 90 under Hoffman’s condition with LiAlH(O-t-Bu)₃ to give an anti-1,2-aminoalcohol, then followed by oxazolidine formation with 2,2-dimethoxypropane gave 91 in 69% yield for two steps. Isomerisation of the terminal olefin with 15 mol% Grubbs-II in methanol at 60 °C gave the internal olefin 92 in an 89% yield 1:1 Z:E isomers. Oxidative cleavage followed by reduction of the corresponding aldehyde to a primary alcohol 93 and subsequent Appel reaction gave bromide 94 in a 54% yield over 3 steps.

Scheme 7. O’Reilly and Murphy’s synthesis of sphingosine derivative 94. (Reprinted with permission from Organic Letters, Copyright 2011, American Chemical Society)
With the bromide 94 and α-glycosyl thiol 87 in hand, coupling of the two intermediates with sodium hydride gave α-glycolipid 95 in a moderate 35% yield (Scheme 8). Treatment of the protected thio glycolipid 95 with formic acid removed both the Boc and oxazolidine to give an amino alcohol. Coupling the amino alcohol with succinate 96 gave the desired amide 97 (54% over two steps). A two-step deprotection was successful to prevent any formation of the eliminated by-product. Selective conversion of the methyl ester to the corresponding carboxylic acid was achieved with lithium iodide in ethyl acetate at reflux. The formation of an enolate followed by elimination does not occur for this intermediate as the H-5’ proton is now less acidic than the carboxylic acid proton. The acetate protecting groups were then removed with guanidine and guanadinium nitrate in CH₂Cl₂-MeOH to give thiol glycolipid 98 in a 58% yield over two steps. The glucuronic acid derivative 99 was obtained through a similar sequence.

Scheme 8. Completing the thioglycolipid synthesis. (Reprinted with permission from Organic Letters, Copyright 2011, American Chemical Society)

1.6 Aims of this Thesis and Target Compounds
The first section of this thesis will be an expansion of the work carried out by Pilgrim, O’Reilly and Murphy. There has been recent development within the Murphy group for the anomerisation of β-glycosyl azides based on uronic acids to their α-anomer. Glycosyl azides have been used in the preparation of carbohydrate derivatives, however work has focused
mostly on β-azides with there being few investigations in the use of α-glycosyl azides as synthetic intermediates. Therefore the aim will be to explore these intermediates in the synthesis of α-1,2,3-triazoles linked at the anomeric position (glycosyl triazoles). Azides can undergo 1,3-dipolar cycloaddition reactions with alkynes to give either 1,4- or 1,5-triazoles. Glycolipids with an α-triazole at the anomeric carbon have not been reported, therefore target compounds AMD289-AMD291 will be of interest in investigating their synthesis and ability to stimulate NKT cells.

Scheme 9. Target triazole analogues of bacterial glycosphingolipids.

1.7 References


Chapter 1


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Chapter 2: Synthesis of α-Galactosyl Ceramide Analogues with an α-Triazole at the Anomeric Carbon

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2.1 Retrosynthetic Analysis

Scheme 10 illustrates the retrosynthetic analysis of each target glycolipid (AMD289-AMD291). It was envisioned that 1,5- and 1,4-triazoles 100-103 could be isolated from copper azide-alkyne cycloaddition (CuAAC) and ruthenium azide-alkyne cycloaddition (RuAAC) between either α-glycosyl azide 104 or 105 and alkyne 106. Alkyne 106 would be generated from aldehyde 107 through the Seyferth-Gilbert homologation followed by deprotection and amide formation. Aldehyde 107 is an intermediate in O’Reilly and Murphy’s synthesis of α-S-glycosphingolipids. α-Glycosyl azide 104 could be generated through chelate induced anomerisation of the corresponding β-glycosyl azide 108 and reduction of 104 would give the alternative azide 105.

Scheme 10. Retrosynthetic analysis of the target glycolipids.

2.2 Lewis Acid Promoted Anomerisation: An Overview

Glycosylation reactions may involve the following mechanism: (i) activation of a glycosyl donor (trichloroacetimidate, glycosyl halide, thio glycoside) with an activating agent (Lewis or Bronsted acid); (ii) formation of an oxocarbenium ion; (iii) nucleophilic attack of a glycosyl acceptor on the resulting cationic intermediate to give the desired glycoside. The choice of protecting group at C-2 can affect the stereochemistry of the reaction (Scheme 11). For instance, 2-O-acyl protected donors often lead to isolation of 1,2-trans glycosides (β-anomer) as a result of neighbouring group participating with the oxocarbenium ion. This interaction prevents nucleophilic attack from the face that would lead to isolation of the 1,2-cis anomer (α-anomer). The use of non-participating groups at C-2 has an alternative outcome. Nucleophiles can now approach from either face of the oxocarbenium ion resulting in formation of either 1,2-cis glycosides, 1,2-trans glycosides or a mixture of each. The
choice of saccharide, leaving group, solvent and other protecting groups can promote bias between the anomeric selectivity.

**Scheme 11.** Illustrating the choice of protecting group on glycosylation outcome.

The stereochemical configuration of a glycosidic bond can influence both its chemical and biological properties. The 1,2-\textit{cis}-glycoside is a necessary linkage in certain drug molecules such as carbohydrate based vaccines and also plays an important role in providing the correct structural configuration for recognition by biological receptors. It can be noted from above that 1,2-\textit{cis} glycosides are more challenging to prepare than the corresponding 1,2-\textit{trans} glycoside. Therefore rather than screening multiple conditions such as choice of donor, promoter, solvent, protecting group and careful optimization of each to give a 1,2-\textit{cis} glycoside, it would be ideal to have a straightforward protocol to give the product from the more readily and easily accessible 1,2-\textit{trans} glycoside.

The anomeric effect is a general phenomenon where in carbohydrate chemistry refers to the increased preference for electron withdrawing substituents on the anomeric carbon to adopt an axial orientation\(^1\). If exploited, it could be used for the stereoselective synthesis of glycosides. Lewis acids have been discovered as efficient promotors in the stereoselective isolation of 1,2-\textit{cis} glycosides from 1,2-\textit{trans} glycosides. This transformation, where equilibrium is established, is labelled anomerisation and is defined as an epimerisation of anomers. The anomeric effect plays a key role in the equilibrium distribution and therefore the thermodynamic 1,2-\textit{cis} product is favoured due to stabilising electrostatic/hyperconjugation interactions which are not observed for the \(\beta\)-anomer (Scheme 12).
Scheme 12. Antiperiplanar lone pair hypothesis as an explanation for the anomeric effect.

Two mechanistic interpretations have been proposed for anomerisation, endocyclic\(^2\) and exocyclic\(^3\) cleavage. The former involves coordination of the Lewis acid to the pyranose ring oxygen resulting in reversible cleavage of the anomeric carbon to ring oxygen bond. The acyclic intermediate allows for bond rotation and facilitates the formation of the \(\alpha\) or \(\beta\) anomer by ring closure. The latter mechanism involves cleavage of the anomeric C to aglycon O bond resulting in an intermediate ion pair where on collapse can result in product formation (Scheme 13).

Scheme 13. Anomerisation pathways: endocyclic (top) and exocyclic cleavage (bottom).

The Lewis acid promoted anomerisation reactions presented in the literature have included mostly benzylated and acetylated saccharides (Scheme 14). Pascu first disclosed SnCl\(_4\) and TiCl\(_4\) anomerisation of various alkyl glycosides to their \(\alpha\)-anomer, noting that TiCl\(_4\) had a superior conversion over SnCl\(_4\)\(^4\). Later Lemieux and Hindsgaul observed a significant rate enhancement of an acetylated isopropyl glucoside when acetic acid was present with SnCl\(_4\)\(^5\). Koto achieved anomerisation of methyl 2,3,4,6-tetra-\(O\)-benzyl-\(\beta\)-D-glucopyranoside \(\textbf{111}\) with
TiCl₄ and applied the conditions to achieve anomerisation of two fully benzylated disaccharides in 14-46% yield respectively. A combined TiBr₄ and MgBr₂-OEt₂ protocol presented by Mukaiyama and co-workers anomerised benzylated disaccharide 115 in good yield. Kishi and co-workers reported the use of the Mukaiyama catalyst (SnCl₃ClO₄) in the anomerisation of β-mannosyl containing disaccharide 109. They attributing the success of the reaction was due to chelation of both the axial 2- and ring oxygen to the catalyst and in turn induced endocyclic cleavage. Rotondo and co-workers illustrated the anomerisation of deprotected D-galacturonic acid 117 in D₂O catalysed by Me₂Sn(OH)₂. This allowed the rate of anomerisation for the four main sugar forms of the deprotected saccharide (a/β pyranoside and a/β furanoside) to be measured. The authors noted the presence of the carboxylic acid caused a 10000 fold increase in the rate of anomerisation due to assistance of the carboxylic acid tail in holding the metal in close proximity to the reactive centre. Investigation into glycosylation reactions of 2,3-trans carbamates or 2,3-trans carbonate glycosyl donors by the Kerns, Crich, Oscarson, Manabe/Ito showed that such donors give 1,2-cis glycosides. The presence of the cyclic protecting group imposes an inner ring strain that raises the ground

Scheme 14. Literature examples of anomerisation. (Adapted from, Lewis acid promoted anomerisation: recent developments and application, Carbohydrate Chemistry, Vol 41, 2015, 90-123, RSC publishing 2015)
state energy leading to a lower barrier to endocyclic cleavage and increase the rate of anomerisation. In recent years, the Murphy group has studied anomerisation reactions for acetylated saccharides. Earlier investigations into the stereoselective SnCl$_4$ promoted glycosidation of glucuronic acid lactone derivatives found only $\alpha$-glycosides could be isolated even in the presence of 2-O-acyl groups, which are known to give $\beta$-glycosides (vide supra)$^{15}$. Further investigation gave evidence of a glycosidation-anomerisation reaction where the $\beta$-glycoside was initially formed followed by in situ SnCl$_4$ anomerisation. A structure activity relationship study for SnCl$_4$ or TiCl$_4$ anomerisation then observed an increased rate for glucuronides when compared to glucosides$^{16}$. A key structural feature of uronic acids when compared to other pyranoses is the existence of a C-6 carbonyl. Chelation of both the C-6 carbonyl and the ring oxygen to the Lewis acid gives a five membered chelate (pyranosides gives a less stable seven membered chelate) and facilities anomerisation via endocyclic cleavage (Scheme 15).

![Scheme 15](image)

**Scheme 15.** Chelate induced anomerisation of uronic acid derivatives via endocyclic cleavage.

Next, Pilgrim and Murphy carried out a systematic investigation into the electronic and structural features that influence anomerisation$^{17}$. The rate of SnCl$_4$ anomerisation for eighteen $\beta$-S and $\beta$-O-glycosides were measured with each substrate following 1$^{st}$ order equilibrium kinetics. The relative rates were calculated in each case and compared to butyl 2,3,4,6-tetra-O-acetyl-D-glucopyranoside 130 which was given a value of 1 (Figure 15). It was found that glucose and galactose uronic acid derivatives had a faster rate than their corresponding pyranosides and gave a larger portion of $\alpha$-anomer at equilibrium. These results are consistent with the C-6 carbonyl chelating to the Lewis acid leading to a rate enhancement. Allyl ester derivative 146 was 5 times faster than methyl ester 145 while the free acid derivative 147 was an order of magnitude faster than 146. Acid 147 was fastest of all the examples and is in line with initial observations by Lemieux and Hindsgaul (vide supra) whereby a combination of SnCl$_4$ and a carboxylic acid significantly increase the rate of anomerisation. Allyl esters have an additional coordination site and act as a $\pi$-donor to
coordinate to the Lewis acid. This can therefore hold the Lewis acid closer to the reactive centre and in turn, increase the rate of anomerisation. Stereochemistry of the sugar was found to have an effect on the anomerisation outcome. \(O\)-Galactoside derivatives were found to have a higher rate than for \(O\)-glucosides. Equatorial substituents are more inductive than when axial, therefore the electron releasing ability of \(C\)-4 in galactose provides more electron density for the pyranose ring oxygen to interact with the Lewis acid and favour cation formation during the rate determining step. The same trend for a rate increase was observed for \(S\)-glycosides with there being one exception, whereby a benzoyl \(S\)-galactoside 143 was slower than its corresponding \(S\)-glucoside 139, reasons for which are unclear. The electron releasing ability of the aglycon increases the rate of anomerisation. \(S\)-glycosides were faster than \(O\)-glycosides and cyclohexyl glycosides were faster than butyl glycosides. The portion of \(\alpha\)-anomer at equilibrium however for both \(S\)- and cyclohexyl glycosides were generally lower. Sulfur is less electronegative than oxygen and therefore more electron releasing. The electron releasing nature can facilitate cation formation and can account for the increased rate. The anemic effect is therefore lower when compared to \(O\)-glycosides attributing to the reduced anemic ratio. A steric effect may also contribute, as sulfur is larger and would prefer to be equatorial. This can also explain the reduced anemic ratio for cyclohexyl vs butyl glycosides. A rate increase was observed on substituting acetyl groups for benzoyl groups. Reasons for this observation are not yet clear as benzoyl groups are more electron withdrawing (\(pK_a\) benzoic acid = 4.19, \(pK_a\) acetic acid = 4.78) and should in theory decrease the rate. The anemic ratio for benzoyl protection was also higher at equilibrium.

Pilgrim and Murphy then observed the effect of Lewis acid concentration, choice of Lewis acid and temperature on the anemic equilibrium ratio. It was found decreasing the temperature increased the portion of \(\alpha\)-anomer. The \(\alpha:\beta\) ratios were higher at 0 °C when compared to reactions carried out at higher temperatures (20, 30, 40 °C). Changing the Lewis acid to \(TiCl_4\) for the anomerisation of both \(O\)- and \(S\)-glucuronides increased the ratio and rate of anomerisation. For these substrates \(TiCl_4\) was superior over \(SnCl_4\). Increasing the concentration of either Lewis acid showed and increased portion of \(\alpha\)-anomer and an increase in rate. This observation is consistent with the saccharide and the Lewis acid forming a complex and not the free glycoside. To provide further evidence of endocyclic cleavage, Pilgrim and Murphy carried out a trapping experiment on the intermediate cation. Gratifyingly the use of excess sodium cyanoborohydride in the presence of \(TiCl_4\) gave an acyclic product consistent with an endocyclic mechanism.
Figure 15. Effect of structure on the SnCl$_4$ promoted β to α anomerisation of acylated saccharides.

Following this study, O’Reilly and Murphy later investigated the anomerisation of glycosyl thiol glucuronides for application in the synthesis of sulfur containing glycolipids$^{19}$. Unlike sugar hemiacetals (glycosyl alcohols), glycosyl thiols do not undergo mutarotation easily under basic conditions, allowing for their stereochemistry to be maintained during alkylations. The best conditions to give the α-glycosyl thiol were a combination of low temperature (0 °C) and TiCl$_4$ (2.5 equiv.). A peak ratio of α-thiol was reached with 2.5 equivalents of Lewis acid. Any deviation gave a decline in the α:β ratio (Table 1).
To gain further insight into the scope of the anomerisation, Farrell, Zhou and Murphy next turned their attention to the anomerisation of disaccharides (Figure 16). A series of compounds were explored with 1→6, 1→4, 1→3 & 1→2 glycosidic linkages. Gratifyingly, 2.5 equivalents of TiCl₄ isomerised each linkage with high yield (> 75%) and selectivity (> 9:1, α:β). The choice of protecting group was found to facilitate the success of the reaction. Benzoyl protected saccharides underwent anomerisation readily while applying identical conditions to acetylated derivatives were unsuccessful. A site specific anomerisation was also noted by the authors. When both saccharides had initial β-linkages only the uronide linkage was isomerised, highlighting further evidence of the rate enhancing ability of the C-6 carbonyl. Anomerisation of glycosyl azides was also presented by Farrell and Murphy (Figure 16). Previous investigations into the anomerisation of glucuronide and pyranose glycosyl azides were unsuccessful with SnCl₄. However, when a series of uronic acid based β-glycosyl azides were reacted with TiCl₄ (2.5 equiv.) at -15 to -18 °C for 48 hours, the corresponding α-glycosyl azides could be isolated in high yield (> 87%) and stereoselectivity (> 9:1, α:β).

Table 1. Effect of Lewis acid concentration on the anomerisation of glycosyl thiols.

<table>
<thead>
<tr>
<th>TiCl₄ equiv.</th>
<th>α:β ratio</th>
</tr>
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<tr>
<td>0.5</td>
<td>60:40</td>
</tr>
<tr>
<td>1.5</td>
<td>80:20</td>
</tr>
<tr>
<td>2.5</td>
<td>88:11</td>
</tr>
<tr>
<td>3.5</td>
<td>83:17</td>
</tr>
<tr>
<td>4.5</td>
<td>78:22</td>
</tr>
</tbody>
</table>
Figure 16. Products obtained from the TiCl₄ promoted anomerisation of various disaccharides (top) and glycosyl azide (bottom).

2.3 Synthesis of α-Glycosyl Azides

The investigation commenced with the synthesis of both galactose and galacturonic acid α-glycosyl azides 104 and 105 (Scheme 16). D-galacturonic acid monohydrate 164 was acetylated with acetic anhydride and a catalytic amount of perchloric acid to give 1,2,3,4-tetra-O-acetyl-α-D-galacturonic acid 165. Esterification of the crude acid intermediate 165 with methyl iodide gave methyl ester derivative 166 in 69% yield for two steps. Next the introduction of the azide at the anomeric centre using SnCl₄ (3 equiv) and TMSN₃ (2.5 equiv) was investigated in order to try to effect glycosylation and anomerisation in one pot. Substitution of the azide for the acetate can usually be achieved in the presence of lower amount of SnCl₄ (0.5 equiv)\textsuperscript{15a}. However for this example increased equivalents of Lewis acid were necessary to ensure full conversion to the glycosyl azide. The glycosylation under these conditions provided an inseparable 4:1 α:β mixture of anomers 104 and 108. To increase the portion of α-anomer, the mixture was treated with TiCl₄ (2.5 equiv) for 48 h at -15 °C as described by Farrell and Murphy to anomerise the remaining β-azide and give exclusively α-azide 104 in an 82% isolated yield\textsuperscript{20}. Next selective cleavage of the methyl ester on 104 with lithium iodide in refluxing ethyl acetate gave its carboxylic acid derivative
in 83% yield. Chemoselective reduction of the carboxylic acid to its corresponding primary alcohol was achieved with borane tetrahydrofuran complex. Intramolecular acyl migration to an adjacent OH can be a common side reaction for polyhydroxylic compounds. Therefore in order to maximise the yield and account for any side reactions, treatment of the crude residue after reduction with acetic anhydride and pyridine for 4 hours gave the peracetylated α-glycosyl azide in 65% for two steps.

Scheme 16. Synthesis of α-glycosyl azides 104 and 105. (Reprinted with permission from Tetrahedron, Copyright 2014, Elsevier)

2.4 Synthesis of the Alkyne
Preparation of alkyne was then explored. As mentioned previously, O’Reilly and Murphy developed a novel procedure for the synthesis of sphinganines from pseudoephedrine chiral auxiliaries (see Chapter 1, Scheme 7, page 19). It was envisioned an aldehyde intermediate from this synthesis could be a precursor for the synthesis of the desired alkyne. Thus the synthesis commenced form pseudoephedrine glycaminide to give internal alkyne in five steps (Scheme 17). Oxidative cleavage of the 1:1 E:Z mixture with osmium tetroxide, NMO and BAIB gave, after chromatography, the desired aldehyde intermediate in 61% yield.

Scheme 17. Synthesis of aldehyde 107. (Reprinted with permission from Tetrahedron, Copyright 2014, Elsevier)
With the desired aldehyde in hand the homologation was next explored. The Seyferth-Gilbert homologation provides one step conversion of aldehydes and ketones to alkynes under basic conditions using α-diazophosphonates\textsuperscript{23}. However, the substrate scope is limited as the diazophosphonate requires deprotonation with strong bases such as alkyllithiums or potassium tert-butoxide. The Ohira-Bestmann reagent was developed to overcome these caveats\textsuperscript{24}. In this modification the reagent, dimethyl-1-diazo-2-oxopropylphosphonate 169 is added to a solution of potassium carbonate and the aldehyde in methanol at room temperature, where upon stirring for several hours gives the alkyne in excellent yield. The reaction conditions are mild and enantiopure aldehydes do not undergo racemization. This was therefore the reagent of choice for the investigation. Gratifyingly treatment of aldehyde 107 with the Ohira-Bestmann reagent in anhydrous potassium carbonate and methanol gave alkyne 170 in an excellent 87% yield (Scheme 18).

\textbf{Scheme 18.} The Ohira-Bestmann transformation of 107 to 170. (Reprinted with permission from Tetrahedron, Copyright 2014, Elsevier)

To complete the synthesis, treatment of 170 with formic acid removed both the Boc and oxazolidine protecting groups. Treatment of crude 171 with succinate 96 gave the desired alkyne 106 in 71% yield over two steps.

\textbf{Scheme 19.} Completing the synthesis of 106. (Reprinted with permission from Tetrahedron, Copyright 2014, Elsevier)
2.5 Synthesis of α-Glycosyl Triazoles

2.5.1 Introduction to ‘Click’ Chemistry

The cycloaddition of azides and alkynes selectively gives 1,2,3-triazoles. The classical mechanism, initially proposed by Huisgen in 1969, involves the concerted formation of 5-membered heterocycles from a 1,3-dipole and a dipolarophile. Unfortunately, the transformation requires elevated temperature and, when using asymmetric alkynes, often produces mixtures of 1,4- and 1,5-regioisomers (Scheme 20).

**Huisgen 1,3-dipolar cycloaddition**

\[
R-N_3 + \equiv \equiv R' \xrightarrow[\Delta, \text{slow}]{} N=N=O + N=N=O
\]

1,5-triazole 1,4-triazole

Scheme 20.

The ‘click’ reaction has emerged in recent years as a popular tool for the selective formation of 1,4- or 1,5-disubstituted triazoles. In 2002, the research groups of Fokin-Sharpless and Meldal independently disclosed the Huisgen 1,3-dipolar cycloaddition to triazoles could be achieved with copper(I) catalysts. The presence of the catalyst accelerated the rate of the cycloaddition by \(10^7\) times and only produced the 1,4-disubstitued-1,2,3-triazole (Scheme 21). This copper azide-alkyne cycloaddition (CuAAC) is simple to perform, applicable to both organic and aqueous systems and is almost independent on the substituents of both the azide and the alkyne; i.e. neither electronic nor steric effects influence the outcome of the transformation. A range of copper catalysts can be used for the transformation provided copper(I) species are generated. Copper(II) salts are often selected and easily reduce \textit{in situ} to the active Cu(I) in the presence of a reducing agent. Copper(I) salts are less commonly used due to their instability under aerobic conditions. If selected, stabilizing ligands have to be added to prevent oxidation.

**Copper Azide-Alkyne Cycloaddition**

\[
R-N_3 + \equiv \equiv R' \xrightarrow{\text{Cu(I) cat.}} \text{no 1,5-triazole}
\]

Scheme 21. Formation of 1,4-triazoles.
The mechanism for CuAAC has been investigated, however has yet to be proven. Details on how the Cu(I)-species complexes and the origin of the selectivity are still unknown\textsuperscript{27}. The proposed mechanism involves copper(I) acetylide formation from the alkyne and the active copper species. Next the azide coordinates to the Cu(I) acetylide. Following bond formation, (resulting in an unusual 6-membered Cu(III) vinylidene metallacycle) ring contraction and proteolysis, the 1,4-disubstituted-1,2,3-triazoles can be formed (Scheme 22).

\textbf{Scheme 22.} Proposed mechanism for CuAAC. (Reprinted with permission from Coordination Chemistry Reviews. Copyright 2011, Elsevier)

More recently, the ruthenium azide-alkyne cycloaddition (RuAAC) has emerged to compliment CuAAC, as the formation of 1,5-disubstituted-1,2,3-triazoles can be achieved\textsuperscript{28}. A unique feature of RuAAC enables triazoles to be formed from both terminal and internal alkynes, offering the possibility for fully substituted triazoles (Scheme 23). Many aprotic solvents can accommodate RuAAC. However, unlike the complementary CuAAC, protic solvents such as water, methanol etc. are not tolerated resulting in diminished yield and formation of side products.

\textbf{Ruthenium Azide-Alkyne Cycloaddition}

\[
\text{R-N}_3 + X \equiv \text{R'} \xrightarrow{\text{Ru (II) cat.}} \begin{align*}
\text{N} & \equiv \text{N} \equiv \text{N} \equiv \text{R} \\
\text{R} & \equiv \text{R'} \\
\text{or} \\
\text{N} & \equiv \text{N} \equiv \text{N} \equiv \text{R} \\
\text{R} & \equiv \text{R''} \\
\text{no 1,4-triazole}
\end{align*}
\]

\[
X = \text{R''} \text{ or H}
\]

\textbf{Scheme 23.} 1,5- or fully-substituted triazole formation via RuAAC.
The choice of catalyst is central to the success of the reaction. Ru(II) species containing a η⁵-pentamethylcyclopentadienyl (Cp*) ligand are the only systems to show high yield and selectivity. It has been suggested the Cp* stabilises higher oxidation states of the metal centre and is therefore irreplaceable. The most popular catalysts are Cp*RuCl(COD) and Cp*RuCl(PPh₃)₂. The former is the most advantageous as the high lability of the spectator COD (cyclooctadiene) ligand enables reactions to be carried out at room temperature. The latter requires elevated temperatures and longer reaction times. The success of RuAAC is virtually unaffected by nature of the alkyne but sensitive to the nature of the azide. Primary azides give high yields and rates while secondary react more slowly with diminished yields. Tertiary azides are not clickable under RuAAC. The proposed catalytic cycle involves forming the active [Cp*RuCl] species from displacing the spectator ligands by the azide and the alkyne (Scheme 24). Oxidative coupling of the azide and the alkyne, in which the new carbon-nitrogen bond is formed between the most electronegative carbon on the alkyne and the terminal electrophilic nitrogen of the azide, followed by reductive elimination gives the triazole product.

**Scheme 24.** Proposed mechanism for RuAAC. (Reprinted with permission from Journal of the American Chemical Society. Copyright 2008, American Chemical Society)
2.5.2 Coupling of the Azide and the Alkyne

The synthesis of the triazole linkage was next explored. To identify optimal conditions, test reactions were first carried out with commercial alkynes and α-glycosyl azides 104-105. Opatz and co-workers have recently reported microwave CuAAC and RuAAC conditions for the coupling of β-glycosyl azides and glycosyl alkynes. Microwave irradiation of azide 104 and 2-ethynylanisole with Cp*RuCl(COD) gave 1,5-triaozles 172 in a 62% yield (Scheme 25). A dimeric scaffold 173 could also be synthesised by this approach. The RuAAC for aliphatic alkynes was however, less successful. Due to the aliphatic nature of lipid alkyne 106, it was necessary to screen conditions and not attribute overall success on aromatic derivatives. Identical conditions with azide 104 and either 1-hexyne or 5-chloro-1-pentyne failed to provide any 1,5-triazole. Focusing attention with lipid alkyne 106 and either α-glycosyl azide gave no triazole glycolipid. Investigating multiple conditions, catalyst, temperature, concentrations and solvents were undesirable with decomposition observed in most cases. The reasons for this observation are yet unclear but it can be concluded, the nature of the alkyne (aromatic vs aliphatic) dictates the outcome of the RuAAC with α-glycosyl azides.

![Scheme 25. Results of the RuAAC investigation.](image)

The CuAAC had a better outcome. The regioisomeric 1,4-triazole glycolipids 102-103 could be isolated through microwave irradiation using a catalytic amount of copper(I) iodide with either α-glycosyl azide and alkyne 106 (Scheme 26). Aromatic alkynes were also successful.
2.6 Final Step: Deprotection

As mentioned in chapter 1, due to the acidity of the H-5’ proton, protected uronic acid derivatives do not undergo saponification easily as the corresponding pyranosides. To prevent the E1cB elimination of protected glycolipid 102, the deprotection protocol recently described by Pilgrim and Murphy was attempted. Gratifyingly using hydroperoxide, generated in n-propanol from sodium propoxide and hydrogen peroxide gave deprotected glycolipid AMD292 in 57% yield. Identical conditions were applied to the corresponding protected glycolipid 103 to give AMD291 in 86% yield.

Scheme 27. Deprotection of protected glycolipids 102 and 103. (Reprinted with permission from Tetrahedron, Copyright 2014, Elsevier)
2.7 Biological Evaluation of AMD291 and AMD292

Both 1,4-triazole glycolipids prepared were evaluated for iNKT cell stimulation in collaboration with Dr. Derek Doherty at Trinity College Dublin (Figure 17). The glycolipids were administered to iNKT cells in three ways: just added directly (bars denoted ‘medium’), bound to mock-transfected HeLa cells (denoted ‘mock’) and bound to HeLa cells expressing transfected CD1d (denoted ‘CD1d’). As controls, no glycolipid was administered (medium), phorbol myristate acetate with ionomycin was administered to stimulate all cells (PMA/iono) and α-GalCer (GC) was administered as a prototype glycolipid at 100 ng/mL to stimulate iNKT cells. When no glycolipid was administered, iNKT cells failed to produce cytokines except for low levels when CD1d was present, possibly due to an endogenous glycolipid. PMA/iono stimulated all cells and GC stimulated the production of both IFN-γ and IL-4 only when HeLa-CD1d cells were present. The cytokine levels for AMD291 and AMD292 at each concentration are similar to those in the control where no glycolipid was administered (medium-stimulated cells). These results, although disappointing, clearly show AMD291 and AMD292 do not stimulate iNKT.

Figure 17. Biological evaluation of AMD291 and AMD292.
2.8 Conclusion

In summary, of the four initial target glycolipids, only the 1,4-disubstituted-1,2,3-triazoles based on galactose and galacturonic acid were successful. The route included anomerisation to generate the α-azide of galacturonic acid, which was subsequently reduced to the galactose derivative. The Ohira-Bestmann homologation of an aldehyde intermediate followed by deprotection and amide formation gave the lipid alkyne. RuAAC conditions were evaluated to identify alkynes of an aromatic nature reacted with α-glycosyl azides to give 1,5-triazoles, while aliphatic alkynes gave no product. Microwave assisted CuAAC lead to generation of the 1,4-triazole linkage and deprotection under mild basic conditions gave the target glycolipids. Biological evaluation showed neither glycolipid stimulated iNKT cells. Nonetheless a successful route, which was published in *Tetrahedron* ([doi:10.1016/j.tet.2014.03.029](https://doi.org/10.1016/j.tet.2014.03.029)), to synthesise 1,4-triazole glycolipids form α-glycosyl azides has been achieved. Despite these specific glycolipids (lipid chain length, sugar stereochemistry) not stimulating iNKT, the route has a potential application in synthesising a novel library of glycosyl triazole glycolipids for a SAR study. An investigation of this nature would hopefully identify glycolipids to stimulate iNKT with a bias cytokine production.

2.9 References


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### Chapter 3: Lewis Acid Induced Anomerisation of Selenium Glycosides

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3.1 Selenium

Selenium is a non-metallic chalcogen with an atomic number of 34 and a relative atomic mass of 78.96 located between sulfur and tellurium in group VI of the periodic table. Six isotopes, $^{74}$Se, $^{76}$Se, $^{77}$Se, $^{78}$Se, $^{80}$Se, $^{82}$Se occur naturally and have a natural abundance of 0.89, 9.37, 7.63, 23.77, 49.61 and 8.73%. Selenium has many allotropic forms, such as red, grey and black selenium and when bonded to other elements, has the common oxidation states of -2, +4 and +6. The most popular industrial uses for selenium are as an additive in glassmaking to tint, colour or decolourise glass and to act as a pigment for paint, plastics and ceramics. Other applications include uses in solar cells, photocells and photocopiers due to its photovoltaic and photoconductive properties. It also has application in anti-dandruff shampoos due to its toxicity towards the scalp fungus that causes dandruff. In biology, selenium is an essential element for life in small quantities. Organoselenium compounds, such as the 21st amino acid selenocystine is an important building blocks in the structure and function of selenoproteins. Examples include, glutathione peroxidase (protect organisms from oxidative damage), thioredoxin reductase, iodothyronine deiodinase (metabolises thyroid hormones) and selenophosphate synthetase 2 (involved in selenoprotein biosynthesis). The chemical properties of selenium are somewhat comparable to sulfur when considering nomenclature, similar reaction transformations and electronegativity (O = 3.5, S = 2.5, Se = 2.4). However, selenium is less basic and more nucleophilic than sulfur, has a larger atomic radius, more polarizable and forms both weaker and less polar bonds with carbon. Selenium reagents are involved in organic synthesis for a number of transformations such as addition, elimination (e.g. Grieco elimination), substitution, oxidation, rearrangement (e.g. Pummerer rearrangement), reduction and cyclization reactions. Incorporating selenium into molecules can be advantageous since the isotopic distribution of the element can give a fingerprint for identifying compounds in mass spectrometry and the nuclear spin of $\frac{1}{2}$ for $^{77}$Se allows for studying and identifying compounds via nuclear magnetic resonance (NMR) spectroscopy. A further application is to help solve the phase problem in X-ray crystallography. Taking advantage of the anomalous dispersion of selenium in response to X-ray irradiation, protein structures can be more easily determined with multi-wavelength anomalous dispersion (MAD) phasing.
3.2 Selenium in Carbohydrate Chemistry

A common technique in carbohydrate chemistry is to substitute oxygen atoms with other elements such as carbon, sulfur, selenium and tellurium. The purpose of this strategy is to develop novel compounds with more interesting biological properties compared to the native species or to simply create reactive intermediates for further transformations (e.g. glycosyl donors). Introducing selenium within carbohydrates is less encountered than carbon and sulfur but has received considerable attention in recent years. The most common selenium modified carbohydrates included:

- Selenoglycosides - where the selenium atom has been introduced at the anomeric linkage.
- Selenosugars - where the intrinsic ring oxygen has been replaced by selenium.
- Selenoether pseudocarbohydrates - where any of the external hydroxyls are replaced by selenium.

![Scheme 28](image)

**Scheme 28.** Selenium modified carbohydrates.

3.2.1 Selenoether Pseudocarbohydrates

Selenoether pseudodisaccharides, where two monosaccharide units are non-glycosidically linked by a selenium atom, are presented in the literature. In 2010, Fournière and Cumpstey synthesised pyranose and furanose disaccharide selenoethers by reacting nucleophilic selenium carbohydrates, generated from an in situ reduction of the corresponding diselenides, with a carbohydrate sulfonate 179 (Scheme 29)\textsuperscript{12}. The authors investigated the reactivity of
primary C-6 and secondary C-3 selenides. The reactions were successful, however a drawback of the methodology was the formation of a reduced by-product 183 in all cases, resulting from sulfonate 179 reacting with sodium borohydride. 183 was found to be inseparable from some pseudodisaccharide products such as 182. Additionally, decomposition of the starting material was observed when attempting a Birch type reduction (Na\(^0\), liq. NH\(_3\)) to cleave the benzyl ethers on intermediate 180. However, this challenge could be overcome by carrying out the selenium alkylation with deprotected diselenides.

Scheme 29. Fournière and Cumpstey’s synthesis of selenoether pseudodisaccharides.

Later Lüdtke and co-workers synthesised both selenoether pseudodisaccharides and neoglycoconjugates\(^{13}\). The pseudodisaccharides were C-6 linked and were synthesised through the similar procedure described above, for the in situ reduction of diselenides. The selenium-linked neoglycoconjugates were synthesised through a nucleophilic ring opening of chiral N-Boc aziridines. As a comparison, the reaction with an amino diselenide 184 and a carbohydrate sulfonate 185 was investigated. The isolated yield was slightly lower for this route, suggesting the ideal combination is to use the sugar diselenide as the nucleophilic partner and the aziridine as the electrophile (Scheme 30).
Scheme 30. Selenoether pseudodisaccharides and neoglycoconjugates form Lüdtke and co-workers.

Seleno-pseudomonosaccharides have also been reported. Selenols (RSeH) are rather susceptible to oxidation to give diselenides (RSeSeR) under aerobic conditions and therefore cannot be analogues to hydroxyl (ROH) groups in carbohydrates. In order to prevent oxidation selenols must be converted to selenoethers. In 2001, Huang and co-workers replaced the 2'-hydroxyl in uridine with methylselenide (SeMe) to serve as an anomalous scattering centre in X-ray crystallography\textsuperscript{14}. The selenium labelled nucleoside was converted to the phosphoramidite 190 and used as a monomer in solid phase DNA/RNA synthesis (Scheme 31). A DNA-octamer, decamer and an RNA-hexamer were synthesised and their structure determined by X-ray crystallography using MAD phasing. Likewise, a 6-SeMe lactose derivative 191 was synthesised by Kiso and co-workers in 2014 to act as a molecular tool in the X-ray structural determination of human galectin-9 NCRD (Scheme 31)\textsuperscript{15}. The carbohydrate-protein complex with 191 and galectin-9 NCRD co-crystallized and the structure could be determined with SAD/MAD phasing. Various 6-Se aryl, propargyl, allyl, acyl and alkyl analogues (194–201) of galactose were synthesised by Lüdtke and co-workers as potential intermediates for the synthesis of complex selenium-containing carbohydrates. The seleno-carbohydrates were prepared by either reacting commercial diselenides with carbohydrate sulfonates or carbohydrate diselenides with commercial alkyl halides (Scheme 32)\textsuperscript{16}.
Scheme 31. Synthesis of selenium labelled phosphoramidite 190 and structure of the 6-SeMe lactose ligand 191 that co-crystallized with galactin-9 NCRD.

Scheme 32. Lüdtke and co-workers synthesis of 6-Se modified carbohydrates.

3.2.2 Selenosugars

The replacement of the sugar ring oxygen by selenium has been the subject of numerous studies. 4'-selenoribonuelcosides show different sugar puckering (2’-endo/3’exo, S-type) compared to 4'-oxo- and 4'-thionucleosides (2’-exo/3’endo, N-type) due to steric effects induced the bulky selenium atom (Scheme 33). These conformational differences have opened investigations into 4'-selenonucleosides as biological tools or drugs. The groups of Jeong\textsuperscript{17}, Pinto\textsuperscript{18} and Matsuda\textsuperscript{19} independently disclosed the first synthesis of these derivatives with a common methodology for both the selenosugar and selenonucleoside (Scheme 33). The general chemistry involved displacing a dibromide or dimesylate with sodium selenide to
give the selenosugar and a Pummerer type rearrangement with a selenoxide and the nucleobase to give the 4’-selenonucleoside.

![Scheme 33](image)

Scheme 33. (Left) N-type and S-type conformations for O, S and Se nucleosides. (Right) General synthesis for 4’-selenoribonucleosides.

Application of these DNA/RNA building blocks have included synthesis of oligonucleotides and investigating their properties as antiviral and anticancer agents\(^\text{20}\). However, unlike 4’-oxo- and 4’-thionucleosides, most 4’-selenonucleosides were shown to have no significant antiviral or anticancer activity\(^\text{21}\). Taking into account the S-type conformation, it was hypothesised the lack of activity was due to the bulky selenium atom preventing phosphorylating of the 5’-hydroxyl group by cellular kinases. To overcome this issue, Jeong and co-workers synthesised 5’-homo-4’-selenonucleosides, envisioning that a one carbon-homologation would relieve the steric effects and allow for phosphorylating\(^\text{22}\). Gratifyingly after synthesising the 5’-homo analogues via a seleno-Michael reaction, potent antiviral activity was observed, indicating that they could be phosphorylated by cellular kinases. A more versatile route to 5’-homo-4’-selenonucleosides was also recently reported by Jeong and co-workers\(^\text{23}\).

Selenosugars can also behave as antioxidants. Oxidative stress in biological systems, whereby an imbalance between the production of reactive oxygen species and antioxidant systems are met, can result in tissue damage and be linked to a number of inflammatory diseases and neurodegenerative disorders. The role of selenoproteins, for example glutathione peroxidase, is to behave as a line of cellular defence against oxidative damage by catalysing the reduction of reactive oxygen species (ROS). To mimic these properties, and have application in treating diseases associated with oxidative damage, simple 5-selenopyranose
and 4-selenofuranose monosaccharides were prepared as water soluble scavengers for hypohalous acids and peroxides. In 2011, Storkey et al. synthesised sulfur and selenium derivatives of L-1-deoxynojirimycin and D-1-deoxymannojirimycin and evaluated their properties as scavengers for hypochlorous acid (HOCl)\textsuperscript{24}. Selenosugars \textbf{202} and \textbf{203} were found to be potent scavengers than their corresponding thiosugars. Following this, the researchers presented the scavenging ability of 5-selenopyranose and 5-selenofuranose derivatives for hypohalous acids (HOCl, HOBr, HOSCN)\textsuperscript{25}. A kinetic study reviled the sugar stereochemistry unaffected the rate of reaction and derivatives reacted with HOCl at a comparable rate to an endogenous antioxidant glutathione. The rate constants with HOBr were somewhat slower (~8 times lower) while reactions with HOSCN were the slowest. All selenosugars were found to protect amino acid residues on both bovine serum albumin and human plasma proteins against HOCl mediated oxidation. In 2012, Merino-Montiel et al. synthesised L-isofucoselenofagomine derivatives to imitate the function of glutathione peroxidase by scavenging H\textsubscript{2}O\textsubscript{2}\textsuperscript{26}. Protected selenosugar \textbf{204} was found to catalyse the reduction of H\textsubscript{2}O\textsubscript{2} in the presence of dithiothreitol. The purpose of the thiol was to mimic glutathione, the cofactor for glutathione peroxidase.

![Scheme 34](image)

**Scheme 34.** General reaction for scavenging reactive oxygen species (ROS) by selenosugars and illustrating examples of selenosugar antioxidants.

### 3.2.3 Selenoglycosides

Selenoglycosides have found many applications in carbohydrate chemistry. They are useful intermediates for the preparation of C-glycosides\textsuperscript{27}, glycosyl halides\textsuperscript{28}, hemiacetals\textsuperscript{29}, functionalised glycals\textsuperscript{30}, orthoesters\textsuperscript{31}, deoxyglycosides\textsuperscript{31} and glycoconjugates\textsuperscript{32}. As glycosyl donors, aryl selenoglycosides can be chemoselectively activated in the presence of thioglycosides, which makes them a powerful tool in oligosaccharide synthesis for multistep or iterative glycosylations\textsuperscript{33}. Their application as carbohydrate mimetics are less explored.
Like S-glycosides, Se-glycosides are stable to the hydrolytic action of glycosidase enzymes, suggesting they could have a potential application in developing new carbohydrate based drugs\textsuperscript{34}.

The introduction of selenium at the anomeric position can be achieved through several methods. In 1995, Pinto and co-workers glycosylated 4-selenol 207 with trichloroacetimidate donor 206 to give selenodisaccharide 208 as a 4.5:1 α:β mixture in a combined yield of 57\%\textsuperscript{34}. Selenol 207 was found to be unstable however, as any attempts to purify the compound resulted in oxidation to a diselenide. 207 also showed some air stability but oxidised to the diselenide over a number of days. 207 was used in the glycosylation immediately after preparation. Subsequently, Czernecki and Randriamandimby synthesised 1→6 selenodisaccharides by reducing symmetric diglycosyl diselenides and reacting the corresponding seleno\textsuperscript{35}lates with either 6-O-tosyl or 6-deoxy-6-iodo monosaccharides. However, acetyl groups were unstable to the reaction conditions and reacetylation was necessary after the transformation.

![Scheme 35](image)

**Scheme 35.** Pinto’s (top) and Czernecki’s (bottom) synthesis of selenodisaccharides.

Alkyl and aryl selenoglycosides can be prepared by reacting alkyl/aryl selenolates (generated upon treating the corresponding diselenides with a hydride reducing agent) with glycosyl halides\textsuperscript{36}. The drawback of this procedure is again the incompatibility of acyl protecting groups. Tiwari and Misra have developed an efficient method for preparing selenoglycosides\textsuperscript{37}. The procedure involves an indium(I) iodide assisted cleavage of aryl diselenides and reacting with α-glycosyl bromides. The protocol is effective for a number of acetylated saccharides and yields were excellent in all cases. Retention of configuration at the anomeric carbon was also observed in every reaction, indicating glycosylselenide formation.
proceeds through a radical mechanism. A recent procedure reported by Chandrasekaran and co-workers involved cleaving aryl diselenides with rongalite (HOCH$_2$SO$_2$Na) and reacting with acetylated $\alpha$-glycosyl bromides to give aryl $\beta$-selenoglycosides$^{38}$.

\[
\text{RS}_2\text{Se} \rightarrow 1. \text{NaBH}_4 \text{ or Inl or Rongalite} \rightarrow \begin{array}{c} \text{PO} \end{array} \text{Br} \rightarrow 2. \text{PO} \begin{array}{c} \text{O} \end{array} \text{SeR}
\]

**Scheme 36.** Synthesis of selenoglycosides from diselenides and glycosyl bromides.

Ishihara and co-workers have reported the synthesis of peracetylated galactose and glucose $\beta$-selenoglycosides from a $\beta$-glycosyl $p$-methylbenzoselenoate, which behaves as a selenolate anion precursor$^{39}$. The mechanism involves selectively activating the acyl selenoglycoside with a secondary amine to produce a $\beta$-selenolate anion which is then stabilized by a cesium counterion. Addition of an electrophile followed by a subsequent nucleophilic displacement then yields the $\beta$-selenoglycoside. The reaction has a wide substrate scope and can prepare alkyl and aryl selenoglycosides, selenodisaccharides and selenoglycosyl amino acids. Ishihara and co-workers have also reported the synthesis of $\alpha$-selenoglycosides$^{40}$. The protocol is identical to that above, except involves the production of an $\alpha$-selenolate anion. The $\alpha$-glycosyl $p$-methylbenzoselenoate was synthesized by reacting a $\beta$-glycosyl chloride with potassium $p$-methylselenobenzoate. The reaction produced a large variety of $\alpha$-selenoglycosides in excellent yields and had a substrate scope similar to that for $\beta$-selenoglycosides.

\[
\begin{array}{c}
\text{AcO} \begin{array}{c} \text{Se} \\
\text{O} \end{array} \text{S}_{\text{e}} \text{Se} \text{R} \\
\text{Cs}_2\text{CO}_3 \text{ Amine}
\end{array}
\rightarrow
\begin{array}{c}
\text{AcO} \begin{array}{c} \text{Se} \\
\text{O} \end{array} \text{Se} \text{R} \\
\text{R-X}
\end{array}
\]

$\beta$-selenide

\[
\begin{array}{c}
\text{AcO} \begin{array}{c} \text{Se} \\
\text{O} \end{array} \text{Se} \text{R} \\
\text{Cs}_2\text{CO}_3 \text{ Amine}
\end{array}
\rightarrow
\begin{array}{c}
\text{AcO} \begin{array}{c} \text{Se} \\
\text{O} \end{array} \text{Se} \text{R} \\
\text{R-X}
\end{array}
\]

$\alpha$-selenide

R = Alkyl, Aryl, Sugar, Amino Acid

**Scheme 37.** Ishihara and co-workers synthesis of $\beta$ and $\alpha$-selenoglycosides.
Glycosyl-isoselenonium salts were reported by Szilágyi and co-workers as convenient starting materials for synthesising β-selenoglycosides\(^{41}\). The isoselenonium salts are prepared on treating α-glycosyl bromides with selenourea in refluxing acetone and a subsequent reaction with an electrophile in the presence of acetonitrile and triethylamine gives the β-selenide. The reaction can be used to prepare acyl, alkyl, aryl selenoglycosides and selenodisaccharides with yields varying between 49-98%. Selenoglycosides can also be prepared from a transacetalization reaction between a glycosyl trichloroacetimidate and a selenoacetal\(^{42}\). The glycosyl imidate is activated with TMSOTf and the resulting oxocarbenium ion reacts with a benzyloxymethyl selenide to give the selenoglycoside. Acetylated saccharides undergo the transformation to give exclusively alkyl and aryl β-selenoglycosides.

![Scheme 38. Synthesis of β-selenoglycosides using isoselenonium salts (top) and transacetalization (bottom).](attachment:Scheme_38.png)

3.3 Aims and Objectives

It can be noted from above that there are various procedures for preparing β-selenoglycosides and a lesser number reported for the corresponding α-anomer. The indium(I) iodide protocol reported by Tiwari and Misra does give α-selenides but is only applicable to aryl substituents on the aglycon. Ishihara’s method, through the in situ production of an α-selenolate anion, has a wide substrate scope but involves unstable β-glycosyl chlorides. Another drawback is that both procedures don’t show any application to synthesise more complex targets of interest for their bioactivity. To overcome these limitations, it was envisioned that Lewis acid promoted anomerisation could give α-selenoglycosides from the corresponding β-anomer. Discussed below are the results of this investigation.
3.4 Investigating the Anomerisation of Selenium Glycosides

The investigation commenced with the synthesis of propyl β-Se-glycosides, which were based on both galactopyranose and the corresponding galacturonic acid $210\beta$, $211\beta$, $216\beta$ and $217\beta$ (Scheme 39). Using the protocol reported by Ishihara and co-workers for the synthesis of β-selenides from β-glycosyl p-methylbenzoselenoates (vide supra), the first β-selenide $210\beta$ could be obtained by treatment of benzoselenoate $209$ with piperazine followed by the addition of 1-bromopropane. The second β-selenide $211\beta$ was then isolated through a protecting group exchange of the acetyl groups on $210\beta$ for benzoyl esters. Synthesis of methyl esters $216\beta$ and $217\beta$ were more challenging. Attempts to form benzoselenoates $214$ and $215$ from α-glycosyl bromides $84$ and $212$ using Ishihara’s conditions gave the desired products in low yields. However, ultrasonication of potassium p-methylbenzoselenoate $213$ in the presence of $84$ or $212$ for 15 min in DMF lead to improved yields (63-70%). Subsequent reaction of $214$ and $215$ with propyl bromide then gave $216\beta$ and $217\beta$. The selenium alkylation for methyl ester derivatives was found to be slower when compared to pyranose derivatives (15 min vs 60 min). Induction from the C-5 methyl ester can decrease selenium’s nucleophilic ability towards alkylation and in turn, decrease in rate of reaction.

Scheme 39. Synthesis of equatorial selenium glycosides. (Reprinted with permission from Organic Letters, Copyright 2016, American Chemical Society)

Next, attention was turned to the anomerisation reaction (Table 2). Since previous investigations showed 2.5 equiv of Lewis acid (SnCl$_4$ or TiCl$_4$) and CH$_2$Cl$_2$ to be effective for the anomerisation of O- and S-glycosides, these conditions were chosen as a starting point for the investigation. When galactopyranosides $210\beta$ and $211\beta$ were treated with SnCl$_4$ or TiCl$_4$ at various temperatures no α-glycoside could be observed or isolated. Reactions carried out at
room temperature gave the corresponding α-glycosyl chlorides, resulting from exocyclic cleavage and low temperature reactions lead only to recovery of unreacted starting material. Galacturonates 216β and 217β were then investigated. Gratifyingly it was found that carrying out the reaction at low temperature in the presence of TiCl₄ lead to the isolation of α-selenides 216α and 217α in moderate yield. Higher temperature reactions with TiCl₄ lead to the isolation of the α-glycosyl chlorides in these cases. Reactions with SnCl₄ at various temperatures gave no α-anomer with only the α-glycosyl chloride being isolated. There is evidence that α-selenocarbenium ions are generated from O,Se acetals/ketals in the presence of TiCl₄ while this is not the case for SnCl₄ where oxocarbenium ions are preferred due to Sn(IV) affinity for Se⁴³. Therefore the results observed are consistent with TiCl₄ promoting endocyclic cleavage to give α-selenocarbenium ions and in turn anomerisation, while reactions with SnCl₄ proceed by breaking the anomeric C-Se bond leading to glycosyl chloride formation (Scheme 40).

Increasing the isolated yield of the α-selenide was next investigated. Reasons that are yet unclear, it was found however that on lowering the concentration of all reactants, the yields increased for both 216α and 217α to 94%. The stereochemistry of the α-selenide was confirmed by ¹H NMR (J₁₂ = 4-5 Hz) and through single X-ray crystal diffraction of 217α. The C-Se bond length (1.98 Å), the C-Se-C angle (97°) and the C-Se-C distance (2.97 Å) were in agreement with predicted values⁴⁴. These values can be compared to typical C-O, C-S bond lengths (1.4, 1.8 Å), C-O-C, C-S-C bond angles (115°, 95°) and C-O-C, C-S-C distances (2.4, 2.9 Å).

Scheme 40. Effect of TiCl₄ and SnCl₄ on O,Se acetals.
Table 2. Investigating reagents and conditions for the anomerisation to α-selenides. (Reprinted with permission from Organic Letters, Copyright 2016, American Chemical Society)

<table>
<thead>
<tr>
<th>entry</th>
<th>α-Se-glycoside</th>
<th>Lewis acid</th>
<th>$t$ (°C)</th>
<th>Concentration (µM)</th>
<th>outcome (isolated yield)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>210β or 211β</td>
<td>TiCl₄</td>
<td>-15</td>
<td>100</td>
<td>no 210α/211α</td>
</tr>
<tr>
<td>2</td>
<td>210β or 211β</td>
<td>TiCl₄</td>
<td>rt</td>
<td>100</td>
<td>no 210α/211α</td>
</tr>
<tr>
<td>3</td>
<td>211β</td>
<td>SnCl₄</td>
<td>rt</td>
<td>100</td>
<td>no 211α</td>
</tr>
<tr>
<td>4</td>
<td>216β</td>
<td>SnCl₄</td>
<td>rt</td>
<td>90</td>
<td>no 216α</td>
</tr>
<tr>
<td>5</td>
<td>216β</td>
<td>SnCl₄</td>
<td>4</td>
<td>90</td>
<td>no 216α</td>
</tr>
<tr>
<td>6</td>
<td>216β</td>
<td>SnCl₄</td>
<td>-20</td>
<td>90</td>
<td>no 216α</td>
</tr>
<tr>
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<td>216β</td>
<td>TiCl₄</td>
<td>rt</td>
<td>90</td>
<td>no 216α</td>
</tr>
<tr>
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<td>216β</td>
<td>TiCl₄</td>
<td>4</td>
<td>90</td>
<td>216α (58%)</td>
</tr>
<tr>
<td>9</td>
<td>216β</td>
<td>TiCl₄</td>
<td>-20</td>
<td>7</td>
<td>216α (94%)</td>
</tr>
<tr>
<td>10</td>
<td>217β</td>
<td>TiCl₄</td>
<td>0</td>
<td>80</td>
<td>217α (46%)</td>
</tr>
<tr>
<td>11</td>
<td>217β</td>
<td>TiCl₄</td>
<td>-40</td>
<td>70</td>
<td>217α (68%)</td>
</tr>
<tr>
<td>12</td>
<td>217β</td>
<td>TiCl₄</td>
<td>-20</td>
<td>15</td>
<td>217α (86%)</td>
</tr>
<tr>
<td>13</td>
<td>217β</td>
<td>TiCl₄</td>
<td>-20</td>
<td>7</td>
<td>217α (94%)</td>
</tr>
</tbody>
</table>

Figure 19. Structure of 216α and 217α. (Reprinted with permission from Organic Letters, Copyright 2016, American Chemical Society)
3.5 Substrate Scope: Synthesis and Investigation

With the optimal conditions identified, a series of benzoylated β-selenides were synthesised to examine the wider scope of the anomerisation reaction. Benzoylated derivatives were selected as they have been found to be generally superior to the corresponding acetylated saccharides, in particular for the anomerisation of more structurally complex substrates such as glycolipids or disaccharides. First a series of commercially available alkyl halides were reacted with 215 to give β-selenides 218β-225β (Scheme 41).

![Scheme 41. Synthesis of β-selenides 218β-225β from commercial alkyl halides.](image)

Next, to investigate the anomerisation of disaccharides, benzoyl protected 1→6 and 1→4 β-Se-linked substrates (226β-228β) were synthesised. Triflates 229-231 were therefore necessary for the Se-alkylations (Scheme 42). Regioselective trityl protection of the primary hydroxyl in methyl α-D-glucopyranoside 232 followed by benzoyl ester formation and subsequent removal of the trityl group with FeCl₃ gave intermediate 233 in a 53% yield over three steps. Primary alcohol 233 was then treated with trifluoromethanesulfonic anhydride to give 6-O-trflate 229 in quantitative yield. To synthesise the 4-O-trflate 230, regioselective protection of the primary alcohol in 232 with TIPS followed by benzoyl ester formation and a silyl mediated protection-deprotection gave 4-hydroxyl 234 in a 50% yield over three steps⁴⁵.
Triflate formation then gave 230 in quantitative yield. The 4-O-triflate 231 was synthesised through a similar sequence from methyl β-D-galactopyranoside 235.

The Se-alkylations to give either 1→6 or 1→4 disaccharides were however, unsuccessful. It has been reported for carbohydrate derivatives that neighbouring group participation of ester protecting groups can displace adjacent triflates, resulting in the formation of stable acetoxonium intermediates. This intramolecular mechanism can compete with an S_N2 displacement in polar solvents such as DMF. However, in non-polar solvents such as toluene the intramolecular displacement is slow and can favour the intermolecular S_N2 reaction. Since the Se-alkylation solvent medium is DMF, acetoxonium ion formation could account for the poor reaction outcome. Gratifyingly, on switching the solvent medium to toluene the desired 1→6 and 1→4 disaccharides 226β-228β could be obtained in good to excellent yields (62-90%) (Scheme 43).
The last β-selenide for the substrate scope involved the synthesis of a steroidal glycoside 237β. The synthesis involved an Apple type reaction of the secondary alcohol on cholestanol 238 with triphenylphosphine, iodine and imidazole to give the alkyl iodide 239 in an 82% yield. Alkyl halide 239 was found to be insoluble in DMF on attempting the Se-alkylation. To overcome this caveat, 239 was dissolved in toluene and added via cannula to the activated mixture of selenide 215 in DMF. The alkylation proceeded smoothly and gave β-selenide 237β in a 77% isolated yield.

Scheme 43. Synthesis of β-Se-disaccharides for substrate scope.

Scheme 44. Synthesis of steroidal glycoside 237β.
Scheme 45. Anomerisation of various alkyl glycosides. (Reprinted with permission from Organic Letters, Copyright 2016, American Chemical Society)
With all substrates in hand, the anomerisation reactions were then attempted (Scheme 45 & Scheme 46). All alkyl glycosides anomerised to give the corresponding axial anomers 218α-225α in good to excellent yields and selectivity. Allyl glycoside 224β was found to require extra TiCl₄ (5 equiv) at -20 °C to ensure complete conversion of the starting material, while anomerisation of propargyl glycoside 225β required both elevated temperature (0 °C) and increased equivalents of TiCl₄ (5 equiv). The double anomerisation reaction of bivalent saccharide 223β proceeded smoothly at -20 °C with a higher quantity of promoter (5 equiv) and longer reaction time being needed. Anomerisation of steroidal glycoside 237β only required 2.5 equivalents of TiCl₄ and anomerised rapidly at -20 °C to give the corresponding α-anomer 237α in an excellent 90% yield. The 1→6 disaccharide 226β gave a low conversion at -20 °C with 2.5 equivalents of TiCl₄. After screening various temperatures and equivalents of promoter, it was found that both additional TiCl₄ (10 equiv) and room temperature were necessary to maximise the yield of the axial anomer. The requirement for higher temperature and more Lewis acid can be rationalised on the basis of increased electron withdrawing properties of the aglycon and an increase in the number of sites around the molecule that can coordinate to the Lewis acid.

Scheme 46. Anomerisation of 1→6 disaccharide 226β and steroidal glycoside 237β.

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It is worth noting that when screening reaction conditions for the 1→6 disaccharide anomerisation, the diselenide by-product 240 was observed, resulting from exocyclic cleavage of the glycoside. This suggests that 1→6 disaccharides of this nature have a competing exocyclic pathway and can account for the moderate yield (67%). In an attempt to overcome the undesired mechanism it was envisioned, converting the C-5 methyl ester to an allyl ester would predominately favour an endocyclic pathway. The $\eta^2$ π-donating ability of the allyl group can provide an additional coordination site and hold the metal more effectively to the reactive centre compared to the corresponding methyl ester and result in an increased rate for anomerisation. The β-selenide ally ester derivative 241β was synthesised for methyl ester 226β (Scheme 47). Treatment of 226β with lithium iodide in ethyl acetate at reflux selectively cleaved the methyl ester to give carboxylic acid derivative 242. Ester formation under basic conditions from acid 242 gave allyl derivative 241β in an 89% yield for two steps. Treating 241β with TiCl₄ (10 equiv) gratifyingly gave the α-anomer 241α in a higher yield (80%) compared to the methyl ester derivative 226β.

Scheme 47. Synthesis and anomerisation of allyl ester 1→6 derivative 241β and illustrating the coordinating ability of the allyl group to the Lewis acid.

Anomerisation of the 4-Se-linked disaccharides 227β and 228β were then attempted (Scheme 48). Unfortunately neither glycoside underwent the transformation and instead, diselenide products were observed. Various temperatures, Lewis acid concentrations and quenching times were screened for disaccharide 227β with no positive outcome. Converting to the allyl
ester derivative \(243\beta\) gave no \(\alpha\)-anomer. A combined electronic and steric factor may contribute to favour exocyclic cleavage. The electron withdrawing ability of the benzoyl protecting groups on the aglycon can destabilise cation formation in the rate determining step for endocyclic cleavage and decrease the rate for anomerisation. Moreover, since selenium forms weak bonds with carbon, the glycosidic bond can become even weaker from the inductive effects and contribute to the exocyclic pathway. Steric repulsion in the transition state for the 4-Se-linkage may lead to a higher energy barrier for endocyclic cleavage. Steric crowding exists about the secondary glycosidic linkage and since selenium is larger compared to oxygen and sulfur, it could interact with the bulky OBz protecting groups on the aglycon in the transition state. An interaction with the 6'-OBz and the metal chelate could also be possible. These interactions are not observed for the 6-Se-linkage since the primary C-Se bond is unhindered and minimises steric repulsion. This can therefore attribute success of the anomerisation reaction for the 6-Se-linkages.

**Scheme 48.** Unsuccessful anomerisation of 4-Se-linked disaccharides.
3.6 Conclusion

To conclude, a convenient chelation induced anomerisation reaction for the generation of axial or α-Se-glycosides form the corresponding equatorial or β-anomers has been presented. Galacturonic acid derivatives were found to undergo the transformation over galactopyranose derivatives and low temperature (-20 °C), low concentration (7 µM) and TiCl₄ were identified as the optimal conditions. Reactions with SnCl₄ gave no α-anomer with only the glycosyl chloride being obtained. A variety of β-selenides with increasing complexity on the aglycon (alkyl, alkene, alkyne, saccharide and steroid) were synthesised for the substrate scope. Competing intramolecular acetoxonium ion formation in DMF made disaccharide synthesis challenging but could be prevented on switching to toluene. The anomerisation was found to have a wide scope and accommodate a variety of functionality on the glycoside. Increasing the complexity of the aglycon lead to the requirement to increase the amount of Lewis acid and with some examples, increase the temperature. A diselenide by-product was observed during the anomerisation of methyl ester 1→6 disaccharides but could be prevented on substituting to a C-5 allyl ester. The anomerisation of 1→4 disaccharides were unsuccessful giving only diselenide products. Nevertheless in the fortunate examples described, a high degree of conversion was achieved and the reactions were highly stereoselective. It is believed the methodology is not limited to galactopyranuronates but could potentially be applied to other glycuronic acid derivatives. Key α-selenide intermediates could be accessed through this method to synthesise therapeutic neoglycoconjugates and pseudo-oligosaccharides with an incorporated selenium atom. This methodology along with the substrate scope was published in Organic Letters (doi:10.1021/acs.orglett.5b03591). The unsuccessful anomerisation for the 1→4 disaccharides was not included in this publication as further investigation is required to achieve an optimal reaction outcome.

3.7 References


(18) Jayakanthan, K., Johnston, B. D., Pinto, B. M. *Carbohydrate Research* **2008**, *343*, 1790.


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4.1 Retrosynthetic Analysis

In light of the success for the selenium anomerisation reaction, it was envisioned that selenoglycoside analogues of biological relevant molecules could be synthesised by this approach. As mentioned previously in chapter 1, the synthetic glycolipid immunostimulant α-GalCer (KRN7000) can stimulate iNKT cells to produce high levels of IFN-γ and IL-4 cytokines. Due to the lack of bias, various analogues have been synthesised to favour IFN-γ or IL-4 production. The C- and S-glycoside analogues have shown interesting properties to date, however the Se-glycoside analogue has yet to be reported. Since an important feature of α-GalCer is the axial orientation of the glycosidic linkage, selenium anomerisation would be an ideal candidate to generate the axial linkage and consequently α-Se-GalCer.

![Scheme 49. Retrosynthetic analysis of α-Se-GalCer.](image_url)

The retrosynthetic analysis of the target glycolipid 245 is illustrated above (Scheme 49). Hence, α-Se-GalCer could be synthesised from methyl ester derivative 246 via a regioselective reduction of the C-5’ methyl ester to its primary alcohol followed by a subsequent global deprotection. It was envisioned protected ceramide 246 could be synthesised in two step form glycolipid derivative 247 and the key α-linkage in 247 could be generated through the recently developed chelate induced anomerisation reaction via β-selenide 248. Selenium alkylation with benzoselenoate 215 and bromo-phytosphingosine...
derivative 249 would give 248 and a series of transformations from commercially available phytosphingosine 56 would give alkyl halide 249.

4.2 Synthesis

The synthesis of α-Se-GalCer commenced from phytosphingosine 56 (Scheme 50). Copper-catalysed diazo transfer with trifluoromethanesulfonfyl azide gave azido-triol 52 in excellent yield. Regioselective trityl protection of the primary alcohol followed by protection of the resulting diol with benzoyl esters and subsequent trityl deprotection under acidic conditions gave intermediate 250 in a 71% yield for three steps. Alcohol 250 was converted to a mesylate intermediate and subsequent S_N2 displacement with KBr in DMF at 90 °C gave alkyl bromide 249. Treatment of benzoselenoate 215 with piperidine followed by addition of alkyl halide 249 then gave β-selenide glycolipid derivative 248 in 82% yield.

![Scheme 50](image.png)

Scheme 50. Synthesis of β-selenide 248 from phytosphingosine 56.

With the β-selenide 248 in hand the anomerisation reaction was then attempted. On carrying out the anomerisation with 2.5 equivalents of TiCl4 at -20 °C the α-anomer could be isolated in a low 46% yield. TLC indicated poor conversion of starting material and formation of an unidentified by-product. The C-5’ methyl ester was converted to an allyl derivative 252 and 2-hydroxyethyl ester 253 to investigate whether full conversion could be achieved at -20 °C. As mentioned previously allyl esters can increase the rate of anomerisation and prevent the formation of diselenide by-products. More recently, unpublished research in the Murphy group carried out by fellow PhD student Louise Kerins led to observation that 2-hydroxyethyl esters have a faster rate of anomerisation than allyl esters. It was therefore anticipated, derivative 253 could potentially give both a higher conversion and isolated yield of the α-
anomer. Surprisingly no conversion of 252 or 253 to the axial anomer or any product could be observed at -20 °C. The anomerisations were found only to take place when raising the reaction temperature. Room temperature and 10 equivalents of TiCl₄ were identified as the optimal conditions for these glycolipid substrates, giving 254 and 255 in 82% and 90% yield respectively. Gratifyingly treating methyl ester 248 with the optimal conditions gave the corresponding α-anomer 247 in an 87% isolated yield (Scheme 51).

**Scheme 51.** Investigating the anomerisation of β-Se-glycolipid derivatives.

Since conditions were identified to selectively reduce the methyl ester on C-5’ to its carboxylic acid (i.e. lithium iodide)⁴, which is a key step in the proposed synthesis of α-Se-GalCer, α-selenide 247 was selected for further transformations. Reduction of the azide was then investigated (Scheme 52). The Staudinger reaction with tributylphosphine gave the amine, however migration of the benzoyl group from either the 3 or 4 positions on the phytosphingosine gave the corresponding benzamide as the major product. A thio acid/azide amidation was then envisioned to prevent migration since it enables the acylation of azides with thio acids to give amides³. Unfortunately azide 247 failed to react with thio acid 260 to give amide 246. The next strategy involved a global deprotection followed by reduction of the azido group and subsequent N-acylation. Removal of both the methyl and benzoyl esters with NaOMe-MeOH gave 258 in a 96% yield without any E1cB by-product formation. However, the azide reduction was again troublesome. Various reagents were screened such as
PMe$_3$, Pb$_3$, zinc-acetic acid, H$_2$S, NaBH$_4$, hydrogenation and 1,3-propanedithiol with no successful outcome. The polarity of both the starting material 258 and the amine product 259 made reaction monitoring difficult via TLC, NMR and mass spectrometry. On assuming reduction of the azide to the corresponding amine, N-acylation was attempted. However no α-Se-GalCer could be observed or isolated.

Scheme 52. Investigating the reduction of the azido intermediate.

In order to attempt an anomerisation-deprotection in one pot, substituting the base sensitive benzoyl esters on the phytosphingosine for acid sensitive protecting groups was next explored. This strategy has been previously successful for bacterial glycolipids where one benzyl ether on a sphinganine lipid chain was removed during the anomerisation with TiCl$_4$. If successful for phytosphingosine glycolipids, a diol would be produced and azide reduction would hopefully occur with ease. The three β-glycolipids 261-263, with acetonide, silyl ether and benzyl ether protecting groups were synthesised from alkyl iodides 53, 264 and 265.
Lewis acid promoted anomerisation reactions for isopropylidene and tert-butylidimethylsilyl ether protecting groups are not reported. It would therefore be interesting to see if application can be found in an anomerisation-deprotection. However, the TiCl$_4$ anomerisation for both the acetonide and silyl ether derivatives 261 and 262 were unsuccessful with decomposition observed for each reaction. Treating the benzyl ether derivative 263 with TiCl$_4$ removed both protecting groups; however anomerisation of the diol intermediate was slow and failed to go to completion. Decomposition was also observed and despite some α-anomer being produced, the reproducibility of the reaction was an issue. It was therefore decided to abandon investigating the reduction of the azide intermediate and any route thereof.

**Scheme 53.** Investigating the anomerisation-deprotection of β-glycolipids 261-263.

### 4.3 Revised Retrosynthetic Analysis

With the disappointing results for the azide reduction, a new route to synthesise α-Se-GalCer was presented (Scheme 54). It was envisioned amide bond formation at the beginning from phytosphingosine 56 would be advantageous, as any azide intermediate could be excluded. Selenium alkylation followed by subsequent selenium anomerisation of β-glycolipid 269
would give the corresponding α-anomer 246 and reduction of the C-5’ methyl ester on the saccharide followed by a global deprotection would then give α-Se-GalCer 245.

Scheme 54. Revised retrosynthesis.

4.4 A Successful Route to α-Se-GalCer

The redesigned synthesis commenced from phytosphingosine 56. N-Acylation of the amine on 56 with the N-hydroxysuccinimide ester of cerotic acid 271 gave ceramide 272 in excellent yield\(^{12}\). Treating 272 with triphenylchloromethane in pyridine gave O-trityl formation on the primary alcohol. The remaining two hydroxyl groups were then treated with benzoyl chloride in pyridine and the resulting intermediate was subjected to detritylation under acidic conditions to give intermediate 273\(^{13}\). Alkyl bromide formation was successful from primary alcohol 273 via the Appel reaction, however a spontaneous intramolecular cyclisation decomposed the bromide to an oxazoline by-product 275. The introduction of other leaving groups such as iodine, chlorine and triflate gave an identical observation, however the rate of oxazoline formation was found to be slow for the mesylate derivative 274. It was hopeful that this decrease in rate would allow the Se-alkylation to take place. Gratifyingly, reaction of the primary alcohol on 273 with methane sulfonyl chloride provided 274 and this was immediately coupled with 215 to give β-glycolipid 269 in a moderate 48% yield. Anomerisation of 269 with TiCl\(_4\) (10 equiv) at room temperature for 3.5 hours gave the
Scheme 55. Synthesis of α-Se-GalCer 245 and acid analogue 277. (Reprinted with permission from Organic Letters, Copyright 2016, American Chemical Society)

axial anomer 246 in an excellent 80% yield. Next the selective cleavage of the methyl ester on 246 with lithium iodide in refluxing EtOAc gave the carboxylic acid intermediate 276. Removal of all the benzoate ester from 276 with NaOMe-MeOH gave the galacturonic acid
analogue of $\alpha$-Se-GalCer 277, which can contribute to SAR studies$^{14}$. On the other hand, chemoselective activation of the carboxylic acid on 276 with PyBOP followed by a one-pot reduction of the resulting hydroxybenzotriazole ester with sodium borohydride furnished primary alcohol 278$^{15}$. Removal of all the benzoyl groups form 278 completed the synthesis to give $\alpha$-Se-GalCer 245 in an excellent 95% yield.

4.5 Biological evaluation

For the biological evaluation a third glycolipid, $\beta$-Se-GalCer 279 was synthesised (Scheme 56). All three selenium glycolipids 245, 277 and 279 were sent to The Scripps Research Institute, La Jolla, California in collaboration with Prof. Luc Teyton and Dr. Paul B. Savage. Results so far have indicated neither glycolipid stimulates mouse iNKT cells. This outcome is consistent with $\alpha$-S-GalCer where the sulfur analogue was a poor stimulate for mouse iNKT cells but was found to induce cytokine production for human iNKT cells. The selenium glycolipids are now being tested on human iNKT and results will be reported in due course.

![Scheme 56. Synthesis of $\beta$-Se-GalCer 279.](image)

4.6 Conclusion

In summary, the first selenium glycoside analogues of the potent immunostimulant KRN7000 have been synthesised. The initial route proposed was unsuccessful due to complications encountered during the reduction of an azido intermediate. All other routes deviated to resolve this issue also failed. A revised retrosynthesis redesigned the route to omit any azide intermediate and to have the amide linkage formed at the beginning of the synthesis. Introducing a leaving group to the primary positon of the ceramide caused a spontaneous cyclisation which made the Se-alkylation challenging. The rate of cyclisation was found to be slowest for a methanesulfonate derivative and allowed the alkylation to take place. Anomerisation gave the axial linkage and deprotection gave $\alpha$-Se-GalCer and an acid analogue. Biological evaluation was disappointing for mouse iNKT cells but the Se-glycolipids may have success in stimulating human iNKT cells. Nonetheless, an application
of selenium anomerisation to synthesise compounds with biological relevance has been demonstrated. The route to $\alpha$-Se-GalCer is short, attractive and allows access to both $\alpha$ and $\beta$ galactopyranose and galacturonic acid analogues. A library of Se-glycolipid analogues could be synthesised via this method and contribute to identifying glycolipid antigens with a bias cytokine profile. The successful route for $\alpha$-Se-GalCer was published in *Organic Letters* with the selenium anomerisation methodology described in the previous chapter (doi:10.1021/acs.lett.5b03591).

### 4.7 A Note on Future Work

The first section of this thesis described the synthesis of bacterial glycolipids with a triazole at the anomeric carbon. The initial synthesis proposed both 1,4- and 1,5-triazole analogues, however only the former 1,4-derivatives were successful. It would be ideal to reinvestigate the synthesis of the 1,5-derivatives and identify new conditions to synthesise the 1,5-triazole. Likewise, since the 1,4-triazole analogues failed to stimulate iNKT cells, an SAR study could discover compounds with a bias cytokine profile. A triazole analogue of KRN7000 would also be ideal to synthesise.

The selenium anomerisation described in Chapter 3 had a wide substrate scope but failed for 1→4 disaccharide derivatives. The reasons for this observation are not yet clear. An investigation to understand why these structures failed to anomerise is required. A study of this nature may lead to 1→4 disaccharides that preform the anomerisation to the corresponding $\alpha$-selenide successfully.

Regarding $\alpha$-Se-GalCer, an SAR study would be necessary since preliminary showed the compound, and analogues thereof, to be poor stimulants for mouse iNKT cells.

### 4.8 References


Pilgrim, W., Murphy, P. V. \textit{Organic Letters} \textbf{2009}, \textit{11}, 939.


Chapter 5: Experimental

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5.1 General Experimental Conditions

NMR spectra were recorded using a 500 MHz & 600 MHz spectrometer. Chemical shifts are reported relative to internal Me₄Si in CDCl₃ (δ 0.0) or CDCl₃ (δ 7.26), pyridine-d₅ (δ 8.74, 7.58, 7.22), (CD₃)₂SO (δ 2.50), HOD for D₂O (δ 4.65) and CD₂HOD (δ 3.31) for ¹H and CDCl₃ (δ 77.16) pyridine-d₅ (δ 150.0, 136.0, 124.0), (CD₃)₂SO (δ 39.5) and CD₂OD (δ 49.0) for ¹³C. ¹H-NMR signals were assigned with the aid of COSY. ¹³C signals were assigned with the aid of DEPT-135, HSQC and HMBC. Coupling constants (J) are reported in Hertz and are reported uncorrected. Low and high resolution mass spectra were measured in positive and/or negative mode as indicated using MeCN, H₂O and/or MeOH as solvent using a Waters LCT Mass Spectrometry instrument. FT-IR spectra were recorded using a polarized UATR (Universal Attenuated Total Reflectance) Accessory. Optical rotations were determined at the sodium D line at 23 ºC using CHCl₃ as indicated. TLC was performed on aluminium sheets pre-coated with Silica Gel 60 (HF₂54, E. Merck) and spots visualized by UV and charring with cerium (IV) molybdate solution or ninhydrin solutions. Flash column chromatography was generally employed and was carried out using silica gel 60 (0.040-0.630 mm) using a stepwise solvent polarity gradient correlated with TLC mobility. Chromatography solvents used were petroleum ether (40-60 ºC, Fisher Scientific), diethyl ether, toluene, cyclohexane, EtOAc, CH₂Cl₂ and MeOH (Sigma Aldrich). Anhydrous pyridine was used as purchased from Sigma-Aldrich. THF, toluene, CH₂Cl₂, Et₂O, DMF and methanol were used as obtained from a Pure-Solv™ solvent purification system. When necessary, solvents were degassed prior to use by bubbling argon through the solvent for 15 minutes followed by three freeze-thaw cycles. The numbering scheme for NMR assignments is indicated in some places below.
5.2 Chapter 2-Experimental

1,2,3,4-Tetra-O-acetyl-α-D-galactopyranosiduronic acid, methyl ester (166). To a stirred solution of HClO$_4$ (220 µL) in Ac$_2$O (57 mL) at 0 °C was added D-galacturonic acid monohydrate 164 (10 g, 52 mmol) in one portion. The reaction was warmed to room temperature and stirred for 3 h. The reaction was then re-cooled to 0 °C and MeOH was added cautiously. After stirring for a further 30 min 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 Na$_2$SO$_4$ and the solvent was removed under reduced pressure. The resulting residue was azeotroped several times with toluene to remove acetic acid. Once removed, the crude acid intermediate 165 was taken up in DMF (58 mL). NaHCO$_3$ (8.3 g, 100 mmol) and iodomethane (8.1 mL, 130 mmol) were added and the solution was stirred overnight. The reaction was diluted with H$_2$O and extracted into EtOAc. The combined organic layers were washed with H$_2$O, brine, dried over Na$_2$SO$_4$, filtered and the solvent was removed under reduced pressure. Flash chromatography of the residue (petroleum ether-EtOAc 2:1) gave 166 (13.5 g, 69% over two steps) as a white solid. The $^1$H and $^{13}$C NMR data for 166 were in good agreement with those previously reported in the literature$^1$; R$_f$ 0.18 (petroleum ether-EtOAc 2:1); IR (film) cm$^{-1}$: 2966, 1747, 1440, 1371, 1208, 1068, 942; $^1$H NMR (500 MHz, CDCl$_3$) δ 6.52 (1H, d, $J$ 2.6, H-1), 5.83 (1H, m, H-4), 5.42 – 5.36 (2H, overlapping signals, H-2, H-3), 4.75 (1H, d, $J$ 1.4, H-5), 3.77 (3H, s, OMe), 2.16 (3H, s), 2.12 (3H, s), 2.02 (6H, s) (each OAc); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 170.0, 169.6 (2s), 168.4, 166.5 (each C=O), 89.6 (C-1), 70.7 (C-5), 68.6 (C-4), 66.9, 66.0 (C-2 and C-3), 52.8 (OMe), 20.8, 20.6, 20.5 (2s) (each OAc); ESI-HRMS calcd. for C$_{15}$H$_{20}$O$_{10}$Na 399.0903, found m/z 399.0916 [M+Na]$^+$. 
1-Azido-1-deoxy-2,3,4-tri-O-acetyl-α-D-galactopyranuronic acid, methyl ester (104 & 108). Methyl ester 166 (10 g, 26.6 mmol) and trimethylsilyl azide (8.7 mL, 67 mmol) were taken up in dry CH₂Cl₂ (100 mL) and cooled to 0 °C. Tin (IV) chloride (9.4 mL, 80 mmol) was added dropwise. The resulting solution was warmed to room temperature and stirred for 24 h. The mixture was diluted with CH₂Cl₂ washed with 1 M KHSO₄, satd. NaHCO₃, water and brine, dried over Na₂SO₄ and the solvent was removed under reduced pressure. Flash chromatography of the residue (cyclohexane-EtOAc 2:1) provided the title compound 104 and 108 (5.92 g, 62%) as a mixture of anomers (α:β, 4:1). NMR data for both anomers (¹H and ¹³C) were in good agreement with the reported literature data²; IR (film) cm⁻¹: 2959, 2119, 1744, 1349, 1271, 1129, 1063; ¹H NMR (500 MHz, CDCl₃) δ 5.76 (1H, d, J 3.9, H-1α), 5.75 (1H, dd, J 3.1, 1.6, H-4α), 5.72 (1H, dd, J 3.4, 1.4, H-4β), 5.28 (1H, dd, J 10.9, 3.0, H-3α), 5.24 (1H, dd, J 10.7, 3.9, H-2α), 5.18 (1H, dd, J 10.4, 8.7, H-2β), 5.08 (1H, dd, J 10.2, 3.5, H-3β), 4.76 (1H, d, J 1.6, H-5α), 4.67 (1H, d, J 8.7, H-1β), 4.39 (1H, d, J 1.4, H-5β), 3.76 (3H, s, OMe), 3.75 (3H, s, OMe), 2.11 (6H, s), 2.10 (3H, s), 2.09 (3H, s), 2.08 (3H, s), 1.99 (3H, s, each OAc); ¹³C NMR (125 MHz, CDCl₃) δ 169.9, 169.8, 169.7, 169.6, 169.5, 169.1, 166.6, 165.8 (each C=O), 88.4 (C-1β), 86.9 (C-1α), 74.0 (C-5β), 70.3 (C-3β), 70.1 (C-5α), 68.6 (C-4α), 68.0 (C-4β), 67.6 (C-2β), 66.9, 66.7 (C-2α and C-3α), 52.9 (OMe), 52.8 (OMe), 20.6 (2s), 20.5 (3s), 20.4 (each OAc); ESI-HRMS calcd. for C₁₃H₁₇N₃O₉Na 382.0862, found m/z 382.0869 [M+Na]⁺.

1-Azido-1-deoxy-2,3,4-tri-O-acetyl-α-D-galactopyranuronic acid, methyl ester (104). To a stirred solution of the mixture of anomers 104 and 108 (1.2 g, 3.34 mmol) in dry CH₂Cl₂ (15 mL) at 0 °C was added 1 M TiCl₄ in CH₂Cl₂, (8.35 mL) dropwise. The solution was stirred at 0 °C for 10 min and then placed in a fridge freezer at −15 °C for 48 h. The reaction mixture was then diluted with CH₂Cl₂, washed twice with satd. NaHCO₃, water, brine, dried over NaSO₄ and the solvent was removed under reduced pressure. Flash chromatography of
the residue (petroleum ether-EtOAc 2:1) provided the title compound 104 (987 mg, 82%) as a white foam; NMR data ($^1$H and $^{13}$C) were in good agreement with the reported literature data$^2$; $R_f$ 0.54 (cyclohexane-EtOAc 1:1); mp 107–110 °C; IR (film) cm$^{-1}$: 2959, 2114, 1768, 1744, 1441, 1368, 1250, 1213, 1057; $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 5.78 (1H, d, $J$ 3.9, H-1), 5.77 (1H, dd, $J$ 3.0, 1.6, H-4), 5.30 (1H, dd, $J$ 10.8, 3.1, H-3), 5.26 (1H, dd, $J$ 10.7, 3.9, H-2), 4.78 (1H, d, $J$ 1.5, H-5), 3.77 (3H, s, OMe), 2.12 (3H, s), 2.11 (3H, s), 2.01 (3H, s) (each OAc); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 170.0, 169.7, 169.6, 166.6 (each C=O), 86.0 (C-1), 70.1 (C-5), 68.7 (C-4), 66.9, 66.8 (C-2 and C-3), 52.9 (OMe), 20.6 (2s), 20.5 (each OAc); ESI-HRMS calcd. for C$_{15}$H$_{17}$N$_3$O$_9$Na 382.0862, found m/z 382.0864 [M+Na]$^+$. 

1-Azido-1-deoxy-2,3,4-tri-O-acetyl-$\alpha$-D-galactopyranuronic acid (167). LiI (1.6 g, 8.3 mmol) was added to a solution of methyl ester 104 in anhydrous EtOAc and the reaction mixture was heated to reflux for 16 h. Upon cooling the reaction mixture was quenched with 10% HCl and the aqueous layer extracted into EtOAc. The combined organic layers were washed with sat. Na$_2$S$_2$O$_3$ dried over Na$_2$SO$_4$ and the solvent was removed under reduced pressure to give 167 (397 mg, 83%) as a yellow solid. The compound was used without further purification; IR (film) cm$^{-1}$: 3484 (br), 2998, 2121, 1744, 1678, 1619, 1371, 1212, 1122; $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 5.85 – 5.76 (2H, overlapping signals, H-1 and H-4), 5.39 – 5.10 (3H, overlapping signals, H-2, H-3 and OH), 4.82 (1H, s, H-5), 2.12 (6H, s, each OAc), 2.00 (3H, s, OAc); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 170.1, 169.7, 169.6, 166.6 (each C=O), 86.9 (C-1), 69.8 (C-5), 68.5 (C-4), 66.8 (C-2 or C-3), 66.7 (C-2 or C-3), 20.6, 20.5 (2s) (each OAc); ESI-HRMS calcd. for C$_{12}$H$_{14}$N$_3$O$_9$ 344.0730, found m/z 344.0733 [M-H]$^-$. 

2,3,4,6-Tetra-O-acetyl-$\alpha$-D-galactopyranosyl azide (105). Acid 167 (100 mg, 0.29 mmol) was taken up in dry THF (3 mL) and cooled to 0 °C. To this was added BF$_3$.THF complex (0.9 mL, 1M in THF) dropwise. The reaction was warmed to room temperature and stirred overnight. Methanol was added and the solvents were removed under reduced pressure. The
crude residue was taken up in Ac₂O (2 mL) and pyridine (2 mL) and stirred for a further 4 hours. The reaction was diluted with EtOAc, washed with 1M HCl, water, brine, dried over Na₂SO₄ and the solvent was removed under reduced pressure. Flash chromatography of the residue (petroleum ether-EtOAc 2:1) gave 105 (70 mg, 65% over two steps) as a crystalline solid. The ¹H and ¹³C NMR data for 105 were in good agreement with those previously reported in the literature³; [α]D +180.2 (c 1.0, CHCl₃); Rf 0.3 (cyclohexane-EtOAc 2:1); IR (film) cm⁻¹: 2981, 2118, 1744, 1373, 1207, 1122, 1064; ¹H NMR (500 MHz, CDCl₃) δ 5.66 (1H, d, J 4.0, H-1), 5.46 (1H, dd, J 3.2, 1.3, H-4), 5.25 (1H, dd, J 10.8, 3.1, H-3), 5.20 (1H, dd, J 10.7, 4.1, H-2), 4.36 (1H, td, J 6.9, 1.2, H-5), 4.16 – 4.09 (2H, m, H-6a & H-6b overlapping signals), 2.14 (3H, s), 2.11 (3H, s), 2.06 (3H, s) (each OAc); ¹³C NMR (125 MHz, CDCl₃) δ 170.4, 170.1, 170.0, 169.8 (each C=O), 86.7 (C-1), 68.5 (C-5), 67.6, 67.4, 67.2 (C-3 & C-2), 20.6 (4s, each OAc); ESI-HRMS calcd. for C₁₄H₁₉N₃O₉ Na 396.1019, found m/z 396.1036 [M+Na]⁺.

N-Boc-2-amino-N-((1R,2R)-1-hydroxy-1-phenylpropan-2-yl)-N-methylacetamide  (88). Trimethyl acetyl chloride (0.75 mL, 6.05 mmol) was added dropwise to a stirred solution of N-Boc-glycine (1.2 g, 6.8 mmol) and triethylamine (0.9 mL, 6.8 mmol) in dry CH₂Cl₂ (15 mL) at 0 °C. The solution was stirred for 40 min, then a second portion of triethylamine (0.9 mL, 6.8 mmol) was added, followed by the rapid addition of (R,R)-(−)-pseudoephedrine (1 g, 6.05 mmol). The reaction was stirred for 1 h, diluted with 1 M HCl solution, water, brine. Phases were separated and the aqueous layer extracted into EtOAc. The combined organic phases were washed with satd. K₂CO₃, dried over Na₂SO₄ and the solvent was removed under reduced pressure. Flash chromatography of the crude residue (EtOAc-Petroleum ether 1:1) gave 88 (1.8 g, 92%) as a viscous oil. The ¹H and ¹³C NMR data for 88 were in good agreement with those previously reported in the literature⁴; ¹H NMR (500 MHz, 1.5:1 rotamer ratio, CDCl₃) δ 7.39-7.26 (5H, m, Ar-H), 5.59 (1H, m), 4.61-4.52 (1H, m), 4.16 (1H, dd, J 16.6, 3.8), 4.01 (1H, dd, J 16.5, 5.0), 3.90 (1H, dd, J 4.5, 2.1, CHHNH₂), 3.87-3.79 (1H, m, CHHNH₂), 2.94 (3H, s, NMe), 2.81 (3H, s, NMe), 1.44 (9H, s, Boc), 1.05 (3H, d, J 6.3, CH₃), 0.96 (3H, d, J 6.8, CH₃); ¹³C NMR (125 MHz, CDCl₃) δ 170.3, 169.6, 156.1, 155.0, 141.9, 141.2, 128.9, 128.7 (2s), 128.1, 127.0, 126.6, 79.8, 79.7, 76.2, 75.4, 57.8, 57.6, 43.0,
N-Boc-(S)-2-amino-N-((1R,2R)-1-hydroxy-1-phenylpropan-2-yl)-N-methylpent-4-enamide (89). n-BuLi (20.4 mL of 2.3 M in hexanes) was added dropwise to a stirred suspension of lithium chloride (3.85 g, 91 mmol) and diisopropylamine (6.8 mL, 48.4 mmol) in dry THF (92 mL) at -78 °C. After stirring for 10 minutes a solution of 88 (5 g, 15.12 mmol) in dry THF (87 mL) was added via cannula. The resulting yellow solution was stirred for 10 minutes at -78 °C, transferred to an ice bath and stirred at 0 °C for 20 minutes. Allyl bromide (2.1 mL, 23.3 mmol) was added dropwise and the reaction mixture was stirred for a further 2 h. The reaction was terminated by the addition of 1 M aqueous HCl. The phases were separated and the aqueous layer was extracted into ethyl acetate. The combined organic phases were washed with NaHCO₃, dried over Na₂SO₄ and the solvent was removed under reduced pressure. Flash chromatography of the residue (petroleum ether-EtOAc, 1:1) gave 89 (4.5 g, 82%) as a yellow oil. The ¹H and ¹³C NMR data for 89 were in good agreement with those previously reported in the literature⁴; ¹H NMR (500 MHz, 2:1 ratio of rotamers, the asterisk denotes signals of the minor rotamer, CDCl₃) δ 7.40 – 7.27 (5H, m, Ar-H), 5.83* (1H, ddt, J 17.2, 10.0, 7.1, CH=CH₂), 5.69 (1H, ddt, J 17.2, 10.0, 7.2, CH=CH₂), 5.31 (1H, d, J 8.3), 5.13 – 5.06 (2H, m), 4.73* (1H, m), 4.59 (3H, m), 4.20 (1H, m), 2.96 (3H, s, NMe), 2.62 (1H, m), 2.47 (1H, m), 2.31 (2H, m), 1.43 (9H, s), 1.41* (9H, s), 1.06 (3H, d, J 6.9), 0.99* (3H, d, J 6.7); ¹³C NMR (125 MHz, CDCl₃) δ 173.8, 172.6*, 155.7, 155.4*, 141.0, 141.2*, 133.9*, 132.8, 129.0, 128.8*, 128.6, 128.0, 127.1*, 126.7, 118.8, 118.2*, 80.0, 79.7*, 75.9, 75.6*, 58.1, 50.8, 50.1*, 37.6*, 37.3, 28.5, 27.2*, 15.7*, 14.6; ESI-HRMS calcd. for C₂₀H₃₀N₂O₄Na 385.2103, found m/z 385.2108 [M+Na]⁺.
(S)-\textit{t}-Butyl-5-oxodocos-1-en-4-ylcarbamate (90). 1-Heptadecanol (14 g, 55 mmol) and triethylamine (23 mL, 168 mmol) were dissolved in dry CH$_2$Cl$_2$ (110 mL) and cooled to 0 °C. To this was added methanesulfonyl chloride (5.1 mL, 65 mmol) dropwise. The solution was warmed to room temperature and stirred for 4 h. The reaction was diluted with water and washed with 1 M aqueous HCl. The organic phase was washed with brine, dried over Na$_2$SO$_4$ and the solvent was removed under reduced pressure. The crude mesylate was dissolved in dry acetone (140 mL) and NaI (41 g, 274 mmol) was added and the reaction was heated at reflux overnight. H$_2$O and EtOAc were added and the phases separated. The organic phase was washed with water, brine, dried over Na$_2$SO$_4$ and the solvent was removed under reduced pressure. Flash chromatography of the crude residue (petroleum ether) gave 1-iodoheptadecanol (16.7 g, 83%) as a white solid; $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 3.19 (2H, t, $J$ 7.1, CH$_2$I), 1.89 – 1.76 (2H, m, CH$_3$), 1.45 – 1.33 (2H, m, CH$_2$), 1.26 (26H, s, each CH$_2$), 0.88 (3H, t, $J$ 6.9, CH$_3$); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 33.6, 31.9, 30.5, 29.7 (2s), 29.6, 29.5, 29.4, 28.5, 22.7 (each CH$_2$), 14.1 (CH$_3$), 7.3 (CH$_2$I). To a stirred solution of the above alkyl iodide (6.1 g, 16.5 mol) in pentane-Et$_2$O (33 mL, 3:2) at -78 °C was added $t$-BuLi (23 mL, 1.6 M solution in hexane) dropwise. The solution was stirred for 10 minutes at -78 °C, warmed to room temperature and stirred for 1 h. The resulting suspension was added via cannula to a stirred solution of 89 (2 g, 5.51 mmol) in dry THF (15 mL) at -78 °C. After 10 minutes at -78 °C the reaction flask was transferred to an ice bath and stirred for 2 h. The reaction mixture was poured slowly onto a mixture of crushed ice and NH$_4$Cl and extracted into EtOAc. The combined organics were dried over Na$_2$SO$_4$ and the solvent was removed under reduced pressure. Flash chromatography of the residue (petroleum ether-EtOAc 15:1) gave 90 (1.2 g, 50%) as a white solid. The $^1$H and $^{13}$C NMR data for 90 were in good agreement with those previously reported in the literature$^4$; IR (film) cm$^{-1}$: 3337, 2916, 2848, 1723, 1691, 1523, 1472, 1322, 1169 cm$^{-1}$; $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 5.70 – 5.60 (1H, m, H-2), 5.21 (1H, d, $J$ 7.6, NH), 5.11 (2H, d, $J$ 13.7, H-1), 4.36 (1H, q, $J$ 6.4 , H-4), 2.64 – 2.54 (1H, m, H-3a), 2.48 (2H, td, $J$ 7.4, 3.6, H-6), 2.42 – 2.33 (1H, m, H-3b), 1.62 – 1.55 (2H, m, H-7), 1.43 (9H, s, Boc), 1.25 (28H, s, each CH$_2$), 0.88 (3H, t, $J$ 6.9, CH$_3$); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 208.8 (C=O), 155.3 (C=O), 132.4 (C-2), 118.9 (C-1), 79.7 (t-Bu), 58.6 (C-4), 40.0 (C-6), 36.0 (C-3), 31.9, 29.7 (2s), 29.6 (2s), 29.4, (2s), 29.3, 29.2 (each CH$_2$), 28.3 (t-
Bu), 23.4, 22.7 (each CH₂), 14.1 (CH₃); ESI-HRMS calcd. for C₂₇H₅₁NO₃Na 460.3767, found m/z 460.3780 [M+Na]⁺.

(4S,5R)-t-Butyl-4-allyl-5-heptadecyl-2,2-dimethyloxazolidine-3-carboxylate (91). Ketone 90 (575 mg, 1.31 mmol) was taken up in dry EtOH (10 mL) and cooled to -78 °C. LiAl(O-t-Bu)₃H (2 g, 8 mmol) was added portion-wise over 1 h. After stirring for 24 h at -78 °C the reaction was diluted with CH₂Cl₂ (15 mL) and 10% citric acid (18 mL). The mixture was stirred at room temperature for 2 h and extracted into CH₂Cl₂. The combined organic layers were washed with water, brine, dried over Na₂SO₄ and the solvent was removed under reduced pressure. Flash chromatography of the residue (petroleum ether-EtOAc 6:1) gave t-Butyl-(4S,5R)-5-hydroxydocos-1-en-4-ylcarbamate (395 mg, 69%) as a white solid. The ¹H and ¹³C NMR data for the anti-amino alcohol were in good agreement with those previously reported in the literature⁴; IR (film) cm⁻¹: 3344, 2917, 2849, 1684, 1525, 1466, 1251, 1171, 1015; ¹H NMR (500 MHz, CDCl₃) δ 5.86 – 5.77 (1H, m, H-2), 5.15 – 5.04 (2H, m, H-1), 4.66 (1H, d, J 7.1, -NH-), 3.65 (2H, overlapping signals, H-4 & H-5), 2.36 – 2.28 (1H, m, H-3a), 2.18 (1H, m, H-3b), 1.72 (1H, brs, OH), 1.44 (11H, overlapping signals, CH₂ & t-Bu), 1.25 (30H, s, each CH₂), 0.88 (3H, t, J 6.9, CH₃); ¹³C NMR (125 MHz, CDCl₃) δ 156.3 (C=O), 135.0 (CH=CH₂), 117.6 (CH=CH₂), 79.6 (t-Bu), 74.3, 54.8 (-CH-), 34.1(CH₂-CH=CH₂), 33.3, 32.0, 29.7 (3s), 29.6 (3s) (each CH₂), 28.4 (t-Bu), 26.0, 22.7 (each CH₂), 14.1(CH₃); ESI-HRMS calcd. for C₂₇H₅₃NO₃Na 462.3923, found m/z 460.3916 [M+Na]⁺.

The above amino alcohol (426 mg, 0.97 mmol) was taken up in dry toluene (2 mL). To this was added 2,2-dimethoxypropane (0.3 mL, 2.4 mmol) and p-toluenesulfonylic acid (3 mg). The resulting suspension was stirred at 85 °C for 6 h. Upon cooling the reaction was neutralised with solid NaHCO₃ (4 mg) and the solvent was removed under reduced pressure. Flash chromatography of the residue (petroleum ether-EtOAc 50:1) gave 91 (420 mg, 90%) as a colourless oil. The ¹H and ¹³C NMR data for 91 were in good agreement with those previously reported in the literature⁴; IR (film) cm⁻¹: 2923, 2854, 1698, 1384, 1365, 1255, 1178, 1075; ¹H NMR (500 MHz, CDCl₃) δ 5.77 (1H, ddt, J 17.3, 10.1, 7.3, H-2), 5.03 – 4.89 (2H, m, H-1), 3.99 – 3.87 (1H, m, H-5), 3.75 (1H, q, J 5.8, H-4), 2.39 – 2.25 (1H, m, H-3a), 2.14 (1H, m, H-3b), 1.72 (1H, brs, OH), 1.44 (11H, overlapping signals, CH₂ & t-Bu), 1.25 (30H, s, each CH₂), 0.88 (3H, t, J 6.9, CH₃); ¹³C NMR (125 MHz, CDCl₃) δ 156.3 (C=O), 135.0 (CH=CH₂), 117.6 (CH=CH₂), 79.6 (t-Bu), 74.3, 54.8 (-CH-), 34.1(CH₂-CH=CH₂), 33.3, 32.0, 29.7 (3s), 29.6 (3s) (each CH₂), 28.4 (t-Bu), 26.0, 22.7 (each CH₂), 14.1(CH₃); ESI-HRMS calcd. for C₂₇H₅₃NO₃Na 462.3923, found m/z 460.3916 [M+Na]⁺.
2.23 – 2.08 (1H, m, H-3b), 1.51 (6H, s), 1.47 (1H, s), 1.45 (1H, s), 1.39 (9H, s, t-Bu), 1.19 (30H, s, each CH₂), 0.81 (3H, t, J 6.9, CH₃); ¹³C NMR (125 MHz, CDCl₃) δ 152.3*, 151.7 (C=O), 135.8 (CH=CH₂), 116.7 (CH=CH₂), 116.4*, 92.5, 92.1*, 79.8*, 79.4 (t-Bu), 77.2 (CHO), 59.0 (CHN), 58.8*, 34.8, 34.4* (CH₂-CH=CH₂), 31.9, 29.7 (2s), 29.6 (2s), 29.5, 29.4, 29.1, 29.0 (each CH₂), 28.5, 28.4, 27.8, 27.0, 26.4 (2s), 24.9, 23.6 (each CH₃), 22.7 (CH₂), 14.1 (CH₃); ESI-HRMS calcd. for C₃₀H₅₇NO₃Na 502.4236, found m/z 502.4213 [M+Na]⁺.

(4S,5R)-t-Butyl-5-heptadecyl-2,2-dimethyl-4-(prop-1-enyl)oxazolidine-3-carboxylate (92). Oxazolidine 91 (0.42 g, 0.86 mmol) was taken up in methanol (12 mL) and the Grubbs II catalyst (0.11 g, 0.13 mmol) was added. The reaction mixture was placed in a pre-heated oil bath at 60 °C and stirred for 12 h. Upon cooling the solvent was removed under reduced pressure and the crude residue was purified by flask chromatography (petroleum ether-EtOAc 50:1) to give 92 (0.35 g, 83%) as a colourless oil. The ¹H and ¹³C NMR data for 92 were in good agreement with those previously reported in the literature⁴; IR (film) cm⁻¹: 2923, 2854, 1698, 1456, 1383, 1375, 1364, 1251, 1178, 1073; ¹H NMR (500 MHz, CDCl₃, 1:1 E,Z mixture) δ 5.57 (1H, dq, J 13.1, 6.3, H-2), 5.47 (1H, dq, J 13.1, 6.4, H-3), 5.26 (1H, dd, J 8.8, 1.7), 5.23 (1H, dd, J 8.8, 1.7), 4.17 (1H, t, J 6.9), 4.00 (1H, dd, J 9.1, 5.0), 3.89 (2H, q, J 6.2), 1.65 (3H, d, J 1.6, CH₃), 1.64 (3H, d, J 1.7, CH₃), 1.55 (2H, s), 1.50 (2H, s), 1.44 (5H, s), 1.41 (7H, s), 1.34 (9H, s), 1.18 (51H, s), 0.81 (6H, t, J 6.9, each CH₃); ¹³C NMR (125 MHz, CDCl₃) δ 151.9, 129.0*, 128.8, 126.6*, 126.3, 92.7, 92.3*, 79.8*, 79.1, 76.8, 62.6, 62.2*, 56.56, 31.9, 29.7 (3s), 29.6 (2s), 29.5, 29.4, 29.2, 28.5, 27.3, 25.8, 25.6, 24.9, 23.8, 22.7, 17.8*, 17.7, 14.1; ESI-HRMS calcd. for C₃₀H₅₇NO₃Na 502.4236, found m/z 502.4235 [M+Na]⁺.
To a solution of Alkene 92 (194 mg, 0.41 mmol) in 10:1 acetone-water (3.9 mL) were added, 2,6-lutidine (0.1 mL, 0.82 mmol), 4-methylmorpholine-N-oxide (72 mg, 0.615 mmol) and a catalytic amount of osmium tetroxide (0.1 mL, 2 mol%). The solution was stirred vigorously for 2 days. Then PhI(OAc)$_2$ (264 mg, 0.82 mmol) was added and the solution stirred for a further 3 h. The reaction was quenched with aqueous sodium thiosulphate and extracted into EtOAc. The combined organics were washed with water, brine, dried over Na$_2$SO$_4$ and the solvent removed under reduced pressure. Flash chromatography of the residue (petroleum ether-EtOAc 30:1) gave 107 (117 mg, 61%) as a colourless oil; IR (film) cm$^{-1}$: 2923, 2854, 1738, 1712, 1466, 1366, 1256, 1176, 1112; $^1$H NMR (500 MHz, 3:2 rotamer ratio, the asterisk denotes the signals of the minor rotamer, CDCl$_3$) $\delta$ 9.51* (1H, d, J 3.0, H-1), 9.44 (1H, d, J 4.0, H-1), 4.19 – 4.11 (1H, m, H-3), 3.99 (1H, dd, J 6.6, 3.9, H-2), 1.66 (3H, s, CH$_3$), 1.60* (3H, s, CH$_3$), 1.50 (5H, s, overlapping signals, CH$_2$ & CH$_3$), 1.46* (3H, s, CH$_3$), 1.43* (9H, s, t-Bu), 1.33 (9H, s, t-Bu), 1.19 (48H, s, each CH$_2$ & CH$_2$*), 0.81 (5H, t, J 6.9, each CH$_3$ & CH$_3$*); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 200.2, 200.0* (C=O), 152.5*, 151.4 (C=O), 94.4, 93.7*, 81.1*, 80.8 (t-Bu), 77.2*, 76.4 (CHO), 67.6, 67.5* (CHN), 31.9, 29.8, 29.7 (3s), 29.6, 29.5, 29.4 (3s) (each CH$_2$), 28.3*, 28.3, 27.5*, 26.7, 26.2*, 26.1, 24.8*, 23.8 (each CH$_3$), 22.7 (CH$_2$), 14.1 (CH$_3$); ESI-HRMS calcd. for C$_{28}$H$_{53}$NO$_4$Na 490.3872, found m/z 490.3889 [M+Na]$^+$. 

Under an argon atmosphere, Aldehyde 107 (111 mg, 0.24 mmol) and Bestmann reagent 169 (0.1 mL, 0.72 mmol) was taken up in dry methanol (1.1 mL) and cooled to 0 °C. K$_2$CO$_3$ (133 mg, 0.96 mmol) was added in one portion. The reaction mixture was stirred at 0 °C for 1 h, warmed to room temperature and stirred for a further 16 h. The
reaction was diluted with satd. NH₄Cl and extracted into EtOAc. The combined organic layers were dried over Na₂SO₄ and the solvent was removed under reduced pressure. Flash chromatography of the residue (petroleum ether-EtOAc 30:1) gave 170 (96 mg, 87%) as a colourless oil; [α]D +23 (c 1, CHCl₃); IR (film) cm⁻¹: 3313, 2923, 2854, 1704, 1457, 1375, 1365, 1246, 1176, 1142, 1089; ¹H NMR (500 MHz, 2.5:2 rotamer ratio, the asterisk denotes the signals of the minor rotamer, CDCl₃) δ 4.50* (1H, dd, J 5.1, 2.1, H-2), 4.35 (1H, dd, J 5.1, 2.1, H-2), 3.91 – 3.86 (2H, m, overlapping signals, H-3* & H-3), 2.24* (1H, d, J 2.2, H-1), 2.22 (1H, d, J 2.1, H-1), 1.77 – 1.64 (4H, m), 1.57 (3H, s, CH₃), 1.53* (3H, s, CH₃), 1.49* (3H, s, CH₃), 1.44 (3H, s, CH₃), 1.43* (9H, s, t-Bu), 1.42 (9H, s, t-Bu), 1.19 (60H, s, each CH₂ & CH₃*), 0.81 (6H, t, J 6.9, CH₃ & CH₃*); ¹³C NMR (125 MHz, CDCl₃) δ 151.8,* 151.3 (C=O), 93.8, 93.3*, 80.8*, 80.2 (t-Bu), 79.9, 79.5* (-C≡CH), 76.1, 75.8* (CHO), 72.8*, 72.3 (-C≡CH), 52.2, 52.0* (CHN), 31.9, 30.3, 30.1, 29.7 (3s), 29.6, 29.5, 29.4 (2s) (each CH₂), 28.4, 27.5*, 26.5, 25.6, 25.5*, 25.3*, 24.4 (each CH₃), 22.7 (CH₂), 14.1 (CH₃); ESI-HRMS calcd. for C₂₉H₅₃NO₃Na 486.3923, found m/z 486.3945 [M+Na].

2,5-Dioxopyrrolidin-1-yl nonadecanoate (96). N-hydroxysuccinimide (0.27 g, 2.34 mmol) and EDC (0.45 g, 2.34 mmol) were added to a solution of nonadecanoic acid (0.7 g, 2.34 mmol) in CH₂Cl₂ (46 mL). After stirring the mixture overnight, the solvent was concentrated under reduced pressure and the resulting residue was dissolved in CH₂Cl₂. The solution was washed with water, dried over Na₂SO₄ and the solvent was removed under reduced pressure to give 96 (0.84 g, 90%) as a white solid. The compound was used without further purification. The ¹H and ¹³C NMR data for 96 were in good agreement with those previously reported in the literature⁴; ¹H NMR (500 MHz, CDCl₃) δ 2.83 (4H, s, O=CCH₂CH₂C=O), 2.60 (2H, t, J 7.5, CH₃C=O), 1.74 (2H, m, CH₂), 1.45 – 1.35 (2H, m, CH₂), 1.25 (29H, s, each CH₂), 0.88 (3H, t, J 6.9, CH₃); ¹³C NMR (125 MHz, CDCl₃) δ 169.1, 168.7 (each C=O), 31.9, 31.0, 29.7 (2s), 29.6 (2s), 29.4, 29.1, 28.8, 25.6, 24.6, 22.7 (each CH₂), 14.1 (CH₃).
N-((3S,4R)-4-Hydroxyhenicos-1-yn-3-yl)nonadecanamide (106). Alkyne 170 (90 mg, 0.19 mmol) was taken up in formic acid (6 mL) and stirred vigorously for 2 h. Toluene (15 mL) was added and the solvents were removed under reduced pressure. The resulting residue was taken up in water and basified with solid NaHCO₃, extracted into CH₂Cl₂, dried over Na₂SO₄ and the solvent was removed under reduced pressure. The resulting residue was taken up in CH₂Cl₂ (6 mL) and DIPEA (0.12 mL, 0.68 mmol) was added. To this was added a solution of 96 (192 mg, 0.49 mmol) in CH₂Cl₂ (3 mL) and the mixture stirred for 24 h. Upon completion the reaction mixture was diluted with CH₂Cl₂, washed with satd. NaHCO₃, and the aqueous layers extracted into CH₂Cl₂. The combined organics were washed with water, brine, dried over dried over Na₂SO₄ and solvent removed under reduced pressure. Flash chromatography of the residue (petroleum ether-EtOAc 4:1) gave 106 (83 mg, 71% over two steps) as a white solid; [α]D +5.5 (c 0.8, CHCl₃); Rf 0.3 (cyclohexane EtOAc 3:1); mp 86-90 °C; IR (film) cm⁻¹: 3341 br, 3228, 2917, 2849, 1631, 1532, 1472; ¹H NMR (500 MHz, CDCl₃) δ 6.07 (1H, d, J 8.5, -NH-), 4.81 (1H, dt, J 8.5, 2.6, H-3), 3.62 (1H, td, J 6.7, 2.8, H-4), 2.30 (1H, d, J 2.4, H-1), 2.20 (2H, t, J 7.7, CH₂), 1.67-1.56 (4H, m, each CH₂), 1.53-1.42 (1H, m, CH), 1.34-1.21 (59H, m, each CH₂), 0.88 (6H, t, J 6.7, each CH₃); ¹³C NMR (125 MHz, CDCl₃) δ 172.6 (C=O), 79.7 (-C≡CH), 73.8 (C-4), 73.3 (-C≡CH), 46.9 (C-3), 36.8, 34.5, 32.1, 29.9 (2s), 29.83, 29.8, 29.7, 29.6 (2s), 29.5 (2s), 29.4, 25.7 (2s), 22.9 (each CH₂), 14.3 (CH₃); ESI-HRMS calcd. for C₄₀H₇₈NO₂ 604.6033, found m/z 604.6025 [M+H]+.

1-(5-(S)-methoxycarbonyl-2,3,4-tri-O-acetyl-β-l-arabinopyranosyl)-5-(3-methoxyphenyl)-1H-1,2,3-triazole (172). 3-Ethynylanisole (25 µL, 0.2 mmol) was dissolved in N, N-dimethylacetamide (1.5 mL). After addition of azide 104 (144 mg, 0.4
mmol) and Cp*RuCl(COD) (5 mg, 0.012 mmol, 6 mol%), the reaction mixture was placed in a microwave reactor for 30 min at 100 °C and 120 W. The resulting solution was diluted with EtOAc and washed with H₂O (x3). The organic layer was dried over Na₂SO₄ and the solvent was removed under reduced pressure. Flash chromatography of the resulting residue (petroleum ether-EtOAc 1:1) gave 172 (61 mg, 62%) as a white solid; ¹H NMR (500 MHz, CDCl₃) δ 7.75 (1H, s, triazole-H), 7.40 (2H, t, J 8.0, Ar-H), 7.03 (1H, ddd, J 8.4, 2.6, 0.9, Ar-H), 6.96 (1H, ddd, J 7.6, 1.7, 0.9, Ar-H), 6.92 (1H, dd, J 2.6, 1.6, Ar-H), 6.47 (1H, d, J 6.3, H-1), 6.42 (1H, dd, J 10.7, 3.6, H-3), 6.01 (1H, dd, J 3.7, 1.4, H-4), 5.59 (1H, dd, J 10.7, 6.3, H-2), 5.14 (1H, d, J 1.3, H-5), 3.85 (3H, s, OMe), 3.73 (3H, s, OMe), 2.13 (3H, s, OAc), 2.01 (3H, s, OAc); ¹³C NMR (125 MHz, CDCl₃) δ 169.9, 169.6, 169.4, 166.6 (each C=O), 160.1 (Ar-C), 140.3 (triazole C), 132.5 (triazole CH), 130.6, 126.7, 121.1, 115.7, 114.6 (each Ar-C), 79.1 (C-1), 71.6 (C-5), 68.9 (C-4), 67.8 (C-3), 66.5 (C-2), 55.4 (OMe), 52.7 (OMe), 20.6, 20.5, 20.3 (each CH₃); ESI-HRMS calcd. for C₂₂H₂₅N₃O₁₀Na 514.1438, found m/z 514.1420 [M+Na⁺].

1,4-bis(1-(5-(S)-methoxycarbonyl-2,3,4-tri-O-acetyl-β-L-arabinopyranosyl)-1H-1,2,3-triazol-5-yl)benzene (173). 1,4-Diethynylbenzene (12.6 mg, 0.1 mmol) was dissolved in N,N-dimethylacetamide (1.5 mL). After addition of azide 104 (144 mg, 0.4 mmol) and Cp*RuCl(COD) (4.5 mg, 0.012 mmol, 12 mol%), the reaction mixture was placed in a microwave reactor for 1 h at 100 °C and 120 W. The resulting solution was diluted with EtOAc and washed with H₂O (x3). The organic layer was dried over Na₂SO₄ and the solvent was removed under reduced pressure. Flash chromatography of the resulting residue (petroleum ether-EtOAc 1:3) gave 173 (53 mg, 63%) as a white solid; ¹H NMR (500 MHz, CDCl₃) δ 7.84 (2H, s, triazole-H), 7.58 (4H, s, each Ar-H), 6.48 (2H, d, J 6.3, H-1), 6.45 (2H, dd, J 10.7, 3.7, H-3), 6.02 (2H, dd, J 3.7, 1.3, H-4), 5.59 (2H, dd, J 10.7, 6.3, H-2), 5.08 (2H, d, J 1.3, H-5), 3.76 (6H, s, OMe), 2.14 (6H, s), 2.03 (6H, s), 1.87 (6H, s)(each OAc); ¹³C NMR (125 MHz, CDCl₃) δ 170.0, 169.6, 169.4, 166.5 (each C=O), 139.2 (triazole C), 133.0 (triazole CH), 130.0, 127.5 (each Ar-C), 79.3 (C-1), 71.5 (C-5), 68.8 (C-4), 67.8 (C-3), 66.6
(C-2), 52.9 (OMe), 20.6, 20.5, 20.3 (each OAc); ESI-HRMS calcd. for C_{36}H_{40}N_{6}O_{18}Na 867.2297, found m/z 867.2285 [M+Na]^+.

1-(5-(S)-methoxycarbonyl-2,3,4-tri-O-acetyl-β-L-arabinopyranosyl)-4-(3-methoxyphenyl)-1H-1,2,3-triazole (176). 3-Ethynylanisole (18 µL, 0.14 mmol) was dissolved in N, N-dimethylformamide (1.5 mL). After addition of azide 104 (50 mg, 0.14 mmol), copper(I) iodide (5.3 mg, 0.025 mmol, 20 mol%) and N,N-diisopropylethylamine (48 µL), the reaction mixture was placed in a microwave reactor for 60 min, at 80 °C and 120 W. The resulting solution was diluted with EtOAc and washed with H_{2}O (x3). The organic layer was dried over Na_{2}SO_{4} and the solvent was removed under reduced pressure. Flash chromatography of the residue (petroleum ether-EtOAc 2:1) gave 176 (45 mg, 65%) as a white solid; ^1H NMR (500 MHz, CDCl_{3}) δ 7.85 (1H, s, triazole-H), 7.45 – 7.41 (1H, m, Ar-H), 7.37 – 7.31 (2H, m, each Ar-H), 6.90 (1H, dt, J 6.8, 2.5, Ar-H), 6.60 (1H, d, J 6.0, H-1), 6.29 (1H, dd, J 10.7, 3.6, H-3), 5.99 (1H, dd, J 3.6, 1.5, H-4), 5.56 (1H, dd, J 10.7, 6.0, H-2), 5.14 (1H, d, J 1.4, H-5), 3.86 (3H, s, OMe), 3.72 (3H, s, OMe), 2.15 (3H, s), 2.02 (3H, s), 1.86 (3H, s)(each OAc); ^13C NMR (125 MHz, CDCl_{3}) ^13C NMR (125 MHz, CDCl_{3}) δ 170.4, 169.5, 169.3, 166.5 (each C=O), 160.1 (Ar-C), 147.1 (triazole C), 130.8, 130.0 (each Ar-C), 122.1 (triazole CH), 118.2, 114.7, 110.9 (each Ar-C), 81.7 (C-1), 72.0 (C-5), 68.7 (C-4), 67.4 (C-3), 66.9 (C-2), 55.3 (OMe), 52.8 (OMe), 20.6, 20.5, 20.4 (each OAc); ESI-HRMS calcd. for C_{22}H_{25}N_{5}O_{10}Na 514.1438, found m/z 514.1424 [M+Na]^+. 
1,4-bis((1-(5-(S)-methoxycarbonyl-2,3,4-tri-O-acetyl-β-L-arabinopyranosyl)-1H-1,2,3-triazol-4-yl)methoxy)benzene (177). 1,4-Bis(2-propynyloxy)benzene (19 mg, 0.1 mmol) was dissolved in N, N-dimethylformamide (0.6 mL). After addition of azide 104 (72 mg, 0.2 mmol), copper(I) iodide (4 mg, 0.02 mmol, 10 mol%) and N,N-diisopropylethylamine (34 µL), the reaction mixture was placed in a microwave reactor for 60 min, at 80 °C and 120 W. The resulting solution was diluted with EtOAc and washed with H₂O (x3). The organic layer was dried over Na₂SO₄ and the solvent was removed under reduced pressure. Flash chromatography of the residue (petroleum ether-EtOAc 2:1) gave 177 (76 mg, 84%) as a white solid; ¹H NMR (500 MHz, CDCl₃) δ 7.67 (2H, s, triazole-H), 6.89 (4H, s, Ar-H), 6.54 (2H, d, J 6.0, H-1), 6.20 (2H, dd, J 10.7, 3.5, H-3), 5.98 (2H, dd, J 3.6, 1.6, H-4), 5.52 (2H, dd, J 10.7, 6.0, H-2), 5.18 (4H, s, CH₂), 5.14 (2H, d, J 1.5, H-5), 3.73 (6H, s, OMe), 2.15 (6H, s), 2.02 (6H, s), 1.80 (6H, s)(each OAc); ¹³C NMR (125 MHz, CDCl₃) δ 170.3, 169.5, 169.3, 166.5 (each C=O), 152.6 (Ar-C), 144.0 (triazole C), 125.1 (triazole CH), 115.8 (Ar-C), 81.7 (C-1), 72.1 (C-5), 68.6 (C-4), 67.3 (C-3), 66.8 (C-2), 62.3 (CH₂), 52.8 (OMe), 20.6, 20.5, 20.2 (each OAc); ESI-HRMS calcd. for C₃₈H₄₅N₆O₂₀Na 905.2689, found m/z 905.2669 [M+H]⁺.
N-((1S,2R)-2-Hydroxy-1-(1-(5-(S)-methoxycarbonyl-2,3,4-tri-O-acetyl-β-1-arabinopyranosyl)-1H-1,2,3-triazol-4-yl)nonadecyl)nonadecanamide (102). Alkyne 106 (15 mg, 0.025 mmol) was dissolved in N, N-dimethylformamide (1.5 mL). After addition of azide 104 (14 mg, 0.038 mmol), copper(I) iodide (1 mg, 0.005 mmol) and N,N-diisopropylethylamine (9 µL), the reaction mixture was placed in a microwave reactor for 60 min, at 80 °C and 120 W. The resulting solution was diluted with EtOAc and washed with H₂O (3 x 10 mL). The organic layer was dried over Na₂SO₄ and the solvent was removed under reduced pressure. Flash chromatography of the residue (cyclohexane-EtOAc 2:1) gave 102 (15.5 mg, 65%) as a white solid; [α]D +58.4 (c 1.3, CHCl₃); Rf 0.3 (cyclohexane-EtOAc 1:1); IR (film) cm⁻¹: 3298 br, 2918, 2850, 1752, 1651, 1467, 1371, 1215, 1072, 720; ¹H NMR (500 MHz, CDCl₃) δ 7.68 (1H, brs, triazole-H), δ 6.65 (1H, d, J 7.9, -NH-), δ 6.54 (1H, d, J 5.9, H-1), δ 6.13 (1H, dd, J 10.6, 3.5, H-3), δ 5.97 (1H, dd, J 3.5, 1.6, H-4), δ 5.52 (1H, dd, J 10.5, 5.9, H-2), δ 5.19 (1H, d, J 1.6, H-5), δ 5.09 (1H, dd, J 8.0, 3.1, H-1’), δ 3.87 (1H, brs, H-2’), δ 3.74 (3H, s, OMe), δ 2.18 (2H, t, J 7.7, CH₂), δ 2.15, 2.02 (3H, s) (each OAc), δ 1.91 (1H, brs, OH) δ 1.84 (3H, s, OAc), δ 1.67-1.52 (2H, m, CH₂), δ 1.51-1.44 (1H, m, CH), δ 1.37-1.16 (60H, m, each CH₂), δ 0.88 (6H, t, J 6.8, each CH₃); ¹³C NMR (125 MHz, CDCl₃) δ 172.8, 169.2, 169.5, 169.4, 166.5 (each C=O), 145.0 (triazole C), 125.6 (triazole CH), 81.7 (C-1), 74.4 (C-2’), 72.3 (C-5), 68.5 (C-4), 67.2 (C-3), 66.8 (C-2), 52.8 (OMe), 48.8 (C-1’), 36.6, 34.6, 31.9, 29.7 (2s), 29.62 (3s), 29.5, 29.5 (2s), 29.3 (2s), 25.8, 25.5, 22.7 (each CH₃), 20.6, 20.5, 20.2, 14.1 (each CH₃); ESI-HRMS calcd. for C₅₃H₉₃N₄O₁₁ 961.6841, found m/z 961.6847 [M-H]⁻.
N-((1S,2R)-2-Hydroxy-1-(1-(2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl)-1H-1,2,3-triazol-4-yl)nonadecyl)nonadecanamide (103). Alkyne 106 (10 mg, 0.017 mmol) was dissolved in N, N-dimethylformamide (0.5 mL). After addition of azide 105 (9.3 mg, 0.025 mmol), copper(I) iodide (1 mg, 0.005 mmol) and N,N-diisopropylethylamine (9 µL), the reaction mixture was placed in a microwave reactor for 60 min, at 80 °C and 120 W. The resulting solution was diluted with EtOAc and washed with H₂O (3 x 10 mL). The organic layer was dried over Na₂SO₄ and the solvent was removed under reduced pressure. Flash chromatography of the residue (cyclohexane-EtOAc 2:1) gave 103 (11 mg, 68%) as a white solid; [α]₀ +44.8 (c 0.9, CHCl₃); Rₓ 0.4 (cyclohexane-EtOAc 1:1); IR (film) cm⁻¹: 3337 br, 2917, 2850, 1747, 1631, 1530, 1371, 1217, 1070, 915, 729; ¹H NMR (500 MHz, CDCl₃) δ 7.65 (1H, s, triazole-H), 6.67 (1H, d, J 8.0, -NH-), 6.38 (1H, d, J 6.0, H-1), 6.10 (1H, dd, J 10.7, 3.5, H-3), 5.66 (1H, dd, J 3.4, 1.2, H-4), 5.48 (1H, dd, J 10.7, 6.0, H-2), 5.08 (1H, dd, J 8.0, 3.2, H-1'), 4.69 (1H, td, J 6.6, 1.3, H-5), 4.11 (1H, dd, J 11.4, 6.8, H-6a), 4.05 (1H, dd, J 11.4, 6.4, H-6b), 3.87 (1H, ddt, J 9.8, 7.1, 4.1, H-2'), 3.49 (1H, d, J 10.1, OH), 2.20 – 2.16 (5H, overlapping signals, CH₂ & OAc), 2.01 (3H, s), 2.00 (3H, s), 1.83 (3H, s)(each OAc), 1.59 (2H, s, CH₂), 1.51 – 1.45 (1H, m, CH), 1.33 – 1.18 (61H, m, each CH₂), 0.88 (6H, t, J 6.9, each CH₃); ¹³C NMR (125 MHz, CDCl₃) δ 172.9, 170.3, 170.1, 170.0, 169.5 (each C=O), 144.8 (triazole, CH=C), 125.5 (triazole, CH=C), 81.9 (C-1), 74.4 (C-2′), 70.7 (C-5), 67.6 (C-3), 67.4 (C-4), 67.2 (C-2), 61.1 (C-6), 48.7 (C-1′), 36.6, 34.6, 31.9, 29.7 (3s), 29.6 (3s), 29.5, 29.4, 29.3 (2s), 25.8, 25.5, 22.7 (each CH₂), 20.6 (3s), 20.2. 14.1 (each CH₃); ESI-HRMS calcd. for C₅₄H₉₅N₄O₁₁ 975.6997, found m/z 975.7020 [M-H]⁻
N-((1S,2R)-2-Hydroxy-1-(1-(5-(S)-hydroxycarbonyl-β-L-arabinopyranosyl)-1H-1,2,3-triazol-4-yl)nonadecyl)nonadecanamide (AMD292). Protected lipid 102 (13.6 mg, 14 µmol) was dissolved in n-PrOH (3.4 mL) and H₂O₂ (30%, 0.34 mL). To this was added n-PrONa/n-PrOH (0.1 N, 6 eq, 84.6 µmol, 846 µL) dropwise at a rate of 100 µL/h. After final addition the reaction was stirred for a further 1 h. Water (9 mL) was added and the solution centrifuged at 15000 rpm for 15 min. The supernatant was removed and the precipitate treated a further two times with water. The precipitate was lyophilised to give AMD292 (6.6 mg, 57%) as a white solid; ¹H NMR (600 MHz, CD₃CO₂D/DMSO-D₆, 60°C) δ 7.92 (1H, s, triazole-H), 6.22 (1H, d, J 5.2, H-1), 4.99 (1H, d, J 5.9 Hz, H-1’), 4.51 (1H, d, J 2.3 Hz, H-5), 4.27 – 4.23 (2H, overlapping signals H-3 & H-4), 4.14 (1H, dd, J 8.7, 5.2, H-2), 3.70 (1H, p, J 4.4, 3.8, H-2’), 2.14 – 2.05 (2H, m, CH₂), 1.49 – 1.41 (2H, m, CH₂), 1.39 – 1.31 (1H, m, CH), 1.16 (61H, s, each CH₂), 0.76 (6H, t, J 6.9, each CH₃); ¹³C NMR (150 MHz, CD₃CO₂D/DMSO-D₆, 60°C) δ 174.3, 170.9 (each C=O), 146.0 (triazole, CH=C), 126.3 (triazole, CH=C), 85.7 (C-1), 74.6 (C-5), 73.6 (C-2’), 70.6 (C-3 or C-4), 70.2 (C-3 or C-4), 68.5 (C-2), 51.3 (C-1’), 36.7, 34.4, 32.5, 30.2 (4s) , 30.1, 26.5, 26.2, 23.2 (each CH₂), 14.6 (CH₃); ESI-HRMS calcd. for C₄₆H₈₅N₄O₈ 821.6367, found m/z 821.6379 [M-H]⁻.
**N-((1S,2R)-2-Hydroxy-1-(1-α-D-galactopyranosyl)-1H-1,2,3-triazol-4-yl)nonadecyl)nonadecanamide (AMD291).** Protected lipid **103** (7 mg, 7.2 µmol) was dissolved in n-PrOH (1 mL) and H₂O₂ (30%, 0.12 mL). To this was added n-PrONa/n-PrOH (0.1 N, 6 eq, 43 µmol, 430 µL) dropwise at a rate of 100 µL/h. After final addition the reaction was stirred for a further 1 h. Water (4 mL) was added and the solution centrifuged at 15000 rpm for 15 min. The supernatant was removed and the precipitate treated a further two times with water. The precipitate was lyophilised to give **AMD291** (5 mg, 86%) as a white solid; ^1^H NMR (600 MHz, pyridine-d_5) δ 9.01 (1H, d, J 8.9, -NH-), 8.71 (1H, s, triazole-H), 7.64 (1H, bs, OH), 6.99 (1H, bs, OH), 6.86 (1H, d, J 4.7, H-1), 6.80 (1H, d, J 4.2, OH), 6.54 (2H, bs, J 6.6, each OH), 6.08 (1H, dd, J 8.8, 5.0, H-1’), 5.24 – 5.14 (2H, m, overlapping signals, H-2 & H-3), 4.89 (1H, td, J 5.9, 1.5, H-5), 4.83 (1H, s, H-4), 4.50 (1H, td, J 7.7, 5.9, 3.4, H-2’), 4.47 – 4.43 (1H, m, H-6a), 4.37 (1H, dt, J 10.8, 5.0, H-6b), 2.50 – 2.38 (2H, m, CH₂), 1.96 – 1.76 (5H, m, overlapping signals, CH & CH₂), 1.59 (1H, tdd, J 13.1, 10.6, 9.2, 5.3, CH), 1.33 – 1.23 (58H, m, each CH₂), 0.88 (6H, t, J 6.9, CH₃); ^1^C NMR (150 MHz, pyridine-d_5) δ 172.7 (C=O), 146.4 (triazole, CH=C), 126.3 (triazole, CH=C), 87.5 (C-1), 77.2 (C-5), 73.8 (C-2’), 71.6 (C-2 or C-3), 70.2 (C-4), 69.5 (C-2 or C-3), 62.0 (C-6), 51.8 (C-1’), 36.8, 35.1, 32.3, 30.2 (2s), 30.1 (2s), 30.0, 29.9 (2s), 29.8, 26.8, 26.5, 23.1 (each CH₂), 14.4 (CH₃); ESI-HRMS calcd. for C₄₆H₈₇N₄O₇ 807.6575, found m/z 807.6579 [M-H]⁻.
**5.3 Chapter 3-Experimental**

**Potassium p-methylselenobenzoate (213).** To a suspension of black selenium powder (12 g, 152 mmol) in freshly distilled ethanol (150 mL) at 0 °C was added NaBH₄ (6.9 g, 182.39 mmol) by portion with care. Then a solution of p-methylbenzoyl chloride (20 mL, 152 mmol) in dry THF (50 mL) was added dropwise over 30 min and stirring was continued for another 30 min at 0 °C. To the resulting mixture was added a solution of iodine (19 g, 76 mmol) and potassium iodide (5.1 g, 30.4 mmol) in distilled ethanol (50 mL) dropwise over 30 min. Once added stirring was continued for a further 30 min. The reaction mixture was partitioned between water and CH₂Cl₂ and the aqueous was extracted into CH₂Cl₂ (x2). The combined organic layers were washed with 1% NaHCO₃, dried over Na₂SO₄, filtered and the solvent was removed under reduced pressure to afford an orange crystalline solid. Recrystallization of the residue with CH₂Cl₂ and n-hexane (1:1) gave diselenide (21 g, 70%).

To a solution of diselenide (21 g, 53 mmol) in toluene (125 mL) was added 1M potassium hydroxide in MeOH (53 mL). The resulting solution was shaken vigorously for 5 min (Caution: exothermic) and concentrated. The resulting residue was filtered and washed with n-hexane to afford the title compound 213 (15.7 g, 94%, 1:1 mixture of potassium p-methylselenobenzoate and selenium) as a dark green crystalline solid.

**2,3,4,6-Tetra-O-acetyl-1-Se-p-toluoyl-β-D-selenogalactopyranose (209).** To 1-bromo-1-deoxy-2,3,4,6-tetra-O-acetyl-α-D-galactopyranose⁵ (1.69 g, 4.10 mmol) in EtOAc (82 mL) were added potassium p-methylbenzoselenoate 213 (1.95 g, 8.2 mmol), tetra-n-butylammonium hydrogen sulfate (2.78 g, 8.2 mmol) and 1M Na₂CO₃ (17 mL). The resulting mixture was stirred for 1.5 h and extracted into EtOAc (x 2). The combined organic layers were washed with water, brine, dried over Na₂SO₄, filtered and the solvent was removed under reduced pressure. Flash chromatography of the residue (cyclohexane-EtOAc 3:1) gave
209 (1.64 g, 76%) as a white foam. The \(^{1}\)H and \(^{13}\)C NMR data for 209 were in good agreement with those previously reported in the literature\(^6\); \(^{1}\)H NMR (500 MHz, DMSO) \(\delta\) 7.76 (2H, d, \(J\) 8.3, Ar-H), 7.40 (2H, d, \(J\) 8.0, Ar-H), 5.65 (1H, d, \(J\) 10.4, H-1), 5.40 (1H, dd, \(J\) 10.0, 3.6, H-3), 5.37 (1H, dd, \(J\) 3.6, 1.2, H-4), 5.28 (1H, t, \(J\) 10.0, H-2), 4.42 (1H, td, \(J\) 6.2, 1.2, H-5), 4.08 – 3.97 (2H, m, H-6a & H-6b), 2.39 (3H, s, \(\text{CH}_3\)), 2.16 – 1.91 (12H, m, each OAc); \(^{13}\)C NMR (125 MHz, DMSO) \(\delta\) 190.3, 169.9, 169.8, 169.4, 169.3 (each C=O), 145.7, 134.9, 130.0, 127.3 (Ar-C), 79.4 (C-1), 75.0 (C-5), 70.8, 67.6, 67.3 (each C-2, C-3 or C-4, overlapping signals), 61.3 (C-6), 21.3 (CH\(_3\)), 20.4 (2s), 20.3 (2s) (each OAc); ESI-HRMS calcd. C\(_{22}\)H\(_{26}\)O\(_{10}\)NaSe, 553.0589 found m/z 553.0579 [M+Na]\(^+\).

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\text{2,3,4-Tri-}\text{-O-acetyl-1-Se-p-toluoyl-\text{-}\beta-D-selenogalactopyranosiduronic acid, methyl ester (214). To a solution of 84}^2 (300 mg, 0.62 mmol) \text{in dry DMF (2 mL) was added potassium } p-\text{-methylbenzoselenoate 213 (890 mg, 3.75 mmol). The reaction vessel was sealed and the mixture was then sonicated for 15 min after which time it was diluted with EtOAc and washed with water. The aqueous layer was extracted into EtOAc and the combined organic layers were washed with brine, dried over Na\(_2\)SO\(_4\), filtered and the solvent was removed under reduced pressure. Flash chromatography of the residue (cyclohexane-EtOAc 3:1) gave 214 (222 mg, 69%) as a white foam; [\(\alpha\)]\(_D\) +50 (c 1.0, CHCl\(_3\)); \(R_f\) 0.25 (cyclohexane-EtOAc 2:1); IR (film) cm\(^{-1}\): 2954, 1747, 1687, 1604, 1368, 1214, 1174, 879, 783; \(^{1}\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 7.77 (2H, d, \(J\) 6.2, Ar-H), 7.28 (2H, d, \(J\) 6.4, Ar-H), 5.83 (1H, broad signal, H-4), 5.62 – 5.53 (2H, H-1 & H-2, overlapping signals), 5.30 – 5.19 (1H, broad signal, H-3), 4.52 (1H, d, \(J\) 1.3, H-5), 3.85 – 3.66 (3H, s, OMe), 2.42 (3H, s, \(\text{CH}_3\)), 2.14 (3H, s, OAc), 2.02 (3H, s, OAc), 2.00 (3H, s, OAc); \(^{13}\)C NMR (125 MHz, CDCl\(_3\)) \(\delta\) 190.7, 169.8 (2s), 169.6, 166.2 (each C=O), 145.6, 135.4, 129.7, 127.7 (each Ar-C), 80.1 (C-1), 77.2 (C-5), 71.6 (C-3), 68.6 (C-4), 66.9 (C-2), 52.8 (OMe), 21.8 (CH\(_3\)), 20.8, 20.6 (2s) (each OAc); ESI-HRMS calcd. C\(_{21}\)H\(_{24}\)O\(_{10}\)NaSe, 539.0432 found m/z 539.0425 [M+Na]\(^+\).
2,3,4-Tri-O-benzyol-1-\textit{Se}-p-toluoyl-\textit{β}-d-selenogalactopyranosiduronic acid, methyl ester (215). To a solution of 213 (500 mg, 0.86 mmol) in dry DMF (3 mL) was added potassium \( p \)-methylselenobenzoate 213 (816 mg, 3.44 mmol). The reaction vessel was sealed and the mixture was then sonicated for 15 min after which time it was diluted with EtOAc and washed with water. The aqueous layer was extracted into EtOAc and the combined organic layers were washed with brine, dried over Na\(_2\)SO\(_4\), filtered and the solvent was removed under reduced pressure. Flash chromatography of the residue (cyclohexane-EtOAc 7:1-6:1-5:1) gave 215 (382 mg, 63%) as a white solid; \([\alpha]_D^{+} +159\) (c 1.0, CHCl\(_3\)); \( R_f \) 0.25 (cyclohexane-EtOAc 3:1); mp 181-184 °C; IR (film) cm\(^{-1}\): 2923, 1763, 1719, 1667, 1452, 1260, 1090, 701; \(^1\)H NMR (500 MHz, CDCl\(_3\)) \( \delta \) 8.06 – 8.01 (2H, m, Ar-H), 7.92 – 7.87 (2H, m, Ar-H), 7.85 – 7.78 (2H, m, Ar-H), 7.71 (2H, d, \( J \) 8.1, Ar-H), 7.60 (1H, t, \( J \) 7.4, Ar-H), 7.50 – 7.41 (4H, m, Ar-H), 7.31 (2H, t, \( J \) 7.8, Ar-H), 7.27 (2H, t, \( J \) 7.8, Ar-H), 7.21 (2H, d, \( J \) 8.0, Ar-H), 6.27 (1H, dd, \( J \) 3.5, 1.3, H-4), 6.08 (1H, t, \( J \) 10.2, H-2), 5.88 (1H, d, \( J \) 10.5, H-1), 5.76 (1H, dd, \( J \) 9.9, 3.5, H-3), 4.79 (1H, d, \( J \) 1.4, H-5), 3.71 (3H, s, OMe), 2.37 (3H, s, CH\(_3\)); \(^{13}\)C NMR (125 MHz, CDCl\(_3\)) \( \delta \) 191.0, 166.2, 165.4, 165.3, 165.2 (each C=O), 145.4, 135.4, 133.5, 133.4, 133.3, 130.1, 129.8, 129.8, 129.6, 128.9 (2s), 128.8, 128.6, 128.4, 128.3, 127.6 (each Ar-C), 80.3 (C-1), 77.7 (C-5), 72.4 (C-3), 69.5 (C-4), 67.9 (C-2), 52.9 (CH\(_3\)), 21.7 (CH\(_3\)); ESI-HRMS calcd. C\(_{38}\)H\(_{30}\)O\(_{10}\)NaSe, 725.0902 found \( m/z \) 725.0934 [M+Na]\(^+\).

Propyl 2,3,4,6-tetra-O-acetyl-1-\textit{β}-seleno-D-galactopyranoside (210\(\text{β}\)). To a solution of 209 (1.64 g, 3.10 mmol) in dry degassed DMF, were added Cs\(_2\)CO\(_3\) (2.02 g, 6.2 mmol), piperazine (320 mg, 3.72 mmol) and propyl bromide (6.2 mmol). The resulting mixture was stirred for 15 min and diluted with water. The aqueous phase was extracted into EtOAc (x2) and the combined organic layers were washed with 2M HCl, sat. NaHCO\(_3\), brine, dried over Na\(_2\)SO\(_4\) and the solvent was removed under reduced pressure. Flash chromatography of the residue (cyclohexane-EtOAc 2:1) gave 210\(\text{β}\) (1.34 g, 95%) as a colourless oil; \([\alpha]_D^{-} -17\) (c
0.3, CHCl$_3$); R$_f$ 0.24 (cyclohexane-EtOAc 2:1); IR (film) cm$^{-1}$: 2965, 1742, 1367, 1214, 1079, 1047; $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 5.44 (1H, dd, $J$ 3.4, 1.1, H-4), 5.30 (1H, t, H-10.0, H-2), 5.03 (1H, dd, H-11.0, 3.4, H-3), 4.72 (1H, d, J 10.1, H-1), 4.16 (1H, dd, J 11.3, 6.6, H-6a), 4.10 (1H, dd, J 11.3, 6.6, H-6b), 3.91 (1H, td, J 6.7, 1.1, H-5), 2.82 – 2.75 (1H, $dt$ J 11.8, 7.3, H-1’a), 2.70 (1H, $dt$ J 11.8, 7.4, H-1b), 2.16 (3H, s, OAc), 2.06 (3H, s, OAc), 2.04 (3H, s, OAc), 1.98 (3H, s, OAc), 1.80 – 1.70 (2H, m, CH$_2$), 1.00 (3H, t, J 7.3, CH$_3$); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 170.4, 170.2, 170.1, 169.6 (each C=O), 78.2 (C-1), 75.5 (C-5), 71.7 (C-3), 68.1 (C-2), 67.3 (C-4), 61.5 (C-6), 26.2 (SeCH$_2$), 23.8 (CH$_2$), 20.9, 20.7 (2s), 20.6 (each OAc), 14.5 (CH$_3$); ESI-MS calcd. C$_{17}$H$_{26}$O$_9$NaSe, 477.0640 found m/z 477.0637 [M+Na]$^+$. 

Propyl 2,3,4,6-tetra-O-benzoyl-1-$\beta$-seleno-D-galactopyranoside (211$\beta$). Peracetylated selenide 210$\beta$ (500 mg, 1.1 mmol) was taken up in dry methanol (4.5 mL) and NaOMe (4.5 mL, 1 M in MeOH) was added dropwise. The solution was stirred for 20 min and quenched with Amberlite IR 120H$^+$. The reaction mixture was filtered and the solvent was removed under reduced pressure to give crude deprotected sugar (300 mg, 96%) as a crystalline solid. $^1$H NMR (500 MHz, MeOD) $\delta$ 4.60 (1H, d, J 9.8, H-1), 3.92 (1H, d, J 3.1, H-4), 3.75 (1H, dd, J 11.5, 6.5, H-6a), 3.70 (1H, dd, J 11.5, 5.4, H-6b), 3.65 (1H, t, J 9.5, H-2), 3.52 (1H, t, J 6.1, H-5), 3.45 (1H, dd, J 9.3, 3.2, H-3), 2.90 – 2.78 (1H, $dt$ J 12.2, 7.3, H-1’a), 2.77 – 2.68 (1H, $dt$ J 11.9, 7.3, H-1’b), 1.85 – 1.69 (2H, m, CH$_2$), 1.03 (3H, t, J 7.3, CH$_3$); $^{13}$C NMR (125 MHz, MeOD) $\delta$ 82.5 (C-1), 81.8 (C-5), 76.1 (C-3), 72.5 (C-2), 70.6 (C-4), 62.6 (C-6), 26.0 (CH$_2$), 25.1 (CH$_2$), 14.9 (CH$_3$); Deprotected sugar (606 mg, 2.13 mmol) was taken up in dry pyridine (24 mL) and cooled to 0 °C. Benzoyl chloride (1.5 mL, 12.8 mmol) was added dropwise and the reaction mixture was warmed to room temperature and stirred for 16 h. Et$_3$O was added and the organic layer was washed with 1M HCl, water, brine, dried over Na$_2$SO$_4$ and the solvent was removed under reduced pressure. Flash chromatography of the residue (cyclohexane-EtOAc 6:1) gave 211$\beta$ (1.31 g, 74%) as a white foam; [a]$_D$ +35 (c 0.25, CHCl$_3$); R$_f$ 0.5 (cyclohexane-EtOAc 2:1); IR (film) cm$^{-1}$: 2962, 1720, 1602, 1451, 1315, 1257, 1092, 1067, 702, 685; $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 8.11-7.21 (20H, m, each Ar-H), 6.05 (1H, d, J 3.3, H-4), 5.91 (1H, t, J 10.0, H-2), 5.62 (1H, dd, J 9.9, 3.4, H-2), 5.11
(1H, d, J 10.1, H-1), 4.66 (1H, dd, J 11.3, 6.6, H-6a), 4.41 (1H, dd, J 11.3, 6.3, H-6b), 4.34 (1H, t, J 6.5, H-5), 2.91 (1H, ddd, J 11.8, 8.4, 6.4, H-1’a), 2.80 (1H, ddd, J 11.8, 8.4, 6.7, H-1’b), 1.88 – 1.71 (2H, m, CH₂), 0.98 (3H, t, J 7.3, CH₃); ¹³C NMR (125 MHz, CDCl₃) δ 166.0, 165.5, 165.5, 165.4 (each C=O), 133.6, 133.3 (3s), 129.9, 129.8, 129.7 (2s), 129.4, 129.2, 129.1, 128.8, 128.6, 128.4 (2s), 128.3 (each Ar-C), 78.2 (C-1), 76.1 (C-5), 72.5 (C-3), 69.0 (C-2), 68.5 (C-4), 62.3 (C-5), 26.2 (CH₂), 24.0 (CH₂), 14.6 (CH₃); ESI-HRMS calcd. C₃₀H₂₇O₉NaSe, 725.1266 found m/z 725.1269 [M+Na]⁺.

### 1-Se-Propyl-2,3,4-tri-O-acetyl-β-seleno-D-galactopyranosiduronic acid, methyl ester (216β)

To a solution of 214 (150 mg, 291 μmol) in dry degassed DMF (3 mL) under argon were added Cs₂CO₃ (189 mg, 582 μmol), piperidine (58 μL, 582 μmol) and 1-bromopropane (110 μL, 1160 μmol). The reaction mixture was stirred for 60 min at room temperature. EtOAc was added and the organic layer was washed with water. The aqueous phase was extracted with EtOAc (x2) and the combined organic layers were washed with water, brine, dried over Na₂SO₄, filtered and the solvent was removed under reduced pressure. Flash chromatography of the residue (toluene-EtOAc 14:1) gave 216β (101 mg, 79%) as a colourless oil; [α]D -7 (c 0.7, CHCl₃); Rᵣ 0.22 (cyclohexane-EtOAc 2:1); IR (film) cm⁻¹: 2961, 1746, 1438, 1368, 1212, 1082, 1049; ¹H NMR (500 MHz, CDCl₃) δ 5.76 (1H, dd, J 3.5, 1.4, H-4), 5.33 (1H, t, J 10.0, H-2), 5.08 (1H, dd, J 10.0, 3.4 H-3), 4.73 (1H, d, J 10.1, H-1), 4.28 (1H, d, J 1.4, H-5), 3.75 (3H, s, OMe), 2.85 (1H, ddd, J 11.7, 8.0, 6.5, H-1’a), 2.74 (1H, ddd, J 11.9, 8.0, 7.0, H-1’b), 2.12 (3H, s, OAc), 2.06 (3H, s, OAc), 2.00 (3H, s, OAc), 1.76 (2H, dq, J 14.2, 7.0, CH₂), 1.01 (3H, t, J 7.3, CH₃); ¹³C NMR (125 MHz, CDCl₃) δ 170.0, 169.8, 169.5, 166.2 (C=O), 78.0 (C-1), 76.7 (C-5), 71.4 (C-3), 68.5 (C-4), 67.7 (C-2), 52.7 (OMe), 26.4 (CH₂), 23.7 (CH₂), 20.9, 20.6, 20.6 (each OAc), 14.5 (CH₃); ESI-HRMS calcd. C₁₆H₂₄O₉NaSe, 463.0483 found m/z 463.0480 [M+Na]⁺.
1-Se-Propyl-2,3,4-tri-O-benzoyl-β-seleno-D-galactopyranosiduronic acid, methyl ester (217β). To a solution of 215 (120 mg, 171 μmol) in dry degassed DMF (1.7 mL) under argon were added Cs$_2$CO$_3$ (111 mg, 342 μmol), piperidine (35 μL, 342 μmol) and 1-bromopropane (62 μL, 684 μmol). The reaction mixture was stirred for 60 min at room temperature. EtOAc was added and the organic layer was washed with water. The aqueous phase was extracted with EtOAc (x2) and the combined organic layers were washed with water, brine, dried over Na$_2$SO$_4$, filtered and the solvent was removed under reduced pressure. Flash chromatography of the residue (toluene-EtOAc 14:1) gave 217β (96 mg, 90%) as a white foam; [α]$_D$ +100 (c 1, CHCl$_3$); IR (film) cm$^{-1}$: 2958, 1767, 1725, 1602, 1452, 1257, 706, 685; $^1$H NMR (500 MHz, CDCl$_3$) δ 8.04 – 7.25 (15H, m, each Ar-H), 6.24 – 6.19 (1H, dd, J 3.5, 1.5, H-4), 5.90 (1H, t, J 10.0, H-2), 5.60 (1H, dd, J 9.9, 3.4, H-3), 5.05 (1H, d, J 10.0, H-1), 4.58 (1H, d, J 1.3, H-5), 3.71 (3H, s, OMe), 2.98 (1H, ddd, J 11.8, 8.5, 6.2, H-1'a), 2.83 (1H, ddd, J 11.8, 8.4, 6.8, H-1'b), 1.92 – 1.73 (2H, m, CH$_2$), 1.02 (3H, t, J 7.3, CH$_3$); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 166.3, 165.5, 165.2, 165.1 (each C=O), 133.5, 133.3 (2s), 129.9, 129.8 (2s), 129.2, 129.0, 128.8, 128.6, 128.4, 128.3 (each Ar-C), 77.6 (C-1), 77.1 (C-5), 72.2 (C-3), 69.4 (C-4), 68.3 (C-2), 52.8 (OMe), 26.0 (CH$_2$), 23.9 (CH$_2$), 14.6 (CH$_3$); ESI-HRMS calcd. C$_{31}$H$_{30}$O$_9$NaSe, 649.0953 found m/z 649.0948 [M+Na]$^+$.

1-Se-Propyl-2,3,4-tri-O-acetyl-α-seleno-D-galactopyranosiduronic acid, methyl ester (216α). β-Selenide 216β (35 mg, 80 μmol) was taken up in dry CH$_2$Cl$_2$ (11.7 mL) and cooled to -20 °C. TiCl$_4$ (0.2 mL, 2.5 eq, 1M in CH$_2$Cl$_2$) was added dropwise and the resulting solution was stirred at -20 °C for 20 min. NH$_4$Cl (1M, 2 mL) was added and the mixture was partitioned between 1M NH$_4$Cl and EtOAc. The aqueous phase was extracted into EtOAc (x2) and the combined organic layers were washed with water, brine, dried over Na$_2$SO$_4$, filtered and the solvent was removed under reduced pressure. Flash chromatography of the residue (cyclohexane-diethyl ether 1:1) gave 216α (33 mg, 94%) as a colourless oil; [α]$_D$
1-Se-Propyl-2,3,4-tri-O-benzoyl-α-seleno-D-galactopyranosiduronic acid, methyl ester (217α). β-Selenide 217β (35 mg, 56 µmol) was taken up in dry CH₂Cl₂ (8.2 mL) and cooled to -20 °C. TiCl₄ (0.14 mL, 2.5eq, 1M in CH₂Cl₂) was added dropwise and the resulting solution was stirred at -20 °C for 20 min. NH₄Cl (1M, 2 mL) was added and the mixture was partitioned between 1M NH₄Cl and EtOAc. The aqueous phase was extracted into EtOAc (x2) and the combined organic layers were washed with water, brine dried over Na₂SO₄, filtered and the solvent was removed under reduced pressure. Flash chromatography of the residue (cyclohexane-diethyl ether 2:1) gave 217α (33 mg, 94%) as a white foam; [α]D +229 (c 1.8, CHCl₃); Rf 0.46 (cyclohexane-EtOAc 3:1); IR (film) cm⁻¹: 2972, 2931, 1760, 1720, 1452, 1281, 1252, 711, 702; ¹H NMR (500 MHz, CDCl₃) δ 8.05 – 7.26 (15H, m, each Ar-H), 6.44 (1H, d, J 3.9, H-1), 6.23 (1H, d, J 1.4, H-4), 5.83 – 5.73 (2H, m, H-2 & H-3, overlapping signals), 5.31 (1H, d, J 1.6, H-5), 3.73 (3H, s, OMe), 2.72 (1H, dt, J 12.0, 7.5, H-1’a), 2.67 – 2.58 (1H, dt, J 11.9, 7.4, H-1’b), 1.76 – 1.66 (2H, m, CH₂), 0.96 (3H, t, J 7.5, CH₃); ¹³C NMR (125 MHz, CDCl₃) δ 167.2, 165.5, 165.4, 165.1 (each C=O), 133.6, 133.5, 133.3, 129.9, 129.7, 129.0, 128.9 (2s), 128.6, 128.5, 128.3 (each Ar-C), 79.6 (C-1), 70.5 (C-5), 69.4, 69.4, 68.7 (C-2, C-3 & C-4, overlapping signals), 52.8 (OMe), 26.1 (CH₂), 23.7 (CH₂), 14.4 (CH₃); ESI-HRMS calcd. C₃₃H₃₃NO₉NaSe, 690.1218 found m/z 690.1207 [M+MeCN+Na]⁺.
1-Se-Methyl-2,3,4-tri-O-benzoyl-β-seleno-D-galactopyranosiduronic acid, methyl ester (218β). To a solution of 215 (50 mg, 70 μmol) in dry degassed DMF (0.7 mL) under argon were added Cs₂CO₃ (46 mg, 140 μmol), piperidine (14 μL, 140 μmol) and methyl iodide (18 μL, 280 μmol). The reaction mixture was stirred for 60 min at room temperature. EtOAc was added and the organic layer was washed with water. The aqueous phase was extracted with EtOAc (x2) and the combined organic layers were washed with water, brine, dried over Na₂SO₄, filtered and the solvent removed under reduced pressure. Flash chromatography of the residue (toluene-EtOAc 14:1) gave 218β (38 mg, 90%) as a white foam; [α]D +174 (c 1, CHCl₃); Rf 0.29 (cyclohexane-EtOAc 2:1); IR (film) cm⁻¹: 2955, 1725, 1602, 1257, 1092, 1067, 706, 685; ¹H NMR (500 MHz, CDCl₃) δ 8.02 – 7.26 (15H, m, each Ar-H), 6.22 (1H, dd, J 3.4, 1.4, H-4), 5.90 (1H, t, J 10.0, H-2), 5.63 (1H, dd, J 10.0, 3.3, H-3), 4.99 (1H, d, J 10.1, H-1), 4.60 (1H, d, J 1.4, H-5), 3.71 (3H, s, OMe), 2.30 (3H, s, SeMe); ¹³C NMR (125 MHz, CDCl₃) δ 166.2, 165.5, 165.3, 165.1 (each C=O), 133.6, 133.4, 133.3, 129.9, 129.8 (2s), 129.1, 129.0, 128.8, 128.6, 128.4, 128.3 (each Ar-C), 77.0 (C-5), 76.8 (C-1), 72.1 (C-3), 69.4 (C-4), 67.7 (C-2), 52.8 (OMe), 2.6 (SeMe); ESI-HRMS calcd. C₂₉H₂₆O₉NaSe, 621.0640 found m/z 621.0646 [M+Na]⁺.

1-Se-Ethyl-2,3,4-tri-O-benzoyl-β-seleno-D-galactopyranosiduronic acid, methyl ester (219β). To a solution of 215 (120 mg, 171 μmol) in dry degassed DMF (1.7 mL) under argon were added Cs₂CO₃ (111 mg, 342 μmol), piperidine (35 μL, 342 μmol) and ethyl bromide (50 μL, 684 μmol). The reaction mixture was stirred for 60 min at room temperature. EtOAc was added and the organic layer was washed with water. The aqueous phase was extracted with EtOAc (x2) and the combined organic layers were washed with water, brine, dried over Na₂SO₄, filtered and the solvent removed under reduced pressure. Flash chromatography of the residue (cyclohexane-EtOAc 2:1) gave 219β (95 mg, 91%) as a white foam; [α]D +155 (c 1, CHCl₃); Rf 0.17 (cyclohexane-EtOAc 3:1); IR (film) cm⁻¹: 2957, 1724, 1451, 1250, 1092, 1067, 705, 685; ¹H NMR (500 MHz, CDCl₃) δ 8.05 – 7.22 (15H, m, each Ar-H), 6.22 (1H,
dd, J 3.5, 1.3, H-4), 5.91 (1H, t, J 10.0, H-2), 5.62 (1H, dd, J 9.9, 3.4, H-3), 5.08 (1H, d, J 10.1, H-1), 4.59 (1H, d, J 1.4, H-5), 3.71 (3H, s, OMe), 3.01 (1H, dq, J 11.7, 7.5, CHH), 2.88 (1H, dq, J 11.7, 7.5, CHH), 1.51 (3H, t, J 7.5, CH3); 13C NMR (125 MHz, CDCl3) δ 166.3, 165.5, 165.2, 165.1 (each C=O), 133.5, 133.3 (2s), 129.9, 129.8 (2s), 129.2, 129.0, 128.8, 128.6, 128.4, 128.3 (each Ar-C), 77.6 (C-1), 77.1 (C-5), 72.2 (C-3), 69.4 (C-4), 68.3 (C-2), 52.8 (OMe), 17.6 (CH2), 15.8 (CH3); ESI-HRMS calcd. C30H28O9NaSe, 635.0796 found m/z 635.0785 [M+Na]+.

1-Se-Butyl-2,3,4-tri-O-benzoyl-β-seleno-D-galactopyranosiduronic acid, methyl ester (220β). To a solution of 215 (120 mg, 171 μmol) in dry degassed DMF (1.7 mL) under argon were added Cs2CO3 (111 mg, 342 μmol), piperidine (35 μL, 342 μmol) and 1-iodobutane (80 μL, 684 μmol). The reaction mixture was stirred for 60 min at room temperature. EtOAc was added and the organic layer was washed with water. The aqueous phase was extracted with EtOAc (x2) and the combined organic layers were washed with water, brine, dried over Na2SO4, filtered and the solvent removed under reduced pressure. Flash chromatography of the residue (cyclohexane-EtOAc 4:1-3:1) gave 220β (91 mg, 84%) as a white foam; [α]D +117 (c 1, CHCl3); Rf 0.3 (cyclohexane-EtOAc 3:1); IR (film) cm⁻¹: 2957, 1764, 1725, 1452, 1253, 1092, 1067, 705, 685; 1H NMR (500 MHz, CDCl3) δ 8.06 – 7.23 (15H, m, each Ar-H), 6.21 (1H, dd, J 3.4, 1.4, H-4), 5.90 (1H, t, J 10.0, H-2), 5.61 (1H, dd, J 9.9, 3.3, H-3), 5.05 (1H, d, J 10.1, H-1), 4.58 (1H, d, J 1.4, H-5), 3.71 (3H, s, OMe), 3.00 (1H, ddd, J 11.6, 8.7, 6.3, CHH), 2.86 (1H, ddd, J 11.7, 8.7, 6.8, CHH), 1.86 – 1.70 (2H, m, CH2), 1.49 – 1.38 (2H, m, CH2), 0.92 (3H, t, J 7.4, CH3); 13C NMR (125 MHz, CDCl3) δ 166.3, 165.5, 165.2, 165.1 (each C=O), 133.5, 133.3 (2s), 129.9, 129.8 (2s), 129.2, 129.0, 128.8, 128.6, 128.4, 128.3 (each Ar-C), 77.6 (C-1), 77.1 (C-5), 72.2 (C-3), 69.4 (C-4), 68.3 (C-2), 52.8 (OMe), 32.5, 23.6, 23.0 (each CH2), 13.5 (CH3); ESI-HRMS calcd. C32H32O9NaSe, 663.1109 found m/z 663.1116 [M+Na]+.

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1-Se-Isopropyl-2,3,4-tri-O-benzoyl-β-seleno-D-galactopyanosiduronic acid, methyl ester (221β). To a solution of 215 (120 mg, 171 µmol) in dry degassed DMF (1.7 mL) under argon were added Cs₂CO₃ (111 mg, 342 µmol), piperidine (35 µL, 342 µmol) and 2-bromopropane (64 µL, 684 µmol). The reaction mixture was stirred for 60 min at room temperature. EtOAc was added and the organic layer was washed with water. The aqueous phase was extracted with EtOAc (x2) and the combined organic layers were washed with water, brine, dried over Na₂SO₄, filtered and the solvent removed under reduced pressure. Flash chromatography of the residue (toluene-EtOAc 14:1) gave 221β (79 mg, 74%) as a white foam; [α]D +145 (c 1, CHCl₃); Rf 0.2 (cyclohexane-EtOAc 3:1); IR (film) cm⁻¹: 2957, 1725, 1602, 1250, 1092, 1067, 706, 685; ¹H NMR (500 MHz, CDCl₃) δ 8.04–7.23 (15H, m, each Ar-H), 6.22 (1H, dd, J 3.5, 1.4, H-4), 5.91 (1H, t, J 10.0, H-2), 5.62 (1H, dd, J 9.9, 3.4, H-3), 5.14 (1H, d, J 10.2, H-1), 4.57 (1H, d, J 1.4, H-5), 3.71 (3H, s, OMe), 3.57 (1H, hept, J 7.0, CH), 1.56 (3H, d, J 6.8, CH₃), 1.46 (3H, d, J 7.0, CH₃); ¹³C NMR (125 MHz, CDCl₃) δ 166.3, 165.5, 165.2, 165.2 (each C=O), 133.5, 133.3, 130.0, 129.8, 129.2, 129.0, 128.8, 128.5, 128.4, 128.3 (each Ar-C), 78.1 (C-1), 77.1 (C-5), 72.3 (C-3), 69.4 (C-4), 68.5 (C-2), 52.8 (OMe), 31.2 (CH), 25.4, 24.3 (each CH₃); ESI-HRMS calcd. C₃₁H₃₀O₉NaSe, 649.0953 found m/z 649.0964 [M+Na]⁺.

1-Se-Cyclopentyl-2,3,4-tri-O-benzoyl-β-seleno-D-galactopyanosiduronic acid, methyl ester (222β). To a solution of 215 (120 mg, 171 µmol) in dry degassed DMF (1.7 mL) under argon were added Cs₂CO₃ (111 mg, 342 µmol), piperidine (20 µL, 205 µmol) and bromocyclopentane (73 µL, 684 µmol). The reaction mixture was stirred for 60 min at room temperature. EtOAc was added and the organic layer was washed with water. The aqueous phase was extracted with EtOAc (x2) and the combined organic layers were washed with water, brine, dried over Na₂SO₄, filtered and the solvent removed under reduced pressure. Flash chromatography of the residue (cyclohexane-EtOAc 3:1) gave 222β (104 mg, 87%) as
a white solid; \([\alpha]_D^{+} +123\) (c 1, CHCl3); Rf 0.26 (cyclohexane-EtOAc 3:1); IR (film) cm\(^{-1}\): 2952, 1770, 1727, 1714, 1451, 1283, 1256, 1093, 1067, 708, 685; \(^1\)H NMR (500 MHz, CDCl3) \(\delta\) 8.04 – 7.23 (15H, m, each Ar-H), 6.22 (1H, dd, J 3.4, 1.4, H-4), 5.92 (1H, t, J 10.1, H-2), 5.61 (1H, dd, J 9.9, 3.4, H-3), 5.11 (1H, d, J 10.1, H-1), 4.58 (1H, d, J 1.3, H-5), 3.71 (3H, s, OMe), 3.71 – 3.63 (1H, m, CH), 2.29 – 2.17 (1H, m, CH), 2.17 – 2.07 (1H, m, CH), 1.85 – 1.54 (6H, m, each CH\(_2\)); \(^{13}\)C NMR (125 MHz, CDCl3) \(\delta\) 166.3, 165.5, 165.2, 165.2 (each C=O), 133.5, 133.3 (2s), 130.0, 129.8, 129.3, 129.0, 128.8, 128.5, 128.4, 128.3 (each Ar-C), 78.3 (C-1), 77.1 (C-5), 72.3 (C-3), 69.4 (C-4), 68.6 (C-2), 52.8 (OMe), 38.6 (CH), 35.3, 33.8, 24.7 (2s) (each CH\(_2\)); ESI-HRMS calcd. C\(_{33}\)H\(_{36}\)NO\(_9\)Se, 670.1555 found m/z 670.1541 [M+NH\(_4\)]\(^+\).

1,4-bis-(5-(S)-methoxycarbonyl-2,3,4-tri-O-benzoyl-\(\alpha\)-L-arabinopyranosylseleno)-butane (223β). To a solution of 215 (185 mg, 264 \(\mu\)mol) in dry degassed DMF (2.7 mL) under argon was added Cs\(_2\)CO\(_3\) (171 mg, 528 \(\mu\)mol), piperidine (31 \(\mu\)L, 319 \(\mu\)mol) and 1,4-dibromobutane (16 \(\mu\)L, 132 \(\mu\)mol). The reaction mixture was stirred for 60 min at room temperature. EtOAc was added and the organic layer was washed with water. The aqueous phase was extracted with EtOAc (x2) and the combined organic layers were washed with water, brine, dried over Na\(_2\)SO\(_4\), filtered and the solvent removed under reduced pressure. Flash chromatography of the residue (cyclohexane-diethyl ether 1:1-1:3) gave 223β (106 mg, 63%) as a white solid; [\(\alpha\]D] +132 (c 1, CHCl3); Rf 0.13 (cyclohexane-EtOAc 2:1); IR (film) cm\(^{-1}\): 2927, 1724, 1451, 1248, 1091, 1067, 704; \(^1\)H NMR (500 MHz, CDCl3) \(\delta\) 8.04 – 7.23 (30H, m, each Ar-H), 6.20 (2H, dd, J 3.4, 1.4, H-4), 5.88 (2H, t, J 10.0, H-2), 5.63 (2H, dd, J 9.9, 3.4, H-3), 5.09 (2H, d, J 10.1, H-1), 4.60 (2H, d, J 1.3, H-5), 3.70 (6H, s, OMe), 3.06 – 2.92 (2H, m, H-1’a), 2.91 – 2.78 (2H, m, H-1’b), 2.03 – 1.88 (4H, m, CH\(_2\)); \(^{13}\)C NMR (125 MHz, CDCl3) \(\delta\) 166.3, 165.5, 165.3, 165.1 (each C=O), 133.6, 133.4, 133.3, 129.9, 129.8 (2s), 129.2, 129.0, 128.8, 128.6, 128.4, 128.3 (each Ar-C), 77.9 (C-1), 77.1 (C-5), 72.2 (C-3), 69.5 (C-4), 68.4 (C-2), 52.8 (OMe), 30.4 , 23.4 (each CH\(_2\)); ESI-HRMS calcd. for C\(_{60}\)H\(_{54}\)O\(_{18}\)NaSe\(_2\), 1245.1538 found m/z 1245.1553 [M+Na\(^+\)].
1-Se- Allyl-2,3,4-tri-O-benzoyl-β-seleno-D-galactopyranosiduronic acid, methyl ester (224β). To a solution of 215 (120 mg, 171 μmol) in dry degassed DMF (1.7 mL) under argon were added Cs₂CO₃ (111 mg, 342 μmol), piperidine (35 μL, 342 μmol) and allyl bromide (60 μL, 684 μmol). The reaction mixture was stirred for 60 min at room temperature. EtOAc was added and the organic layer was washed with water. The aqueous phase was extracted with EtOAc (x2) and the combined organic layers were washed with water, brine, dried over Na₂SO₄, filtered and the solvent removed under reduced pressure. Flash chromatography of the residue (toluene-EtOAc 14:1) followed by a second chromatography (cyclohexane-EtOAc 4:1) gave 224β (97 mg, 91%) as a white foam; [α]D +193 (c 1, CHCl₃); Rf 0.2 (cyclohexane-EtOAc 3:1); IR (film) cm⁻¹: 2956, 1725, 1451, 1250, 1092, 1067, 706; ¹H NMR (500 MHz, CDCl₃) δ 8.04–7.22 (15H, m, each Ar-H), 6.21 (1H, dd, J 3.4, 1.5, H-4), 6.03–5.94 (1H, m, CH₂CH=CH₂), 5.91 (1H, t, J 10.1, H-2), 5.63 (1H, dd, J 9.9, 3.5, H-3), 5.15 (1H, d, J 16.9, CH₂CH=CHH), 5.09 (1H, d, J 9.9, CH₂CH=CHH), 5.02 (1H, d, J 10.2, H-1), 4.54 (1H, d, J 1.4, H-5), 3.72 (3H, s, OMe), 3.63 (1H, dd, J 12.0, 8.7, CHHCH=CH₂), 3.41 (1H, dd, J 12.0, 6.7, CHHCH=CH₂); ¹³C NMR (125 MHz, CDCl₃) δ 166.2, 165.5, 165.3, 165.2 (each C=O), 134.4 (CH₂CH=CH₂), 133.5, 133.4, 133.3, 130.0, 129.8 (2s), 129.1, 128.9, 128.7, 128.6, 128.4, 128.3 (each Ar-C), 117.2 (CH₂CH=CH₂), 77.3 (C-1), 77.1 (C-5), 72.2 (C-3), 69.4 (C-4), 68.5 (C-2), 52.8 (OMe), 26.2 (CH₂CH=CH₂); ESI-HRMS calcd. C₃₁H₂₉O₉NaSe, 647.0796 found m/z 647.0790 [M+Na]⁺.

1-Se-Propargly-2,3,4-tri-O-benzoyl-β-seleno-D-galactopyranosiduronic acid, methyl ester (225β). To a solution of 215 (120 mg, 171 μmol) in dry degassed DMF (1.7 mL) under argon were added Cs₂CO₃ (111 mg, 342 μmol), piperidine (35 μL, 342 μmol) and propargyl bromide (76 μL, 684 μmol). The reaction mixture was stirred for 60 min at room temperature. EtOAc was added and the organic layer was washed with water. The aqueous phase was extracted with EtOAc (x2) and the combined organic layers were washed with water, brine, dried over Na₂SO₄, filtered and the solvent removed under reduced pressure. Flash
chromatography of the residue (toluene-EtOAc 1:4:1) gave 225β (104 mg, 98%) as a white foam; [α]D +151 (c 1, CHCl3); Rf 0.3 (cyclohexane-EtOAc 2:1); IR (film) cm⁻¹: 2952, 1723, 1602, 1452, 1248, 1091, 1066, 705; 1H NMR (500 MHz, CDCl3) δ 8.06 – 7.23 (15H, m, each Ar-C), 6.23 (1H, dd, J 3.5, 1.4, H-4), 5.92 (1H, t, J 10.0, H-2), 5.68 (1H, dd, J 9.9, 3.4, H-3), 5.34 (1H, d, J 10.1, H-1), 4.61 (1H, d, J 1.4, H-5), 3.72 (3H, s, OMe), 3.65 (1H, dd, J 15.3, 2.7, H-1’a), 3.41 (1H, dd, J 15.4, 2.7, H-1’b), 2.31 (1H, t, J 2.6, H-3’); 13C NMR (125 MHz, CDCl3) δ 166.1, 165.5, 165.4, 165.1 (each C=O), 133.6, 133.5, 133.4, 130.0, 129.8 (2s), 129.0, 128.9, 128.7, 128.6, 128.4, 128.3 (each Ar-C), 80.0 (CH₂C≡CH), 77.6 (C-1), 77.2 (C-5), 72.1 (C-3 or CH₂C≡CH), 72.0 (C-3 or CH₂C≡CH), 69.3 (C-4), 68.5 (C-2), 52.8 (OMe), 7.6 (CH₂C≡CH); ESI-HRMS calcd. C₃₁H₂₆O₉NaSe, 645.0640 found m/z 645.0625 [M+Na]⁺.

Methyl 2,3,4-tri-O-benzyol-α-D-glucopyranoside (233). Methyl α-D-glucopyranoside 232 (500 mg, 2.58 mmol), trityl chloride (1.44 g, 5.16 mmol) and 4-dimethylaminopyridine (156 mg, 1.29 mmol), which had been previously dried under high vacuum for 3 h, were taken up in dry pyridine (5 mL) and heated to 50 °C until TLC indicated complete consumption of the starting material indicated by TLC. Then benzoyl chloride (1.2 mL, 10.32 mmol) was added and the reaction mixture was stirred for a further 24 h at 50 °C. After completion, pyridine was removed under reduced pressure. The resulting residue was diluted with CH₂Cl₂, washed with sat. NaHCO₃ (x2), brine, dried over NaSO₄, filtered and the solvent removed under reduced pressure. The crude residue was taken up in CH₂Cl₂ (26 mL) and FeCl₃.6H₂O (1.4 g, 5.16 mmol) was added. The mixture was stirred at room temperature for 1h. Water (20 mL) was added and the mixture was diluted with CH₂Cl₂. The organic layer was dried over NaSO₄, filtered and the solvent was removed under reduced pressure. Flash chromatography of the residue (petroleum ether-EtOAc 11:1-9:1) gave 233 (700 mg, 53% over 3 steps) as a white foam. The 1H and 13C NMR data for 233 were in good agreement with those previously reported in the literature⁷; IR (film) cm⁻¹: 1721, 1602, 1451, 1257, 1091, 1025, 705, 686; 1H NMR (500 MHz, CDCl3) δ 7.98 (4H, dt, J 8.5, 1.6), 7.88 (2H, dd, J 8.3, 1.4), 7.57 – 7.49 (2H, m), 7.45 – 7.36 (5H, m), 7.29 (2H, t, J 7.8) (each Ar-H), 6.23 (1H, t, J 9.7, H-3), 5.50 (1H, t, J 9.8, H-4), 5.29 (1H, dd, J 9.9, 3.7, H-2), 5.26 (1H, d, J 3.7, H-1) 4.04 (1H, ddd, J 10.2, 3.7, 2.2, H-5), 3.83 (1H, dd, J 13.0, 2.2, H-6a), 3.74 (1H, dd, J 12.9, 3.7, H-6b), 3.48
(3H, s, OMe); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 166.4, 165.8, 165.8 (each C=O), 133.7, 133.4, 133.1, 130.0, 129.9, 129.6, 129.2, 129.0, 128.6, 128.5, 128.4, 128.3 (each Ar-C), 97.1 (C-1 or C-2), 72.0 (C-1 or C-2), 70.1 (C-3), 69.8 (C-5), 69.6 (C-4), 61.1 (C-6), 55.7 (OMe); ESI-HRMS calcd. C$_{27}$H$_{27}$O$_9$Na, 529.1475 found m/z 529.1473 [M+Na]$^+$. 

Methyl 2,3,4-tri-O-benzoyl-6-O-trifluoromethanesulfonyl-α-D-glucopyranoside (229). To a solution of alcohol 233 (150 mg, 0.3 mmol) in dry CH$_2$Cl$_2$ (2 mL) at 0 °C under an argon atmosphere were added, pyridine (50 µL) and trifluoromethanesulfonic anhydride (70 µL). The resulting mixture was stirred for 10 minutes at 0 °C and concentrated under reduced pressure. The resulting residue was taken up in EtOAc, washed with 1 M HCl, sat. NaHCO$_3$, brine, dried over Na$_2$SO$_4$ and the solvent was removed under reduced pressure to give the crude triflate 229. The residue was used without further purification. The $^1$H and $^{13}$C NMR data for 229 were in good agreement with those previously reported in the literature$^8$; $^1$H NMR (500 MHz, CDCl$_3$) δ 8.00 – 7.27 (15H, m, each Ar-H), 6.19 (1H, t, J 9.6, H-3), 5.46 (1H, t, J 9.9, H-4), 5.32 – 5.23 (2H, m, H-1 and H-2 overlapping siganls), 4.68 (1H, dd, J 11.2, 6.7, H-6a), 4.61 (1H, dd, J 11.3, 2.3, H-6b), 4.38 (1H, ddd, J 9.4, 6.7, 2.2, H-5), 3.50 (3H, s, OMe), 1.72 (1H, s, OH); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 165.7, 165.6, 165.5 (each C=O), 133.8, 133.5, 133.3, 129.9, 129.7, 128.9, 128.8, 128.6, 128.4, 128.3, 128.2 (each Ar-C), 97.1 (C-1), 73.9 (C-6), 71.6 (C-2), 69.8 (C-3), 69.0 (C-4), 67.5 (C-5), 56.0 (OMe).

Methyl 2,3,6-tri-O-benzoyl-α-D-glucopyranoside (234). Methyl α-D-glucopyranoside 232 (500 mg, 2.58 mmol), which had been previously dried under high vacuum for 1 h, were taken up in dry DMF (9 mL). Imidazole (437 mg, 6.43 mmol) and TIPSCI (0.7 mL, 3.08 mmol) were added and the reaction mixture was stirred at room temperature for 1 h. Upon completion the reaction mixture was concentrated and the resulting residue was taken up in CH$_2$Cl$_2$ (25 mL), washed with H$_2$O, brine, dried over Na$_2$SO$_4$, filtered and the solvent was removed under reduced pressure. The residue was taken up in dry pyridine (15 mL) cooled to
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0 °C and benzoyl chloride (2.4 mL, 20.56 mmol) was added dropwise. The reaction mixture was warmed to room temperature and stirred overnight. The solution was quenched with MeOH (1 mL), diluted with CH₂Cl₂, washed with 1M HCl, sat. NaHCO₃, H₂O, brine, dried over NaSO₄, filtered and the solvent was removed under reduced pressure. Flash chromatography of the residue (petroleum ether-EtOAc 19:1-11:1) gave methyl 2,3,4-tri-O-benzoyl-6-triisopropylsilyl-α-D-glucopyranoside (1.03 g, 61% over 2 steps) as a viscous oil; IR (film) cm⁻¹: 2943, 2866, 1725, 1452, 1249, 882, 706, 684; ¹H NMR (500 MHz, CDCl₃) δ 8.01 – 7.84 (6H, m), 7.53 – 7.48 (2H, m), 7.45 – 7.34 (5H, m), 7.28 (2H, t, J 7.8) (each Ar-H), 6.14 (1H, t, J 9.5, H-3), 5.52 (1H, t, J 9.9, H-4), 5.26-5.21 (2H, m, H-1 & H-2 overlapping signals), 4.14 (1H, ddd, J 10.3, 5.3, 3.1, H-5), 3.95 – 3.83 (2H, m, H-6a & H-6b overlapping signals), 3.48 (3H, s, OMe), 1.07 – 0.98 (21H, m, each CH & CH₃); ¹³C NMR (125 MHz, CDCl₃) δ 166.0, 165.9, 165.3 (each C=O), 133.3, 133.2, 133.0, 129.9, 129.8, 129.7, 129.4, 129.3, 129.2, 128.4, 128.3, 128.2 (each Ar-C), 96.7 (C-1 or C-2), 72.3 (C-1 or C-2), 70.8 (2s) (C-3 or C-5), 69.6 (C-4), 62.9 (C-6), 55.3 (OMe), 17.9, 11.9 (each CH & CH₃); ESI-HRMS calcd. for C₃7H₄₆O₉NaSi, 685.2809 found m/z 685.2789 [M+Na]⁺.

Under an argon atmosphere, the above silyl ether (660 mg, 1 mmol) was taken up in dry THF (10 mL) and cooled to 0 °C. TBAF (0.5 mL, 2M in THF) was added and the reaction mixture was stirred at 0 °C for 15 min. A second portion of TBAF (0.5 mL, 2M in THF) was added and the mixture was stirred for a further 30 min at 0 °C. The reaction mixture was diluted with Et₂O and washed with brine (x2). The aqueous phase was extracted with Et₂O (x3) and the combined organic layers were dried over NaSO₄, filtered and the solvent was removed under reduced pressure. Flash chromatography of the crude residue (cyclohexane-EtOAc 4:1) gave 234 (412 mg, 81%) as a white foam. The ¹H and ¹³C NMR data for 234 were in good agreement with those previously reported in the literature⁹; IR (film) cm⁻¹: 3472, 2941, 1719, 1451, 1267, 707, 686; ¹H NMR (500 MHz, CDCl₃) δ 8.09 (2H, d, J 8.3), 7.96 (4H, dt, J 8.4, 1.5), 7.58 (1H, t, J 7.4), 7.52 – 7.44 (4H, m), 7.37-7.32 (4H, m), 5.75 (1H, t, J 9.7), 5.25 (1H, dd, J 10.1, 3.7), 5.12 (1H, d, J 3.6), 4.79 (1H, dd, J 12.1, 4.4), 4.61 (1H, dd, J 12.1, 2.3), 4.09 (1H, ddd, J 9.8, 4.4, 2.1), 3.85 (1H, t, J 9.6), 3.44 (3H, s, OMe), 3.26 (1H, s, OH); ¹³C NMR (125 MHz, CDCl₃) δ 167.6, 167.2, 166.2 (each C=O), 133.7, 133.6, 133.5, 130.1 (2s), 129.9, 129.4, 129.3, 128.7, 128.6 (2s), (each Ar-C), 97.3 (C-1), 74.2 (C-3), 71.5 (C-2), 70.3 (C-5), 70.0 (C-4), 63.6 (C-6), 55.7 (OMe); ESI-HRMS calcd. for C₂₈H₂₇O₉, 507.1655 found m/z 507.1675 [M+H]⁺.
Methyl 2,3,6-tri-O-benzoyl-4-O-trifluoromethanesulfonyl-α-D-glucopyranoside (230). To a solution of alcohol 234 (616 mg, 1.2 mmol) in dry CH₂Cl₂ (8.1 mL) at 0 °C under an argon atmosphere were added, pyridine (0.2 mL) and trifluoromethanesulfonic anhydride (0.29 mL). The resulting mixture was stirred for 10 min at 0 °C and concentrated under reduced pressure. The resulting residue was taken up in EtOAc, washed with 1 M HCl, sat. NaHCO₃, brine, dried over Na₂SO₄ and the solvent was removed under reduced pressure to give the crude triflate 230 (758 mg, 98%) as a yellow foam and was used in the next step without further purification; ¹H NMR (500 MHz, CDCl₃) δ 8.17 – 7.32 (15H, m, each Ar-H), 6.22 (1H, t, J 9.3, H-3), 5.34 (1H, t, J 9.7, H-4), 5.23 – 5.17 (2H, m, H-1 & H-2, overlapping signals), 4.87 (1H, dd, J 12.6, 2.1, H-6a), 4.48 (1H, dd, J 12.6, 3.8, H-6b), 4.42 (1H, ddd, J 10.0, 3.9, 2.1, H-5), 3.47 (3H, s, OMe); ¹³C NMR (125 MHz, CDCl₃) δ 165.9, 165.6, 165.2 (each C=O), 133.6, 133.5, 133.4, 130.0, 129.9, 129.8, 129.3, 128.7, 128.6, 128.5 (2s), 128.4 (each Ar-C), 96.9 (C-1), 79.2 (C-4), 72.0 (C-2), 69.1 (C-3), 66.7 (C-5), 61.6 (C-6), 56.0 (OMe); ESI-HRMS calcd. C₂₉H₂₅O₁₁F₃NaS, 661.0967 found m/z 661.0980 [M+Na]⁺.

Methyl 2,3,6-tri-O-benzoyl-β-D-galactopyranoside (236). Methyl β-D-galactopyranoside 235 (500 mg, 2.58 mmol), which had been previously dried under high vacuum for 1 h, was taken up in dry DMF (9 mL). Imidazole (437 mg, 6.43 mmol) and TIPSCl (0.7 mL, 3.08 mmol) were added and the reaction mixture was stirred at room temperature for 1 h. Upon completion the reaction mixture was concentrated and the resulting residue was taken up in CH₂Cl₂ (25 mL), washed with H₂O, brine, dried over NaSO₄, filtered and the solvent was removed under reduced pressure. The residue was taken up in dry pyridine (15 mL) cooled to 0 °C and benzoyl chloride (2.4 mL, 20.56 mmol) was added dropwise. The reaction mixture was warmed to room temperature and stirred overnight. The solution was quenched with MeOH (1 mL), diluted with CH₂Cl₂, washed with 1M HCl, sat. NaHCO₃, H₂O, brine, dried over NaSO₄, filtered and the solvent was removed under reduced pressure. Flash
chromatography of the residue (petroleum ether-EtOAc 11:1) gave methyl 2,3,4-tri-O-benzoyl-6-trisopropylsilyl-β-D-galactopyranoside (1.10 g, 65% over 2 steps) as a white foam; IR (film) cm⁻¹: 2943, 2866, 1728, 1451, 1282, 1261, 1094, 1068, 705, 685; ¹H NMR (500 MHz, CDCl₃) δ 8.06 (2H, dd, J 8.2, 1.4), 7.96 (2H, dd, J 8.3, 1.4), 7.80 – 7.74 (2H, m), 7.61 – 7.57 (1H, m), 7.52 – 7.35 (6H, m), 7.23 (2H, t, J 7.8)(each Ar-H), 5.96 (1H, dd, J 3.4, 1.2, H-4), 5.73 (1H, dd, J 10.4, 7.9, H-2), 5.60 (1H, dd, J 10.4, 3.4, H-3), 4.71 (1H, d, J 8.0, H-1), 4.02 (1H, ddd, J 7.3, 6.0, 1.2, H-5), 3.97 – 3.88 (2H, m, H-6a & H-6b overlapping signals), 3.59 (3H, s, OMe), 0.99 (21H, m, each CH & CH₃); ¹³C NMR (125 MHz, CDCl₃) δ 165.6, 165.4, 165.3 (each C=O), 133.2, 133.1 (2s), 129.9, 129.8 (2s), 129.5, 128.5, 128.3, 128.2 (each Ar-C), 102.5 (C-1), 74.3 (C-5), 72.0 (C-3), 70.0 (C-2), 68.0 (C-4), 61.4 (C-6), 57.2 (OMe), 17.9, 11.8 (each CH & CH₃); ESI-HRMS calcd. for C₃₇H₄₆O₉NaSi, 685.2809 found m/z 685.2824 [M+Na]⁺. Under an argon atmosphere, the above silyl ether (1.08 g, 1.62 mmol) was taken up in dry THF (16 mL) and cooled to 0 °C. TBAF (0.8 mL, 2M in THF) was added and the reaction mixture was stirred at 0 °C for 15 min. A second portion of TBAF (0.8 mL, 2M in THF) was added and the mixture was stirred for a further 30 min at 0 °C. The reaction mixture was diluted with Et₂O and washed with brine (x2). The aqueous phase was extracted with Et₂O (x3) and the combined organic layers were dried over NaSO₄, filtered and the solvent was removed under reduced pressure. Flash chromatography of the residue (cyclohexane-EtOAc 9:2) gave 236 (269 mg, 33%) as a white solid. The ¹H and ¹³C NMR data for 236 were in good agreement with those previously reported in the literature¹⁰. IR (film) cm⁻¹: 3511, 2892, 1734, 1717, 1698, 1451, 1297, 1270, 707, 682; ¹H NMR (500 MHz, CDCl₃) δ 8.08 – 8.04 (2H, m), 7.98 (4H, dt, J 8.4, 1.6), 7.61 – 7.56 (1H, m), 7.54 – 7.48 (2H, m), 7.46 (2H, t, J 7.8), 7.41 – 7.35 (4H, m)(each Ar-H), 5.76 (1H, dd, J 10.3, 7.9, H-2), 5.36 (1H, dd, J 10.3, 3.2, H-3), 4.71 (1H, dd, J 11.4, 6.6, H-6a), 4.66 (1H, d, J 7.9, H-1), 4.62 (1H, dd, J 11.4, 6.4, H-6b), 4.35 (1H, d, J 3.1, H-4), 4.08 (1H, td, J 6.5, 1.1, H-5), 3.55 (3H, s, OMe), 2.47 (1H, s, OH); ¹³C NMR (125 MHz, CDCl₃) δ 166.4, 165.8, 165.4 (each C=O), 133.5, 133.3, 133.1, 129.9, 129.8 (2s), 129.5 (2s), 128.9, 128.5, 128.3 (each Ar-C), 102.3 (C-1), 74.1 (C-3), 72.3 (C-5), 69.5 (C-2), 67.3 (C-4), 62.6 (C-6), 56.9 (OMe); ESI-HRMS calcd. for C₂₈H₂₆O₉Na, 529.1475 found m/z 529.1479 [M+Na]⁺.
Methyl 2,3,6-tri-O-benzoyl-4-O-trifluoromethanesulfonyl-β-D-galactopyranoside (231). Triflate was prepared from alcohol 236 according to the identical procedure described for 230. The $^1$H and $^{13}$C NMR data for 231 were in good agreement with those previously reported in the literature $^{11}$. $^1$H NMR (500 MHz, CDCl$_3$) δ 8.07 – 7.35 (15H, m, each Ar-C), 5.74 (1H, dd, J 10.4, 7.9, H-2), 5.56 (1H, dd, J 10.4, 3.0, H-3), 5.53 (1H, d, J 3.0, H-4), 4.81 (1H, dd, J 10.8, 5.6, H-6a), 4.73 (1H, d, J 7.9, H-1), 4.38 – 4.29 (2H, m, H-5 & H-6b overlapping signals), 3.57 (3H, s, OMe); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 165.9, 165.7, 164.9 (each C=O), 133.9, 133.6, 133.4, 130.1, 129.8 (2s), 129.0, 128.9, 128.6, 128.5, 128.4, 128.0 (each Ar-C), 102.4 (C-1), 80.9 (C-4), 70.7 (C-3), 70.3 (C-5), 68.6 (C-2), 60.9 (C-6), 57.3 (OMe).

Methyl 6-(2,3,4-tri-O-benzoyl-5-(S)-methoxycarbonyl-α-L-arabinopyranosylseleno)-6-deoxy-2,3,4-tri-O-benzoyl-α-D-glucopyranose (226β). To a solution of 215 (390 mg, 560 μmol) in dry degassed toluene (1.6 mL) under argon were added Cs$_2$CO$_3$ (365 mg, 1.12 mmol), piperidine (66 μL, 672 μmol) and a solution via cannula of 229 (1.43 g, 2.24 mmol) in dry degassed toluene (4.1 mL). The reaction mixture was warmed to 50 °C and stirred for 1 h. The mixture was cooled and diluted with EtOAc. The organic layer was washed with water. The aqueous phase was extracted with a portion of EtOAc and the combined organic layers were washed with brine, dried over Na$_2$SO$_4$, filtered and the solvent removed under reduced pressure. Flash chromatography of the residue (toluene-EtOAc 14:1) followed by a second purification with (cyclohexane-EtOAc 3:1) gave 226β (495 mg, 83%) as a white foam. Any unreacted triflate was recovered and recycled; [α]$_D$ +131 (c 1, CHCl$_3$); R$_f$ 0.36 (cyclohexane-EtOAc 2:1); IR (film) cm$^{-1}$: 2951, 1769, 1723, 1452, 1249, 704, 686; $^1$H NMR (500 MHz, CDCl$_3$) δ 8.03 – 7.24 (30H, m, each Ar-H), 6.22 (1H, d, J 3.4, H-4’), 6.11 (1H, t, J 9.8, H-3), 5.82 (1H, t, J 10.0, H-2’), 5.65 (1H, dd, J 9.9, 3.4, H-3’), 5.47 (1H, t, J 9.7, H-4).
5.35 (1H, d, J 10.2, H-1’), 5.23 (1H, dd, J 10.2, 3.6, H-2), 5.18 (1H, d, J 3.6, H-1), 4.52 (1H, d, J 1.4, H-5’), 4.41 (1H, td, J 9.6, 2.7, H-5), 3.70 (3H, s, OMe), 3.35 (3H, s, OMe), 3.14 (1H, d, J 3.6, H-1), 4.52 (1H, d, J 1.4, H-5’), 4.41 (1H, td, J 9.6, 2.7, H-5), 3.70 (3H, s, OMe), 3.35 (3H, s, OMe), 3.14 (1H, dd, J 13.4, 9.2, H-6a), 3.04 (1H, dd, J 13.3, 2.7, H-6b); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 166.1, 165.8, 165.7, 165.5, 165.4, 165.3, 165.1 (each C=O), 133.5, 133.4 (2s), 133.3 (2s), 133.1, 132.9 (2s), 129.8 (3s), 129.6, 129.2, 129.0 (2s), 128.9, 128.7, 128.5, 128.4 (3s), 128.3 (3s), 96.7 (C-1), 78.5 (C-1’), 77.1 (C-5’), 72.9 (C-4), 72.2 (C-2 or C-3’), 72.1 (C-2 or C-3’), 70.3, 70.2, 69.3, 69.2, 55.4 (OMe), 52.7 (OMe), 24.8 (C-6); ESI-HRMS calcd. C$_{56}$H$_{48}$O$_{17}$NaSe, 1095.1954 found m/z 1095.1980 [M+Na]$^+$.

Methyl 4-(2,3,4-tri-O-benzoyl-5-(S)-methoxycarbonyl-α-L-arabinopyranosylseleno)-4-deoxy-2,3,6-tri-O-benzoyl-α-D-galactopyranose (227β). To a solution of 215 (275 mg, 392 μmol) in dry degassed toluene (1.2 mL) under argon was added Cs$_2$CO$_3$ (255 mg, 784 μmol), piperidine (46 μL, 470 μmol) and a solution via cannula of 230 (500 mg, 784 μmol) in dry degassed toluene (1.4 mL + 0.6 mL wash) was added. The reaction mixture was warmed to 50 °C and stirred for 1.5 h. The reaction mixture was diluted with EtOAc and the organic layer was washed with water. The aqueous phase was extracted with a portion of EtOAc and the combined organic layers were washed with brine, dried over Na$_2$SO$_4$, filtered and the solvent removed under reduced pressure. Flash chromatography of the crude residue (toluene-EtOAc 14:1) gave 227β (379 mg, 90%) as a white solid. Any unreacted triflate was recovered and reused; [α]$_D$ $+140$ (c 1, CHCl$_3$); R$_f$ 0.39 (cyclohexane-EtOAc 2:1); IR (film) cm$^{-1}$: 2932, 2915, 1763, 1710, 1602, 1450, 1252, 703, 682; $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 8.17 – 7.20 (30H, m, each Ar-H), 6.12 – 6.04 (1H, d, J 3.5, H-4’), 5.88 (1H, dd, J 10.4, 4.4, H-3), 5.75 (1H, t, J 10.0, H-2’), 5.44 (1H, dd, J 10.4, 3.9, H-2), 5.31 (1H, dd, J 9.9, 3.5, H-3’), 5.18 (1H, d, 3.9, H-1), 5.16 (1H, d, J 10.3, H-1’), 4.87 (1H, dd, J 12.3, 7.8, H-6a), 4.84 – 4.80 (1H, H-6b), 4.75 – 4.62 (1H, m, H-5), 4.26 (1H, dd, J 4.5, 1.8, H-4), 4.16 (1H, d, J 1.4, H-5), 3.65 (3H, s, OMe), 3.37 (3H, s, OMe); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 166.1, 165.8 (2s), 165.7, 165.4, 165.3, 165.2 (each C=O), 133.8, 133.5, 133.4, 133.3, 133.2, 133.0, 130.1, 130.0, 129.8 (2s), 129.7 (2s), 129.6, 129.3, 129.2, 128.9, 128.8, 128.7, 128.6 (2s), 128.4 (3s), 128.3 (each Ar-C), 97.2 (C-1), 77.1 (C-5’), 76.8 (C-1’), 71.9 (C-3’), 71.1 (C-2), 70.5 (C-3), 69.6 (C-2’), 69.0
Methyl 4-(2,3,4-tri-O-benzoyl-5-(S)-methoxycarbonyl-α-L-arabinopyranosylseleno)-4-deoxy-2,3,6-tri-O-benzoyl-β-D-glucopyranose (228β). To a solution of 215 (66 mg, 94 μmol) in dry degassed toluene (0.25 mL) under argon were added Cs$_2$CO$_3$ (60 mg, 188 μmol), piperidine (11 μL, 113 μmol) and a solution via cannula of 231 (240 mg, 376 μmol) in dry degassed toluene (0.7 mL). The reaction mixture was stirred at room temperature for 2 h. The mixture diluted with EtOAc and the organic layer was washed with water. The aqueous phase was extracted with a portion of EtOAc and the combined organic layers were washed with brine, dried over Na$_2$SO$_4$, filtered and the solvent removed under reduced pressure. Flash chromatography of the residue (cyclohexane-EtOAc 4:1-3:1) gave 228β (63 mg, 62%) as a white foam. Any unreacted triflate was recovered and recycled; $^1$H NMR (500 MHz, CDCl$_3$) δ 8.13 – 7.14 (30H, m, each Ar-H), 6.24 (1H, dd, J 3.3, 1.5, H-4’), 5.77 (1H, dd, J 11.6, 9.6, H-3), 5.72 (1H, t, J 10.1, H-2’), 5.63 (1H, d, J 10.3, H-1’), 5.60 (1H, dd, J 10.1, 3.3, H-3’), 5.41 (1H, t, J 8.6, H-2), 5.08 (1H, dd, J 12.1, 3.8, H-6a), 4.96 (1H, dd, J 12.1, 1.8, H-6b), 4.68 (1H, d, J 7.5, H-1), 4.58 (1H, d, J 1.4, H-5’), 4.54 – 4.46 (1H, m, H-5), 3.72 (3H, s, OMe), 3.57 – 3.49 (4H, m, H-4 and OMe, overlapping signals); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 166.2, 166.2, 165.9, 165.4, 165.3, 165.2, 165.1 (each C=O), 133.6, 133.3, 133.2, 133.1, 133.0, 130.1, 130.0, 129.8 (2s), 129.7 (2s), 129.4, 129.0, 128.8, 128.7, 128.5 (2s), 128.3 (3s), 128.2 (each Ar-C), 101.9 (C-1), 76.7 (C-5’), 76.5 (C-1’), 74.2 (C-5), 73.5 (C-2), 72.1 (C-3’), 71.2 (C-3), 68.8 (2s)(C-2’ and C-4’), 65.0 (C-6), 57.0 (OMe), 52.8 (OMe), 41.5 (C-4); C$_{56}$H$_{48}$O$_{17}$NaSe, 1095.1954 found m/z 1095.1936 [M+Na]$^+$.

Methyl-6-(2,3,4-tri-O-benzoyl-5-(S)-prop-1-ene-3-oxycarbonyl-α-L-arabinopyranosylseleno)-6-deoxy-2,3,4-tri-O-benzoyl-β-D-glucopyranose (241β).
LiI (265 mg, 1.98 mmol) was added to a solution of methyl ester 226β (352 mg, 0.33 mmol) in anhydrous EtOAc (3.3 mL) and the reaction mixture was heated to reflux for 16 h. Upon cooling the reaction mixture was diluted with water. The aqueous layer was extracted into EtOAc (x2) and the combined organic layers were washed with 10% Na$_2$S$_2$O$_3$, dried over Na$_2$SO$_4$, filtered and the solvent removed under reduced pressure to give the acid intermediate 242 (347 mg, 99%) as an off white solid. The compound was used without further purification; [α]$_D$ +127 (c 1, CHCl$_3$); R$_f$ 0.4 (CH$_2$Cl$_2$-MeOH 9:1); IR (film) cm$^{-1}$: 3591, 3425, 3067, 1721, 1628, 1602, 1260, 1091, 1067, 1025, 704, 685; $^1$H NMR (500 MHz, DMSO/AcOD (1 drop)) δ 7.89–7.33 (30H, m, each Ar-H), 6.07–6.02 (1H, bs, H-4’), 5.87 (1H, t, J 9.8, H-3), 5.72–5.63 (1H, apt m, H-3’), 5.57 (1H, t, J 9.9, H-2’), 5.51–5.39 (2H, m, H-1’ & H-4, overlapping signals), 5.33 (1H, dd, J 10.2, 3.5, H-2), 5.21 (1H, d, J 3.5, H-1), 4.37–4.14 (2H, m, H-5 & H-5’ overlapping signals), 3.29 (3H, s, OMe), 3.11–2.99 (2H, each H-6a & H-6b overlapping signals); ESI-HRMS calcd. C$_{55}$H$_{45}$O$_{17}$Se, 1057.1822 found m/z 1057.1804 [M-H]$^+$.

Acid 242 (120 mg, 113 µmol) was taken up in DMF (1.7 mL) and were added, NaHCO$_3$ (20 mg, 226 µmol) and allyl iodide (26 µL, 283 µmol). The mixture was stirred for 2 h, at which point EtOAc (20 mL) was added and the organic phase was washed with water, brine, were dried over Na$_2$SO$_4$, filtered and the solvent removed under reduced pressure. Flash chromatography of the residue (cyclohexane-EtOAc 2:1) gave 241β (111 mg, 90%) as a white solid; [α]$_D$ +91 (c 1, CHCl$_3$); R$_f$ 0.21 (cyclohexane-EtOAc 3:1); IR (film) cm$^{-1}$: 2934, 1724, 1602, 1451, 1257, 705, 686; $^1$H NMR (500 MHz, CDCl$_3$) δ 8.01–7.24 (30H, m, each Ar-H), 6.22 (1H, dd, J 3.4, 1.4, H-4’), 6.09 (1H, t, J 9.8, H-3), 5.80 (1H, t, J 10.0, H-2’), 5.75 (1H, m, CH$_2$CH=CH$_2$), 5.63 (1H, dd, J 9.9, 3.4, H-3’), 5.45 (1H, t, J 9.7, H-4), 5.33 (1H, d, J 10.4, H-1’), 5.27–5.19 (2H, m, H-2 & CH$_2$CH=CH/H), 5.16 (1H, d, J 3.6, H-1), 5.09 (1H, d, J 10.4, CH$_2$CH=CH/H), 4.62–4.52 (2H, m, CH$_2$CH=CH$_2$), 4.51 (1H, d, J 1.4, H-5’), 4.44–4.35 (1H, td, J 9.5, 2.4, H-5), 3.34 (3H, s, OMe), 3.13 (1H, dd, J 13.3, 9.2, H-6a), 3.02 (1H, dd, J 13.2, 2.8, H-6b); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 165.8, 165.7, 165.5 (2s), 165.3, 165.2, 165.1 (each C=O), 133.5, 133.4, 133.3 (2s), 133.0 (each Ar-C), 130.9 (CH$_2$CH=CH$_2$), 130.0, 129.9, 129.8 (3s), 129.6, 129.3, 129.1, 129.0, 128.9, 128.8, 128.5, 128.4 (3s), 128.3 (2s) (each Ar-C), 119.8 (CH$_2$CH=CH$_2$), 96.7 (C-1), 78.4 (C-1’), 77.2 (C-5’), 72.9 (C-4), 72.2 (C-3’), 72.1 (C-2), 70.3 (C-3), 70.2 (C-5), 69.3 (C-4’), 69.2 (C-2’), 66.5 (CH$_2$CH=CH$_2$), 55.4 (OMe), 24.8 (C-6); ESI-HRMS calcd. C$_{58}$H$_{50}$O$_{17}$NaSe, 1121.2111 found m/z 1121.2117 [M+Na]$^+$. 


Methyl-4-(2,3,4-tri-\(\text{O}\)-benzoyl-5-(S)-prop-1-ene-3-oxycarbonyl-\(\alpha\)-L-arabinopyranosylseleno)-4-deoxy-2,3,6-tri-\(\text{O}\)-benzoyl-\(\alpha\)-D-galactopyranose (243\(\beta\)). LiI (112 mg, 840 \(\mu\)mol) was added to a solution of methyl ester 227\(\beta\) (150 mg, 330 \(\mu\)mol) in anhydrous EtOAc (1.4 mL) and the reaction was heated to reflux for 16 h. Upon cooling the reaction mixture was diluted with water. The aqueous layer was extracted into EtOAc (x2) and the combined organic layers were washed with 10% \(\text{Na}_2\text{S}_2\text{O}_3\), dried over \(\text{Na}_2\text{SO}_4\), filtered and the solvent removed under reduced pressure to give the acid intermediate as a yellow solid. The crude acid intermediate (120 mg, 113 \(\mu\)mol) was taken up in DMF (2.1 mL) and were added, NaHCO\(_3\) (24 mg, 280 \(\mu\)mol) and allyl iodide (33 \(\mu\)L, 350 \(\mu\)mol). The mixture was stirred for 2 h, at which point EtOAc (20 mL) was added and the organic phase was wash with water, brine, were dried over \(\text{Na}_2\text{SO}_4\), filtered and the solvent removed under reduced pressure. Flash chromatography of the residue (cyclohexane-EtOAc 3:1) gave 243\(\beta\) (139 mg, 90% for two steps) as a white solid; \(R_f\) 0.52 (cyclohexane-EtOAc 2:1); IR (film) \(\text{cm}^{-1}\): 3062, 2922, 1765, 1719, 1603, 1248, 1097, 1068, 1205, 704, 686; \(^1\text{H NMR}\) (500 MHz, CDCl\(_3\)) \(\delta\) 8.13 – 7.18 (30H, m, each Ar-\(\text{C}\)), 6.10 (1H, dd, \(J\) 3.6, 1.5, H-4'), 5.88 (1H, dd, \(J\) 10.4, 4.4, H-3), 5.78 – 5.67 (2H, m, H-2' & CH\(_2\)CH=CH\(_2\), overlapping signals), 5.43 (1H, dd, \(J\) 10.4, 3.9, H-2), 5.31 (1H, dd, \(J\) 9.9, 3.5, H-3’), 5.19 – 5.14 (3H, m, CH\(_2\)CH=CH\(_2\), H-1 & H-1’, overlapping signals), 5.02 (1H, dt, \(J\) 10.5, 1.2, CH\(_2\)CH=CH\(_2\)), 4.90 (1H, dd, \(J\) 12.2, 8.2, H-6a), 4.78 (1H, dd, \(J\) 12.3, 2.8, H-6b), 4.72 – 4.66 (1H, m, H-5), 4.55-4.50 (2H, m, CH\(_2\)CH=CH\(_2\)), 4.26 (1H, dd, \(J\) 4.5, 1.7, H-4), 4.17 (1H, d, \(J\) 1.5, H-5’), 3.36 (3H, s, OMe); \(^{13}\text{C NMR}\) (125 MHz, CDCl\(_3\)) \(\delta\) 166.1, 165.8, 165.7, 165.4 (2s), 165.1, 165.0 (each C=O), 133.8, 133.5, 133.4, 133.3, 133.2, 133.0 (each Ar-C), 131.0 (CH\(_2\)CH=CH\(_2\)), 130.1 (2s), 129.8 (2s), 129.7 (2s), 129.6, 129.3, 129.2, 128.9, 128.8, 128.7 (2s), 128.6, 128.4 (2s), 128.4, 128.3 (each Ar-C), 119.6 (CH\(_2\)CH=CH\(_2\)), 97.2 (C-1), 77.1 (C-5’), 76.8 (C-1’), 71.9 (C-3’), 71.2 (C-2), 70.5 (C-3), 69.6 (C-2’), 69.0 (C-4’), 68.3 (C-5), 67.0 (C-6), 55.2 (CH\(_2\)CH=CH\(_2\)), 44.6 (OMe); ESI-HRMS calcd. C\(_{58}\)H\(_{50}\)O\(_{17}\)NaSe, 1121.2111 found \(m/z\) 1121.2107 [M+Na]\(^+\).
3β-(5-(S)-methoxycarbonyl-2,3,4-tri-O-benzoyl-α-L-arabinopyranosylseleno)-5α-cholestan (237β). To dry CH₂Cl₂ (5 mL) were added triphenylphosphine (510 mg, 1.94 mmol), imidazole (132 mg, 1.94 mmol) and iodine (492 mg, 1.94 mmol) followed by a solution of cholestanol 238 (500 mg, 1.29 mmol) in dry CH₂Cl₂ (2 mL). The resulting mixture was stirred at room temperature for 3 h and concentrated under reduced pressure. Flash chromatography of the residue (pentane) gave 3α-iodo-5α-cholestane 239 (530 mg, 82%) as a white solid; ¹H NMR (500 MHz, CDCl₃) δ 4.96 (1H, q, J 3.0, CH), 2.06 – 0.60 (46H, m, CH, CH₂ & CH₃); ¹³C NMR (125 MHz, CDCl₃) δ 56.4, 56.3, 53.8, 42.6, 42.0, 39.9, 39.5, 38.8, 38.3, 36.5, 36.2, 35.8, 35.4, 34.4, 32.7, 31.8, 28.2, 28.0, 27.8, 24.2, 23.9, 22.8, 22.6, 20.8, 18.7, 13.4, 12.1. To a solution of 215 (120 mg, 171 μmol) in dry degassed DMF (1.7 mL) under argon were added Cs₂CO₃ (111 mg, 342 μmol), piperidine (20 μL, 205 μmol) and a solution of 239 (170 mg, 342 μmol) via cannula, in dry degassed toluene (1 mL + 1 mL wash). The reaction mixture was stirred for 60 min at room temperature. EtOAc was added and the organic layer was washed with water. The aqueous phase was extracted with EtOAc (x2) and the combined organic layers were washed with water, brine, dried over Na₂SO₄, filtered and the solvent removed under reduced pressure. Flash chromatography of the residue (cyclohexane-EtOAc 8:1-3:1) gave 237β (126 mg, 77%) as a white foam; [α]D +79 (c 1, CHCl₃); Rf 0.45 (cyclohexane-EtOAc 4:1); IR (film) cm⁻¹: 2931, 2848, 1765, 1761, 1259, 705, 685; ¹H NMR (500 MHz, CDCl₃) δ 8.05 – 7.23 (15H, m each Ar-H), 6.21 (1H, dd, J 3.6, 1.4, H-4), 5.91 (1H, t, J 10.0, H-2), 5.60 (1H, dd, J 10.0, 3.4, H-3), 5.12 (1H, d, J 10.1, H-1), 4.57 (1H, d, J 1.4, H-5), 3.70 (3H, s, OMe), 3.39 – 3.28 (1H, tt, J 12.4, 4.1, CH), 2.11 – 0.54 (46H, m, each CH, CH₂ & CH₃); ¹³C NMR (125 MHz, CDCl₃) δ 166.3, 165.5, 165.2, 165.1 (each C=O), 133.5, 133.3 (2s), 130.0, 129.8, 129.8 (2s), 129.3, 129.0, 128.8, 128.6, 128.4, 128.3 (each Ar-C), 77.6 (C-1), 77.2 (C-5), 72.3 (C-3), 69.5 (C-4), 68.7 (C-2), 56.6, 56.3,
54.5, 52.8 (OMe), 48.0, 42.6, 39.7, 39.5, 38.0, 36.2, 35.8, 35.6, 35.4, 32.1, 29.9, 28.6, 28.2, 28.0, 24.2, 23.8, 22.8, 22.6, 20.9, 18.7, 12.2, 12.1; ESI-HRMS calcd. C_{53}H_{70}O_{9}NaSe, 977.4083 found m/z 977.4041 [M+Na]^+.

**1-Se-Methyl-2,3,4-tri-O-benzoyl-α-seleno-D-galactopyranosiduronic acid, methyl ester (218α).** β-Selenide 218β (70 mg, 117 μmol) was taken up in dry CH₂Cl₂ (17 mL) and cooled to -20 °C. TiCl₄ (0.29 mL, 2.5 eq, 1M in CH₂Cl₂) was added dropwise and the resulting solution was stirred at -20 °C for 20 min. NH₄Cl (1M, 10 mL) was added and the mixture was partitioned between 1M NH₄Cl and EtOAc. The aqueous phase was washed with water, brine dried over Na₂SO₄, filtered and the solvent removed under reduced pressure. Flash chromatography of the residue (cyclohexane-EtOAc 5:1) gave 218α (57 mg, 81%) as a white foam; [α]D +315 (c 0.5, CHCl₃); Rf 0.39 (cyclohexane-EtOAc 3:1); IR (film) cm⁻¹: 1746, 1722, 1601, 1264, 1249, 714; ¹H NMR (500 MHz, CDCl₃) δ 8.05 – 7.26 (15H, m, each Ar-H), 6.36 (1H, d, J 4.0, H-1), 6.26 – 6.23 (1H, broad signal, H-4), 5.84 – 5.77 (2H, H-3 & H-2, overlapping signals), 5.27 (1H, d, J 1.5, H-5), 3.73 (3H, s, OMe), 2.05 (3H, s, SeMe); ¹³C NMR (125 MHz, CDCl₃) δ 167.2, 165.4 (2s), 165.1 (each C=O), 133.6, 133.6, 133.3, 130.0, 129.9, 129.7, 128.9, 128.8, 128.6, 128.5, 128.3 (each Ar-C), 79.9 (C-1), 70.4 (C-5), 69.4 (2s), 68.7 (C-2, C-3 & C-4, overlapping signals), 52.8 (OMe), 3.2 (SeMe); ESI-HRMS calcd. C_{31}H_{29}NO_{9}NaSe, 662.0905 found m/z 662.0904 [M+MeCN+Na]^+.

1-Se-Ethyl-2,3,4-tri-O-benzoyl-α-seleno-D-galactopyranosiduronic acid, methyl ester (219α). β-Selenide 219β (30 mg, 49 μmol) was taken up in dry CH₂Cl₂ (7 mL) and cooled to -20 °C. TiCl₄ (0.12 mL, 2.5 eq, 1M in CH₂Cl₂) was added dropwise and the resulting solution was stirred at -20 °C for 20 min. NH₄Cl (1M, 2 mL) was added and the mixture was partitioned between 1M NH₄Cl and EtOAc. The aqueous phase was extracted into EtOAc
(x2) and the combined organic layers were washed with water, brine dried over Na₂SO₄, filtered and the solvent removed under reduced pressure. Flash chromatography of the residue (cyclohexane-EtOAc 4:1) gave 219α (25 mg, 83%) as a white foam; [α]D +249 (c 1.3, CHCl₃); Rf 0.42 (cyclohexane-EtOAc 3:1); IR (film) cm⁻¹: 2970, 2919, 1761, 1719, 1602, 1452, 1261, 1111, 1095, 1071, 711; ¹H NMR (500 MHz, CDCl₃) δ 8.05 – 7.26 (15H, m, each Ar-H), 6.50 – 6.46 (1H, broad signal, H-1), 6.24 (1H, d, J 1.4, H-4), 5.82 – 5.75 (2H, m, H-2 & H-3, overlapping signals), 5.32 (1H, d, J 1.5, H-5), 3.73 (3H, s, OMe), 2.78 – 2.61 (2H, m, CH₂), 1.42 (3H, t, J 6.9, CH₃); ¹³C NMR (125 MHz, CDCl₃) δ 167.2, 165.4 (2s), 165.1 (each C=O), 133.6, 133.5, 133.3, 130.0, 129.9, 129.7, 129.0 (2s), 128.9, 128.6, 128.5, 128.3 (each Ar-C), 79.3 (C-1), 70.5 (C-5), 69.5, 69.4, 68.6 (C-2, C-3 & C-4, overlapping signals), 52.8 (OMe), 17.5 (CH₂), 15.7 (CH₃); ESI-HRMS calcd. C₃₂H₃₁NO₉NaSe, 676.1062 found m/z 676.1055 [M+MeCN+Na]⁺.

1-Se-Butyl-2,3,4-tri-O-benzoyl-α-seleno-D-galactopyranosiduronic acid, methyl ester (220α). β-Selenide 220β (30 mg, 47 μmol) was taken up in dry CH₂Cl₂ (6.7 mL) and cooled to -20 °C. TiCl₄ (0.12 mL, 2.5 eq, 1M in CH₂Cl₂) was added dropwise and the resulting solution was stirred at -20 °C for 20 min. NH₄Cl (1M, 2 mL) was added and the mixture was partitioned between 1M NH₄Cl and EtOAc. The aqueous phase was extracted into EtOAc (x2) and the combined organic layers were washed with water, brine dried over Na₂SO₄, filtered and the solvent removed under reduced pressure. Flash chromatography of the residue (cyclohexane-EtOAc 4:1) gave 220α (28 mg, 93%) as a white foam; [α]D +192 (c 0.6, CHCl₃); Rf 0.5 (cyclohexane-EtOAc 3:1); IR (film) cm⁻¹: 2956, 2928, 1762, 1717, 1452, 1322, 1251, 704; ¹H NMR (500 MHz, CDCl₃) δ 8.08 – 7.26 (15H, m, each Ar-H), 6.44 (1H, d, J 4.9, H-1), 6.23 (1H, d, J 1.4, H-4), 5.83 – 5.73 (2H, m, H-2 & H-3, overlapping signals), 5.31 (1H, d, J 1.5, H-5), 3.73 (3H, s, OMe), 2.73 (1H, dt, J 12.0, 7.5, CH), 2.64 (1H, dt, J 12.0, 7.5, CH), 1.72 – 1.61 (2H, m, CH₂), 1.37 (2H, h, J 7.4, CH₂), 0.87 (3H, t, J 7.3, CH₃); ¹³C NMR (125 MHz, CDCl₃) δ 167.2, 165.5, 165.4, 165.1 (each C=O), 133.6, 133.5, 133.3, 130.0, 129.9, 129.7, 129.0 (2s), 128.6, 128.5, 128.3 (each Ar-C), 79.6 (C-1), 70.5 (C-5), 69.5, 69.4, 68.7 (C-2, C-3 & C-4, overlapping signals), 52.8 (OMe), 32.4 (CH₂), 23.8
(CH₂), 23.0 (CH₂), 13.5 (CH₃); ESI-HRMS calcd. C₃₄H₄₅NO₉NaSe, 704.1375 found m/z 704.1366 [M+MeCN+Na]+.

1-Se-Isopropyl-2,3,4-tri-O-benzoyl-α-seleno-D-galactopyranosiduronic acid, methyl ester (221α). β-Selenide 221β (30 mg, 48 µmol) was taken up in dry CH₂Cl₂ (7 mL) and cooled to -20 °C. TiCl₄ (0.12 mL, 2.5 eq, 1M in CH₂Cl₂) was added dropwise and the resulting solution was stirred at -20 °C for 25 min. NH₄Cl (1M, 2 mL) was added and the mixture was partitioned between 1M NH₄Cl and EtOAc. The aqueous phase was extracted into EtOAc (x2) and the combined organic layers were washed with water, brine dried over Na₂SO₄, filtered and the solvent removed under reduced pressure. Flash chromatography of the residue (cyclohexane-EtOAc 4:1) gave 221α (29 mg, 97%) as a white foam; [α]D +217 (c 0.7, CHCl₃); Rf 0.46 (cyclohexane-EtOAc 3:1); IR (film) cm⁻¹: 2958, 2861, 1760, 1789, 1452, 1252, 1111, 1095, 1084, 1070, 712, 702; ¹H NMR (500 MHz, CDCl₃) δ 8.06–7.24 (15H, m, each Ar-H), 6.54 (1H, d, J 4.7, H-1), 6.23 (1H, dd, J 3.0, 1.6, H-4), 5.78 (1H, dd, J 10.6, 4.8, H-2), 5.75 (1H, dd, J 10.6, 3.1, H-3), 5.34 (1H, d, J 1.7, H-5), 3.72 (3H, s, OMe), 3.28 (1H, hept, J 6.9, CH), 1.46 (3H, d, J 6.8, CH₃†), 1.44 (3H, d, J 7.0, CH₃†); ¹³C NMR (125 MHz, CDCl₃) δ 167.3, 165.5, 165.4, 165.1 (each C=O), 133.6, 133.5, 133.3, 130.0, 129.9, 129.7, 129.0 (2s), 128.9, 128.6, 128.5, 128.3 (each Ar-C), 79.3 (C-1), 70.6 (C-5), 69.6 (C-3 or C-4), 69.4 (C-3 or C-4), 68.7 (C-2), 52.8 (OMe), 30.8 (CH), 24.8, 24.4 (each CH₃); ESI-HRMS calcd. C₃₃H₃₅NO₉NaSe, 690.1218 found m/z 690.1217 [M+MeCN+Na]+.

1-Se-Cyclopentyl-2,3,4-tri-O-benzoyl-α-seleno-D-galactopyranosiduronic acid, methyl ester (222α). β-Selenide 222β (30 mg, 46 µmol) was taken up in dry CH₂Cl₂ (7 mL) and
cooled to -20 °C. TiCl$_4$ (0.12 mL, 2.5 eq, 1M in CH$_2$Cl$_2$) was added dropwise and the resulting solution was stirred at -20 °C for 20 min. NH$_4$Cl (1M, 1 mL) was added and the mixture was partitioned between 1M NH$_4$Cl and EtOAc. The aqueous phase was extracted into EtOAc (x2) and the combined organic layers were washed with water, brine dried over Na$_2$SO$_4$, filtered and the solvent removed under reduced pressure. Flash chromatography of the residue (cyclohexane-EtOAc 4:1) gave 222α (29 mg, 97%) as a white foam; [α]$_D$ +236 (c 0.4, CHCl$_3$); R$_f$ 0.5 (cyclohexane-EtOAc 3:1); IR (film) cm$^{-1}$: 2958, 1757, 1720, 1452, 1267, \(^1\)H NMR (500 MHz, CDCl$_3$) δ 8.07 – 7.25 (15H, m, each Ar-H), 6.51 (1H, d, $J$ 4.5, H-1), 6.27 – 6.21 (1H, dd, $J$ 3.0, 1.5, H-4), 5.78 (1H, dd, $J$ 10.3, 4.5, H-2), 5.76 (1H, dd, $J$ 10.3, 3.0, H-3), 5.36 (1H, d, $J$ 1.5, H-5), 3.74 (3H, OMe), 3.37 (1H, p, $J$ 7.0, CH), 2.18 – 2.05 (2H, m, CH$_2$), 1.81 – 1.47 (6H, m, each CH$_3$); \(^1\)C NMR (125 MHz, CDCl$_3$) δ 167.3, 165.5, 165.4, 165.1 (each C=O), 133.6, 133.5, 133.3, 130.0, 129.9, 129.7, 129.0 (3s), 128.6, 128.5, 128.3 (each Ar-C), 80.2 (C-1), 70.6 (C-5), 69.5 (2s), 68.7 (C-2, C-3 & C-4, overlapping signals) 52.8 (CH$_3$), 38.6 (CH), 34.6, 34.3, 24.9, 24.5 (each CH$_2$); ESI-HRMS calcld. C$_{35}$H$_{35}$NO$_9$NaSe, 716.1375 found m/z 716.1378 [M+MeCN+Na]$^+$. 

1,4-bis-(5-(S)-methoxycarbonyl-2,3,4-tri-O-benzoyl-β-L-arabinopyranosylseleno)-butane (223α). β-Selenide 223β (30 mg, 24.6 μmol) was taken up in dry CH$_2$Cl$_2$ (3.6 mL) and cooled to -20 °C. TiCl$_4$ (120 μL, 5 eq, 1M in CH$_2$Cl$_2$) was added dropwise and the resulting solution was stirred at -20 °C for 1 h. NH$_4$Cl (1M, 2 mL) was added and the mixture was partitioned between 1M NH$_4$Cl and EtOAc. The aqueous phase was extracted into EtOAc (x2) and the combined organic layers were washed with water, brine dried over Na$_2$SO$_4$, filtered and the solvent removed under reduced pressure. Flash chromatography of the residue (cyclohexane-diethyl ether 3:2) gave 223α (19 mg, 63%) as a white solid; [α]$_D$ +219 (c 1.2, CHCl$_3$); R$_f$ 0.21 (cyclohexane-EtOAc 2:1); IR (film) cm$^{-1}$: 2926, 1761, 1722, 1452, 1250, 1093, 1069, 703, 685; \(^1\)H NMR (500 MHz, CDCl$_3$) δ 8.06 – 7.26 (30H, m, each Ar-H), 6.41 (2H, s, H-1), 6.23 (2H, s, H-4), 5.78 – 5.72 (4H, m, each C-2 and C-3, overlapping signals), 5.26 (2H, d, $J$
1.5, H-5), 3.72 (6H, s, OMe), 2.74 – 2.63 (2H, m, H-1’a), 2.63 – 2.54 (2H, m, H-1’b), 1.80-1.68 (4H, m, CH2); 13C NMR (125 MHz, CDCl3) δ 167.1, 165.4, 165.4, 165.1 (each C=O), 133.6 (2s), 133.3, 129.9 (2s), 129.8, 128.9, 128.8, 128.6, 128.5, 128.3 (each Ar-C), 79.8 (C-1), 70.6 (C-5), 69.4, 69.3, 68.7 (each C-2, C-3 and C-4, overlapping signals), 52.8 (OMe), 30.4, 23.2 (each CH2); ESI-HRMS calcd. for C60H54O18NaSe2, 1245.1538 found m/z 1245.1539 [M+Na]+.

1-Se-allyl-2,3,4-tri-O-benzoyl-α-seleno-D-galactopyranosiduronic acid, methyl ester (224a). β-Selenide 224β (30 mg, 48 μmol) was taken up in dry CH2Cl2 (7 mL) and cooled to -20 °C. TiCl4 (0.24 mL, 5 eq, 1M in CH2Cl2) was added dropwise and the resulting solution was stirred at -20 °C for 30 min. NH4Cl (1M, 2 mL) was added and the mixture was partitioned between 1M NH4Cl and EtOAc. The aqueous phase was extracted into EtOAc (x2) and the combined organic layers were washed with water, brine dried over Na2SO4, filtered and the solvent removed under reduced pressure. Flash chromatography of the residue (cyclohexane-diethyl ether 5:2) gave 224a (22 mg, 73%) as a waxy solid; [α]D +245 (c 0.5, CHCl3); Rf 0.46 (cyclohexane-EtOAc 3:1); IR (film) cm⁻¹: 2956, 1764, 1721, 1452, 1248, 1095, 1068, 703; 1H NMR (500 MHz, CDCl3) δ 8.06 – 7.24 (15H, m, each Ar-C), 6.37 (1H, d, J 4.6, H-1), 6.24 (1H, dd, J 3.0, 1.6, H-4), 5.85 (1H, m, CH2CH=CH2), 5.81 (1H, dd, J 10.3, 4.6, H-2), 5.78 (1H, dd, J 10.3, 3.0, H-3), 5.28 (1H, d, J 1.6, H-5), 5.18 (1H, dt, J 16.9, 1.4, CH2CH=CHH), 5.04 (1H, dd, J 9.9, 1.4, CH2CH=CHH), 3.73 (3H, s, OMe), 3.34 (1H, dd, J 12.3, 8.8, CHHCH=CH2), 3.23 (1H, dd, J 12.1, 6.4, CHHCH=CH2); 13C NMR (125 MHz, CDCl3) δ 167.1, 165.4, 165.3, 165.1 (each C=O), 133.6 (Ar-C), 133.5 (CH2CH=CH2), 133.5, 133.3, 130.0, 129.9, 129.7, 128.9 (2s), 128.8, 128.6, 128.5, 128.3 (each Ar-C), 117.8 (CH2CH=CH2), 79.3 (C-1), 70.7 (C-5), 69.7, 69.3, 68.5 (C-2, C-3 or C-4, overlapping signals), 52.8 (OMe), 25.5 (CH2CH=CH2); ESI-HRMS calcd. C33H31NO9NaSe, 688.1062 found m/z 688.1066 [M+MeCN+Na]+.
1-Se-Propargyl-2,3,4-tri-O-benzoyl-α-seleno-d-galactopyranosiduronic acid, methyl ester (225a). β-Selenide 225β (30 mg, 48 μmol) was taken up in dry CH₂Cl₂ (7 mL) and cooled to 0 °C. TiCl₄ (0.24 mL, 5 eq, 1M in CH₂Cl₂) was added dropwise and the resulting solution was stirred at 0 °C for 90 min. NH₄Cl (1M, 2 mL) was added and the mixture was partitioned between 1M NH₄Cl and EtOAc. The aqueous phase was extracted into EtOAc (x2) and the combined organic layers were washed with water, brine dried over Na₂SO₄, filtered and the solvent removed under reduced pressure. Flash chromatography of the residue (cyclohexane-diethyl ether 2:1) gave 225a (18 mg, 60%) as a white foam; R_f 0.35 (cyclohexane-EtOAc 3:1); IR (film) cm⁻¹: 2973, 1757, 1721, 1452, 1278, 1251, 1105, 1093, 1081, 1068, 702, 683; ¹H NMR (500 MHz, CDCl₃) δ 8.07–7.24 (15H, m, each Ar-H), 6.66 (1H, d, J 5.5, H-1), 6.26 (1H, dd, J 3.6, 1.5, H-4), 5.87 (1H, dd, J 10.5, 5.5, H-2), 5.78 (1H, dd, J 10.5, 3.4, H-3), 5.29 (1H, d, J 1.5, H-5), 3.73 (3H, s, OMe), 3.37 (1H, dd, J 15.7, 2.7, H-1'a), 3.23 (1H, dd, J 15.6, 2.7, H-1'b), 2.22 (1H, t, J 2.4, H-3'); ¹³C NMR (125 MHz, CDCl₃) δ 166.9, 165.4, 165.2, 165.1 (C=O), 133.6, 133.3, 130.0, 129.9, 129.7, 128.8 (2s), 128.7, 128.6, 128.5, 128.3 (each Ar-C), 80.1 (C-1), 80.0 (CH₂C=CH), 71.8 (CH₂C=CH), 71.0 (C-5), 69.5 (C-3), 69.3 (C-4), 68.4 (C-2), 52.8 (OMe), 7.2 (CH₂C=CH); ESI-HRMS calcd. C₃₃H₂₉NO₉NaSe, 686.0905 found m/z 686.0916 [M+MeCN+Na]⁺.

Methyl 6-(2,3,4-tri-O-benzoyl-5-(S)-methoxycarbonyl-β-L-arabinopyranosylseleno)-6-deoxy-2,3,4-tri-O-benzoyl-α-D-glucopyranose (226a). β-Selenide 226β (30 mg, 28 μmol) was taken up in dry CH₂Cl₂ (4.1 mL) and cooled to 0 °C. TiCl₄ (0.28 mL, 10 eq, 1M in CH₂Cl₂) was added dropwise and the resulting solution was warmed to room temperature and stirred for 15 min. NH₄Cl (1M, 2 mL) was added and the mixture was partitioned between 1M NH₄Cl and EtOAc. The aqueous phase was extracted into EtOAc (x2) and the combined
organic layers were washed with water, brine dried over Na₂SO₄, filtered and the solvent removed under reduced pressure. Flash chromatography of the residue (cyclohexane-EtOAc 7:2) gave 226α (20 mg, 67%) as a waxy solid; [α]D° +157 (c 1.8, CHCl₃); Rₜ 0.4 (cyclohexane-EtOAc 2:1); IR (film) cm⁻¹: 2922, 1722, 1602, 1452, 1259, 707; ¹H NMR (500 MHz, CDCl₃) δ 8.06 – 7.26 (30H, m, each Ar-H), 6.56 (1H, d, J 5.1, H-1’), 6.23 (1H, dd, J 3.2, 1.5, H-4’), 6.07 (1H, t, J 9.9, H-3), 5.82 (1H, dd, J 10.6, 3.2, H-3’), 5.78 (1H, dd, J 10.6, 5.1, H-2’), 5.46 (1H, t, J 9.7, H-4), 5.36 (1H, d, J 1.5, H-5’), 5.15 (1H, d, J 3.7, H-1), 5.04 (1H, dd, J 10.2, 3.7, H-2'), 4.24 (1H, ddd, J 10.1, 7.4, 3.0, H-5), 3.64 (3H, s, OMe), 3.38 (3H, s, OMe), 3.08 (1H, dd, J 13.1, 3.1, H-6a), 2.85 (1H, dd, J 13.1, 7.4, H-6b); ¹³C NMR (125 MHz, CDCl₃) δ 167.0, 165.7, 165.6, 165.5, 165.4, 165.3, 165.1 (each C=O), 133.5, 133.4, 133.3, 133.0, 130.1, 129.9 (2s), 129.8, 129.6, 129.2, 129.1, 128.9, 128.8, 128.6, 128.5, 128.4 (2s), 128.3, 128.2, 110.0 (each Ar-C), 96.9 (C-1), 79.8 (C-1’), 72.3 (C-4), 72.1 (C-2), 70.7 (C-5’), 70.2 (C-3), 69.4, 69.2, 68.7 (each C-2’, C-3’ and C-4’, overlapping signals), 68.5 (C-5), 55.7 (OMe), 52.7 (OMe), 24.3 (C-6); ESI-HRMS calcd. C₅₆H₄₈O₁₇NaSe, 1095.1954 found m/z 1095.1958 [M+Na]+.

3β-(5-(S)-methoxycarbonyl-2,3,4-tri-O-benzoyl-β-L-arabinopyranosylseleno)-5α-cholestan (237α). β-Selenide 237β (30 mg, 46 μmol) was taken up in dry CH₂Cl₂ (4.6 mL) and cooled to -20 °C. TiCl₄ (80 μL, 2.5 eq, 1M in CH₂Cl₂) was added dropwise and the resulting solution was stirred at -20 °C for 20 min. NH₄Cl (1M, 1 mL) was added and the mixture was partitioned between 1M NH₄Cl and EtOAc. The aqueous phase was extracted into EtOAc (x2) and the combined organic layers were washed with water, brine dried over Na₂SO₄, filtered and the solvent removed under reduced pressure. Flash chromatography of
the residue (cyclohexane-EtOAc 6:1) gave 237α (27 mg, 90%) as a white solid; [α]D +126 (c 1, CHCl3); Rf 0.48 (cyclohexane-EtOAc 4:1); IR (film) cm⁻¹: 2928, 2850, 1766, 1724, 1451, 1260, 705, 686; ¹H NMR (500 MHz, CDCl3) δ 8.05 – 7.24 (15H, m, each Ar-H), 6.56 (1H, d, J 4.4, H-1), 6.24 – 6.21 (1H, dd, J 3.0, 1.6, H-4), 5.75 (2H, m, H-2 & H-3, overlapping signals), 5.35 (1H, d, J 1.6 H-5), 3.72 (3H, s, OMe), 3.04 (1H, tt, J 12.4, 4.2, CH), 2.04 – 0.57 (46H, m, each CH, CH₂ & CH₃); ¹³C NMR (125 MHz, CDCl3) δ 167.3, 165.5, 165.4, 165.1 (each C=O), 133.5, 133.4, 133.2, 130.0, 129.9, 129.7, 129.0 (2s), 128.6, 128.4, 128.3 (each Ar-C), 79.0 (C-1), 70.6 (C-5), 69.6, 69.5, 68.7 (each C-2, C-3 & C-4, overlapping signals), 56.4, 56.3, 54.3, 52.8 (OMe), 48.0, 42.6, 40.0, 39.8, 39.6, 39.5, 36.7, 36.2, 35.8, 35.6, 35.4, 32.0, 30.7, 30.2, 28.6, 28.2, 28.0, 26.9, 26.9, 24.2, 23.8, 22.8, 22.6, 20.9, 18.7, 12.3, 12.1; ESI-HRMS calcd. C₅₇H₇₃NO₉NaSe, 1018.4348 found m/z 1018.4345 [M+Na]+.

Methyl-6-(2,3,4-tri-O-benzoyl-5-(S)-prop-1-ene-3-oxocarbonyl-β-L-arabinopyranosylseleno)-6-deoxy-2,3,4-tri-O-benzoyl-β-D-glucopyranose (241α). β-Selenide 241β (30 mg, 27 μmol) was taken up in dry CH₂Cl₂ (4 mL) and cooled to 0 °C. TiCl₄ (0.27 mL, 10 eq, 1M in CH₂Cl₂) was added dropwise and the resulting solution was warmed to room temperature and stirred for 10 min. NH₄Cl (1M, 2 mL) was added and the mixture was partitioned between 1M NH₄Cl and EtOAc. The aqueous phase was extracted into EtOAc (x2) and the combined organic layers were washed with water, brine dried over Na₂SO₄, filtered and the solvent removed under reduced pressure. Flash chromatography of the residue (cyclohexane-diethyl ether 2:1) gave 241α (24 mg, 80%) as a white solid; [α]D +106 (c 0.8, CHCl3); Rf 0.29 (cyclohexane-EtOAc 3:1); IR (film) cm⁻¹: 2967, 2851, 1724, 1602, 1451, 1248, 706, 686; ¹H NMR (500 MHz, CDCl3) δ 8.07 – 7.25 (30H, m, each Ar-H), 6.57 (1H, d, J 5.2, H-1’), 6.26 (1H, dd, J 3.3, 1.6, H-4’), 6.08 (1H, t, J 9.9, H-3), 5.83 (1H, dd, J 10.6, 3.3, H-3’), 5.78 (1H, dd, J 10.6, 5.4, H-2’), 5.76 – 5.70 (1H, m, CH₂CH=CH₂), 5.47 (1H, t, J 9.6, H-4), 5.38 (1H, d, J 1.6, H-5’), 5.22 (1H, dd, J 17.1, 1.5, CH₂CH=CHH), 5.16 (1H, d, J 3.7, H-1), 5.07 (1H, dd, J 10.5, 1.4 CH₂CH=CHH), 5.04 (1H, dd, J 10.3, 3.7,
H-2), 4.59 – 4.50 (2H, m, CH₂CH=CH₂), 4.28 – 4.21 (1H, td, J 9.6, 7.3, 3.1, H-5), 3.39 (3H, s, OMe), 3.09 (1H, dd, J 13.1, 3.1, H-6a), 2.87 (1H, dd, J 13.2, 7.3, H-6b); ¹³C NMR (125 MHz, CDCl₃) δ 166.2, 165.7, 165.6, 165.5, 165.4 (2s), 165.1 (each C=O), 133.5 (2s), 133.4, 133.0 (each Ar-C), 130.9 (CH₂CH=CH₂), 130.1, 130.0, 129.9 (2s), 129.8, 129.6, 129.2, 129.1, 129.0 (2s), 128.8 (2s), 128.5, 128.4 (2s), 128.3, 128.2 (each Ar-C), 119.8 (CH₂CH=CH₂), 96.9 (C-1), 79.9 (C-1’), 72.3 (C-4), 72.1 (C-2), 70.8 (C-5’), 70.2 (C-3), 69.4 (C-4’), 69.2 (C-3’), 68.7 (C-2’), 68.5 (C-5), 66.4 (CH₂CH=CH₂), 55.7 (OMe), 24.3 (C-6); ESI-HRMS calcd. C₅₈H₅₀O₁₇NaSe, 1121.2111 found m/z 1121.2137 [M+Na]⁺.

5.4 Chapter 4-Experimental

(2S,3S,4R)-2-Azido-1,3,4-octadecanetriol (52). Trifluoromethanesulfonyl azide was prepared as follows: NaN₃ (2.23 g, 34.6 mmol) was dissolved in H₂O (7 mL) and cooled to 0 °C. With vigorous stirring, CH₂Cl₂ (7 mL) was added followed by the dropwise addition of trifluoromethanesulfonic anhydride (2.9 mL, 17 mmol). The flask was stoppered and stirring was continued at 0 °C for 2 h. A solution of NaHCO₃ (6 mL) was then added while stirring was continued until gas evolution ceased. The reaction mixture was transferred to a separation funnel and the phases were separated. The aqueous was extracted into CH₂Cl₂ (x2) and the combined organic phases were washed with a solution of NaHCO₃ (6 mL). The resulting solution of trifluoromethanesulfonyl azide was used in the next step without further purification. Phytosphingosine 56 (1.8 g, 5.7 mmol) and CuSO₄·5H₂O (9 mg, 57 µmol, 1 mol%) were taken up in H₂O (18 mL). The solution of freshly prepared trifluoromethanesulfonyl azide was added with vigorous stirring followed by the addition of MeOH (54 mL) over 5 min. After 18 h the reaction was diluted with water (50 mL) and the white crystals were collected and washed with aqueous MeOH (1:1) to give 52 (1.8 g, 92%) as a white solid. The ¹H and ¹³C NMR data for 52 were in good agreement with those previously reported in the literature¹²; ¹H NMR (500 MHz, MeOD) δ 3.89 (1H, dd, J 11.6, 3.3, H-1a), 3.72 (1H, dd, J 11.6, 8.0, H-1b), 3.56 (1H, ddd, J 7.9, 4.5, 3.3, H-2), 3.53 – 3.46
(2H, m, H-3 and H-4, overlapping signals), 1.69 – 1.59 (1H, m, CH), 1.57 – 1.47 (1H, m, CH), 1.27 (24H, m, each CH₂), 0.87 (3H, t, J 6.9, CH₃); ¹³C NMR (125 MHz, CDCl₃) δ 74.6 (C-3 or C-4), 71.4 (C-3 or C-4), 65.3 (C-2), 61.1 (C-1), 32.5, 31.6, 29.4 (2s), 29.0, 25.3, 22.3 (each CH₂), 13.0 (CH₃).

(2S,3S,4R)-2-azido-3,4-di-O-benzoyl-1,3,4-octadecanetriol (250). Triol 52 (740 mg, 2.15 mmol) trityl chloride (840 mg, 3.01 mmol) and 4-dimethylaminopyridine (132 mg, 1.08 mmol), which had been previously dried under high vacuum for 3 h, were taken up in dry pyridine (5 mL) and heated to 50 °C for 16 h. Then benzoyl chloride (1.25 mL, 10.8 mmol) was added and the reaction mixture was stirred for a further 4 h at 50 °C. The reaction mixture was then cooled, diluted with EtOAc, washed with sat. NaHCO₃, brine, dried over NaSO₄, filtered and the solvent removed under reduced pressure. The resulting residue was taken up in CH₂Cl₂-MeOH (1:2) 50 mL. To this was added H₂SO₄ (conc) (0.7 mL) and the reaction mixture was allowed to stir at room temperature for 16 h. The mixture was washed with water, sat. NaHCO₃, water, brine, dried over NaSO₄, filtered and the solvent removed under reduced pressure. Flash chromatography of the residue (cyclohexane-EtOAc 8:1) gave 250 (840 mg, 71% over 3 steps) as a white solid; [α]D +13.4 (c 1, CHCl₃); Rf 0.4 (cyclohexane EtOAc 3:1); IR (film) cm⁻¹: 3426, 2920, 2851, 2106, 1723, 1260, 1248, 706, 686; ¹H NMR (500 MHz, CDCl₃) δ 8.06 – 7.98 (4H, m), 7.62 (1H, t, J 7.5), 7.57 (1H, t, J 7.4), 7.48 (2H, t, J 7.8), 7.42 (2H, t, J 7.8)(each Ar-H), 5.60 – 5.50 (2H, m, H-3 & H-4 overlapping signals), 3.99 (1H, q, J 6.0, H-1a), 3.81 (2H, dd, J 10.2, 5.6, H-1b & H-2 overlapping signals), 2.00 – 1.83 (2H, m, CH₂), 1.50 – 1.14 (24H, m, each CH₂), 0.88 (3H, t, J 6.9, CH₃); ¹³C NMR (125 MHz, CDCl₃) δ 166.1, 165.8 (each C=O), 133.7, 133.3, 129.9, 129.7 (2s), 129.1, 128.6, 128.5 (each Ar-H), 73.3 (C-3 or C-4), 72.9 (C-3 or C-4), 63.1 (C-2), 62.2 (C-1), 31.9, 29.8, 29.7 (2s), 29.6 (3s), 29.5, 29.4 (2s), 25.5, 22.7 (each CH₂), 14.1 (CH₃); ESI-HRMS calcd. C₃₂H₄₅N₃O₅Na, 574.3257 found m/z 574.3258 [M+Na]⁺.
(2R,3S,4R)-2-azido-1-bromo-3,4-di-O-benzoyl-3,4-octadecanediol (249). Under a nitrogen atmosphere alcohol 250 (824 mg, 1.49 mmol) was taken up in dry CH$_2$Cl$_2$ (11 mL) and cooled to 0 °C. Triethylamine (0.6 mL, 4.47 mmol) and methanesulfonyl chloride (0.2 mL) were added. The reaction mixture was warmed to room temperature and stirred for 2 h. CH$_2$Cl$_2$ was added and the mixture was washed with water, brine, dried over Na$_2$SO$_4$, filtered and the solvent removed under reduced pressure to give the crude mesylate as a yellow oil. Mesylate and KBr (3.6 g, 30 mmol) which had been previously dried under high vacuum for 6 h, were taken up in dry DMF (22 mL) and heated to 90 °C for 7 h. After cooling the reaction mixture, Et$_2$O was added and the mixture was washed with water (x3), dried over Na$_2$SO$_4$, filtered and the solvent was removed under reduced pressure. Flash chromatography of the residue (cyclohexane-EtOAc 9:1) gave 249 (804 mg, 88% over 2 steps) as a colourless oil which spontaneously solidified over time; [α]$_D$ - 2.4 (c 1, CHCl$_3$); R$_f$ 0.7 (cyclohexane-EtOAc 9:1); IR (film) cm$^{-1}$: 3273, 2920, 2851, 2125, 1715, 1245, 706, 685; $^1$H NMR (500 MHz, CDCl$_3$) δ 8.01 (4H, t, J 7.4), 7.60 (2H, m), 7.46 (4H, m)(each Ar-H), 5.57 – 5.48 (2H, m, H-3 & H-4 overlapping signals), 4.06 (1H, ddd, J 8.9, 5.6, 3.3, H-2), 3.75 (1H, dd, J 11.1, 3.2, H-1a), 3.50 (1H, dd, J 11.0, 9.0, H-1b), 1.94 – 1.80 (2H, m, CH$_2$), 1.49 – 1.17 (24H, m, each CH$_2$), 0.88 (3H, t, J 6.8, CH$_3$); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 165.7, 165.1 (each C=O), 133.7, 133.3, 129.9, 129.7, 129.5, 129.0, 128.6, 128.5 (each Ar-C), 74.2 (C-3 or C-4), 72.9 (C-3 or C-4), 63.4 (C-2), 31.9 (C-1), 31.7, 30.2, 29.7 (2s), 29.6 (3s), 29.5, 29.4 (2s), 29.3 (2s), 22.7 (each CH$_2$), 14.1 (CH$_3$); ESI-HRMS calcd. C$_{32}$H$_{44}$N$_3$O$_4$NaBr, 636.2413 found m/z 636.2407 [M+Na]$^+$. 

1-Se-((2R,3S,4R)-2-azido-3,4-di-O-benzoyl-3,4-octadecyldiol)-2,3,4-tri-O-benzoyl-β-seleno-D-galactopyranosiduronic acid, methyl ester (248). To a solution of 215 (403 mg, 575 μmol) in dry degassed DMF (11 mL) at 0 °C, under argon were added Cs$_2$CO$_3$ (375 mg, 1.15 mmol), piperidine (85μL, 862 μmol) and a solution via cannula of bromide 249 (530 mg, 862 μmol) in dry degassed DMF (4 mL + 2 mL wash). The reaction mixture was stirred for
60 min at 0 °C. Et₂O was added and the organic layer was washed with water. The aqueous phase was extracted with Et₂O (x3) and the combined organic layers were washed with brine, dried over Na₂SO₄, filtered and the solvent removed under reduced pressure. Flash chromatography of the residue (cyclohexane-EtOAc 6:1-4:1) gave 248 (529 mg, 82%) as a white foam. Unreacted bromide was recovered and recycled; Rf 0.38 (cyclohexane-EtOAc 3:1); IR (film) cm⁻¹: 2924, 2853, 2113, 1721, 1451, 1246, 1093, 1068, 705, 685; ¹H NMR (500 MHz, CDCl₃) δ 8.15–7.20 (25H, m, each Ar-H), 6.24 (1H, dd, J 3.5, 1.4, H-4), 5.85 (1H, t, J 10.0, H-2), 5.61 (1H, dd, J 9.9, 3.5, H-3), 5.59–5.52 (2H, m, H-3’ & H-4’, overlapping signals), 5.15 (1H, d, J 10.2, H-1), 4.66 (1H, d, J 1.4, H-5), 4.12 (1H, dt, J 9.8, 3.8, H-1’b), 1.92–1.78 (2H, m, CH₂), 1.42–1.15 (24H, m, each CH₂), 0.88 (3H, t, J 6.9, CH₃); ¹³C NMR (125 MHz, CDCl₃) δ 166.1, 165.9, 165.4, 165.2, 165.2, 165.1 (each C=O), 133.5, 133.5, 133.3 (2s), 130.0, 129.9, 129.8 (2s), 129.7, 129.5, 129.2, 128.9 (2s), 128.7 (2s), 128.6, 128.5, 128.3 (2s) (each Ar-C), 78.3 (C-1), 77.2 (C-5), 75.1 (C-3’ or C-4’), 72.7 (C-3’ or C-4’), 72.1 (C-3), 69.4 (C-4), 68.4 (C-2), 63.8 (C-2’), 52.7 (OMe), 31.9, 30.4, 29.7 (2s), 29.6 (2s), 29.5, 29.4, 29.3 (2s), 25.3 (each CH₂), 24.6 (C-1), 22.7 (CH₂), 14.1 (CH₃); ESI-HRMS calcd. for C₆₀H₂₇N₃O₁₃NaSe, 1140.3737 found m/z 1140.3756 [M+Na]+.

1-Se-((2R,3S,4R)-2-azido-3,4-di-O-benzoyl-3,4-octadecydiol)-2,3,4-tri-O-benzoyl-β-seleno-d-galactopyranosiduronic acid, allyl ester (252). LiI (604 mg, 4.5 mmol) was added to a solution of methyl ester 248 (420 mg, 376 µmol) in anhydrous EtOAc (4.5 mL) and the reaction was heated to reflux for 16 h. Upon cooling the reaction mixture was diluted with water. The aqueous layer was extracted into EtOAc (x2) and the combined organic layers were washed with 10% Na₂S₂O₃, dried over Na₂SO₄, filtered and the solvent removed under reduced pressure to give the acid intermediate 251 (371 mg, 89%) as a yellow solid. The crude acid intermediate (125 mg, 113 µmol) was taken up in DMF (1.7 mL) and were added, NaHCO₃ (20 mg, 226 µmol) and allyl iodide (26 µL, 283 µmol). The mixture was stirred for 16 h, at which point EtOAc (20 mL) was added and the organic phase was wash with water, brine, were dried over Na₂SO₄, filtered and the solvent removed under reduced pressure. Flash chromatography of the residue (cyclohexane-EtOAc 6:1- 4:1) gave 252 (80 mg, 62%)
as a waxy solid; IR (film) cm⁻¹: 2925, 2854, 2112, 1722, 1452, 1256, 1092, 1067, 704, 685; ¹H NMR (500 MHz, CDCl₃) δ 8.15 – 7.22 (25H, m, each Ar-H), 6.26 (1H, dd, J 3.5, 1.5, H-4), 5.84 (1H, t, J 10.0, H-2), 5.74 (1H, dtd, J 16.7, 10.2, 6.1, OCH₂CH=CH₂), 5.61 (1H, dd, J 9.9, 3.5, H-3), 5.58 – 5.51 (2H, m, H-3’ & H-4’, overlapping signals), 5.21 (1H, dd, J 17.1, 1.5, OCH₂CH=CH₂), 5.16 (1H, d, J 10.2, H-1), 5.05 (1H, d, J 10.3, OCH₂CH=CH₂), 4.67 (1H, d, J 1.5, H-5), 4.58 (1H, dd, J 12.8, 6.0, OCH₂CH=CH₂), 4.53 (1H, dd, J 12.8, 6.2, OCH₂CH=CH₂), 4.12 (1H, dt, J 9.5, 3.8, H-2’), 3.43 (1H, dd, J 13.6, 3.5, H-1’a), 3.01 (1H, dd, J 13.6, 9.7, H-1’b), 1.84 (2H, m, CH₂), 1.41 – 1.15 (24H, m, each CH₂), 0.87 (3H, t, J 6.9, CH₃); ¹³C NMR (125 MHz, CDCl₃) δ 166.0, 165.4, 165.2 (2s), 165.2, 165.1 (each C=O), 133.5, 133.3 (2s)(each Ar-C), 130.9 (OCH₂CH=CH₂), 130.0, 129.9 (2s), 129.8, 129.7, 129.5, 129.2, 128.9 (2s), 128.8, 128.7, 128.5 (2s), 128.3 (2s) (each Ar–C), 119.7 (OCH₂CH=CH₂), 78.2 (C-1), 77.2 (C-5), 75.1 (C-3’ or C-4’), 72.7 (C-3’ or C-4’), 72.1 (C-3), 69.4 (C-4), 68.4 (C-2), 66.4 (OCH₂CH=CH₂), 63.8 (C-2’), 31.9, 30.4, 29.7 (2s), 29.6 (2s), 29.5, 29.4, 29.3 (2s), 25.3 (each CH₂), 24.6 (C-1), 22.7 (CH₂), 14.1 (CH₃); ESI-HRMS calcd. for C₆₂H₇₀N₃O₁₃NaSe, 1166.3893 found m/z 1166.3898 [M+Na]⁺.

1-Se-((2R,3S,4R)-2-azido-3,4-di-O-benzoyl-3,4-octadecyldiol)-2,3,4-tri-O-benzoyl-β-seleno-D-galactopyranosiduronic acid, 2-hydroxyethyl ester (253). To a solution of the crude acid intermediate 251 (125 mg, 113 µmol) in DMF (1.7 mL) were added, NaHCO₃ (20 mg, 226 µmol) and 2-iodoethanol (22 µL, 283 µmol). The mixture was stirred for 16 h, at which point EtOAc (20 mL) was added and the organic phase was wash with water, brine, were dried over Na₂SO₄, filtered and the solvent removed under reduced pressure. Flash chromatography of the residue (cyclohexane-EtOAc 2:1–1:1) gave 253 (74 mg, 57%) as a waxy solid; ¹H NMR (500 MHz, CDCl₃) δ 8.14 – 7.21 (25H, m, each Ar-H), 6.32 (1H, d, J 3.4, H-4), 5.89 (1H, t, J 10.0, H-2), 5.62 (1H, dd, J 9.9, 3.4, H-3), 5.59 – 5.50 (2H, m, C-3’ & C-4’, overlapping signals), 5.16 (1H, d, J 10.1, H-1), 4.68 (1H, s, H-5), 4.39 (1H, ddd, J 11.9, 6.3, 2.5, OCH₂CH₂OH), 4.16 – 4.02 (2H, m, H-2’ & OCH₂CH₂OH, overlapping signals), 3.77 – 3.68 (1H, m, OCH₂CH₂OH), 3.67 – 3.59 (1H, m, OCH₂CH₂OH), 3.44 (1H, dd, J 13.7, 3.4, H-1’a), 3.01 (1H, dd, J 13.6, 9.8, H-1’b), 2.42 (1H, t, J 6.7, OCH₂CH₂OH), 1.82 (2H, m, CH₂), 1.41 – 1.14 (24H, m, each CH₂), 0.87 (3H, t, J 6.9, CH₃); ¹³C NMR (125 MHz, CDCl₃)
δ 166.2, 166.0, 165.4, 165.3, 165.2, 165.2 (each C=O), 133.9, 133.5 (2s), 133.4, 133.4, 130.0, 129.9 (2s), 129.7, 129.5, 129.2, 128.9, 128.7 (2s), 128.5 (2s), 128.4, 128.3 (each Ar-C), 78.1

1-Se-((2R,3S,4R)-2-azido-3,4-di-O-benzoyl-3,4-octadecyldiol)-2,3,4-tri-O-benzoyl-a-seleno-D-galactopyranosiduronic acid, methyl ester (247). β-Selenide 248 (300 mg, 269 μmol) was taken up in dry CH₂Cl₂ (32 mL) and cooled to 0 °C. TiCl₄ (2.3 mL, 10 eq, 1M in CH₂Cl₂) was added dropwise and the resulting solution was stirred at room temperature for 30 min. NH₄Cl (1M, 10 mL) was added and the mixture was partitioned between 1M NH₄Cl and EtOAc. The aqueous phase was extracted into EtOAc (x2) and the combined organic layers were washed with water, brine dried over Na₂SO₄, filtered and the solvent removed under reduced pressure. Flash chromatography of the residue (cyclohexane-EtOAc 6:1) gave 247 (260 mg, 87%) as a white solid; Rf 0.2 (cyclohexane-EtOAc 6:1); IR (film) cm⁻¹: 2926, 2853, 2112, 1724, 1636, 1603, 1451, 1248, 1094, 708, 686; ¹H NMR (500 MHz, CDCl₃) δ 8.02-7.24 (25H)(each Ar-H), 6.57 (1H, d, J 5.3, H-1), 6.25 (1H, d, J 3.1, H-4), 5.77 (1H, dd, J 10.6, 5.0, H-2), 5.73 (1H, dd, J 10.5, 3.3, H-3), 5.56 – 5.46 (2H, m, H-3’ & H-4’ overlapping signals), 5.32 (1H, s, H-5), 4.06 (1H, dt, J 8.8, 4.0, H-2’), 3.64 (3H, s, OMe), 3.18 (1H, dd, J 13.2, 3.5, H-1’a), 2.95 (1H, dd, J 13.1, 10.2, H-1’b), 1.84 (2H, m, CH₂), 1.42 – 1.01 (24H, m, each CH₂), 0.87 (3H, t, J 6.8, CH₃); ¹³C NMR (125 MHz, CDCl₃) δ 166.8, 165.9, 165.3 (2s), 165.2, 165.0 (each C=O), 133.5, 133.3, 133.2, 129.9 (2s), 129.8, 129.7, 129.6, 129.2, 129.0, 128.9, 128.7, 128.6 (2s), 128.5, 128.4, 128.3 (each Ar-C), 81.3 (C-1), 75.1 (C-3’ or C-4’), 72.7 (C-3’ or C-4’), 70.6 (C-5), 69.3 (C-3), 69.1 (C-4), 68.5 (C-2), 63.2 (C-2’), 52.7 (OMe), 31.9, 30.3, 29.7 (2s), 29.6 (3s), 29.5, 29.4, 29.3 (2s), 25.3 (each CH₂), 24.5 (C-1’), 22.7 (CH₂), 14.1 (CH₃); ESI-HRMS calcd. for C₆₀H₆₇N₃O₁₄NaSe, 1140.3737 found m/z 1140.3743 [M+Na]⁺.
1-Se-((2R,3S,4R)-2-azido-3,4-di-O-benzoyl-3,4-octadecyldiol)-2,3,4-tri-O-benzoyl-a-seleno-D-galactopyranosiduronic acid, allyl ester (254). β-Selenide 252 (38 mg, 33 μmol) was taken up in dry CH₂Cl₂ (4 mL) and cooled to 0 °C. TiCl₄ (0.3 mL, 10 eq, 1M in CH₂Cl₂) was added dropwise and the resulting solution was stirred at room temperature for 60 min. NH₄Cl (1M, 10 mL) was added and the mixture was partitioned between 1M NH₄Cl and EtOAc. The aqueous phase was extracted into EtOAc (x2) and the combined organic layers were washed with water, brine dried over Na₂SO₄, filtered and the solvent removed under reduced pressure. Flash chromatography of the residue (cyclohexane-EtOAc 8:1) gave 254 (31 mg, 87%) as a white solid; ¹H NMR (500 MHz, CDCl₃) δ 8.06 – 7.26 (25H, m, each Ar-H), 6.56 (1H, d, J 4.9, H-1), 6.28 (1H, dd, J 3.0, 1.5, H-4), 5.80 – 5.67 (3H, m, H-2, H-3 & OCH₂CH=CH₂, overlapping signals), 5.56 – 5.47 (2H, m, C-3’ & C-4’, overlapping signals), 5.34 (1H, d, J 1.5, H-5), 5.20 (1H, dd, J 17.2, 1.5, OCH₂CH=CH₂ ), 5.06 (1H, d, J 10.3, OCH₂CH=CH₂), 4.57 (1H, dd, J 12.8, 6.0, OCH₂CH=CH₂), 4.50 (1H, dd, J 12.8, 6.2, OCH₂CH=CH₂), 4.11 – 3.98 (1H, m, H-2’), 3.19 (1H, dd, J 13.2, 3.4, H-1’a), 2.96 (1H, dd, J 13.2, 10.3, H-1’b), 1.91 – 1.77 (2H, m, CH₂), 1.28 – 1.18 (24H, m, each CH₂), 0.87 (3H, t, J 6.9, CH₃); ¹³C NMR (125 MHz, CDCl₃) δ 166.0, 165.9, 165.4, 165.3, 165.2, 165.0 (each C=O), 133.5, 133.3, 133.2 (each Ar-C), 130.9 (OCH₂CH=CH₂), 130.0, 129.9, 129.8 (2s), 129.6, 129.2, 129.0, 128.9, 128.7, 128.6, 128.5 (2s), 128.4, 128.3 (each Ar-C), 119.7 (OCH₂CH=CH₂), 81.3 (C-1), 75.1 (C-3’ or C-4’), 72.7 (C-3’ or C-4’), 70.7 (C-5), 69.3, 69.1 (2s), 68.5, 66.4 (OCH₂CH=CH₂), 63.1 (C-2’), 31.9, 30.3, 29.7 (2s), 29.6 (3s), 29.5, 29.4, 29.3 (2s), 25.3 (each CH₂), 24.6 (C-1’), 22.7 (CH₂), 14.1 (CH₃); ESI-HRMS calcd. for C₆₂H₄₉N₃O₁₃NaSe, 1166.3893 found m/z 1166.3872 [M+Na]⁺.
1-Se-((2R,3S,4R)-2-azido-3,4-di-O-benzoyl-3,4-octadecyldiol)-2,3,4-tri-O-benzoyl-α-seleno-D-galactopyranosiduronic acid, 2-hydroxyethyl ester (255). β-Selenide 253 (20 mg, 17 μmol) was taken up in dry CH₂Cl₂ (2.2 mL) and cooled to 0 °C. TiCl₄ (0.3 mL, 10 eq, 1M in CH₂Cl₂) was added dropwise and the resulting solution was stirred at room temperature for 60 min. NH₄Cl (1M, 10 mL) was added and the mixture was partitioned between 1M NH₄Cl and EtOAc. The aqueous phase was extracted into EtOAc (x2) and the combined organic layers were washed with water, brine dried over Na₂SO₄, filtered and the solvent removed under reduced pressure. Flash chromatography of the residue (cyclohexane-EtOAc 3:1) gave 255 (18 mg, 90%) as a white solid; ¹H NMR (500 MHz, CDCl₃) δ 8.07 – 7.24 (25H, m, each Ar-H), 6.57 (1H, d, J 5.4, H-1), 6.35 (1H, dd, J 3.3, 1.5, H-4), 5.81 (1H, dd, J 10.5, 5.4, H-2), 5.76 (1H, dd, J 10.6, 3.3, H-3), 5.54 (1H, t, J 5.2, H-3’), 5.49 (1H, dt, J 9.0, 4.4, H-4’), 5.36 (1H, d, J 1.5, H-5), 4.35 (1H, ddd, J 11.7, 6.5, 2.5, OCH₂CH₂OH), 4.10 – 3.99 (2H, m, H-2’ & OCH₂CH₂OH, overlapping signals), 3.74 (1H, ddt, J 12.9, 6.4, 2.4, OCH₂CH₂OH), 3.62 (1H, ddt, J 13.0, 6.6, 2.4, OCH₂CH₂OH), 3.18 (1H, dd, J 13.1, 3.4, H-1’a), 2.97 (1H, dd, J 13.2, 10.2, H-1’b), 2.41 (1H, t, J 6.7, OCH₂CH₂OH), 1.92 – 1.76 (2H, m, CH₂), 1.27 – 1.15 (24H, m, each CH₂), 0.87 (3H, t, J 6.9, CH₃); ¹³C NMR (125 MHz, CDCl₃) δ 166.1, 166.0 (2s), 165.3, 165.3, 165.2 (each C=O), 133.9, 133.6, 133.6, 133.4, 133.3, 130.0 (2s), 129.9, 129.8, 129.7, 129.6, 129.1, 128.7 (2s), 128.6 (2s), 128.5 (2s), 128.3 (each Ar-C), 81.2 (C-1), 75.1 (C-3’), 72.7 (C-4’), 70.5 (C-5), 69.3 (C-4), 69.2 (C-3), 68.5 (C-2), 67.6 (OCH₂CH₂OH), 63.1 (C-2’), 60.6 (OCH₂CH₂OH), 31.9, 30.2, 29.7 (2s), 29.6 (3s), 29.5, 29.4, 29.3 (3s) (each CH₂), 24.5 (C-1’), 22.7 (CH₂), 14.1 (CH₃); ESI-HRMS calcd. for C₆₁H₄₉N₅O₁₄NaSe, 1170.3842 found m/z 1170.3831 [M+Na]⁺.
1-Se-((2R,3S,4R)-2-azido-3,4-octadecyldiol)-α-seleno-D-galactopyranosiduronic acid (258). LiI (532 mg, 3.9 mmol) was added to a solution of methyl ester 247 (440 mg, 394 μmol) in anhydrous EtOAc (4.5 mL) and the reaction was heated to reflux for 16 h. Upon cooling the reaction mixture was diluted with water. The aqueous layer was extracted into EtOAc (x2) and the combined organic layers were washed with 10% Na2S2O3, dried over Na2SO4, filtered and the solvent removed under reduced pressure to give the acid intermediate 251 (389 mg, 89%) as a yellow solid. To a solution of the crude acid (389 mg, 353 μmol) in a mixture of THF (50 mL) and MeOH (50 mL) was added NaOMe (1 M in methanol, 5 mL). The reaction mixture was stirred for 4 h at room temperature. Water (1.4 mL) was added and the mixture stirred for another 12 h. Acetic acid (0.2 mL) was added and the solvent was removed under reduced pressure. Flash chromatography of the crude residue (Rf 0.23, MeOH-CH2Cl2-H2O 25:65:4) gave 258 (171 mg, 74% for two steps) as a white solid; 1H NMR (500 MHz, CD3CO2D (1 drop)/DMSO-D6) δ 5.79 (1H, d, J 5.3, H-1), 4.52 (1H, s, H-5), 4.07 (1H, dd, J 9.8, 5.2, H-2), 3.62 (1H, dt, J 8.5, 3.8, H-2'), 3.44 (2H, m, H-3 & H-3' overlapping signals), 3.35 (1H, t, J 7.9, H-4'), 2.82 (1H, dd, J 13.0, 3.4, H-1'a), 2.68 (1H, dd, J 13.0, 10.0, H-1'b), 1.51 (1H, m, CH), 1.38 (1H, m, CH), 1.15 (24H, s, each CH2), 0.75 (3H, t, J 6.6, CH3); ESI-HRMS calcd. for C24H44N3O8Se, 582.2294 found m/z 582.2308 [M-H]-.

(2R,3S,4R)-2-azido-1-iodo-3,4-O-isopropylidine-3,4-octadecanediol (53). Sc(OTf)3 (86 mg, 0.175 mmol) was added to a suspension of (2S,3S,4R)-2-azido-octadecane-1,3,4-triol 52 (600 mg, 1.75 mmol) in a 1:1 mixture of acetone and dimethoxypropane (156 mL) at 0 °C resulting in complete dissolution of the solid. The reaction mixture was stirred at room temperature for 20 min, neutralised with triethylamine and the solvent was removed under reduced pressure. The resulting residue was dissolved in CH2Cl2 and washed with sat.
NaHCO₃. The aqueous phase was extracted into CH₂Cl₂ (x3). The combined organic layers were dried over NaSO₄, filtered and the solvent removed under reduced pressure to provide a mixture of acetals. The crude mixture was taken up in CH₂Cl₂/H₂O (78 mL, 10:1) and treated with 50% TFA in water (0.9 mL). After 10 minutes TLC analysis showed complete hydrolysis of the acyclic acetal. The reaction mixture was neutralised with triethylamine and diluted with sat. NaHCO₃. The phases were separated and the aqueous extracted into CH₂Cl₂ (x2). The combined organic layers were dried over NaSO₄, filtered and the solvent was removed under reduced pressure. Flash chromatography of the residue (petroleum ether-EtOAc 9:1) gave (2S,3S,4R)-2-azido-3,4-O-isopropylidene-1,3,4-octadecanetriol. (507 mg, 76%) as a colourless oil. The ¹H and ¹³C NMR data for the compound were in good agreement with those previously reported in the literature. ¹H NMR (500 MHz, CDCl₃) δ 4.18 (1H, dt, J 9.3, 4.1, H-4), 4.02 – 3.93 (2H, m, H-1b & H-3 overlapping signals), 3.87 (1H, dt, J 12.0, 6.3, H-1a), 3.47 (1H, dt, J 9.5, 4.9, H-2), 2.08 (1H, t, J 6.2, OH), 1.65 – 1.51 (3H, m), 1.43 (3H, s, CH₃), 1.30 (26H, m, CH₂ & CH₃), 0.88 (3H, t, J 6.8, CH₃); ¹³C NMR (125 MHz, CDCl₃) δ 108.4 (C CH₃CH₃), 77.7 (C-4), 76.7 (C-3), 64.0 (C-1), 61.2 (C-2), 31.9, 29.7 (4s), 29.6 (3s), 29.5, 29.4 (2s) (each CH₂), 28.0 (CH₃), 26.5 (CH₂), 25.6 (CH₃), 22.7 (CH₂), 14.1 (CH₃). The above intermediate (50 mg, 0.130 mmol) was taken up in dry CH₂Cl₂ (1.2 mL) and cooled to 0 °C. To this was added triethylamine (60 μL, 0.4 mmol) and methanesulfonyl chloride (20 μL, 0.26 mmol). The reaction mixture was warmed to room temperature at stirred for 2 h. The mixture was diluted with CH₂Cl₂, washed with water, brine dried over Na₂SO₄, filtered and the solvent was removed under reduced pressure to give the crude mesylate (57 mg, 95 %) as an orange solid. This intermediate was used without further purification; ¹H NMR (500 MHz, CDCl₃) δ 4.67 (1H, dd, J 10.8, 2.6, H-1a), 4.31 (1H, dd, J 10.8, 7.8, H-1b), 4.23 – 4.14 (1H, m, H-4), 3.90 – 3.82 (1H, m, H-3), 3.71 (1H, td, J 9.3, 8.4, 2.5, H-2), 3.09 (3H, s, CH₃), 1.75 – 1.15 (32H, m, H-5a, H-5b, CH₂ & CH₃), 0.88 (3H, t, J 6.8, CH₃); ¹³C NMR (125 MHz, CDCl₃) δ 108.7 (CCH₂CH₃), 77.6 (C-4), 75.4 (C-3), 69.9 (C-1), 59.4 (C-2), 37.6 (CH₃), 31.9, 29.7 (2s), 29.6 (3s), 29.5, 29.4, 29.2 (each CH₂), 28.0 (CH₃), 26.6 (CH₂), 25.4 (CH₃), 22.7 (CH₂), 14.1 (CH₃); ESI-HRMS calcd. C₂₂H₄₄N₃O₅S, 462.3002 found m/z 462.3015 [M+H]⁺. To a solution of the crude mesylate (458 mg, 0.99 mmol) in dry acetone (20 mL) was added NaHCO₃ (406 mg, 4.7 mmol), Na₂SO₃ (300 mg, 2.38 mmol) and NaI (1.5 g, 9.9 mmol). The resulting mixture was heated at reflux for 16 h. Upon cooling Et₂O and water were added. The layers were separated and the organic layer was washed with brine. The aqueous layer was extracted into Et₂O and the combined organic layers were dried over Na₂SO₄, filtered and the solvent was removed under reduced pressure. Flash
chromatography (cyclohexane-EtOAc 10:1) of the crude residue gave 53 (309 mg, 63%) as a colourless oil. The $^1$H and $^{13}$C NMR data for 53 were in good agreement with those previously reported in the literature$^{13}$; $^1$H NMR (500 MHz, CDCl$_3$) δ 4.15 (1H, ddd, $J$ 9.0, 5.5, 2.9, H-4), 3.82 (1H, dd, $J$ 8.5, 5.3, H-3), 3.65 – 3.53 (1H, m, H-1a), 3.47 – 3.34 (2H, m, H-1b & H-2 overlapping signals), 1.63 – 1.48 (3H, m, CH$_2$ & CH), 1.41 (3H, s, CH$_3$), 1.33 (3H, s, CH$_3$), 1.26 (23H, m, each CH$_2$ & CH), 0.88 (3H, t, $J$ 6.9, CH$_3$); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 108.4 (CCH$_3$CH$_3$), 78.4 (C-3), 77.6 (C-2), 61.2 (C-4), 31.9, 29.7 (4s), 29.6 (2s), 29.5, 29.4 (each CH$_2$), 28.1 (CH$_3$), 26.5 (CH$_2$), 25.5 (CH$_3$), 22.7 (CH$_2$), 14.1 (CH$_3$), 7.3 (C-1).

(2R,3S,4R)-2-azido-1-iodo-3,4-di-O-tetra-tert-butyldimethylsilyl-3,4-octadecanediol (264). To a stirred solution of 52 (300 mg, 0.87 mmol) in 2,6-lutidine (3.2 mL) and CH$_2$Cl$_2$ (3.2 mL) at 0 °C was added TBSOTf (1.2 mL, 5.24 mmol) dropwise. After stirring at room temperature for 2 h the reaction was quenched with MeOH (2 mL). The solvent was removed under reduced pressure and the remaining 2,6-lutidine was azeotropically removed with toluene. The resulting residue was taken up in EtOAc and washed with sat. NaHCO$_3$, brine, dried over Na$_2$SO$_4$, filtered and the solvent was removed under reduced pressure. Flash chromatography of the residue (petroleum ether) gave the silyl ester intermediate (484 mg, 81%) as a colourless oil. The $^1$H and $^{13}$C NMR data for the compound were in good agreement with those previously reported in the literature$^{14}$; $^1$H NMR (400 MHz, CDCl$_3$) δ 3.99 (1H, dd, $J$ 10.4, 2.3), 3.74 – 3.65 (2H, m), 3.60 (1H, dd, $J$ 5.8, 2.6), 3.58 – 3.55 (1H, m), 1.58 – 1.19 (26H, m, each CH$_2$), 0.91 (9H, s), 0.90 (9H, s), 0.89 (9H, s), 0.88 (3H, t $J$ 6.8) 0.10 – 0.06 (18H, m); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 75.8, 74.6, 65.3, 64.2, 32.8, 31.9, 29.9, 29.7 (2s), 29.6, 29.4, 26.0 (2s), 25.8, 25.3, 22.7, 18.3, 18.2 (2s), 14.1, -4.0, -4.1, -4.5, -4.7, -5.5 (2s); ESI-HRMS calcd. C$_{36}$H$_{79}$N$_3$O$_3$Si$_3$, 708.5327 found m/z 708.5306 [M+Na]$^+$. To a stirred solution of the above intermediate (475 mg, 0.69 mmol) in THF (16 mL) at 0 °C was added, 10% aqueous trifluoroacetic acid (v/v 6 mL) dropwise. After 16 h, 15% NaOH was added and the reaction mixture was diluted with EtOAc, washed with sat. NaHCO$_3$, brine, dried over Na$_2$SO$_4$, filtered and the solvent was removed under reduced pressure. Flash chromatography of the residue (petroleum ether-EtOAc 30:1) gave the primary alcohol (308 mg, 78%) as a colourless oil. The $^1$H and $^{13}$C NMR data for the compound were in good
agreement with those previously reported in the literature\textsuperscript{14}; \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) \(\delta\) 3.94 – 3.85 (1H, m), 3.78 – 3.67 (4H, m), 2.37 (1H, t, \(J\ 5.9,\ \text{OH}\), 1.61 – 1.20 (26H, m, each CH\textsubscript{2}), 0.91 (18H, s, CH\textsubscript{3}), 0.88 (3H, t \(J\ 7.2,\ \text{CH}_3\)), 0.13 (3H, s, CH\textsubscript{3}), 0.11 (6H, s, each CH\textsubscript{3}), 0.09 (3H, s, CH\textsubscript{3}); \textsuperscript{13}C NMR (100 MHz, CDCl\textsubscript{3}) \(\delta\) 76.0, 75.5, 64.6, 62.1, 33.8, 31.9, 29.8, 29.7, 29.7 (6s), 29.6 (4s), 29.5, 29.4, 26.0, 25.5, 22.7, 18.2, 14.1, -4.0, -4.2, -4.5, -4.8; ESI-HRMS calcd. C\textsubscript{30}H\textsubscript{66}N\textsubscript{3}O\textsubscript{3}Si\textsubscript{2}, 572.4643 found m/z 572.4642 \([\text{M+H}]^+\). The primary alcohol (50 mg, 0.087 mmol) was taken up in dry CH\textsubscript{2}Cl\textsubscript{2} (0.8 mL) and cooled to 0 °C. To this was added triethylamine (40 μL, 0.26 mmol) and methanesulfonyl chloride (14 μL, 0.17 mmol). The reaction mixture was warmed to room temperature at stirred for 2 h. The mixture was diluted with CH\textsubscript{2}Cl\textsubscript{2}, washed with water, brine dried over Na\textsubscript{2}SO\textsubscript{4}, filtered and the solvent was removed under reduced pressure to give the crude mesylate (50 mg, 88 %) as an orange solid. This intermediate was used without further purification; \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) \(\delta\) 4.62 (1H, dd, \(J\ 10.7,\ 2.7,\ \text{H-1a}\)), 4.21 (1H, dd, \(J\ 10.7,\ 8.9,\ \text{H-1b}\)), 3.97 (1H, ddd, \(J\ 8.8,\ 4.1,\ 2.7,\ \text{H-2}\)), 3.73 – 3.66 (2H, m, H-3 & H-4, overlapping signals), 3.06 (3H, s, CH\textsubscript{3}), 1.61 – 1.45 (2H, m, H-5a & H-5b), 1.26 (24H, s, each CH\textsubscript{2}), 0.92 – 0.90 (18H, m, each CH\textsubscript{2}), 0.88 (3H, s, CH\textsubscript{3}), 0.13 (3H, s, CH\textsubscript{3}), 0.11 (3H, s, CH\textsubscript{3}), 0.10 (3H, s, CH\textsubscript{3}), 0.09 (3H, s, CH\textsubscript{3}); \textsuperscript{13}C NMR (125 MHz, CDCl\textsubscript{3}) \(\delta\) 75.6 (C-3 or C-4), 75.1 (C-3 or C-4), 69.8 (C-1), 62.4 (C-2), 37.5 (CH\textsubscript{3}), 33.9, 31.9, 29.7, 29.7 (2s), 29.6 (2s), 29.5, 29.3, 26.0 (2s), 25.9 (2s), 25.1, 22.7, 18.2, 18.1, 14.1, -4.2, -4.2, -4.5, -4.7; ESI-HRMS calcd. C\textsubscript{31}H\textsubscript{68}N\textsubscript{3}O\textsubscript{5}Si\textsubscript{2}S, 650.4418 found m/z 650.4403 \([\text{M+H}]^+\). To a solution of the crude mesylate (494 mg, 0.76 mmol) in dry acetone (25 mL) was added NaHCO\textsubscript{3} (620 mg, 7.3 mmol), Na\textsubscript{2}SO\textsubscript{3} (460 mg, 3.65 mmol) and NaI (2.3 g, 15.2 mmol). The resulting mixture was heated at reflux for 16 h. Upon cooling Et\textsubscript{2}O and water were added. The layers were separated and the organic layer was washed with brine. The aqueous layer was extracted into Et\textsubscript{2}O and the combined organic layers were dried over Na\textsubscript{2}SO\textsubscript{4}, filtered and the solvent was removed under reduced pressure. Flash chromatography (cyclohexane-EtOAc 15:1) of the crude residue gave 264 (468 mg, 90%) as a colourless oil; \textsuperscript{1}H NMR (500 MHz, CDCl\textsubscript{3}) \(\delta\) 3.75 – 3.68 (2H, m, H-2 & H-4, overlapping signals), 3.64 (1H, dd, \(J\ 5.0,\ 3.3,\ \text{H-3}\)), 3.53 (1H, dd, \(J\ 10.6,\ 2.8,\ \text{H-1a}\)), 3.21 (1H, dd, \(J\ 10.6,\ 8.9,\ \text{H-1b}\)), 1.61 – 1.54 (1H, m, H-5a), 1.52 – 1.44 (1H, m, H-5b), 1.27 (24H, m, each CH\textsubscript{2}), 0.92 (9H, s, each CH\textsubscript{3}), 0.90 (9H, s, each CH\textsubscript{3}), 0.88 (3H, t, \(J\ 7.0,\ \text{CH}_3\)), 0.14 (3H, s, CH\textsubscript{3}), 0.12 (3H, s, CH\textsubscript{3}), 0.11 (3H, s, CH\textsubscript{3}), 0.09 (3H, s, CH\textsubscript{3}); \textsuperscript{13}C NMR (125 MHz, CDCl\textsubscript{3}) \(\delta\) 77.5, 75.0, 65.6, 33.5, 31.9, 29.8, 29.7 (2s), 29.6 (3s), 29.3, 26.0 (2s), 25.2, 22.7, 18.2, 18.1, 14.1 (CH\textsubscript{3}), 7.0 (C-1), -4.0, -4.2, -4.4, -4.5 (each CH\textsubscript{3}).
(2R,3S,4R)-2-azido-1-iodo-3,4-di-O-benzyl-3,4-octadecanediol (265). 52 (500 mg, 1.46 mmol), trityl chloride (550 mg, 1.96 mmol) and 4-dimethylaminopyridine (90 mg, 0.73 mmol), which had been previously dried under high vacuum for 3 h, were taken up in dry pyridine (4 mL) and heated to 50 °C for 16 h. Upon cooling the reaction mixture was poured onto sat. NaHCO$_3$ and extracted into EtOAc (x2) the combined organic layers were dried over Na$_2$SO$_4$ and the solvent was removed under reduced pressure. Flash chromatography of the residue (petroleum ether-EtOAc 9:1-8:2) gave the tritylated intermediate (688 mg, 81%) as a colourless oil. To a solution of this intermediate (676 mg, 1.15 mmol) and benzyl bromide (0.6 mL, 5.06 mmol) in dry dimethylformamide (8 mL) under nitrogen, was added NaH (122 mg, 5.29 mmol). After stirring for 4 h, methanol was added and the reaction mixture was poured onto brine, extracted with petroleum ether (x2), dried over Na$_2$SO$_4$, filtered and the solvent was removed under reduced pressure. Flash chromatography of the residue (petroleum ether-EtOAc 98:2-97:3) gave the protected intermediate (867 mg, 98%) as a colourless oil. The $^1$H and $^{13}$C NMR data for the compound were in good agreement with those previously reported in the literature\textsuperscript{15}; IR (film) cm$^{-1}$: 2924, 2853, 2095, 1492, 1449, 1068, 744, 695; $^1$H NMR (500 MHz, CDCl$_3$) δ 7.46 – 7.06 (25H, m, each Ar-H), 4.60 – 4.53 (2H, m, C$_2$H$_2$C$_6$H$_5$), 4.48 – 4.39 (2H, m, C$_2$H$_2$C$_6$H$_5$), 3.76 (1H, ddd, J 8.3, 5.7, 2.9, H-2), 3.56 – 3.46 (3H, m, H-1b, H-3 & H-4, overlapping signals), 3.37 (1H, dd, J 8.3, 5.7, 2.9, H-2), 3.56 – 3.46 (3H, m, H-1b, H-3 & H-4, overlapping signals), 3.37 (1H, dd, J 10.0, 8.3, H-1a), 1.64 – 1.55 (1H, m, H-5a), 1.49 (1H, m, H-5b), 1.24 (2H, m, each CH$_2$), 0.88 (3H, t, J 6.9, CH$_3$); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 143.7, 138.3 (2s), 137.9, 128.7, 128.4, 128.3, 128.2, 127.9, 127.8 (2s), 127.6 (2s), 127.5, 127.0 (each Ar-C), 87.2, 79.4, 79.2, 73.5, 72.1, 72.0, 64.3, 63.2, 31.9, 29.8 (2s), 29.7 (3s), 29.6 (2s), 29.4, 25.3, 22.7, 14.1 (CH$_3$). The above intermediate (847 mg, 1.11 mmol) and p-toluenesulfonic acid (50 mg) were taken up in dry dichloromethane-methanol (20 mL, 3:1) and stirred at room temperature for 16 h. The reaction mixture was washed with sat. NaHCO$_3$ and extracted with EtOAc. The organic layer was dried over Na$_2$SO$_4$ and the solvent was removed under reduced pressure. Flash chromatography of the residue (petroleum ether-EtOAc 9:1) gave the primary alcohol (496 mg, 86%) as a colourless oil. The $^1$H and $^{13}$C NMR data for the compound were in good agreement with those previously reported in the literature\textsuperscript{16}; IR (film) cm$^{-1}$: 3437, 2923, 2853, 2096, 1455, 1058, 1028, 733, 696; $^1$H NMR (500 MHz, CDCl$_3$) δ 7.39 – 7.27 (10H, m, each Ar-H), 4.71 (1H, d,
J 11.3, 4.67 (1H, d, J 11.3), 4.63 (1H, d, J 11.4), 4.57 (1H, d, J 11.4)(each CH₂C₆H₃), 3.90 (1H, dt, J 11.2, 5.5, H-1a), 3.80 (1H, ddd, J 11.8, 6.9, 5.1, H-1b), 3.73 – 3.62 (3H, m, H-2, H-3 & H-4 overlapping signals), 2.52 – 2.46 (1H, m, OH), 1.74 – 1.64 (1H, m, H-5a), 1.61 – 1.51 (1H, m, H-5b), 1.47 – 1.14 (24H, m, each CH₂), 0.88 (3H, t, J 6.8, CH₃); ¹³C NMR (125 MHz, CDCl₃) δ 137.9, 137.6, 128.5, 128.4, 128.1, 128.0 (2s), 127.8 (each Ar-C), 80.5, 79.0, 73.6 (CH₂C₆H₃), 72.5 (CH₂C₆H₃), 63.1, 62.3 (C-1), 31.9, 30.2, 29.7 (3s), 29.6 (2s), 29.4, 25.5, 22.7 (each CH₂), 14.1 (CH₃); ESI-HRMS calcd. C₃₂H₄₉N₃O₃Na, 546.3672 found m/z 546.3676 [M+Na]⁺.

The primary alcohol (380 mg, 0.726 mmol) was taken up in dry CH₂Cl₂ (5.6 mL) and cooled to 0 °C. To this was added triethylamine (0.4 mL, 2.9 mmol) and methanesulfonyl chloride (85 μL, 1.09 mmol). After 30 min at 0 °C Et₂O and a solution of sat. NaHCO₃ were added and the resulting mixture stirred for a further 30 min. The mixture was then poured onto sat. NaHCO₃ and Et₂O. The organic layer was separated and washed with a further portion of sat. NaHCO₃, brine, dried over Na₂SO₄, filtered and the solvent was removed under reduced pressure to give the crude mesylate; ¹H NMR (500 MHz, CDCl₃) δ 7.38 – 7.28 (10H, m, each Ar-H), 4.65 (2H, s), 4.58 – 4.55 (2H, m)(each CH₂C₆H₃), 4.52 (1H, dd, J 11.0, 2.9, H-1a), 4.30 (1H, dd, J 10.9, 8.3, H-1b), 3.96 (1H, dt, J 7.8, 3.4, H-2), 3.65 – 3.60 (2H, m, H-3 & H-4, overlapping signals), 2.95 (3H, s, CH₃) 1.73 – 1.54 (2H, m, H-5a & H-5b), 1.26 (24H, m, each CH₂), 0.88 (3H, t, J 6.8, CH₃); ¹³C NMR (125 MHz, CDCl₃) δ 137.9, 137.3, 128.5 (2s), 128.1 (2s), 128.0, 127.8 (each Ar-C), 79.7 (C-3 or C-4), 78.4 (C-3 or C-4), 73.5 (CH₂C₆H₃), 72.3 (CH₂C₆H₃), 69.5 (C-1), 61.4 (C-2), 37.4 (CH₃), 31.9, 30.1, 29.7 (5s), 29.6 (2s), 29.4, 25.0, 22.7 (each CH₂), 14.1 (CH₃); ESI-HRMS calcd. C₃₃H₅₁N₃O₄SNa, 624.3447 found m/z 624.3453 [M+Na]⁺.

To a solution of the crude mesylate in dry acetone (15 mL) was added NaHCO₃ (300 mg, 3.5 mmol), Na₂SO₃ (220 mg, 1.74 mmol) and NaI (1.5 g, 7.26 mmol). The resulting mixture was heated at reflux for 16 h. Upon cooling Et₂O and water were added. The layers were separated and the organic layer was washed with brine. The aqueous layer was extracted into Et₂O and the combined organic layers were dried over Na₂SO₄ filtered and the solvent was removed under reduced pressure. Flash chromatography (cyclohexane-EtOAc 15:1) of the crude residue gave 265 (170 mg, 37% over 2 steps) as a colourless oil. ¹H NMR (500 MHz, CDCl₃) δ 7.40 – 7.27 (10H, m, each Ar-H), 4.74 (1H, d, J 11.1), 4.65 (1H, d, J 11.1), 4.61 (1H, d, J 11.6), 4.57 (1H, d, J 11.6)(each CH₂C₆H₃), 3.69 – 3.59 (3H, m, H-2, H-3, & H-4, overlapping signals), 3.49 (1H, dd, J 10.7, 3.0, H-1a), 3.39 (1H, dd, J 10.7, 7.5, H-1b), 1.74 – 1.64 (1H, m, H-5a), 1.62 – 1.55 (1H, m, H-5b), 1.47 – 1.37 (1H, m, CH), 1.29 – 1.23 (23H, m, each CH₂ & CH), 0.89 (3H, t, J 6.8, CH₃); ¹³C NMR (125 MHz, CDCl₃) δ 138.0, 137.6, 128.5, 128.4, 128.1, 128.0, 127.9,
127.8 (each Ar-C), 81.4 (C-2, C-3 or C-4), 78.8 (C-2, C-3 or C-4), 73.9 (CH₂CH₃), 72.1 (CH₂CH₃), 63.8 (C-2, C-3 or C-4), 31.9, 30.2, 29.7 (5s), 29.6 (2s), 25.4, 22.7 (each CH₂), 14.1 (CH₃), 6.5 (C-1).

1-Se-((2R,3S,4R)-2-azido-3,4-O-isopropylidene-3,4-octadecyldiol)-2,3,4-tri-O-benzoyl-β-seleno-D-galactopyranosiduronic acid, methyl ester (261). To a solution of 215 (77 mg, 110 μmol) in dry degassed DMF (1.9 mL) at 0 °C, under argon was added Cs₂CO₃ (72 mg, 220 μmol), piperidine (16 μL, 165 μmol) and a solution via cannula of iodide 53 (217 mg, 440 μmol) in dry degassed DMF (1.9 mL + 1.1 mL wash). The reaction mixture was stirred for 60 min at 0 °C. Et₂O was added and the organic layer was washed with water. The aqueous phase was extracted with a portion of Et₂O and the combined organic layers were washed with brine, dried over Na₂SO₄, filtered and the solvent removed under reduced pressure. Flash chromatography of the crude residue (cyclohexane-EtOAc 5:1) gave 261 (92 mg, 88%) as a yellow oil. Unreacted Iodide was recovered and reused; [α]D +70 (c 1.0, CHCl₃); Rf 0.2 (cyclohexane-EtOAc 6:1); IR (film) cm⁻¹: 2924, 2853, 2114, 1770, 1728, 1452, 1248, 1092, 1067, 1026, 705, 685; ¹H NMR (500 MHz, CDCl₃) δ 8.02 (2H, d, J 7.8), 7.95 (2H, d, J 7.8), 7.79 (2H, d, J 7.9), 7.60 (1H, t, J 7.4), 7.48 (4H, m), 7.38 (2H, t, J 7.7), 7.28 – 7.23 (2H, m)(each Ar-H), 6.23 (1H, d, J 3.4, H-4), 5.89 (1H, t, J 10.0, H-2), 5.64 (1H, dd, J 10.0, 3.4, H-3), 5.14 (1H, d, J 10.1, H-1), 4.60 (1H, s, H-5), 4.14 (1H, dt, J 9.3, 4.5, H-4’), 3.93 (1H, dd, J 9.0, 5.6, H-3’), 3.78 (1H, td, J 8.4, 3.3, H-2’), 3.70 (3H, s, OMe), 3.47 (1H, dd, J 13.2, 3.4, H-1’a), 3.07 (1H, dd, J 13.2, 7.9, H-1’b), 1.58 (3H, m, each CH₂ & CH), 1.39 (3H, s, CH₃), 1.28 (3H, s, CH₃), 1.26 (23H, m, each CH₂ & CH), 0.88 (3H, t, J 6.8, CH₃); ¹³C NMR (125 MHz, CDCl₃) δ 166.0, 165.4, 165.2, 165.2 (each C=O), 133.5, 133.4, 133.3, 130.0, 129.8 (2s), 129.1, 128.9, 128.7, 128.6, 128.4, 128.3 (each Ar-C), 108.2 (CCH₃CH₃), 78.5 (C-3’), 78.0 (C-1), 77.7 (C-4’), 77.3 (C-5), 72.1 (C-3), 69.4 (C-4), 68.5 (C-2), 61.3 (C-2’), 52.7 (OMe), 31.9, 29.7, 29.6 (2s), 29.5, 29.4, 29.3 (each CH₂), 28.1 (CH₃), 26.7, 26.5 (each CH₂), 25.5 (CH₃), 22.7 (CH₂), 14.1 (CH₃); ESI-HRMS calcd. for C₄₉H₆₃N₃O₁₁NaSe, 972.3526 found m/z 972.3517 [M+Na]⁺.
1-Se-((2R,3S,4R)-2-azido-3,4-di-O-tert-butyldimethylsilyl-3,4-octadecyldiol)-2,3,4-tri-O-benzoyl-β-seleno-D-galactopyranosiduronic acid, methyl ester (262). To a solution of 215 (231 mg, 330 μmol) in dry degassed DMF (6 mL) at 0 °C, under argon was added Cs₂CO₃ (215 mg, 660 μmol), piperidine (50 μL, 500 μmol) and a solution via cannula of iodide 264 (342 mg, 500 μmol) in dry degassed DMF (2 mL + 1.2 mL wash). The reaction mixture was stirred for 60 min at 0 °C. Et₂O was added and the organic layer was washed with water. The aqueous phase was extracted with Et₂O (x3) and the combined organic layers were washed with brine, dried over Na₂SO₄, filtered and the solvent removed under reduced pressure. Flash chromatography of the crude residue (cyclohexane-EtOAc 9:1) gave 262 (319 mg, 85%) as a yellow oil. Unreacted Iodide was recovered and reused; [α]D +85 (c 1.0, CHCl₃); Rₚ 0.4 (cyclohexane-EtOAc 6:1); IR (film) cm⁻¹: 2926, 2855, 2108, 1771, 1731, 1452, 1257, 1125, 1092, 1067, 833, 776, 705, 685;¹H NMR (500 MHz, CDCl₃) δ 8.02 (2H, d, J 7.7), 7.94 (2H, d, J 7.8), 7.80 (2H, d, J 7.8), 7.60 (1H, t, J 7.5), 7.50 (1H, t, J 7.5), 7.48 – 7.42 (3H, m), 7.36 (2H, t, J 7.7), 7.26 (2H, d, J 8.0)(each Ar-H), 6.22 (1H, d, J 10.2, H-1), 5.84 (1H, t, J 10.0, H-2), 5.62 (1H, dd, J 9.9, 3.5, H-3), 5.18 (1H, d, J 10.2, H-1), 4.55 (1H, s, H-5), 3.86 (1H, apt-t, J 5.9, H-2'), 3.79 – 3.72 (1H, m, H-4'), 3.70 (3H, s, OMe), 3.60 (1H, dd, J 6.3, 2.9, H-3'), 3.22 (1H, dd, J 13.2, 3.4, H-1’a), 2.90 (1H, dd, J 13.2, 9.0, H-2'b), 1.56 (1H, m, CH), 1.47 (1H, m, CH), 1.26 (24H, s, each CH₂), 0.95 – 0.84 (21H, m, each CH₃), 0.10 (3H, s, CH₃), 0.08 (3H, s, CH₃), 0.06 (6H, s, each CH₃);¹³C NMR (125 MHz, CDCl₃) δ 166.1, 165.5, 165.2, 165.2 (each C=O), 133.5, 133.3, 130.0, 129.8 (2s), 129.1, 129.0, 128.8, 128.6, 128.4, 128.3 (each Ar-C), 78.7, (C-1), 77.6 (C-3’), 77.4 (C-5), 75.1 (C-4’), 72.2 (C-3), 69.4 (C-4), 68.9 (C-2), 65.3 (C-2’), 52.7 (OMe), 33.4, 31.9, 29.8, 29.7 (3s), 29.6 (3s), 29.4, 26.1, 26.0 (2s), 25.5, 22.7, 18.2, 14.1 (CH₃), -3.9 (CH₃), -4.0 (CH₃), -4.5 (CH₃), -4.6 (CH₃); ESI-HRMS calcd. for C₅₈H₈₇Ν₃O₁₁NaSi₂Se, 1160.4942 found m/z 1160.4937 [M+Na]⁺.
1-Se-((2R,3S,4R)-2-azido-3,4-di-O-benzyl-3,4-octadecyldiol)-2,3,4-tri-O-benzoyl-β-
seleno-D-galactopyranosiduronic acid, methyl ester (263). To a solution of 215 (91 mg,
129 μmol) in dry degassed DMF (2 mL) at 0 °C, under argon was added Cs₂CO₃ (84 mg, 258
μmol), piperidine (20 μL, 194 μmol) and a solution via cannula of iodide 265 (123 mg, 194
μmol) in dry degassed DMF (0.8 mL + 0.5 mL wash). The reaction mixture was stirred for 60
min at 0 °C. Et₂O was added and the organic layer was washed with water. The aqueous
phase was extracted with a portion of Et₂O and the combined organic layers were washed
with brine, dried over Na₂SO₄, filtered and the solvent removed under reduced pressure.
Flash chromatography of the crude residue (cyclohexane-EtOAc 15:1-6:1) gave 263 (115 mg,
83%) as a yellow oil. Unreacted Iodide was recovered and reused; [α]D +62 (c 1.0, CHCl₃);
Rf 0.3 (cyclohexane-EtOAc 6:1); IR (film) cm⁻¹: 2924, 2853, 2111, 1768, 1723, 1452, 1259,
1123, 1091, 1067, 1026, 705; ¹H NMR (500 MHz, CDCl₃) δ 8.01 – 7.23 (25H, m, each Ar-
H), 6.16 (1H, dd, J 3.6, 1.4, H-4), 5.87 (1H, t, J 10.0, H-2), 5.57 (1H, dd, J 9.9, 3.5, H-3),
5.06 (1H, d, J 10.1, H-1), 4.66 (1H, d, J 11.5), 4.65 (1H, d, J 11.5), 4.55 (1H, d, J 11.5), 4.50
(1H, d, J 11.5) (each CH₂C₆H₅), 4.33 (1H, d, J 1.4, H-5), 4.07 (1H, dt, J 8.6, 4.4, H-2’), 3.65
(5H, s, H-3’, H-4’ & OMe, overlapping signals), 3.37 (1H, dd, J 13.3, 4.7, H-1’a), 2.99 (1H,
dd, J 13.3, 8.2, H-1’b), 1.70 – 1.54 (2H, m, CH₂), 1.42 – 1.13 (24H, m, CH₂ & CH), 0.88
(3H, t, J 6.9, CH₃); ¹³C NMR (125 MHz, CDCl₃) δ 166.0, 165.4, 165.2, 165.2 (each C=O),
138.3, 137.9, 133.5, 133.4, 133.3, 130.0, 129.8 (2s), 129.1, 128.8, 128.7, 128.5, 128.4 (3s),
128.3, 128.2, 127.8 (2s), 127.7 (each Ar-C), 80.9 (C-3’ or C-4’), 79.4 (C-3’ or C-4’), 78.4 (C-
1), 77.1 (C-5), 73.5 (CH₂C₆H₅), 72.0 (C-3), 71.8 (CH₂C₆H₅), 69.3 (C-4), 68.4 (C-2), 63.9 (C-
2’), 52.7 (OMe), 31.9, 30.1, 29.8, 29.7 (3s), 29.6, 29.4 (each CH₂), 25.7 (C-1’), 25.3 (CH₂),
22.7 (CH₂), 14.1 (CH₃); ESI-HRMS calcd. for C₆₀H₇₁N₃O₁₁NaSe, 1112.4152 found m/z
1112.4181 [M+Na]⁺.
Hexacosanoic acid 2,5-dioxo-pyrrolidin-1-yl ester (271). To a solution of hexacosanoic acid (1 g, 2.52 mmol) in CH₂Cl₂ (60 mL) were added EDC (483 mg, 2.52 mmol) and N-hydroysuccinimide (290 mg, 2.52 mmol). The reaction mixture was heated at reflux for 16 h and then the mixture was poured into water (20 mL) and extracted into Et₂O (60 mL). The organic layer was washed with brine, dried over Na₂SO₄ and the solvent was removed under reduced pressure to give 271 (1.13 g, 91%) as a white solid. The ¹H and ¹³C spectra were in good agreement with reported procedures¹⁷; ¹H NMR (500 MHz, CDCl₃) δ 2.83 (4H, s), 2.60 (2H, t, J 7.5, CH₂), 1.79 – 1.70 (2H, m, CH₂), 1.40 (2H, m, CH₂), 1.25 (42H, m, each CH₂), 0.88 (3H, t, J 6.8, CH₃); ¹³C NMR (125 MHz, CDCl₃) δ 169.1, 168.7 (each C=O), 31.9, 30.9, 29.7 (3s), 29.6 (2s), 29.4, 29.1, 28.8, 25.6, 24.6, 22.7 (each CH₂), 14.1 (CH₃).

(2S,3S,4R)-2-(N-hexacosanoylamino)-1,3,4-octadecanetriol (272). To a solution of ester 271 (1.3 g, 2.63 mmol) in dry THF (20 mL) were added phytosphingosine 56 (695 mg, 2.19 mmol) and NEt₃ (0.9 mL, 6.57 mmol). The reaction was stirred at 50 °C for 16 h. Upon cooling EtOAc (50 mL) was added and the resulting suspension was centrifuged (3000 rpm, 30 min). The upper liquid layer was removed to give 272 (1.39 g, 91%) as a white solid. IR spectra was in good agreement with the reported procedures¹⁷. The insolubility of the title compound at room temperature prevented any further characterisation; IR (film) cm⁻¹: 3313, 2917, 2850, 1709, 1641, 1541, 1470, 1220, 1085, 981, 719.
(2S,3S,4R)-2-(N-hexacosanoylamino)-3,4-di-O-benzoyl-1,3,4-octadecanetriol (273). To a solution of ceramide 272 (500 mg, 718 µmol) in dry pyridine were added triphenylchloromethane (2.8 g 10.2 mmol) and 4-dimethylaminopyridine (6 mg). The reaction mixture was heated at 40 °C for 3 h were upon cooling, water was added and the aqueous phase was extracted into EtOAc (x3). The combined organic layers were washed with NaHCO₃, water, brine, dried over Na₂SO₄ and the solvent was removed under reduced pressure. Flash chromatography of the residue (cyclohexane-EtOAc 8:1-3:1) gave the 1-O-tritylated intermediate (502 mg, 75%) as a white foam. The ¹H and ¹³C spectra were in good agreement with reported procedures¹⁸; ¹H NMR (500 MHz, CDCl₃) δ 7.44 – 7.22 (15H, m, each Ar-H), 6.00 (1H, d, J 8.3, NH), 4.25 (1H, dd, J 8.3, 4.2, -2), 3.60 – 3.53 (1H, m, -3), 3.50 (1H, dd, J 9.9, 3.6, -1a), 3.44 – 3.30 (2H, m, -1b & -4), 3.07 (1H, d, J 8.5, OH), 2.18 – 2.12 (3H, m, CH₂ & OH), 1.74 – 1.54 (3H, m, CH₂ and CH), 1.51 – 1.18 (69H, m, each CH₂ and CH), 0.88 (6H, t, J 6.9, each CH₃); ¹³C NMR (125 MHz, CDCl₃) δ 173.2 (C=O), 143.2, 128.4, 128.1, 127.4 (each Ar-C), 87.7, 75.6, 73.3, 62.9, 50.4, 36.9, 33.3, 31.9, 29.7 (3s), 29.6, 29.5, 29.4 (2s), 29.3, 25.8, 25.7, 22.7, 14.1. To a solution of the above intermediate (792 mg, 844 µmol) in dry pyridine (11 mL) was added benzoyl chloride (440 µL, 5.06 mmol) and 4-dimethylaminopyridine (8 mg). After stirring for 16 h, the reaction mixture was diluted with EtOAc, washed with sat. NaHCO₃, brine, dried over NaSO₄, filtered and the solvent removed under reduced pressure. The resulting residue was taken up in CH₂Cl₂-MeOH (2:1, 22 mL) and p-toluenesulfonic acid monohydrate (80 mg, 420 µmol) was added. After stirring at room temperature for 4 h the solvent was removed under reduced pressure and flash chromatography of the residue (cyclohexane-EtOAc 7:1-4:1-2:1) gave 273 (503 mg, 66% for two steps) as a white solid. The ¹H and ¹³C NMR data for 273 were in good agreement with those previously reported in the literature¹⁸; Rf 0.43 (cyclohexane-EtOAc 4:1); IR (film) cm⁻¹: 3288, 2917, 2850, 1721, 1649, 1544, 1467, 1451, 1265, 708; ¹H NMR (500 MHz, CDCl₃) δ 8.07 – 7.33 (10H, m, each Ar-H), 6.36 (1H, d, J 9.3, NH), 5.41 (1H, dd, J 9.5, 2.4, H-3), 5.37 (1H, dt, J 9.6, 3.0, H-4), 4.39 (1H, tt, J 9.4, 2.8, H-2), 3.70 – 3.56 (2H, m, H-1a & H-1b), 2.79 (1H, bs, OH), 2.29 (2H, t, J 7.6, CH₂), 2.02 (2H, tq, J 13.8, 4.8, 3.8,
CH$_2$), 1.77 – 1.66 (2H, m, CH$_2$), 1.25 (68H, m, each CH$_2$), 0.88 (6H, t, $J$ 6.8, each CH$_3$); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 173.2, 167.2, 166.3 (each C=O), 133.8, 133.1, 130.0, 129.9, 129.6, 129.0, 128.7, 128.4 (each Ar-C), 73.9 (C-3 or C-4), 73.8 (C-3 or C-4), 61.6 (C-1), 49.9 (C-2), 36.9 (CH$_2$), 31.9, 29.7 (3s), 29.6 (4s), 29.5, 29.4 (2s), 29.3 (2s), 28.4, 25.8, 25.7, 22.7 (each CH$_2$), 14.1 (each CH$_3$); ESI-HRMS calcd. for C$_{58}$H$_{97}$NO$_6$Na, 926.7214 found m/z 926.7197 [M+Na$^+$].

1-Se-((2R,3S,4R)-2-hexacosanoylamino-3,4-di-O-benzoyl-3,4-octadecyldi)l)2,3,4-tri-O-benzoyl-$\beta$-seleno-$\alpha$-galactopyranosiduronic acid, methyl ester (269). 273 (102 mg, 113 µmol) was taken up in dry CH$_2$Cl$_2$ (1 mL) and cooled to 0 °C. To this was added triethylamine (48 µL, 340 µmol) and methanesulfonyl chloride (18 µL, 230 µmol). The reaction mixture was warmed to room temperature at stirred for 5 minutes. Et$_2$O (20 mL) was added and the organic layer was washed with water, brine, dried over Na$_2$SO$_4$, filtered and the solvent removed under reduced pressure to give crude mesylate 274. The crude material was dried under high vacuum for 2 h prior to use. To a solution of glycosyl selenium benzoate 215 (40 mg, 57 µmol) in dry degassed toluene (0.2 mL) under argon was added Cs$_2$CO$_3$ (37 mg, 114 µmmol), piperidine (7 µL, 68 µmol) and a solution via cannula of freshly prepared mesylate 274 (vide supra) in dry degassed toluene (0.2 mL + 0.2 mL wash). The reaction mixture was stirred for 3 h at 50 °C. Et$_2$O was added and the organic layer was washed with water. The aqueous phase was extracted with a portion of Et$_2$O and the combined organic layers were washed with brine, dried over Na$_2$SO$_4$, filtered and the solvent removed under reduced pressure. Flash chromatography of the residue (cyclohexane-EtOAc 5:1) gave 269 (40 mg, 48%) as a colourless oil; [$\alpha$]$_D$ +51 (c 1.0, CHCl$_3$); R$_f$ 0.3 (cyclohexane-EtOAc 4:1); IR (film) cm$^{-1}$: 2922, 2852, 1725, 1452, 1260, 1093, 1068, 1026, 707; $^1$H NMR (500 MHz, CDCl$_3$) δ 8.04 – 7.22 (25H, m, each Ar-H), 6.85 (1H, d, $J$ 9.2, NH), 6.21 (1H, dd, $J$ 3.3, 1.2, H-4), 5.77 (1H, t, $J$ 10.0, H-2), 5.61 – 5.55 (2H, H-2’ & H-3’, overlapping signals), 5.42 (1H, dt, $J$ 8.6, 3.8, H-4’), 5.16 (1H, d, $J$ 10.1, H-1), 4.76 – 4.66 (1H, m, H-2’), 4.58 (1H, d, $J$ 1.4, H-5), 3.57 (3H, s, OMe), 3.35 (1H, dd, $J$ 13.6, 5.1, H-1’a), 2.93 (1H, dd, $J$ 13.9, 8.0, H-1’b), 2.34 – 1.05 (74H, m, each CH$_2$), 0.90 – 0.85 (6H, m, each CH$_3$); $^{13}$C NMR (125 MHz,
CDCl$_3$ $\delta$ 173.7, 166.2, 166.0, 165.5, 165.4, 165.3, 165.0 (each C=O), 133.6, 133.4, 133.3 (2s), 133.1, 133.0, 129.9 (2s), 129.8 (2s), 129.7 (2s), 128.8 (2s), 128.7, 128.6, 128.5, 128.4 (2s), 128.3 (each Ar-C), 77.7 (C-1), 76.7 (C-5), 75.3 (C-3 or C-3’), 73.4 (C-4’), 71.9 (C-3 or C-3’), 69.2 (C-4), 68.7 (C-2), 52.7 (OMe), 47.3 (C-2’), 36.5, 31.9, 29.7 (6s), 29.6 (2s), 29.5 (3s), 29.4 (2s), 25.7 (each CH$_2$), 25.4 (C-1’), 22.7 (CH$_2$), 14.1 (each CH$_3$); ESI-HRMS calcd. for C$_{86}$H$_{119}$NO$_{14}$NaSe, 1492.7693 found $m/z$ 1492.7676 [M+Na]$^+$. 

![Image](1-Se-((2R,3S,4R)-2-hexacosanoylamo-3,4-di-O-benzoyl-3,4-octadecyldiol)-2,3,4-tri-O-benzoyl-a-seleno-D-galactopyranosiduronic acid, methyl ester (246). β-glycolipid 269 (40 mg, 27 μmol) was taken up in dry CH$_2$Cl$_2$ (3.6 mL) and cooled to 0 °C. TiCl$_4$ (0.27 mL, 10 eq, 1M in CH$_2$Cl$_2$) was added dropwise and the resulting solution was warmed to room temperature and stirred for 3.5 h. NH$_4$Cl (1M, 10 mL) was added and the mixture was extracted into EtOAc. The organic layer was washed with 1M NaHCO$_3$, water, brine dried over Na$_2$SO$_4$, filtered and the solvent removed under reduced pressure. Flash chromatography of the residue (cyclohexane-EtOAc 6:1) gave 246 (32 mg, 80%) as a colourless oil; [α]$_D$ +37 (c 0.4, CHCl$_3$); R$_f$ 0.7 (cyclohexane-EtOAc 4:1); IR (film) cm$^{-1}$: 2923, 2852, 1725, 1451, 1259, 1093, 1025, 707; $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 8.04 – 7.23 (25H, m, each Ar-H), 6.60 (1H, d, $J$ 9.9, NH), 6.35 (1H, d, $J$ 4.3, H-1), 6.30 (1H, d, $J$ 2.0, H-4), 5.74 – 5.65 (2H, m, H-2 & H-3, overlapping signals), 5.58 (1H, dd, $J$ 8.1, 3.2, H-3’), 5.41 (1H, d, $J$ 1.5, H-5), 5.28 (1H, dt, $J$ 8.3, 3.8, H-4’), 4.87 – 4.74 (1H, m, H-2’), 3.74 (3H, s, OMe), 3.05 (1H, dd, $J$ 13.7, 2.8, H-1’a), 2.92 (1H, dd, $J$ 13.6, 7.3, H-1’b), 2.34 (2H, t, $J$ 7.6, CH$_2$), 1.87 (2H, q, $J$ 7.8, CH$_2$), 1.70 (2H, m, CH$_2$), 1.51 – 1.05 (68H, m, each CH$_2$), 0.87 (6H, m, each CH$_3$); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 173.2, 166.9, 166.3, 165.4, 165.3, 165.2, 165.0 (each C=O), 133.5 (2s), 133.4, 133.2, 133.0 (2s), 129.9 (2s), 129.7 (2s), 129.4, 129.1, 128.9, 128.7, 128.6, 128.4, 128.3 (2s) (each Ar-C), 81.8 (C-1), 75.1 (C-3’), 73.8 (C-4’), 70.8 (C-5), 69.1 (2s), 68.8 (each C-2, C-3 & C-4), 52.8 (OMe), 47.9 (C-2’), 36.6, 31.9, 29.8, 29.7 (3s), 29.6 (3s), 29.5 (2s), 29.4 (2s), 29.3 (each CH$_2$), 28.7 (C-1’), 25.8, 25.7, 22.7 (each CH$_2$), 14.1 (each CH$_3$); ESI-HRMS calcd. for C$_{86}$H$_{119}$NO$_{14}$NaSe, 1492.7693 found $m/z$ 1492.7699 [M+Na]$^+$. 

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1-Se-((2R,3S,4R)-2-hexacosanoylamino-3,4-di-O-benzoyl-3,4-octadecyldiol)-2,3,4-tri-O-benzoyl-α-seleno-D-galactopyranosiduronic acid (276). LiI (29 mg, 218 μmol) was added to a solution of methyl ester 246 (32 mg, 21.8 μmol) in anhydrous EtOAc (1.4 mL) and the reaction mixture was heated to reflux for 16 h. Upon cooling the reaction mixture was diluted with EtOAc and the organic layer was washed with brine. The aqueous layer was extracted into EtOAc (x2) and the combined organic layers were dried over Na₂SO₄, filtered and the solvent removed under reduced pressure gave 276 (29 mg, 91%) as a yellow waxy solid. The compound was used without further purification; IR (film) cm⁻¹: 3341, 2922, 2852, 1724, 1451, 1260, 707; ¹H NMR (500 MHz, CDCl₃) δ 8.04 – 7.23 (25H, m, each Ar-H), 6.51 (1H, d, J 9.7, NH), 6.41 (1H, d, J 4.2, H-1), 6.34 – 6.28 (1H, broad signal, H-4), 5.71 – 5.64 (2H, H-2 & H-3, overlapping signals), 5.49 (1H, dd, J 7.1, 4.1, H-3’), 5.37 (1H, dt, J 8.7, 4.1, H-4’), 5.31 (1H, d, J 1.5, H-5), 4.85 – 4.77 (1H, m, H-2’), 3.06 (1H, dd, J 13.7, 3.4, H-1’a), 2.82 (1H, dd, J 13.7, 8.1, H-1’b), 2.31 (2H, t, J 7.6, CH₂), 1.90 – 1.83 (2H, m, CH₂), 1.24 (68H, d, J 8.8), 0.90 – 0.85 (6H, m, each CH₃); ¹³C NMR (150 MHz, CDCl₃) δ 173.5, 166.3 (HMBC) 165.4, 165.2, 164.9 (each C=O), 133.6, 133.6, 133.5, 133.3, 133.2, 130.0, 129.9, 129.9, 129.8 (2s), 129.7, 129.2, 128.9, 128.7, 128.6, 128.5 (2s), 128.3 (each Ar-C), 82.0 (C-1), 75.5 (HMBC, C-3’), 73.6 (C-4’), 70.7 (C-5), 69.1, 68.7, 68.6 (each C-2, C-3 & C-4, overlapping signals), 48.3 (C-2’), 36.7, 31.9, 29.8, 29.7 (4s), 29.6, 29.5 (2s), 29.4 (3s), 29.3 (each CH₂), 27.9 (HMBC, C-1’), 25.7, 25.5, 22.7 (each CH₂), 14.1 (each CH₃); ESI-HRMS calcd. for C₈₅H₁₁₆NO₁₄Se, 1454.7561 found m/z 1454.7554 [M-H]⁻.
μmol) and PyBOP (22 mg, 46 μmol) in THF (0.5 mL) was added DIPEA (8 μL, 42 μmol).

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The resulting solution was stirred at room temperature for 5 min, after which time NaBH₄ (3 mg, 79 μmol) was added. After stirring for 20 min, Et₂O (20 mL) was added and the organic layer was washed with 5% HCl, sat. NaHCO₃, brine, dried over Na₂SO₄, filtered and the solvent removed under reduced pressure. Flash chromatography of the residue (cyclohexane-EtOAc 6:1) gave 278 (37 mg, 67%) as a waxy solid; [α]D +65 (c 0.46, CHCl₃); Rf 0.3 (cyclohexane-EtOAc 6:1); IR (film) cm⁻¹: 3329, 2922, 2853, 1726, 1655, 1451, 1277, 1259, 707; ¹H NMR (500 MHz, CDCl₃) δ 8.13 – 7.20 (26H, m, each Ar-H & NH), 6.26 (1H, d, J 5.6, H-1), 5.89 (1H, d, J 3.4, H-4), 5.86 (1H, dd, J 9.2, 3.0, H-3’), 5.72 (1H, dd, J 10.7, 5.5, H-2), 5.63 (1H, dd, J 10.8, 3.4, H-3), 5.23 (1H, dt, J 10.6, 2.8, H-4’), 4.89 – 4.80 (2H, m, H-2’ and H-5, overlapping signals), 4.34 (1H, dd, J 9.5, 5.3, OH), 3.99 (1H, dt, J 12.4, 4.2, H-6a), 3.80 (1H, dt, J 12.4, 9.2, H-6b), 3.03 (1H, dd, J 14.5, 2.7, H-1’a), 2.98 (1H, dd, J 14.4, 5.6, H-1’b), 2.32 (2H, t, J 7.6, CH₂), 1.99 – 1.81 (2H, m, CH₂), 1.75 – 1.65 (2H, m, CH₂), 1.24 (68H, s, each CH₂), 0.89 – 0.85 (6H, m, each CH₃); ¹³C NMR (125 MHz, CDCl₃) δ 173.6, 167.4, 165.6, 165.5, 165.2, 165.1 (each C=O), 133.6, 133.4, 133.3, 133.2, 129.9, 129.8, 129.7 (2s), 129.4, 129.1, 129.0, 128.9, 128.6 (2s), 128.4, 128.3, 128.2 (each Ar-C), 83.5 (C-1), 74.6 (C-4’), 73.8 (C-3’), 73.3 (C-2’ or C-5), 69.6 (C-2), 69.4 (C-3), 69.0 (C-4), 61.6 (C-6), 47.5 (C-2’ or C-5), 36.5, 31.9, 30.3, 29.8, 29.7 (3s), 29.6, 29.5 (4s), 29.4, 29.2, 28.0, 25.8, 25.6, 22.7 (each CH₂), 14.1 (each CH₃); ESI-HRMS calcd. for C₅₈H₁₁₉NO₁₃NaSe, 1464.7744 found m/z 1464.7743 [M+Na]+.

1-Se-((2R,3S,4R)-2-hexacosanoylamino-3,4-octadecyldiol)-α-seleno-D-galactopyranosiduronic acid (277). Protected glycolipid 276 (12 mg, 8 μmol) was taken up in a mixture of dry THF (1.2 mL) and MeOH (1.2 mL). NaOMe (1M in MeOH, 0.12 mL) was added and the reaction mixture was stirred for 4 h. Water (1 drop) was added and the mixture was stirred for a further 16 h. AcOH (1 drop) was added and the solvent was removed under reduced pressure. The resulting residue was taken up in MeOH and the suspension was transferred to a 2 mL centrifuge tube and centrifuged at 15000 rpm for 5 min. The supernatant was removed and the precipitate was washed with a further portion of
methanol followed by two portions of water. Lyophilisation gave 277 (6 mg, 80%) as a white amorphous solid; $^1$H NMR (600 MHz, CDCl$_3$/MeOD/TFA 2:1:1 drop) δ 5.67 (1H, d, $J = 5.2$, H-1), 4.57 (1H, s, H-5), 4.11 (1H, s, H-4), 4.04 (1H, s, H-2'), 3.80 (1H, dd, $J = 9.8$, 5.2, H-2), 3.41 (1H, dd, $J = 9.7$, 3.3, H-3), 3.36 – 3.28 (2H, m, H-3' & H-4', overlapping signals), 2.80 – 2.64 (2H, m, H-1’a & H-1’b, overlapping signals), 2.01 (2H, t, $J = 7.5$, CH$_2$), 1.42 (3H, m, CH$_2$ & CH), 1.34 (1H, s, CH), 1.07 (6H, s, each CH$_3$), 0.69 (6H, t, $J = 6.8$, each CH$_3$);

$^{13}$C NMR (150 MHz, CDCl$_3$/MeOD/AcOD 2:1:1 drop) δ 174.4 (C=O, HMBC), 171.3 (C=O, HMBC), 84.2 (C-1), 76.0 (C-3' or C-4'), 72.4 (C-5), 71.9 (C-3' or C-4'), 71.1 (C-3), 69.4 (C-4), 68.1 (C-2), 51.3 (C-2'), 36.2, 32.2, 31.6, 29.4, 29.3 (2s), 29.1 (2s), 29.0 (2s), 25.6, 25.5 (each CH$_2$), 23.9 (C-1'), 22.3 (CH$_2$), 13.7 (each CH$_3$); ESI-HRMS calcd. for C$_{50}$H$_{96}$NO$_9$Se, 934.6250 found m/z 934.6267 [M-H]$^-$.

$^{1}$Se-((2R,3S,4R)-2-hexacosanoylamino-3,4-octadecyldiol)-α-seleno-D-galactopyranoside (245). Protected glycolipid 278 (12 mg, 8.3 μmmol) was taken up in THF (1.2 mL), MeOH (1.2 mL) and water (2 drops). NaOMe (1M in MeOH, 120 μL) was added and the reaction mixture was stirred for 3 h. AcOH (2 drops) was added and the solvent was removed under reduced pressure. The resulting residue was taken up in MeOH and the suspension was transferred to a 2 mL centrifuge tube and centrifuged at 15000 rpm for 5 min. The supernatant was removed and the precipitate was washed with a further portion of methanol followed by two portions of water and a final portion of methanol to give 245 (7.6 mg, 95%) as a white amorphous solid; $^1$H NMR (500 MHz, pyridine-$d_5$/D$_2$O (1drop)) δ 6.53 (1H, d, $J = 5.4$, H-1), 5.30 (1H, dt, $J = 10.4$, 5.1, H-2'), 4.91 (1H, dd, $J = 9.7$, 5.3, H-2), 4.82 (1H, t, $J = 6.0$, H-5), 4.60 (1H, dd, $J = 11.4$, 7.2, H-6a), 4.53 (1H, d, $J = 2.9$, H-4), 4.49 – 4.37 (3H, m, H-3, H-3' & H-6b, overlapping signals), 4.31 (1H, t, $J = 7.5$, H-4'), 3.73 – 3.66 (2H, m, H-1’a & H-1’b), 2.59 (2H, dd, $J = 8.2$, 6.1, CH$_2$), 2.31 (1H, s, CH), 2.06 – 1.85 (3H, m, CH$_2$ & CH), 1.75 (1H, s, CH), 1.53 – 1.23 (67H, m), 0.98 – 0.89 (6H, m); $^{13}$C NMR (125 MHz, pyridine-$d_5$) δ 174.0 (C=O), 86.5 (C-1), 78.5 (C-3'), 75.5 (C-5), 73.6 (C-3), 73.2 (C-4'), 71.1 (C-4), 70.8 (C-2), 63.4 (C-6), 53.9 (C-2'), 37.4, 34.6, 32.6 (2s), 30.8, 30.7, 30.5, 30.5 (6s), 30.4 (2s), 30.3 (2s),

![Chemical structure](image.png)
30.1 (2s), 27.5, 27.1, 27.0 (each CH₂), 24.1 (C-1’), 23.4 (CH₂), 14.8 (each CH₃); ESI-HRMS calcd. for C₅₀H₉₈NO₈Se, 920.6458 found m/z 920.6468 [M-H]⁻.

1-Se-((2R,3S,4R)-2-hexacosanoylamino-3,4-octadecyldiol)-β-seleno-D-galactopyranoside (279). Alcohol 273 (70 mg, 77.4 µmol) was taken up in dry CH₂Cl₂ (0.7 mL) and cooled to 0 °C. To this was added triethylamine (33 µL, 232 µmol) and methanesulfonyl chloride (12 µL, 155 µmol). The reaction mixture was warmed to room temperature at stirred for 5 minutes. Et₂O (20 mL) was added and the organic layer was washed with water, brine, dried over Na₂SO₄, filtered and the solvent removed under reduced pressure to give crude mesylate 274. The crude material was dried under high vacuum for 2 h prior to use. To a solution of 209 (40 mg, 57 µmol) in dry degassed toluene (0.14 mL) under argon was added Cs₂CO₃ (37 mg, 114 µmol), piperidine (7 µL, 68 µmol) and a solution via cannula of freshly prepared mesylate 274 (vide supra) in dry degassed toluene (0.14 mL + 0.14 mL wash). The reaction mixture was stirred for 3 h at 50 °C. Et₂O was added and the organic layer was washed with water. The aqueous phase was extracted with a portion of Et₂O and the combined organic layers were washed with brine, dried over Na₂SO₄, filtered and the solvent removed under reduced pressure. The protected β-glycolipid was found to be difficult to purify and was taken onto the next step without further purification. The crude material was taken up in THF (3.3 mL), MeOH (3.3 mL) and water (5 drops). NaOMe (1 M in MeOH, 300 µL) was added and the reaction mixture was stirred for 3 h. AcOH (5 drops) was added and the solvent was removed under reduced pressure. The resulting residue was taken up in MeOH and the suspension was transferred to a 2 mL centrifuge tube and centrifuged at 15000 rpm for 5 min. The supernatant was removed and the precipitate was washed with a further portion of methanol followed by two portions of water and a final portion of methanol to give 279 (6.5 mg, 17% for two steps) as a white amorphous solid; ¹H NMR (500 MHz, pyridine-d₅) δ 8.59 (1H, d, J 8.6, NH), 7.38 (1H, s, OH), 6.90 (1H, s, OH), 6.68 (1H, d, J 5.6, OH), 6.61 (1H, s, OH), 6.44 (1H, s, OH), 6.02 (1H, d, J 6.9, OH), 5.37 (1H, d, J 9.5, H-2), 5.33 – 5.22 (1H, m, H-2’), 4.63 (1H, t, J 9.8, H-3), 4.56 – 4.46 (2H, m, H-4 & H-6a, overlapping signals), 4.38 (1H, d, J 6.4, H-3’), 4.35 – 4.24 (1H, m, H-6b), 4.24 – 4.14 (1H, m, H-4’), 4.10 – 4.01 (2H, m, H-3 & H-5,
overlapping signals), 3.95 (1H, d, J 12.9, H-1’a), 3.59 – 3.53 (1H, m, H-1’b), 2.51 (2H, tt, J 14.1, 6.8, CH$_2$), 2.32 – 2.21 (1H, m, CH), 1.87 (4H, m, CH$_2$), 1.67 (1H, s, CH), 1.47 – 1.15 (66H, m, each CH$_2$), 0.87 (6H, t, J 6.8, each CH$_3$); $^{13}$C NMR (125 MHz, pyridine-d$_5$) δ 174.0 (C=O), 83.0, 82.8, 78.1 (C-3’), 77.0, 73.7 (C-2), 73.2 (C-4’), 71.3 (C-4), 63.4 (C-6), 52.6 (C-2’), 37.4, 34.7, 32.6, 30.8, 30.6, 30.5 (2s), 30.4 (2s), 30.3 (2s), 30.1 (2s), 27.1, 26.9 (each CH$_2$), 25.3 (C-1’), 23.4 (CH$_2$), 14.8 (each CH$_3$); ESI-HRMS calcd. for C$_{50}$H$_{98}$NO$_8$Se, 920.6458 found m/z 920.6466 [M-H]$^-$.

5.5 References

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